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Histopathologic findings in erythromelalgia are nonspecific: special studies show decreased small nerve fiber density

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Erythromelalgia is a rare disorder characterized by burning pain, warmth and redness of the extremities. There have been few reports of the histopathologic findings in skin biopsies from the affected areas. Our objective is to report the results of skin biopsies of patients with erythromelalgia seen at Mayo Clinic. The Mayo Clinic patient database was retrospectively searched for the records of patients fulfilling the criteria for the diagnosis of primary erythromelalgia and who had skin biopsy of the affected area. Thirty-three skin biopsy specimens from 29 patients with a confirmed clinical diagnosis of primary erythromelalgia were reexamined. Vascular thrombi were not identified on any of the histologic sections of the 33 skin biopsies examined. Perivascular lymphocytic inflammation (73%), perivascular edema (52%), smooth muscle hyperplasia (87%) and swelling (78%), thickening of the arteriolar basement membrane (65%) were noted. Endothelial swelling (67%), and endothelial nuclear enlargement (73%), capillary hyperplasia (55%) and capillary ectasia (36%) were also observed. Using confocal microscopy, a relative decrease in nerve density surrounding superficial dermal blood vessels was observed. The histopathologic changes in primary erythromelalgia are subtle and non-specific; intraluminal thrombi are not observed. The most consistent findings include mild perivascular lymphocytic inflammation and edema, capillary hyperplasia and ectasia, arteriolar smooth muscle cell alteration (hyperplasia and swelling), and enlarged endothelial cell nuclei. However, these findings are subtle and may also be observed in venous hypertension and chronic lower-extremity edema. Our present morphological observations that epidermal nerve density was decreased in 81% of the patients evaluated supports our previous reports that, functionally, patients with erythromelalgia have a small fiber neuropathy.

2

Epidermal VEGF is required for permeability barrier homeostasis, dermal angiogenesis and development of epidermal hyperplasia: implications for the provocation of psoriasis

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Primary abnormalities in permeability barrier function appear to underlie atopic dermatitis; and increasingly, barrier dysfunction is seen as the 'driver' of other inflammatory dermatoses, including psoriasis. Central to this 'outside-inside' disease pathogenesis view is epidermal generation of cytokine/growth factors, which signal downstream, epidermal repair mechanisms, with sustained stimulus ultimately generating epidermal hyperplasia and inflammation. Psoriasis is often provoked by external trauma (isomorphic (Koebner) phenomenon), and is associated with excess VEGF production, prominent angiogenesis, and epidermal hyperplasia. We found here that epidermal-derived, vascular endothelial growth factor (VEGF) is required for normal permeability barrier homeostasis, dermal angiogenesis, and development of epidermal hyperplasia. Specifically, acute barrier disruption (simulating the Koebner phenomenon) rapidly up-regulates epidermal VEGF-A mRNA expression in normal hairless mice, a specific response to permeability barrier requirements, as up-regulation is blocked by application of a vapor-impermeable membrane. Epidermal-VEGF-/- mice also display: 1) abnormal permeability barrier homeostasis, attributable to decreased lamellar body production; 2) a paucity of dermal capillaries; and 3) reduced vascular permeability. Finally, epidermal VEGF-/- mice develop neither angiogenesis nor epidermal hyperplasia after repeated tape stripping (a model of psoriasiform hyperplasia). These results support: 1) a central role for epidermal VEGF in epidermal permeability barrier homeostasis; and 2) a link between epidermal VEGF production, dermal angiogenesis, and development of epidermal hyperplasia. These results could provide an 'outside-inside' mechanistic basis for the provocation of psoriasis.

3

Activation of VEGFR-3 signaling attenuates UVB-induced edema formation and inflammation by inducing lymphangiogenesis

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We have previously demonstrated that systemic blockade of vascular endothelial growth factor (VEGF) receptor-3 signaling prolonged the ultraviolet-B (UVB)-induced edema formation and inflammation in the skin, and that UVB irradiation resulted in impaired function of cutaneous lymphatic vessels. These results suggested a crucial role of lymphatic vessel function in the mediation of UVB-induced cutaneous inflammation. Nonetheless, the molecular mechanisms of lymphatic involvement in cutaneous inflammation induced by UVB have remained unclear. Here, we show that VEGF-C expression is downregulated after an acute UVB irradiation, associated with enlargement of lymphatic vessels and with an increase of macrophage infiltration in the dermis. To determine whether activation of VEGF-C/VEGFR-3 signaling might reduce UVB-induced inflammation, mice were exposed to a single dose of UVB irradiation together with intradermal injection of PBS or of mutant VEGF-C (Cys156Ser), which specifically binds to VEGFR-3 on lymphatic endothelium. We found that the activation of VEGFR-3 attenuated UVB-induced edema formation, associated with a decreased number of CD11b-positive macrophages in the dermis as compared to the vehicle treated mouse ears. To elucidate the mechanisms of prevention of UVB-induced inflammation by treatment with mutant VEGF-C, immunohistochemical and quantitative image analyses of lymphatic and blood vessels were performed. We found that UVB-induced enlargement of lymphatic vessels was inhibited in the ears treated with mutant VEGF-C, compared to vehicle-treated mouse ears, and that the number of lymphatic vessels was increased. Treatment with mutant VEGF-C had no effect on blood vessel size or number. These results indicate that UVB-induced lymphatic impairment is mediated by downregulation of VEGF-C expression and that the activation of the VEGF-C/VEGFR-3 pathway might be a suitable target for prevention of UVB-induced inflammation by promoting lymphangiogenesis.

4

Sphingosine 1-Phosphate attenuates H_2O_2 -induced apoptosis in cultured vascular endothelial cells -Roles of caspase-3 protease and p38 MAP kinase-

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Background Reactive oxygen species, notably H_2O_2, lead vascular endothelial cells to undergo apoptosis, associated with loss of vascular integrity and function. Sphingosine 1phosphate (S1P) is a platelet-derived sphingolipid mediator that elicits various (patho)physiological responses in endothelial cells. Objectives We aimed to explore whether and how S1P modulates endothelial apoptotic responses induced by H_2O_2. Methods Cultured bovine aortic endothelial cells (BAEC) were used as a model. The degrees of intracellular DNA fragmentation were determined using ELISA. The degrees of DNA nick formation were determined *in situ* by means of TUNEL staining. Activity of caspase-3 as well as MAP kinases p38 and JNK were analyzed by immunoblot analyses (IB) using antibodies specific to active (cleaved or phosphorylated) forms of these proteins. Results Treatment of BAEC with H_2O_2 (750 μmol L^{-1} for 6 hours) led to DNA fragmentation (ELISA), DNA nick formation (TUNEL stainig), and cleavage of caspase-3 (IB), key features of endothelial apoptosis. These responses induced by H_2O_2 were alike markedly attenuated by pretreatment with S1P (1 μmol L^{-1}, 30min). When BAEC were treated with H_2O_2, both p38 and JNK MAP kinases underwent robust phosphorylation. However, pretreatment with S1P decreased phosphorylation of only p38 MAP kinase, but not that of JNK; conversely, an inhibitor of p38 MAP kinase, but not that of JNK, attenuated H_2O_2-induced caspase-3 activation. Conclusions S1P attenuates H_2O_2-induced apoptosis of cultured bovine aortic endothelial cells, at a level of, or proximal to, p38 MAP kinase. These results may identify a novel point of control at which ROS and sphingolipids determine endothelial cell fate.

5

An ethanol extract of *Grifola frondosa* (Maitake mushroom) enhances the anti-angiogenic and anti-tumorigenic actions of polymethoxyflavonoid, nobiletin, *in vitro*

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Flavonoids from medicinal plants possess pharmacological effects for preventing tumor progression by inhibiting tumor-cell proliferation, neoplastic angiogenesis, and tumor invasion. We reported that a citrus polymethoxyflavonoid, nobiletin (5,6,7,8,3′,4′-hexamethoxy flavone), exhibits anti-tumor-invasive and antitumorigenic actions *in vivo* and *in vitro*. In addition, water extract of *Grifola frondosa* (Maitake mushroom) has been reported to possess antitumor effects by enhancing the immune system. However, the anti-angiogenic actions of nobiletin and Maitake mushroom extract remain unclear. In the present study, we examined whether nobiletin and an ethanol extract of the Maitake mushroom (Maitake extract) influenced the tube formation of human microvascular endothelial cells (HMVEC). Nobiletin dose-dependently inhibited tube formation, whereas the Maitake extract did not show any effect on HMVEC. When HMVEC were co-treated with both nobiletin and Maitake extract, the nobiletin-mediated inhibition of tube formation was enhanced. Another citrus polymethoxyflavonoid, tangeretin, did not inhibit tube formation by HMVEC even in the presence of Maitake extract. On the other hand, nobiletin slightly but significantly inhibited the proliferation of human melanoma Mewo cells in a dose-dependent manner, whereas the effect of Maitake extract on melanoma cell growth was negligible. The combination of nobiletin and Maitake extract synergistically inhibited tumor cell proliferation. In contrast, there was no change in cell proliferation in tangeretin and/or Maitake extract-treated Mewo cells. Thus, these results suggest that nobiletin exhibits an anti-angiogenic effect by inhibiting the tube formation of vascular endothelial cells. Moreover, the combination of Maitake extract and nobiletin is likely to be a novel clinical strategy for cancer therapy against melanoma to prevent both angiogenesis and tumorigenesis.

6

Treatment of bone marrow-derived mesenchymal stromal cells with the artificial dermis accelerates skin wound healing of diabetic rats

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Diabetes causes delayed or impaired wound healing, leads to chronic ulcer formation and treatment often requires long-term hospitalization. Based on current advances in stem cell therapy, the use of autologous bone marrow-derived mesenchymal stromal cells (MSCs) is considered for the treatment of diabetic wound sites. However, little information is available concerning the behavior of self MSCs used during diabetic wound healing. To understand the role of transplanted MSCs to the wound site under diabetic conditions, we investigated the relationship between the fate and healing effect of transplanted MSCs in a diabetic rat skin wound model. Diabetic rats were induced by intravenous injection of streptozotocin. A full-thickness skin (2×2 cm^2 in size) was excised on the head of diabetic rats, and covered with an artificial dermis impregnated with either bone marrow cells (BMCs) or bone marrow-derived MSCs from firefly luciferase (luc) transgenic (Tg) rats or LacZ Tg rats. The effect of wound healing was assessed using planimetry and immunohistochemistry, and the fate of transplanted MSCs was chased using *in vivo* luminescent imaging. The diabetic wound site treated with MSCs-impregnated artificial dermis was significantly smaller than that treated with artificial dermis alone at 1 week post-operation. Luminescence intensity of luc+ MSCs was detected at the transplanted site during healing (3 weeks), whereas that of luc+ BMCs faded out only after 1 week post-implantation. In immunohistochemistry, we demonstrated that CD31+ vessels at the wound site treated with LacZ+ MSCs were increased with expression of vascular endothelial growth factor, suggesting that MSCs accelerate angiogenesis. These findings suggest that transplanted MSCs could be held at wound sites during the healing process in a diabetic rat model, and that they promote wound healing through angiogenesis.

7

Expression and functional study of vascular endothelial growth factor receptors in normal human and psoriatic epidermal keratinocytes

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Angiogenesis is the complex process by which new blood vessels arise from the pre-existing vasculature under physical or pathological conditions. Angiogenesis is controlled by some growth factors and their receptor tyrosine kinases. Among them, the most important is vascular endothelial growth factors and their receptors, VEGF receptors (VEGFRs), which include VEGFR-1, VEGFR-2, VEGFR-3 and their co-receptors, neuropilins (including NRP-1 and NRP-2). We investigated the expression of VEGFR in normal human skin and psoriatic nonlesional, perilesional and lesional skin, the effect of VEGF on proliferation, migration and adhesion of epidermal keratinocytes, the functional changes of VEGFRs in different stages of psoriasis and the regulating effect of calcium and VEGF on the expression of VEGFRs in psoriasis. Our results showed that VEGFR-1, VEGFR-2, VEGFR-3 and their co-receptors, NRP-1 and NRP-2 were expressed in epidermal keratinocytes *in vitro* at mRNA and protein levels. VEGFRs and NRPs were expressed in normal epidermis except for the corny layer. VEGFR-1 and VEGFR-2 were localized primarily in basal cell layer and adjacent spinosum stratum, while VEGFR-3, NRP-1 and NRP-2 were uniformly expressed in the epidermis. VEGF could enhance the proliferation and migration, while inhibit the adhesion of epidermal keratinocytes partly through VEGFR-2. Furthermore, VEGFR-1, VEGFR-2 and VEGFR-3 were overexpressed by psoriatic lesional keratinocytes, as compared with normal epidermal keratinocytes, at mRNA and protein levels. Our results suggested that the distribution pattern of VEGFR-1, VEGFR-2 and VEGFR-3 be associated with proliferation, migration and differentiation of psoriatic epidermis and that both VEGF and calcium be two factors enhancing expression of VEGFR-1, VEGFR-2 and VEGFR-3 in psoriasis, whereas the effect of calcium be independent of VEGF.

8

SDF-1α/CXCR4-enhanced angiogenesis of human basal cell carcinoma cells involves transcriptional regulation of interleukin-6 (IL-6) via ERK1/2-NF-κB pathway

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Tumor growth and metastasis depend upon the ability to induce its own blood supply. SDF-1α (CXCL12) has been found that can enhance tumor angiogenesis. We have previously demonstrated human basal cell carcinoma (BCC) tissues and a BCC cell line had significant expression of CXCR4, which was higher in invasive than non-invasive BCC types. In this study, we studied the expression profiles of SDF-1α/CXCR4- regulated angiogenesis-associated genes in BCC cells by using a cDNA oligonucleotide microarray and also elucidate the underlying mechanism. We found that human BCC tissues with high expression of CXCR4 had higher vascularity. Further, among 71 BCCs diagnosed during 2004-2006, BCCs with high CXCR4 expression had higher microvessel density (MVD) as compared to those with low CXCR4 expression (15.9 ± 4.6 vs. 7.8 ± 3.1, $P < 0.0001$). We found that SDF-1α induced significant *in vitro* and *in vivo* angiogenic activity in human BCC cells, evidenced by more tube formation of HUVECs and high MVD in mouse xenografts. SDF-1α significantly up-regulated several angiogenesis-associated genes, including *IFI27*, *IL-6*, *BMP-6*, *SOCS2*, and *PTGS2 (COX-2)* in human BCC cells. IL-6 was the earliest up-regulated pro-angiogenic factor and major determinant of SDF-1α-induced BCC angiogenesis. The angiogenic activity induced by SDF-1α was mediated by ERK/NF-κB/IL-6 pathway in human BCC cells. The mouse tumorigenicity study also revealed co-expression of CXCR4 and IL-6 in the xenografts.

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Stimulatory effect of adenosine on cutaneous post pressure hyperemia in the rat

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When pressure is applied directly to the skin to occlude blood flow, a brisk and prolonged increase in blood flow follows. This may represent a mechanism to protect the skin from pressure induced ischemic damage. We infused several vasoactive agents subcutaneously to assess a potential effect on post pressure hyperemia. We used an infusion pump and subcutaneous microdialysis tubing to administer saline solutions containing the agents. After obtaining baseline readings with a laser Doppler probe for five minutes, we applied pressure using a vise clamp directly to the probe, occluding flow for 10 minutes. We then released pressure and measured flow post pressure for 20 minutes. The area under the flow curve was determined and the ratio of the area post pressure to the area during the baseline period was computed. Saline infusion without the agent was used as the control condition. We compared the ratios with the agent to those with saline. A number of agents were infused. Epinephrine suppressed hyperemia from 4.3±0.5 to 2.7±0.4*. Norepinephrine and L-NAME, a suppressor of nitric oxide synthetase, had no effect. Adenosine infusion stimulated the hyperemia ratio from 3.6±0.3 to 4.5±0.4*. DMPX, an adenosine 2 receptor antagonist, suppressed the ratio to 1.9±0.2**. DXPX, an adenosine 1 receptor antagonist had no effect. * $p<0.05$; ** $p<0.01$. We concluded that Adenosine appears to stimulate post pressure cutaneous hyperemia perhaps through the adenosine type 2 receptor. This effect may be of therapeutic benefit in patients with cutaneous ischemia.

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Clinicopathologic differentiation of arteriovenous malformation of early stage with capillary malformation of port-wine stain

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During early stage such as stage I, arteriovenous malformation (AVM) may not be apparent or even mimic port-wine stain (PWS) clinicopathologically and therefore they may be misdiagnosed as PWS. So we herein suggest the clinicopathological differential clues of early AVM from PWS in earlier time. We selected a set of radiologically-proven 10 AVMs of stage I mimicking PWS and 10 age and sex matched PWSs as a control. We reviewed their clinical features and analyzed H&E, CD31, smooth muscle actin (SMA) immunohistochemistry. We found 2 clinical and 4 pathologic points for their differentiation. Among clinical differential point, vivid color of AVM and PWS were found in 40% and 20%, respectively ($p>0.05$). AVM and PWS showing telangiectasia around or on the lesions were 30% and 20%, respectively ($p>0.05$). As for histopathologic differential points, first, the capillary density of AVM (35.9/HPF) was significantly higher than that of PWS (12.3/HPF) by CD31 result ($p<0.05$). Second, more elongated and haphazardly branching vessels were found significantly frequent by SMA stains in the superficial dermis of AVM (90%) than in PWS (20%) ($p<0.05$) and some of them had antler shaped. And the vessel lumen of superficial dermis contained no or few RBC in AVM (10%), but PWS (80%) did RBC lump ($p<0.05$). Finally, 80% of AVM contained the SMA-positive thickened vessel's wall, but 10% in PWS ($p<0.05$). So we propose several differential clues of AVM of stage I from PWS. Furthermore there might be some possibilities that early stage AVM may be misdiagnosed as PWS and tried unwise management like laser therapy.

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Nuclear translocation of phosphorylated STAT3 is essential for lymphatic endothelial cell migration induced by VEGF-A

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Recent studies indicate a major role of the VEGF-A/VEGFR-2 signaling pathway in lymphangiogenesis. VEGF-A induces proliferation of lymphatic endothelial cells (LEC). However, the mechanism of VEGF-A in LEC migration has not been well characterized. We studied the signal transduction pathway of VEGF-A induced migration in human dermal microvascular lymphatic endotherial cells (HDMLEC) and report here the involvement of signal transducers and activators of transcription 3 (STAT3) in HDMLEC migration. First, the effect of VEGF-A on HDMLEC was examined by Boyden chamber assay. VEGF-A enhanced HDMLEC migration in a dose-dependent manner, with a 2 fold optimum at 33ng/ml in 6 hrs. Then, nuclear translocation of phosphorylated STAT3 was assessed by Wesetrn Blotting and con-focal microscopy using anti- phosphorylated STAT3 antibody. No phosphorylated STAT3 was observed in nuclei before the addition of 33ng/ml of VEGF-A. However, translocation of phosphorylated STAT3 into nuclei was found 30 min after the addition of VEGF-A and reached an optimum at 60 min. To prove the functional involvement of STAT3 phosphorylation in HDMLEC migration, transfection of dominant negative STAT3 adenovirus vector was performed. Transfection of dominant negative STAT3 adenovirus at M.O.I. of 10 almost completely abolished VEGF-A-induced HDMLEC migration as well as nuclear translocation of phosphorylated STAT3. In conclusion, nuclear translocation of phosphorylated STAT3 is essential for VEGF-A-induced HDMLEC migration.

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Topical application of anti-angiogenic peptides based on pigment epithelium-derived factor can improve psoriasis

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Although the number of capillary vessels is increased in psoriatic lesions, the role of angiogenesis in psoriasis has not been fully elucidated. Pigment epithelium-derived factor (PEDF) has been shown to exhibit strong anti-angiogenic activity. We previously determined that PEDF is expressed in skin, and that angiogenic factors, such as vascular endothelial growth factor (VEGF), can overcome PEDF anti-angiogenic activity in psoriatic skin. The purpose of this study was to identify low-molecular weight PEDF peptides with significant anti-angiogenic effects that can penetrate the epidermis and demonstrate efficacy in the treatment of psoriasis. Initially, we identified three potential PEDF peptides (P5-1, 2, 3) (MW <850D) that exhibited anti-angiogenic properties using screens of proteolytic fragmented peptides derived from PEDF. When biotin-labeled PEDF peptides were applied to skin, these PEDF peptides were subsequently detected in the dermis and colocalized to endothelial cells. We then assessed the therapeutic potential of the PEDF peptides after topical application on to human psoriatic skin grafted onto severe combined immunodeficient mice. The PEDF peptides (1 mM) were applied to the grafted site each day for 10 days. After two weeks treatment, topical application of all these peptides significantly reduced the epidermal thickness of the grafted area. The number of CD31-positive capillary endothelial cells in the papillary dermis was also significantly reduced after treatment. These data suggest that certain low-molecular weight PEDF peptides might be useful in novel therapeutic strategies to treat psoriasis.

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Evidence for co-dependence within the cutaneous neurovascular niche: immunocyte, nerve and blood vessel interactions

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Skin cells communicate through tightly regulated signaling pathways which control keratinocyte (KC) proliferation, angiogenesis, nerve innervation and immunocyte response. Using mice genetically engineered to overexpress either angiopoietin-1 (Ang1) or its receptor Tie2 in KCs or endothelial cells (ECs) we analyzed the phenotypic characteristics of epidermal, vascular, neural and immune systems in KC-Ang1, KC-Tie2, and EC-Tie2 mice. Each mouse line showed unique modifications to their cutaneous anatomy compared with each other and littermate controls, including a 4 fold increase in epidermal thickening in KC-Tie2 mice and a 2, 1.8, and 1.4 fold increase in number of blood vessels in KC-Tie2, KC-Ang1, and EC-Tie2 mice respectively (all $p<0.05$). Concurrent with vascular variation, distinctive changes in peripheral nerve patterning were found: KC-Tie2 mice had thinner more numerous fibers, KC-Ang1 mice had fewer but thicker fibers and EC-Tie2 mice had thinner more highly branched nerves. Changes were also observed in all mice for location and number of $CD4^+$ and $CD8^+$ T cells, $CD11c^+$ dendritic cells and $F4/80^+$ macrophages (KC-Tie2>KC-Ang1≥EC-Tie2≥control). Microarray analyses comparing KC-Tie2 and control mouse skin identified a 2-6 fold increase in expression of genes involved in KC proliferation and differentiation (EGF, epiregulin, epithelial mitogen) and angiogenesis (HIF1α, VEGFR1), a 5-30 fold increase in genes involved in inflammation (interleukins, S100s) and a 2-20 fold increase in neural synaptic genes (synaptotagmin, neurexin, synapsin). Interestingly, a 3-5 fold increase in proinflammatory and proangiogenic signaling (CXCL7, CXCL1) was found concurrent with a 2-4 fold decrease in anti-inflammatory and angiostatic responses (CXCL9, CXCL10). These data provide evidence that regardless of the cellular source of altered gene expression (KC vs EC) physiological alteration in other tissues occurs. This suggests that targeted therapeutic strategies need to consider multiple cutaneous tissue responses.

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p120-catenin plays an essential role in vascular development

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VE-cadherin (VE-cad) is a central regulator of angiogenesis and endothelial barrier function. p120-catenin (p120) binds to the VE-cad cytoplasmic domain and regulates VE-cad endocytosis. In addition, p120 plays a key role in regulating the actin cytoskeleton and is implicated in cell migration and proliferation. To define the role p120 in vascular development, a conditional gene ablation system was used. Mice harboring a "floxed" p120 allele were mated with transgenic animals expressing Cre recombinase via the endothelial-specific Tie2 promoter. Mice lacking endothelial p120 frequently died embryonically. At E9.5, major blood vessels in the yolk sac and embryo appeared normal in p120 null animals, suggesting that initial stages of vascuogenesis proceeded normally. However, by E12.5 small vessels in the placenta, yolk sac, brain, and other regions of the embryo exhibited striking abnormalities that included defects in vessel branching and patterning. Overall vessel density was also decreased in p120 null embryos, and p120 deleted embryos were typically smaller and paler than controls. Furthermore, at E12.5 p120 null embryos exhibited hemorrhages in the brain and other organs, suggesting defects in vessel barrier properties. To determine the mechanism underlying these vascular defects and embryonic lethality, we examined VE-cad levels in mice lacking endothelial p120. Interestingly, VE-cad was detectable but expression levels were reduced in vessels lacking p120. In addition, endothelial cells were isolated from the p120 floxed mice and adenoviral delivery of Cre recombinase was used to excise the p120 gene. Cultured endothelial cells lacking p120 exhibited striking decreases in VE-cad levels and dramatically reduced barrier function. Collectively, these results demonstrate an essential role for p120 in regulating VE-cad functions in the context of mouse vascular development.

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AMP-activated protein kinase-β1 overexpression alters the senescence profile in human dermal microvascular endothelial cells

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Previously, using proteomic study, we reported that expression of AMP-activated protein kinase-β1 (AMPK-β1), one of the AMP-activated protein kinases, was increased with age in human dermal microvascular endothelial cells (HDMECs). Also in an *in vivo* study using Caenorhabditis elegans (C. elegans) knockdown system, inhibition of AMPK-β1 gene by RNA interference led to short lifespan and earlier accumulation of lipofucsin with increasing age when compared with the wild type. However, it remained unknown how the senescence profile would be altered by overexpression or knockdown of AMPK-β1 in HDMECs *in vitro*. The objective of this study was to generate HDMEC cell lines with AMPK-β1 and AMPK-β1 short hairpin RNA to study their molecular profile as well as their ability to respond to cellular senescence. We have used lentivirus to infect HDMECs with AMPK-β1 and AMPK-β1 short hairpin RNA (shRNA) to investigate their effects on essential cell cycle proteins, all of which take significant parts in the proliferation and senescence of cells. AMPK-β1 was overexpressed with lentivirus in early passage HDMECs and subcultured until parental HDMECs senesced, and the following results were obained: 1) senescence-associated reduction in β-galactosidase activity, 2) increased proliferative lifespan, and 3) reduced G0/G1 ratio of the cell cycle through activation of a p53/p21 and p16 dependent pathways, 4) activation of extracellular signal-regulated kinase (ERK) as well as p38 and JNK mitogen-activated protein kinase (MAPK) signaling pathways and no effect on Akt phosphrylation. In contrast, knockdown of AMPK-β1 with AMPK-β1 short hairpin RNA (shRNA) lentivirus in young HDMECs showed opposite results. In conclusion, these results indicate that in HDMECs, the AMPK-β1 may play an important role in the defense mechanism against cellular aging process.

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The effect of moesin overexpression on aging of human dermal microvascular endothelial cells

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Senescence of endothelial cells seems to have a significance in the understanding of aging pathophysiology. However, the precise mechanism for senescence and implicated genes have not been known clearly. A high-throughput screening from proteomics disclosed a variety of major aging-related proteins such as moesin, Rho GTP dissociation inhibitor, and actin. Among them, moesin was the most promising protein for aging, because the expression of moesin was the most significantly changed in senescent endothelial cells and was recovered rapidly by an anti-aging agents. In addition, moesin knock-down in dermal microvascular endothelial cells (HDMECs) resulted in the induction of senescence caused by the increase of p16 and arrest of cellular cycle. To study the effects of moesin overexpression on cellular senescence and metabolism, HDMECs were transfected with lentivirus to overexpress moesin gene. Aging retardation in moesin-overexpressed HDMEC was displayed using SA-β-gal staining. Moreover, aging-related proteins such as p16, cyclin D1, and cdk4 were low-expressed and the decrease of metabolic activity was low in the moesin-overexpressed HDMECs. This results revealed that moesin overexpression could suppress functionally the progress of cellular senescence. On the basis of these results, we could suggest that the expression of moesin might induce the delay of the senescence of HDMECs and this study provides a useful model for developing anti-aging treatments.

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MMP-13 increases processing of VEGF-C and regulates vascular patterning of squamous cell carcinomas

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Cutaneous squamous cell carcinomas (SCCs) are malignant tumors with high ability to invade locally and metastasize to lymph nodes. SCCs express high levels of matrix metalloproteinase-13 (MMP-13) which promotes invasion capacity and tumor growth. Vascular endothelial growth factor-C (VEGF-C) is a member of VEGF family which is proteolytically processed to the active form. VEGF-C binds to VEGF receptors 2 and 3 on the endothelial cells, and stimulates both angiogenesis and formation of lymphatic vessels. Genome-wide expression profiling of 8 cutaneous SCC cell lines and 5 normal human epidermal keratinocyte cell lines revealed that expression of VEGF-C was markedly (3-fold) increased in SCC cell lines as compared to normal keratinocytes. Expression of VEGF-C mRNA was further analyzed in 48 different head and neck SCC cell lines by quantitative real-time RT-PCR (Taqman), and compared to the expression levels in normal human epidermal keratinocytes. Average expression level of VEGF-C was increased by 3-fold in SCC cell lines confirming the data from gene expression profiling. Adenovirus mediated expression of MMP-13 in cutaneous SCC cell lines resulted in increased release of processed soluble VEGF-C into the cell culture media, whereas the MMP inhibitors inhibited amount of processed VEGF-C in the cell culture media. Human skin SCC tumor xenografts were established in SCID mice and injected with a specific adenovirus expressing MMP-13 antisense ribozyme. The CD-31 immunostaining of tumor sections showed that dilation of vessels in the xenografts treated with the MMP-13 antisense ribozyme were decreased as a result of MMP-13 inhibition revealing a role of MMP-13 in the formation of new blood or lymphatic vessels. These results show the first time that MMP-13 can regulate in VEGF-C processing and thus facilitate tumor growth and metastasis by regulating vessel formation.

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Identification of a novel mechanism that controls the activity of VEGF protein family members

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VEGF family members are key mediators in vascular remodeling. Structure-function analysis of this protein family is not complete and a detailed analysis is mandatory for a comprehensive understanding of this essential growth factor family in processes such as tissue repair and cancer. A common structural feature of VEGF proteins is differential mRNA splicing, which gives raise to different protein isoforms, that differ primarily in the absence or presence of a C-terminal domain of highly basic-amino acids. This so called heparin-binding domain (HBD) has been identified as the epitope for neuropilin and proteoglycans, both of which are central receptor molecules to control vascular growth. However, it is still unclear how cell functions are specifically modulated by neuropilin/proteoglycan and HBD-interactions in different VEGF proteins. Furthermore, beside genetic mechanisms no other process has been identified that controls these interactions. We performed systematic structure-function analysis of different VEGF family members (Vascular endothelial growth factor-A, VEGF-A; Placenta growth factor, PlGF-1/-2). Using screens for protease sensitivity, MALDI-TOF-mass-spectrometry, BIAcore analysis and functional *in vitro* and *in vivo* angiogenic assays, we revealed novel chemotactic and molecular binding activities of the C-terminal domain of PlGF isoforms. Furthermore, in different VEGF proteins (VEGF-A, PlGF) we identified a specific plasmin-cleavage site, which leads to loss of the HBD and significant alterations in functional properties of VEGF proteins. Differential analysis of the amino acid sequence of the plasmin cleavage site in VEGF proteins across different species revealed a high conservation. Our data strongly support the significant impact of the HBD of different VEGF proteins on vascular remodeling and emphasize plasmin-mediated cleavage as a general mechanism that controls interactions of different VEGF family members with neuropilins and proteoglycans.

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Platelets support vascular integrity in angiogenesis and inflammation

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Platelets are required for plug formation upon traumatic vessel injury. They have also been known to support vessel stability – the precise mechanism still being a matter of current research. This study investigated the role of platelets for vessel stability during inflammation and angiogenesis in the microcirculation of the skin. We made the surprising discovery that, during thrombocytopenia, inflammation (Irritant contact dermatitis; reverse Arthus reaction) and tumor angiogenesis (melanoma) led to severe skin hemorrhage. By imaging the cutaneous Arthus reaction and melanoma growth through a skin window, we observe in real-time the loss of vascular integrity and the kinetics of skin hemorrhage in thrombocytopenic mice. Bleeding – mostly observed from small venules – occurred as early as 20 minutes upon challenge, pointing to a fundamental continuous need for platelets to maintain vascular integrity in inflamed and angiogenic microcirculation. Platelet transfusion experiments showed that 10% of platelet count is sufficient to prevent inflammatory hemorrhage. Genetically engineered mice revealed that maintenance of vessel integrity is independent of the major platelet adhesion receptors, indicating that this platelet function completely differs from that in primary hemostasis. Our studies reveal that platelets play a crucial role for the maintenance of vascular integrity during inflammation and angiogenesis.

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Antiangiogenic effect of anthocyanin extracts

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Angiogenesis, the process of new blood vessel growth from pre-existing capillaries, is involved in physiological and pathological processes such as tumor growth and metastasis. Thus, suppression of abnormal angiogenesis may provide therapeutic strategies for the treatment of angiogenesis-dependent disorders. There is increasing evidence that polyphenols found in natural products may interfere with several processes involved in cancer and metastasis. In the present study, we investigated whether anthocyanin extracts (from dietary black bean) modulate angiogenesis in both *in vitro* and *in vivo* models. *In vitro* studies were performed using human umbilical vein endothelial cells (HUVEC) and human dermal microvascular endothelial cells (HDMEC) cultures. After inducing angiogenesis by vascular endothelial cell growth factor (VEGF) on those cultured cells, we analyzed dose-dependent changes before- and after-anthocyanin extracts treatment. Angiogenesis was observed according to the stages: endothelial cell proliferation, migration, and tube formation. *In vivo* studies, using Chorioallantoic membrane (CAM) assay was done to check developmental effect on embryo vasculature. Anthocyanin extracts had statistically significant inhibitions on endothelial cell migration and tube formation, in dose dependant manner and also inhibited embryological vascular development. Dietary anthocyanin extracts from black bean revealed inhibitory effect on angiogenesis *in vivo* and *vitro* study. Further therapeutic approaches to anti-cancer drug could be possible on the aspect of angiogenesis.

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VEGF expression from the cultured keloid fibroblasts in various anatomic locations under the hypoxia

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Keloids are excessive scar formation during healing of wounds. Capillary proliferation and localized hypoxia have been assumed to occur in keloids. In the previous reports, higher levels of hypoxia-inducible factor 1α protein and VEGF than the adjacent normal skins providing evidences of abnormal angiogenesis in keloids. Also, there is a preference for occurrence and recurrence in anatomic location. Usually anatomic locations with higher regional tension and more sebaceous glands are inclined toward pathogenesis of keloids. In this study, we investigated VEGF activity of keloid fibroblasts in various anatomic locations of body. Fibroblasts derived from excised keloid were placed in hypoxia for varying periods of time. The locations of keloids were the ear (n=3), shoulder (n=3), and chest (n=3). Expression of VEGF in oxygen tensions was measured with RT-PCR. It was observed that a time-dependent VEGF mRNA upregulation was seen under the both hypoxic and normoxic conditions. In keloid fibroblasts, the expression of VEGF was different according to anatomic locations under the both hypoxia and normoxia. Keloid fibroblasts from the ear demonstrated a time-dependent downregulation at 6, 12, and 24 h but was then upregulated at 48 h under the normoxia. However under the hypoxia the VEGF expression was similar at 6, 12, and 24 h in keloid fibroblasts from the ear. Keloid fibroblasts from the shoulder had nearly no change under the both hypoxia and normoxia. The expression of VEGF from keloid fibroblasts of the chest was changed dynamically at varying periods of time under the both hypoxia and normoxia. These results suggest that the property of keloid fibroblasts to oxygen tensions was specific in anatomic locations of the body.

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Firm adhesion of melanoma cells to endothelial cell derived ultra large von Willebrand factor fibres under shear flow conditions

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Tumor endothelial crosstalk is the basis of efficient tumor cell extravasation, a pivotal step in hematogenous metastasis. Melanoma cell supernatant induced PAR1 activation is – at least partially – mediated by tumor secreted MMP-1. Endothelial cell activation leads to a prompt luminal secretion of ultra large von Willebrand factor (VWF) fibres, P-selectin and IL-8. Our data indicate that this haemostatic active VWF released by endothelial cells (EC) and immobilized at the vessel wall occurs within seconds upon melanoma cell exposure. Surprisingly, high molecular weight multimers of VWF were found to form elongated fibre-like structures (>200µm in length) on the EC cell surface. After adding platelet-poor plasma during a 5 minute stimulation time cell surface attached platelets were found to be almost exclusively sticking to VWF fibres. What is more, these VWF fibres are capable to anchor melanoma cells under flow conditions. Therefore, luminal immobilized VWF network plays a pivotal role in firm adhesion of melanoma cells to the vessel wall under shear flow conditions. This report provides evidence for a new concept of efficient and instantaneous tumor-endothelial communication via a functional MMP-1/PAR1 axis. Melanoma cell derived MMP-1 canonically cleaves endothelial expressed PAR1 and apparently paves the metastatic path generating a prothrombotic, proinflammatory and cell adhesive endothelial surface. Moreover, we show that von Willebrand factor firmly binds melanoma cells under high shear flow conditions and therefore may play a pivotal role in tumor progression.

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The anti-angiogenic effects of topical epigallocatechin-3-galate (EGCG) on human skin

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EGCG, the major polyphenolic component of green tea, is of therapeutic interest due to its anti-inflammatory and anti-carcinogenic effects. Previous studies have shown that EGCG also has anti-angiogenic properties, and may be useful in treatment of dermatoses with predominantly inflammatory and angiogenic pathologies. We evaluated skin biopsies from volunteers with underlying facial erythema/telangiectasias due to rosacea,. Patients applied EGCG cream (10% w/w) and placebo to separate halves of the face twice daily for 6 weeks. Both investigators and subjects were blinded to the identity of the two creams. A total of 10 subjects completed the study. Punch biopsies of rosacea affected areas (n=10) and control/untreated areas (n=10) were obtained at the end of the 6-week treatment. Sections were immunostained with antibodies against vascular endothelial growth factor (VEGF) and hypoxia inducible factor-1-alpha (HIF-1α). The percentage area of positive staining in the epidermis for both markers was determined using Image-Pro image analysis software on 15 measures/sample. We observed significant decreases in HIF-1α expression, such that 15.4% of the epidermis showed positive staining in vehicle control vs. 7.3% in EGCG treated sites ($p \leq 0.005$). Significant decreases were also found for VEGF expression (2.5% vs. 15.9% in EGCG treated skin relative to vehicle-treated skin ($p \leq 0.005$). These decreases in VEGF and HIF-1α levels were also significant in EGCG treated sites in comparison to the baseline control (17.2% and 18.6%, respectively.) These results suggest that at the molecular level, topical ECGC can down-regulate HIF-1α and VEGF expression. Increases in the length of time of cream application could further enhance the anti-angiogenic effects of topical ECGC on patients with rosacea.

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Pregnancy does not result in enhanced angiogenesis in cutaneous inflammation and wound healing

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Pregnancy results in physiological skin modifications such as spider angiomas, palmoplantar erythema and edema, suggestive of changes in the function and structure of the blood and lymph microvasculature. To further analyze these observations we conducted a histological quantitative analysis of angiogenesis in normal, inflammatory and wounded skin between pregnant and non pregnant mice. Wild-type C57BL/6 female mice were bred to syngenic males. Skin inflammation was obtained by inducing a contact hypersensitivity reaction of the right ear using oxazolone. The left ear was used as normal skin. Animals had eczema at day 16 and were sacrificed at day 19 of gestation for analysis. Surgical wounds were created at day 8-10 and animals were sacrificed at day 12-14 of gestation. Non-pregnant female controls matched for age received similar procedures in parallel. Assessment criteria were *in vivo* laser doppler imaging and computer-assisted morphometric analysis of lymphatic and blood vessels respectively stained by Lyve-1 and CD31 antibodies. We compared 35 pregnant to 48 non pregnant mice for angiogenesis during skin inflammation. The level of inflammation was similar between both groups by histology, ear thickness measurement, draining lymph node cell count or flow-cytometry. *In vivo* laser doppler imaging found increased perfusion of inflamed compared to non-inflamed ears ($p=0.004$). However, there were no differences between the skin of pregnant and non-pregnant mice. Vessel density and size was increased in inflamed skin compared to normal skin, however, we found no differences in blood or lymphatic vessel density, diameter or surface, in inflammatory or healthy skin between pregnant and non-pregnant mice. Finally, in cutaneous wounds, 12 pregnant and 26 non pregnant controls did not differ in morphometric analysis. In conclusion, cutaneous angiogenesis is not modified during mouse gestation. The clinical observations may be due to increased blood flow.

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Ultrastructural evaluation of cutaneous ingrowth within the micropores of implanted porous poly[hydroxyethyl methacrylate] using a percutaneous mouse model recapitulates the overall structural organization of normal skin

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Percutaneous medical devices such as catheters penetrate the skin, disrupt the protective barrier and create an avenue for bacterial invasion. We hypothesize that forming a seal at the interface between the skin and biomaterial could prevent these infections. We have previously shown using a rafted organ culture model that precision-engineered 40μm pore size poly[hydroxyethyl methacrylate] (HEMA) implants placed through newborn foreskin induced epidermal growth, migration and incorporation as opposed to marsupialization. Ultrastructural evaluation revealed a basement membrane-like structure had formed at the interface between the keratinocytes and the inner surface of the biomaterial pore. The organ culture model did not, however, support dermal ingrowth nor did it address the inflammatory response. In our endeavor to understand the *in vivo* response to percutaneous porous biomaterials, we used a C57BL/6 mouse implant model to examine the interface between the skin and porous polyHEMA at the ultrastructural level. We found that unlike the rafted organ culture model, many cell types were incorporated within the micropores including endothelial cells, fibroblasts, keratinocytes and leucocytes. We observed that the cell types and locations were influenced on two levels: first, on a macro level in which the normal stratification of the skin dictates which cell types are available adjacent to the biomaterial for incorporation; and second, on a micro-niche level in which the cell types present within each pore organize themselves into zones of preferential location that in some ways recapitulates the organization of normal skin. This initial ultrastructural exploration of these microniches is an important step in determining the influences that pore size, throat size and surface modification of porous biomaterials have on cutaneous incorporation.

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Inhibition of melanoma angiogenesis by telomere homolog oligonucleotides

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Telomere homolog oligonucleotides (T-oligos) are understood to activate an innate program that leads to multiple anti-cancer effects including apoptosis and/or senescence of cancer cells. To determine if T-oligos affect tumor angiogenesis, paired dishes of human melanoma (MM-AN) cells, human microvascular endothelial cells (HMVEC) and human umbilical vein endothelial cells (HUVEC) were treated with 40 μM T-oligo (GTTAGGGTTAGGGTTA) or diluent alone and processed for qRT-PCR, gel shift assay, and western blot. By 24 and 48h, T-oligo decreased the protein levels of the angiogenic factors VEGF and angiopoeitin-1 in MM-AN cells by 40-70%. T-oligo also decreased the expression of VEGF receptors 1 (by 45-95%) and 2 (by 45-75%) in HMVEC. Within 48 to 72h, T-oligo increased the level (4-5-fold) and activity (2-fold) of E2F1, a transcription factor known to promote apoptosis as well as to negatively regulate NFκB, a pro-angiogenic and pro-survival transcription factor. At 1-16h DNA binding activity of transcription factors identified as major positive regulators of VEGF transcription were decreased 98% for NFκB, 90-95% for hypoxia-inducible factor-1α and 30-60% for Ap1 in T-oligo-treated vs. control cells. Over 72 hours, T-oligo treatment reduced MM-AN cell release of VEGF into culture medium (measured by ELISA) by 30-40% ($p<0.01$). In a 22h *in vitro* assay T-oligo markedly inhibited HMVEC tubulogenesis. Over 22 hours, compared to diluent-supplemented medium, conditioned medium from MM-AN cells treated with T-oligo for 72 hours inhibited invasion of fresh MM-AN cells into Matrigel by 96% ($p<0.03$). In SCID mice, IV administration of T-oligos beginning 3 days after IV injection of MM-AN cells reduced the number and volume of melanoma metastases identified at autopsy on day 34 by 59% ($p<0.003$) and 66% ($p< 0.04$) respectively, consistent with the anti-angiogenic effects observed *in vitro*. Decreased tumor angiogenesis complements other previously reported anti-cancer effects of T-oligos.

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Immunization with different adjuvants alters the disease progression in mice with experimental epidermolysis bullosa acquisita (EBA)

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The deposition of autoantibodies against type VII collagen at the dermal-epidermal junction leads to a blistering phenotype of the skin and is called epidermolysis bullosa acquisita (EBA). In EBA, a dominance of CD4+ T cells, which preferentially produce IFN-g (T-helper1 cells; Th1), has been reported. We have previously established an active disease model of EBA by immunizing mice with recombinant murine type VII collagen. In the present study, we addressed the question, whether a shift from Th1 to Th2 cells (preferentially producing IL-4) would ameliorate the course of diseased mice. In this active mouse model of EBA, Th2 cells were induced *in vivo* using alum as an adjuvant. Three major observations were made: 1) The course of the disease was drastically improved as shown by the significant reduction of the clinical score. 2) However, in the blood, the concentration of type VII collagen-specific antibodies of the Th1 subtype (IgG2a) and the Th2 subtype (IgG1) were identical among the 2 groups. 3) Interestingly, in the spleen of alum-treated mice, the number of type VII collagen-specific B cells was altered. These cells were increased in the red pulp and decreased in the follicles of the spleen. Our results indicate that the affinity of antibodies to type VII collagen in the alum-treated group is reduced thereby causing a milder disease. This conclusion is supported by the observation that the localization of the antibody producing cells in the spleen is different in alum-treated compared to non-treated animals and by reports that the affinity of antibodies is influenced by the localization of the antibody producing cells. Our results may provide a basis for novel strategies to treat EBA, i.e. by the use of appropriate adjuvants inducing anti-type VII collagen antibodies which are not pathogenic. Ultimately, these findings may also have implications for the treatment of other autoantibody-mediated diseases.

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T cells are required for initiation of autoimmunity in experimental epidermolysis bullosa acquisita

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Epidermolysis bullosa acquisita (EBA) is a prototypical organ-specific autoimmune disease caused by autoantibodies against type VII collagen, the major constituent of the anchoring fibrils. While mechanisms of autoantibody-induced blister formation were extensively characterized, the initiation of autoimmunity and autoantibody production in autoimmune blistering diseases are still poorly defined. In the present study, we addressed the role of T cells for the production of blister-inducing autoantibodies in mice immunized with 100 μg of recombinant form of the non-collagenous 1 domain of type VII collagen. To detect autoreactive type VII collagen-specific T cells, lymph node cells from immunized SJL mice (n=6) were stimulated *in vitro* with recombinant antigen and their proliferation was measured by both radioactive thymidine incorporation and flowcytometry analysis of carboxy-fluorescein diacetate succinimidyl ester-labeled cells. Interestingly, using 20mer overlapping synthetic peptides of the immunogen, different patterns of T and B cell epitopes in mice immunized with type VII collagen were demonstrated. In contrast to wild type SJL mice (n=6), immunization of SJL athymic nude mice (n=6) lacking T cells with type VII collagen did not induce an autoimmune response and blistering disease. Importantly, 1 x 107 lymphocytes from immunized wild type mice (n=6) passively transferred into SJL nude mice (n=6) produced autoantibodies over 3 months resulting in subepidermal blistering disease in these animals. Our results demonstrate that T cells are required for the initiation of autoimmunity against type VII collagen in experimental EBA and provide a basis for developing T cell-directed immunomodulatory strategies for this and related autoimmune diseases.

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Psoriatic fibroblasts demonstrate distorted suppression of reactive oxygen species production by neutrophils

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Neutrophils-fibroblasts interactions are regarded to be involved in psoriatic plaques development. The aim of the study was to evaluate reactive oxygen species (ROS) production by neutrophils both in monocultures and in co-cultures with fibroblasts. Neutrophils were obtained from the peripheral blood of both psoriatic patients and healthy subjects by isolation on Gradisol G. Fibroblasts were obtained from psoriatic lesions, perilesional skin and healthy subjects skin by enzymatic dispersion. Cells from passages 3-4 were used for experiments. For co-culture experiments neutrophils density was 10^6 cells/ml and fibroblasts 10^5 cells/ml. ROS production was assessed by chemiluminescence (CL) enhanced by luminol using MLX Microtiter Plate Luminometer (Dynex, USA). Neutrophils were stimulated by N-formyl-methionyl-leucyl-phenyloalanine (FMLP; concentration $2x10^6$M). Moreover, the cells were primed with TNF-α (10 ng/ml). The results were expressed as Relative Light Units (RLU). In co-cultures of both normal healthy neutrophils or psoriatic ones with either normal, lesional or perilesional psoriatic fibroblasts, decrease in ROS production was observed in comparison to respective neutrophil controls ($p<0.05$). Of note, suppression of ROS production was the most pronounced in normal fibroblasts co-cultures in comparison to lesional and perilesional fibroblastas/neutrophils co-cultures ($p<0.05$). In conclusion, weaker suppression of ROS production in neutrophils/psoriatic fibroblasts co-cultures could lead to inflammatory response maintenance in psoriatic skin.

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Psoriatic neutrophils and fibroblasts stimulate interleukin-10 production

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Neutrophils-fibroblasts interactions seem to play an active role in psoriatic lesion development. The aim of the study was to evaluate interleukin 10 (IL-10) levels in supernatants of psoriatic neutrophils and fibroblasts. Fibroblasts were obtained from psoriatic lesions, perilesional skin and healthy subjects skin by enzymatic dispersion. Cells from passages 3-4 were used for experiments. Neutrophils were obtained from the peripheral blood of both psoriatic patients and healthy subjects by isolation on Gradisol G. For co-culture experiments neutrophils density was 10^6 cells/ml and fibroblasts 10^5cells/ml. Experiments were performed in Eagle medium supplemented with 20% fetal calf serum and stimulated with either IL-8 (0.1 μg/ml) or TNF-α (10 ng/ml). IL-10 was evaluated by ELISA (BD Biosciences Pharmingen, USA; sensitivity below 1 pg/ml). In all the examined supernatants of both normal and psoriatic fibroblasts and psoriatic neutrophils monocultures, IL-10 levels were below the detection levels. However, interactions between all the studies fibroblast and psoriatic neutrophils led to significantly increased IL-10 production in co-cultures ($p<0.05$). Furthermore, the lowest IL-10 production was noted in psoriatic neutrophils/lesional psoriatic fibroblasts co-cultures in all the studied settings (controls, under IL-8 and TNF-α stimulation). In conclusion, defect in IL-10 production by both psoriatic neutrophils and fibroblasts could result in decreased IL-10 production in psoriasis and be at least in part responsible for insufficient anti-inflammatory activity observed in the course of this disease.

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HLA class II allele distribution in pemphigus vulgaris in white Europeans and Indo-Asians from the United Kingdom: association with a novel HLA class II allele DRB1*1454.

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Population studies of pemphigus vulgaris (PV) have consistently shown a link with Class II HLA alleles according to ethnic group. However, little is currently known about the distribution of these alleles in the UK pemphigus population. We therefore sought to determine HLA DRB1 and DQB1 allele frequency in White Europeans (WE) and Indo-Asians (IA) with PV living in the U.K using high-resolution PCR-SSP. Desmoglein (Dsg) 1 and 3 antibody profile in both patient groups was analyzed using specific ELISA assays. 153 patients (96 WE and 57 IA patients) with PV were recruited with 159 ethnically-matched healthy controls. There was a significant increase in frequency of HLA DRB1 *0402,*1404 and DQB1*0503 and *0302 alleles in both ethnic groups. DRB1*1404 was the strongest risk factor in the IA group ($p<0.00001$, OR 10, CI 4.2, 24.5) and DRB1*0402 the strongest risk factor in the WE group ($p<0.00001$, OR 75, CI 11.1, 1478). In the WE patient group there was a significant association with a novel allele DRB1*1454 ($p=0.00007$, OR 4.8, CI 2.05, 11.65) which has not been previously reported. In addition there was a positive association with DRB1*15 and DQB1*0602 in WE patients who were Dsg3 antibody positive/ Dsg1 antibody negative suggesting these 2 alleles may confer protection against the development of Dsg1 antibodies in PV. Our study represents the largest White European and Indo-Asian cohort of patients with PV with class II HLA allele association to date. We have also revealed a novel DRB1*1454 allele association with pemphigus vulgaris in White Europeans

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IL-12, IL-23, and IL-27 enhance human β-defensin-2 production in human keratinocytes

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IL-12, IL-23, and IL-27, which are produced by antigen-presenting cells, modulate innate and adaptive immunities. Human β-defensin-2 (hBD-2) produced by epidermal keratinocytes promotes cutaneous antimicrobial defense and inflammation. We examined the *in vitro* effects of IL-12, IL-23, and IL-27 on hBD-2 production in human keratinocytes. IL-12, IL-23, and IL-27 enhanced IL-1β-induced hBD-2 secretion and mRNA expression in keratinocytes. The stimulatory effects of IL-12, IL-23, and IL-27 were suppressed by antisense oligonucleotides against NF-κB p50 and p65. In addition, the effects of IL-12 and IL-27 were suppressed by antisense STAT3 and STAT1, respectively. All the 3 IL enhanced the basal and IL-1β-induced transcriptional activities of NF-κB, while IL-12 and IL-27 enhanced STAT3 and STAT1 activities, respectively. Further, IL-12, IL-23, and IL-27 promoted basal and IL-1β-induced phosphorylation of IκBα. IL-12 and IL-23 tyrosine phosphorylated STAT3 and STAT1, respectively; IL-12, IL-23, and IL-27 tyrosine phosphorylated Janus kinase-2 (JAK2) and tyrosine kinase-2; and IL-27 tyrosine phosphorylated JAK1. These results suggest that IL-12, IL-23, and IL-27 may enhance IL-1β-induced hBD-2 production in keratinocytes by activating NF-κB. STAT3 and STAT1 are involved in the effects of IL-12 and IL-27, respectively. Thus, IL-12, IL-23, and IL-27 may promote cutaneous antimicrobial defense and inflammation via hBD-2.

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Eosinophilic skin reactions to glycolipids in mycobacterial infection represent a novel form of delayed-type hypersensitivity

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Delayed-type hypersensitivity (DTH) elicited in the antigen (Ag)-challenged skin is a hallmark of high levels of protein Ag-specific TH1 responses induced by mycobacterial infection. Besides protein Ags, we have recently shown that a substantial T-cell immunity directed against glycolipid Ags is also mounted in mycobacteria-infected humans and guinea pigs (J. Invest. Dermatol. 121: 517, 2003; Vaccine 24: 5700, 2006), but DTH responses to this class of Ags have not been fully assessed. To address this issue directly, glycolipid-specific skin reactions were evaluated in guinea pigs infected with Mycobacterium avium complex (MAC). We found that a MAC-derived glycolipid, trehalose dimycolate (TDM), elicited DTH skin reactions with marked infiltration of eosinophils in MAC-infected, but not uninfected, guinea pigs. DTH reactions to TDM with eosinophilic infiltration were also observed in BCG-immunized guinea pigs, which contrasted sharply with those to the purified protein derivative (PPD) with mononuclear cell infiltration. Consistent with this, splenocytes isolated from BCG-immunized guinea pigs upregulated the TH2 cytokine (interleukin-5) expression in response to TDM, but not in response to PPD, underscoring a skewed TH2 response to glycolipids. Thus, these findings identify a new form of DTH reactions that is distinct from the classical DTH response to protein Ags. The "eosinophilic DTH" to glycolipids may represent a critical aspect of skin inflammation that has never been appreciated previously.

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Gene expression features of Lichen planopilaris and Pseudopelade de Brocq

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Lichen planopilaris (LPP) and Pseudopelade de Brocq (PPB) are two scarring alopecia diagnoses that often exhibit similar clinical features. However, the pathogenesis of these two diseases is not clear. Even the most experienced clinicians have difficulty in diagnosing between these cicatricial alopecias. Some suggest LPP and PPB are not distinct conditions, but rather different clinical presentations in a spectrum derived from the same underlying pathogenetic mechanism. We sought to identify differential gene expression patterns associated with the pathogenesis and diagnosis of LPP and PPB. We wanted to explore the degree of similarity between LPP and PPB gene expression patterns and the potential for common and unique gene pathways and gene activation in each diagnosis. Microarray analysis, using a 21K cDNA set was performed on pairs of biopsies obtained from the effected and unaffected scalp of previously untreated patients (4 LPP and 3 PPB biopsy pairs). Diagnosis was confirmed by routine histopathology. Significantly differentially expressed genes were identified by analysis of microarray results in various data sets and screened for signaling pathway involvement. Selected genes were validated by real-time PCR using the same set of samples and subsequently by immunohistology. The global gene expression profiles in LPP and PPB versus comparative intra-control scalp tissue were distinguishable by Significance Analysis of Microarrays (SAM). Specific genes, such as MMP11, were identified with significantly differential expression in association with LPP versus PPB and vice versa. There was almost no commonality in the gene expression profiles between LPP and PPB. Our findings may have important implications for understanding the pathogenesis of LPP and PPB at the molecular level. Results suggest LPP and PPB may involve distinct mechanisms of disease development and can be regarded as biologically distinct diagnoses. Genes that we have identified may potentially be useful as markers of the respective diagnoses.

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Cytokine mediated growth inhibition rather than cell mediated apoptosis may be the dominant effector mechanism of alopecia areata in the C3H/HeJ mouse model

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Alopecia areata (AA) is a chronic inflammatory disease of hair follicles manifesting as patchy areas of hair loss on the scalp and body. Development of AA is associated with peri- and intra-follicular inflammation of anagen stage hair follicles, primarily by CD4+ and CD8+ cells. To study whether cell mediated cytotoxicity against the follicular epithelium is a component of the hair loss disease mechanism, we examined gene and product expression profiles typical of cytotoxic cells in the C3H/HeJ mouse model for AA. mRNA expression levels of granzyme A, granzyme B, perforin, Fas, Fas ligand, TNF-α, TNF-αR1 and R2, TRAIL, TRAILR and TRAMP, plus multiple cytokines were compared between the skin, draining lymph nodes, thymus and spleens of normal and AA-affected mice using quantitative reverse transcription PCR. FasL, granzyme A, granzyme B, pro- and anti-inflammatory cytokines were all highly up-regulated in the skin of AA-affected mice. Immunohistochemical studies of the skin revealed that, although greater numbers of granzyme B and FasL expressing cells were present in AA affected skin, the cells were morphologically diffusely distributed and not exclusively located within the focal peri- and intra-follicular infiltrates of CD4+ and CD8+ cells. Almost no perforin expressing cells were identified in AA affected mouse skin and TUNEL staining suggested relatively limited apoptosis activity in hair follicle keratinocytes. While granzymes and FasL may play important roles in disease development, the profiles and patterns of expression are not consistent with direct lymphocyte cell mediated cytotoxic action against the follicular epithelium in chronic mouse AA. Potentially, hair growth inhibiting cytokines may play a more dominant role in AA development than previously thought.

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Human skin explants release inflammatory cytokines following stimulation with *Staphylococcus aureus*

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Staphylococcus aureus is thought to play a role in exacerbating inflammatory skin disorders such as atopic dermatitis. We have examined the release of inflammatory cytokines from human skin explants exposed to heat-killed *S.aureus*. Human skin was obtained from patients undergoing mastectomy. Subcutaneous fat was removed and skin cut into explants of around $2mm^2$. The explants (n=29) were stimulated with 10^6 heat-killed colony-forming units(CFU)/ml *S.aureus*, buffer control or 100ng/ml LPS for 24 hours. The supernatant was recovered, stored at –70°C and cytokines measured by ELISA. The explants were weighed and cytokine release corrected for tissue weight. Human skin explants released little or no TNFa or IL-1b in the absence of a stimulus (0.07±0.02 pg TNFa/mg tissue and 0.12±0.05 pg IL-1b/mg tissue), but heat-killed *S.aureus* induced TNFa release reaching a peak at 24 hours (1.51±0.29 pg/mg tissue, $P<0.05$), while IL-1b reached 0.46±0.11 pg/mg tissue at 24 hours, $P<0.05$ and increased further at 48 hours. Unstimulated explants released substantial amounts of both IL-6(198±54pg/mg tissue) and IL-8(478±56pg/mg tissue). However, exposure to *S.aureus* increased IL-6 release to 759±325pg/mg tissue, $P<0.05$ and IL-8 release to 1341±308pg/mg, $P<0.05$ at 24 hours. The response to *S.aureus* was similar in magnitude to the inflammatory stimulus LPS for all four cytokines. Preliminary experiments (n=12) suggest that exposure to the non-pathogenic bacteria *S.epidermidis* resulted in the release of lower amounts of TNFa. 10^6CFU/ml heat-killed *S.epidermidis* caused release of 0.46±0.13pg TNFa/mg tissue compared with 0.87± 0.22 pg TNFa/mg tissue for *S.aureus* ($P<0.05$ versus *S.epidermidis*) and 0.03±0.01 pg TNFa/mg tissue for control. In summary, human skin explants release significant levels of inflammatory cytokines 24 hours after exposure to *S.aureus*, but the response is muted in non-pathogenic *S.epidermidis*. The skin explants may be a useful model to explore inflammatory responses *in vitro* and may offer insights into inflammatory skin disease.

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Linear IgA bullous dermatosis in a patient with nasopharynx carcinoma after radiotherapy

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We describe a 59-year-old Chinese woman who developed linear IgA bullous dermatosis 10 years after she was diagnosed with nasopharynx carcinoma. Her nasopharynx carcinoma had been controlled by radiotherapy. The patient came with complaining of recurrent bullae all around her body accompanied by itching. On her presentation in Aug. 20, 2007, histological examination revealed subepidermal blister formation with neutrophils and eosinophils infiltration. Direct immunofluorescence (DIF) demonstrated deposition of IgA along the dermal-epidermal junction, while C3, IgG and IgM were not detected. Therapy with dapsone at doses 100mg per day was effective. The occurrence of nasopharynx carcinoma and linear IgA bullous dermatosis in the same patient is a rare event. We referred a possibility that tumor may play a role in the pathogenesis of autoimmune blistering disease.

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CD200-CD200R Signaling in cutaneous immune homeostasis

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Immunosuppressive molecules expressed on tissue-resident cells have the potential to regulate tissue-specific inflammation and autoimmunity. CD200 is a cell surface glycoprotein that transmits an immunosuppressive signal by ligating its receptor, CD200R. Previously, we have shown that CD200 and CD200R are expressed in murine skin. CD200 is expressed on Langerhans cells (LCs) and a subset of keratinocytes (KCs). CD200-expressing KCs preferentially localized to the outer root sheath of hair follicles (HF). In functional studies, CD200 expression had a dramatic effect on protecting HFs from inflammation and autoimmune attack. Given that basal cell carcinoma (BCC) is thought to arise from follicular epithelium, we hypothesize that CD200 is expressed on BCC. Using the murine BCC cell line ASZ001, we show that CD200 is expressed on a subset of murine BCC cells. We also show that CD200 is expressed on several other murine and human cutaneous and non-cutaneous tumor cell lines. Taken together, our results suggest that CD200-CD200R signaling pathway plays a role in establishing and maintaining cutaneous immune homeostasis. This pathway may be especially important in attenuating tumor-specific immune responses in the skin.

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Prostaglandin E_2-EP3 signaling, as a negative regulator of skin immune responses by suppressing cutaneous dendritic cell functions

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PGE_2 exerts its physiological functions through four subtypes of receptors, EP1, EP2, EP3, and EP4. When PGE_2 is highly expressed under robust inflammation, PGE_2-EP4 signaling initiates skin immune response. On the other hand, EP3 signaling was known to suppress murine allergic asthma reaction. EP3 is highly expressed on dendritic cells (DCs) and Langerhans cells (LCs), however, the role of EP3 signaling for DCs/LCs and skin immune response remains unknown. Here we assessed the effect of PGE_2-EP3 interaction on DC/LCs functions by using a novel EP3 receptor-selective agonist, AE-248. Initially, epidermal cell (EC) suspensions were prepared from BALB/c mice and incubated for 24 hours. $CD11c^+$ MHC class II^+ LCs among EC suspensions showed enhanced the expression of co-stimulatory molecules including CD54, CD80 and CD86, which was inhibited by EP3 agonist. To evaluate the direct effect of EP3 agonist on dendritic cells, we prepared bone marrow-derived DCs (BMDCs) by the incubation of bone marrow cells with GM-CSF for 7 days. The addition of EP3 agonist did not affect the total number of BMDCs, but significantly decreased the mature (defined as MHC class II high positivity) population. Moreover, the chemotaxis of BMDCs to CCL21 assessed using transwells was enhanced by a high concentration of PGE_2, but attenuated by low dose PGE_2 added to the upper chamber, suggesting that PGE_2 has biphasic activity to DC migration. Furthermore, the addition of EP3 agonist decreased the culture-promoted migration of the mature DC subset. The affinity of PGE_2 to EP3 receptor is the highest among all PGE_2 receptor. Consistent with the *in vitro* results, EP3 agonist treated mice showed reduced DNFB-induced contact hypersensitivity response compared to non-treated mice. Therefore, in contrast to PGE_2-EP4 signaling, PGE_2-EP3 signaling suppresses the cutaneous DC maturation and migration, thereby keeping homeostasis of the skin immunity.

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A new mouse model of polymyositis indicates IL-6 as a therapeutic target

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Polymyositis (PM) and dermatomyositis (DM) are chronic autoimmune inflammatory myopathies of unknown etiology. Quite a few pathological features and responses to specific therapeutic agents are shared by the two diseases, suggesting that they represent a spectrum of diseases. Research studies of PM/DM have been hampered by lack of appropriate animal models. Experimental autoimmune myositis of SJL/J mice that has been used as a PM model seems to be mediated by CD4 T cells. Moreover, SJL/J mice carries a dysferlin gene mutation that causes spontaneous muscle necrosis and secondary inflammation. Recently, we established a new mouse model of PM, termed C-protein induced myositis (CIM) in B6 mouse. Analogous to PM muscles, CIM muscles had preferential infiltration of CD8- and perforin-expressing cells in endomysium. Adoptive transfer of the myositis into naïve mice could be mediated primarily by CD8 cells, and was observed only in the limbs treated with complete Freund's adjuvant. Thus, effector CD8 T cells and activation of local innate immunity must act in concert to induce myositis. Presence of interleukin(IL)-6 producing cells in the PM/DM muscles prompted us to investigate pathological roles of IL-6 on CIM. Immunohistological analysis disclosed that IL-6 was also expressed by infiltrating mononuclear cells in the CIM muscles. IL-6-null mice were resistant to CIM induction. Treatment with anti-IL-6 receptor antibody ameliorated histological findings of CIM preventively as well as therapeutically. Notably, IL-17A null mice, which are resistant to a couple of autoimmune models, were fully susceptible to CIM. These results suggested that IL-6 blockade is a new option in treating PM/DM, and is not mediated by inhibition of autoreactive IL-17 producing cells induced by IL-6 and TGFβ.

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Redefining the phenotype of macrophages in psoriasis

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Macrophages are abundant tissue resident cells that play an important role in innate immunity. We have recently re-characterized macrophages in normal skin, and found that the best marker is CD163, the hemoglobin/haptoglobin scavenger receptor, considered to be a marker of "alternatively activated" macrophages that polarize Th2 cells. Factor XIIIA, which has been previously used to identify "dermal dendrocytes" in the skin, was co-expressed by macrophages, rather than dendritic cells (DCs). We have now turned our attention to characterize macrophages in psoriasis, as they are increased in this disease, and return to normal levels with therapy. We studied a large panel of antibodies by in situ two-color immunofluourescence (n=6). Macrophages were HLA-DR+, CD206/MMR+, and CD209/DC-SIGN+, but these are not exclusive macrophage markers. The only antibodies studied that co-localized with nearly all CD163+ cells were RFD7 and CD107b/LAMP2, although occasional CD11c+ myeloid dermal DCs were also positive. CD68, long considered an exclusive macrophage marker, actually identifies both DCs and macrophages. Macrophages were negative for the DC markers CD83, CD205/DEC205, CD208/DC-LAMP, and few cells co-stained with the co-stimulatory markers CD40 and CD86. We have recently identified an inflammatory DC population that produce TNF and iNOS (Tip-DCs) in psoriasis. Macrophages produced little TNF and no iNOS co-localization was seen. Thus in psoriasis, macrophages are abundant, and can be identified by CD163, RFD7, and LAMP-2.

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Pathogenic pemphigus foliaceus (PF) and vulgaris (PV) antibodies share a consensus amino acid motif in their CDR3 regions

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Autoantibodies in PF and PV bind to desmoglein (Dsg) 1 and 3, respectively, and cause loss of keratinocyte adhesion. We previously used phage display to clone monoclonal antibodies (mAbs) from a PF patient (PF1) and found anti-Dsg1 antibodies using only 5 heavy chain genes (VH). The only strongly pathogenic antibody [PF1(3-30)] used VH 3-30. To analyze whether pathogenic antibodies in PF share homologous features we cloned antibodies from a second PF patient (PF2). The only pathogenic mAb from PF2 [PF2(3-53)] used VH3-53, not used in any PF1 mAbs. These results demonstrate that PF pathogenic antibodies are not restricted to one VH gene. However, blocking ELISA assays show that PF1(3-30) and PF2(3-53) bind at or near the same epitope. Furthermore, binding to EDTA-treated Dsg1 and chimeric Dsg1/Dsg3 molecules indicate that both pathogenic mAbs bind to a calcium-sensitive conformational epitope at the N-terminus of Dsg1, the same findings for Dsg3 binding noted for the only two pathogenic mAbs previously cloned from a PV patient [PV1(3-07) and PV1(VH1-4M28)]. Because these 4 pathogenic mAbs shared these immunochemical properties, we hypothesized that, even though their VH gene usage is different, their heavy chain CDR3 regions, which are known to contain the major antigen contact sites, might have conserved motifs. Indeed, all 4 pathogenic antibodies had a conserved D/E-x-x-x-W amino acid motif, where D/E is an acidic amino acid and W is tryptophan. None of 16 non-pathogenic anti-Dsg antibodies with unique CDR3 regions from the pemphigus patients had this sequence. Additionally, the CDR3 region of AK23, a highly pathogenic anti-Dsg3 mouse mAb, had the consensus motif. Homology to classical cadherins suggests that these pathogenic mAbs may insert their W in the N-terminal calcium-stabilized W-acceptor pocket used for trans adhesion, thereby blocking it. This conserved CDR3 sequence should be valuable to target pathogenic pemphigus antibodies.

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A collaborative web-based database for cutaneous manifestations of lupus erythematosus: initial analysis of 113 prospectively enrolled patients

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Skin disease, the second most frequent clinical manifestation of Lupus Erythematosus (LE), can present with either lupus-specific (Cutaneous LE [CLE]) or lupus-nonspecific findings. Lupus-specific skin manifestations are subclassified into chronic CLE (CCLE), subacute CLE (SCLE), and acute CLE (ACLE). This prospective study was designed to assess disease prevalence and severity in various subsets of CLE by using outcome and quality of life (QoL) measures, and to determine treatment responsiveness by establishing a web-based database of patients with CLE. A linked CLE tissue bank will enable future genetic studies. We enrolled and followed 113 patients who met the criteria for having CLE and presented to our outpatient clinic in a one-year period. We used the CLE Disease Activity and Severity index (CLASI) to evaluate cutaneous disease. At each visit, patients completed the SKINDEX 29+3. Predominant diagnosis in 7 patients (6.1%) was ACLE, in 21 patients (18.5%) was SCLE, and in 77 patients (68.1%) was CCLE (58 Classic DLE [24 generalized DLE, 34 localized DLE], 2 hypertrophic DLE, 13 lupus tumidus, 1 chilblain LE, 3 lupus panniculitis), and 8 patients (6.8%) had SLE or only lupus non-specific skin lesions. The mean SKINDEX score in ACLE, SCLE, and CCLE patients at their first visit was 76.2, 79.4, and 82.6, respectively (ns). The mean 1st SKINDEX score in generalized and localized DLE was 90.7 and 77.5, respectively (p=0.06). The mean 1st CLASI activity-damage score in ACLE, SCLE, and CCLE patients was 6.4-5.1, 11-1.5, and 7.4-10.2, respectively. Eleven patients (9.7%) were considered refractory to current therapies. This web-based database is the first systematic multicenter epidemiologic study of cutaneous LE in the United States, and should allow collection of data related to disease activity, QoL, and response to therapy at multiple centers.

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Oral chymase inhibitor SUN13834 ameliorates skin inflammation and pruritus in a 5-week mouse model for atopic dermatitis

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Chymase is a chymotrypsin-like serine protease exclusively stored in secretory granules of mast cells and has been thought to participate in allergic diseases. SUN13834 is a specific inhibitor for chymase, i.e. Ki for human chymase and mouse chymase (mouse mast cell protease-4) are 0.161 μM and 0.063 μM, respectively. Previously, we have reported that SUN13834 improves dermatitis in two animal models, NC/Nga mouse and acute biphasic dermatitis models. In the present study, effect of SUN13834 was examined using a mouse dermatitis model induced by repeated painting with 2,4-dinitrofluorobenzene (DNFB). Oral administration of SUN13834 once a day for 5 weeks inhibited increase of skin thickness in the model, with statistical significance for the inhibition at doses of 2 mg/kg and higher. The SUN13834 treatment also inhibited accumulation of mast cells and eosinophils in the skin of the mice. In addition, SUN13834 significantly decreased the number of scratching behavior induced by the DNFB challenge at 10 mg/kg and higher. SUN13834 appeared to inhibit the scratching behavior at 2 mg/kg as well, while statistical significance was not observed. These findings suggest that mast cell chymase might participate in skin inflammation as well as pruritus induction, and that SUN13834 is thought to be useful for treatment of the patients with atopic dermatitis.

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Involvement of keratinocyte-derived semaphorin 3A in epidermal innervation of atopic dermatitis

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Cutaneous nerve fibers are present at higher densities in the epidermis of patients with atopic dermatitis (AD). The increase of nerve fibers is partly responsible for the intense itch sensations in atopic skin. It is now generally accepted that keratinocyte-derived NGF is one of the mediators determining skin innervation density. On the other hand, the number of epidermal nerve fibers in AD patients is decreased by phototherapy, but the underlying mechanisms are poorly understood. Interestingly, semaphorin 3A (Sema3A), which is a diffusible molecule important in repulsive axon guidance, inhibits NGF-induced sprouting of sensory afferents in adult mammalian spinal cord. Therefore, Sema3A might be involved in the modulation of epidermal innervation in AD. To address this issue, quantitative RT-PCR and immunohistochemistry were performed on skins from healthy volunteers and AD patients before and after PUVA therapy. Nerve fibers in the skins were also stained with an anti-PGP9.5 antibody, and the number of epidermal nerve fibers was counted. The analyses revealed that both epidermal Sema3A mRNA and protein levels were decreased in AD patients compared with those in healthy volunteers, while the number of epidermal nerve fibers was significantly increased in the AD patients. After PUVA therapy, both epidermal Sema3A mRNA and protein levels were upregulated in the treated group compared with those in the non-treated group. Moreover, epidermal nerve densities were decreased in the treated group, concomitant with a decrease of VAS scores. Thus, the epidermal innervation may be regulated by Sema3A levels in the atopic skin, and not only by neurotrophin levels.

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Significance of interleukin-27 in the skin inflammation in MRL/lpr mice

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We examined the significance of interleukin (IL)-27 signaling in skin inflammation in vivo using WSX-1 knockout (KO)- and WSX-1 transgenic (Tg) MRL/lpr mice. IL-27 is known to play two distinct roles as an initiator of Th1 responses and as an immune modulator. WSX-1 is an IL-27 receptor subunit which confers the ligand specificity, and is expressed on various leukocytes including T cells. It has been reported that deletion of WSX-1 in MRL/lpr mice, a model for systemic lupus erythematosus, converts the Th1-type nephritis into Th2-type nephritis, while CD2-promoted overexpression of WSX-1 in MRL/lpr mice (WSX-1 Tg) ameliorates the nephritis and autoantibody production. Using these mice, we examine how such IL-27 signaling alteration could affect skin inflammation as it is known that the background MRL/lpr mice spontaneously develop an inflammatory skin disease. Approximately 90% of WSX-1 KO- and control MRL/lpr mice (hetero littermates) developed inflammatory skin disease at 6 months of age, while WSX-1 Tg mice developed no apparent skin symptoms. Histologically, epidermal hyperplasia and massive dermal infiltration of mononuclear cells were observed in the affected skin of both the WSX-1 KO- and control mice. The ratio of IL-4 positive cells and mast cells per total dermal infiltrates in the WSX-1 KO mice (16.0%±3.8% and 29.0%±5.9%, respectively) were significantly higher than those (9.0%±4.2%,12.9%±8.8%) of control mice. Lupus band test was positive in the affected skin in both the KO- and control mice, but deposition of Th1-associated immunoglobulin subclass (IgG2a), was observed only in control mice. Overall, we found that absence of IL-27 signaling skew Th1-type skin inflammation into that of Th2-type, and that the CD2-promoted WSX-1 overexpression was sufficient to abolish the development of skin inflammation in MRL/lpr mice. These indicates that WSX-1 KO mice might serve as a model for Th2-deviated skin inflammation such as atopic dermatitis, and that application of IL-27 might have therapeutic- as well as preventive effects on inflammatory skin diseases.

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Semaphorin3A alleviates skin lesions and scratching behavior in NC/Nga mice, an atopic dermatitis model

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Topical steroids and antihistamines are commonly used for the treatment of atopic dermatitis (AD). However, a substantial number of patients with AD are resistant to the existing treatments. In AD patients, the C-fiber extends and grows into the epidermis, inducing hypersensitivity to itch followed by scratching, which is considered to aggravate and prolong the disease. Semaphorin3A (Sema3A), an axon guidance molecule, is a potent inhibitor of neurite outgrowth of sensory neurons. To investigate the effect of Sema3A on AD, we administered recombinant Sema3A intracutaneously into the skin lesions of NC/Nga mice, an animal model of AD. Sema3A dose-dependently improved skin lesions and attenuated the scratching behavior in NC/Nga mice. Histological examinations revealed a decrease in the density of invasive nerve fibers in the epidermis; a decrease in the infiltration of inflammatory cells, including lymphocytes and mast cells; and a decrease in the epidermal thickness in the Sema3A-treated lesions. Because the interruption of the itch-scratch cycle likely contributes to the improvement of the AD-like skin lesions, Sema3A is promising in the treatment of patients with refractory AD, as well as overall itching dermatosis.

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Platelet P-selectin is required for leukocyte recruitment to skin in the late phase of immediate hypersensitivity reactions

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In addition to their role in hemostasis and thrombosis, platelets are important for assisting and modulating inflammatory reactions, but their function in the immediate hypersensitivity reaction in skin, such as that occurring in atopic dermatitis, remains unclear. The aim of this study was to investigate the role of platelets in a mouse model of IgE-mediated immediate hypersensitivity. Mice were sensitized by intraperitoneal injection of ovalbumin and alum and then challenged by intradermal injection of ovalbumin into the ears. Some groups of mice were depleted of platelets to a level of about 1000/μl by administration of antiplatelet antibody or busulfan, but levels of circulating leukocytes were unchanged. Sensitized mice developed a biphasic response at the site of ovalbumin injection, which was characterized by immediate and late phase reactions induced via a rapid increase in capillary permeability and leukocyte infiltration, respectively. In the immediate phase, the distribution and degranulation of mast cells in the ear skin did not differ between platelet-depleted mice and controls. Platelet depletion inhibited the late phase reaction (i.e. ear swelling, neutrophil infiltration and eosinophil infiltration) at 24 hours after ovalbumin challenge, but the immediate phase reaction was not suppressed. In platelet-depleted mice, the late phase reaction was restored by infusion of platelets from normal mice. Furthermore, this reaction was blocked by platelets from P-selectin-deficient mice or by pretreatment of platelets with anti-P-selectin antibody. These results suggest that platelet P-selectin plays an important role in the late phase reaction in a murine IgE-mediated skin inflammation model by mediating inflammatory cell adhesion to the endothelium, although platelets may not be involved in the immediate phase reaction. Therefore, platelet P-selectin may be a potential therapeutic target for regulating leukocyte infiltration in human allergic skin inflammation.

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Tacrolimus downregulates cell proliferation through inhibiting autocrine loop of vascular endothelial growth factor and its receptors in HaCaT cells

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Keratinocyte is considered as one of the major cell types which could secrete vascular endothelial growth factor (VEGF, an angiogenic growth factor in physiological and pathological conditions) and express VEGF receptors. In order to study the effect of tacrolimus on cell proliferation and on the expression of VEGF and VEGFR-2 in HaCaT cells, we determined the effect of VEGF165 (at 0,1,5,10,25,50, 100 ng/ml) and avastin (at 0,0.063, 0.13, 0.25, 0.50, 1.0, 2.0 mg/ml) on the proliferation of HaCaT cells by MTT assay and the effect of VEGF165 and avastin in different concentrations on the migrating activity of HaCaT cells by migration assay. The effect of VEGF165 (10ng/ml) on phosphorylation of ERK1/2 was also checked in HaCaT cells in pretreated or not pretreated with avastin (0.5mg/ml). The results showed that VEGF could significantly enhance the proliferation and migration of HaCaT cells in a dose-dependent manner, whereas avastin could inhibit the proliferation and migration of HaCaT cells in a dose-dependent manner. VEGF165 at 10ng/ml could induce the phosphorylation of ERK1/2 in HaCaT cells, but this effect was diminished in the cells pretreated with 0.5mg/ml avastin for 1 hour. Tacrolimus inhibited the mRNA and protein expression of VEGF and VEGFR-2 on HaCaT cells in a dose dependent manner, as determined by RT-PCR and Western-blot respectively. Downregulation effect of Tacrolimus on the proliferation of HaCaT cells could be partly reversed by adding VEGF165 at 10ng/ml. It is concluded that the inhibiting effect of tacrolimus on the proliferation of keratinocytes is partly due to the inhibition on the VEGF-VEGFR-2 autocrine loop in keratinocytes.

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Autoantibodies against the linker subdomains of envoplakin and periplakin play an important role in the pathogenesis of paraneoplastic pemphigus

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Circulating anti-Dsg3 antibody, the major pathogenic autoantibody in PV, has been proved pathogenic in PNP. Because of many differences between PNP and PV, we speculate autoantibodies against other molecules may also play roles in the pathogenesis of PNP. Envoplakin and periplakin are recognized by most PNP sera. Their linker subdomains are highly homologous and necessary for the association of intermediate filaments. We characterized the autoantibodies against the linker subdomains of envoplakin (EPL) and periplakin (PPL) in PNP patients' sera and their associated tumors by ELISA. Ligand-receptor assay were used to probe the specific immunoglobulin receptors on B lymphocytes in tumors. We studied the effects of these autoantibodies on keratinocytes in vitro by immunofluorescence and adhesion assay. Autoantibodies against EPL and PPL were detected in most PNP patients, and the decrease of these autoantibodies after removal of the tumors was roughly comparable to the improvement of clinical symptoms. Cultured Tumor cells from PNP patients secreted these autoantibodies. Specific immunoglobulin receptors for EPL and PPL were found on B lymphocytes in tumors from PNP. Purified anti-EPL and anti-PPL autoantibodies from PNP sera were capable of dissociating cultured HEK. Our results demonstrate autoantibodies against EPL and PPL may be primarily produced and are important factors in the pathogenesis of PNP.

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Utility of the DNA microarray scanner to simplify the diagnosis of autoimmune bullous diseases

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We tested the utility of a DNA microarray scanner as a digital fluorescence microscope for the diagnosis of autoimmune bullous diseases. We substituted the more stable cyanine dyes, Cy3 and Cy5, in place of fluorescein, and then utilized the higher sensitivity of a DNA microarray scanner in place of a microscope. Frozen sections of skin biopsies were taken from three patients with bullous pemphigoid, one patient with lichen planus pemphigoides, one patient with linear IgA disease, and one patient with dermatitis herpetiformis. The sections were incubated with diagnostic antibodies including anti-IgG directly conjugated with Cy3, anti-IgA antibody followed by a secondary antibody conjugated to Cy5, and anti-complement C3 followed by a secondary antibody conjugated to Cy5. A DNA microarray scanner was used to digitally scan the tissue sections at a resolution of five microns. Significantly, the entire tissue could be imaged in one large field of view with immune deposits highlighted within that view, thereby obviating difficulties of tissue orientation associated with standard fluorescence microscopy. The images were all diagnostic, and they included a linear pattern along the basement membrane zone (BMZ) using antibodies to IgG and C3 in all cases of bullous pemphigoid; a linear pattern of IgG along the BMZ in lichen planus pemphigoides; and a linear pattern of IgA along the BMZ in linear IgA dermatosis. The expected pattern of IgA deposition along the dermal papillary tips was found in dermatitis herpetiformis, though the fine granular pattern was not discernable at the resolution of this scanner. Immune deposits were absent in all normal controls. The advantages of the microarray scanner over standard fluorescence microscopy include the technical ease, the large field of view allowing easy confirmation of immune deposits, the potential for visualizing multiple antibodies simultaneously in the same tissue, and the convenience of digital image archiving.

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Expression of somatostatin receptor subtypes 1 to 5 in normal skin, psoriasis and atopic dermatitis

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This study describes the presence and distribution of somatostatin receptors (SSTR) in normal human skin, psoriasis and atopic dermatitis analyzed with immunohistochemistry. Somatostatin immunoreactive nerve fibers and cells with dendritic morphology are found in normal human skin. Somatostatin mediates its actions by G-protein-coupled receptors, which consists of five distinct subtypes (SSTR 1-5). Normal human skin from healthy volunteers, psoriasis patients and atopic dermatitis showed a lot of similarities but also some differences with respect to SSTR expression. SSTR 1-3 was strongly expressed in the epidermis of normal skin, psoriatic skin and in atopic dermatitis. SSTR 4 and 5 was strongly expressed in the epidermis of psoriasis patients, but showed a weak expression in atopic dermatitis and normal skin. The intensity of the staining also varied notably between the different keratinocyte layers in the epidermis, especially in psoriasis patients. Dendritic cells, which were mostly distributed in the papillary and upper reticular dermis, showed a strong expression of SSTR 1-4, but a weak expression of SSTR 5 in all cases. SSTR 1-5 were strongly expressed in sweat glands in all skin biopsies. Hair follicles and sebaceous glands expressed all five SSTR. Striated muscle fibres showed an intense positive expression of SSTR 1-4, but a weak or negative expression for SSTR 5. Inflammatory skin diseases such as psoriasis and atopic dermatitis have a neurogenic component and neuropeptides can directly modulate functions of keratinocytes, dendritic cells, mast cells, endothelial cells and infiltrating immune cells in the skin. Somatostatin is a neuropeptide with several immmunomodulating actions and it has been reported to inhibit lymphocyte proliferation, chemokine-induced T-lymphocyte infiltration and to block the release of various growth factors.

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Vitamin D treatment upregulates the antimicrobial protein hCAP18/LL-37 in atopic dermatitis

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Low levels of antimicrobial peptides was recently proposed to contribute to susceptibility for skin infections in atopic dermatitis (AD). Focussing on the human cathelicidin antimicrobial protein, hCAP18, the aim of the present study was to explore whether reduced hCAP18 expression is a constitutive trait in AD and/or whether known inducers of hCAP18 expression are capable of upregulating hCAP18 in skin of atopic dermatitis. First we compared levels of hCAP18 mRNA between lesional skin in AD and psoriasis and verified a significantly lower expression in lesional AD skin. However, when comparing non-lesional skin from both groups with healthy skin, there was no difference between groups indicating that there is no constitutive defect in basal production of hCAP18 in AD patients. In skin, strong upregulation of hCAP18 normally occurs following injury and in addition, current research has identified vitamin D as a direct and potent inducer of hCAP18mRNA and protein. In psoriasis, there was significant upregulation of hCAP18 expression in non-lesional as well as in lesional skin following topical application of vitamin D as well as following injury. In AD however, there was upregulation of hCAP18 mRNA only in non-affected skin and not in eczema lesions in response to wounding. However, topical vitamin D treatment upregulated hCAP18 expression in lesional as well as in non-lesional AD skin. Cultured primary keratinocytes from non-lesional skin of psoriasis, AD and healthy skin, all upregulated hCAP18mRNA following treatment with vitamin D in vitro. Taken together, our data indicate that there is normal basal production of hCAP18 in the skin of AD patients, but a failure to respond adequately to trauma in eczema lesions, likely due to the suppressive inflammatory profile. In contrast, vitamin D was able to effectively overcome this suppression and upregulated hCAP18 also in the eczema lesions. This suggests that topical treatment with vitamin D might aid in reducing skin infections in AD.

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Eotaxin, TARC, MCP-1, and CCR-1, CXCR-1, CXCR-2 expression in patients with dermatitis herpetiformis and bullous pemphigoid

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Bullous pemphigoid (BP) and dermatitis herpetiformis (DH) are skin diseases associated with eosinophilc and neutrophilic infiltrations. There are few findings concerning complex assessment of chemokines and their receptors in skin lesions and perilesional skin in these diseases. The aim of the study was to asses differences in expression of chemokines and their receptors in DH and BP. Skin biopsies were taken from 10 patients with DH, 14 with BP and from 8 healthy subjects. The localization and expression of eotaxin, TARC, MCP-1, CCR-1, CXCR-1 and CXCR-2 in skin lesions and perilesional skin were studied by immunohistochemistry. Moreover the serum concentration of selected cytokines was measured by immunoassays. Expression of eotaxin and TARC in the epidermis as well as in inflammatory influxed cells in dermis was detected in BP and DH patients. Expression of MCP-1 was confirmed only in epidermis and infiltration cells in BP patients. Examined chemokines expression was detected in perilesional skin of all patients but it was much lower than in lesional skin. CCR-1, CXCR-1 i CXCR-2 expression was confirmed in lesional as well as perilesional skin. The expression of neither examined chemokines nor their receptors was observed in healthy people. Our data suggest that the difference in chemokine pattern expression directly contributes to variations in the cellular infiltration in DH and PH.

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Effectiveness of IVIg used with or without a cytotoxic drug in the treatment of pemphigus.

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As IVIg works by lowering serum levels of pemphigus antibodies, this study was conducted to examine whether co-administration of a cytotoxic drug could improve the clinical effectiveness of this treatment. Thirteen patients with pemphigus vulgaris (PV) or foliaceus (PF) were treated with 16 courses of IVIg given alone (n=5) or concurrently (n=11) with cyclophosphamide or azathioprine. Each course consisted of three cycles of IVIg given 2 weeks apart. Each cycle consisted of 400 mg Ig/kg given IV daily for 5 days. Response was evaluated based on changes in intercellular antibody serum levels and in disease severity 1 week after the third cycle. Disease severity was graded using a severity score based on disease activity, extent, and intensity of treatment. Intercellular antibodies decreased by a median of 69% following eleven course of IVIg given with cyclophosphamide or azathioprine compared to 38% following five courses of IVIg given without a cytotoxic agent, p=0.005. Median disease severity score in patients treated with IVIG + cyclophosphamide or azathioprine decreased almost three times more rapidly (by 31.5% and 36.5% respectively) than treatment with IVIg alone (12.5%), though the difference was not statistically significant in this small group of patients. There was no significant difference in effectiveness between the two cytotoxic agents used. These findings indicate the therapeutic effectiveness of IVIg when used to treat pemphigus, and possibly other autoantibody-mediated diseases, may be improved by the concurrent administration of a cytotoxic agent.

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Telomerase reverse transcriptase transgenic rats develop severe dermatitis

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Atopic dermatitis is a chronic inflammatory skin disease. Its development is primarily determined by unknown genetic factors, leading to the accumulation of activated T lymphocytes in the skin. There is emerging evidence that peripheral blood T lymphocytes from patients with atopic dermatitis significantly increase the telomerase activity and reduce the telomere length, which are specialized nucleoprotein structures that are capped at the end of each chromosome. The evidence suggests that a majority of the lymphocytes have undergone a high number of cell divisions *in vivo*, although the relationship between increased telomerase activity and chronic inflammatory skin disease remains unclear. Herein, we demonstrate that telomerase reverse transcriptase (TERT)-expressing transgenic (Tg) rats spontaneously develop eczematous skin inflammation during infanthood, and that peripheral blood T lymphocytes of TERT Tg rats with severe dermatitis show significant TERT expression and activity. TERT Tg rats developed visible dermatitis in 40% of new-born rats, in which the skin lesion microscopically showed spongiosis and acanthosis with infiltrates of lymphocytes and mast cells. TERT Tg rats with severe dermatitis exhibited an increased cell number in CD4 (2.5 fold) and CD8 (5 fold) T cells, compared with Tg rats without dermatitis. TERT activity was stronger in peripheral lymphocytes of dermatitis Tg rats than non-dermatitis Tg rats ($p<0.05$). RT-PCR analysis revealed that interferon-g was expressed in the dermatitis lesion of Tg rats, but IL-2 and IL-4 were not detected. Moreover, skin grafting of TERT Tg rats with severe dermatitis onto T-cell deficient nude rats demonstrated that the inflamed skin lesions could not be maintained. Thus, the results from TERT Tg rats suggest that increased telomerase activity in T lymphocytes plays a critical role in developing eczematous skin inflammation.

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Development of an active disease model for bullous pemphigoid

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Bullous pemphigoid (BP) is the most common autoimmune blistering skin disease caused by autoantibodies against collagen XVII (COL17). Recent studies in passive transfer neonatal BP mouse models using injection of anti-COL17 IgG antibody have made significant advances to understand the pathomechanisms of BP. However, the investigations into novel therapeutic strategies have been limited because these passive transfer models only demonstrate transient disease activity. The purpose of this study is to establish an active, stable BP mouse model that continuously produces anti-COL17 antibodies in vivo and demonstrates BP disease phenotype. In order to develop such BP model mice, we crossed COL17-humanized ($COL17^{m-/-,h+}$) mice that we recently generated *(Nat Med 2007)* with Rag-$2^{-/-}$ mice to make Rag$^{-2-/-}$/$COL17^{m-/-,h+}$ mice. Splenocytes from wild type mice, that had been immunized by grafting of hCOL17 transgenic ($COL17^{m+/+,h+}$) mouse skin, were intravenously transferred into Rag-$2^{-/-}$/$COL17^{m-/-,h+}$ mice. In the recipient mice anti-human COL17 (hCOL17) IgG was continuously produced without the need for boosting at least for over five weeks. The recipient mice developed severe erythema and erosions mainly on the chest and face. Histological examination demonstrated separation of the epidermis from the dermis with a dermal inflammatory cell infiltration. Direct immunofluorescence studies showed linear deposition of mouse IgG and C3 along the epidermal basement membrane zone. To further elucidate the pathogenic role of anti-hCOL17 IgG obtained from immunized mice, we performed passive transfer of this IgG into $COL17^{m-/-,h+}$ neonatal mice. These mice developed diffuse erythema and epidermal detachment after gentle friction. In conclusion, we have successfully developed the first active BP adult mouse model that continuously produces pathogenic anti-hCOL17 IgG and demonstrates BP clinical phenotype. This BP model could be used not only for further study of the BP pathomechanisms, but also to establish novel therapeutic strategies for BP.

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Modulation of inflammation in two clinically relevant humanized mouse models of psoriasis

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We here describe two approaches to identify and evaluate candidate drugs in a humanized mouse model of psoriasis using either non-lesional or lesional skin from psoriasis vulgaris patients. Full-thickness punch skin biopsies of these patients were transplanted onto the back of hairless bg-nuxid mice lacking B, T, and NK cells. In the non-lesional skin graft model the psoriatic process was initiated by intradermal injection of 0.5 - 1x10E6 autologous T cells, after their activation *in vitro* by SEB superantigen. In this model the effects of prophylactic treatment with various compounds on early events of psoriasis development were evaluated. As an alternative, we employed a lesional skin graft model to evaluate the effect of candidate drugs on an ongoing disease process. In both models the prevention or inhibition of skin inflammation could be achieved as measured by decreased proliferation and differentiation of keratinocytes – evident from decreased expression of Ki-67 and CK-16 - and a decreased number of Langerhans cells in the skin graft. This coincided with decreased expression of HLA-DR and diminished production of inflammatory cytokines like TNF-α. Epidermal hyperplasia being the most important read-out for the development of a psoriatic lesion was evaluated to demonstrate a potential therapeutic effect of new drug candidates. In multiple studies these two models were shown to represent a reliable platform for the functional pre-clinical testing of new therapeutic compounds. Inhibitory effects of a wide range of compounds including inhibitors of TNF-α, antibodies directed against growth factors, MMP inhibitors, calcipotriol, metothrexate, betamethasone, and cyclosporine A were demonstrated. Our findings show that the two humanized mouse models for psoriasis represent a powerful tool for the identification or validation of compounds with potential for the treatment of psoriasis.

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Cryopyrin-associated IL-1β secretion in mast cells and a role played by mast cells in autoinflammatory disorders

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Cryopyrin-associated periodic syndrome (CAPS) is a hereditary periodic autoinflammatory diseases characterized by urticarial rush, neurological symptoms and arthritis. The responsible gene is CIAS1 and its product, cryopyrin, belongs to a member of the NOD-LRR protein family that is a key molecule for host defense by recognizing molecular patterns of pathogens and internal danger signals. Upon physical association with ASC and procaspase-1 to form inflammasome, cryopyrin activates caspase-1, leading to processing of proIL-1β into its active form. CIAS1 mutants in CAPS can spontaneously activate inflammasomes leading to the excessive IL-1β production, which is believed to be involved in the clinical symptoms. In fact, IL-1β receptor antagonist but not any antihistamines is effective to urticarial rush in CAPS. Therefore, we hypothesized that IL-1β secretion but not the degranulation by mast cells may be responsible for the clinical symptoms. Using RT-PCR, murine bone marrow-derived mast cells (BMMC) and fetal skin-derived mast cells expressed little mRNA for CIAS1 and IL-1β, but both were inducible by LPS treatment. Assessed by ELISA, LPS did not induce an active form of IL-1β in both cell types. However, R837, a cryoprin activator, dramatically induced IL-1β secretion only if the cells were treated with LPS. Interestingly, both stimuli did not induce the degranulation from mast cells. We then introduced several CIAS1 mutants corresponding to reported mutations in CAPS, which can induce ASC-dependent spontaneous NF-κB activation in reporter assay, into BMMC using a retrovirus vector system. As expected, all mutant CIAS1-transfected mast cells secreted active IL-1β after LPS treatment even without R837. These results suggest that mast cells are also a responsible cell type in CAPS for the pathophysiological aspects by producing IL-1β. Furthermore, since we found combinations of stimuli (e.g., LPS plus R837) could render normal mast cells to secrete IL-1β, it is conceivable that cryoprin-mediated IL-1β may be involved in antihistamine-refractory urticaria.

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TGF-beta-dependent suppressive function of regulatory T-cells requires CD18 wild-type levels in a psoriasis murine model

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Dysfunctional regulatory T-cells (Treg-cells) have been identified in human psoriasis. However, their role in its pathogenesis remains unclear. We previously showed that CD18 hypomorphic (CD18hypo) PL/J mice with reduced levels of the common chain of beta2 integrins (CD11/CD18) to only 2–16% of wild-type levels develop a psoriasiform skin disease strongly resembling human psoriasis. Here we explored the CD18 hypomorphic (CD18hypo) PL/J psoriasis mouse model that closely resembles human psoriasis to study the effect of diminished CD18 expression on CD4+CD25+CD127- Treg-cell function. We found that CD18 expression reduced to 2-16% of wild-type levels is responsible for an impaired cell-cell contact between Treg-cells and dendritic cells (DCs). This leads to dysfunctional Treg-cells which fail to suppress pathogenic T-cells, thus promoting onset and severity of the disease. This failure is TGF-beta-dependent, with diminished TGF-beta1 expression by Treg-cells derived from CD18hypo PL/J mice. Adoptive transfer of CD18 wild-type (CD18wt) Treg-cells into affected CD18hypo PL/J mice resulted in a significant improvement of the psoriasiform skin disease, which does not occur upon coinjection with TGF-beta neutralizing antibody. Our data indicate a primary dysfunction of CD18hypo Treg-cells with subsequent hyperproliferation of pathogenic T-cells, which provides a significant step forward in our understanding of the unique role of wild-type CD18 expression levels in avoiding autoimmunity.

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Bioavailability and pharmacological effects of a new pentyl-rhamnoside on inflamed skin

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Atopic dermatitis is a chronic inflammatory skin disease associated with cutaneous hyper-reactivity to environmental triggers. In response to epidermal barrier disruption and bacterial invasion, biological signals are generated by host epidermal cells to raise locally the innate then acquired immune defence system. Keratinocytes initiate skin inflammation and recruit immune inflammatory cells but excessive inflammatory cell influx contributes to the development of this inflammatory disease. We proposed to synthesize a pentyl-rhamnoside (PR) in order to enhance the low epidermal absorption of rhamnose, known to exhibit anti-inflammatory activities. Our objective was to determine the relevance of this chemical structure modification in term of skin absorption enhancement and to demonstrate its improved activity in the modulation of uncontrolled inflammatory processes, as compared to rhamnose. A dermal absorption study showed that less than 15% of rhamnose was found in the stratum corneum and only 3% in the epidermis, whereas about 30% of PR was found in the SC, and up to 8% in the epidermis, with a specific localization in the SC lipid layers and in the keratinocyte membranes, visualized with auto-historadiography. A topical treatment of SEB-stimulated human skin explants with the formulated PR, contributed to inhibit the production of primary pro-inflammatory cytokines, such as IL-1, TNFα, IL-6 or IL-8 by the keratinocytes. Moreover, it lowered their RANTES and ICAM-1 expression. The production of TSLP and Rank-L by the SEB-stimulated skin keratinocytes was also modulated by the PR treatment. In conclusion, we demonstrated that the chemical modification of rhamnose into pentyl-rhamnoside provided a higher epidermal bio-availability. Beyond the improvement of the rhamnose anti-inflammatory activity into the epidermis, the use of PR could contribute to restrain the excessive recruitment of the immune inflammatory cells into the atopic epidermal compartment.

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CC chemokine receptor-6 (CCR6) is essential for IL-23-mediated psoriasiform dermatitis in a murine model of psoriasis

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Recent data implicate IL-23 and Th17 cells in the pathogenesis of psoriasis and other autoimmune diseases. CC chemokine receptor-6 (CCR6)-positive T cells have been found in human psoriatic skin lesions where CCL20, a CCR6 ligand, is abundantly expressed. Th17 cells express CCR6, but it is unknown whether CCR6 is critical for Th17 cell homing or function at sites of inflammation. To address this question, we injected recombinant IL-23 into ear skin of CCR6-knockout (KO) and wild type (WT) control mice every other day for 2 weeks to induce psoriasiform inflammation (i.e., epidermal acanthosis, parakeratosis, and a dermal lymphocytic infiltrate). IL-23-injected WT mice showed a marked increase in ear thickness of ~100 μm compared to PBS-injected controls (N=10/group, P=0.0007), whereas CCR6 knock-out (CCR6 KO) mice showed no increase in thickness after IL-23 treatment. By flow cytometry, increased numbers of CD4+ T cells in ear skin were detected in IL-23-treated WT, but not CCR6 KO, mice. Analysis of dermal cell suspensions by flow cytometry indicated that >95% of IL-17+ dermal T cells from IL-23-treated WT mice also co-expressed CCR6. By quantitative RT-PCR and flow cytometry, CCR6-expressing T cells were increased ~2 fold in IL-23-treated (vs. PBS) skin of WT mice. IL-23-treated WT and CCR6 KO mice showed similar increases in epidermal and endothelial CCL20 expression, suggesting that CCR6 was not required for up-regulation of CCL20 in skin. In vitro, ~45% of T cells, which had been driven from a naïve state toward Th17 differentiation by anti-CD3 mAb + Th17 promoting cytokines, expressed CCR6. Furthermore, sorted CCR6+ T cells expressed more IL-17 than did CCR6- T cells. In summary, IL-23 treatment results in psoriasiform dermatitis and accumulation of CCR6+ T cells in the dermis of WT mice. Mice deficient in CCR6 are resistant to IL-23-mediated psoriasiform dermatitis, suggesting possible critical role(s) for this chemokine receptor in human psoriasis.

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Elevation of IgA anti-epidermal transglutaminase antibodies in dermatitis herpetiformis

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Dermatitis herpetiformis (DH) is a papulo-vesicular eruption caused by ingestion of gluten. It is characterized by the deposition of IgA in the dermal papillae. IgA antibodies directed at tissue transglutaminase are elevated in gluten-sensitive diseases including DH and celiac disease (CD). More recently, antibodies directed at epidermal transglutaminase were identified in patients with DH, and this may be the dominant autoantigen in this disease. Our objectives were to measure IgA antibodies to epidermal transglutaminase and tissue transglutaminase in patients with DH, CD, and control populations. Serum IgA antibodies against tissue transglutaminase and epidermal transglutaminase were measured from adult patients with DH, adult and pediatric CD patients, psoriasis patients, and Red Cross blood donors. Patients with DH and CD had elevated levels of IgA anti-tissue transglutaminase antibodies compared to control populations. The levels in the DH and adult CD patients were similar. IgA anti-tissue transglutaminase antibodies were higher in the pediatric CD patients compared with adult DH and CD patients, and control populations. Patients with DH and adult patients with CD had elevated levels of IgA anti-epidermal transglutaminase antibodies compared with pediatric CD patients and control populations. There was a trend towards higher levels in the DH patients compared with adult CD patients. In conclusion, IgA antibodies to epidermal transglutaminase are elevated in patients with DH and adult patients with CD. The progressive expansion of the epitope binding profile of IgA anti-transglutaminase antibodies in CD patients may explain the development of DH in undiagnosed CD patients during their adult life.

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Induction of both humoral and cellular immune response against desmoglein 3 provides a mouse model mimicking an immunological aspect of paraneoplastic pemphigus

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Pemphigus vulgaris (PV) model mice are generated by adoptive transfer of lymphocytes of $Dsg3^{-/-}$ mice that do not acquire tolerance to Dsg3 both in humoral and cellular immunity. Recipient mice receiving $Dsg3^{-/-}$ lymphocytes persistently produce anti-Dsg3 IgG and develop PV phenotype including loss of cell-to-cell adhesion of oral epithelial cells without lymphocytic infiltration. In this study, we attempted to induce cellular immune response by changing the way of priming to Dsg3 with skin graft. The mortality of $Dsg3^{-/-}$ mice with C57BL/6 (B6) background was rescued by ectopic expression of Dsg1 in the place of Dsg3 by keratin 5 promoter ($Dsg1^{Tg}Dsg3^{-/-}$). When wild type (B6) Dsg3+ skin was grafted on $Dsg1^{Tg}Dsg3^{-/-}$ mice, the graft showed spongiosis, CD4+ and CD8+ T cell infiltration, as well as individual epithelial cell necrosis at day 21. The graft was eventually rejected by day 28. The second Dsg3+ skin graft was more quickly rejected by day 21. No such changes were observed when the skin was grafted on $Dsg1^{Tg}Dsg3^{+/-}$ mice. Furthermore, when splenocytes of the grafted $Dsg1^{Tg}Dsg3^{-/-}$ mice were adoptively transferred to Rag2 immunodeficient mice, the recipient mice showed the cellular infiltration of CD4+ and CD8+ T cells to oral epithelia in addition to the production of anti-Dsg3 IgG and development of PV phenotype. These findings indicate that this protocol can induce inflammatory CD4+ and cytotoxic CD8+ T cells presumably against Dsg3, which in turn attack Dsg3-expressing epithelial cells in oral mucosa, in addition to humoral response against Dsg3. This mouse model provides an important tool to study unclarified immunological mechanisms of paraneoplastic pemphigus that shows the both humoral and cellular autoimmune attack to epithelia.

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Analysis of discoidin domain receptor-1 expression by tissue-infiltrating eosinophils in allergic skin diseases

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Discoidin domain receptor 1 (DDR1) is a receptor tyrosine kinase that is activated on binding to its ligand-collagen a component of the extracellular matrix (ECM) in the organs and is known to be constitutively expressed in the normal tissues such as the lungs, kidneys, colon, and brain. It has been reported that the tissue-infiltrating eosinophils expressed DDR1 and interaction between endogenous DDR1 on eosinophils and collagen in the ECM might affect eosinophil survival in the tissue microenvironment through NFκ-B activation in Churg-Strauss syndrome (CSS) characterized by necrotizing granulomatous angitis with massive eosinophil infiltration, asthma, and hypereosinophilia. However, little is known about the DDR1 expression and its significance in tissue infiltrating eosinophils in allergic diseases. Therefore, we analysed DDR1 expression by tissue-infiltrating eosinophils in allergic skin diseases such as atopic dermatitis, drug eruption and Kimura's disease with eosinophil infiltration. Immunohistochemical staining revealed that tissue-infiltrated eosinophils in CSS skin lesions exhibit intense immunoreactivity for DDR1, while other allergic skin diseases are less intense. Colabeling with eosinophil major basic protein (EMBP) and DDR1 also confirmed that CSS-related skin-infiltrated eosinophils are positive for DDR1. Our current results indicate that DDR1 expression by eosinophils in the local inflammatory sites is specific for CSS and could be the disease marker in allergic skin diseases with eosinophil infiltration. Mechanisms of eosinophil survival and activation might be different between CSS and allergic skin disease such as atopic dermatitis.

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Histological criteria of psoriatic erythroderma

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Erythroderma is severe and sometimes life threatening condition characterized by an universal inflammatory affection of the skin, a generalized lymphadenopathy. Erythroderma may be as a severe form of psoriasis. It can begin as a progress of common psoriasis process under the influence of exter-nal factors. Making a differential diagnosis between different types of erythrodermas is sometimes dif-ficult because of similarity of histological features. We made histology examination of 12 patients with psoriatic erythroderma and 15 patients with common forms of psoriasis. In common psoriasis histological changes are seen usually in epidermis and characterized by parakeratosis, hyperkeratosis, regular acanthosis, Munro microabscesses, papil-lomatosis. In psoriatic erythroderma rete ridges where not only long but also wide, the length was 302,8 mkm (25,2 keratynocytes), width – 269,8 mkm (24,1 keratinocytes). In common psoriasis the length was 283,6 mkm (23,4 keratynocytes; p>0,05), width – 201,3 mkm (16,1 keratinocytes; p<0,05). The quantity of mitotic cells in the progressive stage of common psoriasis was up to 13,1‰ in the basal layer, and up to 11,8‰ in suprabasal layers. In psoriatic erythroderma - 15,7‰ and 13,6‰. In psoriatic erythroderma intercellular and intracellular edema is seen. Granular and corneum layers are sometimes absent that may confirm abnormal differential process of the keratynocytes. In both forms perivascular infiltrates in derma was made of lymphocytes, macrophages and fibroblasts. The density of cells in common psoriasis was 2123,5 cells/mm2, in erythroderma – 2856,0 cells/mm2. In erythroderma widening of miscrovessels was more prominent. Destructive changes where seen in cap-illaries of papillary dermis – capillaritis, adhesion of erythrocytes to endothelium, sometimes – des-quamation of endotheliocytes. In the vessels of upper vessels there are phlebitis, trombophlebitis, arte-riolitis. Such changes where not seen in psoriasis vulgaris. As a conclusion, histological signs of psoriasis vulgaris and psoriatic erythroderma may serve as a differential tools for confirming diagnosis.

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Presence of circulating basement membrane antibodies in patients with graft-versus-host-disease

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In the past, 12 cases of bullous autoimmune dermatoses (mostly bullous pemphigoid) have been reported in patients with acute or chronic graft-versus-host-disease (cGvHD) after allogeneic hematopoetic cell transplantation (HCT). These cases raise the question whether GvHD and bullous autoimmune dermatoses represent related disorders or just a mere coincidence. To assess the prevalence of circulating antibodies to basement membrane (BM) proteins in HCT patients, we analysed sera of 42 HCT patients with active cGvHD involving skin or visceral organs, of 18 HCT patients without GvHD and 40 controls by indirect immunofluorescence (IIF) on salt-split-skin, NC16a-ELISA and immunoblot with keratinocyte extracts. While none of the HCT patients without GvHD showed positive results by any of the assays, 24% (10/42) of the cGvHD patients displayed circulating antibodies by immunoblot analysis. The target antigens were collagen VII (3/42), BP230 (1/42), collagen XVII (4/42) and p200 (2/42). When IIF and ELISA were used as assays, nearly all cGvHD-sera were unreactive. Only one serum reactive with collagen XVII in immunoblot showed also IgG binding to the epidermal side by IIF (titer 1:80) and to the NC16a-domain of collagen XVII by ELISA (OD 1,2; normal < 0,36). Of the control sera, only 0,03% (1/40) reacted in immunoblot and none in IIF or ELISA. Of the cGvHD-patients with circulating antibodies, one was diagnosed having epidermolysis bullosa acquisita on the basis of clinical, histological and immunofluorescence criteria. None of the others exhibited symptoms of a bullous autoimmune disorder, although all patients with positive immunoblot reactions showed mucocutaneous involvement in the context of cGvHD. In summary, we hypothesize that recurrent mucocutaneous lesions in cGvHD liberate antigens leading to production of BM antibodies as an epiphenomenon. Additional factors will be needed for the development of a concurrent bullous autoimmune dermatosis.

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Possible pathogenic role of Th17 cells for atopic dermatitis

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The critical role of IL-17 has recently been reported in a variety of conditions. Since IL-17 deeply participates in the pathogenesis of psoriasis and keratinocyte production of certain cytokines, the involvement of Th17 in atopic dermatitis (AD) is an issue to be elucidated. We investigated the percentage of Th17 cells in the peripheral blood of AD by flow cytometry, the infiltration of Th17 cells in the lesional skin of AD by immunohistochemistry, and the effect of IL-17 in combination with IL-22 on the production of cytokines/chemokines and vascular endothelial growth factor (VEGF) by normal human keratinocytes. We successfully detected circulating lymphocytes intracellularly positive for IL-17 by flow cytometry, and the IL-17+ cell population was found exclusively in CD3+CD4+ T cells. The percentage of Th17 cells was increased in peripheral blood of AD patients and associated with the severity of AD. There was a significant correlation between the percentages of IL-17+ and interferon-g+ cells, although the Th17 percentage was not closely related to Th1/Th2 balance. Immunohistochemically, IL-17+ cells infiltrated in the papillary dermis of atopic eczema more markedly in the acute than chronic lesions. Finally, IL-17 stimulated keratinocytes to produce GM-CSF, TNF-a, IL-8, CXCL10, and VEGF. A marked synergistic effect between IL-17 and IL-22 was observed on IL-8 production. In conclusion, the number of Th17 cells is increased in the peripheral blood and acute lesional skin of AD. Th17 cells may exaggerate atopic eczema.

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IgM to IgG isotype switching of anti-desmoglein 3 antibodies is an essential step for pathogenesis of pemphigus vulgaris

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Pemphigus vulgaris (PV) is a tissue specific autoimmune disease caused by pathogenic IgG autoantibodies against a desmosomal adhesion molecule, desmoglein 3 (Dsg3). In a humoral immune response, IgM production preceds that of IgG. Pathogenic role of IgM autoantibodies in PV remains to be elucidated. In this study we explored the role of IgM in PV by converting AK23 pathogenic IgG monoclonal antibody to a IgM form. AK23 IgG reacts with adhesive interface of Dsg3 and can induce PV phenotype including oral erosions with acantholysis in histology in mice. We first generated B cell transgenic (B-Tg) mice using cDNA for variable regions of AK23 in a IgM backbone. AK23 B-Tg mice showed no apparent PV phenotype in spite of AK23 IgM in circulation. To administrate excess amount of AK23 IgM, we isolated hybridoma cells producing AK23 IgM from B-Tg mice. However, no PV phenotype was induced either by passive transfer of AK23 IgM into neonatal mice or hybridoma inoculation in adult mice in contrast to AK23 IgG. In those mice with AK23 IgM, *in vivo* IgM deposition was found on keratinocyte cell surfaces but its intensity was much weaker than that of IgG deposition. AK23 IgM binding was found on cell membrane just at the edges of desmosomes, but not inside of extracellular portion of desmosomes, by immunoelectron microscope. Furthermore, *in vitro* dispase assay demonstrated that AK23 IgM failed to induce apparent keratinocyte dissociation while smaller fragments of F(ab')2 digested from AK23 IgM could do so. These results indicate that AK23 IgM is unable to induce the loss of cell adhesion due to its inaccessibility to native Dsg3 in the core of desmosomes. Thus, IgM to IgG isotype switching is an essential step for pathogenesis of pemphigus, providing an important framework for better understanding of immunological process of autoantibody production.

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FoxP3+ regulatory T cell deficiency in patients with alopecia areata

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Alopecia areata is an inflammatory hair loss disorder, which is characterized by Tcell-infiltration around hair follicles. Although spontaneous regression can occur in most cases with limited patches, the prognosis is miserable in cases with extensive lesions. The accumulating evidence indicates autoimmune etiology: (1) there is increased frequency of other autoimmune disease in alopecia areata; (2) alopecia can be reproduced by the transfer of alopecia skin-derived activated T cell in the human hair follicles grafted in lymphocyte-deficient mice. The current conventional therapies are rather empirical; therefore, further investigation is required for establishing etiology-oriented therapies. Dysfunction of Regulatory T cells (Tregs), a specific subpopulation of T lymphocytes suppressing immune reaction, can contribute to kinds of autoimmune disease such as psoriasis and SLE and, furthermore, the manipulation of Tregs can impede the undesired autoimmune reaction in model mice. Here we report that the numbers of Tregs are diminished in alopecia areata. There is a significant decreased frequency of CD4+FOXP3+ T cells in alopecia patients (n = 37) compared to normal donors (n = 35), respectively, 5.4% versus 6.9%; $P < 0.01$. The clinical data show correlations between lower proportions of peripheral blood Tregs count and disease severity, activity and duration. FOXP3+Tregs are rarely observed in active alopecia lesions of usual patients. In contrast, prominent FOXP3+ Tregs infiltrations around hair follicles were detected in alopecia lesions of alopecia totalis patients who followed exceptional course of self-healing. We analyzed Tregs-associated genes in these patients to clarify the association between gene polymorphisms and these phenotypes. These results provide a novel insight into the pathogenesis of alopecia areata and suggest that manipulation of Tregs could be instrumental for controlling aberrant immune reaction in alopecia areata.

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A novel therapeutic strategy for bullous pemphigoid using recombinant monoclonal Fab antibody against NC16A domain of collagen XVII

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Bullous pemphigoid (BP) is the most common autoimmune blistering diseases. Previous studies have shown that the classic complement cascade activation pathway is required for the development of tissue injury and blister formation after the initial binding of pathogenic autoantibodies against collagen XVII (COL17). The purpose of the present study was to develop a new therapeutic strategy by preventing complement activation via blocking autoantibody binding to the pathogenic epitope (NC16A domain). We have generated Fab monoclonal antibody (Fab-mAb) fragments using antibody repertoires of BP patients by phage display. After functional characterization, two Fab-mAbs, Fab-B4 and Fab-19, showed a marked ability to inhibit BP autoantibody binding and subsequent complement activation in a dose-dependent manner *in vitro*. *In vivo* therapeutic effects of these Fab-mAbs were examined using our newly constructed BP autoantibody-induced COL17 humanized model mice (Nishie et al, Nat Med 13, 378-383, 2007). Injection of BP autoantibodies demonstrated a BP-like clinical phenotype exhibiting extensive erythema and blister formation together with characteristic histological findings including dermal-epidermal separation and the inflammatory cell infiltration. In contrast, these clinical and histological signs did not develop in the mice treated with Fab-B4 (n=23) or Fab-19 (n=12). These results confirm that our Fab-mAb therapy appears to be a novel and effective treatment for BP.

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CD19, a response regulator of B lymphocytes, regulates wound healing through hyaluronic acid-induced TLR4 signaling

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Immune cells are critical to wound-healing process through cytokine and growth factor secretion. Although previous studies have revealed that B lymphocytes are present within wound tissue, little is known about the role of B cells in wound healing. To clarify this, we investigated cutaneous wound healing in mice lacking or overexpressing CD19, a critical positive response regulator of B cells. CD19 deficiency inhibited wound healing, infiltration of neutrophils and macrophages, and cytokine expression, including basic and acidic fibroblast growth factor, interleukin (IL)-6, platelet-derived growth factor, and transforming growth factor (TGF)-β. By contrast, CD19 overexpression enhanced wound healing and cytokine expression. Hyaluronic acid (HA), an endogenous ligand for toll-like receptor (TLR)-4, as well as lipopolysaccharide stimulated B cells, which infiltrated into wounds, to produce IL-6 and TGF-β through TLR4 in a CD19-dependent manner. CD19 expression regulated TLR4 signaling through p38 activation. HA accumulation was increased in injured skin tissue relative to normal skin, and exogenous application of HA promoted wound repair in wild type mice but not in CD19-deficient mice, suggesting that beneficial effect of HA to wound healing process is CD19-dependent. Collectively, these results suggest that increased HA accumulation in injured skin induces cytokine production by stimulating B cells through TLR4 in a CD19-dependent manner. Thus, this study is the first to reveal a critical role of B cells and novel mechanisms in wound healing.

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Psoriasis patients generate increased serum levels of autoantibodies to TNF-α and Interferone-α but not to CCL2, CCL3, CCL5 and IL-17

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Pro-inflammatory cytokines and chemokines are believed to play a pivotal role in the initiation and progression of the inflammatory process in several autoimmune diseases including psoriasis. We have previously demonstrated that during ongoing autoimmunity the immune system generates selective beneficial response against a selective number of gene products that drive the inflammatory process. The present study was performed to determine whether psoriasis patients generate increased serum levels of auto-antibodies (Abs) against several pro-inflammatory cytokines and chemokines. We studied 50 patients with moderate to severe psoriasis and 28 healthy controls. Of the psoriasis patients 11 were studied before and after UVB phototherapy. The titer of cytokine – or chemokine-specific Abs was determined using a direct ELISA assay. ELISA plates were coated with recombinant human TNF-α, IFN-α, CCL2, CCL3, CCL5 and IL-17. Significantly increased titers of autoAbs to TNF-α and IFN-α but not to CCL2, CCL3, CCL5 and IL-17 were detected in the psoriasis patients as compared to healthy controls. UVB-induced remissions were not associated with significant alterations in these titers. We show for the first time that psoriasis patients produce increased amounts of auto Abs against two of the key players in the pathogenesis of psoriasis: TNF-α and IFN-α. This might constitute an attempt to down regulate the inflammatory process, and underscore the importance of these two cytokines in the pathogenesis of psoriasis.

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CCR5 expressing $CD4^+CD25^{high}$ regulatory T cells are both numerically and functionally impaired in patients with psoriasis vulgaris

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CCR5 expression on $CD4^+CD25^+$ regulatory T (Tr) cells has been reported to be crucial for limiting Th1 inflammation via the recruitment of these cells directly to disease sites. Examples include murine models of *Leishmania major* infection and acute graft-versus-host disease. Given the ongoing Th1 response in psoriasis, we asked whether abnormalities of $CCR5^+$ Tr cells might be involved in disease pathogenesis. Indeed, the proportion of cells positive for both Tr-cell markers($CD4^+CD25^{high}FOXP3^+$) and CCR5 was 60.3 ± 9.5% in control Tr cells (n=8), whereas only half as many CCR5 expressing cells were found in psoriatic Tr cells (30.2 ± 2.2%, n=8, $P<0.05$). In both psoriasis and control populations, greater than 95% of $CCR5^+$ Tr cells were also positive for CCR4. Cutaneous Leukocyte Antigen (CLA) bearing Tr cells were also assessed, and similarly, psoriatic skin-homing CLA^+CCR5^+ Tr cells were ~50% that of controls. Functionally, the inhibitory capacity of flow-sorted control $CCR5^+$ Tr cells consistently exceeded that of $CCR4^+CCR5^-$ or unfractionated Tr cells when co-cultured with autologous responder T cells at ratios from 1:1 to 1:4, exhibiting >50% more potency on a cell for cell basis (n=3). On the other hand, psoriatic $CCR5^+$ Tr cells showed only equal or less suppressive capacity compared to $CCR4^+CCR5^-$ or unfractionated Tr cell pools (n=3). Therefore, these data demonstrate that: 1) CCR5 expressing Tr cells are functionally *high potency* regulatory T cells and 2) $CCR5^+$ psoriatic Tr cells are numerically as well as functionally deficient compared to controls. Our previously published observation established that psoriatic Tr cells have a decreased capacity to suppress autologous pathogenic T cell proliferation. In support of this mechanism, we demonstrate here abnormalities of the *high potency* $CCR5^+$ Tr cell population that may contribute to the pathogenesis of psoriasis.

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The brain processing of scratching in patients with atopic eczema

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Previously we demonstrated in healthy subjects that repetitive scratching induces robust activation of the secondary somatosenosory cortex, insular cortex, prefrontal cortex, inferior parietal lobe and cerebellum as well as robust deactivation of the anterior and posterior cingulate cortices (ACC and PCC) using BOLD fMRI. However, no neuroimaging study has been reported on brain processing of scratch in atopic dermatitis patients. In the current study, we have used Arterial Spin Labeling (ASL) fMRI, a new sensitive brain imaging technique, to assess scratch induced brain activity in patients with atopic dermatitis. Repetitive scratch stimuli were applied in lesion skin. All the patients with atopic dermatitis perceived scratch stimuli as aggravating their itch. Our preliminary results show a robust bilateral activation in atopic dermatitis patients in ACC, dorsolateral prefrontal cortex (DLPFC), insula and contralateral basal ganglia. These brain areas activated by scratch are similar to our previous findings with histamine induced itch in atopic dermatitis. In addition no deactivation in ACC and PCC was noted, in contrast to our previous findings of scratching in healthy subjects. These results suggest that brain processing of scratch in atopic dermatitis differs from that of healthy subjects.

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A novel reversible model of psoriasis following epidermal overexpression of Tie2

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Psoriasis is a complex interplay between keratinocytes (KC), endothelial cells and cells of the immune system. Previously we reported the generation of a tetracycline repressible mouse model in which the angiopoietin receptor Tie2 was overexpressed in a KC-specific manner. These mice develop acanthosis in the absence of oncogenesis, increased VEGF expression and increased dermal and sub-dermal angiogenesis. We now report the presence of the classic human psoriasis immunocyte and cytokine profiles in the skin of these mice, including the presence of $CD4^+$ and $CD8^+$ T cells, dendritic cells and macrophages; and increases in IFNγ, TNFα, IL-6, IL-12, IL-22, IL-23, and IL-17 in both ear and back skin (between 2-6 fold increase each; $p<0.05$). These cytokines indicate likely activity of Th1 and Th17 cells, which have been shown to play important roles in mediating psoriasis. Host defense molecules were also massively upregulated in the skin, including a 26 fold increase in cathelicidin, an 84 fold increase in β-defensin and a 13 and 30 fold increase in the calcium binding proteins S100A8 and S100A9, respectively (all $p<0.0002$). Of particular interest was the observation that within 3 days of turning gene expression on, S100A8 was already significantly upregulated compared to controls (2.3 fold, $p<0.001$), indicating an important role for the innate immune system in development of the phenotype, or as a response to initiation of the phenotype. The psoriatic phenotype, including acanthosis, immunocyte infiltration and cytokine expression was completely reversed without scarring following repression of the transgene with doxycycline and was significantly improved following treatment with the classic anti-psoriasis systemic therapeutic, Cyclosporin A. This novel mouse model is a useful resource for understanding the mechanisms underlying psoriasis development, spontaneous regression, and lack of oncogenic progression that occurs in humans and may be useful for studying psoriasis-related co-morbidities.

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Self-peptide administration attenuates the course of disease in a mouse model of autoimmunity

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To provide a model for studying T cell responses against epithelium-derived antigen, we recently reported a spontaneous model of autoimmunity, in which soluble chicken ovalbumin (OVA) is expressed in the squamous epithelia of the tongue, esophagus, skin and thymus of C57BL/6 mice under keratin-14-promotor control. When OT-I cells (CD8 T cells expressing the Vα2/Vβ5-chains with specificity for $OVA_{257-264}$), are adoptively transferred into K14-sOVA mice, these mice die within 5-7 d of transfer. When K14-sOVA mice are crossed to OT-I mice only 17% of K14-sOVA/OT-I pups survive by d21. Death was due to diminished food intake and inflammatory infiltrates in multiple organs. The aim of this study was to attempt to attenuate this disease by the administration of self-peptide. 10ug $OVA_{257-264}$ in PBS was injected i.p. on d5, on d9, or on both d5 and d9. Signs of disease and overall survival were monitored. I.p. injections of peptide on both d5 and d9 increased the survival rate of K14-sOVA/OT-I mice at day 21 to 60%, whereas for PBS alone the survival rate was 0%. Single peptide injections increased the percentage of surviving mice at day 21 (injection on d5- 21% survival, injection on d9- 8% survival) compared to PBS-treated mice (0 survival), and a one to three day delay of disease progression was noted in the peptide-injected mice. A treatment responder at day 25 showed no histological changes, while dying non-responders at d15 showed heavy inflammatory infiltrates in the tongue, esophagus, lung, brain and liver, resembling the findings in untreated K14-sOVA/OT-I mice. Although comparative FACS analysis of healthy survivors at d35-d56 and dying pups at day 13-25 revealed similar numbers and Vα2/Vβ5 staining intensity of OT-I cells persisting in the spleen and lymph nodes, a striking reduction of CD8 expression was observed in healthy mice. Peptide–induced downregulation of the CD8 molecule is likely to contribute as a tolerance mechanism in the disease attenuation of our model, making this treatment approach a potential paradigm for attenuating autoimmune diseases.

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Establishment of a highly sensitive lymphocyte activation test (LAT) to para-phenylenediamine (PPD) to avoid severe patch test reactions in PPD allergic patients

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In patients sensitized to p-phenylenediamine (PPD), allergic reactions in response to black hair dye are increasingly eliciting severe skin reactions including massive cutaneous and subcutaneous edema and lymph node hyperplasia even necessitating hospitalization. We examined 9 patients with severe skin and lymph node reactions of the head and neck, temporary black tattoos in the history and recent use of permanent hair dyes. These patients responded vigorously to epicutaneous patch testing with 0.5% and 0.1% PPD leading to bullous reactions with the risk of scar formation with half of the patients not responding to 0.01% PPD. We therefore established an *in vitro* test system to detect PPD sensitization. To this end, PBMC from these patients as well as 10 negative controls with or without history of using permanent black hair dye were activated with titrated concentrations of PPD with or without IL-2 supplementation. After six days, thymidine was added to the culture and T cell proliferation was determined for 18 hours. In 8 out of 9 patients with severe allergic reactions, LAT was positive with PPD-specific lymphocyte proliferation at least 2-fold above background. In only one patient, PPD-specific LAT was positive with IL-2 supplementation only, in all others, PPD specific LAT was reactive with and without IL-2. None of the control PBMC responded to PPD demonstrating lymphocyte proliferation not above unstimulated PBMC values. Identical results were obtained when the LAT with PPD was repeated several times over a 10-months-period indicating a long-lasting presence of PPD specific memory T cells in peripheral blood of highly sensitized individuals. These data clearly show that LAT with PPD is a highly sensitive and specific *in vitro* test system to prove PPD sensitization in patients with severe reactions. With this sensitive *in vitro* test system we can avoid severe test reactions and booster effects following even titrated patch tests with PPD.

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Investigation of p38 mitogen activated protein kinase in acute skin inflammation

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Four isoforms of the p38 Mitogen Activated Protein Kinase (MAPK) have been described; α, β, δ and γ. Only α, β and δ are expressed in the skin. p38α and –β are believed to play a key role in skin inflammation. In this study p38 β/δ knockout (KO) mice in combination with a p38 α/β inhibitor were investigated in an eczema model in order to elucidate the importance of the p38 MAPK isoforms in skin inflammation. Acute contact eczema was induced with phorbol 12-myristate 13-acetate (TPA) application on the ears of p38 β/δ KO mice and C57BL/6J control mice and changes in ear thickness, as a marker of the degree of oedema, were measured together with neutrophilic cell infiltration estimated by a myelopeoxidase assay. IL-1β and IL-6 protein and mRNA expression were analyzed by ELISA and qRT-PCR. Similar experiments were conducted after topical application of a p38 α/β inhibitor, SB202190. TPA application resulted in a significant increase in ear thickness and neutrophilic cell infiltration compared with vehicle treated mice ($p<0.001$), however no differences were seen between control and p38 β/δ KO mice. When SB202190 was applied a significant inhibition of the increase in ear thickness and neutrophilic cell infiltration was seen in both mice groups ($p<0.001$). These results were also demonstrated by histological examination. The level of IL-1β and IL-6 protein expression was significantly higher in control mice 8 hours after TPA application compared with KO mice ($p<0.05$). However, these findings were not seen on mRNA level, where no significant differences between KO and control mice were seen. There was a significant inhibition of the mRNA and protein expression of IL-1β and IL-6 in both mice groups when SB202190 was applied ($p<0.05$). Based on these results we conclude that the p38α isoform is the most important isoform in the induction of skin inflammation, although the p38β and/or –δ isoforms may play a posttranscriptional role in the regulation of proinflammatory cytokines.

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Development of an IgG4-based classifier/ predictor of endemic pemphigus foliaceus (Fogo Selvagem)

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Fogo Selvagem (FS) is an autoimmune skin disease mediated by pathogenic IgG anti-desmoglein 1-(Dsg1) autoantibodies, which are predominantly IgG4. The clustering of FS cases in certain endemic regions of Brazil strongly suggests an environmental etiology. The sera of 214 FS patients and 261 healthy controls were tested for anti-Dsg1 IgG and IgG subclass autoantibodies by ELISA, and the index values were analyzed. The best prediction model involves only IgG4 and has sensitivity 92% (95% CI: 82-95%), specificity 97% (95% CI: 89-100%) and area under the curve 0.97 (95% CI: 0.94-1.00). The classifier identifies as FS cases, individuals with IgG4 index values above 6.43. This IgG4-based classifier was validated in a group of patients from an endemic region of FS in Brazil and in another group of 60 Japanese patients with pemphigus. The classifier identified 21 individuals from a Brazilian cohort of 96 subjects aged 5 to 20 during preclinical FS. Prospective studies of this cohort will further validate the accuracy of the IgG4-based classifier. HLA typing and the IgG4-based classifier would become powerful tools to identify individuals at risk of developing clinical FS and this in turn, may enhance our ability to identify potential etiological agent(s) involved in triggering the autoimmune disease in FS.

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The influence of systemic immune milieu on cutaneous inflammation of the K14-IL-4-transgenic atopic dermatitis model

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To investigate the influence of systemic immune milieu on cutaneous inflammation, we crossed and backcrossed the IL-4 transgenic (Tg) mice in an outbred CByB6 strain into Th1-dominant C57Bl/6 (C) and Th2-dominant BALB/c (B) strains. Resulting C57Bl/6-Tg (C-Tg) and BALB/c-Tg (B-Tg) mice were observed for cutaneous inflammation clinically. Three weeks after the onset of skin inflammation, specimens were studied and statistically analyzed: 1) Sera were examined for total IgE; 2) Reverse transcription was performed on extracted RNA from skin lesions, and the cytokine cDNAs were semi-quantified by real-time PCR; 3) Skin lesions were investigated for T cell infiltration, angiogenesis, and adhesion molecules by immunofluorescence microscopy. On average, B-Tg mice develop skin lesions much earlier than C-Tg mice (11 vs. 90 days old). Similarly, the disease extent is substantially greater in B-Tg mice (17.7% vs. 5.8% body surface area). While the total IgE in C-Tg mice is significantly increased compared to the non-Tg C mice, there is no such increase in B-Tg mice compared to the non-Tg B group, probably due to a high baseline total IgE in the latter. Real-time PCR showed Th2-cytokines (IL-4, IL-6, IL-10) and VEGF are significantly upregulated in the Tg vs. non-Tg mice of both strains, but these cytokines were increased 3-30 folds in the B-Tg vs. C-Tg mice. IL-1beta is not upregulated in the C-Tg vs. non-Tg C mice, but it is 177 fold greater in B-Tg vs. non-Tg B mice and is increased 21 fold in B-Tg vs. C-Tg mice. No TNF-alpha cDNAs were increased in either Tg strain. While both B-Tg and C-Tg mice have significantly more CD3+, CD4+, and CD8+ T lymphocytes and vessels positive for PECAM, VCAM, and P-selectin vs. non-Tg groups, no increase was found in B-Tg vs. C-Tg mice. We conclude that the Th2 systemic immune milieu strongly influences the rate and extent of cutaneous inflammation in our IL-4-Tg atopic dermatitis model, probably by way of higher expression of local Th2 and other pro-inflammatory cytokines.

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Interleukin-13 and interferon-gamma producing skin resident $CD8^+$ T cells: a vicious circle of barrier disruption of the skin in atopic dermatitis

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Atopic dermatitis (AD) is an inflammatory skin disease characterized by barrier dysfunction of the skin and infiltration of activated T cells. We have isolated the T cells from skin lesions of AD via short-term explant cultures and compared them to cells isolated from normal skin. AD skin lesions contain an expanded population of $CD8^+$ T cells compared to normal skin (mean 20% vs. 4%). Strikingly, 41% of $CD8^+$ T cells produced interferon-gamma (IFNg) and 42% produced interleukin-13 (IL-13); these populations were mutually exclusive. In fact, we found production of IL-13 by up to 60% of the total T cells ($CD4^+$ and $CD8^+$) infiltrating AD lesions. The vast majority of AD $CD8^+$ T cells expressed CD49a, similar to recent findings in psoriasis and suggesting epidermal targeting. Both IFNg and IL-13 have profound but distinct proinflammatory effects in tissue. However, we found co-expression of Tc1 and Tc2 subsets in atopic dermatitis skin. Previous work has shown that IFNg production by T cells renders keratinocytes more susceptible to Fas/FasL-induced apoptosis and may contribute to barrier dysfunction. IL-13 produced by T cells plays a pivotal role in the induction of epithelial apoptosis in ulcerative colitis and induces MMP-9 and chemokine production by keratinocytes. We hypothesize that skin infiltrating IL-13 and IFNg producing $CD8^+$ T cells play a central role in maintaining the disruption of the epidermal barrier, through induction of keratinocyte apoptosis and by facilitating inflammatory cell influx. Epidermal injury renders the skin susceptible to external stimuli resulting in a vicious circle of barrier disruption and inflammation that may be pivotal in both the initial induction as well as the switch to chronicity in AD skin lesions. Our work suggests that agents targeting the activity of $CD8^+$ T cells in AD may improve both the inflammatory and barrier disruption aspects of the disease.

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On the origin of the Virchow cell in leprosy – role of host-derived lipids

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Intracellular pathogens can evade the host immune system, yet access host metabolic pathways for their growth, which for mycobacteria depends on the utilization of host-derived lipids. The purpose of these studies was to examine to the role of these lipids in innate immunity in the skin. In the lesions from disseminated/lepromatous form of human leprosy, there was preferential expression of host lipid metabolism genes, including a group of phospholipases, which by comparative domain profiling were virtually absent from the mycobacterial genome. Host-derived oxidized phospholipids were increased in macrophages within leprosy skin lesions, with one specific oxidized phospholipid, 1-palmitoyl-2-(5,6-epoxyisoprostanse E2)-sn-glycero-3-phsphorylcholine (PEIPC, m/z 828.5) accumulating in infected macrophages. The host-derived oxidized phospholipids inhibited innate immune responses, which could be reversed by the addition of normal high-density lipoprotein (HDL), a scavenger of oxidized phospholipids, but not by HDL from lepromatous patients. These data indicate striking similarities between the inflammatory processes in disseminated leprosy and atherosclerosis, including the accumulation of distinct host-derived oxidized phospholipids at the site of disease, which in mycobacterial infection favors the pathogen by inhibiting innate immunity.

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Preclinical toxicology of AN2728, a novel oxaborole in development for the topical treatment of psoriasis

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AN2728 (5-(4-cyanophenoxy)-1-hydroxy-1, 3-dihydro-2,1-benzoxaborole) is a novel oxaborole compound with anti-inflammatory activity. Preclinical toxicity data is available for *in vitro* studies and *in vivo* studies following systemic and topical administration to shrews, ferrets, rats, and mice. AN2728 (1 μM) was classified as a low potency hERG-channel blocker, minimizing concerns over cardiovascular safety. AN2728 (up to 5000 μg per plate) demonstrated no mutagenic activity in the presence or absence of Aroclor-induced S9 liver fraction against *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 *uvr*A. AN2728 (up to 500 μg/mL), demonstrated no clastogenic activity in the *In Vitro* Mammalian Chromosome Aberration Assay in the presence or absence of Aroclor-induced S9 using human peripheral blood lymphocytes. Following oral administration of 10, 30, and 100 mg/kg to shrews, *(Suncus murinus)*, dose-responsive emetic effect was observed. The no-observed-effect level (NOEL) was 10 mg/kg, and was associated with significant plasma exposure. Following oral administration of 10, 30, and 100 mg/kg to ferrets, no emetic episodes were observed in any dose group. Following oral administration of 30, 100, and 300 mg/kg/day to male and female Sprague-Dawley rats for 14 consecutive days, the NOEL was determined to be 300 mg/kg/day. No mortality, adverse effects on clinical pathology, or microscopic or macroscopic changes were observed in any dose group. AN2728 was formulated as a 1%, 5%, and 10% solution in acetone/ethanol (50:50, v/v) and tested in a local lymph node assay for its potential to induce a hypersensitivity response. Topical treatment with AN2728 to female CBA/J mice did not result in a stimulation index of 3 or greater and therefore AN2728 was not considered to have skin sensitizing activity. AN2728 is a novel compound in development for the topical treatment of psoriasis and has been demonstrated to be safe in various preclinical toxicity studies.

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Phenotypic and genotypic profile of psoriatic patients with asthma

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IL-13 has several biological roles in the pathogenesis of asthma as well as inhibiting the development of Th17 cells. Recently we identified three IL13-linked SNPs (rs1800925, rs20541, rs848) that associate with psoriasis (Ps). The major susceptibility allele singly and in combination with susceptibility variants of HLA-C, IL12B, IL23R confer up to an 11 fold increase risk for psoriasis. The possible overlap of association of IL-13 in asthma and Ps, and reports of decreased frequency of atopic diathesis in subjects with Ps caused us to determine the frequency of asthma in Ps subjects in the Utah Psoriasis Initiative (UPI) and to characterize their phenotypic features of Ps. In addition the frequency of susceptibility alleles, IL-13, HLA-C, IL12B and IL-23R was carried out. The incidence of self-reported asthma was slightly higher in the UPI than in the general population in Utah (9.0% vs. 7.5%). As in the general population, the prevalence of asthma was higher in females than males and the rate of obesity was higher than in the non-asthmatic cohort. Asthmatic patients were more likely to report joint pain (62% vs. 48.5%; P=.05). No difference in the age of onset of Ps, positive family history, severity, frequency of koebnerization, nail involvement, or palmar/plantar disease was noted for the two groups. The effect of common modifiers of psoriasis (i.e. infection, stress, sunlight or pregnancy) was also similar. The allele frequency for susceptibility single nucleotide polymorphisms (SNPs) for IL-13, IL12B and IL23R was similar for the two groups. The incidence of HLA0602 carriers was slightly higher in the asthmatic group (42.2% vs 33.7%) which was not significant (P=.31).

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AN2898, a novel oxaborale compound with anti-inflammatory activity: mechanism of action and *in vitro* cytokine inhibition

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AN2898 is a novel phosphodiesterase 4 (PDE4) inhibitor in development to treat inflammatory dermatological diseases topically. AN2898 is a competitive, reversible inhibitor of PDE4 with a K_i of 65 nM. The AN2898-PDE4B2 catalytic domain co-crystal structure shows that AN2898 binds directly to the metal ions and activated water molecule in the active site. This binding configuration is unique amongst classical PDE4 inhibitors (i.e., rolipram). The IC_{50} value for inhibition of PDE4 activity is 0.06 μM. AN2898 has equal activity against the four PDE4 subtypes. AN2898 does not significantly inhibit PDE 1, 2, 3, 5, or 6; however, it does have activity against PDE7 (IC_{50} = 0.2 μM). Like other PDE4 inhibitors, AN2898 inhibits the release of TNF-α, IL-2, IFN-γ, IL-5, and IL-10 in the low to mid-nanomolar range, and does not inhibit IL-1β, IL-6 and IL-8. Unlike classical PDE4 inhibitors, AN2898 also inhibits IL-12 (IC_{50} = 0.016 μM) and IL-23 (IC_{50} = 2.1 μM). Because of its unique mechanism of action and anti-inflammatory activity, AN2898 has the potential to be useful in the treatment of psoriasis and other immune-mediated diseases.

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Rapid suppression of IL-20-related cytokines by etanercept decreases regenerative hyperplasia *in vivo*

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Psoriasis is characterized by regenerative hyperplasia, as reflected by increased keratin 16 (K16) expression. Recently, several structurally related cytokines, including IL-19, IL-20, IL-22, and IL-24, have been found to stimulate keratinocyte hyperproliferation and directly upregulate K16 in reconstituted human epidermis. To further investigate these cytokines, referred to here as "IL-20-related cytokines," we obtained skin samples from psoriasis patients treated with etanercept. Based on Physicians' Global Assessment, clinical improvement of patients began at 2 weeks of therapy and was maximal at 12 weeks (all P<0.05). Based on RT-PCR, mRNA expression of IL-19 (N=21), IL-20 (N=21), IL-22 (N=20), and IL-24 (N=20) in untreated lesional skin was increased 3874, 119, 55, and 130 fold, respectively, compared with uninvolved skin (all P<0.05). After 1 week of therapy, mRNA expression of these cytokines decreased 59%, 68%, 39%, and 74%, respectively (all P<0.05). Similarly, mRNA expression of STAT3, which is activated by IL-20-related cytokines, decreased 20% at 1 week (N=18, P<0.05). Markers of myeloid immunocytes, which are a source of IL-20-related cytokines, also decreased significantly (P<0.05) at 1 week (e.g., IL-23p19 and IL-23p40). In comparison, mRNA expression of K16 was increased 49 fold in baseline lesional skin (N=21, P<0.05). Although no significant changes were seen at week 1 of therapy (P>0.05), K16 mRNA expression diminished significantly at week 2 (62%, P<0.05). Based on immunostaining, K16 protein (N=13) in lesional skin was elevated 1549 fold at baseline and demonstrated near-normalization at week 4 (all P<0.05). These data indicate that the therapeutic mechanism of etanercept may involve suppression of IL-20-related cytokines prior to clinical improvement. Since suppression of these cytokines preceded that of K16 expression, the IL-20-related cytokines may represent an important bridge between inflammation (leukocyte activation) and phenotypic changes (epidermal hyperplasia) in psoriasis.

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Structure-activity studies of AN2728 and AN2898, novel oxaborole compounds with anti-inflammatory activities

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The structure-activity relationships of novel boron-containing anti-inflammatory agents was investigated. AN2728 (5-(4-cyanophenoxy)-1-hydroxy-1,3-dihydro-2,1-benzoxaborole) is a broad spectrum anti-inflammatory compound that is currently under development for the topical treatment of plaque psoriasis. AN2728 inhibited release of TNF-α from peripheral blood mononuclear cells (PBMCs) stimulated by lipopolysaccharide (LPS). AN2728 was found to inhibit the phosphodiesterase 4 (PDE4) enzyme derived from human U937 cells. Since the PDE4 inhibition was considered to be a part of its mechanism of action to inhibit the TNF-α release, investigation of structure-activity relationships around this compound was carried out to identify a more potent PDE4/TNF-α inhibitor. Compounds were screened by a biochemical assay against PDE4 enzyme and cell-based cytokine release assays using PBMCs stimulated by LPS or phytohemagglutinin (PHA). The results showed that electron-withdrawing groups, such as cyano and *N,N*-dialkylcarbamoyl, at the *para*-position to the oxygen atom were important for the activity. Ester and pyridine analogs also showed potent activity. However, carboxy derivatives lost most of the activity in both assays. AN2898, an analog of AN2728, showed potent inhibition against PDE4 (IC_{50} 60 nM), while the IC_{50} of AN2728 and rolipram were 490 nM and 1700 nM, respectively. AN2898 also inhibited the release of cytokines, such as TNF-α (IC_{50} 120 nM), IL-2 (IC_{50} 140 nM), and interferon-γ (IC_{50} 50 nM). AN2898 did not show cytotoxicity against L929 and HepG2 cell lines up to 100 μM.

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***In vitro* activity and mechanism of action of AN2728, a novel oxaborole in development for treatment of psoriasis**

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AN2728 (5-(4-cyanophenoxy)-1-hydroxy-1, 3-dihydro-2, 1-benzoxaborole) is a broad spectrum anti-inflammatory compound currently under development for the topical treatment of plaque psoriasis. AN2728 inhibits TNF-α secretion from peripheral blood mononuclear cells (PBMCs) stimulated by lipopolysaccharide (LPS) and IL-2 and IFN-γ secretion after stimulation with phytohemagglutinin (PHA) in the nanomolar range. AN2728 inhibits TNF-α secretion with an IC_{50} of 770 nM; however the pro-inflammatory cytokines IL-1 and IL-6 are not affected by AN2728. IL-2 and IFN-γ are inhibited with IC_{50}s of 460 nM and 270 nM, respectively. IL-5 and IL-10 are inhibited by AN2728 in the low micromolar range. One mechanism of action of AN2728 to inhibit a broad range of cytokine secretion may be through inhibition of phosphodiesterase 4 (PDE4). AN2728 inhibits PDE4 enzyme activity in human U937 cells with an IC_{50} of 0.49 μM, inhibiting all four PDE4 isoforms equally. AN2728 has been shown to be a competitive inhibitor of the substrate cAMP, as determined by enzyme kinetic analysis. AN2728 also inhibits PDE7 with an IC_{50} of 0.73 μM but does not significantly inhibit PDE1, 2, 3, or 5. In contrast to classic PDE4 inhibitors, for example rolipram, AN2728 inhibits production of IL-23 from human THP-1 macrophages with an IC_{50} of 2.25 μM. This activity, with its inhibition of TNF-α and TH1 cytokines, suggests potential for AN2728 in the treatment of psoriasis.

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AN2898, a novel oxaborale compound with anti-inflammatory activity: results of *in vivo* efficacy and preclinical safety studies

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AN2898 is the second in a series of novel oxaborale compounds that decreases TNF-α release through potent inhibition of phosphodiesterase 4 (PDE4). This new drug is currently in preclinical development for psoriasis and atopic dermatitis. AN2898 demonstrates significant *in vivo* activity in a model of acute skin irritation. In an acetone/ethanol vehicle, 5% AN2898 inhibits PMA-induced ear edema greater than 15% dexamethasone (82% vs 73% inhibition, respectively, p <0.01) and is active in this model in an ointment vehicle at concentrations as low as 0.01%. Topical AN2898 also significantly inhibits oxazolone-induced ear swelling (a model of allergic contact dermatitis). *In vitro* screening shows AN2898 to have no significant hERG or p450 enzyme inhibition. Pharmacokinetic analysis in the rat after oral dosing at 100 mg/kg demonstrates relatively low bioavailability (7%) and, after intravenous dosing at 20 mg/kg, a mean plasma residence time of 0.64 h. AN2898 is safe in a rat 14-day oral repeated dose study at 10, 100, and 300 mg/kg/day; no clinical chemistry or hematology effects were seen at any dose. It also shows less emetic effect than rolipram. The no effect Cmax plasma level of AN2898 in a shrew emesis assay was 12.4 μg/ml. Dermal application of 5% AN2898 ointment or cream over 15% body surface area on the Göttingen minipig results in undetectable plasma levels of the compound (LLOQ = 0.01 μg/ml) and no dermal irritation after 24 hours of exposure. These early studies suggest that AN2898 has the required *in vivo* biological activity and good preclinical safety profile to enable clinical trials.

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IgE and IgG4 anti-desmoglein 1 autoantibodies in endemic pemphigus foliaceus

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Pathogenic anti-Dsg1 autoantibodies in endemic pemphigus foliaceus [Fogo Selvagem (FS)] are IgG4 restricted. In the course of sensitization to allergens or during immunotherapy of allergic diseases the appearance of IgE antibodies is followed by a sustained production of IgG4 antibodies ("blocking antibodies"). Additionally, IgE antibodies are known to develop in patients with parasitic diseases or individuals exposed to insect bites, i.e. bees. These allergens are thought to be risk factors for FS. An ELISA assay, using recombinant Dsg1, was used to test the same FS and control sera for IgE and IgG4 anti-Dsg1 autoantibodies and the results were expressed as index values. A ROC curve was plotted to determine sensitivity and specificity [Cut off 100 (61% sensitivity and 92% specificity)]. IgE anti-Dsg1 autoantibodies were found in 61% of FS sera tested (N=143), in 7% of healthy donors from endemic areas (N=125) and in 2% of donors of non-endemic areas (N=99). Control sera including PF exhibited 26% positive IgE anti-Dsg1 (N=39), PV exhibits 20% (N=59) and BP exhibits 3% (N=35). Even after correcting p-values for multiple comparisons, the proportion of positive tests in FS patients is significantly different from any of the other groups with p-value<0.001. IgG4 anti-Dsg1 autoantibodies were detected only in the sera of FS patients. Comparing the anti-Dsg1 IgE and IgG4 autoantibodies in FS sera, we found a weak but statistically significant correlation between these autoantibodies in this FS population (r= 0.27, p-value: 0.001). These results suggest that the IgG4 and IgE anti-Dsg1 autoantibody responses in FS are linked to the same environmental antigen(s). These studies will enhance the likelihood of identifying the etiologic agent that triggers FS.

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Capsiate suppresses UVB-induced inflammatory and angiogenic activity via inhibition of ERK and NF-kB activation in skin

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Capsiate is a non-pungent capsaicin analog, the one of the major capsaicinoids of sweet pepper. We investigated the effect of capsiate on UVB-induced inflammatory response *in vitro* and *in vivo*. Treatment of capsiate inhibited the production of intracellular reactive oxygen species (ROS). As it was demonstrated that UVB-induced oxidative stress mediates activation of mitogen-activated protein kinase (MAPK) and nuclear factor-kB (NF-kB) pathways, we tried to determine the effect of capsiate on the pathways involved in UVB-induced oxidative stress. Capsiate treatment inhibited UVB-induced- COX-2 expression, phosphorylation of ERK1/2, and nuclear translocation of NF-kB. Capsiate also inhibited the expression of NF-kB-targeted genes, such as interleukin-6, -8 (IL-6, -8), TNF-a and potent angiogenic factors, such as vascular endothelial cell growth factor (VEGF) and matrix metalloproteinase-2, 9 (MMP-2, -9). We also studied the photoprotective effect of capsiate in *in vivo* SKH-1 hairless mice. Topical treatment of capsiate significantly decreased UV-induced skin damage and inhibited the activation of ERK1/2. The mice treated with capsiate also exhibited the decreased expression of cyclooxygenase-2 (COX-2), pro-inflammatory cytokines and angiogenic factors including PECAM (CD31) and ICAM-1(CD54) in skin. Collectively, our data demonstrate that capsiate protects the skin from UVB-induced damages and down regulates UVB-induced inflammation and angiogenesis.

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Saponins of tea [Camellia oleifera Abel] alleviates atopic dermatitis(AD)-like skin lesions in NC/Nga mice

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Camellia oleifera Abl. is one of the herbs used in China for the treatment of burning injury and as cooking oil. The active component, saponins(Tea-saponins) were isolated from seed of Tea[Camellia oleifera Abl.]. It have been shown to have anti-inflammatory and anti-oxidative effect in human skin disease. The purpose of this study was to evaluate whether topical treatment of Tea-saponins suppress the development of AD-like skin leisions in NC/Nga mice, which are induced by repeated application of DNCB. The efficacy of Tea-saponins in NC/Nga mice was assessed by measuring symptom severity, serum level of IgE, scratching behavior, inflammatory cell infiltration in the skin, epidermal necrosis, hyperkeratosis, and evaluated histamine release from rat basophilic leukemia cell(RBL-2H3). Topical treatment of Tea-saponins to NC/Nga atopic dermatitis–model mice led to a striking suppression of the development of dermatitis, scratching behavior, and serum IgE elevation. Histologic analysis revealed that infiltration of inflammatory cells, thickening of the epidermis, and prominent hyperkeratosis were significantly reduced. Also, Tea-saponin dose-dependently blocked the release histamine from basophilic leukemia cells. This study demonstrates a novel activity of Tea-saponins on atopic dermatitis–model mice and,*in vitro*. Our results indicate that Tea-saponins treatment could provide an effective theraphy for the management of AD.

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Augmentation of IL-22 receptor expression and IL-20 subfamily production plays a critical role in psoriasis

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IL-17 and IL-22 that are secreted by Th17 lymphocytes, seems to play important roles in psoriasis pathologically. These cytokines act to epidermal keratinocytes, and mediate chemokine production, proliferation, and differentiation of keratinocytes. On the other hand, an expression of IL-22 related cytokines including IL-19, -20, and -24, so-called IL-20 subfamily, is also detected in the lesion skin of psoriasis. A mechanism of IL-20 subfamily production and its role are not fully examined in psoriasis. We hypothesized that Th17 cytokines induce the production of IL-20 subfamily in epidermal keratinocytes. IL-17 stimulation induced IL-19 production in cultured keratinocyte, and IL-22 stimulation induced IL-20 and IL-24 production, that were confirmed by mRNA and protein levels. A combination of IL-17 and IL-22 strongly augmented all these cytokine production. In the lesion epidermis of psoriasis, IL-22 receptor (IL-22R) mRNA expression is augmented, in compared with that of normal epidermis. IL-20, -24, and -22 share this receptor. When IL-22R were transfected into the cultured keratinocytes by adenovirus vector, the phosphorylation of STAT3 by IL-22, -20 and -24 were markedly enhanced. Furthermore, IL-20 subfamily production in response to IL-17 and IL-22 combination was significantly increased about 10-fold. MIP-3α is noted to have a potency to attract Th17 lymphocytes. Interestingly, a significant increase of MIP-3α expression was observed in the lesion skin of psoriasis. MIP-3α production was induced by IL-17 stimulation in epidermal keratinocytes, and IL-20 and -24 enhanced the MIP-3α production at 2- and 4-fold in response to IL-17 in cultured keratinocytes transfected with IL-22R. Taken together, in psoriasis, the increase of IL-22R expression may enhance IL-20 subfamily cytokine production, and augment MIP-3α production in response to IL-17, resulting in the further recruitment of Th17 lymphocytes.

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Cathelicidin antimicrobial peptides inhibit hyaluronan-induced cytokine release and modulate chronic allergic dermatitis

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Low expression of cathelicidin in skin is a risk factor for skin infection in atopic dermatitis. Cathelicidins also inhibit TLR4 mediated activation of dendritic cells, and recent studies show that cathelicidin modulate inflammation by interfering with Toll-like-receptor (TLR) function as cathelicidin deficient mice (Cnlp-KO) show enhancement of acute allergic responses. Hyaluronan (HA) was recently discovered to be an endogenous ligand for TLR4 as well as CD44, and induces cytokine release from macrophages by forming a complex of TLR4-CD44. Here, we examined if cathelicidin modifies chronic allergic dermatitis through inhibition of HA-induced cytokine release. Chronic allergic dermatitis was induced by repeated topical application of DNFB to mouse ears. Cnlp-KO showed significant enhancement of ear swelling (1.6 fold, p<0.01), cell infiltration (2-3 fold, p<0.01) and cytokine expression (MIP-2 : 2.4 fold, IL-1 beta : 2.7 fold, qPCR) compared to wild type mice. There were no significant differences in the expression of IL-4 (ear, lymph node: qPCR) and serum IgE concentration. Since an increase of HA was observed in ears with dermatitis, we evaluated CD44 deficient mice (CD44-KO) in the same dermatitis model. CD44-KO showed significantly diminished ear swelling and cell infiltration (p<0.01, p<0.05, each) compared to wild type mice. HA induced IL-6 release from bone marrow derived macrophages (BMDM) in a TLR4 and CD44 dependent manner, and cathelicidin inhibited the HA-induced IL-6 release (40%:3uM, 80%:10uM). This inhibitory activity was not blocked by pertussis toxin. Meanwhile, cathelicidin did not inhibit the MALP2(TLR2)-induced IL-6 release from BMDM. These results suggest that cathelicidin has anti-inflammatory activity in chronic allergic dermatitis by the blockage of HA-CD44-TLR4 interaction.

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Laser confocal microscopic study in sclerodermatous graft-versus host disease

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Cutaneous involvement in chronic graft-versus host disease (GVHD) includes lichenoid and/or sclerodermatous manifestations resembling generalized scleroderma, morphea and lichen sclerosus (LS). The aim of the study was to compare similarities and differences between cutaneous lesions in chronic sclerodermatous GVHD and idiopathic cutaneous LS and morphea. Three patients with sclerodermatous GVHD (one of them bullous), ten idiopatic LS and ten morphea patients were investigated. Biopsy specimens from the patients' skin were cut into 40 um thick sections and labeled with antibodies against: beta4 integrin and collagen VII (lamina lucida and lamina densa markers of skin basement membrane zone-BMZ), and collagen IV (lamina densa marker of BMZ and of the vessels). Three-dimensional reconstruction (3DR) of the BMZ and cutaneous microvasculature was obtained by computer program. One case of GVHD with bullous lesions showed alterations of BMZ similar to LS at the level of collagen VII: granular material in upper dermis suggestive of partial degradation of lamina densa at the level of anchoring fibers. However in contrast to LS, there were also gross alterations in the lamina lucida of the BMZ. A mapping study showed blister formation through the basal cell layer of the epidermis in bullous GVHD, whereas in three cases of bullous lichen sclerosus blister formed below the lamina densa. In two other cases of GVHD continuity of BMZ was preserved as in morphea. 3DR of cutaneous microvasculature in active idiopatic morphea showed a large number of small tortuous vessels with no orientation either to papillary plexus or transverse connecting vessels reflecting increased angiogenesis, whereas in inactive morphea, lichen sclerosus and GVHD only enlarged vessels were visible with reduplications of the basal lamina. Despite the fact, that sclerodermatous GVHD combines the clinical and histological features of LS and/or morphea, laser confocal microscopic studies revealed differences in alteration of cutaneous BMZ and microvasculature between sclerodermatous GVHD and idiopathic LS or morphea.

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Expression of heme oxygenase-1 in skin tumors and non-tumor dermatoses

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The skin, which is irradiated ceaselessly by ultraviolet (UV) radiation, is exposed to the high level of oxidative stress, and therefore is armed with a set of antioxidants to protect organisms from oxidative damage. Skin cancers are well known to be induced by chronic UV irradiation, and, therefore, they are a good model to study the role of oxidative stress in carcinogenesis (photocarcinogenesis). As an antioxidant, heme oxygenase-1 (HO-1) was reported to be induced by stressful stimuli, such as UV irradiation, hydrogen peroxide, and lipopolysaccharide. Interestingly, HO-1 was reported to perform an important cytoprotective role under various inflammatory conditions in the skin, suggesting the complexity of HO-1 functions besides an antioxidant. A set of studies showed that HO-1 expression was impaired in several malignant tumors, and the expression was related with metastasis and prognosis. With the background, we studied HO-1 expression in various skin diseases, including inflammatory and non-inflammatory conditions, by immunohisotchemical technique. In the normal skin, HO-1 expression was strongly expressed in the granular layer and spinous layer of epidermis. Next, we evaluated the expression level of HO-1 in keratinizing and non-keratinizing skin tumos. HO-1 expression was impaired in non-keratinizing BCC and MM, while HO-1 expression was further accentuated at the keratinzing foci of BCC and SCC. As for non-tumor dermatoses, HO-1 expression was significantly impaired in alopecia, in which hair loss was induced by hair follicle dysfunction via autoimmune mechanism. In contrast, HO-1 expression was accentuated in the psoriatic epidermis. From our study, HO-1 is revealed to pursue multiple roles in the skin besides its known antioxidant role in the skin.

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Serum calcium-binding S100A8/A9 proteins in patients with psoriatic arthritis are derived from not only the skin lesions but also circulating monocytes

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S100A8 and S100A9 proteins are the Ca2+-binding proteins secreted from inflammatory cells such as neutrophils and macrophages. Recently, serum levels of S100A8/A9 heterodimers have been found to correlate with disease activity in psoriasis. We evaluated the correlation between serum levels of S100A8/A9 proteins and each type of psoriasis, and investigated the source of serum S100A8/A9 proteins. Serum samples were collected from patients with psoriasis vulgaris (PV) (n=30), psoriatic arthritis (PA) (n=14), pustular psoriasis (PP) (n=24), healthy control (n=21), and disease control (n=14). Serum concentrations of S100A8/A9 were assessed by ELISA. Skin sections were stained using rabbit anti-human S100A8 and S100A9 antibodies. mRNAs were extracted from freshly isolated monocytes and neutrophils, and processed for quantitative PCR. Serum levels of S100A8/A9 proteins were significantly increased in patients with any type of psoriasis, as compared with those of healthy controls ($P<0.01$), although the levels were different in psoriasis groups: much high in PA and PP. Immunostaining showed both S100A8 and A9 proteins were detected in keratinocytes and infiltrating cells, including neutrophils and other mononuclear cells in psoriatic lesions. Although no difference was found in S100A8/A9 mRNA expression by circulating neutrophils, their expression was increased in monocytes from patients with PA. In conclusion, although serum S100A8/A9 levels were increased in any type of psoriasis, significantly high levels of S100A8/9 were detected in PA and PP. In PA, serum S100A8/A9 proteins were derived from not only psoriatic skin lesions but also circulating monocytes.

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Atopic dermatitis skin-care: impact of a natural sugars blend

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Atopic Dermatitis (AD) is a chronic inflammatory skin disorder, characterized by cutaneous hyperreactivity and pruritic lesions. Moreover AD is frequently aggravated by recurrent infections of skin lesions. AD development can be attributed to genetic, mechanical, immunological and biochemical factors. Mechanical factors inducing AD are linked to a defective skin barrier. Indeed, skin physical barrier is affected by a bad cohesion of keratinocytes, and an abnormal skin lipids synthesis, both leading to the hyper-reactivity and xerosis of AD skin. Further, the biological skin barrier is also affected by a deficiency in the expression of antimicrobial peptides (AMP). Those endogenous peptides play an integral part in innate immunity and are essential for the ability of skin to fight against microbial infection. Thus this lack of AMP can account for the susceptibility of AD patients to skin infection. Immunological factors leading to AD come from an imbalance between TH1 and TH2 lymphocytes resulting in an overexpression of TH2 cytokines. These specific cytokines can inhibit AMP expression and activate IgE production that in turn induces histamine release resulting in the induction of an allergic response. Using *in vitro* studies, we have evaluated the impact of a specific blend of natural sugars on the management of AD. We have shown that these sugars were able to stimulate, in keratinocytes, the expression of desmoglein-1, a constitutive element of corneodesmosomes. The sugars could stimulate the production of AMP hBD2 and hBD3 by keratinocytes in normal condition and also in a model mimicking AD. The sugars were also able to stimulate the production of a TH1 cytokine and to inhibit the production of a TH2 cytokine in peripheral blood mononuclear cells (PBMC). Finally, the sugars inhibited histamine release by IgE-stimulated basophils. Thus these original natural sugars are able to reinforce skin physical as well as biological barrier, to restore the TH1/TH2 balance and to inhibit allergic response. By their *in vitro* specific activities, these sugars could be of a great interest in the management of AD.

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Expression of matrix metalloproteinases-2, 3, and 9 in autoimmune bullous diseases

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Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes degrading extracellular matrix components. They may involve the disruption of epidermal keratinocyte adhesion and degrade collagens of basement membrane zone. This study evaluated the levels and expression of MMP-2, MMP-3 and MMP-9 in the patients with pemphigus vulgaris (PV), pemphgius foliaceus (PF), paraneoplastic pemphigus (PNP), and bullous pemphigoid (BP) and the possible association with the disease severity. Skin biopsy samples and sera of 26 patients with PV (8), PF (8), PNP (2), BP (8) and 6 normal persons as control were investigated. The expression was analyzed by immunohistochemistry (IHC), and levels of these MMPs of the sera of PV, PF, PNP, and BP patients were measured by ELISA. On IHC, MMP-2 was expressed in the epidermis of PV, BP and MMP-9 was expressed in the dermis of BP. With ELISA, the levels of MMP-2 and MMP-3 were increased in the sera of BP patients as compared to those in normal control, and the levels of MMP-2 were closely related with disease severity of BP patients. The level of MMP-3 was increased in all patients' sera rather than that in normal control. In the patients with PNP, MMP-3 and MMP-9 were significantly greater than those in the sera with other patients and normal control, which suggested sera of PNP patients involved these MMPs in other tissues as well as skin in PNP. These results suggest that MMPs may play a role in the induction of blister formation in patients with PV, PF, PNP and BP.

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TACE inhibitor attenuates collagen synthesis and degradation in murine bleomycin-induced skin sclerosis

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Several cytokines including TGF-β, TNF-α are known to play an important role in the pathomechanism of systemic sclerosis (SSc). In this study, we focused on tumor necrosis factor-α converting enzyme (TACE) as a new therapoetic target of SSc. TACE is a member of the adamalysin family of metalloproteinases which is responsible for the processing of pro TNF-α to TNF-α and TNF receptor p55 and p75 to its soluble forms. Recent report described that TACE expression was upregulated in peripheral monocytes of patients with early SSc. We used bleomycin (BLM) induced murine SSc model that demonstrates localized skin fibrosis similar to human SSc. Immunohistochemical staining showed that TACE was expressed in the infiltrating mononuclear cell of BLM treated mice. Also, serum concentration of soluble TNF receptor p55 was significantly increased in BLM treated mice ($P=0.014$). From these findings, we assumed that TACE up regulation in mononuclear cell may play an important role in the pathogenesis in this SSc model. To test this hypothesis, we evaluated the effect of TACE inhibitor, TNF-α processing inhibitor-1 (TAPI-1). Oral administration of TAPI-1 decreased the level of serum soluble TNF receptor p55 ($P=0.028$) and significantly inhibited BLM induced skin thickening (325% vs. 241%, $P=0.034$). Furthermore, it also inhibited the appearance of autoantibody and the number of myofibroblast was fewer in TAPI-1 treated mice. MMP-13 mRNA expression was significantly lower in TAPI-1 treated mice indicating that both collagen synthesis and degradation are attenuated by TACE. From these results, we suggest that TACE might be new therapeutic target in patient with SSc.

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Introducing stereology as a tool to assess the severity of psoriasis

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The purpose of this study was to introduce stereology as a novel tool in assessing the severity of psoriasis. Psoriasis is a well described chronic inflammatory skin disease affecting approximately 2% of the Caucasian population. The severity of psoriasis has been assessed by a multitude of clinical and histological tools in recent medical history. However, studies of the efficacy of potential anti-psoriatic drugs have been hampered by the lack of methods that are both objective and quantitative in nature. Stereology is a microscopy tool based on mathematical statistics applied to histological specimens in order to obtain unbiased and quantitative three-dimensional properties from two-dimensional tissue samples. The psoriasis xenograft model used in this trial is accepted as a leading animal model for psoriasis. Psoriatic skin from psoriatic patients was grafted onto severe combined immunodeficient (SCID) mice. Subsequently, the mice were subjected to intraperitoneal injections five times weekly with either ciclosporin A, a well known anti-psoriatic drug, or PBS (vehicle control) for three weeks. Each group consisted of 3 mice and the treatment groups were blinded to the observers. A punch biopsy from the skin donor was used as baseline control. After completion of the treatment, the mice were sacrificed and punch biopsies taken from the center of the graft. Epidermal thickness was measured using stereological principles. In the ciclosporin A treated group, epidermal thickness was reduced from 0.26 (+/-0.03) mm (baseline) to 0.13 (+/-0.02) mm, corresponding to a 49% decrease whereas the epidermal thickness in the PBS group increased to 0.33 (+/-0.02) mm, corresponding to a 26% increase. This study shows that stereology clearly demonstrates reduction in epidermal thickness during anti-psoriatic treatment compared to the negative control and might prove a very valuable tool when assessing the efficiency of future anti-psoriatic drugs in the psoriasis xenograft model.

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Analysis of chemokine receptors on peripheral memory CD4+ T cells from patients with eosinophilia

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Chemokine receptors have been used to characterize subsets of human memory T cells with distinct migratory capacity and effector functions. For example, CXCR3 is expressed preferentially on human Th1 cells, whereas CCR4 is expressed on Th2 cells. Recently it has been reported that human memory CD4+ T cells selectively producing IL-17 express both CCR6 and CCR4. We examined the expression of these chemokine receptors on the memory CD4+ T cells from patients of various skin diseases. Interestingly, patients with eosinophilia had significantly fewer CCR6+ CD4+ memory T cells in peripheral blood compared to those without eosinophilia. These results suggest that interaction between CCR6+CD4+ memory T cells and eosinophils might be involved in the pathogenesis of some skin diseases.

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PPAR delta is a type 1 interferon target gene and inhibits apoptosis in T cells

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Peroxisome proliferator activater receptor beta/delta (PPAR δ) is a nuclear hormone receptor regulating diverse biological processes including the β-oxidation of fatty acid and epithelial differentiation. The role of PPARδ in the immune system has not been thoroughly studied to date. Here we show that PPARδ is expressed in activated human T cells purified from peripheral blood, as well as in T cells isolated from affected psoriasis skin lesions. PPARδ is induced in T cells upon stimulation with type 1 interferon (IFN). Functionally, PPARδ enhances proliferation of primary T cells and blocks apoptosis induced by type 1 IFN and by serum deprivation. We show that these cellular functions are mediated by activation of ERK1/2 signalling. Our results (1) establish a direct molecular link between type 1 IFN signalling and PPARδ, (2) define a functional role for PPARδ in human T cells, and (3) suggest that the induction of PPARδ by type 1 IFN contributes to the persistence of activated T cells in psoriasis skin lesions.

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TNFα gene polymorphisms in the pathogenesis of acne vulgaris

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Previously we have shown that Propionibacterium acnes (P. acnes) induced the expression of tumor necrosis factor alpha (TNFα) gene in cultured human keratinocytes, suggesting that TNFα might play a role in the pathogenesis of acne. Various single nucleotide polymorphisms (SNP) of the TNFα gene have been shown to be associated with an increased risk to develop chronic inflammatory diseases. In order to find out if known TNFα regulatory SNPs (-1031, -857, -863, -308, -238) have any role in the development of the inflammatory reactions in acne, we analyzed our genomic collection in a case-control study containing 108 control individuals (83 females and 25 males) and 133 acne patients (76 females and 57 males) suffering from more severe papulo-pustular or nodulocystic lesions by PCR-RFLP method, and compared the allele frequencies. There were no significant differences in any genotype or allele frequencies between the control and acne group in case of the -1031, -863, -308, -238 SNPs. However, the TNFα -857 C/C genotype was significantly increased in the acne group (p=0,0444, OR=1,769). The presence of the mutant allele might lead to altered signal transduction events by creating a novel OCT-1 transcription factor binding site that interferes with the regulation of the TNFα gene. These results might help us to gain a better understanding of the role of TNFα in the pathogenesis of acne.

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Studies of the inflammation in palmoplantar pustulosis

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Palmoplantar pustulosis (PPP) is a chronic and intensely inflammatory skin disease with pustules, erythema and scaling localized to the palms and soles. Structure loss in the acrosyringium (the outermost part of the eccrine sweat duct) in the lowest part of stratum corneum and the migration of granulocytes within the acrosyringium indicates that this is the target for the inflammation. Identification of specific features of the PPP cell infiltrate and mediators of inflammation may provide insight into the pathogenesis of the disease. In this study we have further characterized the cellular inflammatory components of palmar skin by use of immunohistochemistry. We observed an enormously strong inflammation in lesional PPP skin, where both the innate and adaptive immune responses were represented and these inflammatory infiltrates were significantly increased in non-involved palmar skin of PPP patients as compared to healthy controls. A characteristic feature of these infiltrates was that Langerhan's cells were gathered around the sweat ducts in PPP skin. We found that specimens from PPP skin with a more pustular activity showed larger numbers of CD4+ T cells and CD8+ T cells (p=0.03) as compared to specimens with no pustules. Macrophages were seen in all specimens, with the largest number in the papillary dermis in sections from pustular PPP skin. Cells of the innate immune system, natural killer (NK) cells, plasmacytoid dendritic cells (pDC) and regulatoric T cells were also observed in the infiltrates. There were significantly more NK cells in reticular dermis in involved PPP skin than in palmar skin from healthy controls (p=0.004) and most pDCs were seen in papillary dermis in sections with large numbers of inflammatory cells. These results further strengthens that PPP is an intensely inflammatory skin disease and indicates involvement of both adaptive and innate immune cells.

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Subchronic allergic contact dermatitis in pigs: a new model for profiling topical anti-inflammatory drugs

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Available models for testing efficacy of topical agents in T cell-mediated inflammatory skin conditions such as atopic dermatitis (AD) or psoriasis have limited predictability, because skin permeability and/or the course of the experimentally induced inflammation in animals differ substantially from the human situation. Here we present a new model of subchronic allergic contact dermatitis (ACD) in pigs that shows a high degree of correlation with clinical efficacy in AD patients when testing various anti-inflammatory drugs. Sensitized young domestic pigs were challenged with 0.2% 2,4-dinitrofluorobenzene at test sites on both sides of the dorso-lateral back on days 1, 3, 6, 8 and 10. The test sites were treated with the active formulations twice on days 6, 8 and 10 (2 and 6 hrs after challenge), followed by clinical (and/or biophysical) examination and scoring of the lesions on days 7, 9 and 11. Additionally, biopsies taken after the last treatment were examined. In comparison with untreated test sites, Protopic® 0.1% (tacrolimus) inhibited inflammation by 77%, Protopic® 0.03% by 49%, Alfason® (0.1% hydrocortisone-17-butyrate) by 53% and Hydroderm® (1.0% hydrocortisone acetate) by 15%. Inflammatory cell infiltration (T cells) was similarly reduced. The degree of anti-inflammatory activity of the compounds in this model closely mirrors the relative efficacy reported in AD patients. The model is stringent because treatment is started when pronounced inflammation is established. This ACD model appears, therefore, to be valuable for assessing the clinical potential of new compounds for the treatment of AD and, possibly, other T- cell mediated skin diseases.

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Laser scanning confocal microscopic study in differentiation of autoimmune subepidermal bullous diseases mediated by IgA

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Epidermolysis bullosa acquisita mediated by IgA (IgA-EBA) may clinically resemble linear IgA bullous dermatosis (LABD) especially at the onset of the disease but they differ in a course and response to the treatment. In this study we applied laser scanning confocal microscopy (LSCM) to determine the localization of the *in vivo* bound IgA at the basement membrane zone (BMZ) in biopsies taken from six patients presenting exclusively IgA at the BMZ. Localization of *in vivo* bound IgA was compared to the localization of collagen type IV. *In vivo* bound IgA were visualized by labeling with FITC-conjugated anti-human antibody, whereas antibody against collagen IV were labeled with anti-mouse Cy5-conjugated antibodies. Excitation of FITC and Cy5 was performed with laser lines of 488 nm and 637 nm respectively. To obtain specific signal of FITC (green color) and Cy5 (red color) and avoid cross-talking channel effect we used dichroic mirror of 560 nm and bandpass filter 500-560 nm for epifluorescence of FITC and longpass filter 660 nm for Cy5. Green and red images were overlaid by an image processing system integrated with the LSCM Radiance 2000 and photographed. Overlay image showed the presence of *in vivo* bound IgA deposits above collagen IV in four of six studied patients. In those patients the disease disappeared within several months. Whereas in two of six *in vivo* bound IgA were found below collagen IV. In retrospect, those two patients were characterized by long-lasting duration of the disease and development of atrophic scars and milia few years after the onset of the disease. Our study showed that patients in whom *in vivo* bound IgA were found above collagen type IV represent milder course of bullous disease as compared to those with IgA localized below collagen type IV. This suggest that studied patients may represent two different entities: LABD and IgA-EBA respectively.

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Myrtacine regulates arachidonic acid cascade, cyclo-oxygenase and lipoxygenase pathways in human keratinocytes : interest in the treatment of inflammatory acne disease.

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Acne vulgaris is an exceptionally common, chronic and recurring disorder affecting many adolescents and adults throughout their lifetimes. The pathogenesis of acne is multifactorial and is thought to involve excess sebum, follicular hyperkeratinization, bacterial colonization and inflammation. Many therapeutic options exist for the treatment of acne, including topical or systemic drugs. These drugs exert their therapeutic effects by not only bacteriocidal actions but also anti-inflammatory actions. Drugs possessing these anti-inflammatory capacities are clinically beneficial for the treatment of inflammatory acne. As in inflammatory skin disorders dysregulation of epidermal eicosanoids biosynthesis is also observed, we focused on these inflammatory potent mediators which represent an heterogeneous family derived from arachidonic acid (AA) metabolism by cyclooxygenase (prostaglandins) and lipooxygenase (leukotrienes) pathways. The aim of the present study was to investigate the activity of Myrtacine, a new extract of Myrtus communis aerial parts containing 1% myrtucommulones, on the AA metabolism of human keratinocyte cell line HaCaT. The Myrtacine (1, 3, 10 µg/ml) was found to inhibit the A23187-triggered increase in the levels of [3H]-labelled metabolites of the cyclooxygenase (-81% to -100%) and 5-lipoxygenase (-52% to -95%) pathways. A closer examination of the activity of Myrtacine on 6KPGF1α, PGF2α, PGE2, PGD2, PGA2 and LTB4, HETEs revealed the dose-dependent inhibition of each AA metabolite. This activity could be associated with a new AA distribution at the membrane lipids level. Our results demonstrate the strong regulator properties of Myrtacine with respect to cutaneous inflammatory response showing therefore a real interest of Myrtacine in the treatment of inflammatory acne disease.

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Localization of B cell epitopes of desmoglein 3 autoantigen in pemphigus vulgaris patients

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Pemphigus vulgaris (PV) is a serious blistering dermatosis. The pathogenesis of acantholysis in the disease is mediated by the specific autoantibodies targeting Desmoglein 3 (dsg3). There is no definite information available on the autoantibody binding sites on Dsg3. In this study, we aimed to express extracellular domain 1 and 2 (EC1-2) of Dsg3 and characterize the immunodominant epitopes with the application of phage display peptide libraries. The EC1-2 specific autoantibodies (IgG) from PV sera were purified with recombinant protein EC1-2. Then the IgG Abs were served as a probe and potential B cell epitopes recognizing by the Abs within EC1-2 were determined by a random phage-peptide library. Based on sequences analysis of selected positive phage clones, the potential B cell epitopes were predicted. Comparison between the sequences of the selected phage clones and the sequence of EC1-2, one B cell epitope was reduced at amino acid residues 176-184 (DREQASSYR) of EC1-2 protein. To confirm the prediction, the 176-184 peptide was displayed on the phage as fusion to the NH2-terminus of the major coat protein pVIII. The phage showed a high affinity with the PV sera and the EC1-2 protein could significantly reduce the binding activity between PV sera and the phage displaying 176-184 peptide of EC1-2 protein with competitive ELISA. Our results suggested that 176-184 peptide (DREQASSYR) within EC1-2 is a potential B cell epitope, which was recognized by the autoantibodies from PV patients sera. The identification of this B cell epitope is helpful to understand the pathogenesis of the disease. Also, it may have potential to be used in clinical diagnosis and immunotherapy.

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Pathogenic pemphigus vulgaris autoantibodies demonstrate variable ELISA activity due to preferential binding of mature versus proprotein isoforms of desmoglein 3

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We have previously reported the cloning of pathogenic and nonpathogenic anti-desmoglein (Dsg) human monoclonal antibodies (mAbs) from a patient with pemphigus vulgaris (PV). Over time we have observed variable ELISA binding by certain pathogenic PV mAbs, despite consistent functionality in pathogenicity assays against endogenously expressed Dsg3 in human keratinocytes. Commercial Dsg3 ELISA plates use baculovirally-produced extracellular ectodomain of Dsg3, which may demonstrate variable ratios of mature protein to proprotein depending on culture methods. To investigate whether the variable ELISA binding of pathogenic PV mAbs was due to differential binding of mature Dsg versus Dsg proprotein, we tested a panel of PV mAbs against baculovirally-produced extracellular Dsg3 ectodomain. Two human pathogenic mAbs, as well as the pathogenic mouse PV mAb AK23, preferentially immunoprecipitate mature protein from baculoviral supernatants. All three of these antibodies recognize a calcium-sensitive conformational epitope in the amino terminal domain of Dsg3. In contrast, two nonpathogenic and one pathogenic human PV mAb bind both mature and proprotein isoforms of Dsg3. These latter antibodies all recognize denatured Dsg3 by immunoblot and bind throughout the ectodomain. Furin proprotein convertase added directly to baculoviral supernatants was able to process Dsg3 proprotein to the mature cleaved form. Solid phase furin treatment of Dsg3 immobilized on commercial ELISA plates resulted in preferentially increased binding of pathogenic and Dsg3 amino-terminal binding PV mAbs. These results suggest that the presence of the proprotein polypeptide sequence alters the conformation of the Dsg3 extracellular domain, resulting in diminished binding of pathogenic antibodies. These findings have important implications for pemphigus researchers using commercial ELISA plates, given the increasing number of studies using pathogenic mouse and human PV mAbs.

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Specific species of glycosaminoglycans accumulate in skin in cutaneous lupus erythematosus, dermatomyositis, and after ultraviolet B irradiation

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Elevated glycosaminoglycans (GAGs) have been observed on Hale's stain of skin in three inflammatory conditions: cutaneous lupus erythematosus (CLE), dermatomyositis (DM), and UVB-irradiation. Hale's stain, however, cannot distinguish among different GAG species, which have distinct effects on recruitment and activation of immune cells. Thus, we used immunohistochemistry to examine two GAG species, hyaluronic acid (HA) and chondroitin sulfate (CS), in CLE, DM, and UVB-irradiated SCLE and healthy skin. Skin sections from patients with DM or one of several types of CLE (subacute cutaneous, SCLE; discoid, DLE; and tumid, TLE), healthy controls, and UVB- or sham-irradiated subjects were separately stained with HA binding protein and the anti-CS antibody CS-56. Total and upper dermal staining among disease subtypes and controls was graded in a blinded fashion from 0 (absent) to 4 (high). Staining of irradiated healthy skin was quantified using automated imaging software. Among CLE subtypes, DLE, but not SCLE, exhibited higher total dermal HA and CS compared to controls ($p<0.05$). In contrast, TLE only showed an upper dermal increase in HA ($p<0.05$). DM also exhibited a distinct pattern: a large, significant increase in total dermal CS ($p<0.05$) but non-significant changes in HA. Among UVB-irradiated samples, HA decreased in the upper dermis 48h post-irradiation but returned after 72h in both SCLE and healthy skin compared to sham-irradiated skin. After one month of irradiation of healthy skin, upper dermal CS was elevated compared to sham-irradiated skin ($p=0.011$). Thus, HA and CS exhibited distinct patterns of altered staining, depending on the condition. We speculate that the increase in HA in DLE and TLE patients may be part of the local inflammatory response. The biologic role for the increase in CS in DLE, DM, and UVB-irradiated healthy skin is unknown. Importantly, the decrease in HA 48h after UVB-irradiation may indicate HA fragmentation, which could augment photosensitivity.

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Influence of TH-1 and TH-17 type cytokines on epidermal differentiation

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In inflammatory skin diseases such as psoriasis and graft vs. host disease, there is altered epidermal barrier function. The process of terminal differentiation and barrier formation is linked to several key proteins including filaggrin, caspase 14 and loricrin. After verifying psoriatic plaques are characterized by reduced expression of these proteins, we sought to determine the mechanism by which cytokines interfere with barrier function of skin. Using a 3-D skin equivalent model system in which human keratinocytes are exposed to cytokines in a submerged state, followed by raising to an air/liquid interface leading to a stratified epithelium resembling normal epidermis, we determined the influence of cytokines on the differentiation process. In the absence of cytokines, there is detection of all three key proteins by immunostaining (resembling normal skin including a granular layer and stratum corneum) and western blot analysis. However, when a relatively low amount (10-20 ng/ml) of a TH-1 cytokine such as IFN-gamma is present, there is suppression of these three proteins by both immunostaining and western blot analysis. Similarly, addition of TH-17 cytokines such as IL-17, IL-22, or IL-17 plus IL-22 also reduced these three proteins. A common signaling pathway shared by these different cytokines was activation of ERK (e.g. pERK). Interestingly pERK is highly expressed in psoriatic plaques. To establish cause:effect relationships, we over-expressed a constitutively active form of MEK, the upstream kinase for ERK, and observed reductions in levels of filaggrin and caspase 14. For all treatments, the histological appearance of epidermis reflected a decrease in the differentiation related proteins by reduction in the granular cell layer. We conclude that both TH-1 and TH-17 type cytokines can directly influence terminal differentiation mediated by activation of ERK, which can suppress expression of several different differentiation-related proteins. Targeting these cytokines or suppressing ERK is indicated to restore barrier function in a variety of inflammatory skin diseases by influencing the terminal differentiation process.

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Induction of memory IL-17+ T cell trafficking and expansion by interferon-gamma: mechanism and pathological relevance in psoriasis

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Psoriasis is a common, immunologically mediated, chronic disease of the skin and joints displaying a strong HLA Class I association. Previous studies implicated Th1 cells in psoriasis pathogenesis and maintenance. An antibody that neutralizes IL-12p40, and thereby both IL-23 and IL-12, has shown potent effects in treating psoriasis. However, genes encoding subunits specific to IL-23 and its receptor, but not those specific to IL-12 and its receptor, are associated with and overexpressed in psoriasis, suggesting specific involvement of the IL-23 signaling pathway. As IL-23 stimulates Th17 cell production, it is possible that IL-17 producing T cells participate in psoriasis pathogenesis. We therefore investigated IL-17 producing T cells in psoriasis and their connection to the Th1 cytokine, IFN-γ. We observed increased numbers of IL-17+ T cells in psoriatic plaques, including a striking increase in epidermal CD8+IL-17+ T cells. Psoriatic plaques also contain high levels of Th1 cells, macrophages, IFN-γ, IL-1, IL-23, and CCL20. Furthermore, we found that blood- and plaque-derived psoriatic macrophages potently induce IL-17+ T cells, and that IFN-γ programs macrophages to expand human memory IL-17+ T cells via IL-1 and IL-23. IFN-γ additionally stimulates macrophage production of CCL20, which promotes Th17 migration via CCR6. Finally, we found that IFN-γ and IL-17 synergize to stimulate production of β-defensin 2 from keratinocytes. Our study reveals a novel mechanistic interaction between Th1 and Th17 cells, challenges the view that Th1 cells suppress Th17 development through IFN-γ, and suggests that Th1 and IL-17+ T cells collaborate in psoriasis pathogenesis and maintenance.

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Role of reactive oxygen species in TNF-alpha-activated signaling cascades in primary human keratinocytes: Implications for psoriasis

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The multifunctional cytokine tumor necrosis factor-α (TNF-α) is known to play an important role in inflammatory and immunological responses in human skin. Although it has been documented that reactive oxygen species (ROS) are involved in TNF-α induced signaling pathways associated with certain inflammatory diseases, its role in TNF-α signaling cascades has not been examined in primary human keratinocytes used as a model of inflammatory skin disease and psoriasis. Employing a series of *in vitro* and *in vivo* approaches, we have demonstrated that in primary human keratinocytes: i. TNF-α rapidly induces ROS generation, IκB degradation, nuclear NF-κB p65 translocation, and ultimately production of inflammatory cytokines; ii. TNF-α- induced cytokine production is mediated both by the mammalian target of rapamycin (mTOR) signaling pathway via NF-κB activation as well as by ROS; iii. ROS-mediated TNF-α -dependent cytokine production is not due to NF-κB activation (that is, IκB degradation and nuclear NF-κB p65 translocation); and iv. a cell-penetrating derivative of the antioxidant enzyme, catalase, as well as taurine and N-acetyl-cysteine (NAC) attenuate the TNF-α -induced production of cytokines. Additionally we conducted a hematological examination of psoriatic and normal patients to determine the relative levels of various antioxidant enzymes. We found in this preliminary analysis that the erythrocyte activity of catalase, glutathione peroxidase, glutathione reductase, and superoxide dismutase is lower in patients with psoriasis as compared to controls. Taken together, these results shed light on the molecular mechanisms and pathophysiology of inflammatory skin diseases, including psoriasis, and may provide targets for therapeutic intervention.

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20-Hydroxycholecalciferol and 20,23-dihydroxycholecalciferol, products of vitamin D3 hydroxylation by P450-scc, decrease NFκB activity by increasing IκBα levels in normal human- and HaCaT-keratinocytes

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It has been shown recently that the side chain of vitamin D3 is hydroxylated in a sequential manner by cytochrome P450scc (CYP11A1) to form 20-hydroxycholecalciferol and 20,23-dihydroxycholecalciferol as the main metabolic products. We have established that these metabolites have biological activity on keratinocytes, inducing growth arrest and differentiation of both normal and HaCaT keratinocytes. Thus, we have identified a useful candidate for the treatment of hyperproliferative skin diseases. Nuclear factor-κB (NFκB) plays a pivotal role in immune and inflammatory responses, and in the regulation of cell proliferation and apoptosis. It regulates the transcription of genes encoding proinflammatory cytokines, chemokines, adhesion molecules, etc. To uncover the mechanism underlying the biological activity of 20-hydroxycholecalciferol, as well as that of 20,23-dihydroxycholecalciferol, we examined their effects on the activity of NFκB and NFκB-cytosolic inhibitor, IκB. NFκB binding activity was determined by EMSA, NFκB-p65 and IκBα proteins were detected by Western blotting and mRNA was determined by RT-PCR. Both 20-hydroxycholecalciferol and 20,23-dihydroxycholecalciferol induced significant increases in mRNA and protein levels of IκBα in a time dependant manner, while no changes in total NFκB-p65 mRNA and protein levels were observed. Comparison of these effects with those of 1,25-dihydrocholecalciferol showed that the novel products of CYP11A1 metabolism of vitamin D3 are compatible in inhibiting NFκB activity with the hormonally active form of vitamin D3. Since NFκB is a major transcription factor of inflammatory mediators, these findings indicate that the 20-hydroxycholecalciferol and 20,23-dihydroxycholecalciferol may be effective as anti-inflammatory therapeutic agents.

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Protein biomarker discovery from skin extra-cellular microenvironment in contact dermatitis

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Allergic contact hypersensitivity (CHS) is a complex inflammatory skin condition that requires the interaction of many different cellular and molecular mediators. While the roles of immune cells and cytokines have been widely studied, the dynamics of protein expression *in vivo* remains unexplored. In this study, we employed two approaches, *ex-vivo* tissue perfusion and *in vivo* capillary ultrafiltration (CUF), to extract interstitial fluids (IF) from the cutaneous extra-cellular microenvironment for proteomic analysis of the proteins secreted into the skin during elicitation of allergic CHS to dinitrofluorobenzene (DNFB) and in irritant contact dermatitis (CD) to TPA in C3H/HeN mice. Tissue perfusion provides IF at the exact time points of disease stages, while the in-vivo CUF sampling allows continuous IF collection from individual live, freely-moving animals during the evolution of the CHS response. The collected IFs were treated with multiple affinity spin cartridge to remove high abundance proteins, such as albumin and transferrin, before analysis by 2-D Fluorescence Difference Gel Electrophoresis (DIGE) for quantitative protein profile analysis. DIGE protein profiles of IFs collected at 24 hours after the induction of CHS and irritation responses clearly showed significant changes of protein regulation. Mass Spectrometry-based protein profiling of these IFs by MALDI-TOF MS also showed major protein pattern changes at m/z 10,183 and 20,375, which indicates the possible protein biomarkers that distinguish irritant from allergic responses. With the protein identification capability of current mass spectrometry techniques, we expect to identify these possible biomarker candidates detected with our current approaches. The outcome of the extra-cellular proteome may provide new information for understanding the role of these proteins involved in the inflammatory responses to these two conditions which may lead to better diagnostic strategies and improved therapeutic strategies.

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Withdrawn

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TACE, ADAM 8, ADAM 10 and ADAM 15 take part in destruction of basement membrane in bullous pemphigoid

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Reports in recent literature reveal the role of metalloproteinases in the pathogenesis of bullous pemphigoid (BP). However there are no data in available literature concerning the expression and role of adamalisynes (ADAMs – a disintegrin and metalloproteinases), a specific family of metalloproteinases, in this process. Recent studies established biochemical properties of these enzymes and integrins and their high affinity to components of the basement membrane. The aim of the study was evaluation of the expression of TACE, ADAM8, ADAM10 and ADAM15 in course of the BP patients (n=20) in active stage through examination of their expression and distribution in lesional skin, normally appearing skin as well as in healthy skin (n=10) by immunohistochemistry (R&D). Expression of tissue inhibitors of metalloproteinases (TIMP1, 2, 3) was evaluated (Novocastra), in order to establish the degree of imbalance between these groups of enzymes. Moderate expression of TACE, ADAM 8, 10 and 15 in skin lesions were observed mostly in the basal keratinocytes as well as focally in the other layers of epidermis. In cellular infiltrate we found a strong expression for the ADAM8 and 10. TIMPs were detected in dermal part of the blister and in the basal keratinocytes. In normally appearing skin from BP patients the expression of TACE, ADAM 8, 10 and 15 was single and weak. In healthy biopsies expression of adamalisynes was single or there was no expression. Obtained results will enlarge the state of knowledge concerning the subepidermal bullous diseases and confirm the role of ADAMs as proteases and adhesive molecules in BP.

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Noninvasive molecular profiling technique for skin irritation assessment using ultrasound extraction: Alterations in protein, lipid, and nucleic acid profiles in NC/Nga and fsn mouse skin

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A variety of epidermal metabolic activities are tightly regulated in the healthy skin to maintain its functional homeostasis while disease pathology can cause not only structural alteration but also various molecules imbalances in skin. However, current methods used to assess skin irritation have relied on qualitative visual examination and subjective evaluation of invasively-biopsied samples, consisting of visual grading of skin based on degrees of erythema, dryness, papules, vesicles, etc. In this presentation, we report a noninvasive and quantitative molecular profiling technique using ultrasound extraction. We ultrasonically extracted biomolecules from NC/Nga and fsn mice for atopic dermatitis and psoriasis, respectively. The extracts were analyzed for proteins including cytokines, lipids, and nucleic acids, and were compared with those in the healthy balb/c mice. Our technique could extract and quantify 71 types of proteins (keratins, interleukins, chemokines, IgG and others), a total of 14 lipids, and nucleic acid (DNA and RNA) from the normal or abnormal skins. Using a high-throughput assay, we analyzed the presence of 62 cytokines in the extracts and found out their specific up/down-regulation patterns by comparative normalization with total extracted protein amount. Lipid analysis of the extracts also revealed significant alterations in the lipid composition of these diseases. Additionally, we also found a qualitative increase of up to 10–fold in RNA and 30–fold in DNA mass from ultrasonic extracts of the two diseased animal models. Our results demonstrated that the present method is a useful noninvasive technique for creating skin's "molecular signature" and assessing skin inflammatory events.

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The presence of functional anti-CD22 antibodies in patients with systemic sclerosis: Autoantibody-mediated B cell activation

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Systemic sclerosis (SSc) is a connective tissue disorder characterized by fibrosis and vascular changes with autoimmune background. Recent studies have demonstrated that B cells play critical roles in the manifestation of SSc. B cell functions are under the collaborative regulation by B cell antigen receptor (BCR) and other cell surface coreceptors including CD22. CD22 is a B cell-specific inhibitory receptor that dampens BCR signaling via tyrosine phosphorylation-dependent mechanism. In this study, we examined the presence of autoantibodies against CD22 in the sera from SSc patients. Serum samples from 50 SSc patients (20 with diffuse cutaneous SSc and 30 with limited cutaneous SSc) were assessed for anti-CD22 antibodies by ELISA using recombinant human CD22. Twenty patients with systemic lupus erythematosus and 17 healthy donors were included as controls. Anti-CD22 antibodies were positive in 26% of SSc patients. Patients with anti-CD22 antibodies showed significantly higher Modified Rodnan skin thickness score compared with those without anti-CD22 antibodies (16±11 vs.8.4±8.6). Additionally, % vital capacity was significantly lower in those positive for anti-CD22 antibodies than those negative (90±25% vs.105±20%). There was no significant difference in frequencies of kidney or heart involvement. Furthermore, anti-CD22 antibodies from patients' sera were capable of reducing BCR-mediated phosphorylation of all four major CD22 tyrosine motifs (Y762, Y807, Y822, and Y842) significantly detected by western blot using phospho-specific antibodies against CD22. Thus, these antibodies can interfere CD22-mediated suppression onto B cell activation, suggesting the possibility of autocrine B cell activation that B cells produce functional autoantibodies that can enhance B cell activation. This novel mechanism of autoantibody-mediated B cell activation may contribute to the pathogenesis of SSc.

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***In vivo* evidence for oxidative damage and accelerated aging of ulcer-adjacent fibroblasts in chronic venous leg ulcers**

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In vivo evidence for oxidative stress-dependent mechanisms of impaired wound healing is scarce. Here we analyzed the oxidative damage in inflammatory cells and its potential pathogenic impact on the dermal fibroblasts in chronic venous leg ulcers. Immunostaining of skin biopsies taken from ulcer margins of patients with non-healing ulcers persisting for more than 6 month despite of adequate compression therapy revealed a persistent inflammatory infiltrate mainly consisting of CD68+ macrophages. These were highly activated with substantial up-regulation of the classical activation markers CD18 and iNOS when compared to unaffected skin. In chronic, but not in acute wounds, macrophages were a major source of ROS at toxic concentrations with strong expression of 8OHdG, indicating oxidative DNA damage. Protein oxidation assessed by the formation of carbonyl derivatives in oxyblot analyses was significantly increased in the ulcer margin, but not in unaffected skin from the same patient. Moreover, a 50kDa protein was highly oxidized in all ulcers but not in unaffected skin suggesting it to be a preferred target for oxidation in non-healing ulcers. Importantly, resident fibroblasts highly expressed the DNA-damage associated γH2AX, indicative of cellular growth arrest and senescence. In addition, immunostaining for p16, which was recently identified as a robust *in vivo* marker for irreversible skin aging, showed a substantially increased number of p16-positive fibroblasts in the dermis adjacent to chronic venous leg ulcers but not in unaffected skin from the same patients. These data suggest that fibroblasts persistently exposed to oxidative stress in the margins of chronic venous leg ulcers undergo an accelerated, premature senescence resulting in functional incapacity to promote wound healing. Rigorous debridement or even shaving of the ulcer margins with irreversibly senescent fibroblasts may be the therapy of choice in recalcitrant leg ulcers.

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Mir-203, a skin-specific microRNA, is upregulated in psoriasis and regulated by keratinocyte differentiation

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MicroRNAs are a recently discovered class of posttranscriptional regulators of gene expression with critical functions in health and disease. MicroRNAs suppress the expression of protein-coding genes by targeting mRNAs for translation repression or less frequently for degradation. Psoriasis is the most prevalent chronic inflammatory skin disease in adults, with a substantial negative impact on the patients' quality of life. Recently, we showed for the first time that psoriasis-affected skin has a specific microRNA expression profile when compared with healthy human skin or with another chronic inflammatory skin disease, atopic eczema. Among the psoriasis-specific microRNAs, we identified leukocyte-derived microRNAs and one keratinocyte-derived microRNA, miR-203. In a panel of 21 different human organs and tissues, miR-203 showed a highly skin-specific expression profile. Among the cellular constituents of the skin, it was exclusively expressed by keratinocytes. Using microRNA-specific quantitative real-time PCR, we showed that miR-203 is regulated during keratinocyte proliferation and differentiation. miR-203 was expressed in cultured primary keratinocytes constitutively and its expression increased more than ten-fold in post-confluent, differentiated keratinocytes with high involucrin expression, compared to subconfluent, proliferating keratinocytes. In accordance with *in vitro* results, microRNA-specific *in situ* hybridization showed that miR-203 was expressed in the suprabasal layers of the epidermis, consisting of differentiated keratinocytes. Computational prediction of the target genes of miR-203 identified several genes involved in the regulation of keratinocyte proliferation and differentiation as well as cytokine responses. Our results suggest that deregulation of microRNAs is involved in the pathogenesis of psoriasis and the overexpression of miR-203 may contribute to keratinocyte dysfunction in psoriasis lesions.

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Human Thy-1 induces secretion of matrix metalloproteinase-9 and CXCL8 from neutrophils

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Neutrophils are one of the first cells arriving at sites of acute inflammation. On their way from blood to the site of inflammation neutrophils have to adhere to endothelial cells (EC), to transverse the basement membrane and subsequently to travel through the interstitial matrix. Recently, we have shown that human Thy-1 is an alternate EC receptor for the leukocyte integrin Mac-1 (CD11b/CD18) that contributes to leukocyte recruitment to sites of inflammation thus providing a new pathway for adhesion and transmigration of neutrophils. Here, we studied the effect of Thy-1 mediated adhesion on neutrophil functions. Since MMP-9 plays an important role for the migration of neutrophils through the basement membrane we analyzed secreted MMP-9 after interaction of neutrophils with Thy-1. Binding of neutrophils to recombinant human Thy-1 stimulated the release of MMP-9 from neutrophils resulting in their enhanced migration through collagen-IV and matrigel. Further, we showed that neutrophil interaction with Thy-1 stimulated secretion of CXCL8 and thus could support the attraction of additional neutrophils to inflammatory sites. Blocking experiments confirmed the pivotal roles of Thy-1 expression on activated dermal EC or fibroblasts and the beta 2 integrins on neutrophils for the regulation of MMP-9 and CXCL8 release from neutrophils. In summary, Thy-1 mediates not only the adhesion of neutrophils to activated dermal EC and fibroblasts but also regulates neutrophil function. Our results support the general concept that the function of 'adhesion molecules' in particular of human Thy-1, may not only be to provide mechanical support but also regulate functions such as transmigration or release of chemotactic factors.

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MicroRNA-mediated regulation of suppressor of cytokine signaling 3 in psoriasis

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Psoriasis is a chronic inflammatory skin disease, affecting approximately 2-3% the population worldwide. MicroRNAs are ~22 nt noncoding RNAs that suppress the expression of protein-coding genes by targeting mRNAs for translation repression or less frequently for degradation. Recently, we identified a skin and keratinocyte –specific microRNA overexpressed in psoriasis, miR-203. To investigate the functions of miR-203 in keratinocytes and the pathogenesis of psoriasis, we predicted genes that can be regulated by miR-203 using bioinformatic tools. One of the evolutionarily conserved target genes we identified was Suppressor of Cytokine Signaling 3 (SOCS-3), a gene involved in the negative feedback loop in cytokine signaling. To validate that SOCS-3 is a direct target of miR203 for translational suppression, we cloned the predicted miR-203 binding site in the 3'UTR of SOCS-3 gene into a pMIR luciferase reporter vector and performed luciferase assays. When the SOCS-3 3'UTR was coupled to a luciferase reporter and cotransfected with miR-203 precursor into HeLa cells, we observed significant repression of the reporter. The repression was alleviated when the predicted binding site was mutated, indicating that this binding site is functional. Taken together; these results indicate that SOCS-3 is a direct target of miR-203. Moreover, we analyzed the expression of SOCS-3 in psoriatic lesional skin and healthy skin using immunohistochemistry, western blotting and real-time PCR. In accordance with the overexpression of miR-203 in psoriasis skin, SOCS-3 protein level was significantly decreased in psoriasis plaques as compared to healthy skin. However, SOCS-3 mRNA expression was not significantly different from healthy skin, suggesting that down-regulation of SOCS-3 in psoriasis occurs at the posttranscriptional level. Taken together, miR-203 suppresses SOCS-3, a negative regulator of cytokine signaling, in keratinocytes. The overexpression of this microRNA may have a role in the increased/elongated cytokine responses in psoriasis skin.

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Laminin gamma-1 is a major autoantigen in anti-p200 pemphigoid

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Anti-p200 pemphigoid is an autoimmune subepidermal blistering disease characterized by autoantibodies to a 200- kDa protein (p200) of the dermal-epidermal junction. Though numerous cases with this novel disease have been reported over the past 12 years, the molecular identity of p200 had not yet been determined. The aim of this study was the characterization of p200 at the molecular level. We performed two-dimensional (2-D) electrophoresis of dermal extracts followed by Western blotting (WB) with patients' sera, and the detected bands were characterized by mass spectrometry. These studies revealed labeling of a unique band which corresponded to laminin gamma-1 (LNγ1). The band was identical to that detected by mouse anti-human LNγ1 monoclonal antibody (mAb) in WB after 2-D electrophoresis of dermal extracts. Sera from 20 patients with anti-p200 pemphigoid showed 75-85% reactivity to LNγ1 in WB with purified human laminin 111, laminin 211/221 and recombinant laminin 111, respectively. All these trimers contain LNγ1 as a subunit. Importantly, none of the healthy control sera reacted with LNγ1. By WB, reactivity of a patient's serum to p200, extracted from dermis, was competitively inhibited by adding anti-LNγ1 mAb or purified laminin 211/221. To determine the responsible epitope on LNγ1, we constructed a recombinant laminin 111 complex in truncated form. All LNγ1-positive sera showed reactivity with this recombinant C-terminal fragment. These results suggest that LNγ1 is a major dermal autoantigen for patients with anti-p200 pemphigoid and the epitope locates on the C-terminal portion of the coiled-coil domain, which seems important for the structural conformation in laminin-integrin interaction.

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Abnormal DNA methylation in subacute cutaneous lupus erythematosus

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Background : It has been shown that impaired T cell DNA methylation might contribute to the development of lupus But there is no similar or related studies about DNA methylation in subacute cutaneous lupus erythematosus (SCLE), a subtype of lupus erythematosus (LE). Our objectives was to investigate whether alterations in DNA methylation occur in SCLE patients. Our methods included Global DNA methylation in CD4+ and CD8+ T cells from 12 SCLE patients and 9 healthy control subjects were assayed using the the Methylamp™ Global DNA Methylation Quantification Kit. The mRNA levels of DNA methyltransferases (DNMTs) and methylated CpG binding proteins (MBDs) were measured by real-time quantitative polymerase chain reaction. Our results showed that SCLE patients had significantly lower global DNA methylation in CD4+ T cells than that in controls (28.90%±9.91% vs. 42.87%±7.60%; P=0.002). DNMT1 and DNMT3a mRNA levels were significantly lower in CD4+ T cells from SCLE patients (1.167±0.334 vs. 1.781±0.802; P=0.027 and 1.050±0.411 vs. 2.206±1.145; P=0.004 respectively). MBD1, MBD3 and MBD4 mRNA were significantly higher in CD4+ T cells from SCLE patients than that in controls (1.189±0.412 vs. 0.291±0.279;p<0.001, 1.296±0.395 versus 0.510±0.257;p<0.001 and 1.379±0.504 versus 0.534±0.327; p<0.001 respectively). MeCP2 and MBD4 mRNA were also significantly increasd in in CD8+ T cells from SCLE patients than that in controls (p<0.001, ;p<0.001 respectively). However, global DNA methylation,DNMT1, DNMT3a and DNMT3b mRNA in CD8+ T cells were no different between patients and controls (P>0.05). In addition, we found that a positive correlation between DNMT1 and methylation indices (P<0.01). This data suggests that aberrant DNA methylation may be implicated in the development of SCLE

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Granular IgG deposition in pemphigus foliaceus: clusters of IgG containing desmoglein 1 and plakoglobin

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Pemphigus foliaceous (PF) is one of the two main variants of pemphigus, and is characterized by subcorneal blistering of the skin, no involvement of the mucous membranes, and immunologically by circulating IgG to desmoglein (Dsg) 1. In a previous study in Dsg3-mediated pemphigus vulgaris (PV) we found IgG deposits in a granular pattern of clusters that colocalize with Dsg3, and plakoglobin (PG) and, depending on the state of pathogenesis, also with other desmosomal components. In the present study we have analyzed the IgG deposition patterns in skin of PF patients. We included biopsies of both lesional and healthy skin of seven PF patients. By Dsg-ELISA we ascertained that the IgG profile was exclusively anti-Dsg1. Double staining immunofluoresence microscopy was performed for IgG and a series of adhesion molecules. As in PV the IgG deposited in a granular ICS pattern in lesional skin, but also in non-lesional skin. In all lesional biopsies the clustered IgG co-localized with the target autoantigen Dsg1, PG, and, depending on the state of pathogenesis, also with other desmosomal components. The clusters were localized in the intermediate layers of the epidermis, and in lesional skin were positioned well below the superficial blister. Hematoxylin-eosin staining of the sections used for immunofluorescence microscopy showed that keratinocytes in the areas with granular depositions exhibited intercellular widening. One of the biopsies demonstrated an exceptional deposition pattern. In addition to the intercellular granular epidermal depositions, it also contained granular clusters of IgG, Dsg1, and PG at the epidermal basement membrane zone. Immunomapping against laminin-332 showed that these clusters were localized outside the basal cells. The observation that clustering of IgG and Dsg1 preceeds the blistering suggests that these clusters represent an early phase in the pathogenesis of PF.

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Anti-desmoglein -3 autoantibodies obtained from pemphigus vulgaris patients in clinical remission may induce apoptosis in HaCaT keratinocytes

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Pemphigus vulgaris is an autoimmune blistering disease, immunologically characterized by the presence of circulating and bound *in vivo* autoantibodies directed either solely against desmoglein 3 (Dsg3) or against both Dsg 3 and Dsg 1. Despite extensive research, the exact mechanism of acantholysis development and immulogical properties of anti-Dsg antibodies are not fully elucidated. Thus the aim of our study was to evaluate the ability of ant-Dsg3 antibodies isolated from PV patients in active stage of disease (a; n=18) and in clinical remission (b, n=14) to initiate apoptosis. Control donors consisted of sera obtained from healthy unrelated subjects age and sex matched (n=5). HaCaT cells were incubated with anti-Dsg3 antibodies and then Bax, Bcl-2 and procaspase -3 expression were determined by Western blot analysis. We found an increased expression of proapoptotic proteins (Bax and procaspase -3) in HaCaT cells incubated with anti –Dsg-3 autoantibodies taken both from active and clinical remission patients. The obtained results indicate that anti-Dsg3 antibodies from PV patients both in active phase of the disease and in the clinical remission have similar ability to induce apoptosis in HaCaT keratinocytes.

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Immunodominant CD4+ T cell epitopes of human desmoglein 3 initiate a pathogenic antibody response in an HLA-class-transgenic mouse model of pemphigus vulgaris

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Pemphigus vulgaris (PV) represents a severe autoimmune blistering disorder which is characterized by IgG autoantibodies (autoAb) predominantly against the epidermal adhesion molecule desmoglein 3 (dsg3). The strong HLA-class II-association provides the rationale for using HLA-class II-transgenic mice to investigate the autoimmune response to human dsg3-protein *in vivo*. We recently demonstrated that immunizing HLA-DRB1*0402-DQ8-transgenic mice with the entire dsg3 ectodomain induces a dsg3-specific CD4+ T cell and B cell response. To explicitly characterize early activation steps of dsg3-reactive CD4+ T cells *in vivo*, we immunized HLA-DRB1*0402-DQ8-transgenic mice with a set of five immunodominant dsg3-epitopes that had previously been identified to be recognized by autoreactive CD4+ T cell clones in PV patients. Sera of dsg3-epitope-immunized mice contain IgG antibodies recognizing human dsg3-protein as detected by dsg3-ELISA and indirect immunofluorescence. To demonstrate the pathogenic relevance of these dsg3-reactive antibodies, we incubated human skin biopsies with serum samples of dsg3-epitope-immunized mice and exfoliative toxin A. Histological exam reveals an acantholytic split formation at the suprabasilar level of the epidermis resembling the histological findings in PV. Moreover, direct immunofluorescence of these skin biopsies shows intercellular deposition of IgG autoAb as found in PV. Animals that were immunized with control, i.e. non-immunodominant human dsg3-peptides, do not develop a pathogenic ab response which demonstrates that HLA-shaped dsg3-derived peptides are able to elicit a complex autoAb-driven immune response against dsg3. This study provides direct *in vivo* evidence for the hypothesis that PV is an HLA class II-restricted, CD4+ T cell-driven autoimmune disease. The present mouse model holds great promise to further investigate crucial steps of the autoimmune cascade in PV.

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Evidence for intra- and inter-molecular epitope spreading involving the bullous pemphigoid 180 (BP180) and BP230 antigens in the immune pathogenesis of BP

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BP is the most common autoimmune bullous skin disease associated with a potentially lethal humoral immune response against the skin basement membrane components, BP180 and BP230. Retrospective analyses demonstrated that these major autoantigens of BP harbor several epitopes which are targeted by both autoaggressive B- and T-cells. The aim of the present prospective multicenter study was to follow the evolution of the IgG autoantibody response against BP180 and BP230 during the clinical course of BP. Several ELISA utilizing BP180 and BP230 recombinants were used to investigate the dynamics of the humoral response against BP180 and BP230 in 37 BP patients over a 24 months follow up period. Overall, epitope spreading phenomena were detected in 14 of 37 (38%) BP patients during the course of disease. Specifically, 12 and 2 of 37 BP patients showed intra- and intermolecular epitope spreading, respectively. Sera from 7 and 4 of 12 BP patients recognized at diagnosis the BP180 immunodominant domain (NC16A) and subsequently also bound to epitopes in the extracellular and intracellular domain, respectively. Six epitope spreading events were observed within 1 month from diagnosis and 9 events within 6 months. Of note, the recognition of intracellular antigenic sites of BP180 and BP230 was regularly preceded by IgG reactivity against the BP180 ectodomain. These findings support the concept that IgG recognition of the BP180 ectodomain is an early event in BP followed by variable intra- and intermolecular epitope spreading which presumably shapes the individual course of BP.

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The level of cytotoxic activity in peripheral blood associates with MHC class I genotypic profile in psoriasis

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HLA-Cw*0602 (Cw6) consistently associates to psoriasis yet little is known about its functional role in this disease. Previously, we showed that HLA-C is differentially expressed between psoriasis and normal skin. We also found evidence that HLA-C and HLA-E interact at the genetic level in psoriasis. In the present study we compared the distribution of lymphocyte populations, cytotoxic activity in blood and the levels of soluble HLA in cell culture supernatants and in serum from patients and control groups that were selected so that 20 individuals in each group were HLA-Cw6(+)/HLA-ER and 20 were Cw6(-)/HLA-EG. All subjects were free of infection and of systemic treatment, and the majority of the patients (95%) had a PASI<7 while 5% had PASI between 7 and 12. Whole blood was mixed with target cells lacking expression of surface MHC class I (K562) or cells expressing Cw6 on the surface (LCL-Cw6). The level of cytotoxicity against K562 target cells was significantly lower in HLA-Cw6(+)/HLA-ER psoriasis patients than in the corresponding group of healthy controls (p=0,04381). However, the level of cytotoxicity against LCL-Cw6 or K562 target cells was not different between psoriasis cases and healthy controls when the two groups were compared regardless of genotype profiles. In addition, increased cytotoxicity in the presence of OK432 was comparable between patients and controls. We also found that target cells that display HLA-Cw6 are killed more readily than K562 targets which completely lack MHC class I surface expression. No differences in the distribution of lymphocyte populations were found between the groups studied. Soluble HLA was increased in serum from psoriasis patients. These results support the concept that class I genetic profiles are related to the outcome of at least some immune functions in psoriasis.

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A promoter polymorphism in the heme oxygenase-1 gene is associated with intrinsic atopic dermatitis

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Chronic recurrent cutaneous inflammation is a hallmark of atopic dermatitis (AD). Recently, heme oxygenase-1 (HO-1), has been shown to be crucial for the regulation of inflammatory responses. Thus we speculated that polymorphic variants of the HO-1 gene determine an individual's susceptibility to AD. To investigate whether an association exists between HO-1 polymorphisms and AD phenotypes, 125 AD patients [extrinsic (n=107) and intrinsic (n=18)] and 142 nonatopic healthy volunteers, all of European descent, were screened for the (GT)n dinucleotide length polymorphism classified as short (<25 repeats, S) and long (≥25 repeats, L) repeat alleles by restriction fragment length polymorphism analysis. Genotype distributions of S/S, S/L and L/L were similar in AD patients (16.0% S/S, 47.3 % S/L and 36.6% L/L) and controls (15.5%, 45.1% and 39.4%). The frequency of the S/S genotype was however significantly higher among intrinsic (39%) when compared to extrinsic AD patients (11%) (p=0.008). These data are the first to show an association of a functional genomic polymorphism/the short (<25) GT repeats in the HO-1 gene promoter with the intrinsic, but not the extrinsic form of AD, which support the concept that the pathophysiologic background is different in the two subtypes of the disease.

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Differential distribution of HLA G and CD160 genotyping profiles in psoriasis patients compared with population matched controls

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Class I molecules are central to the immune system not only for their antigen presenting properties but also for other regulatory functions mediated by a diversity of lymphocyte and NK cell receptors. There is evidence that not only HLA-C but several MHC class I molecules may have a role in inflammation. We found that HLA-C and HLA-E interact at the genetic level in our Swedish Psoriasis Cohort and we are currently investigating the functional relevance of such interaction. HLA-G is another class I molecule that seems to be functionally coupled to HLA-C and E, and that is reported to be overexpressed in psoriasis lesions. Together, these molecules appear to have an immunoregulatory role that minimizes self reactivity. For example, soluble class I molecules are known to reduce maternal NK cell reactivity during pregnancy. HLA-G is known to interact with several NK cell receptors including NKG2 but also it binds CD160 that it is expressed by endothelial cells. Soluble HLA-G was found to reduce angiogenesis through CD160 via apoptotic pathways. We investigated the relationship between a 14 bp ins/del polymorphism in HLA-G and SNP rs1023945 in CD160 and found that patients that are heterozygote for the ins/del are about 2 times more likely to carry the CC genotype at CD160 than other genotype combinations (p=0.003). The functional relevance of this genetic profile on the vascular architecture of psoriasis lesions is under investigation.

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***In vitro* clustering of IgG and desmogleins in a human tissue model of pemphigus**

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Pemphigus vulgaris (PV) and pemphigus foliaceus (PF) are autoimmune diseases in which there is loss of cohesion between keratinocytes (acantholysis) with blistering of skin and mucous membranes. PV is characterized by deep epidermal blistering and autoantibodies to desmoglein (Dsg) 3. PF exhibits superficial blistering of only skin and autoantibodies to Dsg1. In skin of both PV and PF the deposited IgG demonstrates a clustered appearance that is not compatible with the normal distribution of the desmogleins. These IgG clusters also contain desmoglein and plakoglobin and sometimes, depending on the state of pathogenesis, other desmosomal components. As it is impossible to follow the actual pathogenic process *in vivo*, we developed a tissue model in which we could monitor the IgG-induced cellular changes in a time-controlled fashion. Biopsies of normal human skin were incubated in a defined medium to which Dsg-ELISA characterized amounts of purified patient IgG were added. At chosen time intervals biopsies were processed for immunofluorescence. The added IgG bound to keratinocytes in a pattern where, in addition to the normal distribution of the desmogleins, also condensation to clusters was observed. When anti-Dsg3 oral PV patient IgG was added, these clusters were most prominent in the more basal cells. Similar to *in vivo* PV skin these clusters of IgG co-localized with Dsg3 and plakoglobin (PG). Adding anti-Dsg1 PF patient IgG in this *in vitro* model also resulted in partly clustering of IgG, but not mid-epidermal as in the *in vivo* situation in PF skin, but most prominent in the basal cell layer. Staining for desmosomal components demonstrated that besides IgG also PG and the PF antigen Dsg1 were present in these clusters. When IgG from a mucutaneous PV patient, that had a combined anti-Dsg1 and anti-Dsg3 profile, was added, a combination of the above patterns was observed. These results demonstrate the feasibility of the employed model to perform time-controlled experiments to study the pathogenic events that lead to acantholysis and blistering.

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Impact of dragon's blood on an *in vitro* model of cutaneous neurogenic inflammation

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Dragon's blood (DB) or Sangre de drago is a viscous bright red resin collected from trees of species Croton lechleri growing in the Peruvian jungle. This sap is used extensively in the pharmacopeia of Peruvian natives to treat cutaneous disorder. His efficiency as inhibitor of neurogenic inflammation has been recently demonstrated. In order to understand the underlying mechanism of these effects, we examined whether or not DB is able to reduce the substance P (SP) release in an *vitro* model of cutaneous neurogenic inflammation (CNI). This model is based on an enzyme immunoassay (EIA) of SP (marker of CNI) in a porcine co-culture (very close to human response) of dorsal root ganglion neurons (DRGN) and keratinocytes (K). After one week of co-culture, we can measure a basal level of SP release in the medium. This level can be highly increased in a dose-dependent manner by the stimulation of compounds knows to induce the neurogenic inflammation like capsaicin. This induced SP-release can be amplified by pre-treatment with prostaglandin E2 or decreased by the pre-treatment with Ruthenium Red. So, we are able to assay the molecules capacity for modify the basal SP release and modulate an artificial CNI induced by addition of capsaicin. After incubation with different concentrations of DB (n=3) we noted an significant (p<0,01) dose-dependent decrease of SP basal release on average of 32% at 1% (v/v) and 26% at 0,1% (v/v). On the other hand the pre-treatment (72h or 1h) of the co-culture by DB was sufficient to induce a 111% (72h) or 65% (1h) inhibition at 1% (v/v) and a 109% (72h) or 30% (1H) inhibition at 0,1% of the CNI induced by capsaicin. We conclude that DB is a potent inhibitor of sensory afferent nerves by a direct inhibition on the neuropeptides release and support its ethnomedical use for neurogenic inflammation.

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Potential anti-inflammatory properties of a new undecyl-rhamnoside on human keratinocytes

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Inflammatory skin disorders, including acne, psoriasis or the skin of patients with atopic dermatitis are characterized by the expression of central mediators of inflammation such as prostaglandin E2 (PGE2) or cytokines such as IL-8. The nuclear factor kappa B (NFKB) has been shown in skin and other organs to regulate important cellular functions, including cell proliferation and differentiation, as well as inflammatory responses. NFKB is a pivotal transcription factor triggering a cascade of proinflammatory NFkB target genes such as cytokines or prostaglandins. Thus, an investigation was carried out to evaluate whether a new rhamnoside derivative namely the undecyl-rhamnoside was capable of reducing the inflammatory responses in human keratinocytes. The NFKB activation was assessed after TNF-alpha stimulation on human keratinocytes cell line (HaCaT) transfected with a luciferase reporter gene ligated to a NFKB promoter. The NFKB activation and translocation was assessed through the synthesis of luciferase. Productions of PGE2 an IL-8 were performed in HaCaT cell line after various inflammatory stimuli. PGE2 release was mediated by Phorbol 12-myristate 13-acetate (PMA) and IL-8 by a lipopolysaccharide (LPS) stimulation. Our results showed that the undecyl rhamnoside is capable of inhibiting, in a dose-dependent manner, the transcription factor NFKB in TNFalpha-stimulated keratinocytes. Furthermore the overproduction of IL-8 induced by LPS was reduced by pre-treating the keratinocytes with the undecyl-rhamnoside. PMA-mediated PGE2 release was also inhibited by the undecyl-rhamnoside with 30% inhibition. NFKB is a transcription factor which plays a key role in the expression of many genes involved in the inflammatory responses. Therefore, we propose that the undecyl-rhamnoside might contribute to significantly inhibit the pro-inflammatory mediators found in inflammatory skin diseases.

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Altered Hoxb13 expression differentially influences the inflammatory response following cutaneous injury

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Hox genes are best known for their roles in embryonic patterning. Hox genes are also expressed in adult tissues, but their roles in adult skin remain obscure. We have previously shown that loss of Hoxb13 from adult skin results in higher levels of epidermal and dermal hyaluronan, and cutaneous wound healing proceeding with dramatically reduced scar. Because several lines of evidence suggest that a robust inflammatory response following injury to skin (and to other tissues as well) may contribute significantly to scarring, and because hyaluronan is also thought to influence inflammation, we have initiated a study to determine how altered Hoxb13 expression may be affecting the inflammatory profile in skin following injury. We are currently utilizing two mouse models for this purpose: 1) Hoxb13 knockout mice and 2) Hoxb13 transgenic mice that specifically overexpress Hoxb13 in the basal layer of the epidermis via the keratin 14 promoter. Our results thus far indicate that Hoxb13 knockout skin wounds (24 h, full-thickness) express higher levels of anti-inflammatory cytokines (i.e. IL-4, IL-13, sTNFR1) relative to wild-type wounds, and negatively influence molecules thought to be important in neutrophil recruitment/extravasation. In contrast, Hoxb13 transgenic mice subjected to epidermal barrier injury (acetone) exhibit a dramatic increase in dermal cellular infiltrate relative to control mice, suggestive of significant levels of dermal inflammation. Together, these data suggest that loss of Hoxb13 dampens the inflammatory response, while forced expression of Hoxb13 promotes inflammation. Hoxb13 may therefore represent a therapeutic target in cases of protracted inflammation and/or non-healing wounds

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HLA-E*0103 is associated with susceptibility to pemphigus vulgaris

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Non-classical human leukocyte antigen – E (HLA-E) has previously been shown to mediate NK and CD8 cell activity, possibly indicating a role in the regulation of autoimmunity. Thus far, eight alleles have been reported at the HLA-E locus. HLA-E*0101 has been associated with a reduced risk of Behcet's disease and the 0103 allele has been found to cluster in type 1 diabetes patients diagnosed after age 10. While HLA-DR and HLA-DQ associations are well established in Pemphigus vulgaris, the HLA-E locus has not been evaluated. In this study, we investigate HLA-E allele status in Caucasian and Ashkenazi Jewish PV patients. Participant allele status was determined in 51 PV patients and 52 healthy controls by restriction fragment length polymorphism-polymerase chain reaction and amplification refractory mutation system. Associations were determined via Cochran Mantel Hanzel Chi square and logistical regression analysis. HLA-E outcomes included presumed homozygous 0101 (0101/x) or 0103 (0103/x) allele status, or 0101/0103 heterozygous status. PV did not significantly associate with either 0101/x or 0101/0103 allele status in Caucasian or Ashkenazi Jewish populations. Stratification of subjects by age of onset, ethnicity, and six additional clinical parameters did not alter this result. However, we did find that patients significantly presented as 0103/x (presumed homozygotes) when compared to control subjects (p=0.0146, OR=3.730, 95% CI=1.241–11.213). Furthermore, patients reporting mucocutaneous lesion type history (p=0.0254), age of onset in the fourth (p=0.0500), fifth (p=0.0580), and sixth decade (p=0.0278), and Caucasian ethnicity (p=0.1046) all trended towards 0103/x. The role of the HLA-E*0103 allele in altering risk of developing PV requires further investigation.

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Pemphigus IgG and pathogenic monoclonal anti-desmoglein 3 antibody induced cleavage of extracellular domain of membrane-pooled desmoglein 3 during endocytosis

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Pemphigus vulgaris (PV) is an autoimmune blistering disease, characterized by the loss of cell-cell adhesion and autoantibody against desmoglein 3 (Dsg3). Previously, using anti-Dsg3 polyclonal or monoclonal antibodies (Abs), we were able to induce endocytosis of Dsg3 from the non-desmosomal pool, leading to the formation of Dsg3-depleted desmosomes. To investigate the Dsg3-depletion mechanism, we examined the degradation process of Dsg3 by immuoprecipitation method. Human squamous cell carcinoma cell line, DJM-1 cells were treated with PV-IgG, pathogenic (AK23) and nonpathogenic (AK18, AK20) anti-Dsg3 mAbs, and were extracted in 1% Brij buffer, were subjected to immunoprecipitation using anti-Dsg3 c-terminal Ab (AHP319). The resulting immune complexes were subjected to immunoblotting using various anti-Dsg3 Abs, which has different epitope of extracellar (EC) domain of Dsg3, AHP319 and anti-p120ctn Ab. Smaller (110kDa) Dsg3 was detected in addition to full length (130kDa) Dsg3 after 10 min treatment with PV-IgG or AK23 mAb, but not with nonpathogenic AK18 or AK20. Results of immunoblotting with various anti-Dsg3 EC domain Abs showed that Dsg3 was cleaved in EC2/EC3 domain. Cleavage of 130 kDa-Dsg3 and generation of 110kDa occurred after 1 min treatment with AK23 or PV-IgG, and this cleavage was partially inhibited with PP1 (Src inhibitor) and genistein (PTK inhibitor). AK15 mAb, which recognizes a domain inner side than EC3 of the extracellular portion of Dsg3, immunoprecipitated exclusively cleaved Dsg3. AK15 mAb immunoprecipitates did not contain of p120ctn, indicating only intact 130kDa Dsg3 forms complex with p120ctn, but degrading Dsg3 does not. These results suggest that pathogenic anti-Dsg3 Abs induced cleavage of Dsg3, which may be mediated by Src, during endocytosis. p120ctn may play a role as a possible stabilizer of pooled Dsg3 at membrane, and in triggering endocytosis and/or cleavage of Dsg3 in pemphigus.

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Intravenous immunoglobulin (IVIG) is effective in experimental epidermolysis bullosa acquisita

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Type VII collagen, the main component of the anchoring fibrils, is the autoantigen of epidermolysis bullosa acquisita (EBA), a subepidermal autoimmune blistering disease. We have recently shown that either passively transferred antibodies against murine type VII collagen (mCol7), or active immunization of mice with peptides of the immunodominant NC1-domain, cause a subepidermal blistering skin disease reproducing the findings in the skin of EBA patients. Currently, response of EBA patients to treatment is often unsatisfactory and mainly relies on immunosuppressive agents. Due to the low incidence of the disease, controlled clinical trials neither have been performed, nor are underway. However, use of our EBA animal models may allow to evaluate different therapeutic options with regard to their effectiveness and side effects *in vivo*. Therefore, in the active disease model for EBA, SJL mice were immunized with mCol7. Subsequently, animals were weekly monitored for skin lesions. If more than 2% of body-surface area were affected by blisters/erosions, mice were randomly assigned to one of the 4 following treatment protocols for a period of 6 weeks: 1) methylprednisolone (daily 20mg/kg i.p.), 2) IVIG 2g/kg (every second day) or 3) weekly, or 4) left untreated. Interestingly, methylprednisolone and IVIG every second day, but not IVIG once weekly, significantly inhibited further disease progression. These findings were confirmed in the passive transfer model of EBA where mice where BALB/c mice were injected with IgG from rabbits immunized with mCol7 and then treated accordingly. Concisely, our data demonstrate that IVIG is an effective treatment for experimental EBA. Further studies using these animal models will aim at dissecting the mechanisms that mediate the effect of IVIG. Eventually, this may lead to a more targeted therapy in EBA and other autoantibody-mediated diseases in general.

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Multiplexed array analysis supports disease phase related cytokine profiles in pemphigus vulgaris

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Pemphigus vulgaris (PV) is a relatively rare, but potentially life threatening autoimmune condition in which cytokine balance may play a pivotal role in disease pathogenesis. Cytokines involved in regulation of the self-directed immune response in PV have not been fully characterized. The aim of our study was to evaluate the level of circulating cytokines in sera of patients across phases of disease (active, newly remittent and long-term remittent) compared with healthy controls. We performed a quantitative multiplexed array analysis to determine levels of 15 different cytokines in patient and control sera. Several plasma cytokines involved in the Th2 pathway (IL-4, IL-5), TH17 cell differentiation pathway (IL-17A, IL-6), and Treg pathway (IL-10, TGFβ) are differentially expressed in different phases of disease and in patients vs. controls. The Th2 cytokines IL-4 and IL-5 are significantly elevated in active vs newly remittent patients (p=0.012) and active patients vs controls (p=0.46), respectively. Similarly, IL-17A, a pro-inflammatory cytokine known to be secreted by TH17 effector cells, is significantly increased in active patients vs. remittent patients (p=0.027). Conversely, IL-10, an immunosuppressive cytokine, was found to be highly elevated in sera from long-term remittent patients compared to active patients and controls. Our global analysis of multiple cytokines suggests a possible interplay between the pro-inflammatory profile (TH17 pathway) observed in the active phase of disease and the anti-inflammatory response (Treg pathway) revealed in the remittent phase. These data suggest novel biomarkers of disease activity in PV and potential targets for patient-tailored cytokine therapy.

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Development of an ELISA to detect BP180-specific IgE autoantibodies

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Bullous pemphigoid (BP) is an autoimmune disease characterized by a humoral autoimmune reaction directed predominantly against the NC16A domain of BP180, a hemidesmosomal protein. Historically, IgG class autoantibodies have been the focus of studies investigating BP pathogenesis; however, IgE class autoantibodies specific for BP180 are present in approximately 90% of patients and, recently, have been shown to have a pathogenic role in the disorder. The level of IgE in serum is 1/10,000 that of IgG and only a fraction of this is BP180 specific; therefore its detection can be difficult. In this study, recombinant NC16A-GST, a GST control and ε chain specific anti-human IgE, were utilized to develop an IgE specific ELISA. Sera assayed for reactivity to the recombinant protein included: 35 BP sera collected from patients who met the clinical and histologic criteria for pemphigoid and confirmatory direct immunofluorescence; 33 age and gender-matched sera from patients without any known autoimmune disease; 42 other autoimmune sera from patients with lupus (21), HG (6) and EBA (6). Using a TG-ROC analysis, the assay had a specificity of 1.0 and a sensitivity of 0.7. Sixty-two percent of the BP sera, none of the matched sera, lupus or EBA sera had detectable levels of BP180-specific IgE. Interestingly, 4 out of 6 (66%) of the HG patients were positive for IgE autoantibodies. By comparison, 77% of the BP, none of the age-matched, or lupus, and 66% of the HG sera had detectable levels of BP180 specific-IgG using a commercially available ELISA. This ELISA provides a sensitive and highly specific tool for the investigation of BP180-specific IgE in the immunopathogenesis of cutaneous autoimmune diseases.

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Inhibition of the STAT1-ICSBP-IL-12p35/p40 signaling by glutathione depletion induces TH2 immune responses *in vitro* and *in vivo*

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Innate signals that activate dendritic cells (DC) through Toll-like receptors (TLR) modifiy DC maturation and differentiation. Lipopolysaccharid (LPS) activates DC through TLR4 and induces a "DC1" phenotype, characterized by the production of high amounts of interleukin (IL-)12p35/p40. Such DC1 direct antigen-specific T helper (TH) cells into an interferon (IFN-)γ-producing TH1 phenotype. Here we show that the generation of reactive oxygen species (ROS) by glutathione depletion selectively inhibits TLR4-mediated activation of signal transducer and activator of transcription 1 (STAT1) and, down-stream, IFN consensus sequence-binding protein (ICSBP), a transcription factor for IL-12p35/p40. Using STAT1 knockout mice we show that STAT1 activation is required for competent IL-12 production by DC activated through TLR4. Interestingly, high ROS levels also abrogate STAT1 phosphorylation in DC in response to TLR4 signals, strongly reduce ICSBP induction and subsequently prevent IL-12p35/p40 production. Instead IL-10 production is enhanced. Importantly, DC generated under high ROS levels fail to induce IFN-γ-producing TH1 cells, but induce IL-4-producing TH2 cells. Also *in vivo*, glutathione depletion prevents innate induction of IL-12p35/p40-expressing DC and subsequent priming of T-bet-expressing TH1 cells. Instead, high ROS levels promote GATA3-expressing TH2 cell development that protects against TH1 cell driven autoimmune encephalomyelitis. Modulation of the STAT1-ICSBP-IL-12p35/p40 pathway through intracellular glutathione/ROS levels thus determines the outcome of TLR-signaling and ultimately determines the differentiation of TH immune responses towards either a TH1 or a TH2 phenotype.

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Abrogation of IL-23 and TH17-mediated autoimmunity by reactive oxygen species

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T helper (TH) 17 cells produce the pro-inflammatory cytokines interleukin (IL-)17 and IL-21 and are critically implicated in the pathogenesis of organ-specific, inflammatory autoimmune diseases such as psoriasis or multiple sclerosis. Lineage differentiation of TH 17 cells depends on activation by dendritic cells (DC) in the presence of Interleukin (IL-)6, TGF-β and IL-23. Toll-like receptor (TLR)4 or 9-activated DC are the primary source of high amounts of IL-23 that serve as survival and proliferation cytokine of TH17 cells. As various infections strongly induce oxidative stress in DC, we investigated now if oxidative stress affects the capacity of DC to produce IL-6 and IL-23 and subsequently to promote TH17 differentiation. *In vitro*, induction of oxidative stress in DC by either H2O2 or depletion of reactive oxygen scavengers (ROS) strongly modulates their cytokine expression in response to TLR stimulation. The production of IL-6 and IL-23 by DC was abrogated when DC were activated at high ROS level. These DC lost their capacity to induce TH17 cells *in vitro*. All phenomena were reversed by ROS scavengers. Similarly, myelin antigen (PLP)-specific TH17 cells generated under low ROS conditions induce severe encephalomyelitis after transfer in naïve mice. In contrast, PLP-specific TH cells generated by DC at high ROS levels lost their capacity to induce encephalomyelitis. Similarly, we found that *in vivo* immunization of mice with PLP in CFA and pertussis toxin at low ROS level concentrations induced severe encephalomyelitis. In contrast, mice immunized under conditions of high ROS-levels developed either no or only mild disease. This was associated with strong reduction of IL-6 and IL-23 expression and of TH17 development *in vivo*. Thus, high ROS levels inhibit TLR-stimulated production of IL-6 and IL-23 by DC *in vitro* and *in vivo* and abrogate TH17 development; in consequence depletion of ROS scavengers should be useful as therapy for inflammatory autoimmune diseases, such as psoriasis.

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The role of LILRA2 in neutrophil recruitment using erythema nodosum leprosum as a model for type III hypersensitivity reactions

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Expression of the leukocyte immunoglobulin-like receptor A2 (LILRA2) correlates with ineffective host immunity in human leprosy skin lesions, with over 90% of histiocytes expressing LILRA2 protein in the progressive lepromatous form (L-lep) vs <5% in the self-healing tuberculoid form. Skin lesions from patients with erythema nodosum leprosum (ENL), a model of immune complex-mediated hypersensitivity, showed similar levels of LILRA2 expression to L-lep lesions by gene expression profiling studies. Consistent with previous studies of L-lep skin biopsy specimens, double-labeling immunofluorescence shows that cells in ENL lesions expressing LILRA2 also have a macrophage-like phenotype, co-expressing CD68 and but not CD1a. As up to 50% of patients with lepromatous leprosy develop ENL, we hypothesized that LILRA2, expressed in both L-lep and ENL, may play a role in immune-complex mediated tissue injury. Treatment of peripheral blood monocytes with anti-LILRA2 induced CD40 and CD14, did not change CD209 or CD1b, and diminished CD206 and MHC class II expression. Furthermore, anti-LILRA2 treatment resulted in an increase in the neutrophil chemokines, ENA-78, IL-8, and GROα; while mononuclear cell chemokines such as IL-16 and MCP-4 were less or no different from media and isotype control-treated cells. Lastly, LILRA2 activation of monocytes induced TNFα, IL1β, and IL-23, cytokines associated with a TH17-promoting environment. As TH17 cells are implicated in neutrophil recruitment and anti-LILRA2 activation stimulates neutrophil chemokines, these findings provide a potential mechanism for the role of LILRA2 in the recruitment of neutrophils in immune complex-mediated tissue injury.

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JunB in the skin : a key regulator in autoimmunity

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We have previously shown that mice with specific epidermal deletion of the transcription factor JunB (JunB Δep) develop a UV-accelerable autoimmune disease with systemic alterations starting at the age of 3 months. The skin of these mice shows epidermal hyperplasia and subepidermal IgG-band-like deposits, accompanied by epidermal overexpression of IL-6. IL-6 was also elevated in the serum, whereas other important Th1/Th2 cytokines were unaltered. Furthermore JunB Δep mice spontaneously produce antinuclear antibodies (ANA) and anti-histone (H1, H2A, H2B, H3 and H4) antibodies. The systemic autoimmune disease is characterized by enlarged spleen and lymph nodes as well as autoimmune vasculitis in several organs. Mice exhibit a glomerulonephritis with massive subendothelial immune complex deposits containing IgG, IgM and C3, mesangial cell proliferation, and proteinuria. Therefore we postulated that the epidermis acts as endocrine-like organ, where epidermal loss of JunB leads to a complex systemic disease via upregulation of IL-6. We now aimed to disclose the mechanisms, by which the lack of JunB leads to this systemic autoimmune disease. First of all, we found by FACS analysis in lymph nodes elevated levels of CD138+ plasma cells and reduced levels of CD8+ T-lymphocytes. The importance of lymphocytes in JunB Δep mice was further substantiated by crossing with T- and B-lymphocyte-deficient (Rag2-/-) mice - thereby the phenotype was rescued. We then demonstrated by immunoprecipitation studies that JunB binds to the promoter region of IL-6, indicating that IL-6 transcription may be directly regulated by JunB. These findings are significant for human autoimmune disease because in biopsy samples from lupus patients we found reduced JunB protein expression in the skin and increased IL-6 receptor expression in lymph nodes. The JunB Δep mouse model may offer the opportunity to learn more about the pathogenic factors of autoimmune diseases and to develop therapeutic strategies interfering with JunB pathways.

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Development of a novel *in vivo* model to assess skin barrier function

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The following observations in literature are consistent with the view that epicutaneous exposure to allergens play a role in atopic dermatitis (AD): Approximately 80% of patients with AD have elevated levels of IgE and evidence of specific IgE antibodies against a variety of food and environmental allergens has been established. In addition, studies have shown that avoidance of these allergens results in clinical improvement. Finally, allergen specific T-cells have been found in skin lesions isolated from patients with AD. The epicutaneous allergen sensitization model in mice has long been used as a model to assess the ability of compounds to act as contact sensitizers. A typical protocol would involve adult female BALB/c mice that are sensitized by painting 25 µl sensitizing solution (acetone:olive oil 4:1) containing the allergen on the shaved abdomen and 5 µl on each footpad. At day 0 and day 6, ear thickness is measured to establish the baseline. Subsequently, on day 6, 10 µl of a challenge solution (olive oil), containing the same allergen is applied to each side of both ears and ear thickness is measured for 2-3 subsequent days to assess swelling as a measure for inflammation. This poster proposes a modified mouse ear swelling model that can be used as a tool to assess the ability of skin barrier products to protect the skin from environmental contact allergens. The protocol was modified by optimizing the challenging solution in terms of its hydrophilicity and compatibility with skin barrier products tested. In addition, the time frame of pretreatment was assessed by applying the skin barrier product(s) at various time intervals prior to applying the challenging solution to the ears. This model evaluates both the level and duration of protection of the skin from assault by allergens, which makes it particularly useful in the development of novel products aimed to prevent flare-ups in atopic dermatitis patients.

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Dietary gluten challenge increases neutrophil CD11b expression and serum IL-8 levels in patients with dermatitis herpetiformis (DH)

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The association of a gluten sensitive enteropathy (GSE) and DH is well established however the mechanism through which the GSE mediates the classic neutrophilic eruption is not clear. We have previously demonstrated that patients with DH have an increased expression of neutrophil CD11b expression and elevated serum levels of IL-8. The relationship of dietary gluten ingestion and these pro-inflammatory markers however is not known. 3 patients with DH on gluten containing diets and dapsone and 4 normal subjects were fed 20 grams of raw wheat gluten daily for 2 weeks, while maintaining their normal diet (DH patients remained on dapsone). Neutrophil CD11b expression, serum IL-8 levels and serum IgA anti-tissue transglutaminase 2(TTG2) antibodies were studied before gluten challenge, after 1 and 2 weeks of gluten challenge and 1 week after stopping gluten. Cutaneous and gastrointestinal symptoms were recorded. None of the normal subjects reported significant clinical symptoms with gluten challenge. One DH patient reported mild gastrointestinal complaints and none reported a flare of their skin disease. Pre-gluten challenge neutrophil CD11b expression was elevated in patients with DH (Mean CD11b MFSC/ABC: DH = 64,656; Nl = 33,853). After 1 week of gluten challenge neutrophil CD11b expression increased 51% in DH patients but did not increase in normal subjects. After 2 weeks, neutrophil CD11b expression was at baseline in both groups. Serum IL-8 levels increased in DH patients after 1 week (DH mean IL-8 level: Week 0 = 3.49 pg/ml; Week 1 = 6.65 pg/ml) but showed no change in normal subjects. Serum IL-8 levels remained elevated in 2 of 3 DH patients and at or below baseline in the normal subjects. IgA anti-TTG2 antibodies were elevated at baseline in 2 of 3 DH patients and no normal subjects. No change in IgA anti –TTG2 was seen in either group. These findings suggest that gluten challenge can increase the pro-inflammatory profile of patients with DH and this may play a role in the development of DH skin lesions.

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Phenotype and function of CD4+CD25+ regulatory T cells in patients with systemic sclerosis

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Systemic sclerosis (SSc) is a rare multi-system autoimmune disease showing a large variability in the extent of skin and visceral organs involvement. Several groups have demonstrated that CD4+CD25+ regulatory T cells (Treg) play a critical role in suppressing responses of the immune system to self-antigens. Therefore, we were interested to analyze the phenotype and function of CD4+CD25+ Treg in the skin and peripheral blood of patients (n= 15) with the limited cutaneous form and the diffuse cutaneous form of SSc. The number of Treg in skin specimens was investigated using anti-Foxp3 and anti-CD4 monoclonal antibodies for immunohistochemistry. The quantitative analysis of CD4+ T cells in skin lesions of patients with SSc revealed a significantly lower number when compared to other chronic inflammatory skin diseases, such as lupus erythematosus, psoriasis, and atopic eczema. Moreover, the frequency of Treg in the skin expressed as percentages of Foxp3+ cells from CD4+ cells in the inflammatory infiltrate of SSc was similar to lupus erythematosus but significantly reduced compared to other control diseases. Furthermore, characterization of peripheral blood CD4+CD25+ Treg from patients with SSc was carried out by flow cytometry analyzing the expression of Foxp3 and Treg subpopulations. In peripheral blood, our preliminary data show that the number of CD4+CD25highFoxp3+ Treg was normal compared to healthy donors. In addition, CD4+CD25high Treg from patients with SSc were tested for their ability to suppress T-cell proliferation; however, no significant differences were detected in SSc patients compared to healthy donors analyzed in the same assay. In conclusion, our results demonstrate a significant reduction of Treg in the skin of patients with SSc rather than a global peripheral dysfunction. Further investigations will evaluate if this organ-specific abnormality of Treg is limited to the skin of this autoimmune disease.

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Impaired skin homing of regulatory T cells in vitiligo

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Cytotoxic T cells reactive with melanocyte differentiation antigens eliminate melanocytes from vitiligo skin, whereas in healthy individuals, autoimmunity is held in check by regulatory T cells. Treg abundance measured in skin samples by FoxP3/CD3 immuno-double staining revealed a very significantly reduced number of Treg in non-lesional, perilesional and lesional vitiligo skin (n=7 each) compared with control skin (n=5), whereas the proportion of regulatory T cells in lentigo maligna tissue (n=4) was similar to control skin. In the peripheral compartment of vitiligo patients however, the abundance of CD4+FoxP3+ or CD4+CD25high cells was not reduced as measured by FACS analysis, and Treg sorted among control and vitiligo PBMC were equally capable of inhibiting proliferation of autologous CD25- cells (n=3 each). These data suggested a skin homing defect among circulating Tregs in vitiligo patients. Expression of CLA and CCR8 has been implicated in skin homing of Treg. Among these, expression of CCR8 by circulating Treg was significantly reduced in vitiligo patients compared to controls as measured by FACS analysis (n=3 each). Immunostaining of control and vitiligo skin samples with antibodies to CCL-1, the CCR8 ligand considered responsible for chemoattraction of Treg to the skin, did not reveal differences in expression among control and vitiligo skin samples. Thus we compared the ability of Treg among patients and control PBMC (n=3 each) to migrate through 8 μm pores in response to CCL-1, in transwell migration assays. The data indicate that the proportion of migrating Treg among CD3+ T cells is increased in response to CCL-1 in all control samples, whereas the proportion of Treg is reduced in all vitiligo samples. The data support the concept that functionally active Treg found in the circulation of vitiligo patients do not effectively home to the skin, allowing for the unbridled expansion of cytotoxic T cell responses and progressive depigmentation observed in active disease.

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Epilysin (matrix metalloproteinase [MMP]-28) plays a protective role during allergic inflammation in a mouse model of atopic dermatitis.

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Epilysin (matrix metalloproteinase [MMP]-28) is a novel MMP expressed constitutively by keratinocytes and other epithelial cell types. Little is known about epilysin's physiologic substrate(s) and *in vivo* function. However, recent evidence in a murine asthma model has suggested a potential role in modulating Th2-type allergic inflammation. We hypothesized that epilysin would demonstrate a suppressive role in a mouse model of atopic dermatitis (AD), thereby establishing itself as a potential therapeutic target. We used a mouse model based on intraperitoneal sensitization with ovalbumin-alum complex followed by epicutaneous sensitization with ovalbumin, a protocol that we have shown to recapitulate human AD. To evaluate temporal expression during inflammation, we followed cutaneous epilysin mRNA expression by RT-PCR over time and correlated this with stage and degree of inflammation by cellular and cytokine phenotyping. We found that epilysin expression was decreased during acute stages of inflammation, as reflected by increased IL-4, IL-5, and TNF-α and decreased IFN-γ, while its expression increased over time as inflammation proceeded. This later increase in expression was consistent with previous findings that epilysin expression is upregulated by TNF-α. Next, we used epilysin-null mice to determine the effects of loss of epilysin on allergic inflammation in our AD model. Epilysin-null mice are otherwise healthy and phenotypically normal. Upon epicutaneous ovalbumin challenge, however, they showed more robust inflammation compared to challenged wild-type mice. Together, these data support our hypothesis that during the acute stage of Th2-type allergic inflammation, initiators of the inflammatory process cause downregulation of epilysin to allow inflammation to proceed, whereas later, the accumulation of proinflammatory cytokines such as TNF-α, turns expression back on in order to counterbalance the inflammatory response.

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Ultraviolet light mediated induction of systemic lupus erythematosus-like disease in NOD mice: A model of environmentally induced autoimmunity.

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The role of environmental precipitants in autoimmunity such as systemic lupus erythematosus (SLE) remains unclear. Here we demonstrate that ultraviolet light B (UVB) with or without topical application of synthetic Toll like receptor 7 ligand (imiquimod) induces SLE like disease in auto-immune prone non-obese diabetic (NOD) mice but not in the related NOR strain. Repeated weekly exposures of mice to UVB alone (≥ 7500 J/m2) or combined with topical imiquimod (250 ug) induced high levels of auto-antibodies against chromatin, dsDNA, sm-RNA or RNP detectable by indirect immunofluoresence using HEP-2000 cells and antibody against the cutaneous antigen desmoglein 3 (Dsg3) by ELISA. This treatment also resulted in glomerulosclerosis with immune complex deposition. The incidence of apoptotic keratinocytes was significantly increased in the skin 24 hours following UV irradiation. IFNα inducible myxovirus protein A (MxA) expression was detectable in the skin of UV treated mice with or without TLR7 agonist. High mobility group box 1 (HMGB1), a chromatin protein, has been implicated in immune complex mediated autoimmune pathogenesis. In our studies increased HMGB1 was expressed extracellularly in the dermis of skin after treatment with UVB and imiquimod but not in normal control mice. Systemic immune activation was detected following combination (UVB and imiquimod) but not single therapy as evidenced by IL-6 and TNF α production in serum. Combined treatment induced up-regulation of TLR-7 in IgM positive peripheral blood B cells. These studies demonstrate UV induction of systemic autoimmunity in an autoimmune prone mouse strain and suggest synergistic roles of UV induced local inflammation and TLR7 engagement.

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Bullous pemphigoid patients have IgE antibodies to the shed ectodomain of BPAg2

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IgE is believed to potentially play a pathogenic role in bullous pemphigoid (BP). Up to 70% of patients with BP have elevated total IgE levels. We have developed a mouse model of BP using IgE hybridomas directed against the shed ectodomain of BPAg2 (LABD97) that recapitulates the histological inflammatory process in BP. We now have evaluated BP patients for presence of IgG and IgE antibodies to LABD97 to demonstrate that this mechanism may be active in humans. We evaluated sera from 18 new BP patients (9 males and 9females, mean age 74,range 34 to 94). All had clinical disease consistent with BP and direct immunofluorescense (IF)demonstrating linear IgG at the basement membrane zone (BMZ). IgG and IgE antibodies to LABD97 were evaluated by immunoblot to LABD97 extracted from human skin. IgG and IgE BMZ titers were measured by indirect IF. Total IgE was measured by ELISA. IgE antibody specificity in BP sera was evaluated on immunoblot after immunoadsorption, which removed the IgG. 18/18 sera had IgG BMZ by indirect IF (range 1:5 to 1:20480). 4/18 sera had IgE BMZ antibody by indirect IF after immunoadsorption of IgG(range (1:20 to 1:40). Thirteen of the 18 patient sera had IgG antibodies specific for the LABD97 antigen while 11 of the 18 displayed IgE activity for this same antigen on immunoblt. Nine patients had both IgG and IgE antibodies specific for the LABD97 while two had only IgE. 7/18 patients had elevated levels of serum IgE (mean = 492 and range of 33 to 2083IU). There was no correlation between total serum IgE levels and the presence of specific IgE antibodies to LABD97, indicating that patients with normal IgE levels may have potentially pathogenic IgE antibody to LABD97. We conclude that 61% of our patients with BP have IgE antibodies directed against LABD97. Our previous demonstration of pathogenicity of IgE mouse antibodies to this antigen together with this result, demonstrating specific IgE in patient sera makes it likely that IgE antibodies to LABD97 are pathogenic in human BP.

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Application of serum albumin lowers tumour interstitial fluid pressure and increases the uptake of macromolecules in a xenograft tumour model

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Elevated tumour interstitial fluid pressure (TIFP) is a characteristic of most solid tumours. Clinically, TIFP is described as a physical parameter that hampers the uptake of anti-tumour drugs into tumour tissue and thus lack of therapeutic effects. In the present study the impact of an i.v. injection of concentrated human serum albumin (HSA) on the TIFP was investigated. Furthermore, intratumoural distribution of macromolecules in control and HSA-treated animals was shown by i.v. injection of fluorescent-coupled dextrans and fluorescent labeled cetuximab. A431 or A549 epithelial carcinoma cells were s.c. implanted into the back of immunodeficient animals. TIFP was measured directly in the central region of solid tumours. In our experimental setup i.v. injections of hyperosmotic HSA solutions led to an increase of colloid osmotic pressure (COP) in the vascular system in series an efflux of tissue water decreased the TIFP significantly. In addition, fluorescent-dextrans or fluorescent labeled cetuximab were i.v. applied at distinct time-points after the HSA injection. Distribution of dextrans or cetuximab in TIFP-lowered vs untreated tumour tissue was investigated using fluorescence microscopy and time-domain optical imaging techniques. These data demonstrate that an increased COP can lower TIFP and can augment the delivery of therapeutics to tumour tissue. Therefore, lowering of TIFP could enhance the efficiency of anti-tumour therapies utilising monclonal antibodies.

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Low FAS/CD95 expression in CTCL correlates with reduced sensitivity to apoptosis that can be restored by FAS up-regulation

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Several clinicopathologic features of CTCL suggest that its pathogenesis may involve the acquisition of resistance to apoptosis; however, no specific molecular mechanism has been elucidated. It has been reported that CTCL cases vary widely in expression of CD95/FAS protein. We performed immunohistologic analysis of CTCL lesional skin specimens and documented that FAS expression is generally low in CTCL. To explore potential mechanisms for this phenomenon, we determined FAS transcript levels, cell surface FAS protein expression and susceptibility to FAS-mediated apoptosis in viable CTCL cells. Three CTCL lines (MyLa, SeAx, HH), freshly isolated leukemic cells from a patient with Sezary syndrome (SS), Jurkat and JFL (a FAS-low variant of Jurkat) were studied. The results confirmed low FAS expression by the leukemic SS cells, HH and JFL relative to normal peripheral blood mononuclear leukocytes and control cell lines. There was a direct correlation among FAS transcript level, FAS protein level and FAS-mediated apoptotic sensitivity in the CTCL samples. When the FAS-deficient cell lines JFL and HH were transfected with a wild-type FAS construct, FAS cell surface protein expression and sensitivity to FAS-mediated apoptosis were restored. In aggregate, these findings provide evidence that, like normal T cells, CTCL cells exhibit a mechanistic connection between transcriptional regulation of FAS and sensitivity to FAS-mediated apoptosis, point to the development of FAS deficiency as one molecular mechanism responsible for acquired resistance to apoptosis in CTCL, and indicate that restoration of FAS expression can enhance CTCL sensitivity to apoptosis via the FAS pathway.

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Targeting a tumor specific laminin domain critical for human carcinogenesis

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Laminin-332 is critical for SCC tumorigenesis, correlating with tumor invasion and patient prognosis, but targeting it for cancer therapy has been unachievable due to its mediation of tissue integrity. Our study thus focused on the precursor region of laminin-332 alpha3 chain termed G45 that is cleaved shortly after secretion. While G45 is undetectable in normal tissues and transiently detectable at leading wound edges, G45 persisted amply in >75% in our survey of 75 cutaneous and 56 extracutaneous SCCs. We postulated G45 promotes carcinogenesis by activating wound healing processes through its receptor syndecan-1 but without a termination event signaled by wound closure. In our study JEB cells devoid of laminin-332 were transduced with mutant laminin-332 lacking G45 (ΔG45), showing defects in syndecan-1 stability, matrix deposition, hemidesmosomal formation and migration that were restored with G45 expression. *In vitro* Ras/lkB transformation of ΔG45 cells markedly attenuated activation of ERK1/2 and PI3K but were reversed by G45 and activated PI3K retroviral transfer. ΔG45 cells moreover showed impaired invasion through matrigel, consistent with zymographic findings of absent collegenases MMP-1, MMP-9 (MMP-2 unaffected), that was corrected by G45 and PI3K transfer. *In vivo* Ras/lkB-transformed ΔG45 cells failed to sustain SCC tumorigenesis over a 4-week period but were restored by retroviral addition of G45 and PI3K. In a parallel manner, G45 inhibitory antibodies were demonstrated to ablate SCC tumorigenesis *in vivo*. Ki67 and TUNEL analysis of extracted SCCs in both *in vivo* experiments supported G45's promotion of proliferation and survival, respectively. Extensive necropsy and T.E.M. of tissues repeatedly treated with G45 antibodies revealed no vesiculation or BMZ abnormalaties, confirming G45 is not required in normal tissue cohesion. G45's striking pro-tumorigenic activities, prevalent accummulation in SCCs and absence in normal tissues collectively make G45 a specific and attractive target for anti-cancer therapy.

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Basal cell carcinoma gene expression profiles resemble hair follicles but with significant differences in specific pathway activation

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Hair follicles (HF) and BCCs can be regarded as ordered and disordered skin appendages respectively and may utilize similar molecular mechanisms of growth. We wanted to examine the similarities and differences in gene expression patterns between BCCs and HF to define common growth mechanisms and gene expression patterns that distinguish an ordered skin appendage from a disordered skin growth. Nodular BCCs (n= 8) and non-follicular skin epithelium (n=8) were obtained. Scalp hair follicle root sheath was micro dissected from between the sebaceous gland duct and the lower one third of the HF (n=7). Microarray analysis was performed using 21K cDNA arrays and selected genes were validated using qPCR and histochemistry staining. Two differentially expressed gene sets were identified by significance analysis of microarray (SAM) in BCC and HF verses skin epithelium respectively. Based on these two lists, we conducted multiple signaling pathway analyses. The results indicated that Notch, hedgehog, and WNT signaling pathways were involved in regulating formation of both HF and BCCs. However, Notch signaling, including tumor suppressor genes Notch 1, Notch 2, Jagged ligands (JAG 1, 2), notch signaling inhibitor NUMB, Lunatic Fringe (LFNG), Deltex proteins (DTX 1, 2) which serve as important signaling components downstream of Notch, and Notch target genes HES1,RBPSUHL, hairless protein and HES7, all showed selective differential activation in BCCs compared to HF. Our data provides compelling evidence that "tumorgenic" growth signaling pathways are commonly expressed in both HF epithelial progenitor regions and BCCs. However, the Notch signaling pathway was highly suppressed in BCCs as compared to HF.These results suggested differential expression of the Notch signaling pathway might play a crucial role in disordered BCC growth. The components of the notch signaling pathway may be potential new targets in the development of new therapeutic approaches to BCCs.

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Combining ER-stress-induced apoptosis with protein disulphide isomerase inhibition as a novel therapeutic strategy for metastatic melanoma

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Malignant melanoma remains largely untreatable due to the notorious resistance of such tumours to apoptosis. Recent research has shown that targeting endoplasmic reticulum (ER) stress-induced apoptosis with fenretinide or velcade may represent a powerful therapeutic approach. However, this response may be counteracted by the defensive homeostatic unfolded protein response resulting in upregulation of protein disulphide isomerase (PDI) family members, key chaperones which assist in the clearance of unfolded proteins and promotion of cell survival. The aim of the current study was to test the hypothesis that the efficacy of ER stress-induced apoptosis in response to fenretinide or velcade would be increased by combination of these agents with inhibitors of PDI. Dose response and fixed dose ratio experiments with bacitracin or novel PDI inhibitors in combination with fenretinide or velcade confirmed PDI inhibition significantly increased both apoptosis ($P<0.001$) and expression of GADD 153 (the key transcription factor regulating the transition from pro-survival to pro-apoptotic signalling during ER stress). Transient transfection of A375 metastatic melanoma cells with wild-type P4HB PDI or an inactive mutant confirmed the target activity of PDI inhibitors. Over expression of wild-type PDI resulted in a 4-fold induction of cellular PDI activity compared to cells transfected with inactive mutant but abrogated the effects of either bacitracin or novel PDI inhibitors on increased apoptosis in response to fenretinide or velcade. In contrast, the effect of PDI inhibitors to increase ER stress-induced apoptosis was not abrogated in A375 cells over-expressing mutant P4HB. These data support the novel concept of combining ER stress-inducing agents with PDI inhibition as a more effective therapeutic strategy for metastatic melanoma.

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Reactivity of LH7.2 and FNC1 antibodies against collagen VII does not predict the development of squamous cell carcinomas in recessive dystrophic epidermolysis bullosa

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Squamous cell carcinomas (SCCs) occur at a rate 50 times greater than age-matched controls in individuals with generalized recessive dystrophic epidermolysis bullosa (RDEB). Ortiz-Urda et al (Science 2005) proposed that negative staining in RDEB patients with a polyclonal antibody targeting the binding site of the NC1 domain of collagen VII with lam 332, (FNC1) is predictive that no SCCs will develop. This study investigates whether negative staining of FNC1 and monoclonal antibody LH 7.2 correlates with the lack of development of SCCs in 12 unrelated individuals with generalised RDEB. Skin biopsies taken from the upper inner arm were tested by immunofluorescence for reactivity with the monoclonal antibody LH7.2 (Novocastra, 1:20) and the polyclonal antibody FNC1 (kindly provided by Peter Marinkovich, Stanford University,1:400). The degree of reactivity was compared with the presence or absence of SCCs in the 12 individuals and was compared to normal human skin controls. Of the 12 generalized RDEB patients tested, 6/12 were negative to both antibodies; half of these patients (3/6) had not developed any SCCs (current ages 5,9,36), while the other half had (current ages 20,28,33). 6 out of the 12 individuals had positive but reduced staining with both antibodies; 5 out of 6 had not developed SCCs (current ages 7,23,25,33,75), while the remaining one had (age 36). Contrary to what had been reported in the above ras-driven model of oncogenesis, negative reactivity of antibodies LH7.2 and FNC1 against collagen VII in individuals with RDEB did not correlate with the absence of SCCs.The COL7A1 mutations that were known in 9/12 of these patients also did not predict the likelihood or age at which SCCs developed.

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Phenol peel as a novel therapeutic approach to premalignant skin tumours

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Actinic keratosis (AK) and Bowen's disease (BD) have been defined as precancerous lesions or the early phase of squamous cell carcinoma (SCC). Chemical peeling has been used to treat these disorders, but no histological and immunohistochemical studies investigating the effect of peeling have been performed the clinical impression. In our clinic, 46 patients (32 AK and 14 BD) were treated with phenol peeling and followed another 1-year between 2001 and 2007. Complete response (CR) was obtained in 39 cases after treatment with 1-8 sessions, whereas 7 cases progressed the diseases (PD). In this study, various parameters for epidermal thickness, proliferation, differentiation, dysplasia and apoptosis of the skin biopsy specimens of the patients were evaluated by light microscopic and immunohistochemical analyses and assessed in correlation with the efficacy of the treatment, the criteria of selecting patients, and especially the risk for transformation into SCC. As the result, the difference of clinical improvement with the peeling and the treatment sessions was statistically correlated with tumor thickness, cyclin A and abnormal β-catenin expression, but not with horney cell layer thickness, TUNEL positiveness, PCNA, p53, and α-catenin expression. The cases of PD showed relatively increased p53 expression and TUNEL positiveness after treatment. Although the cases with the thicker tumor thickness, those with the higher expression of cyclin A, and those with more abnormal β-catenin expression would require the more attention for transformation into SCC, the cases with thin tumor thickness, low expression of cyclin A and little abnormal β-catenin expression would be good candidates for this therapy.

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The etiopathologic roles of CXCR3/ligands in the tumorigenesis of basal cell carcinomas

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Basal cell carcinoma (BCC), the most common malignancy found in the Caucasian population, is due in part to genetic mutations of the Sonic Hedgehog (Shh) pathway. Recently, it has been suggested that expression of CXCR3/ligands are associated with the progression of advanced-stage tumors, such as malignant melanoma and ovarian carcinoma. Our previous research of immunoregulatory genes in BCCs indicated that expression of mRNA for the chemokines CXCL11, 10, 9 and their receptor CXCR3 were significantly increased as compared to non-lesional skin epithelium. In this study, we examined the biochemical expression of CXCL11, 10, 9 and CXCR3 by immumohistochemistry (IHC) in normal skin and BCC tissues. Subsequently, we conducted functional assays of the chemokines by HaCaT immortalized human keratinocyte cell cultures, IFN-γ treatment, which is a promoter for the expression of the 3 CXC chemokines, and CXCL11 peptide supplementation. The IHC results demonstrated that CXCR3 and its ligands were localized in the keratinocytes of the tumor masses in BCC biopsies. The addition of IFN-γ to HaCaT cell cultures did not result in significant cell proliferation. However, the expression of the Shh pathway downstream targets STAT1, NF-κB1, Gli1, and Gli2 were upregulated in the cultured cells. The addition of CXCL11 peptide to HaCaT cell cultures led to significant increased proliferation associated with significant upregulation of mRNA for STAT1 and NF-κB1 in the cells as analyzed by quantitative PCR. In conclusion, the chemokines CXCL11, 10 and 9 may be significantly involved in the regulation of BCC keratinocyte growth. Such outcomes may ultimately provide an insight into a novel strategy for BCC regression by suppressing the CXCR3/ligand pathway.

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Molecular analysis of the genetic aberrations in dermatofibrosarcoma protuberans

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Dermatofibrosarcoma protuberans (DFSP) is a rare tumour of borderline malignancy; it is locally aggressive, frequently recurs but rarely metastasises. Clinical and histological diagnosis can be problematic as it may be difficult to differentiate DFSP from some common benign lesions. Clinical diagnosis is aided by histology and immunohistochemical analysis with CD34 and Factor XIIIa antibodies. However there may be an overlap in expression between DFSP and some benign lesions. It is therefore important to employ more accurate diagnostic methods to ensure appropriate early management. Parraffin embedded tissues were used for immunohistochemical staining with CD34 and FactorXIIIa antibodies. RT-PCR was used to analyse the presence of a fusion transcript between the platelete derived growth factor beta gene (PDGFB) and the collagen type 1A1 gene (COL1A1). Both frozen and parraffin embedded tissues were used for RNA extraction. The location of the breakpoints was confirmed at a DNA level by direct sequence analysis. All cases showed a variable degree of positivity to CD34, however, 2 cases were positive to FXIIIa immunohistochemical staining. Direct sequence analysis of the 11 cases showed that 8 cases contained the PDGF/COL1A1 fusion transcript. The COL1A1 breakpoint was variable (exons 6, 8, 29 (2 cases), 38 and 47 (3 cases)); however, the PDGF fusion site was constant; at the start of exon 2. In conclusion, DNA sequence analysis is very important for the accurate diagnosis of DFSP.

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Polarized light camera (polCAM) for delineating tumors margins of skin cancer

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Basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) account for the vast majority of cutaneous malignancies and may cause significant disfigurement and disability if left untreated. In a pilot clinical study, a polarized light camera (polCAM) was developed and tested for imaging skin cancer margins in vivo to aid surgical excision. The tumor margins of 35 patients undergoing Mohs micrographic surgery for the treatment of BCC and SCC were imaged. When linearly polarized light propagates through light-scattering media such as the skin, the polarization state of the light is randomized. Superficially scattered light, however, retains the polarization of the incident light. The polCAM creates an image based only on the superficially scattered light, which reveals a fabric pattern in the skin. Skin cancer disrupts this fabric pattern, which allows the surgeon to visually identify the tumor margin. The data obtained from the 35 case studied with the polCAM were compared with the clinical margins of the tumor and then verified with the true skin cancer margins as determined by Mohs micrographic surgery. Normal skin had a unique mesh fabric pattern, which was clearly different from the homogenous pattern of tumor. In several instances, the polCAM images detected tumor involvement beyond that which was appreciated clinically, and predicted margins comparable to those obtained with Mohs micrographic surgery. The ability of the polCAM to identify cancer margins may reduce the need for multiple excisions stages during Mohs surgery.

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Loss of novel tumor suppressors may play a role in the genesis of atypical spitzoid nevomelanocytic proliferations not exhibiting the BRAF mutation

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BRAF is mutated in approximately 70% of melanomas but it paradoxically induces senescence in primary human melanocytes. This indicates requirement for an additional hit in melanoma genesis. Using an unbiased genome-wide human shRNA library screen we have identified 17 candidate genes that are necessary for activated BRAF induced senescence. Knocking down any of these genes rescues primary human melanocytes from senescence. The reported incidence of BRAF mutations in Spitz nevi is relatively low. The purpose of this study was to ascertain the utility of analysis of the BRAF mutational status as a diagnostic adjunct in atypical spitzoid nevomelanocytic proliferations with histologic features that deviate from a typical Spitz but yet are insufficient to qualify for a diagnosis of spitzoid melanoma. Genomic DNA for genotyping was isolated per protocol using techniques that included laser capture microdissection to isolate nevus cells from regular Spitz nevi (4), atypical Spitz nevi (5) and spitzoid melanoma (1). The BRAF gene was analyzed for the V600E mutation, the most common mutation in melanomas. Genotype analysis of all 10 showed absence of this oncogeneic mutation. Given that this group included a spectrum of atypical spitzoid lesions including atypical Spitz nevi and Spitzoid melanoma, our findings indicate that the BRAF mutational status cannot be used to separate Spitz nevi from atypical Spitzoid nevomelanocytic proliferations. Our analyses of the 17 candidate genes revealed loss of expression of some tumor suppressors independent of the BRAF mutation. Extending this to the current study, the presence of BRAF-WT in the six cases with architectural disorder and/or cytologic atypia, suggests that the melanocytic proliferation in atypical Spitz nevi might not be RAF-MEK-ERK pathway-induced but may be initiated by loss of other tumor suppressor/s.

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Ancient origin of a Japanese xeroderma pigmentosum founder mutation

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Xeroderma pigmentosum (XP) patients have sun sensitivity, a 1000-fold increased risk of skin cancer and defective DNA repair. XP is a recessive disease with a frequency of about 1:22,000 in Japan, which is much more common than in the US. There are 8 XP DNA repair genes (*XPA* to *XPG* and XP variant). The *XPA* gene is defective in about 55% of Japanese XP patients. They have a severe form of XP with progressive neurological degeneration. *XPA* is located on 9q22.3 and codes for a 273 amino acid protein. More than 90% of the mutant alleles in Japanese XP-A patients have the same G to C base change mutation. This founder mutation at the 3′ splice acceptor site of intron 3 results in no detectable protein production and markedly reduced DNA repair. Approximately 1% of the Japanese general population, representing about 1 million people, are heterozygous carriers of this mutation. We used haplotype analysis to estimate the age of the most common recent ancestor (MCRA) of this founder mutation in Japan. We studied DNA from 42 XP patients in 38 Japanese families who had a homozygous *XPA* G to C splice mutation. We measured 70 single nucleotide polymorphisms (SNP's) that were located up to 2.5 MB upstream and 2.5 MB downstream of the mutation on chromosome 9 as indicated on the hapmap http://www.hapmap.org. We found a small region of SNP homozygosity in all the XP patients extending about 150 kb upstream and 50 kb downstream from the mutation. The size of this region is a reflection of the relationship among the patients: a large region indicates a close relationship and a small region indicates a more ancient relationship. Using a likelihood-based method that uses multilocus marker data we determined the age of the MRCA of the *XPA* founder mutation to be about 120 generation (95% CI, 71-205 generations) or about 2400 years (95% CI, 1420-4100 years) based on a generation length of 20 years.

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Ascorbic acid inhibits proliferation of melanoma cells by suppression of Aurora-A and -B expression

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Aurora-A and -B are mitotic kinases that localize in centrosome and spindle midbody, respectively, and play important roles during cell division. We investigated the expression of these kinases in human malignant melanoma cell lines. As a result, the messenger RNA levels for Aurora-A and -B were increased in melanoma cells compared to cultured normal human epidermal melanocytes. Ascorbic acid has been reported to have anti-cancer effects inducing apoptosis via activation of caspase-3. Exogenous ascorbic acid inhibited proliferation of cultured melanoma cells and induced apoptosis in dose- and time-depended manners, and decreased Aurora-A and -B expression. These results implicate that Aurora-A and -B are candidates for therapeutic targets in malignant melanoma therapy, and also suggest that ascorbic acid is a potent anti-melamona therapy inhibiting expression of these kinases.

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Nestin is expressed in HMB-45 negative melanoma cells in dermal lesion of nodular malignant melanoma

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Previously, we immunohistochemically examined the expression of three hair follicle stem cell and progenitor cell markers, nestin, keratin 15 (K15), and CD34 in normal human epidermis and hair follicles and in epidermal and follicular tumors, trichilemmoma, basal cell carcinoma (BCC), and squamous cell carcinoma (SCC). We certified that trichilemmoma originates in the nestin-positive/K15-positive/CD34-negative outer-root sheath cells below sebaceous glands, BCC tumor cells from the more mature nestin-negative/K15-positive /CD34-negative outer-root sheath cells, and SCC from the nestin-negative/K15-positive/CD34-negative keratinocytes of the basal cell layer in the epidermis. In the present study, we examined the expression of hair follicle and neural stem cell marker nestin, and S100, HMB-45 in junctional, compound, and dermal nevomelanocytic nevi, malignant melanoma and neurofibroma. Nestin immunoreactivity was observed in all 20 cases of dermal nevomelanocytic nevus, all 13 cases of dermal lesions of nodular malignant melanoma, and all 12 cases of neurofibroma, however nestin expression was negative in the nevus cells of dermal-epidermal junction in 9 of 11 cases junctional and compound nevus and in all 6 of 6 cases of superficial spreading melanoma and dermal-epidermal junction of nodular melanoma. Nestin immunoreactivity was observed in the HMB-45 negative melanoma cells in dermal lesion of all 13 cases nodular malignant melanoma. These results suggest that the nestin is the important marker of HMB-45 negative melanoma cells in dermal lesion of melanoma cells of nodular malignant melanoma.

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Photoprotective effects of chemical peeling agents against ultraviolet-induced photoaging in hairless mice

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Chemical peeling is one of the dermatological treatments available for certain cutaneous diseases and conditions or improvement of cosmetic appearance of photoaged skin. Here, we assessed the photochemopreventive effect of several clinically used chemical peeling agents on the ultraviolet (UV)-irradiated skin of hairless mice. The peeling agents, 35% glycolic acid (GA) dissolved in distilled water, 30% salicylic acid (SA) in ethanol, 10% and 35% trichloroacetic acid (TCA) in distilled water, were applied to the back of UV-irradiated hairless mice every 2 (GA, SA, and 10% TCA) or 4 (35% TCA) weeks for totally 18 weeks after establishment of 2 models of photoaged mice by UVB or UVA irradiation for 14 weeks. Tumor number and incidence were evaluated and wrinkle formation was assessed by replica taking. Skin specimens were taken from treated and non-treated area for evaluation of hydroxyproline amount. All peeling agents used have shown to decrease the total number of tumors as well as tumor incidence. Wrinkle formation was reduced in treated area compared with non-treated area and hydroxyproline amout was also increased in the treated area. Our findings have clearly shown that chemical peeling with GA, SA, and TCA help not only reduce wrinkles by increasing collagen amount but also prevent UV-induced tumor formation in hairless mice. These results indicate that chemical peeling with GA, SA, and TCA could serve as anti-photoaging treatment both in wrinkle reduction and tumor prevention.

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Co-expression of MMP-7, CD44v3 and HB-EGF in recessive dystrophic epidermolysis bullosa –associated squamous cell carcinomas

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In this study, we elucidate the molecular mechanisms of the invasion and growth in recessive dystrophic epidermolysis bullosa (RDEB) –associated squamous cell carcinomas (SCC). Formalin-fixed, paraffin embedded samples of 25 RDEB-associated SCCs, 60 sporadic SCCs and 28 Bowen's diseases were studied by immunohistochemistry using monoclonal antibodies against matrilysin-1 (matrix metalloproteinase-7, MMP-7), CD44 variant 3 (CD44v3) and heparin binding EGF-like growth factor (HB-EGF). MMP-7 was expressed by tumor cells in all RDEB-associated SCCs (25/25) and in most of sporadic SCCs (59/60) studied. Semiquantitative analysis showed stronger cytoplasmic staining for MMP-7 in RDEB-SCCs compared with sporadic SCCs. MMP-7 was also expressed in part of Bowen's diseases but not in normal epidermis. Co-expression of MMP-7, CD44v3 and HB-EGF was noted in both SCC groups. Similar co-expression was noted also in Bowen's disease, but not in normal epidermis where CD44v3 is homogenously present but MMP-7 is not expressed. Interestingly, CD44v3 expression was elevated in part of Bowen's diseases while it was typically dimished in SCCs studied. In conclusion, our findings support the hypothesis that CD44v3 recruits proteolytically active MMP-7 to cleave proHB-EGF *in vivo* in RDEB-associated and sporadic SCCs and also in early stage of tumorigenesis i.e. in Bowen's diseases. These results suggest that MMP-7 can be considered as a potential therapeutic target in these carcinomas.

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Photocarcinogenesis of topical tazarotene and isotretinoin alone and in combination with valproic acid in hairless mice

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Retinoids and the histone deacetylase (HDAC) inhibitor valproic acid have shown anticancer properties, but the photocarcinogenic or photoprotective effect is unclear. Therefore, we investigated the influence of topical isotretinoin, tazarotene, valproic acid, and isotretinoin and tazarotene in combination with valproic acid in topical formulations on squamous cell carcinoma formation in hairless female C3.Cg/TifBomTac immunocompetent mice exposed to solar simulated radiation (SSR). The products were applied on the dorsal skin of 400 mice 5 times weekly followed by SSR (3 standard erythema doses, SED) 3 times weekly 3-4 hours after medication. The retinoids tazarotene and isotretinoin proved to be photocarcinogenic as tumors appeared sooner when combined with SSR compared with control mice (134 and 148 days vs. 197 days, $p<0.0005$). The HDAC inhibitor valproic acid did not show any significant photocarcinogenic effect or protection against SSR carcinogenesis (176 days vs. 197 days, $p=0.065$). When valproic acid was added to tazarotene + SSR or isotretinoin + SSR it did not significantly change their carcinogenicity (134 days for both groups vs. 197 days, $p<0.0005$). In summary, the present study shows that valproic acid alone is not photocarcinogenic or photoprotective in hairless mice. When valproic acid is in combination with tazarotene or isotretinoin it does not change their carcinogenicity significantly. Use of valproic acid for dermal malignancies should be investigated because it is less photocarcinogenic, and has fewer adverse effects in hairless mice than isotretinoin and tazarotene.

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The XRCC1 Arg194Trp polymorphism increases the risk of basal cell carcinoma at sun-exposed sites in Japanese population.

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The X-ray repair cross-complementing groups 1 (XRCC1) gene plays an important role in base excision repair(BER). The protein encoded by this gene interacts with DNA ligase III, polymerase beta and poly(ADP-ribose) polymerase to participate in the BER. At least three common SNPs frequently occur in this gene (Arg399Gln, Arg194Trp, Arg280His). Recent studies reported that these polymorphisms were associated with not only risk of visceral malignancy but also that of skin cancer such as basal cell carcinoma(BCC) and squamous cell carcinoma(SCC). However we have had no study of SNPs of XRCC1 in skin cancer in Japanese population. In our study, we investigated whether these SNPs were associated with the risk of skin cancer in Japanese population. The study population was composed of about 120 patients with skin cancer (BCC, SCC, malignant melanoma, Bowen's disease, actinic keratosis) and 53 control subjects in Japanese population. We genotyped two SNPs(Arg399Gln, Arg194Trp) using polymerase chain reaction-restriction fragments length polymorphism(PCR-RFLP) analysis. We found a statistically significant difference between controls and the cases of BCC with sun-exposed sites (chi-square test=4.22, odds ratio=2.69, 95% confidence interval 1.03-6.98). Our result tentatively suggests that XRCC1 polymorphisms may be involved in photo-induced carcinogenesis of BCC in Japanese population.

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Overexpression of GRIM19 in cancer cells suppressed STAT3-mediated signal transduction

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A number of studies have demonstrated that signal transducer and activator of transcription 3 (STAT3) is constitutively activated in a ligand-independent manner in human and murine cancer cells, leading to cell proliferation and cell survival. Also there have been reported that STAT3-specific inhibitor, such as JSI-124, suppressed cancer growth *in vitro* and *in vivo*. To investigate the antitumor effects by inhibiting STAT3-mediated signal cascade in cancer microenvironment with molecular-targeting approach, we focused on gene associated with retinoid-IFN-induced mortality 19 (GRIM19) molecule. GRIM19 (MW; 16kDa) is reported to bind physically with transactivation-domain of STAT3 and to inhibit STAT3-dependent signal transduction. GRIM19 is also reported as the cell death activator in cancer cells. Previously we have utilized polyarginine (R9)-protein transduction domain (R9-PTD) as protein-carrier for its safer and higher cell-transduciton efficiency *in vitro* and *in vivo*. We generated the R9-PTD-containing GRIM19 fusion proteins (rR9-GRIM19) to induce the overexpressional conditions easily in various types of cancer cells. We confirmed that *in vitro* treatment of STAT3-constitutively activated (STAT3c) cancer cells, including v-Src-NIH3T3, B16 melanoma, and A20 lymphoma cells, with rR9-GRIM19 significantly blocked STAT3-dependent transcription by luciferase assay or by analyses of downstream molecules of STAT3 signal cascade (bcl-XL, cyclin b1). Moreover, intratumoral injections of rR9-GRIM19 to STAT3c cancer-bearing mice significantly suppressed tumor growth *in vivo* as well as *in vitro* treatment, especially in A20 cells. In contrast, rR9-GRIM19 did not alter the viabilities to STAT3-inactivated cells (EL-4 or NIH3T3). These results indicated that intratumoral injections of rR9-GRIM19 might be a novel anti-cancer therapy for STAT3c tumors by inhibiting STAT3-dependent signal transduction without major systemic side effects.

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Aberrantly expressed Fra-2 promotes CCR4 expression and cell proliferation in adult T-cell leukemia

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Human T-cell leukemia virus type-1 (HTLV-1) is associated with the development of adult T-cell leukemia (ATL). CCR4 is highly expressed in primary ATL cells. Since CCR4 expression was not induced by HTLV-1 Tax, its expression may be constitutively activated by unknown transcription factor(s). To examine the transcriptional regulation of CCR4 expression in ATL, we constructed a reporter plasmid carrying the CCR4 promoter region fused with the luciferase reporter gene. We detected an activator protein-1 (AP-1) site in the CCR4 promoter as the major positive regulatory element in ATL cells. We studied the specific binding of AP-1 family members to the AP-1 site in the CCR4 promoter using the NoShift transcription factor assay and the chromatin immunoprecipitation. We identified specific binding of Fra-2, JunB and JunD. With luciferase assay, we showed that CCR4 promoter was activated by heterodimers of Fra-2/JunB and Fra-2/JunD in Jurkat cells. To examine the role of Fra-2, JunB and JunD in CCR4 expression and cell proliferation in ATL cells, we employed the small interfering RNA (siRNA) knockdown technique. CCR4 expression and cell growth were suppressed by Fra-2 siRNA or JunD siRNA, but not JunB siRNA. Conversely, cell growth was promoted by excess expression of Fra-2 or JunD in Jurkat cells. To identify the target gene of Fra-2 in ATL cells, we compared the gene expression profiles of ATL-derived cells transfected with Fra-2 siRNA or control siRNA using the Affymetrix high-density oligonucleotide microallay. We identified 49 genes, including c-Myb, BCL-6 and MDM2, which were downregulated by Fra-2 siRNA in ATL cells. These proto-oncogenes were highly expressed in primary ATL cells but not in normal CD4(+) T cells. Together, aberrantly expressed Fra-2 in association with JunD may play a major role in CCR4 expression and oncogenesis in ATL.

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Steroidogenesis-related proteins expression is decreased in skin cancer

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Skin cells express receptors to numerous steroid hormones. Besides it, skin cells can produce steroid hormones by itself, but their precise role in skin functioning is unclear. Locally synthesized steroid hormones may act as paracrine and autocrine regulators and could be involved in numerous skin pathology developments. The aim of this research was to evaluate levels of proteins associated with the beginning of steroid formation – pregnenolone synthesis: peripheral benzodiazepine receptor (PBR), which functions as a "gate keeper" in cholesterol import to mitochondria, diazepam binding inhibitor (DBI) – endogenous PBR's ligand and cytochrome P450scc – mitochondrial enzyme responsible to conversion of cholesterol to pregnenolone. Cells (normal keratinocytes (NHEK, Clonetics), normal melanocytes (HEM, Cascade biologics), squamous cell carcinoma cells (A431, ATCC), melanoma cells (SK-MEL-2, ATCC)) were cultivated under appropriate conditions. Immunocytochemical assay was accomplished by standard protocols. Number of PBR+ and CYP450scc+ cells was estimated per 100 visible cells. Transfection PBR-GFP was performed to melanoma and squamous cell carcinoma cells and then immunocytochemical assay with anti-CYP450scc antibodies was done. Confocal microscopy was performed by scanning laser confocal microscope Olympus IX-70. DBI levels were evaluated by immunoblotting. PBR, DBI and CYP450scc were present in all skin cells types tested. PBR and CYP450scc expression were significantly diminished in cancer cells in comparison to normal keratinocytes and melanocytes. There was no colocalization of PBR and CYP450scc revealed by confocal microscopy that could be explained by essential participation other proteins in this initial step of steroidogenesis. DBI levels were also suppressed in melanoma and squamous cell carcinoma cells. Similar alterations of these proteins levels in malignant skin cells should be considered as reduced steroid biosynthesis in skin cancer.

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Expression of CYP isoforms and MDR-1 in the skin and cutaneous malignant tumors

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Cytochrome P450 is a member of phase one drug metabolizing enzymes and responsible for drug and xenobiotics metabolism. As the enzymes and drug transporters play important roles in detoxifycation or metabolic activation of such substances, it is of great significance to investigate tissue distribution. Multidrug resistance-1 (MDR-1) which is one of the ABC transporters with the functions as the transmembrane efflux pump for various drugs and materials including anticancer agents and toxins, is known to play an important role in liver, gastrointestinal tract, and kidney. And in the field of the oncology, it is thought that overexpression of MDR-1 in cancer cells induces resistance to multiple anticancer agent. Therefore, extrahepatic distribution of CYPs has been investigated, but to date little information is known in cutaneous tumors. The expression of CYPs and transporter were investigated immunohistochemically; MDR-1, CYP1B1, CYP2E1, and CYP2A6 are checked in normal skin and in the cutaneous malignant tumors including Extramammary Paget 's disease (EMPD), Basal cell carcinoma (BCC), Squamous cell carcinoma (SCC), Bowen' s disease, Malignant melanoma (MM), and Eccrine porocarcinoma (EPC). In normal skin, MDR-1 and CYP2E1 were positive for epidermis, hair follicule, appendage, vascular endothelium, and muscules arrector pili. CYP1B1 was positive for hair follicule and appendage, CYP2A6 was positive for vascular endothelium. MDR-1 and CYP2E1 was also stained in all tumor cells, while CYP1B1 was positive in EMPD, SCC, and EPC. CYP2A6 was positive only in EMPD. Melanoma cells were reported to express mRNA for CYP1B1 but no mRNA for MDR-1; our result shows clear expression of PGP. CYP2A6 were highly expressed in EMPD cells. Excessive expression of CYP2A6 in colon cancer cells, and engagement in outbreak of the lung cancer were reported. Considering the role of CYP2A6 in carcinogenesis and metabolism in antitumor agents such as futrafur. The expression may be clinically important.

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MicroRNA-205 regulates the lipid phosphatase SHIP2 in keratinocytes and may function as an oncogene in squamous cell carcinomas

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MicroRNAs (miRNAs) regulate many cellular processes including cancer; however, few miRNA-targets have been confirmed experimentally. MiRNA-205 (mir-205) is expressed in stratified squamous epithelia and overexpressed in head and neck squamous cell cancer (SCC) cell lines. Loss-of-function studies using an antagomir to mir-205 in human epidermal keratinocytes (HEKs) caused cells to change from a cuboidal to a stellate appearance, along with a striking reorganization of actin. As SHIP2 is a potential target of mir-205 and has been implicated in regulating cytoskeletal organization, we assessed SHIP2 expression in HEKs. A marked increase in SHIP2 protein was observed in HEKs treated with anti-mir-205 compared with controls. To confirm that SHIP2 is a direct target for mir-205, we co-transfected HeLa cells with mir-205 and: (i) a reporter construct carrying the 3′UTR for SHIP2; or (ii) a 205-binding mutant reporter. Accordingly, this resulted in a clear reduction (>60%) in luciferase activity only in the case of the wild-type SHIP2 3′UTR. Consistent with our findings in HEKs, ectopic overexpression of a mir-205 mimic in HeLa cells, which have negligible endogenous levels of mir-205, resulted in a marked reduction in SHIP2 protein, along with increased cortical F-actin staining. The relevance of these data was underscored by our finding of a reciprocal relationship between mir-205 and SHIP2 expression in SCC cell lines. SCC68, an aggressive oral SCC, had a high signal for mir-205 and negligible SHIP2 expression. Conversely, SCCs 9 and 12, which are minimally invasive, had much lower levels of mir-205, but relatively high SHIP2 expression. Our findings establish that: (i) mir-205 regulates SHIP2 in keratinocytes; and (ii) abnormally elevated levels of mir-205 in SCCs downregulate SHIP2. Since SHIP2 is a negative regulator of the AKT pathway and can function in a manner similar to the tumor suppressor protein PTEN, mir-205 may be viewed as an oncogene in the context of SCCs.

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Tumor associated macrophages-induced cyclooxygenase-2 dependent invasion and angiogenesis of human basal cell carcinoma

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Tumor associated macrophages (TAM) and cyclooxygenase-2 (COX-2) were associated with poor prognosis and progression of many human cancers. However, the role TAM in human basal cell carcinoma (BCC) remained elusive. Immunohistochemical analysis of human BCC specimens showed that the number of infiltrated TAM was correlated with vertical depth of invasion and tumor microvessel counts in human BCC. In addition, tumor nests highly infiltrated with TAM showed increased expression of tumor cyclooxygenase-2 (COX-2). Linear regression analysis showed number of TAM and tumor COX-2 expression were independent predictors for invasion and angiogenesis. We hypothesized that TAM might induced COX-2 expression and thus enhance cancer cell invasion and angiogenesis. To test this, we cocultured BCC cells line cells with phorbol myrisate acetate (PMA) activated human THP-1 macrophages. After coculture, we found increasing in vitro invasiveness and angiogenesis, which could be attenuated by specific COX-2 inhibitor celecoxib and COX-2 siRNA. The nuclear factor-κB site on the COX-2 promoter is involved in p38 MAPK-regulated COX-2 expression. These intracellular networks drive the COX-2 dependent matrix metalloproteinase-9, vascular endothelial growth factor-A, basic fibroblast growth factor release and contribute to cell invasion and angiogenesis.

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Infliximab inhibits DNA repair in UVB-irradiated premalignant keratinocytes

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Anti-tumor necrosis factor-a (TNFα) approaches are increasingly used in the therapy of autoimmune diseases. One of the safety concerns is the potential enhancement of skin carcinogenesis. The aim of this study was to investigate if the TNFa neutralizing antibody, infliximab, directly affects the cell cycle and DNA repair in premalignant human keratinocytes after ultraviolet-B (UVB) radiation. We found that infliximab-treated cells exhibited an enhanced G2/M cell cycle arrest and increased apoptosis after 10-20 mJ/cm2 UVB. In spite of this, the level of cyclobutane pyrimidine dimers (CPD) in infliximab-treated cells was significantly higher by 19% both 24 and 48 hours after irradiation with 10 mJ/cm2 UVB. Since we have recently shown that protein kinase B/Akt is involved in the TNFα signalling pathway and promotes cell survival and skin carcinogenesis, we measured activatory phosphorylations of Akt (Ser-473 and Thr-308) and the signalling via related pathways Erk 1/2, p38 and p70-S6K. Infliximab inhibited Akt and its downstream targets p70-S6K and Erk 1/2, and stimulated p38 both in sham-irradiated and UVB-irradiated cells. In conclusion, despite the fact that infliximab blocks Akt and stimulates the G2/M checkpoint and apoptosis in UVB-irradiated keratinocytes, the repair of CPD is impaired. It is conceivable that anti-TNFa treatments may contribute to the accumulation of mutagenic lesions in the epidermis and enhance the early stages of skin carcinogenesis.

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Vertical and horizontal expression of Cx26 in epidermis adjacent to malignant melanoma correlates to tumor depth

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Gap Junctions are communicating cell-cell junctions which are formed by connexins. In human epidermis the main connexin is Cx43, while Cx26 and Cx30 are absent in healthy epidermis but are induced by several pathogenic conditions. Previously we described the absence of Cx43, Cx26 and Cx30 in non-keratinocytic skin tumors (malignant melanoma, Merkel cell carcinoma) and an upregulation of Cx26 and Cx30 as well as a downregulation of Cx43 in keratinocytic skin tumors (e.g. squamous cell carcinoma, Morbus Bowen). Interestingly, we observed an induction of Cx26 and Cx30 in epidermis adjacent to malignant melanoma and Merkel cell carcinoma, while there was no induction in epidermis adjacent to non-malignant tumors. Here we wanted to examine whether the induction of Cx26 and Cx30 correlates to tumor depth and other parameters, e.g. proliferation and ulcer formation. We investigated the localization of Cx26 and Cx30 in the epidermis of 42 cases of malignant melanoma with various Breslow-indices. We found a significant correlation of the dissemination of Cx26 protein expression to tumor depth vertically (Cx26 positive layers) as well as horizontally (horizontal area of Cx26 protein expression). There was no correlation to Cx30. We conclude that malignant melanoma influences Cx26 protein expression in adjacent epidermis in a size dependant manner.

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MC1R variants increase risk of melanomas harboring BRAF mutations

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Melanocortin-1 receptor (*MC1R*) variants have been associated with *BRAF* mutations in non-CSD (chronic solar-damaged) melanomas in an Italian and an American population. We studied an independent Italian population of 330 subjects (165 melanoma patients, 165 controls) to verify and estimate the magnitude of this association and to explore possible effect modifiers. We sequenced *MC1R* in all subjects and exon 15 of *BRAF* in 92/165 melanomas. Patients with *MC1R* variants had a high risk of carrying *BRAF* mutations in melanomas (OR=7.0, 95%CI=2.1-23.8) that increased with number of *MC1R* variants and variants associated with red hair color. Combining these subjects with the originally reported Italian population (513 subjects overall), *MC1R* variant carriers had a 5- to 15-fold increased risk of *BRAF*-mutant melanomas based on carrying 1 or 2 variants, respectively ($p<0.0001$, test for trend) and regardless of signs of chronic solar-damage. In contrast, no association with *BRAF*-negative melanomas was found (OR=1.0, 95%CI=0.6-1.6). No subjects' or melanomas' characteristics, including age, nevus count, pigmentation, and melanoma thickness or location on chronically or intermittently sun-exposed body sites substantially modified this association. This study confirms that the *MC1R*-melanoma risk association is confined to subjects whose melanomas harbor *BRAF* mutations.

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Depletion of ARMS enhances oxidative stress-induced autophagic cell death in melanoma cells

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Autophagy (macroautophagy) is a dynamic process in which cytoplasmic proteins and organelles are sequestered into autophagosomes and subsequently degraded in autolysosomes. Autophagic cell death (type II programmed cell death) is suppressed during tumorigenesis. ARMS (ankyrin repeat-rich membrane spanning), also known as Kidins220 (kinase D-interacting substrate of 220kD), is a transmembrane platform functioning in neurotrophin-mediated prolonged MEK/ERK pathway. We have previously demonstrated that overexpressed ARMS may promote melanoma tumorigenesis through preventing UV-induced apoptotic cell death via regulating the MEK/ERK signaling pathway. To investigate the role of ARMS in the tumorigenesis of melanoma under oxidative stress, we established siRNA-mediated ARMS-knockdown/B16-F0 stable cells and compare its growth characteristics with the control after hydrogen peroxide treatment. We found that depletion of ARMS significantly increased hydrogen peroxide-induced autophagy, as evidenced by autophagic vacuolization examined by electron microscopy, punctate translocation of GFP-LC3 (microtubule-associated protein light chain 3) by immunofluoresence, and increased LC3-II/LC3-I proportion by Western blotting. When autophagy was prevented at an early stage by 3-methyladenine, an inhibitor of class III phosphatidylinositol 3-phosphate kinase, hydrogen peroxide-induced autophagic death was suppressed. Moreover, in the subcutaneous xenograft model of ARMS-knockdown/B16-F0 melanoma cells, autophagic vacuoles were revealed by electron microscopy. These results suggest that, through negatively regulating oxidative stress-induced autophagic cell death, overexpression of ARMS may promote melanoma formation.

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RAGE signaling promotes tumor development by sustaining inflammation

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Experimental and clinical evidence has highlighted the central role of chronic inflammation in promoting tumor development. However, the mechanisms that sustain a tumor-promoting microenvironment remain largely elusive. We demonstrate that mice deficient for the receptor for advanced glycation end-products (RAGE) are resistant to DMBA/TPA-induced skin carcinogenesis. Furthermore, RAGE-deficient mice showed severely reduced TPA-induced inflammatory response accompanied by impaired infiltration with neutrophils, macrophages and mast cells and impaired upregulation of pro-inflammatory genes, such as Ptgs2, MIPs, S100a8 and S100a9 using confocal microscopy and gene expression analysis. S100a8 and S100a9 encoding strong chemoattractants for myeloid cells were induced upon TPA treatment of mouse back skin and overexpressed in advanced stages of mouse and human skin tumors using RQ-PCR and tissue microarrays. Since S100 proteins represent activating ligands of RAGE and ligand expression is RAGE dependent, we propose the existence of an S100/RAGE-driven feed-forward-loop in chronic inflammation and tumor formation. Finally, TPA-induced dermal infiltration and epidermal hyperplasia were restored in wildtype bone marrow chimeric RAGE-deficient mice and Keratin 5-driven RAGE deletion revealed that RAGE expression on immune cells, but not keratinocytes, is essential for a RAGE-dependent inflammatory microenvironment and effective tumor promotion. In conclusion, we demonstrate that RAGE signaling drives the strength and maintenance of an inflammatory reaction during tumor-promotion and provide direct genetic evidence for a novel role for RAGE in linking chronic inflammation and cancer.

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Obligatory role for Bmi1 in Hedgehog-pathway driven medulloblastoma expansion

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Deregulated Hedgehog (Hh) signaling plays a central role in the pathogenesis of nearly all basal cell carcinomas and a subset of medulloblastomas (MBL), the most common pediatric brain tumor. Expansion of Hh-driven cancers likely depends on genes involved in stem cell self-renewal, particularly the transcriptional repressor Bmi1. To test this hypothesis, we modified *Bmi1* gene dosage in transgenic mice we generated to express an oncogenic Hh effector, *SmoA1*, in CNS progenitors. Since $Bmi1^{-/-}$ mice die by ~12 weeks of age, we required rapid tumor formation and complete penetrance not available in other MBL mouse models. By postnatal day 14, 100% of *SmoA1* mice developed MBL, and these tumors exhibited a similar morphology and marker profile as human MBL. While 29/29 $SmoA1;Bmi1^{+/+}$ or $SmoA1;Bmi1^{+/-}$ mice between day 14 and day 26 had typical MBL, 6/6 $SmoA1;Bmi1^{-/-}$ animals did not. Instead, $SmoA1;Bmi1^{-/-}$ mice had small collections of abortive cells in the region of greatest tumor load in *SmoA1* animals, suggesting that in the absence of *Bmi1*, MBL is initiated but cannot progress to frank tumors. Cells within the $Bmi1^{-/-}$ lesions express SmoA1 but are largely quiescent (6.2% PCNA+), in contrast to the rapidly proliferating $Bmi1^{+/+}$ MBL tumor cells (81.9% PCNA+). Ectopic cells do not express the progenitor cell marker Nestin and are highly apoptotic (29.6% TUNEL+), relative to MBL tumor cells (6.3% TUNEL+). In keeping with these differences (and the known downstream targets of Bmi1), *SmoA1* tumors, but not $SmoA1;Bmi1^{-/-}$ abortive lesions, express abundant Cyclin D1, while p19 is upregulated in the $SmoA1;Bmi1^{-/-}$ lesions relative to tumors. Our data provide the first demonstration that Bmi1 is required for *de novo* development of a spontaneously-arising solid tumor; implicate Bmi1 as a key factor in oncogenic Hh signaling; and point to a critical role for Bmi1-dependent, Nestin-expressing progenitor cells in MBL expansion.

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Development of mycosis fungoides after bone marrow transplantation;possible transmission from donor

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Secondary cancer is an established long-term complication of bone marrow transplantation (BMT). It is usually associated with radiation exposure, Epstein-Barr virus and mutagenic preparative regimens resulting in great immune deficiency. We report a patient with a diagnosis of mycosis fungoides(MF) a number of years after successful treatment of chronic myeloid leukaemia. An allogeneic BMT was from a first-degree relative. In the time between the BMT and new diagnosis what was considered to be severe eczema developed. The rash did not respond to topical therapy or even the addition of azathioprine. Repeated biopsies showed a spongiotic dermatitis. The patient developed neck nodules 8 years after the BMT and biopsies confirmed MF. T cell gene rearrangement studies of bone marrow and skin samples demonstrated similar clonal rearrangements in T-cell receptor β and γ regions which supported the diagnosis of MF. On full case review after this diagnosis, it was noted that the donor had a history of a rash. Histopathology of skin biopsies from the donor showed a chronic superficial dermatitis. Clonal studies of these specimens are non-contributary yet clonal rearrangement in the donor's serum has been identified which correspond to those found in the patients' marrow and skin gene rearrangement studies. The risk of a secondary haematologic cancer following BMTs is increased by a factor of 4 to 7. Developing a lymphoproliferative disorder such as MF after BMT is an uncommon complication. It is postulated in this case that MF was transmitted from the donor to the patient via the BMT. The cancer molecular diagnostic studies show matching clonal rearrangements between the donor serum and the patients' skin and serum samples. Donor progenitor lymphoma cells may have been transmitted and presented earlier in the patient due to the previous significant immunosuppression undergone. This case highlights the rare potential complications that may occur due to bone marrow transplantation and the unusual nature of potential transmission of disease.

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Vitamin D3 inhibits proliferation of Ptch1+/- mouse basal cell carcinoma cell lines and tumors

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The pivotal molecular abnormality in basal cell carcinoma is inappropriate activation of the hedgehog (HH) signaling pathway, commonly due to mutational inactivation of the tumor suppressor, Patched1 (Ptch1) gene. Ptch1 is a transmembrane receptor for the hedgehog ligand and is the key inhibitor of HH signaling. Dysregulated HH pathway signaling also has been implicated in tumors of the pancreas, lung, and brain (especially medulloblastoma). Therefore, small-molecule inhibitors of the HH pathway hold promise to prevent BCC development and treat other HH-driven cancers. Recently, statins (HMG-CoA reductase inhibitors) and vitamin D have been shown to inhibit the HH pathway, and observational studies suggest that lipophilic statins and vitamin D may lower breast and prostate cancer risk. Cutaneous synthesis of vitamin D3 depends on UV light to convert 7-dehydrocholesterol to vitamin D3. We investigated whether either atorvastatin, vitamin D3 or its precursor could reduce BCCs in Ptch1+/- mice. *In vitro* addition of 7-dehydrocholesterol and vitamin D3 at 5μM, but not atorvastatin, inhibited the proliferation of BCC cell lines established from Ptch1+/- mice. In fact, vitamin D3 at 5μM was more effective than 5μM cyclopamine, a known inhibitor of HH signaling and BCC carcinogenesis. Vitamin D3 and atorvastatin also inhibited the proliferation of a medulloblastoma cell line from Ptch1+/- mice but not that of normal keratinocytes. In vivo, we applied topically vitamin D3, 7-dehydrocholesterol, atorvastatin, or acetone control to the dorsal skin of IR and tamoxifen treated Ptch1+/-K14-Cre-ER2 p53flox/flox mice, and monitored BCC tumor number and size. Vitamin D3 significantly reduced BCC tumor number and size by 50% ($p<0.05$) while 7-dehydrocholesterol and atorvastatin did not. Thus we find that vitamin D3 inhibits BCC cell lines and BCC tumors in Ptch1+/- mice and hence may be an effective chemopreventive agent against BCC.

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Cooperation of tumor suppressor ING1b and SWI/SNF chromatin remodeling complex in nucleotide excision repair

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UV radiation (UVR) is the major environment risk factor for the development of skin cancers including melanoma. It induces DNA lesions such as cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts (6-4PPs) which are repaired by the nucleotide excision repair (NER) pathway. Efficient NER pathway is important to protect cells from mutation and genomic instability and thus to tumorigenesis of skin cancers. DNA is packed in a compact structure called chromatin which is inhibitory to all DNA transactions including NER. Therefore, it is proposed that chromatin remodeling precedes NER and is necessary to provide favorable environment for recruitment of repair factors to UV lesions. However, the role of chromatin remodeling in NER is not fully understood. We have previously showed that tumor suppressor ING1b enhances NER through histone H4 acetylation and chromatin relaxation. It is also required for the localization of DNA lesion recognition, XPA, to CPD lesions. We recently found that ING1b interacts with SNF5, core subunit of the ATP-dependent chromatin remodeling complex, by immunoprecipitation in melanoma cell line MMRU and also in 293 cells. Unlike ING1b, SNF5 binding to chromatin did not change upon UV radiation. To further study the cooperation of SWI/SNF complex with ING1b in NER, we established stable cell line with SNF5 knockdown using SNF5 short-hairpin RNA in 293 cells. We found that UV-induced chromatin relaxation was abrogated in cells with SNF5 knockdown using Micrococcal nuclease digestion assay. This data shows that ING1b may cooperate with SWI/SNF complex as chromatin remodeling factors in NER. It sheds light on the molecular mechanisms of how UV-damaged DNA is repaired which eventually enables us to design effective strategies for skin cancer prevention.

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Oncogene-induced senescence in skin tumors

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Activation of oncogenes or inactivation of tumor suppressor genes is known to be one of multiple critical steps in skin tumorigenesis. Recently, activation of oncogenes in normal primary cells has been reported to trigger antiproliferative responses preventing progression to malignant transformation. The state of cell cycle arrest in primary cells due to inadequate cell proliferation signals by activation of oncogenes is called oncogene-induced senescence (OIS). However, the state of OIS in human skin tumors is currently unknown. In order to elucidate whether or not the state of OIS might be relevant to human skin tumors, we have examined immunohistochemical staining of antiphosho-Histone H2A.X (γ-H2AX) as a marker of OIS in non-melanoma skin tumors, including squamous cell carcinoma (SCC), basal cell carcinoma (BCC), actinic keratosis (AK), Bowen's disease (BD), and seborrheic keratosis (SK). γ-H2AX is the histone modification produced by DNA damage check point response (DDR) through oncogene-induced DNA hyper-replication. Seventeen of 22 cases (77%) of AK showed moderately to strongly positive expression of γ-H2AX. On the other hand, 8 of 20 cases (40%) of SCC, 3 of 20 cases (15%) of BD, 2 of 20 cases (10%) of BCC, and 3 of 30 cases (10%) of SK showed moderately to strongly positive expression. Immunoreactivity of γ-H2AX in AK was statistically significantly higher than each of those in SCC, BD, BCC, and SK. We suppose that DNA damage check point response due to some oncogenic signals might be involved in the early skin carcinogenesis related to chronic exposure to UV.

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Role of AKT in cell death and survival of different stages of skin squamous cell carcinoma upon treatment with the chemotherapeuticum cisplatin

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Advanced cutaneous squamous cell carcinoma (SCC) has a high potential to metastasize and often demonstrates resistance to chemotherapeutic agents, such as cisplatin (CDDP). Circumventing the apoptotic program is a mechanism of resistance to chemotherapy. We studied the role of survival pathway PI3-K/AKT in cell death/cell survival response following exposure to CDDP in cell lines derived from dysplastic skin (PM1), the primary SCC (MET1), and its lymph node metastasis (MET4) from the same patient. AKT phosphorylation and activity was increased in the SCCs as compared to PM1 and paralleled tumor progression. CDDP induced cell death in all cell lines, but there is an increasing innate resistance to apoptosis in the further tumor stages. While CDDP decreased the phosphorylation and activation status of AKT in PM1 and MET1, it did not affect AKT in MET4. Since it is generally known that the PI3-K/AKT pathway may play an important role in the resistance to genotoxic agents, we investigated whether we could sensitize the different stages of SCC to CDDP using a PI3-K inhibitor. PI3-K/AKT inhibition increased the apoptotic sensitivity of PM1, while surprisingly, it decreased the apoptotic sensitivity of MET1 and MET4 to CDDP. Moreover, the MET4 cells showed a marked morphology after CDDP treatment, which is more pronounced after PI3-K inhibition. Electron microscopic analysis showed an increased amount of autophagic vacuoles. Signs of autophagy were further confirmed at the morphological (accumulation of cells with a punctuated pattern of GFP-LC3) and biochemical level (increase in the amount of Beclin 1 and in LC3-II protein levels). In conclusion, these results show that the response of successive stages of carcinogenesis differs after cisplatin treatment. We will further investigate the mechanism underlying the cell death/survival response to cisplatin in these cell lines.

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Azathioprine-induced microsatellite instability is not seen in cutaneous squamous cell carcinomas

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Immunosuppressive therapy after organ transplantation is associated with an increased risk for cancer, in particular skin carcinomas. Azathioprine, a thiopurine drug, is widely used as an immunosuppressant in organ transplant recipients (OTRs). The incorporation of thioguanine pseudo-bases in genomic DNA is thought to trigger the mismatch repair system. Acute myeloid leukemia (AML) and sebaceous gland carcinoma (SGC) of OTRs with long-term azathioprine treatment have been shown to display microsatellite instability (MSI), a diagnostic marker of defective or insufficient mismatch repair. These results contrasted to those found in immunocompetent patients where AMLs and (not Muir-Torre related) SGCs are microsatellite stable. In immunocompetent patients less than 5% of the cutaneous squamous cell carcinomas (SCCs) display MSI. To investigate if SCCs from azathioprine-treated OTRs display a higher frequency of MSI, DNA was isolated from formalin-fixed paraffin-embedded material of nine SCCs from eight OTRs using azathioprine. The stability of six microsatellite markers was determined using the MSI Analysis System (Promega) and compared to a microsatellite stable control (K652 cell line). None of the nine SCCs of the azathioprine-treated OTRs demonstrated microsatellite instability and showed a similar profile of the microsatellite markers as the microsatellite stable control. Thus, in contrast to AMLs and SGCs of azathioprine-treated OTRs, cutaneous SCCs are microsatellite stable. These results concur with the results found in SCCs of immunocompetent patients. Apparently the role of azathioprine in the development of SCCs is different compared to its role in AML and SGCs.

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Aberrant expression of topoisomerase binding protein 1 in non-melanoma skin cancer

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Ultraviolet radiation is the major aetiology of non-melanoma skin cancer (NMSC) however beta human papillomavirus (HPV) infection may act as a co-carcinogen. Topoisomerase binding protein 1 (TopBP1) is a DNA repair protein that interacts with HPV. TopBP1 is involved in the DNA damage response; it is essential for an intact DNA damage checkpoint and is required for initiation of DNA replication. We have collected samples of normal skin and NMSC and HPV genotyped them. We have found that HPV-24, 38 and 15 are the most frequent HPVs in NMSC in the West of Scotland. Immunohistochemistry of the same lesions revealed aberrant TopBP1 in NMSC compared to normal skin. TopBP1 is restricted to the nuclei in normal skin while in NMSC TopBP1 is also present in the nuclei but additionally is associated with intense cytoplasmic staining with occasional evidence of mis-localisation of TopBP1 to a predominately cytoplasmic pattern. The mechanisms which result in aberrant TopBP1 expression have yet to be elucidated but, broadly, may result from increased expression of the TopBP1 gene or increased protein stability. We have shown, in cultured HaCaT keratinocytes, TopBP1 over-expression in cells stably expressing the E6 and E7 oncoproteins of HPV-38. The TopBP1 promoter region contains three putative E2F binding sites and is responsive to E2F thus may be activated by HPV-38E7 which binds to p105Rb releasing E2F. Co-transfection of cultured cells with the TopBP1 promoter-luciferase reporter plasmid and HPV-38E7 oncoprotein induced a 3 fold increase in TopBP1 promoter activity, but deletion/mutation of the putative E2F-responsive sites in the TopBP1 promoter did not lead to the abrogation of HPV-38E7 trans-activation, suggesting that E2F1 is not involved in HPV-38E7 up-regulation of TopBP1. Future work will concentrate on TopBP1 stability.

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Luteolin, a flavonoid compound, protects primary human keratinocytes but not skin cancer cells against ultraviolet B-induced cell death

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Acute UVB-exposure of the skin may lead to the formation of sunburn (or apoptotic) cells and the induction of sunburn (erythema). Repetitive UVB-induced cellular damage may ultimately give rise to the formation of skin cancer, such as squamous cell carcinoma (SCC). Luteolin (Lut) is a botanical compound with excellent antioxidant properties and also anti-carcinogenic characteristics in some cancer cells and tissues. In this study, we have tested the effects of the flavonoid Lut on normal human epidermal keratinocytes (NHEK) and on a metastatic SCC cell line (MET4). We have shown that Lut increased the survival of NHEK to UVB which goes together with a strong delay and reduction of UVB-induced apoptosis. Notably, Lut can diminish, but does not completely block apoptotic signaling, which may be seen as a final escape mechanism for DNA damaged and potentially mutagenic cells. In contrast to the protective effect of Lut in NHEK, the same dose of Lut did not affect UVB-induced apoptosis in the metastatic SCC cell line MET4. Noteworthy was the changed cellular morphology after Lut treatment in unirradiated MET4 cells. Only a few hours after Lut treatment, the cytoplasm of MET4 cells became highly vacuolated and increasing the dose of Lut exacerbated this effect and resulted in cell death. Morphologic and biochemical features of autophagy were detected, but the underlying mechanisms and the role of autophagy in the Lut induced cell death in cancer cells requires further investigations. Nevertheless, the above results suggest that Luteolin induces a different response in normal versus malignant human skin cells; while Lut is able to protect NHEK from UVB-induced cell death, it becomes cytotoxic in the metastatic skin cancer cell line. This argues for a potential therapeutic use of Lut in skin cancer.

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Microarray analysis of ras-transformed keratinocytes genetically or pharmacologically ablated for the epidermal growth factor receptor (EGFR) reveals genetic signature and off-target drug effects.

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The epidermal growth factor receptor (EGFR, HER1, ErbB1) is activated by mutation, amplification or ligand over-expression in a number of human cancers. Because EGFR is considered a critical mediator of cell proliferation, small molecule pharmacological inhibitors have been introduced for cancer therapy. Cutaneous folliculitis is a dose limiting toxicity from EGFR-targeted therapy, and a similar lesion was detected in the skin of mice where EGFR has been genetically deleted. This mouse model also predicted that genetic ablation of EGFR was not sufficient to prevent cutaneous squamous tumor development after transduction of oncogenic ras allele into keratinocytes or to reduce the proliferation rate in the resulting tumors. Ras transformed mouse keratinocytes were used to evaluate pathways impacted by genetic or pharmacological inhibition of EGFR. The studies were designed to identify alternative pathways for tumor proliferation, changes that might produce cutaneous inflammation and possible off target affects of the EGFR inhibitory drugs. Microarray analysis was performed on cDNA from ras transformed mouse keratinocytes derived from wildtype or EGFR null mice or treated with EGFR tyrosine kinase inhibitors AG1478, PD153035, and a clinically approved EGFR inhibitor. Comparison of significant gene lists generated by pharmacologic and genetic inhibition of EGFR created a 25 gene signature for EGFR ablation. Included in the signature are genes Id2, ryanodine receptor 1, neuregulin 1and CD46. Comparison between EGFR null keratinocytes treated and untreated with inhibitors indicated potential off target effects but no signature was discovered. These findings will contribute to further understanding the role of EGFR in tumor formation, to elucidating alternative proliferation pathways that may be important in the absence of EGFR, and to identifying the networks responsible for the cutaneous adverse reactions when EGFR is pharmacologically ablated.

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GLI1 induces characteristics of a senescent response in N/Tert-1 keratinocytes

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The phenomenon of oncogene-induced senescence (OIS) recently gained credence as a physiological mechanism to maintain tumours in a pre-malignant state with additional genetic hits required for malignant progression. This concept is difficult to reconcile with basal cell carcinoma (BCC) as these tumours do not appear to derive from pre-cancerous lesions and may in fact represent dysplastic hair follicle organogenesis. Indeed, in contrast to other oncogenic proteins such as mutant Ras or Raf, OIS has not previously been attributed to the GLI (GLI1 and GLI2) transcription factors that are associated with the formation/maintenance of several tumour types including BCC. Here we show that retroviral expression of GLI1 in the genomically stable N/Tert-1 epidermal keratinocyte cell line induces compact colony formation and potently inhibits proliferation with cells arrested in the G1 phase of the cell cycle. GLI1 induces characteristics of cellular senescence/hypermitogenic arrest as revealed by the concomitant induction of cyclin D1 (pro-proliferative) and cyclin dependent kinase inhibitors (CDKI) p21 and p27 (anti-proliferative). No increase was observed for p53 and, accordingly, its targeted knockdown by RNAi was insufficient to overcome growth arrest. Regarding BCC, immunohistochemical analyses revealed that p21 expression was predominantly negative in a panel of 12 nodular/superficial tumours whereas the expression of p27 was less consistent with tumours displaying a combination of negative or weak and diffuse areas of staining that varied between and within patient samples. We are currently investigating if targeted knockdown of p21 and/or p27 is sufficient to overcome growth arrest and permit amplification of GLI1 colonies to determine whether or not loss of CDKI function may play a role in the development of GLI-induced BCC.

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Activation of protein kinase D by ultraviolet B irradiation in epidermal keratinocytes

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Skin is the largest organ in the body and is exposed to numerous environmental insults. Exposure to ultraviolet (UV) radiation from the sun is among these and represents the most important risk factor for developing the non-melanoma skin cancers, squamous and basal cell carcinoma. These cancers are the most common malignancy in the United States with a million cases diagnosed each year. With the continued depletion of the ozone layer the incidence of this cancer will likely increase globally. Protein kinase D (PKD) is a recently discovered serine/threonine protein kinase. Previous studies performed in our laboratory show that protein kinase D (PKD) levels are upregulated in human basal cell carcinomas and in a neoplastic mouse keratinocyte cell line, supporting a pro-proliferative and/or antidifferentative role of PKD in keratinocytes. These data, along with the current evidence that PKD localizes not only within the cytoplasm and nucleus but also to the mitochondria, a major UV-responsive organelle, led us to investigate the ability of UVB exposure to activate PKD. Our hypothesis is that irradiation of primary mouse keratinocytes with UVB leads to activation of PKD, and PKD can relay signals by responding to ROS generated within the cell, with the net effect being survival and/or proliferation. Primary mouse keratinocytes were irradiated with UVB at 28 mJ/cm2 or sham irradiated with various doses of UVB (0-60 mJ/cm2). Samples were processed for western blotting employing an antibody for activated PKD (phosphoserine-916) at various time points after UVB exposure (15 min to 4 hrs). Our data suggest that PKD is indeed activated in a time- and dose-dependent manner in primary mouse keratinocytes following UVB exposure. Experiments are in progress to determine the role this activated PKD plays in signaling UVB-induced biological events within the cell.

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Resveratrol is a potent chemopreventive agent against DMBA skin carcinogenesis and acts through the TLR4 pathway

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Resveratrol (trans-3,5,4-trihydroxystilbene), a polyphenol found in red grapes, acts as a chemopreventive agent in selected tumor systems. The signal transduction pathways responsible for its protective effects have not been completely worked out. The purpose of this study was to determine whether resveratrol serves as a chemopreventive agent for dimethylbenz(a)anthracene (DMBA) skin carcinogenesis, and if so, whether toll like receptors (TLR) are involved in its chemopreventive actions. Panels of C3H/HeN mice were treated topically with resveratrol or vehicle and then subjected to a DMBA skin carcinogenesis protocol. The resveratrol treated group displayed significantly fewer tumors/mouse, total cumulative number of tumors and tumors/tumor bearing mouse compared to animals that received vehicle ($p<0.05$). The chemopreventive effects of resveratrol in DMBA skin tumorigenesis in C3H/HeJ and C3H/HeN mice were then compared to evaluate the contribution of the TLR4 in this process. C3H/HeJ mice have a natural mutation in the TLR4 gene that renders the receptor non-functional. In contrast to C3H/HeN mice, there was not a statistically significant difference in tumor development among the resveratrol and vehicle treated C3H/HeJ panels. Since TLR4 is a component of innate immunity that influences cell-mediated immune responses, experiments were next performed to determine whether cell-mediated immunity to DMBA was influenced by resveratrol treatment. Cell-mediated immunity to DMBA has been shown to retard skin tumorigenesis in this system. Resveratrol increased cell-mediated immunity to topically applied DMBA in C3H/HeN mice whereas there was no significant effect on the magnitude of the response in C3H/HeJ mice. The results of this study indicate that resveratrol is a potent chemopreventive agent for DMBA skin tumorigenesis and that it mediates its chemopreventive effects through TLR4.

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Protective function of IL-10 and mast cells in chemical skin carcinogenesis

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Exposure to irritating agents can result in chronic inflammation and skin tumor development. It has been shown that proinflammatory cytokines such as TNF, IL-1, and IL-6 contribute to cutaneous carcinogenesis. Thus, anti-inflammatory signals are thought to be involved in mediating host-protection from skin tumors. However, there is little evidence from *in vivo* studies to support this hypothesis. Here, we subjected mice deficient for IL-10, a prototype anti-inflammatory cytokine, to chemical skin carcinogenesis. We found that IL-10-/- mice exhibit a dramatic increase in the rate of tumor development (e.g. 90% vs. 30% tumor bearing mice). Also, the number of tumors per treatment site was significantly elevated as compared to normal C57BL/6 mice (1.3 ± 0.2 vs. 0.1 ± 0.1 tumors per cm^2 skin at week 18 after tumor induction). Skin mast cells (MCs) are a major source of IL-10 and MC-derived IL-10 has been shown to downregulate skin inflammation in various settings. Therefore, we assessed carcinogen-induced tumor development in MC-deficient $Kit^{W}/Kit^{W\text{-}v}$ mice. Interestingly, $Kit^{W}/Kit^{W\text{-}v}$ mice exhibited similar, albeit less pronounced, responses as IL-10-/- mice, i.e. the induction of skin tumors was significantly increased as compared to normal mice. $Kit^{W}/Kit^{W\text{-}v}$ mice that had been repaired for their cutaneous MC-deficiency showed normal carcinogenesis. These observations suggest that MCs control skin tumor development, at least in part, by their release of IL-10. These findings are in contrast with a recent report showing that IL-10 promotes skin tumor development following UV irradiation. This suggests that the protective effects of IL-10 and MCs may be specific for carcinogen-induced tumors, which is associated with prominent and chronic inflammation. In support of this hypothesis we and others found that mice deficient for TNF, a signature proinflammatory cytokine, exhibit dramatically reduced skin tumor development in response to carcinogen treatment. In summary, these findings provide evidence that IL-10 and MCs contribute to the protection from carcinogen-induced skin tumor development.

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Development of human non-melanoma skin cancer stem cell assays

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In the cancer stem cell (CaSC) hypothesis, tumor growth is dependent upon a sub-population of tumor cells that possess the ability to self-renew; while the more numerous non-CaSC tumor cells have only a limited proliferative capacity. CaSC have now been described in a number of human cancers, including from solid tissues. Yet the study of CaSC in skin squamous and basal cell carcinoma (SCC and BCC), has been hindered by the lack of *in vivo* and *in vitro* assays; which we have now developed. Such assays would depend upon optimal tumor dissociation. We determined that an enzyme cocktail of dispase, collagenase 4, hyaluronidase followed by trypsin and DNase resulted in a cell suspension with up to 70% viability. Initial attempts to recapitulate human SCC and BCC *in vivo* from tumor pieces and cell suspensions were disappointing (5 of 64 SCC, 0 of 42 BCC samples), consistent with previous reports. However, we now demonstrate that SCC growth from tumor pieces can be achieved if first a fibrovascular bed is created by implantation of a glass disc or Gelfoam™ dressing (22 of 25), and from SCC cell suspension if the fibrovascular bed is humanized with primary human fibroblasts (9 of 10). In addition, growth of human BCC *in vivo* from tissue pieces (6 of 8) or cell suspension (2 of 2) was rendered possible after administration of IP etoposide. *In vitro* propagation of tumor cells upon an irradiated 3T3 fibroblast feeder layer, yielded focal colonies. These colonies were spheres tethered to feeder cells, were dependent upon the number of input cells, could be serially passaged, and yielded tumors upon xeno-transplantation. Supporting the hypothesis that the number of colonies formed was related to the number of CaSC input, we determined that MACS® cell separation of tumor cell suspensions using putative CaSC markers led to >10-fold enrichment in colony formation. Currently, both *in vivo* and *in vitro* CaSC assays are being used to screen putative tumor sub-populations in SCC and BCC.

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Oral Polypodium leucotomos extract decreases UV-induced COX-2, accelerates removal of CPDs and 8-ox-dG in Xpc +/- mice

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Chronically irradiated murine skin and UV-induced SCC overexpress the inducible isoform of cyclooxygenase-2 (Cox-2), and Cox-2 inhibition reduces photocarcinogenesis in mice. To determine anti-carcinogenic effects of PL, we fed hairless Xpc +/- mice with 300 mg/kg PL or vehicle alone for 10 days then irradiated with 25 mJ/cm^2 of UVB. By 24h UVB-induced COX-2 levels were increased in both vehicle- and PL-fed mice. Compared to vehicle-fed mice, by 48 and 72h post-UV COX-2 levels were significantly (6-7-fold) lower in PL-fed mice ($p<0.04$ and $p<0.05$, respectively). Compared to vehicle-fed mice, p53 expression and activity (phospho p53^{ser15}) was markedly increased in PL-fed and UV-irradiated mice. PL-fed mice showed marked decrease in UVB-induced inflammation determined by decreased infiltration of neutrophils (MPO-1 + cells) by ~60% ($p<0.001$) and macrophages (CD68 + cells) by ~50% (<0.02) at 24 and 48h. Maximum and comparable CPDs were detected immediately after UVB in vehicle- vs PL-fed skin. By 48h ~ 50% of CPDs were removed in both vehicle and PL-fed mouse skin. However, by 72h 54±5% CPDs remained in vehicle-fed skin vs 31±5% in PL-fed skin ($p<0.003$). The level of 8-ox-dG, a marker of oxidative DNA damage, were decreased before UVB by 36% ($p<0.01$), suggesting that PL reduces the level of constitutive oxidative DNA damage. By 6 and 24h the number of 8-ox-dG (+) cells were 59% ($p<0.01$) and 79% ($p<0.03$) lower in PL vs vehicle-fed mice. These data demonstrate that oral supplementation of PL affords photoprotection by activation of p53 and reduction of acute inflammation through inhibition of COX-2 enzyme as well as by acceleration of UV-induced CPD removal and by reduction of oxidative DNA damage. This may contribute to the ability of PL to reduce photocarcinogenesis by means of systemic therapy.

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Identification of function of RAIDD in UV-induced skin tumor formation

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P53 inducible protein with a death domain (PIDD) is a recently identified protein that forms a signaling complex triggered by UV-induced genotoxic stress. PIDD activation has been suggested to induce, sequentially, two opposing effects. It activates receptor interacting protein (RIP) 1 and the survival factor NF-kB, and then recrtuits and activates caspase-2 via the adaptor protein RIP associated Ich-1/CED homologous protein with death domain (RAIDD), which is believed to result in apoptosis. The balance between these life and death signals has been proposed to act as a DNA damage/repair checkpoint. However, this assumption is based on *in vitro* (mostly over-expression) studies and little is known on the role of PIDD-RAIDD-Caspase-2 / RIP1 function after UV exposure *in vivo*. As the skin is the primary organ affected by UV-induced DNA damage, we initiated studies aimed at characterizing the function of this signaling pathway in the skin. We first verified that PIDD and its molecular partners are expressed in normal, non-irradiated skin. We found that modifying the expression levels of these molecules using siRNA does not affect significantly UV-induced apoptosis nor the rapidity of thymidine dimer excision and DNA repair. We also generated RAIDD deficient mice in the hairless albinos mouse strain and started to address their response to UV treatment. Knockout mice and their heterozygotes littermates were exposed to 80mJ of UVB thrice a week, and the number of skin tumors (squamous cell carcinoma) was assessed every week. Surprisingly, we found that, after 20 weeks of irradiation, mice deficient for RAIDD had significantly less tumor (10.8 vs 1.8). Taken together, these results suggest that the RAIDD caspase-2 signaling pathway does not lead to apoptosis, which is consistent with the observation that apoptosis is not affected in caspase-2 deficient mice. Further follow-up of these irradiated mice and the study of PIDD deficient mice are necessary to further clarify the role of this newly discovered signaling platform in the skin.

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Thrombospondin-2 overexpression in the skin of transgenic mice reduces the susceptibility to chemically-induced multistep carcinogenesis

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We have recently reported stromal upregulation of the endogenous angiogenesis inhibitor thrombospondin-2 (TSP-2) during multistep carcinogenesis, and we found accelerated and enhanced skin carcinogenesis and angiogenesis in TSP-2 deficient mice. To investigate whether enhanced levels of TSP-2 might protect from skin cancer development, we subjected transgenic mice with targeted overexpression of TSP-2 in the skin and wild-type controls to a chemical skin carcinogenesis regimen and compared tumor development. TSP-2 transgenic mice showed a significantly delayed onset of tumor formation compared to wild-type mice, but no inhibition of malignant conversion to squamous cell carcinomas. Computer-assisted morphometric analysis of blood vessels revealed pronounced tumor angiogenesis already in early stages of carcinogenesis. TSP-2 overexpression significantly reduced tumor blood vessels density in transgenic mice but had no overt effect on LYVE-1 positive lymphatic vessels. The antiangiogenic effect of transgenic TSP-2 was accompanied by highly increased number of apoptotic tumor cells in transgenic mice. Our results demonstrate that enhanced levels of TSP-2 in the skin results in reduced susceptibility to chemically –induced skin carcinogenesis and identify TSP-2 as a potent new target for the prevention of skin cancer.

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ΔNp63α modulates keratinocyte growth regulation *via* activation of NF-κB/c-Rel

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Dysregulation of the p53 homologue p63 is observed in a majority of squamous cancers, with *p63* gene amplification and/or overexpression, particularly of the ΔNp63α isoform, reported in squamous cell cancers of the head and neck, lung, cervix and skin. We have previously demonstrated that mimicking ΔNp63α overexpression in cultured primary murine epidermal keratinocytes blocks Ca^{2+}-mediated growth arrest, along with morphological and biochemical differentiation. To gain mechanistic insight into the biological impact of ΔNp63α overexpression, we used a transcription factor profiling system to identify altered transcription factor binding activity in keratinocytes overexpressing ΔNp63α *vs* β-gal. ΔNp63α overexpression activates the NF-κB pathway and results in enhanced nuclear accumulation specifically of the c-Rel subunit of NF-κB, which has been implicated in both solid tumors and hematopoietic malignancies. The c-Rel accumulated in response to ΔNp63α overexpression is transcriptionally active and phosphorylated. Decreasing c-Rel levels with siRNA, or blocking the enhanced translocation of c-Rel in ΔNp63α-overexpressing murine keratinocytes with IκBα superrepressor, restores Ca^{2+}-mediated growth arrest. Elevated levels of ΔNp63α block Ca^{2+}-mediated induction of the CDK inhibitor p21^{WAF1}. Similarly, c-Rel functions as a negative regulator of p21^{WAF1}, as measured by downregulation of a p21^{WAF1} reporter gene. Correspondingly, we show that c-Rel interacts with the p21^{WAF1} promoter in conjunction with ΔNp63α. Consistent with a role for p63 in activating the c-Rel/NF-κB pathway, immunostaining of primary human squamous cell carcinomas of the head and neck revealed associated and expanded expression of ΔNp63 and c-Rel in these tumors. We are currently using microarray-based systems to identify downstream genes influenced by enhanced nuclear c-Rel accumulation in response to ΔNp63α. These findings link two important pathways in cancer development, p53/p63 and NF-κB.

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Notch1 signaling in cutaneous T cell lymphomas

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Notch is a transmembrane receptor comprising four homologues (Notch1-4). The pathway is activated through direct membrane contact with adjacent cells expressing ligands Delta or Jagged and depends on the proteolytic cleavage and release of a signal-transducing Notch intracellular domain (Nic). Notch signaling plays a central role in T-cell development and homeostasis, and pathologic Notch1 signaling has been implicated in the pathogenesis of T-cell leukemias and nodal anaplastic large cell lymphoma. In this study we show that Notch1 and its ligand Jagged are expressed on lymphoma cells in the samples from the patients with mycosis fungoides and primary cutaneous CD30$^+$ lymphoproliferative disorders comprising lymphomatoid papulosis (LyP) and primary cutaneous anaplastic large cell lymphoma (ALCL). Expression of Notch1 and Jagged (but not Delta) was particularly abundant in ALCL whereas in LyP Notch1 was expressed in clusters of atypical cells. Similarly, strongly positive Jagged1 cells tended to be located in clusters. Double-staining for confocal microscopy revealed that Notch1$^+$ cells interact with Jagged$^+$ cells. To elucidate the possible functional role of Notch1 we blocked this signaling pathway by gamma-secretase inhibitors (GSI) that prevent proteolytic cleavage and release of Nic. CTCL- or CD30$^+$ anaplastic lymphoma-derived cell lines SeAx, MyLa, Hut-78 and Karpas expressed Notch1 and treatment with GSI arrested their proliferation accompanied by elevated levels of the negative cell-cycle regulators p21^{Cip1} and p27^{Kip1}. Apoptosis was moderately increased. Moreover, we noticed that GSI-treated cells develop features of macroautophagy including formation of autophagic vacuoles, lysosomal fusion and loss of transmembrane potential in single mitochondria. Taken together, our data indicate the existence of functional Notch1 signaling in cutaneous lymphomas and opens new venues for pharmacological targeting of cutaneous lymphomas by GSI.

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Familial cylindromatosis, Brooke-Spiegler syndrome and multiple famililal trichoepithiliomas: Tumour mapping of 26 patients with CYLD mutations suggest androgen stimulation may play a role in tumourigenesis

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Our objective was to characterise the clinical features and tumour distribution of affected members of two large families with CYLD mutations. We designed a prospective observational study utilising a combination of questionnaire led interviews and clinical examination. Our patients included 50 affected individuals were noted out of 210 members across two families. 26 patients, 12 male and 14 female, were included in the study. Our outcome measures included clinical symptoms, scalp removal, tumour mapping, and associated diseases. We report the clinical features of the largest series of patients with CYLD mutations. A slight female predisposition was noted with a F:M ratio of 1.1:1. 5 females and 1 male had undergone scalp removal, suggesting a more severe phenotype in females. The average age of onset was 16 years (range 8-30). Sites affected by tumours included: Scalp (81%), trunk (69%), pubic region (42%) and ears (24%). Tumour mapping of truncal tumours suggest a predisposition to hair follicles that are stimulated by androgens following puberty. Poor genotype-phenotype correlation is seen in our patients with CYLD mutations. Transmission is variable, with patients with mild phenotypes bearing offspring with a severe phenotype and vice versa. Patients did not show evidence of the associated diseases suggested by novel CYLD knockout mouse models including autoimmune disease, inflammatory bowel disease or a predisposition to cancer. We concluded that CYLD mutations can result in a variety of phenotypes, making genetic counselling difficult. The clinical features and the distribution of tumours on the trunk and pubic regions suggest androgen stimulation may play a key role in tumourigenesis. A significant proportion (24%) of patients underwent scalp removal, highlighting the need for development of non-surgical treatments for this devastating disease.

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Overexpression of RON, a tyrosine kinase receptor, in early stage of arsenic-induced carcinogenesis

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The RON- receptor tyrosine kinase regulated cell proliferation and involved in tumor progression and metastasis. Evidences have showed that increased its activity correlates with increased tumorigenesis in a variety of organs of epithelial origin. Recent study also showed that RON regulated papilloma growth and malignant conversion in skin carcinogenesis. On the other hand, arsenic-induced skin cancer is a multiple-step process, and epidemiological studies suggested the dose-response relationships between cancer risks and the concentration of arsenic in drinking water. The aim of the study was to investigate the expression of RON in arsenic-induced carcinogenesis. HaCaT cell, a spontaneously immortalized human epithelial cell line, was treated with low dose (0.1 and 0.5μM) sodium arsenite for one month. MTT assays were used to observe cell proliferation rate. RNA and protein expression levels of RON were detected by using RT-PCR and western blot. The amounts of RON in arsenic-induced Bowen's disease and squamous cell carcinoma were detected by using western blot and immunohistochemistry.The results demonstrate that the proliferation rates of HaCaT cells were slightly increased after one month of low-dose arsenite treatment and RNA and protein expression of RON were increased after arsenite treatment. Using western blot and immunohistochemistry, we also observed protein levels of RON were increased in arsenic-induced Bowen's disease and squamous cell carcinoma. Therefore, increased RON-receptor activity occurred in the very early stage of arsenic-induced carcinogenesis, and suggested the possible important role of RON- receptor tyrosine kinase in the initiation and progression of arsenical cancers.

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Immunosuppression impairs quantity and quality of peritumoral inflammatory infiltrate in squamous cell carcinoma of the skin

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Incidence of epithelial skin cancer is increasing. Organ transplant recipients (OTRs) are at a much higher risk than immunocompetent patients to develop squamous cell carcinoma of the skin. Histologically, peritumoral inflammatory infiltrate is a frequent feature and is believed to be a correlate of antitumoral immune response. Our aim was to characterize composition of peritumoral inflammatory infiltrate in intraepidermal (actinic keratosis or Bowen's disease) and subsequent invasive squamous cell carcinoma in the same patient in organ transplant recipients (n = 31) and immunocompetent patients (n = 32). Thus, in 126 formalin fixed, paraffin-embedded tissue samples peritumoral inflammatory infiltrate was characterized by CD123+ plasmacytoid dendritic cells and by the T-cell markers CD3, CD4, CD8 and FOXP3. In immunocompetent patients invasive lesions showed higher rates of CD123+ plasmacytoid dendritic cells than intraepidermal forms of squamous cell carcinoma ($p = 0.040$), whereas in OTRs CD123 expression was comparable between invasive and intraepidermal forms ($p = 0.426$). There were comparable CD123+ cell proportions in OTRs and immunocompetent patients ($p = 0.704$). Immunocompetent subjects showed higher CD3+ ($p = 0.015$) and CD8+ ($p = 0.038$) cell proportions across all lesions but comparable CD4 immunoreactivity ($p = 0.169$) to OTRs. In invasive lesions, immunocompetent patients showed higher levels of FOXP3 protein expression than OTRs ($p = 0.049$). In conclusion we found a pronounced inflammatory infiltrate during an individual's tumor progression. Peritumoral inflammatory infiltrate showed higher rates of CD3, CD8 and FOXP3 expression in immunocompetent patients than in OTRs but comparable CD123 expression. This distinct inflammatory infiltrate may contribute to the increased cutaneous carcinogenesis and more aggressive behaviour of squamous cell carcinoma in OTRs.

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Physicochemical characteristics of transdermal ionic liquid-vesicles and its cytotoxicity on human skin cells

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Vesicles are prepared by using phosphatidylcholines (PCs) incorporated Ionic liquid and hydrated with pure water for the first time in this study. Ionic liquid which utilized here are 1-hexadecy-l-3-methylimidazolium bromide (C16MIMBr) and 1-hexadecy-l-3-methylimidazolium fluorophosphates, (C16MIMPF6). Additionally, the saturated zwitterionic phospholipid, hydrogenated soybean phosphatidylcholine (HSPC), and egg phosphatidylcholine (EPC) with unsaturated fatty acid side chains are individually chosen to prepare vesicles. The particle size and zeta potential of binary mixed PCs/ionic liquid vesicles are examined, and storage-stability tests of these ionic liquid-lipid vesicles are also carried out. Transmission Electronic Microscopy (TEM) defined vesicles incorporated ionic liquid as spherical unilamellar structures. Finally, an investigation of the composition effect influencing the cytotoxicity of ionic liquid-vesicles on skin cultured cell lines is performed.

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CSN5 overexpression inhibits UVB-induced epidermal apoptosis *in vivo*

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The COP9 signalosome is an evolutionarily conserved multi-protein complex that is required for light-regulated development in Arabidopsis. CSN5 is the catalytic subunit of the COP9 signalosome, and the CSN5 gene resides on the long arm of human chromosome 8, which is frequently amplified in human squamous cell carcinomas (SCC). Dysregulation of CSN5 has a dramatic effect on diverse cellular functions, demonstrating the critical role that CSN5 plays in maintaining homeostasis. However, the role of CSN5 in skin biology remains unclear. Here we show that CSN5 protein is overexpressed in 60% of human cutaneous SCCs. To better define the roles of CSN5 in stratified epidermis in vivo, we generated K5-CSN5 and K5-CSN5(D151N) transgenic mice that express CSN5 or the catalytically inactive CSN5(D151N) in murine epidermis. CSN5 overexpression results in decreased UVB-induced epidermal apoptosis in vivo, an effect that required CSN5 catalytic isopeptidase activity. However, there was not a difference in the repair rate of UVB-induced cyclobutane pyrimidine dimers. These studies suggest a role for CSN5 in the apoptotic response to UVB-mediated DNA damage and carcinogenesis. CSN5 emerges as a potential therapeutic target for human epidermal neoplasia and light-regulated dermatoses.

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Identification and characterization of a novel centrosomal protein

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Centrosomes function as the major microtubule (MT)-organizing center in most animal cells. During mitosis, they also contribute to cell cycle progression which are regulated by check points, such as G1 and G2/M check points. The G1 check point is organized in a p53-dependent manner. On the other hand, G2/M checkpoint is mediated in either a p53-dependent or a p53-independent manner. Previous reports showed that some centrosomal proteins, including Pericentrin2, Centrin2, or, GCP2, induced G1 arrest and inhibited the progression from G1 into S phase, when they were knocked down. However, the relationship between cetrosomal proteins and cell cycle is obscure. Recently, we find a novel centrosomal protein and tentatively named it p70, which was calculated molecular weight of about 70,000. In this study, we showed the roles and modes of action of p70 through cell cycle. Northern and Western blot analyses showed that p70 was ubiquitously expressed in several organs. Immunofluorescence microscopy demonstrated that endogenous p70 as well as GFP-fused p70 was localized at centrosomes in cultured cells. When we knocked down p70, the mitotic index was reduced, whereas the quantification of BrdU incorporation was unexpectedly not reduced in both hTRET-RPE1 and HeLa cells, which contained active and inactive p53, respectively. The flow cytometry experiments revealed that the knockdown of p70 induced the G2 arrest and inhibited the progression from G2 into M phase in both cell lines. Thus, these results indicate that p70 is a widespread centrosomal protein and suggeste that p70 is implicated in G2/M checkpoint in a p53-independent manner.

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ADAM-10, but not MMP14 is critically involved in CD44 shedding from human melanoma cells

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CD44 belongs to a family of plasma membrane glycoproteins involved in adhesion processes and cell movement. It is the functional cellular receptor of hyaluronic acid (HA), a major component of the extracellular matrix. The interaction of HA and CD44 on malignant and non-malignant cells has impact on cell proliferation, cell motility or cell survival. We showed, that CD44-HA interactions promote cell proliferation of malignant melanoma (MM) cells. CD44 can be shed from the cell surface by proteolytic cleavage. Studies with MM cell lines demonstrated, that the promoting effect of HA on cell proliferation could be abolished by secretion of soluble CD44 into the culture supernatant *in vitro* and *in vivo*. Therefore, the mechanism of CD44 shedding is of major interest for tumor biology and consequently putative shedding proteases are studied towards their ability to cleave CD44 from the surface of tumor cells. Among them MMP14, ADAM10 and ADAM17 are the most interesting candidates and here we demonstrate their expression in human melanoma tissues. Further we show the involvement of ADAM-10 but not MMP14 or ADAM-17 in the shedding of native CD44 from human MM cell lines using inhibitor studies and siRNA techniques. All, ADAM10-, ADAM17- and MMP14-expression could be inhibited by siRNA techniques on mRNA and protein levels, but only ADAM-10 blocking was able to reduce the constitutive CD44 shedding from the MM cells. These data support own inhibitor studies indicating, that ADAM-10 is the critical protease for the release of CD44 from human melanoma cells. Using immunfluorescense we show a co-localization of CD44 and ADAM10 giving structural support for putative direct interaction. Overexpression of ADAM10 in the MM cells enhanced solCD44 shedding significantly. Compared to earlier studies using recombinant MMP14 and CD44 we conclude, that ADAM-10 but not MMP14 is the native and essential protease for CD44 shedding from MM cells.

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Tumor immune escape by the loss of homeostatic chemokine expression

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The keratinocyte-specific chemokine CCL27 plays a critical role in the organization of skin-associated immune responses by regulating T cell homing under homeostatic and inflammatory conditions. Here we demonstrate that human keratinocyte-derived skin tumors may evade T cell-mediated immune responses by down-regulating expression of CCL27 through activation of EGFR–Ras–MAPKsignaling pathways. Compared with healthy skin, CCL27 expression was progressively lost in transformed keratinocytes of actinic keratoses, basal and squamous cell carcinomas. *In vivo*, precancerous skin lesions and cutaneous carcinomas showed significantly elevated levels of phosphorylated ERK compared with normal skin, suggesting the activation of EGFR Ras signaling pathways in keratinocyte-derived malignancies. *In vitro*, exogenous stimulation of the EGFR–Ras signaling pathway through EGF or transfection of the dominant-active form of the Ras oncogene suppressed whereas an EGFR tyrosine kinase inhibitor increased CCL27 production in keratinocytes. In mice, neutralization of CCL27 led to decreased leukocyte recruitment to cutaneous tumor sites and enhanced primary tumor growth. Collectively, our data identify a mechanism of skin tumors to evade host antitumor immune responses.

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The association between actinic keratosis and cell-cycle protein expression

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Actinic keratosis (AK) is a precancerous skin lesion or an early phase in the evolution of squamous cell carcinoma (SCC) which arises in fair-skinned people in body areas long-term sun exposed. AK frequency correlates with cumulative UV dose. DNA analysis of the cells within AKs shows characteristic UV-induced mutations in key genes. Literature data indicate that appropriate cell-cycle proteins expression is critical for the normal cell proliferation while the abnormal control of the cell cycle is closely linked to carcinogenesis. The aim of present study was to determine the expression of selected cell-cycle proteins in AK. The study group consisted of 7 histologically confirmed AK samples and 5 skin biopsies taken from normal unaffected skin (control group). In all samples immunohistochemical staining was performed using antibodies directed against p53, p63, p16, p18, p21 and p27.Then the mean rate of positively stained cells for selected protein (nuclear pattern of staining) was calculated in each case. Expression of p53, p63, p16, p18, p21 and p27 was found in all AK cases and the mean rates (85% for p53, 60% for p16, 90% for p18, 50% for p21 and 85% for p27) of positively stained cells were significantly higher when compared to control group (2% for p53, 1% for p16, 2% for p18, 0% for p21 and 0% for p27), except p63 which rates did not differ between AK (90%) and normal skin specimens (90%). The obtained results indicate increased expression of analyzed cell-cycle proteins and suggest its role in AK development.

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***In vitro* evidence for a role of ultraviolet (UV)-A radiation in the pathogenesis of melanoma**

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Melanoma cell lines exposed to UVA irradiation show increased lactate levels and increased levels of the transketolase-like-1 enzyme, which is an important enzyme of the pentose phosphate pathway. These findings indicate an increase of aerobic glycolysis after repetitive UVA exposure. This phenomenom has been observed in many carcinomas and is known as the Warburg effect. Due to the fact that enhanced glucolysis is a considerable part of the Warburg effect, we veryfied this hypothesis by measuring other endpoints to approve this effect focusing on melanoma progression and invasiveness after repetitive UVA exposure. For this purpose we used three different melanoma cell lines with different progression levels: WM 35 (radial growth phase), WM 115 (vertical growth phase) and 1205 LU (metastasizing melanoma). For a time period of three weeks, cells were exposed to UVA (6J/cm2) three times daily. Applying FACS measurements we assessed the adhesion molecule L1 (CD171) which is involved in tumor migration. Applying western blot we assessed Proteinkinase B (Akt) which is a marker for melanoma progression. Due the fact that enhanced carcinogenesis is accomplished by reduced oxidative phosphorylation, we also measured oxygene consumption of melanoma cells after UVA exposure using the Clark Type electrode. Furthermore we assessed the mitochondrial membrane potentiol applying FACS analysis. UVA exposure results in increased L1 protein levels, suggesting increased invasiveness of melanoma cells after UVA exposure. Western blot analysis showed increased protein levels of phosphorylated Akt (pAkt) giving evidence to UVA exposure being involved in melanoma progression. UVA exposure of melanoma cells declined respiration compared to the sham irradiated cells. This effect could be reversed by addition of 75µg/ml caffeine. In line with these results, mitochondrial membrane potential (JC-1 measurement) was increased. Taken together, we present clear evidence for the involvement of repetitive exposure of sublethal UVA radiation on melanoma progression and invasiveness.

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Identification of genomic and epi-genomic lesions in squamous cell carcinomas using expression profiling of quiescent cultured keratinocytes

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Non-melanoma skin cancers are the commonest cancers in the UK and present a major public health problem. Squamous cell carcinoma (SCC) is estimated to affect over 11,500 people in the UK each year. In order to understand the molecular events that contribute to this carcinoma we compared global gene expression of primary cultures of SCC keratinocytes (n=9) to primary cultures of epidermal keratinocytes (n=7) using Illumina gene expression arrays. Criteria based on absolute expression level, relative fold-change and statistical significance identified 289 unique genes specific for SCC cells. 56 % of SCC-specific genes with known function harbour a documented role in carcinogenesis, thus validating the filtering criteria as highly suitable for the identification of cancer-associated genes. We then applied the identified set of 289 genes to rank all tumours according to their relative deviation from normal across the expression of all genes. This analysis predicted the presence of highly aggressive vs. non-invasive SCC cells. Indeed, invasiveness in all SCC samples characterized by Transwell assays correlated strongly with the degree of gene dysregulation (% of invading cells vs. number of highly dysregulated genes was 30.4 / 194; 24 / 156; 12 / 57; 11 / 52 in n = 4 primary cultures, respectively; r = 0.99). Chromosomal mapping of all 289 differentially expressed genes showed that 75% co-localise with at least one other gene to the same or adjacent cytogenetic band. When compared with SNP mapping data, 39% of the gene clusters correspond to regions that show either loss or gain of genetic material, suggesting that the co-ordinate expression of gene clusters not mapping to these regions is being influenced by epi-genetic mechanisms. In conclusion, we describe the novel use of expression profiling of cultured keratinocytes to (a) identify both genomic and epi-genomic lesions in SCC and (b) predict tumour invasiveness.

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Identification of a potential melanoma tumor suppressor gene with differential analysis of transcripts with alternative splicing technology

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Melanoma is an aggressive cancer with a propensity to cause widespread metastatic disease. New methods based on the study of global gene expression are needed in order to understand the events underlying melanoma carcinogenesis. Alternative splicing events in benign nevus and metastatic melanomas were studied using a new global gene profiling technology: Differential Analysis of Transcripts with Alternative Splicing (DATAS). This genomic analysis confirmed by quantitative PCR assays revealed that the expression of a long isoform of BCSC-1 was consistently downregulated in patients with metastatic melanoma. The analysis of BCSC-1 expression in a cohort of 70 skin biopsies from patients with benign nevus, atypical nevus, primary melanoma or metastatic melanoma revealed that BCSC-1 expression was decreased in melanoma patients in comparison with healthy donors. Comparable results were obtained in melanoma cell lines. We show that ectopic expression of the long isoform of BCSC-1 in several human melanoma cell lines decreases their proliferation and induces a block in the G2/M phase of the cell cycle. In contrast the short isoform of BSCS-1 is not downregulated in tumors and does not affect cell proliferation. Invasion assays are ongoing to establish if BCSC-1 regulates only proliferation or affects also invasion potential. Using immunofluorescence analysis, BCSC-1 appears to be predominantly a cytosolic protein. Experiments in the B16 murine melanoma model demonstrate that the ectopic expression of the long isoform of BCSC-1 decreases metastasis <>in vivo. *We conclude that the long isoform of BCSC-1 is downregulated in metastatic melanoma and may have a role in the pathogenesis of melanoma.*

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Differential expression of TRAF1 aids in cutaneous CD30-positive lymphoproliferations and transformed mycosis fungoides

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Lymphomatoid papulosis (LyP), primary cutaneous anaplastic large cell lymphoma (cALCL), and cutaneous infiltrates of systemic anaplastic large cell lymphoma (sALCL) and cases of transformed mycosis fungoides are CD30-positive lymphoproliferative disorders of the skin that overlap clinically, histopathologically, immunophenotypically, and genetically but differ considerably in their prognosis. In particular, lesions of LyP regress spontaneously, while those of cALCL and sALCL persist and may progress and spread to extracutaneous sites. In contrast to patients with cALCL, LyP patients do not benefit from an aggressive radio- and/or chemotherapeutic approach. We generated a novel TRAF1 antibody that recognizes a formalin-resistant epitope (Ber-TRAF1A) and investigated the expression of TRAF1 (tumor necrosis factor receptor (TNFR)-associated factor 1), an intracellular component of TNFR signaling, in LyP and ALCL. We could show a strong TRAF1 expression in the tumor cells of most LyP cases (42/49, 84%). In contrast, tumor cells of primary and secondary cALCL revealed TRAF1 expression in only a few cases (3/41, 7%) as shown for sALCL without skin manifestation. However, cases of transformed Mf exhibiting tumor cells CD30+cells show TRAF1 expression in most cases (16/20.) The data indicate that TRAF1 expression reliably distinguishes LyP from primary or secondary cALCL, which is of crucial diagnostic importance with impact on the treatment decision for for patients with cALCL and LyP.

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DMBA-induced epidermal hyperplasia and inflammation in the absence of keratinocyte-derived VEGF-A

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We have previously shown that the inactivation of Vascular Endothelial Growth Factor A (VEGF) in murine epidermal keratinocytes (KC) abrogates papilloma development induced by 9,12-dimethyl 1,2-benzanthracene (DMBA), applied as both tumor initiator and promoter. Instead, the treated skin of mutant mice showed evidence of inflammation, epidermal hyperplasia and a parakeratotic stratum corneum. To investigate the mechanism(s) underlying these observations we have now focused on the early phases of carcinogen treatment. DMBA (50µg/50µl ethanol) was applied to the backs of 1 day old mice and then once a week for 4, 6, 10 and 15 weeks. Treated skin was harvested for histological analysis one week after the last treatment. After only 6 treatments, mutant mice developed significant epidermal hyperplasia, loricrin and fillagrin expression appeared to be increased, and CD31 stained vessels were situated significantly further from the epidermal-dermal junction. In addition, accumulation of FITC-avidin staining cells, presumably mast cells, was significantly enhanced under the epidermis of mutant mice at this time point. This vessel free zone and the increased FITC-avidin positive cells were present at all the later time points as well. Confirming our earlier results, mutant mice did not develop papillomas, but their skin displayed extreme follicular hyperplasia, parakeratosis, and hyperkeratotic cysts after 15 treatments, and many had an increased invasion of I-A positive cells into both the dermis and epidermis. In contrast, the control mice developed a total of 14 papillomas/20 mice, and their uninvolved skin appeared normal until week 15 when it merely displayed mild focal hyperplasia. These preliminary data indicate that carcinogen treatment in the absence of KC-derived VEGF leads to an early and sustained regression of the vasculature in the vicinity of the epidermis. The loss of the upper vascular plexus may be responsible for the compromised epidermal differentiation, the enhanced inflammatory cell infiltrate and the failure to develop papillomas.

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A novel mouse model of enhanced NF kappa B activity

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The importance of NF kappa B in inflammation and tumorigenesis is well established. Most inflammatory cytokines activate NF-kB and once activated NF-kB can upregulate not only proteins important for the innate and adaptive immunity, but also proteins important for cellular proliferation and apoptosis. It was the role of NF-kB as a pro-survival factor that initially led to its link to a long list of cancers, including those of the skin. However it is now known that NF-kB can promote carcinogenesis in a number a ways, including its effects on proliferation, survival, angiogenesis, and metastasis. The genetic study of NF kappa B mutants have been limited in mouse models due to embryonic lethality and redundancy among members of the NF kappa B family of proteins. We have constructed a novel mouse model for activated basal NF-kB, which will allow us to study the role of NF-kB in inflammation and cancer of the skin. This mouse has a mutant form of the NF-kB subunit p65 knocked-in, which leads to constituitive activation of NF-kB. These mice develop hyperplasia and dysplasia of the skin. Although homozygous knock-in mice die within 10 days of birth, heterozygotes live for over a year. Remarkably, crossing the homozygous PD knock-in with mice lacking the TNF receptor 1 leads to a complete rescue of the hyperinflammatory phenotype. However these mice, probably as a result of continuous upregulation of basal NF-kB activity in all cells and tissues, begin to develop spontaneous tumors in a wide variety of tissues, suggesting that NF-kB activation, even in the absence of additional factors (including overt signs of inflammation), is able to prime cells to transformation. This suggests that despite previous reports suggesting an inhibitory role for NF-kB in the development of skin tumors, activation of NF-kB in skin cells may lead to an increased incidence of skin tumors.

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A role for the receptor tyrosine kinase, Axl, in squamous cell carcinoma invasion and survival

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Axl is a transmembrane receptor tyrosine kinase (RTK) whose downstream targets include the survival protein, Akt. We have identified by Affymetrix chip analysis a 12-fold increase in Axl expression in a squamous cell carcinoma (SCC) cell line compared to a keratinocyte line and demonstrated that Axl is over-expressed, specifically, in SCC (Green et al, Br J Cancer, 2006). In this study, we examined the role of Axl in SCC invasion and survival. The SCC line, MET1, was transduced with the pSuperior retroviral vector alone or two distinct shRNA sequences resulting in 76% and 97% Axl mRNA knockdown, respectively, by real-time PCR, also confirmed at the protein level. Migration and invasion were assessed using in vitro scratch assays and 3-D collagen gels. Apoptosis was induced using UVB and expression of pro- and anti-apoptotic markers was analyzed and quantified using FACS, confocal microscopy and Western blotting. Axl shRNA significantly inhibited migration and invasion correlating with the degree of Axl knock-down. EGF receptor expression and phosphorylation, known mediators of migration, were also markedly inhibited in Axl shRNA cells. Phosphorylation of Akt on Ser473 and its downstream pro-apoptotic substrate Bad on Ser136, resulting in Bad deactivation, were sustained during UV treatment in vector alone control as compared to Axl shRNA cells. Axl knockdown cells were more sensitive to UV-induced apoptosis and exhibited increased caspase 3 (3-fold) and 8 (2-fold) activity with concomitant cytochrome c release. Abrogation of Axl expression induced mitochondrial activation of Bax (confocal microscopy and IP of active monomer) and Bak (FACS staining). These data demonstrate that Axl plays a major role in SCC invasion, probably linked to EGF receptor signalling, and SCC survival, by acting as a negative regulator of the mitochondrial apoptosis pathway. The Axl pathway is a target for chemotherapeutic development in high-risk cutaneous SCC.

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Recruitment of long term engrafted fetal microchimeric cells in chemically induced maternal skin carcinogenesis

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Microchimerism has been described in several situations such as solid organ transplantation, red blood cell transfusion or pregnancy. We and others have recently described the capacity of solid organ donor derived cells to participate and in rare cases to form various benign and malignant skin tumours. During all human pregnancies fetal cells enter maternal circulation and may persist for decades. In the present study, we wondered whether fetal derived cells acquired during pregnancy by mothers could have a similar role in maternal skin tumours in an animal model. In female mice that gave birth to EGFP transgenic pups, chemically induced skin tumours were created by two-stage carcinogenesis using DMBA and TPA. 14 weeks after the protocol and 40 weeks after gestation, animals were sacrificed and tumours and healthy skin were collected. EGFP+ fetal cells were detected in 9 out of 12 (75%) tumours and in none of the normal skin samples from 11 mice. The fetal cells were more frequent in tumours as compared to healthy skin from the same mice (p=0.0034). No tumour entirely derived from fetal cells. Among EGFP+ fetal cells, 36% expressed endothelial and 17% leukocyte markers. Of note 10% of fetal cells expressed cytokeratin using an anti -pan-cytokeratin antibody but none of them expressed keratin 14 or keratin 1, suggesting that they were not keratinocytes. Other markers such as alpha smooth muscle actin did not stain any fetal cell either. In conclusion, long term engrafted fetal microchimeric cells are recruited to maternal skin tumours and are part of the tumour stroma. These results are in line with previous studies describing fetal progenitor cell recruitment in maternal lesional tissues.

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Mechanisms of increased invasion of cutaneous squamous cell carcinoma with loss of type VII collagen

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Type VII collagen (ColVII) is the main component of anchoring fibrils, attachment structures within the basement membrane. Mutations in the ColVII gene cause the severe blistering disorder, recessive dystrophic epidermolysis bullosa (RDEB), associated with increased risk of metastatic squamous cell carcinoma (SCC). We used siRNA to examine the effect of loss of ColVII on SCC tumorigenesis in two SCC cell lines. Experiments included migration and invasion assays, generation and immunostaining of 3-D epidermal models and Illumina gene expression arrays. ColVII-knock-down cells (siCOL7) showed increased expression and activity of MMP-2, as well as increased migration and invasion, when compared to control. siCOL7 cells had reduced and disorganised expression of epithelial differentiation markers including keratin (K)10, K2e, transglutaminase, and involucrin in 3-D epidermis. Changes in the epithelial-mesenchymal transition (EMT) markers E-cadherin (↓), vimentin (↑), and in the vimentin-associated αvβ3 integrin (↑) were also observed. Analysis of Illumina data revealed a significant increase in chemokine, CXCL10 (+5.3) and phospholipase, PLCB4 (+7.0) gene expression. Increased expression of PLCβ4, CXCL10, and its receptor, CXCR3, were confirmed by immunostaining in siCOL7 invading cells. A CXCR3 blocking antibody reduced siCOL7 cell migration to control levels. Our findings suggest that loss of ColVII increases cutaneous SCC invasion through a mechanism that involves disorganised keratinocyte differentiation and EMT linked to CXCR3/PLC signalling and provides an explanation for the accelerated carcinogenesis in RDEB.

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Gene expression analysis of tazarotene's efficacy against a murine basal cell carcinoma cell line

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Basal cell carcinoma (BCC) is the most common cancer, which is generally caused by hyperactive Hedgehog (HH) pathway activation. We have previously shown that the retinoid tazarotene can inhibit BCC carcinogenesis by approximately 85% in our murine model of BCC. Tazarotene's effects against BCC carcinogenesis are most likely via retinoic acid receptor (RAR) γ-mediated transcriptional regulation of target genes in BCC tumor cells. To identify downstream target pathways of tazarotene's action, we treated the murine BCC cell line ASZ001 with 10 µM tazarotene, a concentration that inhibits ASZ001 cell proliferation, or DMSO vehicle, and carried out Affymetrix gene expression analysis on cells treated for 10 h or 24 h. Differentially-expressed gene targets were validated by Taqman Real-time PCR analysis and their expression compared to that in a cell line Med1 (established from a medulloblastoma that grew in a *Ptch1*+/- mouse) that was not growth-inhibited by tazarotene. We found that at 10 h, many known targets of all-trans retinoic acid (ATRA) signaling are upregulated, consistent with the idea that tazarotene can regulate RAR-mediated transcription in these cells. HH pathway target genes, *Gli1* and *FoxM1* were down-regulated and genes involved in skin homeostasis were differentially regulated. ASZ001 cells treated for 24 h showed down-regulation of cell cycle effectors, suggesting that tazarotene causes cell cycle arrest in ASZ001. Differential expression of genes involved in the insulin pathway was seen at both time-points suggesting that tazarotene inhibits this pathway. Since we find that a small molecule inhibitor of IGF/IF signaling also inhibits growth of our murine BCC cell line, inhibition of this pathway may well be a primary mechanism for tazarotene's anti-BCC effect.

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Analysis of cutaneous squamous cell carcinoma by array-based comparative genomic hybridization

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Complete understanding of the genetic basis of cutaneous squamous cell carcinoma (SCC) is lacking. To gain further insight into genetic changes that contribute to its pathogenesis, we performed array-based comparative genomic hybridization (aCGH) on a panel of 25 SCCs. Unsupervised hierarchical clustering determined that our samples grouped into two distinct subsets; one displayed numerous chromosomal aberrations, while the other showed fewer changes. Using a modified t-test built into Significance Analysis of Microarrays (SAM) software, 61 loci showed copy number changes in one group compared to the other. Gains were more commonly observed than losses. Gains at 18q21.1, 20p11.21, 9q34-34.3, 13q34, 21q22.3, 1p36.32, and 2q37.3 were seen in 44% or more of our samples. Losses at 4p14-p12 and 7q11.23 were observed in up to 28% of SCCs studied. Copy number gains were seen at the RXR-alpha locus (9q34.3) in 52% of our tumors. High-level amplification was detected at 7p12 in 2 samples (EGFR locus), 11q13 in 2 samples (Cyclin D1 locus), 5p15.33 in 2 samples (TERT locus), and 18q21.1 in 3 samples (SMAD7 locus). Overall gains at the SMAD7 locus were found in 52% of our tumors. Furthermore, deletion of the TGF-beta receptor II (TGFBR2) locus was seen in 12% of SCCs examined. Given that SMAD7 and TGFBR2 influence TGF-beta signaling, these findings indicate that genomic aberrations involving genes in this pathway are common in SCC and may contribute to tumor pathogenesis. Similarly, copy number changes commonly were seen at loci harboring nucleotide excision repair (NER) pathway genes, and at loci encoding genes involved in p38 MAPK signaling. Our results suggest that cutaneous SCCs have variable propensity for developing chromosomal copy number aberrations. However specific loci and pathways may be targeted and influence tumorigenesis in a subset of tumors.

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Inhibition of Fyn-induced cutaneous neoplasia by Srcasm

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Src-family tyrosine kinases (SFKs) are conserved non-receptor tyrosine kinases that play important roles in regulating cell proliferation and adhesion. Srcasm is a known SFK substrate that downregulates levels of activated SFKs. We have generated two independent transgenic mouse lines bearing a keratin 14-Fyn Y528F transgene, which expresses an activated mutant of Fyn; these mice exhibit epidermal hyperplasia and spontaneous formation of cutaneous tumors in a C57/B6 background, which is typically tumor resistant. The K14-Fyn Y528F mice exhibit hyperplasia more frequently than controls ($p < 0.0001$) or K14-Fyn transgenic mice ($p=0.09$); the K14-Fyn Y528F mice also develop spontaneous cutaneous tumors more frequently than controls ($p= 0.0007$) and K14-Fyn mice ($p=0.0016$). The cutaneous transgenic tumors exhibit histologic features consistent with well-differentiated squamous cell carcinoma (SCC). Therefore, we analyzed the levels of activated Src-kinases and Srcasm in the murine tumors and homologous human tumors. Western blot analysis of lysates from human cutaneous SCC and unremarkable epidermis demonstrated that human SCCs exhibit increased SFK activity and decreased Srcasm levels compared to unremarkable epidermis. Immunohistochemical analysis of well differentiated human cutaneous SCC (N=10) demonstrates that tumor cells exhibit increased SFK activity and decreased Srcasm levels compared to unremarkable epidermis; this data confirms the western blot data. Immunohistochemical data from the murine tumors corresponded with the human tumors. To evaluate the effect of Srcasm upon tumor formation in vivo, we generated double transgenic mice with K14-Fyn Y528F and K14-Srcasm trangenes. Co-expression of the Srcasm transgene decreased the incidence of SCC formation ($p=0.05$) in the Fyn Y528F line. These data support the hypothesis that cutaneous SCC formation is associated with increased levels of SFK activity and that increasing levels of Srcasm can inhibit tumor formation.

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Resveratrol suppresses UVB-induced skin carcinogenesis by modulating epithelial to mesenchymal transition (EMT) through Transforming Growth Factor-β2 (TGF-β2)

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EMT defines a process crucial for the formation of new tissues and organs during embryogenesis, and it is also considered to be a critical determinant of cancer invasion and metastasis. Resveratrol (trans-3,4′,5-trihydroxystilbene), a phytoalexin found in grape skin, arrests the growth of human epidermoid squamous cell carcinoma A431 cells, lacking functional p53. Here we show that the *in vitro* invasiveness and migratory capacity of A431 cells is largely abrogated by resveratrol, and is associated with increased expression of the epithelial cell marker, E-cadherin and downregulation of the mesenchymal marker, alpha smooth muscle actin. In addition, transforming growth factor (TGF)-β2, p-Smad 2/3, and p-ERK1/2 are decreased in resveratrol-treated cells. These responses are all reversible by pretreatment of the cells with TGF-β2. In further studies we assessed the *in vivo* effect of resveratrol on EMT mediators in both p53 null and p53+/-/SKH-1 hairless mice. Oral administration of resveratrol inhibited UVB-induced skin tumorigenesis. At week 25, resveratrol-treated mice showed a 43% reduction in skin tumor number and a 79% decrease in tumor size as compared to UVB-irradiated control mice. Furthermore, the number of SCCs in the resveratrol-treated mice was 70% less than in the UVB-only group. Immunohistochemical studies revealed TGF-β2 in the basal layer of normal human skin, whereas it was overexpressed in both human and UVB-induced murine SCCs, suggesting the involvement of TGF-β2 in the pathogenesis of SCCs. TGF-β2 expression was down-regulated and E-cadherin was increased in SCCs excised from resveratrol-treated mice, suggesting that resveratrol may suppress UVB-induced skin tumorigenesis and malignant tumor progression by modulating EMT mediators and diminishing the expression of TGF-β2.

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Anti-cytokeratin enrichment increases the sensitivity of CTC detection in patients with metastatic breast cancer

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Circulating tumor cells (CTCs) can be detected in various cancer patients' blood samples and may provide useful clinical information for screening, prognostication, monitoring cancer patients during a course of treatment. CTCs are very heterogeneous. Anti-EpCAM based enrichment method may loss tumor cells that have no or low EpCAM. We developed an anti-cytokeratin based method for CTC enrichment and detection. We have successfully applied it to metastatic breast cancer patients' blood samples and obtained superior results. In this study, anti-cytokeratin alone or in combination with anti-EpCAM antibodies linked to microbeads was used for CTC enrichment. Fluorescence labeled antibodies (anti-cytokeratins, anti-CD45), DAPI and HE staining were used for CTC identification. An automated image analysis platform Ariol® SL-50 was used for image capture and characterization of CTCs on slides. CellSearch™ System was used as a control method. The blood samples from metastatic breast cancer patients and normal individuals were processed with our method and CellSearch™ System. Our method showed a significantly higher CTC detection rate (51% vs. 30%) and a wider dynamic range (1-571 vs. 1-270) than CellSearch™ system. Some CTC clusters were detected by our method but not in the CellSearch™ system. No CTCs were detected in blood samples from healthy individuals. The high quality combined images from three fluorescent channels plus brightfield on the same cells were successfully applied to our auto scanning and imaging system for cell analysis. Our data suggested that the four channel capability (three for fluorescence and one for brightfield) cell analysis method can identify tumor cell better. CTC enrichment with anti-cytokeratin alone or in the combination of anti-EpCAM and anti-cytokeratin antibodies increases the sensitivity of CTC detection. Changes of CTC counts in individual patients blood correlate over time with changes of CA27.29 levels.

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Molecular characterization of tumor precursor cells during Noggin-mediated hair follicle tumorogenesis

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Bone morphogenetic proteins (BMPs) are members of the transforming growth factor-beta (TGF-beta) superfamily that play pivotal roles in the control of skin development, hair growth and stem cell maintenance. Here we report that overexpression of the physiologic BMP antagonist Noggin under control of a Keratin 14 promoter leads to de novo formation of bud-like structures from the upper outer root sheath in postnatal hair follicles, followed by formation of trichofolliculomas. To determine the differences in gene expression between the neoplastic bud epithelium containing the tumor progenitor cells and the bulge of telogen hair follicles containing normal epithelial stem cells, laser capture microdissection (LCM), global microarray and real-time PCR analyses were employed. In contrast to the telogen bulge, cells residing in the neoplastic epithelial buds showed elevated expression levels of several components of the Wnt and Shh signaling pathways (Wnt10b, Wnt5b, Tcf4, Shh, Ptch1, Ptch2); growth factor receptors and transcription regulators (Edar, Klf4, Dlx4, Dlx2, Krox20, Krox24, Hes2, Fhl2, Idb3, Idb4, Sox2, Sox13), while expression levels of the Wnt inhibitors Dickkopf-1 and 2 were downregulated. In addition, expression of genes encoding the extacellular matrix degrading enzymes (Adam18, Adamts1, Catepsin C) was increased in the neoplastic epithelial buds versus the telogen bulge. Our data demonstrate that inhibition of the BMP signaling pathway in the hair follicle epithelium results in abnormal activation of the pro-oncogenic signaling pathways and extracellular matrix degrading enzymes, suggesting a complex regulation of the initiation phase of the neoplastic process in the hair follicle keratinocytes by BMP antagonists.

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p38 MAPK can function as a tumor suppressor by regulating JNK-c-Jun proliferation signaling in UVB-induced skin tumorigenesis

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p38 mitogen-activated protein kinase (MAPK) is activated in response to a wide range of extracellular stimuli, including UV irradiation, growth factors, and inflammatory cytokines, and implicated in cell growth and survival, inflammation, differentiation, development, and apoptosis. We have previously demonstrated that p38 MAPK activation in UVB-irradiated normal murine keratinocytes results in cyclin D1 upregulation and that inhibition of p38 abrogates UVB-induced cyclin D1 expression, indicating its growth-promoting role in normal keratinocytes. In contrast, p38 MAPK may function as a tumor suppressor in tumor tissue. Oral administration of the p38 MAPK inhibitor SB203580 (50 mg/kg body weight, twice a week) resulted in accelerated skin tumor growth in $p53^{-/-}$/SKH-1 mice following chronic UVB irradiation (180 mJ/cm^2 twice a week). At week 22, inhibition of p38 caused a 4-fold increase in skin tumor number and a 3-fold increase in average tumor size compared to that occurring in untreated UVB-irradiated mice, suggesting that p38 MAPK may function as a tumor suppressor during UVB-induced skin carcinogenesis. To probe the anti-proliferative role of p38, we inhibited p38 MAPK either pharmacologically or genetically which resulted in upregulation of JNK kinase using c-jun as a JNK kinase substrate in human squamous cell carcinoma (SCC) cells with mutant p53. Endogenous levels of cyclin D1, Cdc25B/C, COX-2, and survivin increased in association with p38 inhibition, suggesting that p38 suppresses the JNK-c-Jun axis for proliferative signaling in human SCC cells. These in vitro results were confirmed in vivo in UVB-induced SCCs and adjacent skin that showed increased JNK-cJun activity, which was further augmented by SB203580. Our data demonstrate that p38 MAPK functions as a tumor suppressor and that crosstalk exists between p38 and JNK signaling pathways during UVB-induced skin carcinogenesis.

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Defective autophagy as a mechanism for the survival of cancer cells

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Macroautophagy is a process for lysosomal degradation of cellular organelles and long-lived proteins based on the formation of an autophagosome, a double-membrane cytosolic vesicle, in response to various types of environmental stress. Defective macroautophagy has been linked to neurodegenerative diseases and cancer. Autophagy has been found to participate in certain forms of cell death, termed Type II programmed cell death and mammalian target of rapamycin (mTOR) is implicated as an upstream negative regulator of the autophagic process. Here we show that resveratrol, a natural phytoalexin, induces a senescence-like state in human epidermoid squamous cell carcinoma (SCC) A431 cells, associated with expression of senescence-associated β-galactosidase (SA-β-Gal) activity. Resveratrol-induced senescence is mediated by copper-dependent generation of reactive oxygen species (ROS), leading to the accumulation of senescence-like apoptosis-resistant A431 cells. Pretreatment with neocuproine, a copper chelator, blocked ROS-mediated senescence and inhibited SA-β-Gal activity. Interestingly, we observed an increase in lysosomal contents in senescent cells, suggesting an aberration in the lysosome-mediated degradation pathway. Further studies verified blockade of autophagolysosome formation in senescent cells, as assessed by accumulation of lipidated light chain 3 (LC3II) of microtubule-associated proteins, a well-characterized autophagic marker. Resveratrol-mediated suppression of autophagy involved mTOR activation and the mTOR specific inhibitor rapamycin restored autophagic activity, reflected by co-localization of autophagosome and lysosome as well as increased cell death in rapamycin-treated cells. These data indicate that resveratrol induces autophagic defects via mTOR, contributing to the survival of cancer cells, and agents targeting autophagy-lysosomal degradation may prove effective in cancer treatment.

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The isolation and characterization of non-melanoma skin cancer stem cells

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Recent studies of leukemias and some solid tumors have identified a population of cancer cells that share some characteristics of tissue-specific stem cells. To date, cancer stem cells have not been identified in non-melanoma skin cancer. Using a mouse model of skin cancer, we provide evidence for the existence of cancer stem cells that generate and maintain squamous cell carcinomas (SCC). Initially, we isolated cells from mouse SCCs on the basis of CD34 and CD49f (α6-integrin) expression, a strategy previously used to isolate stem cells residing in the bulge region of mouse hair follicles. Using an *in vivo* tumor formation assay, we found that $CD34^{+}/CD49f^{high}$, $CD34^{+}/CD49f^{-}$ and $CD34^{-}/CD49f^{high}$ cells were able to generate secondary tumors which retained the same cellular heterogeneity as the original SCC from which they were derived. These tumor cells could be serially passaged in nude mice. In contrast, $CD34^{-}/CD49f^{-}$ and $CD34^{-}/CD49f^{intermediate}$ cell populations failed to generate tumors. Utilizing a sorting method which isolates cells based on their ability to efflux Hoechst dye, termed "side population" (SP) cells, we demonstrate that SCCs contain SP cells which have robust tumor forming capacity. Most SP cells isolated from mouse SCCs express CD49f, but none of them express CD34. Interestingly, SP cells which are $CD34^{-}$ give rise to tumors containing $CD34^{+}$ cells, suggesting that SP cells are a more primitive type of skin cancer stem cell. The isolation and characterization of SCC cell populations with stem-cell like properties are the first steps towards the development of more effective skin cancer therapies.

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Thermal bystander effect: Medium-mediated induction of DNA double strand breaks and apoptosis after thermal stress via intercellular communication

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Thermal exposure causes protein degradation and DNA damage, which can leads to genetic alteration and cell death. In comparison, little is known about the surrounding tissue, which was not heated. So far, the loss of viability in non-heated surrounding cells is explained due to heat diffusion. Our results demonstrate the first time that in absence of temperature diffusion, due to a special insert/well system, surrounding non-heated cells develop DNA damage and loss in viability. This so called "thermal bystander effect" seems to be induced by surviving heat exposed cells. We used human fibroblasts and HeLa cells in a transwell insert system, where heated and non-heated cells share the same medium without cell to cell contact and no heat diffusion. Viability of heated cells 24 h after heat exposure, decreased significantly at temperatures above 44 C and slowed down at 50 C. We also observed a significant increase of DNA double strand breaks (DSBs) and apoptosis in heated cells. Interestingly, non-heated cells, which shared the same medium with heated ones, also showed a loss in viability and increased DNA damage/apoptosis, even though to a much lower extent. The viability decreased up to 10 % starting at a temperature of 40 C, and came back to control level for higher temperatures (60C). In addition, we observed an increased level of DSBs by 2,5x and the induction of apoptosis (1.5x), which returns to control level at higher temperatures around 50 C. Our data indicates that both heat exposed and bystander cells, while sharing the same medium, can generate DSBs and undergo apoptosis, which results in loss of viability. The disappearance of this thermal bystander effect at higher temperatures indicates that the heat exposed cells need to be viable to produce the signal to damage the surrounding cells. For the future, it needs to be investigated when and how long the bystander signal is produced and what molecule (ROS, or protein) is responsible for the thermal bystander effect.

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Transdifferentiation of circulating stem cells and monocytes into keratinocyte-like cells

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Transdifferentiation is a process where the original cell commitment is changed to give rise to unexpected mature cells. Circulating bone marrow-derived stem cells (BMSCs) and monocytes might transdifferentiate into keratinocyte-like cells (KLCs). These KLCs might release promoting factors such as 14-3-3 proteins to modulate the function of dermal fibroblasts. We have transdifferentiated BMSCs and monocytes into KLCs in culture. KLCs were characterized by using immunocytochemistry, immunofluorescence, confocal microscopy, western blotting, mass spectrometry, and gene expression microarray (35,000 oligos). Co-culture systems, immunoprecipitation and exosome purification were used to demonstrate the KLC-modulating effects on dermal fibroblasts. Transdifferentiated KLCs exhibit a phenotype 14-3-3 sigma+, type I and II cytokeratins+, and involucrin+. They also lost specific markers from their original phenotypes (e.g. CD34 and CD14). KLC-derived releasable factors such as 14-3-3 proteins promoted the MMP-1 expression in dermal fibroblasts in a time-dependent manner. We identified exosomes as carriers by which KLCs release more than 100 proteins. These proteins contribute to cytoskeleton structure, cell adhesion, signal transduction, and vesicle trafficking. These findings exhibited high similarity compared to that in keratinocyte-derived exosomes and low similarity compared to that in exosomes purified from PBMC conditioned media on day 1. Therefore, the exosome purification demonstrates the high specialization reached by circulating BMSC/monocytes when they follow cell transdifferentiation into KLCs. Moreover, exosomes seem to be an important mechanism used by KLCs in cell-to-cell interaction. Findings from this research study will provide us new insights into the wound healing that would facilitate not only the production of skin substitutes for burned patients, but also the treatment of chronic non-healing wounds as well as those associated with over-healing wounds such as hypertrophic scarring.

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UV irradiation decreases dermal decorin, and the decorin reduction is inhibited by D-xylose

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Decorin, a member of small leucine-rich proteoglycans, is a dominant dermatan sulfate proteoglycan distributed on the surface of collagen fibrils in the skin. Decorin appears to have high affinity binding site for type I collagen and some studies have suggested that decorin plays important roles in remodeling and/or maturation of collagen fibrils and dermal extracellular matrix. It has already been known that in aged-skin reduction in the length of decorin glycosaminoglycan causes the distances among the collagen fibrils to shrink, but little has been known about the relationship between decorin and photo-aging. The study was made to discover any changes to decorin in photo-aged skin and to evaluate their influences on dermal functions. A western blot analysis of UV-irradiated dorsal skin of hairless mice showed no change in molecular weight of decorin but reduction in quantity, and an analysis of UVA-irradiated human skin fibroblasts gave the same result. The analysis of the UVA-irradiated human skin fibroblasts further showed that there were no changes in the decorin mRNA levels in the fibroblasts. We then tested some materials and found that D-xylose inhibited UVA-induced decorin reduction in human dermal fibroblasts. Topical applies of D-xylose to UV-irradiated murine skin inhibited the reduction of decorin and in addition, UV-induced wrinkle on murine dorsal skin was improved. Also, while decorin is known to suppress the activity of transforming growth factor beta and to reduce fibroblast proliferation, D-xylose showed no such effects. In a collagen gel contraction assay culturing human skin fibroblast in a medium containing type 1 collagen, addition of D-xylose inhibited UV-induced formation of loose matrix. These results suggest that reduction in quantity of decorin is strongly related to UV-induced wrinkle formation and that D-xylose takes part in a restoration of UV-damaged skin.

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Pemphigus autoantibodies disrupt desmosome assembly in human keratinocytes

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Autoantibodies against the desmosomal cell adhesion proteins desmoglein (Dsg) 3 and Dsg1 cause suprabasilar blisters in the skin and mucous membranes of patients with pemphigus vulgaris (PV). How pemphigus autoantibodies lead to acantholysis, or loss of epidermal cell adhesion, remains enigmatic. Previously, we reported the cloning of pathogenic and nonpathogenic anti-Dsg monoclonal antibodies (mAbs) from a PV patient. We sought to determine whether these antibodies affect Dsg3 incorporation into the desmosome during desmosome assembly and disassembly. During calcium-induced desmosome assembly, treatment of primary human keratinocytes with pathogenic but not nonpathogenic PV mAbs leads to a decrease of Dsg3 but not desmocollin (Dsc) 3 in the Triton-insoluble fraction of cell lysates as early as 2 hours after antibody treatment. Consistent with these results, immunofluorescence studies demonstrate that pathogenic but not nonpathogenic antibodies decrease Dsg3 on the cell surface, with a concurrent increase of cytosolic Dsg3 staining. Loss of Dsg3 from the cell membrane is due to antibody-induced internalization, as supported by colocalization of Dsg3 and the early endosomal marker EEA1. In agreement with these findings, pathogenic antibodies are more rapidly cleared in the culture medium of human keratinocytes than nonpathogenic antibodies. While calcium-induced assembly of Dsg3 into desmosomes is rapidly and specifically blocked by PV mAbs, disruption of established desmosomes by PV mAbs shows a delayed timecourse, with both Dsg3 and Dsc3 demonstrating a decrease in the Triton soluble fraction at 24 hours after antibody treatment, consistent with the timeframe for physiologic turnover of the desmosome. In summary, these studies show that pathogenic PV mAbs preferentially cause internalization of non-desmosomal Dsg3, thereby specifically preventing Dsg3 but not Dsc3 assembly into desmosomes. These data suggest that interference with desmosome assembly is a primary pathogenic mechanism leading to acantholysis in PV.

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Hyaluronan is a signal of skin injury: recognition through CD44–TLR4 and hyaluronidase–Cryopyrin/NLRP3-dependent pathway

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Hyaluronan(HA) is a major component of the extracellular matrix in the dermis and epidermis. Our prior work has shown that HA is degraded after injury to small oligosaccharides (sHA) that subsequently stimulate chemokines release from endothelia and macrophages (Mø). We hypothesized that this event is essential to recognize trauma and tested this by defining elements of HA recognition that lead IL1β release. Analysis of mouse BM-derived Mø or the Mø cell line MHS showed that IL1β release by HA is absent in *Tlr4–/–*Mø, inhibited by anti-CD44 antibodies, partially inhibited (50%) in *Cd44–/–*Mø, and IL1β protein release but not mRNA is absent in cryopyrin/NLRP3-deficient Mø. *in vivo* models of sterile injury were developed by freezing mouse skin with liquid nitrogen or sc injection of sterile cytodex beads. This avoided potential microbial stimuli and resulted in HA deposition at the injured site and increased MIP2 and IL1β (4.0 and 2.5 ng/lesion, respectively). MIP2 induction by HA injection or sterile injury was blocked in *Tlr4–/–* mice and was decreased (37%) in *Cd44–/–* mice. *Nlrp3–/–* mice showed greatly decreased inflammation after sterile injury, decreased IL1β, but no change in MIP2 induction. Use of specific inhibitors to block HA endocytosis, lysosomal acidification and hyaluronidase activity showed HA activation of intracellular cryopyrin and IL1β is dependent on the pathway. The events of HA internalization to activate IL1β could be bypassed by intracellular administration of sHA (10–18 mer), thus showing that the presence of sHA in the cell could directly activate cryopyrin. These findings further define the signaling system of HA recognition, confirmed the hypothesis that HA is an important signal of injury *in vivo*, and suggest that HA may participate in the pathology of familial cold urticaria syndrome, as disease caused by an activation mutation in cryopyrin/NLRP3.

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Fibronectin growth factor-binding domains are required for fibroblast survival and growth in response to PDGF-BB: Implications in wound healing

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During normal wound healing, fibroblast ingrowth is the rate-limiting step for granulation tissue formation and appears to require fibronectin (FN), which is absent in chronic wounds and burns. The failure of chronic wounds to heal and the progression of injury in burns are both associated with fibroblast apoptosis. We have found that FN contains 4 growth factor-binding (FN-GFB) sites for PDGF that are required for fibroblast survival and proliferation. The 4 FN-GFB sites are homologous and each binds PDGF with a KD of ~10 nM. To determine the mechanisms by which fibroblasts fail to survive and grow in the absence of FN-GFB sites, FN-null fibroblasts were utilized. Null-cells cultured in DMEM on recombinant RGD cell-binding domains, but not any FN-GFB site, died by apoptosis within three days, in the presence or absence of 1 nM PDGF. Attachment, spreading and focal contact formation occurred within 1 hr and continued to appear morphologically normal for 24 hrs. However, the metabolic response even in the presence of PDGF was minimal at 24 hrs and was associated with autophagy as judged by the appearance of LC3. At 48 hrs apoptosis was first noted, and by 72 hrs most cells had died. In contrast, FN-null cells, when plated on any FN-GFB site in contiguous or noncontiguous arrays with RGD cell-binding domains, survived for 7 days without PDGF and proliferated in its presence. In the absence of FN-GFB sites, PDGF-receptor-β was suboptimally phosphorylated in response to PDGF. Nevertheless, Akt, ERK1/2, p38 and JNK phosphorylation appeared normal. These data are consonant with downstream dysregulation of the mTOR pathway. In summary, fibroblasts required FN-GFB sites in addition to the RGD cell-binding domain to survive. In the absence of FN-GFB sites, cells developed a starvation response including autophagy and finally died by apoptosis. The discovery of these FN-GFB sites has important therapeutic potential for FN-deficient wounds, such as burns and chronic ulcers.

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Two pathogenic anti-desmoglein 1 (Dsg1) monoclonal antibodies isolated from one pemphigus foliaceus (PF) patient define major Dsg1 epitopes recognized by most pemphigus patients

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Pemphigus foliaceus (PF) is an autoimmune blistering disease caused by autoantibodies against Dsg1. Epitopes are believed to be an important factor in determining pathogenicity of autoantibodies in pemphigus. We previously isolated 67 anti-Dsg1 monoclonal antibodies (mAbs) as single-chain variable fragments (scFvs) from a PF patient by phage display. Two anti-Dsg1 mAbs, PF1(3-30) and PF1(3-7), caused typical PF blisters in mouse and/or human skin, while others did not. To investigate whether other pemphigus sera target epitopes defined by the isolated anti-Dsg1 mAbs, we performed competition ELISA to determine if the mAbs block the binding of these sera to Dsg1. The strongly pathogenic mAb PF1(3-30) and the weakly pathogenic PF1(3-7) blocked >20 % binding of 20 and 15, respectively, out of 30 PF sera from patients with active disease. Only three PF sera were not blocked by either pathogenic mAb. Furthermore, the pathogenic mAbs also blocked the binding to Dsg1 of 4 or 6 pemphigus vulgaris sera which contained both anti-Dsg3 and anti-Dsg1 antibodies. In contrast, two representative non-pathogenic mAbs inhibited the binding of only 8 of the 30 PF sera. These data indicate that two mAbs isolated from one PF patient define major pathogenic epitopes targeted by most pemphigus sera with anti-Dsg1 autoantibodies, whereas non-pathogenic epitopes are less restricted. Furthermore, the degree of antibody response between pathogenic and non-pathogenic epitopes did not change significantly in each of 6 PF patients when measured over time, in spite of changes in disease activity, suggesting that intramolecular epitope shift of autoantibodies is not common. In sum, competition ELISA is useful to evaluate antibody activity against shared pathogenic epitopes in pemphigus and should prove valuable to monitor disease activity.

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LOX and LOXL2 expressions are reciprocally regulated during cell growth and differentiation in cultured keratinocytes

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Lysyl oxidase (LO) is an extracelluler enzyme involved in the crosslinking reaction of dermal collagen and elastin. Five genetically distinct species of LOs (LOX, LOXL1, LOXL2, LOXL3 and LOXL4) have been identified, but their functions are still unclear. The purpose of this study is to investigate the regulation of LO isoform expression during cell growth and differentiation in cultured kerationocytes. Relative amount of their mRNAs were determined in cultured keratinocytes by use of real-time PCR. LOX and LOXL2 expression of keratinocytes was reciprocally regulated during terminal differentiation induced by the suspension culture. LOX expression was markedly increased and LOXL2 expression was decreased. When the cells which had been arrested by suspension were allowed to adhere onto the various extracellular matrices-coated culture dishes, fibronectin-coated dishes stimulated LOXL2 mRNA level. This was confirmed by immunoblot analysis of cell extract and immunofluorescent observation of cultured cells using monoclonal anti-LOXL2 antibody. Distinct localization of LOXL2 mRNA was seen in the lower epidermis by in situ hybridization analysis on normal epidermal layer, whereas LOX mRNA was seen mostly in the upper layer of epidermis, and LOXL1 throughout the epidermis. These results suggest that LOX expression in cultured keratinocytes is related to cell growth arrest whereas LOXL2 expression is related to the cell growth mediated by the interaction of fibronectin.

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Desmoglein 1 engages an actin remodeling pathway via the Src-family kinase substrate cortactin during keratinocyte stratification

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Desmoglein 1 (Dsg1) is most highly expressed in the upper granular layers of the epidermis, where its adhesive properties are required for tissue integrity. However, Dsg1 is first expressed and recruited to the cell surface as basal cells commit to differentiation and transition into the suprabasal layers, a process that relies on cytoskeletal remodeling. Our previous data demonstrated that Dsg1 directs the suprabasal morphogenesis of keratinocytes within organotypic raft cultures, leading us to test the hypothesis that Dsg1 modulates signaling pathways regulating the cytoskeleton during stratification. Intriguingly, co-immunoprecipitation studies revealed a complex containing Dsg1 and cortactin, a Src-family kinase (SFK) substrate governing actin dynamics during cell motility. Cortactin was reduced in the membrane fraction of keratinocytes transduced with an miRNA targeting Dsg1 and was also less prevalent at intercellular junctions within raft cultures having Dsg1 silenced, suggesting a role for Dsg1 in localization of the actin modifier during keratinocyte stratification. Further, reconstitution of Dsg1 in knock-down raft cultures using a silencing-refractory construct restored differentiation and promoted phosphorylation of cortactin at the SFK consensus site. Finally, ectopic expression of the cadherin in keratinocytes revealed that (1) wild-type Dsg1, (2) a Dsg1 mutant deficient in binding to plakoglobin and (3) the Dsg1 cytoplasmic domain alone all enhanced cortactin phosphorylation, indicating that Dsg1 domains mediating catenin binding and adhesion are dispensable for cortactin regulation. From these results, we propose a novel, adhesion-independent role for Dsg1 in the localization and phosphorylation of cortactin, which may in turn facilitate actin re-organization required for epidermal stratification and morphogenesis.

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Observation of photo-aged, dermal collagen fiber using polarization-resolved second-harmonic-generation microscopy

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Since dermal collagen fiber plays an important role to determine the morphology and function of skin, there is a considerable need in skin cosmetics for an optical probe method that can reveal the structure and/or orientation of dermal collagen fiber in situ. When ultrashort pulse light is incident to collagen-rich tissues such as dermis, second-harmonic-generation (SHG) light with a half wavelength of the incident light is observed as a result of nonlinear optical property of collagen molecule. This SHG light provides unique imaging modality: selective visualization of collagen fiber, high spatial resolution, low invasion, deep penetration, and staining-free. Furthermore, a combination of polarization methods can lead to additional insights. The efficiency of SHG light is sensitive to collagen orientation when the incident light is polarized, and hence polarization measurements of SHG light are effective in probing the collagen orientation in tissues. In this study, polarization-resolved SHG microscopy is applied to evaluate the photo aging in the dermal collagen fiber. To this end, we visualized orientation of dermal collagen fiber in photo-aged mouse skin (16 weeks old, UVB irradiation for 10 weeks) and control one (16 weeks old, no UVB irradiation) based on the SHG anisotropy. From comparison of SHG anisotropy between them, we confirmed a correlation between collagen fiber orientation and direction of skin wrinkle in the photo-aged skin, i.e. collagen fiber orientation is parallel to the direction of skin wrinkle. Furthermore, the result of depth-resolved imaging of SHG anisotropy indicated that the SHG anisotropy in dermal collagen near the epidermis is higher than that in deep dermal collagen in the photo-aged skin, implying the depth dependence of the photo-aging degree. The proposed method will be a powerful tool for in vivo assessment of photo aging in human dermis.

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Desmosome disassembly and loss of keratinocyte adhesion in response to PV IgG occurs in sequential phases and can be reversed by expression of exogenous Dsg3

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Pemphigus vulgaris (PV) is an epidermal blistering disorder in which antibodies target the desmosomal cadherin desmoglein 3 (Dsg3) and disrupt desmosomes through unknown mechanisms. Previous work demonstrated that PV autoantibodies trigger internalization of Dsg3 from the cell surface. Using biochemical approaches and live cell imaging we find that Dsg3 internalization and desmosomal disassembly occur in three discrete but overlapping phases. Phase I of disassembly is internalization of Triton soluble Dsg3 through a clathrin and dynamin independent pathway which is likely lipid raft mediated. Entry of Dsg3 into the pathway is specified by sequences in the Dsg3 cytoplasmic tail. Phase II is defined by reorganization of Dsg3 into linear arrays perpendicular to cell-cell borders. Dsg3 in these linear structures is at the cell surface and associated with the keratin cytoskeleton. Time lapse fluorescence imaging and retrospective immunolocalization show that Dsg3 buds off of these arrays, dissociates from desmoplakin, and enters a vesicular compartment. Concomitantly, both the Triton soluble and insoluble pools of Dsg3 decrease. Phase III is characterized by desmosome disassembly, loss of steady state levels of Dsg3, and loss of adhesion strength. Two approaches were used to determine if inhibiting the early phases of disassembly prevented subsequent phases including loss of adhesion (phase III). First, Dsg3 internalization was blocked using a tyrosine kinase inhibitor, genistein. Second, the down-regulation of Dsg3 in response to PV IgG was counteracted by expressing exogenous Dsg3.GFP. Both approaches prevented desmoplakin mislocalization, Dsg3 down-regulation and loss of adhesion caused by PV IgG. These findings suggest that PV IgG binding to Dsg3 disrupts the kinetics of desmosome turnover, and that blocking endocytosis or enhancing Dsg3 biosynthesis can compensate for the increased turnover of Dsg3 caused by PV IgG.

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Regulation of p63 through the expression of IGFBP in the reconstruction of skin equivalents

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Dermal matix is known to play a key role in regulating the growth and differentiation of epidermis in skin equivalents (SEs). The regeneration of epidermis is regulated by a large number of growth factors produced by dermal fibroblasts (Fb). Dermal sheath cells (DSCs) and dermal papilla (DP) cells are the two major dermal cells in a hair follicle. In this study, to compare the effects of dermal components on the evolution of SEs *in vitro*, human keratinocytes were seeded on different dermal substitutes. SEs, produced using DSCs and DP cells, showed a thick epidermis and higher expression of β1- and α6-integrin than SEs using Fb cells. Thus, we compared cytokines secreted by DSCs and Fb. Using RayBio® human cytokine antibody array C (series 1000), one-hundred twenty cytokines were tested and revealed that DSCs produced a much greater amount of IGFBP-2, angiogenin, and BMP-6 than Fb. On the basis of the cytokine antibody array, we next investigated whether IGFBP-2, angiogenin, or BMP-6 has effects on SEs reconstruction. The addition of IGFBP-2 showed a thick epidermis and high expression of β1- and α6-integrin, whereas BMP-6 exhibited little effect. In summary, IGFBP-2 is a major factor from DSCs which affects epidermal regenerative capacity of skin. "Grant from Stem Cell Research Center (SC3260)"

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The stimulating effect of a laminin-like heptapeptide on cell adhesion and the extracellular matrix

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Laminin is a widely present protein that contributes to the synthesis of the extracellular matrix (ECM) and is involved in many cell activities, including cell adhesion, migration, and differentiation. We were interested in investigating the effects of a laminin-like heptapeptide on the expression of various key ECM proteins in cultured human cells and skin. Immunofluorescence studies of β1-integrin expression revealed that the addition of 1% of the new peptide significantly increased β1-integrin expression in cultured human keratinocytes and fibroblasts, compared to control cells. Parallel studies were performed on *ex vivo* human skin, treated for 48h and 72h with 1% of the new peptide. These studies demonstrated an enhancement of β1-integrin expression in peptide-treated skin samples. Fibronectin expression studies on cultured human fibroblasts showed a net increase in fibronectin expression after cells were treated with the new peptide. Time course studies included assessment of collagen synthesis in ECM molecules of human fibroblasts and human skin biopsies. These studies revealed an interesting increase of collagen I and collagen III after 48 and 72 hours of treatment with 1% of the new peptide. These results suggest that this new peptide contributes to cellular cohesion of the basement membrane and to extracellular matrix organization. Moreover, in order to characterize the effect of the new peptide on epidermal structure and differentiation, keratin expression was evaluated by immunostaining of human skin biopsies. The results of the study showed that peptide-treated skin exhibited a clear increase in pan-keratin expression, compared to the untreated control skin. Taken together, these studies demonstrate the important properties that this new laminin-like peptide exhibits on extracellular matrix synthesis, suggesting that this new active ingredient can be of great use in anti-aging skin care and wound healing products.

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Spatial regulation of collagen biding proteins, decorin and lumican should contribute to the tight formation of collagen bundles at the palmoplantar skin

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Human skin shows various morphological and biochemical characteristics and also obtains unique physiological features depending on the body-sites. These spatial distinctions of human body sites have been essentially explained from the variance of epidermal keratinocytes and skin appendages, however, the contribution of dermal fibroblasts has been poorly understood. We used the subtractive mRNA screening to identify genes specifically or selectively expressed by the dermal fibroblasts derived from various body sites, including trunk, palm and sole skin and oral mucosa. Consequently, we found that trunk fibroblasts expressed fibronectin more abundantly compared to palmoplantar or oral mucosa fibroblasts. Furthermore, we identified three cDNA clones encoding decorin, tenascin X and chemokine receptor 11, whose mRNAs were more selectively or abundantly expressed by palmoplantar fibroblasts. Since decorin, a member of small leucine-rich proteoglycan (SLRP) and tenascin X are both recognized to regulate fibrillogenesis, we then examined the fine morphology and constituent of the various body sites of human and rat skin. While lumican was abundantly expressed by the palmoplantar fibroblasts compared to the trunk skin or oral mucosal fibroblasts, thrombspondin 2 and dermatopontin were expressed more selectively by trunk fibroblasts. Preferential expression of decorin and lumican in the palm and sole were also confirmed at the protein level. Furthermore, electron microscopic analyses revealed that the diameter of collagen fibrils of sole skin (73 $\pm$ 14 nm) was significantly thinner and more scattering than those of back skin (80 $\pm$ 13 nm) in both rat and human skin. The present study indicates that distinct spatial expression of these collagen-binding proteins should regulate the collagen bundle morphology and contribute to acquisition of the dermal function specific to sole skin.

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Antifibrotic actions of N-methylethanolamine (MEA) in human dermal fibroblasts

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Tissue fibrosis develops when dysregulation of extracellular matrix turnover favors deposition of ECM proteins over degradation. Fibrosis may then lead to organ dysfunction as observed in systemic sclerosis or keloid. In the present study, we investigated the antifibrotic properties of N-methylethanolamine (MEA) in human dermal fibroblasts. MEA suppresses the expression of COLα2(I) mRNA and its protein levels in a dose- and time- dependent manner. MEA inhibited fibroblast collagen production via both transcriptional and post-transcriptional mechanisms. MEA enhances the expression of MMP-1 mRNA and its protein levels. TGF-β, a master cytokine leading to fibrosis, induce type-I collagen production and inhibit MMP-1 production in fibroblasts. Effect of MEA could be explained by antagonizing TGF-β signaling. But MEA did not inhibit TGF-β induced typeI collagen induction and MMP-1 suppression. To further confirmation, we tested wheter MEA affect SMAD signaling, but MEA did not influence SMAD2/3 phophorylation. Together, antifibrotic action of MEA is independent with TGF-β signaling. We found that the ERK signaling was involved in the MEA-induced inhibition of COLα2(I). MEA induced sustained activation of ERK signaling. The sustained activation of ERK signaling induced by MEA leads to reducing the mRNA stability of type I collagen. We have shown MEA positively regulated MMP-1 production via ERK and JNK signaling and negatively regulated via p38 MAPK signaling. The low dose MEA activated ERK and JNK signalings, but not influence p38 MAPK signaling, whereas the high dose MEA strongly inhibited p38 MAPK signaling. Our data suggest that total inhibition of p38MAPK signaling by high dose MEA strongly induce MMP-1 gene expression. The low dose MEA induce MMP-1 by activating ERK and JNK signalings, whereas high dose MEA induce MMP-1 by inhibiting p38 MAPK signaling. In conclusion, our study demonstrates that MEA has an antifibrotic action. MEA can be a promising candidate for the treatment of diseases characterized by excessive ECM deposition such as systemic sclerosis or keloid.

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The effect of human adipose-derived stem cells on the cutaneous wound healing

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Many treatments have been developed for the deteriorated cutaneous wound healing such as diabetic foot and bed sore, but there is still much need for more effective therapy. Adipose-derived stem cells belong to the mesenchymal stem cells which are self-renewing and pluripotent. Recently mesenchymal stem cells have been applied for the tissue repair and regeneration. We investigated the effects of human adipose-derived stem cells (hADSC) on the healing of experimental cutaneous wound which occurred in Balb/c nude mice *in vivo*, and also confirmed the effects of hADSC on the keratinocytes and the human dermal fibroblasts (hDF), which have essential roles in wound healing, *in vitro*. Full-thickness skin defects of 1 cm-sized round wound, not including the panniculus carnosus, were made and covered with collagen lattice with 5 x 10^5 hADSC on the back of nude mice. The collagen lattice without hADSC was used as the control. Wound size was evaluated at 8, 12, 16 and 20 days after injury. The wound size was significantly smaller in the hADSC-treated mice compared to that of control. The proliferation of HaCaT cells and hDF increased by the conditioned medium of hADSC (ADSC-CM) in a concentration-dependant manner. The contractility of hDF was evaluated with *in vitro* hDF-populated collagen lattice contraction model and ADSC-CM stimulated the contraction of hDF-populated collagen lattice. The reverse transcription and the polymerase chain reaction showed that the expression of type I collagen α1 chain gene was upregulated in hDF by ADSC-CM. These data suggest that hADSC might have stimulatory effects on the healing of skin defects and that they could be an innovative treatment for cutaneous wound-healing disorders.

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Transmembrane collagen XVII Is expressed by podocytes of the glomerular filtration barrier

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Collagen XVII is a component of the dermoepidermal hemidesmosomal anchoring complex, which mediates the adhesion of epithelial cells, such as basal keratinocytes, to the underlying basement membrane. The purpose of this study was to investigate the localization and function of collagen XVII in the normal kidney glomerulus, especially in podocytes which form the most important component of the glomerular filtration barrier (GBM). To identify in detail the cell types that express collagen XVII in kidneys, we carried out in situ hybridization and immunohistochemical studies. Podocytes expressed clearly both collagen XVII mRNA and protein. In filtration barrier collagen XVII ultrastructural localization was investigated by immunoelectron microscopy where gold labelling was shown to be present within pedicle and close to the cell membranes of the pedicle soles in the lamina rara externa of GBM. Some labelling was also present in the slit pore or slit diaphragm between neighbouring pedicles. Labelling could also be observed in the lamina rara interna of GBM close to the cell membranes of endothelial cytoplasmic processes. The expression of collagen XVII in podocytes was confirmed further by analyzing conditionally immortalized mouse podocyte cell line. The presence of collagen XVII expression in both proliferating and differentiating podocytes was demonstrated by RT-PCR and subsequent sequencing. Interestingly, TaqMan quantitative real-time PCR analysis showed that collagen XVII mRNA levels were higher in differentiated podocytes than in undifferentiated ones. However, the mRNA levels were not as high as compared to normal mouse keratinocytes. Our results suggest that collagen XVII is one of the molecules that attach endothelial cells and podocyte foot processes to the GBM. It is probably also a component of the slit pore or slit membrane. The pattern of localization suggests that collagen XVII may have some contribution to the glomerular filtration.

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Tobacco smoke extract induces matrixmetalloprotienase (MMP-1)-1 expression in human skin fibroblasts through the arylhydrocarbon receptor (AhR) pathway

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It is well established that smoking has deleterious effects on skin. Accordingly, epidemiological studies suggest a link between smoking and extrinsic skin aging, and we have previously reported that matrix metalloproteinases (MMPs) mediate connective tissue damage in skin exposed to tobacco smoke extracts. Tobacco smoke contains at least 3,800 constituents including numerous, water insoluble polycyclic aromatic hydrocarbons which are known to be capable of triggering the aryhydrocarbon receptor (AhR) signaling pathway. In order to further analyse the molecular mechanisms involved in tobacco smoke–induced skin aging, in the present study, primary human dermal fibroblasts were therefore exposed to a hexane-soluble tobacco smoke extract. We here report that the hexane-soluble extract (3-6 μl/ml) significantly induced MMP-1 mRNA and protein expression in cultured human dermal fibroblasts. This was associated with a significant upregulation of the expression of cytochrome P1B1(CYP1B1), which is well established marker of AhR pathway activation. Both, CYP1B1 as well as MMP-1 induction were abrogated if fibroblasts were treated with the flavonoids, 3-methoxy-4-nitroflavone and α-naphthoflavone, i.e. well known inhibitors of the AhR pathway. Interestingly, treatment of tobacco-smoke extract stimulated cells with two different antioxidants, N-acetyl L-cysteine or NaN_3, inhibited MMP-1, but not CYP1B1 upregulation. These studies suggest that a hexane-soluble tobacco smoke extract induced MMP-1 expression in human skin fibroblasts via activation of the AhR pathway. Thus, the AhR pathway may be pathogenetically involved in extrinsic skin ageing.

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Flavonoids-rich Passiflora alata extract is effective to reduce skin micro-inflammation process in aging

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The relevance of chronic micro-inflammation has been recently proposed as a new significant skin aging mechanism, which is triggered by factors such as UV radiation and external agents. In response to those stimuli, skin cells release several pro-inflammatory signals, mainly interleukins (ILs) and prostaglandin-E2 (PG-E2). Also, it has been postulated that micro-inflammation upregulate ICAM-1 expression in endothelium, causing infiltration of inflammatory cells to dermis, thus amplifying the process. This sub-clinical and silent action, which activates matrix metalloproteinases (MMPs), leads to continuous dermal extracellular matrix degradation. The net result is accelerated skin aging process. Natural compounds such as flavonoids have anti-inflammatory properties. In our study, extracts from leaves of Passiflora alata, were evaluated for anti-inflammatory purposes. Using *in vitro* cultures of normal human dermal fibroblasts (NHDF) exposed to LPS and UVB we showed that P. alata extracts containing the flavonoid vitexin-2-O-rhamnoside reduced significantly the levels of IL-6 (91% and 85%), IL-8 (90 and 86%) and PG-E2 (68% and 98%), by ELISA, respectively. These results were confirmed by RT-PCR. We determined also that collagenase MMP-1 was down-regulated by P. alata extract on NHDF *in vitro* and by RT-PCR. *In vivo* studies were carried out with a panel of 105 volunteers showing that a cosmetic formulation containing P. alata extract reduced the perception of pain caused by lactic acid stimulation comparing to a placebo cream both if applied before or after treatment. Moreover, the formulation also showed potential to inhibit erythemal reaction when skin was treated before UV radiation stimulus. Thus, P. alata extract is suitable for controlling and treating the micro-inflammatory process that leads to aged skin.

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Biphasic p38 MAPK activation in the pemphigus foliaceus mouse model

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In pemphigus, autoantibodies against desmogleins induce loss of cell-cell adhesion. Activation of keratinocyte transmembrane signaling by pathogenic pemphigus IgG is emerging as an important component of pemphigus acantholysis. Activation of p38 mitogen-activated protein kinase (MAPK) by pemphigus IgG may mediate acantholysis since inhibition of p38 blocks pemphigus IgG induced cytoskeletal reorganization in tissue culture and blistering in mouse models of pemphigus vulgaris (PV) and pemphigus foliaceus (PF). In addition to signaling to the cytoskeleton, p38 mediates stress response signaling and apoptosis. A number of investigators have observed induction of keratinocyte apoptosis by pemphigus IgG raising the possibility that p38 activation by pemphigus IgG may also lead to apoptosis. In this study, the PF passive transfer mouse model was used to investigate the relationship of p38 activation to apoptosis in pemphigus IgG mediated acantholysis. Neonatal C57BL/6J mice were injected with purified PF or control IgG for 1-50 hrs after which mice were examined clinically and skin biopsies obtained for histology, immunohistochemical staining, and biochemical analyses. PF IgG treated mice developed blistering and skin extracts showed increased phosphorylation of p38 and HSP25. Two peaks of p38 activation were observed. Inhibition of the early peak of p38 activity with the inhibitor SB202190 blocked blister formation; whereas, inhibition of the second later peak failed to block blistering in vivo. Marked increases in caspase 3 activation and TUNEL staining, markers of apoptosis, were late events occurring after the second peak of p38 activity. These observations indicate a biphasic activation of p38 in which the early peak is induced directly by pathogenic IgG, correlates with cytoskeletal reorganization, and is part of the mechanism by which acantholysis is induced. In contrast, the second later peak of p38 may represent signaling via stress response pathways activated as a consequence of acantholytic keratinocyte injury.

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Dynamic regulation of epidermal Eph receptor signaling complexes

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Eph receptor tyrosine kinases (RTK) are activated by ephrin ligands present on an adjacent cell surface and regulate cellular patterning events during brain, vascular, and gut development. Intact cell-cell adhesion is required in order to bring Eph RTKs into close proximity with their endogenous ligands but little is known about the organization of these signaling complexes in adult epithelial tissues. As a first step to understanding the regulation of epidermal Eph RTK complexes, we focused on EphA2 since it was recently shown to limit skin tumor growth in a mouse model. Similar to E-cadherin, EphA2 was abundantly expressed in freshly isolated foreskin keratinocytes and was recruited to nascent cell-cell contacts which formed in high calcium. As ephrin A1, a high affinity ligand for EphA2, was also found in undifferentiated keratinocytes, we postulated that these partners activate native signaling complexes when epidermal cells first come into contact with one another. To study the fate of the EphA2 receptor upon stimulation, we bypassed its endogenous ligand using recombinant ephrin A1-Fc chimeric peptides. EphA2 activation, as confirmed by RTK phosphorylation and downstream inhibition of ERK 1/2, led to a rapid and sustained redistribution of this RTK away from cell-cell borders and an overall reduction in EphA2 levels. Indeed, EphA2 was normally down-regulated in differentiated keratinocytes where stable cell-cell contacts were maintained over extended periods. These data led us to speculate that novel Eph RTK complexes may be present in these more differentiated keratinocyte populations. Profiling of the entire family of Eph receptors in submerged keratinocytes and organotypic epidermal raft cultures revealed the presence of six additional Eph RTKs (EphA1/A4/A6/B3/B4/B6) as well as their respective ephrin A and B ligands. Collectively, these studies suggest that distinct Eph RTK signaling complexes are sequentially engaged as basal keratinocytes detach from the basement membrane and establish new contacts with cells in the upper epidermal layers.

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Increased elastin levels in skin after topical administration of zinc complexes in a murine model

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Elastin is a protein that functions in conjunction with collagen in connective tissue to provide elasticity to the skin and other vital organs. Post–fetal development, significant elastin production does not occur in humans. As a result, elastin levels in the skin progressively decrease with maturity, producing an aged, wrinkled appearance. No therapeutic compound has heretofore been identified that will induce functional elastin production. Zinc, at specific ionic concentrations, appears to be involved in the regulation of elastin. The primary objective of this study was to evaluate the effects of a zinc-containing topical formulation on elastin and elasticity in a murine model. A preclinical murine histology study was performed to evaluate specific endpoints of the component pathways for elastin production including epidermal thickness, hypodermal fat, and elastin. Application of the zinc formulation in the murine model resulted in significant increases in epidermal thickness, hypodermal fat, and elastin (P=0.0001). This zinc-based formulation is the first therapeutic entity discovered to increase functional elastin content *in vivo*.

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Syndecan-binding peptide PEP75 derived from laminin α3 stimulates integrin β1 dependent cell migration and accelerates wound healing *in vivo*

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We previously demonstrated that the synthetic peptide (PEP75) containing syndecan-binding sequence within laminin-5 (Lm-332) α3 chain LG4 module induced keratinocyte migration by colloidal gold phagokinetic assay. In this study, PEP75-induced cell migration was further analyzed by various assays *in vitro* and its effects on wound healing were estimated *in vivo*. Scattering assay showed that PEP75 induced cell migration and suppressed formation of focal contact and actin stress fibers. Scratch assay and colloidal gold phagokinetic assay demonstrated that intgerin β1 was involved in the PEP75-induced cell migration. PEP75 triggered villous-like protrusion (VP) structure formation containing syndecan 4 and integrin β1 as well as a transition of syndecan 4 from basal to apicolateral surface. Flow cytometry analyses showed no increase in surface syndecan 4 and integrin β1 with PEP75. Pulse-chase study disclosed that PEP75 treatment retained activated form of integrin β1 at cell-cell contact, although it did not impair internalization of integrin β1. Intgerin β1 was functionally activated by PEP75, evidenced by the facts that cell adhesion to extracellular matrices was enhanced following incubation with PEP75. Taken all these data together, it is concluded that PEP75 binding to syndecan induced integrin β1 dependent cell migration, being associated with various changes such as a suppression of formation of focal contact and actin stress fibers, VP structure formation, transition of syndecan 4 distribution, retaining of activated integrin β1 at peripheral part of the cell, and enhancement of intgerin β1 function. PEP75 was topically applied on skin wound of mouse and rabbit. PEP75 promoted wound closure in mouse and wound reepithelialization in rabbit. These results propose a possibility that PEP75, laminin 5-derived synthetic peptide, could accelerate reepithelialization of chronic skin wound, where keratinocytes are unable to migrate over.

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Effect of onion extract, quercetin, on expressions of type I collagen, MMP-1, and TIMP-1 in human skin fibroblasts

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Type I collagen is a major component of extracellular cellular matrix in tissue. The pathologic processes of type I collagen synthesis or degradation is partly contributed in connective tissue disease such as keloid and scleroderma. Thus it is important to modulate the expression of type I collagen, matrix metalloprotenases-I (MMP-1), and tissue inhibitors of metalloproteinases (TIMP-1), to improve the clinical course of connective tissue disease. Quercetin, extracted from onion, has properties modulation of type I collagen expression *in vitro*. However, the effective method of regulation of type I collagen synthesis and degradation by quercetin is still under investigated. The purpose of this study was to investigate the regulation of type I collagen, MMP-1, and TIMP-1 expressions in human skin fibroblasts using quercetin treatment. Human skin fibroblasts were treated by quercetin (0, 10, 20, 30, 40 uM) for 24 hr. The expressions of type I collagen, MMP-1, and TIMP-1 protein were analyzed by RT-PCR, Western blot analysis, zymography, and CAT assay. Our data showed that the expression of type I collagen protein and TIMP-1 was not markedly modulated by quercetin treatment. Interestingly the expression of MMP-1 was markedly up-regulated by quercetin treatment. However, in case of wound healing animal model, the apply of ointment containing quercetin (0.1%) impaired the wound healing process in hairless nude mouse, due to constitutively increased MMP-1 expression. Thus, our data indicated that the expression of MMP-1 protein in human skin fibroblasts was increased by quercetin treatment, implying that topical agent using quercetin may be effective to reducing sclerosis of tissue by up-regulation of MMP-1.

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Effect of erythromycin A and its new derivative, EM201 on type I collagen synthesis in cultured dermal fibroblasts

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Thin of dermis is a principal histological change of skin atrophic disorders or aged skin. It is reported that amount of collagen in the skin is decreased duaring aging. It is also known that productions of type I collagen are decreased and collagenase expression is increased in *in vitro* and *in vivo* aged fibroblasts. Macrolides have been reported to show various pharmacological activities in addition to antimicrobial activity including anti inflammatory activity, inhibition of tumor angiogenesis, and inhibition of the growth of fibroblasts. We would like to present effects of erythromycin A and it's new derivative,EM201 on type I collagen synthesis in cultured dermal fibroblasts. Methods: Dermal fibroblasts were cultured with routine methods. When the cell layers had become confluent, the cells were incubated for 24 hours at 37oC in DMEM containing 0.2% FBS, supplemented with 10-8 M ~10-5 M of erythromycin A or 10-7 M ~10-5 M of EM201. After additional incubation with radioactive proline for 24 hours, collagen synthetic activity was measured by fluorography with extracted protein of the culture supernatant. Total RNA was isolated from cultured fibroblasts by extraction in guanidium isothiocyanate then northern blots analysis were performed using radioactive cDNA of α1(I) collagen and GAPDH. Luciferase assay was also performed. Results: Both erythromycin A and EM201 enhanced collagen synthesis and mRNA levels of type I collagen(to 200%with erythromycin A and 250%with EM201 in maximum) in a dose dependent manner in cultured dermal fibroblasts. The transcription of type I collagen gene was also enhanced by both macrolides. These results suggest that erythromycin and EM201 have potential to improve the thin of dermis of skin atrophic disorders or aged skin.

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Observations of epidermal grafts derived from keratinocytes expressing selectively engineered mutant laminin-332 molecules

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Laminin 332 is an important basement membrane zone (BMZ) component which promotes epidermal-dermal cohesion. The laminin-332 beta 3 chain contains a number of distinct domains, including domain V-III, and domain VI. While domain V-III has been shown to bind collagen VII, the role of laminin beta 3 chain domain VI is currently unknown. In an effort to further investigate the function of individual b3 chain domains, we produced mutant b3 chain cDNAs lacking either domain VI (ΔVI) or lacking domain VI and V-III (ΔVI-III), and stably introduced these cDNAs into laminin beta 3 null epidermolysis bullosa keratinocytes. Both ΔVI and ΔVI-III cells were transferred to skin equivalents and analyzed 3-4 weekds after grafting to immunodeficient mice. Grafts were analyzed by light, immunofluorescence and electron microscopy. We found that both ΔVI and ΔVI-IIII epidermis showed significant adhesive abnormalities and peeled easily from dermis similar to the fragile skin of an epidermolysis bullosa patient. Immunofluoresence microscopy showed laminin-332 and type VII collagen localized at the BMZ in both ΔVI and ΔVI-IIII grafts, suggesting that the laminin b3 chain is not essential in the localization of collagen VII to the BMZ. These studies demonstrate that laminin beta 3 domains VI and VI-IIII each play critical roles in dermal-epidermal cohesion of human skin equivalents and introduce a novel method of molecular BMZ engineering that is widely applicable to *in vivo* analysis.

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Intrinsic aging- and photoaging-dependent changes of glycosaminoglycans in human skin *in vivo*

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Glycosaminoglycans (GAGs) are ones of major components in the human skin tissue, however, their aging-related or photoaging-related changes in skin are not well-defined and controversial. GAGs have been thought to have structural functions, but their physiological functions are recently being reported. To investigate intrinsic aging- and photoaging-dependent changes of GAG in human skin, sun-protected buttock and sun-exposed forearm skins were biopsied from healthy young (20-39 year, n=8) and old (70-89 year, n=8) male volunteers and young (20-39 year, n=8) and old (70-89 year, n=8) female volunteers (total n=32). Using the dermis and epidermis of biopsied skin tissues, wet and dry tissue weights were measured for tissue water contents, and total uronic acid amount, total sulfated GAG amount, and HA amount were measured. The skin elasticity was examined by Dermaflex, and skin hydration by Corneometer, for their correlation with GAG changes. Total uronic acid, HA amount, and tissue water content of buttock dermis showed no change in old groups, but showed significant increase in the forearm dermis of old group. However, total sulfated GAG amount was decreased in both old buttock and forearm dermis. In epidermis, total uronic acid, tissue water content, and total sulfated GAG amount showed decrease in both buttock and forearm of old group, but did not decreased in the buttock of old males. HA amount of the epidermis decreased in all the groups except in the forearm of old females. Measured total uronic acid and HA amounts showed correlation to tissue water contents (R2=0.25~0.6) in both dermis and epidermis, but not correlated with Dermaflex- or Corneometer-measured data. These series of investigations about GAG changes in skin aging process probably provide more fundamental understandings about the functions of GAGs in human skin.

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Effect of PUVA on type I and type III collagen synthesis in normal and scleroderma fibroblasts

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Recent studies have demonstrated that PUVAtherapy is effective to sclerotic skin of scleroderma. UVA irradiation is known to induces collagenase in human dermal fibroblasts *in vitro* and *in vivo*. We investigated that effects of UVAirradiation on gene expression of collagenase, type I collagen and type III collagen in cultured normal and scleroderma fibroblasts under the presence of 8-methoxypsoralen. Methods: Dermal fibroblasts were cultured with routine methods. When the cell layers had become confluent, the cells were incubated for 24 hours at 37oC in DMEM containing 10% FBS, supplemented with 10 –4 M 8-methoxypsoralen. Subsequently cells were irradiated with 0.3-2.4J/cm2 of UVA, and were incubated for another 24 hours with radioactive proline. Then collagen synthetic activity was measured by fluorography with extracted protein of the culture supernatant. Total RNA was isolated from cultured fibroblasts by extraction in guanidium isothiocyanate then northern blots analysis were performed using 32P labeled cDNA of α1(I) collagen,α1(III) collagen and GAPDH. Results: UVAirradiation reduced collagen production(to 40% in maximum) and mRNA levels of α1(I) collagen(to 30% in maximum) and α1(III) collagen(to 30% in maximum), and enhanced gene expression of collagenase (to 200% in maximum)in a dose dependent manner in normal and SSc fibroblasts under the presence of 8-methoxypsoralen *in vitro*. These results suggest that direct effect of UVAirradiation under the presence of 8-methoxypsoralen to fibroblasts of collagen and collagenase expression is one of the mechanism for effectiveness to sclerotic skin of scleroderma by PUVA therapy.

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Identification of a factor which binds to a DNA element between -332 and -402 of the human COL1A1 promoter that elevates the transcription of COL1A1

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The regulation of type I collagen gene expression plays an important role in fibrosis and skin atrophy. However, a deletion analysis of the 5-flanking region of the humanα1(I) collagen gene (COL1A1) to investigate the transcriptional activity of human COL1A1 has not been satisfactorily performed. We report the existence of a DNA element located between -332 and -402 that elevates promoter activity and the existence of a factor that binds to this region. Methods: Dermal fibroblasts were plated and cultured to 50% confluence in 60-mm dishes, then co-transfected using a COL1A1 luciferase construct and a CMV renilla luciferase construct using a FuGene 6 kit. COL1A1-luciferase constructs containing 2,300, 804, 610 and 332 bp of the 5-flanking region of COL1A1 were used. Plasmids containing COL1A1 luciferase constructs with -610, -529, -481, -402 and -332 bp deletions of the 5-flanking region were also used. Forty-eight hours later, the cells were harvested and lysates were prepared; the luciferase activity was then determined. Gel mobility shift assays were performed with nuclear extract. Competition assays using DNA fragment containing short substitution mutation of candidate binding site were also performed. Results: The relative luciferase activity remained unchanged when DNA containing -804 bp or -610 bp of COL1A1 were transfected, whereas when DNA containing 332 bp of COL1A1 was transfected, the relative luciferase activity was remarkably reduced. We then analysed the promoter activity using more precise deletion constructs and found a clear reduction when the -402 to -332 promoter region was deleted. We also identified a binding factor which binds to the DNA around –386 to-371(CTTCCACCTTTGGAAG) of COL1A1. These results suggest that the presence of a DNA element between -332 and -402 of the COL1A1 5-flanking region that activates the transcription of COL1A1 and the existence of a DNA binding factor which binds to the DNA around –386 to-371 related to this activity.

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Protein oxidation during aging: protective effects of a natural extract

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Increased accumulation of oxidatively modified proteins has been described as a hallmark of cellular aging. This accumulation is the result of the deregulation of the balance between the rate of protein oxidative damage and the rate of oxidized protein elimination. Removal of oxidized proteins is achieved by specialized degradation and repair pathways. The only repair mechanisms of oxidized proteins are the specific enzymatic systems thioredoxin / thioredoxin reductase and methionine sulfoxide reductases, which can reverse oxidative damage affecting sulfur-containing amino acids. The elimination of all other oxidative modification of amino acids is mediated by various proteolytic pathways such as the proteasome. The proteasomal pathway is mainly responsible for intracellular protein degradation, including basal protein turnover and elimination of damaged or misfolded proteins. It has been shown that proteasome proteolytic activity is affected with aging, and this decline can be explained by decreased expression of proteasome catalytic subunits. The protective effect of a natural extract against oxidative related degradations during aging has been investigated. First, it has been shown that this natural extract was able to protect cells from oxidative stress by inhibiting lipid peroxides production induced by hydrogen peroxide. Secondly, the effects of this extract were studied on the oxidized proteins repair and degradation systems. In this way, we have shown that the extract was able to stimulate the expression of thioredoxin as well as one proteasome catalytic subunit by dermal fibroblasts. Moreover, in a model of replicative senescence of dermal fibroblasts, the extract was able to counteract the senescence-induced downregulation of this proteasome subunit and to restore a normal level of the latter. We have demonstrated that this natural extract has anti-oxidant properties and is able to stimulate elimination of oxidized protein by stimulation of the repair and degradation pathways. Thus, this extract is able to counteract the accumulation of oxidized proteins that occur during aging.

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A new C-xylopyranoside induces skin expression of heparan sulphate proteoglycans

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Severe structural changes, including deterioration of the mechanical properties of the dermis, occur during skin aging. Whereas many studies have been devoted to age-related alterations of collagen fibrils, far less attention has been paid to another major family of extracellular matrix components, the glycosaminoglycans (GAGs) and proteoglycans (PGs). Heparan sulphate-proteoglycans (HS-PGs), a subclass of the PG family that decreases during aging, may have important functions in skin. The purpose of this study was to demonstrate the potential of a new C-xylopyranoside derivative (simplified as C-Xyloside) to improve HS-PGs expression in human atrophic skin model. Human skin samples were obtained from 12 patients undergoing plastic surgery (Caucasian Fitzpatrick skin type 1-3, women 35 to 55 years old). Atrophic skin model was achieved by applying Diprosone® (bethamethasone at 0.05%) to the surface of skin sample. Skin pieces were maintained in culture for three days in the presence of C-Xyloside (3mM) and were frozen immediately in liquid nitrogen. Cryosections (5µm) were stained with antibodies directed against syndecan 1, syndecan 4 and perlecan. Labeling scoring was performed by semi quantitative grading using scores from 0 (negative) to 4 (maximum). Our experimental model of atrophic skin revealed alterations of syndecan 1 and syndecan 4 at the cell surface of keratinocyte. When C-Xyloside was applied onto atrophic skin samples, syndecan 1 and syndecan 4 depositions increased significantly (3.13 and 3.42 versus 2.6 and 2.7 before treatment). In the same way we observed a decrease in perlecan expression at the level of keratinocyte in atrophic skin samples (1.29 versus 1.9 in normal skin). The treatment of skin samples with C-Xyloside increased expression level in atrophic skin from 1.65 to 1.29. In an organotypic model of corticosteroid atrophic human skin, characterized by decrease of PGs expression, treatment with C-Xyloside improves expression of HS-PGs. In dermo-cosmetology, these PGs may represent important targets to prevent skin aging.

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Effects of natural peptides on cutaneous reepithelialization

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Cutaneous wound healing is a highly coordinate physiological process that rapidly and efficiency restores skin integrity. Skin lesion initiates a series of events including inflammation, granulation tissue formation, reepithelialization and matrix remodelling. Reepithelialization is a crucial step, which involves migration, proliferation and maturation of keratinocytes to restore epidermal integrity. This process is associated with events that require the action of matrix metalloproteinases (MMPs), Laminin-5, some integrins and growths factors (GF), which are essential for the correct epidermal regeneration. We have investigated the *in vitro* effects of natural patented peptides on cutaneous reepithelialization. We have examined these effects on regulating molecular and cellular mechanisms that control reepithelialization process. Firstly, we have studied cell migration by the scratch-Assay and we have evaluated target markers of migration (Laminin-5, MMP-9) by using real time RT-PCR and ELISA tests. Then, we have examined keratinocytes proliferation and KGF release by fibroblasts using ELISA assay. We have shown that theses natural peptides were able to significantly stimulate the keratinocytes migration in the wound. These peptides acted directly on migration markers, laminin-5 and MMP-9. Indeed, they significantly increased the expression of genes encoding for chains composing the laminin-5. In addition, these peptides strongly induced secretion of MMP-9 in keratinocytes via an overexpression of the MMP-9 gene. Moreover, we have demonstrated that these peptides increased significantly keratinocytes proliferation. Furthermore, keratinocytes proliferation is sustained by GF like KGF, which are released by fibroblasts. We have demonstrated that these peptides strongly induced the secretion of KGF by dermal fibroblasts. In this study, we have shown that natural patented peptides can enhance cutaneous reepithelialization. Thus, this new ingredient could be of a great interest to restore rapidly epidermal integrity after superficial cutaneous injuries (scars, post-peeling, post-laser…).

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HIF1 transcription factor regulates laminin-332 expression and keratinocytes migration during wound healing

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Epidermal wound repair is a complex process involving a fine orchestrated regulation of crucial cell functions (proliferation, adhesion, migration). Using an *in vitro* model that recapitulates central aspects of epidermal wound healing, we demonstrate that the transcription factor HIF1alpha is strongly stimulated in keratinocyte cultures submitted to mechanical injury. Signals generated by scratch-wounding stabilize the HIF1alpha protein, which requires activation of the PI3K pathway independently of the oxygen availability. We further show that up-regulation of HIF1alpha plays an essential role in keratinocyte migration during the *in vitro* healing process, because HIF1alpha inhibition dramatically delays the wound closure. In this context, we demonstrate that HIF1 controls the expression of laminin-332, one of the major epithelial cell adhesion ligands involved in cell migration and invasion. Indeed, HIF1alpha silencing abrogates the injury-induced laminin-332 expression, and we provide evidence that HIF1 directly regulates the promoter activity of the laminin alpha3 chain. Our results suggest that HIF1 contributes to keratinocyte migration and thus to the reepithelialization process by regulating laminin-332.

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Evidence that PI3K, Rac, Rho and Rho kinese are involved in basic fibroblast growth factor-stimulated fibroblast-collagen matrix contraction

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Fibroblast-collagen matrix contraction has been used as a model system to study how cells organize connective tissue. Our previous works showed that lysophosphatidic acid (LPA)-stimulated floating collagen matrix contraction is independent of Rho kinase while platelet-derived growth factor (PDGF)-stimulated contraction is Rho kinase-dependent. The current studies were carried out to determine the signaling mechanisms of basic fibroblast growth factor (bFGF)-stimulated fibroblast-collagen matrix contraction. Both bFGF and LPA equally stimulated collagen matrix contraction. Three different inhibitors, LY294002 for phosphatidylinositol-3-Kinase (PI3K), C3 exotransferase for Rho and Y27632 for Rho kinase, suppressed the bFGF-stimulated fibroblast-collagen matrix contraction, respectively. With bFGF stimulation, fibroblasts spread with prominent stress fiber network formation and focal adhesions. In the presence of Rho kinase inhibitor, focal adhesions and stress fiber network formation were mostly lost. We also found that bFGF stimulation for fibroblast caused transient Rac and Rho activation but did not activate Cdc42. In addition, bFGF enhanced fibroblast migration in wound healing assay. The present study implicates that PI3K, Rac, Rho and Rho kinase are involved in bFGF-stimulated collagen matrix contraction. The elucidation of bFGF-triggered signal transduction may be an important clue to understand the roles of bFGF in wound healing.

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Palladin expression in healthy and scleroderma skin fibroblasts.

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A crucial event in wound contraction and fibrosis is conversion of dermal fibroblasts to myofibroblasts, which results from prolonged exposure to TGF-β. Palladin is an actin-associated protein, which localizes in a punctuate manner along stress fibers and controls their integrity. It is expressed in at least five alternatively spliced isoforms, termed by the number of Ig domains, in a cell-type specific manner. These include major 3Ig (90 kDa) and 4Ig (130 kDa) isoforms. Palladin is phosphorylated on tyrosine by Src kinase and on serine/threonine residues. Here, we have studied the expression of palladin in comparison with the classic myofibroblast-specific cytoskeletal protein, α-smooth muscle actin, (α-SMA), in normal and scleroderma skin fibroblasts in culture and *in vivo*. In normal skin, fibroblasts do not produce palladin. In 11 scleroderma skin specimens studied, expression of palladin 4Ig isoform was noted in fibroblasts within dense collagenous matrix by immunohistochemistry. Also keratinocytes and erector pili muscle cells showed palladin staining. Normal human dermal fibroblasts were treated with TGF-β1 (5 ng/ml). Palladin 3Ig and 4Ig isoforms were upregulated already by 12 hours, prior to α-SMA appearance by 24 hours. α-SMA production persisted longer than that of both palladin isoforms. The up-regulatory effect of TGF-β1 on the palladin expression was further augmented by over-expressing wild-type Smad3 construct and it could be inhibited by dominant negative Smad3 in both normal and scleroderma skin fibroblasts. In normal skin fibroblasts, also inhibitory Smad7 inhibited this upregulatory effect, whereas in scleroderma skin fibroblasts Smad7 expression enhanced the expression of palladin 3Ig and 4Ig isoforms. MAPK pathways did not seem to regulate palladin expression in normal or scleroderma skin fibroblasts. In conclusion, TGF-β induced expression of palladin in scleroderma fibroblasts is regulated differently from normal human skin fibroblasts. This may enable an earlier diagnosis and a specific therapy of cutaneous scleroderma.

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p53-knockout mice are resistant to the induction of skin blisters and acantholysis by pemphigus autoantibodies

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Pemphigus vulgaris (PV) and Pemphigus foliaceus (PF) are autoantibody-mediated skin diseases characterized by acantholysis. The molecular mechanisms of acantholysis are the focus of intense investigation in different laboratories. We have shown that PV and PF IgG autoantibodies increased the expression of p53 in the epidermis of the mouse model. Moreover, administration of pifithrin-alpha, a synthetic p53 inhibitor, blocked skin blisters and acantholysis in these animals (JID 127, Abs 64, 2007). We hypothesize that P53, a key regulator of cell proliferation, cell cycle and apoptosis may be involved in pathogenesis of acantholysis. In the present study we tested whether genetic deletion of p53 provides any protection against PF autoantibody induced skin blistering using the p53 knockout mice. Pathogenic PF IgG was injected blindly into neonatal mice (n=19) born from a p53 (+/-) mating pair. The extent of skin disease was evaluated and scored 20 hours later. Mice were sacrificed and skin specimens were harvested for histological evaluation and the tails were used for DNA extraction and PCR genotyping. Of the 19 offspring tested, 5 showed normal skin and 14 had skin blisters. Subsequent genotyping revealed that 3 of the 5 mice without skin disease were p53 (-/-). In contrast, the 14 mice developing skin blisters were either p53 (+/+) or p53 (+/-). Histological analysis of the skin specimens of the p53 (-/-) mice revealed no blisters or minimal acantholysis. Most p53 (+/+) or p53 (+/-) mice showed extensive histological cleft. These data provide direct evidence that p53 plays a key role in the mechanism of acantholysis in pemphigus.

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C5a-C5a receptor signaling pathway is critical for experimental bullous pemphigoid

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Bullous pemphigoid (BP) is an autoimmune skin blistering disease characterized by autoantibodies against two major hemidesmosomal proteins, BP230 and BP180. In the IgG passive transfer mouse model of BP, subepidermal blistering is triggered by anti-BP180 antibodies and depends on the complement system, mast cell (MC) degranulation, and neutrophil infiltration. Activation of the p38 mitogen-activated kinase (MAPK) signaling pathway in MCs is also critical to the development of experimental BP. Binding of anti-BP180 IgG to its target activates the classical pathway of complement system and leads to generation of the anaphylatoxin C5a. C5a in turn triggers MC activation, but the events that link C5a generation and MC activation are not well defined. In this study, we sought to identify the events that connect the activation of the complement system and mast cell degranulation. We found that mice deficient in MCs or C5a receptor (C5aR) injected with pathogenic anti-BP180 IgG failed to develop subepidermal blisters and exhibited a drastic reduction in p38 phosphorylation compared to the diseased wild-type (WT) mice. Local reconstitution of MCs from WT but not C5aR-deficient mice restored high levels of p38 phosphorylation and subsequent subepidermal blistering in MC- and C5a-deficient mice. Local injection of rC5a induced phosphorylation of p38 MAPK in WT, but not MC-deficient mice. Cultured mouse MCs treated with recombinant C5a (rC5a) exhibited a significant increase of p38 MAPK phosphorylation, MC degranulation, and TNF-α release. Furthermore, anti-BP180 autoantibodies from BP patients' sera induced p38 phosphorylation and blistering in humanized BP180NC16A mice. Lesional skin of BP patients also exhibited increased level of p38 phosphorylation. Taken together, these data suggest that C5a interacts with C5aR on MCs, and that this C5a-C5aR interaction triggers activation of the p38 MAPK pathway and ultimately induces MC degranulation and subsequent BP blistering.

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Effect of curcumin on the epidermal basement membrane proteins following chronic radiation injury

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The escalating role of radiation therapy in the treatment of malignant tumors demands development of new agents for prophylaxis, mitigation and treatment of radiation injury. A wide variety of dietary phytochemicals have been studied for radiation-induced injury since they are inexpensive and can be safely manipulated for human use. Curcumin, a natural, biologically active compound extracted from Curcuma species has been shown to have potent anti-inflammatory, anti-apoptotic and anti-fibrotic properties. The objective of this study was to investigate the mitigation effect of curcumin on radiation induced chronic cutaneous injury in rats. WAG/Rij/MCW 35-day-old rats were used for the total body irradiation (TBI). Experimental animals (n=8 in each group; group 1=TBI, 2=TBI + curcumin, 3=curcumin, 4=normal control rats) were exposed to a skin dose of 12.3 Gy. One to two hours later, rats were given fresh isogenic bone marrow cells. The regular rat diet was then supplemented with 5% curcumin starting 5 days after TBI until 120 days post TBI, when all animals were sacrificed. *In vitro* experiments were performed 120 days post TBI. Rats exposed to radiation developed signs of chronic radiation cutaneous injury such as diffuse alopecia and mild stiffness of the skin. In contrast, animals irradiated and then fed with curcumin diet did not show such alterations. Immunohistochemical evaluation of dorsal skin from irradiated rats revealed increased intensity of staining for collagen type IV and VII, while staining for laminin 332 was significantly diminished. Interestingly, the group of animals which received TBI and the addition of curcumin to rat diet had normal expression of laminin 332 in the epidermal basement membrane (EBM). Our studies demonstrate that ionizing radiation alters EBM proteins and may affect cell to extracellular matrix interactions. Moreover, curcumin may serve as a mitigator in the radiation induced chronic cutaneous injury.

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The hair follicle contains fibroblast subpopulations that exhibit preferential wound-healing characteristics, which can be modulated by proopiomelanocortin peptides and corticotropin-releasing factor

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Recent data support significant diversity, topographic differentiation, and positional memory in human fibroblast subpopulations throughout the body. Similarly, human skin contains several distinct fibroblast subpopulations and clinical evidence indicates improved wound healing in terminal haired body sites compared to glaborous or vellus-haired sites. This may reflect the proportionately greater numbers of dermal sheath (DS) and follicular papilla (DP) fibroblasts in the former skin type. This *in vitro* study investigated fully-matched DS, DP and dermal fibroblasts (DF) isolated from healthy individuals for proliferative, migratory, collagen-producing and contractile capacity in the presence and absence of proopiomelanocortin (POMC) peptides and corticotropin-releasing factor (CRF). While DF exhibited greatest baseline proliferation rates and DS exhibited greatest baseline migration ability, ACTH, α-MSH and CRF all significantly increased both parameters for all cutaneous fibroblasts subtypes. DS cells contracted collagen gels to a significantly greater extent than DF or DP cells, reflecting a higher incidence/expression of α-SMA in DS cells. Under wounded conditions DS cells secreted significantly more collagen than DF cells, though this level was significantly increased for both in the presence of TGF-β1. Importantly, TGF- β1 effect was antagonised by ACTH, α-MSH and CRF. Similarly, ACTH, α-MSH and CRF (but not βEND) raised cAMP levels. b-endorphin (b-END) had no effect on any of these parameters. Thus, follicular and interfollicular fibroblasts exhibit very different behaviors *in vitro*, with greater wound-healing features exhibited by DS cells. As POMC peptides antagonized TGF β1-induced collagen production in these cells, these peptides may have a role in treatment of hypertrophic and keloid scars. Our data indicates that DS cells may be more effective wound-healing cells than DF or DP.

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P120-catenin associates indirectly with Desmoglein 3, recruits it to the plasma membrane and stabilizes it at the cell surface

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p120-catenin (p120ctn) is an armadillo-repeat protein that directly binds to the intracytoplasmic domains of classical cadherins. p120ctn binding promotes the stabilization of cadherin complexes on the plasma membrane and thus positively regulates the adhesive activity of cadherins. Previously, we reported that the membrane proximal region (corresponding to residues 641-714) in the IA region of Desmoglein (Dsg) 3 is necessary for complex formation with p120ctn. Interestingly, Δ641-714Dsg3, that lacks p120ctn-binding site, found in the cytoplasm, and not at the plasma membrane. We considered the possibility that association with p120ctn affects Dsg3 expression and/or stability at the cell surface. To address this issue, we performed a biotin surface-labeling experiment in order to examine the fate of mutant Dsg3 molecules with respect to their arrival at the cell surface. The result showed that biotinylated Δ 641-714Dsg3 was turned over more rapidly than wild-type Dsg3. Immunohistochemically, endogenous p120ctn was not co-localized with desmoplakin, but expressed as linear pattern along cell-cell contact together with Dsg3, suggesting that p120ctn associate with pooled Dsg3 at cell surface, but not with Dsg3 at desmosome. To examine the way in which p120ctn binds to Dsg3, we performed an *in vitro* pull down assay using pure recombinant p120ctn-GST and Dsg3-Flag. Purified Dsg3-Flag did not bind to p120ctn-GST in the pull-down assay, which suggests that another protein or proteins, or a physiological three-dimensional molecular structure that is formed *in vivo*, may be required for efficient binding between Dsg3 and p120ctn. A possible mechanism for maintaining Dsg3 at the cell surface is that proteins, including p120ctn, which associate with the membrane proximal region (residues AA641-714) of Dsg3 may stabilize or prevent Dsg3 being endocytosed, or this region of Dsg3 may include a motif that is important to allow Dsg3 to remain at the plasma membrane.

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Matrix metalloproteinase function in laminin-511-mediated keratinocyte migration

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Matrix metalloproteinase (MMP) family has been shown to play a major role in the process of extracellular matrix degradation which is critical in the regulation of keratinocyte migratory activities. Laminin-511, also called laminin-10 (alpha5, beta1, gamma1), is a major extracellular matrix component of epidermal basement membrane of the skin. Although structural important, its function in the keratinocyte functions has not been revealed. Our previous study suggested that laminin-511 involved in the properties of keratinocyte mobility. This study was to examine the involvement of MMP family in the laminin-511 mediated cell migration. In this study we first evaluated the role of laminin-511 in kerationocyte migration by gene knockdown with RNA interference. Laminin alpha5 chain specific small interfering RNA (La5 siRNA) was used to transfect normal human skin keratinocytes. After 72 hours of transfection, reverse transcription and polymerase chain reaction (RT-PCR) was used to examine the mRNA expression of laminin alpha5 and to confirm the gene knockdown. Then RT-PCR was used to evaluate the mRNA expression levels of a panel of MMPs. At the same time, keratinocyte migration assay was performed. 2×105 cells/well were placed on the top of migration membrane in 12-well plate, cells migrated through the membrane were stained and observed under microscope. The results demonstrated that La5 siRNA transfected cells migrated at markedly reduced rate by 91%, 88%, 64%, 52% and 45% at 6, 12, 18, 24 and 36 hours of incubation, respectively, compared with the control cells. RT-PCR analysis showed that the La5 siRNA transfected cells had 42% of laminin alpha5 gene knockdown. At the same time, their mRNA expression levels of MMP-1, MMP-9 and membrane-type 1 (MT1) MMP and MT2-MMP were significantly down. Our data suggest that laminin-511 plays an important role in keratinocyte migration, while the activities of MMP proteins may have regulatory function in this laminin-511-mediated cell migration.

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Caveolin-1 increases basal and TGF-β1-induced expression of type I procollagen through PI-3 kinase/Akt/mTOR pathway in human dermal fibroblasts

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Caveolin-1 (Cav-1) is a major structural protein of caveolae and plays an important role as a negative regulator of various signaling pathways such as the transforming growth factor-β (TGF-β)/smad pathway. In this study, we investigated the role of cav-1 on basal and TGF-β1-induced expression of type I procollagen in human dermal fibroblasts. Our results demonstrated that basal and TGF-β1-induced expression of type I procollagen were significantly increased by adenoviral cav-1 (Ad-cav-1) overexpression, while the basal level of type I procollagen was decreased by cav-1 siRNA. Overexpression of cav-1 inhibited TGF-β1-induced phosphorylation of smad3 and transcription of 3TP-Lux and SBE luciferase reporters, suggesting that cav-1 may inhibit the TGF-β1/smad signaling pathway. On the other hand, TGF-β1 treatment increased the phosphorylation of Akt, and Ad-cav-1 infection augmented this TGF-β1-induced phosphorylation of Akt. Ad-myr-Akt infection significantly increased the basal expression of type I procollagen, while the PI3-kinase inhibitor, LY294002, inhibited the TGF-β1-induced type I procollagen expression and also inhibited the cav-1-induced expression of type I procollagen. In conclusion, our results suggest that cav-1 increases the basal and TGF-β1-induced expression of type I procollagen by regulating two opposite signaling pathways: inhibiting TGF-β1/smad signaling and activating a PI-3 kinase/Akt/mTOR-dependent pathway in human dermal fibroblasts, ultimately resulting in increased type I procollagen expression.

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Tissue-engineered human embryonic extracellular matrix (ECM) for therapeutic applications

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The embryonic extracellular matrix (ECM) creates an environment conducive to rapid cell proliferation and healing without the formation of scars or adhesions. We hypothesized that the growth of human neonatal fibroblasts in 3 dimensions under conditions that simulate the early embryonic environment prior to angiogenesis (hypoxia and reduced gravitational forces) would generate an ECM and soluble proteins with fetal properties. Gene chip array analysis showed the differential expression of over 5000 genes under the hypoxic versus traditional tissue culture conditions. The ECM produced was similar to fetal mesenchymal tissue in that it is relatively rich in collagens type I, III, IV, V, VI, and glycoproteins such as fibronectin, SPARC, thrombospondin, and hyaluronic acid. In addition, metalloproteases were significantly reduced or absent. Since the ECM also plays an important regulatory role in binding and presenting growth factors in putative niches, which support regenerative stem cell populations with key growth factors, we evaluated the effects of hypoxia on growth factor expression during the development of the fetal-like ECM in culture. We show that hypoxia can also enhance expression of factors, which regulate wound healing and organogenesis, such as VEGF, FGF-7, and TGFb, as well as multiple wnts including wnts 2a, 5a, 5b, and 11. The embryonic human ECM also stimulated an increase of metabolic activity in human fibroblasts in vitro, as measured by increased enzymatic activity using the MTT assay. Additionally, we detected an increase in cell number in response to human ECM. These results support the use of this human ECM as a biological surface coating, and tissue filler treatment for various therapeutic applications where new tissue growth and healing without scarring or adhesions would be highly beneficial. Clinical evaluations are currently underway to assess the potential benefits of utilizing the embryonic ECM, wnts, and growth factors in accelerating healing post laser resurfacing.

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Temporary expression of versican in skin wound healing and its possible involvement in keloid pathogenesis

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Keloids are a refractory disease characterized by excessive deposition of extracellular matrices whose etiology remains to be elucidated. We previously found that keloid lesions contained large amount of chondroitin sulfate proteoglycan versican. In this study, we first examined versican expression in normal skin wound healing process using C57BL mice. Immunohistochemistry showed that versican transiently upregulated and reached the maximum at 5 days post-wounding and thereafter decreased. To trace the destination of versican-expressing cells in skin wounds, we generated transgenic mice expressing versican promoter-Cre recombinase/rosa26. The number of LacZ positive cells in the wounds, which were αSMA negative, reached the maximum at 5 days post-wounding, thereafter decreased and disappeared within 14 days. Based on these observations, we hypothesized that excessive ECM deposition of human keloids is developed by persistent survival and proliferation of these versican-expressing cells, which otherwise disappear in normal wound healing process. Next, versican gene regulation was analyzed by RT-PCR and versican-promoter luciferase assay, which demonstrated two-fold higher transcription level in cultured keloid cells. Screening of Wnt, βcatenin, TGF-β, androgen, IL-1β and PI3K showed that the latter two signals were involved in versican expression. Establishment of a human keloid model is required to utilize the results of versican gene regulation for development of keloid therapy. To this end, we implanted cultured keloid cells with collagen sponge scaffolds in nude mice. Collagen sponges with keloid cells deposited versican and weighed significantly heavier than normal fibroblasts sponges after 4 weeks. Utilization of this model may support the development of novel keloid treatment by testing cytokine or reagents, which had been identified to be effective for down-regulation of versican in *in vitro* assays.

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Investigation of adhesive ability of corneodesmosome in stratum corneum

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The stratum corneum (SC) plays essential roles in barrier function, which is the most important role of the skin. Appropriate function of the SC is provided by well-organized SC structure, which is maintained by proper adhesion and desquamation of corneocytes. However, the mechanism of their adhesion and desquamation has not yet been fully clarified although corneodesmosome (CDS) has been thought to be a key factor in its mechanism. To date, we have shown that desmoglein 1 (Dsg1) which was thought to contribute to the adhesion of corneocytes with each other was localized at CDS and its distribution pattern on the corneocytes was diverse in different regions of the body. These results suggested that difference of Dsg1 distribution was involved in the adhesion and desquamation of corneocytes. The purpose of this study is to confirm the adhesion function of CDS and to elucidate the molecular organization of CDS to clarify its role in adhesion and desquamation of corneocytes. First, to confirm the adhesive ability of CDS, we investigated the CDSs in basket weave structure of the SC by retrospective electron microscopy of formalin–fixed and paraffin-embedded skin specimens. As a result, the corneocytes were adhered to each other via CDSs mainly observed in the edge of the cells, whereas CDSs were hardly observed in non-adhered region of the corneocytes. The result demonstrates that CDS in the SC possesses the adhesive ability. To clarify the adhesion and desquamation mechanism of corneocytes, elucidation of precise molecular organization of CDS is inevitable. Consequently, we focused on the adhesion molecules which are known to localize in desmosomes of keratinocytes in living layer, and performed immunoelectron microscopy of the molecules. In the present study, we showed the evidence that CDS in the SC possessed the adhesive ability through adhesion molecule, suggesting contribution of CDS to the SC organization. Our results give a clue to clarification of the role for CDS in the SC organization.

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Role of Macrophage chemoattractant protein-1 in a mouse fibrosis model induced by basic fibroblast growth factor and connective tissue growth factor

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Skin fibrotic disorders as systemic sclerosis are considered to develop under influence of certain growth factors. We have previously established a first animal model of persistent skin fibrosis by subcutaneous injection of transforming growth factor-β(TGF-β) in the first 3 days and connective tissue growth factor (CTGF) in the following 4 days. Here, we have established second skin fibrosis model by simultaneous subcutaneous injection of basic fibroblast growth factor (bFGF) and CTGF. Furthermore we have demonstrated that macrophages and macrophage related proteins are involved in the process of this fibrotic model. Newborn B6 and MCP-1$^{-/-}$ mice were injected with bFGF and CTGF simultaneously for 7 days. Simultaneous injection of bFGF and CTGF caused fibrotic tissue formation, as same as our first fibrotic model. To assess the degree of fibrosis, one portion of the tissue samples obtained from the site of injections on day 8 were stained with hematoxylin and eosin. The amounts of collagen contents were evaluated using the other portion of the tissue samples. The numbers of macrophages, mast cells and CD4+ T lymphocytes were assessed by immunostaining. The up-regulated collagen contents in fibrotic lesion were reduced by MCP-1 deficiency by 55% ($p<0.005$). The number of accumulated macrophages seen in wild type mice on day 8 was decreased in MCP-1$^{-/-}$ mice by 43% ($p<0.05$). Similarly, the numbers of accumulated mast cells and CD4+ T lymphocytes seen in wild type mice were decreased in MCP-1$^{-/-}$ mice by 50% ($p<0.05$), 20% ($p<0.005$) respectively. These results suggest that pro-fibrotic cytokines as bFGF and CTGF not only stimulate fibroblasts directly, but also activate macrophage and enhance MCP-1 production through fibroblast activation.

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Effects of chondroitin sulfate on matrix metabolism and inflammaging in H2O2-induced senescent fibroblasts and keratinocytes

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The ability of human skin to rejuvenate diminishes with time, resulting in increased fragility, which reflects loss of connective tissue. Ageing is also characterized by a chronic, low-grade inflammatory status, called inflammaging. Chondroitin sulfate (ACS) is a glycosaminoglycan composed of sulphated D-glucuronic acid and D-N-acetyl-galactosamine. ACS has been shown to exert anti-inflammatory and anabolic effects in articular cartilage, but little is known about its biological properties in dermis and epidermis. In order to evaluate ACS on skin aging, we have developed an *in vitro* model of stress-induced premature senescence (SIPS), which is closely related to chronological-aging. We have evaluated the effects of ACS on matrix metalloproteinases, interleukins and collagen in SIPS fibroblasts and SIPS keratinocytes. We have also studied the effect of ACS on collagen synthesis in keratinocytes. By using quantitative RT-PCR, we clearly showed that MMP-1 and -3 are up regulated in SIPS fibroblasts and MMP-9 up regulated in SIPS keratinocytes. On the other hand, we observed a decrease of collagen IVa2 mRNA level in SIPS keratinocytes and an increase of IL-6 and -8 in both cell types. We have also showed that pre-incubation with ACS strongly induced a strong inhibition of MMP (-1, -3, -9), IL-6, and IL-8 and an induction of type IVa2 collagen. On the other hand, we have also showed in keratinocytes, that ACS stimulated type IV collagen synthesis, a component of basal membrane, which is quantitatively reduced during aging. Alterations of skin observed during skin aging are due to modification of dermal matrix loss, changes in the basal membrane, and proprobably inflammaging. Our findings illustrated the anti-aging properties of ACS on those three different targets. ACS is able to reduce the exacerbated catabolic activity and inflammaging, and to stimulate the anabolic activity in epidermis, by stimulating collagen synthesis.

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Dermal reconstitution following injury requires integrin-linked kinase

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The extracellular matrix is a key regulator of cell functions. Binding of matrix macromolecules to integrins initiates the assembly of an intracellular multiprotein complex, the focal adhesion, of which integrin-linked kinase (ILK) is a central component. ILK binds to the intracellular tail of β1 integrins and recruits adaptor proteins, thus connecting the outside environment to the actin cytoskeleton. Focal adhesions are force-transducing structures important for cell adhesion/migration and to counteract stress from the environment, such as during the contraction of wound granulation tissue. We showed previously that fibroblasts plated on collagen require integrin α2β1 for proper focal adhesion architecture and collagen lattice contraction *in vitro*. However, in mice, formation and contraction of granulation tissue proceed largely unaltered in the absence of collagen binding integrins α2β1 or α11β1. By contrast, absence of ILK in fibroblasts profoundly not only disturbs focal adhesion and stress fiber formation, collagen lattice contraction and migration *in vitro*, but also results in strongly impaired granulation tissue formation in mice that we generated with inducible, fibroblast-restricted ablation of ILK. Wounds contain fewer myofibroblasts and express lower α-smooth muscle actin levels, due to reduced proliferation and/or impaired TGF-β production and signalling. In line with abnormal TGF-β response, greatly disturbed granulation tissue formation was observed. We believe that fibroblasts deficient in ILK, but not in collagen-binding integrins, are unable to transmit external forces, which in wild type fibroblasts induce an activated, collagen-producing myofibroblastic phenotype. Thus, our results strongly indicate that ILK plays an important role in fibroblasts as mechanical link in dermal cell-matrix interactions and in conveying environmental force cues.

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Interleukin-1 alpha up-regulates expression of MUC18 in melanoma cells

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Metastatic dissemination of melanoma is accompanied by substantial changes of adhesion receptors. Notably, intravasation and extravasation mediated by interaction with endothelial cells are important steps in metastasis cascade. Mucins (MUCs) are produced either as membrane-bound or as secreted gel-forming glycoproteins, and certain type of MUC serves as an adhesion molecule. MUC18, also designated as MCAM or CD146, is a representative transmembrane mucin which is currently thought to contribute to invasion, angiogenesis and metastasis of malignant melanoma. MUC18 is hardly expressed in benign melanocytes and melanocytic nevus cells but abundantly expressed in melanoma cells, especially in metastatic lesions, and represents an independent prognostic marker inversely correlated with patients' survival. The present study showed that interleukin-1 (IL-1) alpha elicited MUC18 expression on human melanoma cell surface. Adhesion assay showed the enhanced adhesion of IL-1-stimulated melanoma cells to HUVEC cells. Invasion assay using modified Boyden chamber also revealed the IL-1-mediated enhancement of melanoma cells invasion thorugh HUVEC monolayer. The results suggest that IL-1 could be in favor of the metastasis potential of melanoma cells. On the other hand, mitogenic acitivity was not affected by IL-1, which was confirmed by MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide] colorimetric bioassay and cell cycle analysis. Furthermore, IL-1-stimulated and nonstimulated melanoma cells failed to generate MUC18-specific CTL line unlike MUC1- or MUC2-specific CTL against some adenocarcinoma cells, which was our first aim.

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Basement membranes in mouse skin are differently affected by the loss of nidogen 1 and 2 or the nidogen binding site on the laminin γ1 chain.

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Nidogen 1 and 2 are ubiquitous basement membrane (BM) proteins which share a common domain structure and comparable binding activities. Studies in mice lacking both nidogens showed severe BM changes in certain organs. However, in some tissues normal BMs form suggesting that nidogens function in a tissue-specific and developmental context. In embryonal skin (E18.5) the lack of both nidogens did not interfere with formation of the dermal-epidermal BM and overall skin morphology. However, formation of the capillary BMs was severely affected as shown by electron microscopy (EM). In vessel BMs deposition of other BM proteins was dramatically decreased demonstrating that nidogens are needed for the retention of these components within the capillary walls. Mice lacking the nidogen-binding site upon the laminin γ1 chain showed reduced and discontinous nidogen 1 staining of the junctional BM whereas nidogen 2 staining was unaltered. The residual nidogen 1 could be colocalized with the laminin γ3 chain. Ultrastructurally no alterations of the junctional BM could be observed. However, in contrast to nidogen double null mice, the capillary BMs of mice lacking this nidogen-binding site were not affected as shown by immunofluorescence and EM. Although both nidogens have been shown to bind in vitro with comparable affinities to this site, only nidogen 2 is retained within the capillary and junctional BMs while nidogen 1 is completely absent from capillary BMs and strongly reduced in the junctional BM. This suggests that in vivo nidogen 1 is retained in the BM by binding to the nidogen-binding site on the laminin γ1 and γ3 chains. In contrast, nidogen 2 appears to be retained by interaction with another site on laminin or another binding partner.

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Retinol suppresses CYR61/CCN1, a negative regulator of collagen homeostasis, in chronologically aged and photoaged human skin *in vivo*

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Natural aging and chronic exposure to solar ultraviolet irradiation (photoaging) cause increased expression of collagen-degrading matrix metalloproteinases (MMP) and reduced production of new collagen. This imbalance of collagen homeostasis, characterized by accumulation of disorganized, fragmented collagen fibers, contributes to decline of skin structure and function. Age-dependent derangement of dermal collagen homeostasis is mediated in part by CCN family member, Cysteine-rich protein 61 (CYR61/CCN1). CCN1 alters collagen homeostasis by inhibiting type I collagen production and promoting MMP-1 expression. CCN1 is predominantly expressed by fibroblasts in the dermis, and is significantly elevated in both chronologically aged and photoaged human skin *in vivo*. Retinoids have been shown to improve aged skin by promoting deposition of new collagen. We therefore investigated regulation of CCN1 by retinol in human skin *in vivo*. Topical treatment of human skin *in vivo* with retinol (vitamin A, 0.4%) for seven days reduced CCN1 mRNA expression in aged skin (80+years) and photoaged skin by 65% (n=10, $p<0.05$) and 70% (n=7, $p<0.05$), respectively, compared to vehicle-treated skin. Immunohistology revealed that topical retinol treatment reduced CCN1 protein expression in fibroblasts in aged and photoaged dermis 64% (n=4, $p<0.05$) and 47% (n=4, $p<0.05$), respectively. To investigate its mechanism of action, CCN1 was over-expressed in cultured human dermal fibroblasts. Elevated CCN1 was found to alter collagen homeostasis (i.e. reduce collagen production and increase MMP-1 expression) by interference with the actions of TGF-β through down-regulation of the type II TGF-β receptor protein (59%, $p<0.05$, n=3). Suppression of CCN1 expression by retinol (or potentially other agents) represents a novel therapeutic mechanism to improve the health of aged and photoaged human skin.

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Pemphigus vulgaris is a disease of impaired desmosome remodeling

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We have shown that Pemphigus vulgaris (PV)-IgG depletes desmosome of Dsg3, which weakened the cell adhesive-strength, in keratinocyte culture. Since this Dsg3-depleting activity was observed more clearly at 30~40% confluence than the complete confluent culture, we thought that migrating-out cells, which are undergoing an active desmosome-remodeling, may be more susceptible to PV-IgG stimulation. To address this hypothesis, we produced a dynamic condition of *in vitro* wound healing model by scratching linearly 120 times crossing the confluent-colonies with a pipette chip. Before and 30 min, 4 hr and 48 hr after wounding the culture and wounding + PV-IgG (2mg/ml) stimulation with or without a Src inhibitor (PP1) and a protein kinase C (PKC) inhibitor (GO6976), cells were subjected to immunoblotting studies. No cells were yet migrating out from the cutting edge at 30 min, but many cells started migrating out 4 to 8 hr, and completed healing at 48 hr. After wounding, all of Dsg3, Dsg1/2, desmoplakin and plakoglobin, but not E-cadherin, were reduced 40 to 60% in content between 30 min to 24 hr, but recovered to the approximately original levels at 48 hr, whereas PV-IgG treatment reduced 40 to 70% only Dsg3 still at 48 hr. Combination of wounding and PV-IgG stimulation decreased extensively (more than 90%) only Dsg3 levels at any time points, suggesting wounded-colony cells are much more susceptible to PV-IgG than confluent-colony cells. Src, but not PKC, inhibitor inhibited almost completely the Dsg3-decrease by any of treatments in the cytoskeleton fraction (Triton X-100 insoluble). These results suggest that Dsg3-decreases caused by wound-healing and PV-IgG are Src dependent and the resultant imbalance of the ratios of Dsg3 to other desmosomal proteins may impair the desmosome remodeling. This may lead to an idea that pemphigus may be a disease of impaired desmosome remodeling, since imbalance of Dsg1/3 ratio in epidermis generated by gene transfection demonstrated previously impairment of keratinocyte differentiation by other researchers.

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Oxidative stress reduces collagen production by impairment of transforming growth factor-beta signaling in human dermal fibroblasts

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Excessive levels of reactive oxygen species (ROS) result in phenotypic and functional changes that are associated with numerous age-dependent human diseases. Prominent deleterious features of aged human skin include thinning and fragility due to reduced production of type I collagen (COL). We report here that low level, sub-toxic, short-term, repetitive oxidative exposure causes long-lived (greater than 28 days) elevation of endogenous production of ROS in human dermal fibroblasts. Fibroblasts were exposed to hydrogen peroxide (200μM) for one hour on two consecutive days. Elevated endogenous production of ROS initially increased two days following oxidative exposure and reached maximum (50-fold, $p<0.05$, n=4) at seven days. Increased ROS down-regulated COL production (reduced 89%, $p<0.01$, n=8) through impairment of transforming growth factor-beta (TGF-β) signaling pathway. Elevated ROS levels down-regulated TGF-β type II receptor (TβRII, reduced 60%, $p<0.05$, n=8), and its downstream effector SMAD3 (reduced 55%, $p<0.05$, n=5), by specifically accelerating their degradation. Treatment with anti-oxidant N-acetylcysteine (NAC), one day after oxidative exposure (i.e. prior to increased ROS production) markedly prevented ROS production. In the absence of elevated endogenous ROS production, expression of TβRII, Smad3, or COL was not reduced. Furthermore, addition of NAC seven days after oxidative exposure (i.e. when ROS levels were maximally elevated) substantially reduced ROS levels (reduced 80%, $p<0.05$, n=4), and largely restored TβRII, Smad3, and COL protein levels (77.4%, 72%, 88.2% of control, respectively, $p<0.05$, n=4-6). These data demonstrate that low level, repetitive oxidative stress leads to stable increase of ROS production, which impairs TGF-β signaling, thereby suppressing COL expression, in human dermal fibroblasts. Anti-oxidants or other agents that prevent down-regulation of TGF-β signaling may retard and treat collagen loss in aged human skin *in vivo*.

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Withdrawn

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Decreased expression of follicular transglutaminases: A possible cause for loose anchoring of anagen hair in active primary cicatricial alopecia

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Anagen hair, confirmed by 4-dimethylaminocinnamaldehyde (DACA) staining, can be extracted with a gentle hair pull in many patients with active primary cicatricial alopecia (CICAL). Although this clinical finding has long been observed, the mechanism has been unclear. Studies have shown that desmogliens 1 and 3 anchor mouse anagen hair in the follicle, and if not functional, there is separation between the inner root sheath (IRS) and outer root sheath (ORS). Since anagen hairs from patients with active CICAL always have an ORS, the cause of the abnormal anchoring is unlikely due to desmogliens. Instead, we hypothesize that transglutaminase (TGM) dysfunction in the IRS leads to abnormal anchoring in CICAL. In the IRS, TGM is the essential enzyme involved in crosslinking trichohyalin to itself and to the intermediate filament. This crosslinking provides the stable, hardened structure of the IRS. TGM genes (TGM 1 - 4) were identified as target genes of PPAR gamma using MAPPER, a platform for the computational identification of transcription factor binding sites (TFBSs) in multiple genomes. We have previously shown that there is loss of PPAR gamma - regulated pathways in patients with CICAL. In addition, hair follicle stem cell - specific PPAR gamma deletion causes scarring alopecia in mice. Gene expression profiling of unaffected and affected scalp biopsies from 20 patients with lymphocytic and neutrophilic CICAL revealed that the expression of follicular TGMs (TGM 1 - 4) are decreased from 2 to 12 fold. TGM 1 and 2 expression is also decreased significantly in the mouse model and an irregular IRS is seen on histology. In addition, Ingenuity Pathway Analysis identified TGMs as part of the immune response, cell death and immunological disease networks. These data suggest that loss of PPAR gamma signaling in CICAL induces decreased TGM expression in the IRS which may lead to abnormal anchoring of anagen hair.

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Both BPAG1 and plectin contribute to keratinocyte motility through the regulation of cofilin activity

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α6β4 integrin is known to stabilize keratinocyte adhesion to matrix via nucleation of the assembly of hemidesmosomes. However, α6β4 integrin also plays a role in keratinocyte migration. β4 integrin-deficient keratinocytes display aberrant motility patterns; these cells move in a circular manner defined by arrays of laminin-332 (laminin-5) in their matrix, as opposed to the back and forward motion of wild-type keratinocytes on linear trails of laminin-332. Moreover, integrin β4 regulates keratinocyte motility behavior by signaling through Rac1 to the actin-severing protein cofilin. The link between β4 integrin and the regulation of cofilin activity has led us to speculate previously that cytoskeleton linkers are key to the mechanism via which β4 integrin determines keratinocyte migration. β4 integrin is known to bind two such linker proteins, plectin and the BP230. To determine if either of these proteins is involved in the functional linkage of β4 integrin and cofilin, we transiently knocked down plectin expression by siRNA transfection of keratinocytes and derived a stable line exhibiting a knockdown in BP230 levels using lentiviral-mediated shRNA technology. Cells exhibiting a knockdown in plectin or BP230 levels express comparable levels of α6β4 integrin on their cell surface. However, keratinocytes deficient in either BP230 or plectin move in a circular path over arrays of laminin-332 in a manner identical to β4 integrin-deficient cells. Furthermore, cofilin activity is significantly lower in knockdown cells compared to wild type keratinocytes. Consistent with this, knockdown of either protein leads to aberrant cell polarity and concomitant differences in their actin cytoskeleton. These data show that both the BP230 and plectin are required for efficient regulation of keratinocyte migration, and function as scaffolding proteins for the regulation of downstream signaling events.

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A 684 helical domain of type VII collagen promotes *in vivo* skin wound closure by enhancing fibroblast and keratinocyte migration

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Type VII collagen (C7) has a central collagenous triple helical domain (TH) flanked by globular non-helical domains, NC1 and NC2. In this study, we examined the effects of topically applied C7 on the healing of wounds in a mouse model *in vivo*. The wounds of mice treated with C7 demonstrated dramatically accelerated wound healing compared with control mice that were treated with vehicle alone. We next sought to determine the mechanism of the C7-induced skin wound healing and assessed whether C7 had an effect on the migration of human dermal fibroblasts (HDFs) and human keratinocytes (HKs) using two independent, computer-assisted, cell migration assays and compared C7 with a type I collagen positive control known to maximally stimulate cell motility. Surprisingly, recombinant C7 vigorously promoted human fibroblast and keratinocyte migration to 95% and 120% of that of type I collagen. C7-driven cell motility was not RDG-dependent but was divalent cation dependent since it was inhibited by EDTA. C7-mediated cell migration was completely inhibited by functional monoclonal antibodies to integrin subunits α2, α3 and β1, but not to α5, α6, and β4. To further localize the responsible C7 domain, we subjected purified NC1, NC2 and a truncated minicollagen that lacked half of the TH domain to the migration assays and mapped the migration promoting activity to 684 amino acids (residues 1920-2603) within the TH domain. Further, two generated G2049E and R2063W mutations associated with recessive dystrophic epidermolysis bullosa, showed significantly reduced ability to promote cellular migration. The NC1 and NC2 domains, as well as two DEB mutants, G2049E and R2063W, that failed to promote skin cell migration *in vitro*, also did not enhance the closure of skin wounds *in vivo*. We conclude that the mechanism by which C7 enhances skin wound closure is via a 684 AA domain within the TH that potently drives cellular motility – HKs participating in re-epithelialization and HDFs participating in the generation of a neo-dermis.

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Extracellular matrix genes regulation: a micro-array approach

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Skin extracellular matrix synthesis and hydrolysis are fundamental events of the cutaneous aging that induce various changes such as loss of firmness or wrinkle formation. A defined amber extract, has been studied to determine whether it could increase extracellular matrix synthesis, inhibit its hydrolysis and or prevent the skin aging. Biological properties of a vegetal amber extract were investigated using human skin fibroblast cultures and specific microarray. Cells were incubated with and without amber extract for 4 hours, followed by gene expression analysis using DNA microarray technology reflecting the expression profile of more than 1300 aging involved genes expressed in healthy skin. We show that after a 4 hours amber extract treatment, modulations in fibroblasts gene expression were observed for 77 genes compared to the non treated cells. The main proportion of the regulated genes can be related to extracellular matrix metabolism. The expression level of type I, III and V collagen genes increased about 3.4, 2.2 and 1.9 fold respectively after amber extract treatment compared to control, whereas the expression level of collagenase 1 or MMP-1 decreased 0.65 fold in the same experimental conditions. Moreover, the expression of Small Leucin Rich Proteoglycans (SLRP) such as biglycan, versican increased 3 and 2.1 fold respectively over control after amber extract treatment. These data show that amber extract not only directly stimulates fundamental components of extracellular matrix biosynthesis by fibroblasts, but also modulates the specific molecules involved in its assemblage and degradation.

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Lysyl oxidase-like gene transcription is down-regulated in aged skin fibroblasts through a Sp-1-rich promoter region

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The formation of elastic fibers takes place mainly during embryogenesis and continues throughout growth. They are then gradually degraded over time. The aim of our study was to determine the changes in expression that could affect the lysyl oxydases LOX and LOXL, responsible for the cross-linking of tropoelastin. LOXL, but not LOX, was less detectable in the skin of adult donors, particularly at the dermo-epidermal junction. This drop in LOXL was reflected at mRNA and protein levels, expressed in fibroblasts in adult donor skin, as well as the level of activity of the human LOXL gene promoter. The LOXL promoter contains a proximal region (-712/-1) that strongly activates transcription in fibroblasts from young donors, but whose activity drops in relation to donor age, especially after 80 years old. This region, and notably the -442/-342 region, indispensable for promoter activity, carries numerous binding sites for the transcription factor Sp-1. EMSA and supershift experiments confirmed the binding of Sp-1. In conclusion, reduced LOXL expression observed in donor skin and fibroblasts as a function of age could be explained by a drop in activity of the LOXL promoter, notably through the activity of the transcription initiating region, rich in Sp-1 transcription factor binding sites.

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Does desmocollin 3 play a role in epidermal differentiation and hair follicle formation?

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Desmocollin 3 (Dsc3) is a desmosomal cadherin that is thought to function as a cell adhesion molecule in the basal layers of the epidermis. Previous results from our laboratory have demonstrated that a conventional null mutation in the Dsc3 gene is embryonically lethal. We have recently generated mice with a conditional null mutation in the Dsc3 gene. Ablation of Dsc3 expression in the skin and other stratified epithelia is compatible with life. Nevertheless, a significant percentage of newborn mice with Dsc3 null skin die shortly after birth with severe intra-epidermal blistering. We now demonstrate that Dsc3 null keratinocytes behave normally in cell culture with respect to cell proliferation and migration. However, as expected, cell adhesion is severely affected in mutant keratinocytes. It has been proposed that Dsc3 expression is related to epidermal stratification. To test this hypothesis, we analyzed the expression of a large panel of differentiation markers in the mutant epidermis. Our results indicate that loss of Dsc3 does not interfere with epidermal differentiation. The experiments described thus far induced a Dsc3 null mutation after the induction of hair follicle formation. To determine whether loss of this gene prior to hair follicle induction (at around embryonic day 13.5; E13.5) affects hair development, we induced an epidermal null mutation at around E9.5. Our results demonstrate that hair follicles are formed normally in these mice. However, adult mutant mice develop a cyclic telogen hair loss phenotype. In summary, our data demonstrate that Dsc3 is critical for cell adhesion in the epidermis, but is not required for proper keratinocytes differentiation.

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The non-collagenous 1 (NC1) domain of type VII collagen enhances the "stemness" of mesenchymal stem cells and their osteogenic potential: implications for bone regenerative repair

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Mesenchymal stem cells (MSC) are pluripotent cells that can differentiate into osteoblasts and provide an excellent source for bone regenerative repair. However, MSC-based cell therapy for skeletal defects has been hindered by a limited life-span of MSC and the fact that they progressively lose their osteogenic potential during *ex vivo* expansion. Type VII collagen (C7) mediates skin dermal-epidermal adhesion and regulates cell attachment and motility. Children with RDEB develop bone-related diseases. In this study, we examined the possible role of C7 in the regulation of the osteogenic potential of MSC. We showed that C7 is expressed in MSC in culture and in bone tissue *in vivo* and that C7 itself strongly supported cell-matrix attachment of both human and mouse MSC. Incubation of human or mouse MSC with osteogenic medium supplemented with recombinant C7 for 4 days significantly increased the osteogenic differentiation of MSC by more than 20 fold, as demonstrated by increased alkaline phosphatase activity, bone nodule formation, and calcium accumulation. The enhanced bone-forming ability of MSC correlated with increased expression of osteoblast markers including alkaline phosphatase and osteocalcin. The ability of C7 to enhance the osteogenic potential of MSC resides within the NC1 domain that contains the homology to cartilage matrix protein. Further, over-expression of C7 in MSC using lentiviral vectors significantly extended their life-span, and not only maintained, but also enhanced their osteogenic potential. Most remarkably, C7 induced bone formation in late passage MSC cultures that otherwise lost their osteogenic differentiation potential. Our data provide the first evidence that C7 may play a role in osteogenic differentiation of MSC. The ability of C7 to maintain and improve the osteogenic potential of MSC may provide a useful strategy for MSC-based cell therapy for bone regeneration and repair.

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Quantifying skin incorporation into porous polyHEMA in a mouse implant model

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Stable skin attachment to percutaneous devices, such as prosthetic limb anchors and central venous catheters, is one approach to addressing the high rates of infection that complicate the use of such devices. We previously described a mouse model of percutaneously implanted porous poly 2-hydoxyethyl methacrylate (polyHEMA) that demonstrated incorporation of keratinocytes and dermal cells into polyHEMA pores, indicating the possibility of stable skin attachment to this biomaterial. Precision-engineered porous polyHEMA rods of consistent shape and pore size were placed through the skin of C57BL/6 mice using a needle-implantation technique. In order to determine if pore size and surface treatment of polyHEMA affect skin incorporation and attachment, we selected an image analysis method for quantifying cutaneous incorporation into implant pores. The polyHEMA rod and surrounding skin tissue were excised on day three or seven, oriented cross-sectional with regard to the rod, and frozen in O.C.T. embedding medium. The tissue was serially sectioned, and a subset of sections was stained for keratinocytes (pankeratin antibody) or endothelial cells (PECAM antibody) using standard peroxidase immunohistochemistry. Sections were imaged using a light microscope and Adobe Photoshop with Fovea Pro Image Analysis plug-ins was used to determine the area of cellular incorporation within the porous implant. The volume of cellular incorporation was then calculated using the area data and thickness between sections. This method can be used as a tool to quantitatively evaluate keratinocyte and endothelial cell incorporation into porous polyHEMA implants, which allows us to determine if pore size and surface modification affect cellular response to the implants. We hypothesize that identifying biomaterial properties that promote sealing the interface between skin and percutaneous devices will be a critical step in reducing device-related infections.

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14-3-3ζ regulates keratinocyte cell migration and matrix organization

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Wound healing in the skin is a complex process involving modulation in cell adhesion and cytoskeleton components. Recent data implicate α6β4 integrin as a key regulator of keratinocyte motility via its ability to determine the laminin-332 tracks along which cells migrate. Moreover, α6β4 integrin determines keratinocyte cell polarity by regulating the activity of Rac1 and the assembly of lamellipodia at the leading edge of migrating cells. In keratinocytes, α6β4 integrin-mediated signaling to Rac1 results in dephosphorylation/activation of the actin severing protein cofilin via members of the slingshot family of phosphatases. Since 14-3-3 proteins bind to α6β4 integrin in actively migrating keratinocytes and slingshot proteins in stationary cells and because 14-3-3 proteins are known to contribute to the regulation of various signaling pathways, we next asked whether 14-3-3 proteins are central to controlling keratinocyte cell migration. We initiated our analyses using adenoviral constructs expressing the amino or carboxy terminal regions of 14-3-3ζ. This isoform has been shown by others to bind both the β4 integrin subunit and slingshot 1, a member of the slingshot family that is expressed in keratinocytes. Expression of the amino terminal half of 14-3-3ζ has no effect on cofilin phosphorylation in human keratinocytes. However, there was a dramatic increase in the level of phosphorylated cofilin in keratinocytes expressing the carboxy terminal half of 14-3-3ζ. These results suggest that the amino terminal portion of the 14-3-3ζ isoform is crucial for mediating signaling events that control keratinocyte cell migration and thus, laminin-332 matrix organization.

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Dermal response to porous poly(2-hydroxyethyl methacrylate) [poly(HEMA)] percutaneous implants in C57Bl/6 mice

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Long-term percutaneous medical devices, such as attachment posts for prosthetics or percutaneous leads for implanted electrical devices, not only need to preclude infection but must establish stable anchorage with the skin. We used a mouse model in which 2mm x 2mm x 10mm poly(HEMA) rods with 40μm pores and ~ 15μm interconnecting throats, were implanted with entry and exit sites through dorsal skin. Rods were implanted below the panniculus carnosus, rested directly on a thin fascial layer, and remained in situ for 7, 14 or 28 days. The poly(HEMA) rods were either left untreated, surface-modified with carbonyldiimidazole (CDI) to enhance protein and cell attachment, or CDI-treated with adsorption of partially purified laminin 332 (laminin 5). Using immunohistochemical techniques and tissue stains, we evaluated pattern and progression of vessel formation and collagen deposition within the poly(HEMA) pores. Endothelial cells (PECAM-1 antibody) migrated into the pores of 7 day implants from 1) dermal regions immediately adjacent to the epidermal interfaces, and 2) regions adjacent to the panniculus carnosus. Endothelial cells were evident throughout the pores in the 14 and 28 day implants, forming vessel-like structures, filled with red blood cells. Fine collagen fibers identified by picro-sirius red staining showed deposition into the pores of 7 day implants adjacent to the entry/exits sites, the panniculus carnosus and thin fascial layer. Large collagen bundles were present throughout the pores of 14 day implants and abundant by 28 days of implantation. The integrity of the cutaneous incorporation is necessary for prevention of permigration, reduction of microtrauma and enhanced functionality of long-term percutaneous devices.

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In a cellular model of adhesion, collagen XVII (BP180) binds to laminin 332

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Collagen XVII (also known as BP180) is a key transmembrane constituent of the epidermal anchoring complex that was first identified and characterized as an autoantigen associated with the blistering skin disease bullous pemphigoid. Mutations in collagen XVII are responsible for a subtype of the inherited blistering disease junctional epidermolysis bullosa. Based on investigations into the biology and pathology of this protein, collagen XVII has long been thought to play an important role in maintaining dermal-epidermal adherence. To perform this function, it has been hypothesized that collagen XVII interacts with one or more components of the epidermal basement membrane. Based on results published by another group using a protein-protein binding assay, laminin 332 (la-5) has been identified as one candidate ligand for collagen XVII. In the present study, we further explore this possibility through the use of a cellular model of adhesion. The non-adherent myelogenous leukemia cell line, K562, was transduced with a retroviral expression vector for wild-type human collagen XVII. Appropriate transmembrane expression of the transgene product was confirmed by flow cytometry. The experimental cells were sorted using an antibody-based magnetic bead system, and cell lines with different levels of expression were characterized. The parental and transduced K562 cells were then assayed for their ability to attach to wells coated with la-5 and other extracellular matrix proteins (laminin 111, collagen I, and fibronectin). Collagen XVII-positive cells did attach to la-5 at levels significantly higher (2 to 5 times) than attachment levels to other matrix proteins. The level of specific cell attachment to immobilized la-5 was positively correlated with both the amount of la-5 coated in the well and the level of collagen XVII expressed by the transduced cells. These findings support the hypothesis that the function of collagen XVII involves binding of its ectodomain to la-5.

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Desmosomal control of microtubule organization in the epidermis

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Desmosomes are button-like cell adhesion structures that provide strength to tissues undergoing mechanical stresses. They are particularly important in the function of the epidermis and the heart. Desmosomes accomplish this function by anchoring the intermediate filament cytoskeleton. Recently, desmosomes were also found to organize microtubules in differentiating epidermis. In basal epidermal cells, microtubules emanate from the centrosome forming an astral network. In suprabasal cells, microtubules are localized cortically at cell–cell junctions. Loss of desmoplakin (DP), a constitutive desmosomal component, prevents accumulation of cortical microtubules *in vivo* and *in vitro*. The mechanism underlying how desmosomes recruit microtubules is not yet understood. Here, we show that ninein, a centrosomal protein, becomes localized to cell junctions when cultured keratinocytes form desmosomes. The junctional localization of ninein is DP-dependent. We have identified and are characterizing other novel proteins which might also participate in the process of cortical microtubule rearrangement. Our work suggests a new role for DP and ninein in organizing microtubules in the developing epidermis.

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Dsg3 antibodies as modulators of epidermal stem cells in pemphigus vulgaris

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Multipotent epidermal stem cells provide the proliferative cell pool for epidermal renewal. Located in the hair follicle bulge and the basal layer of the interfollicular epidermis, they are responsible for tissue homeostasis and wound repair. In the mouse, research on cell signaling pathways regulating stem cell proliferation and differentiation implicated constitutive c-Myc expression in stem cell activation and subsequent depletion of the epidermal stem cell compartment. As reported by our laboratory, c-Myc overexpression and epidermal hyperproliferation is triggered by binding of pathogenic antibodies to Desmoglein 3 (Dsg3) in the epidermis and appendices of human Pemphigus vulgaris (PV) patients. In a passive transfer mouse model for PV, we now find that Dsg3 antibody-induced c-Myc upregulation coincides with altered tissue homeostasis. Specifically, pathogenic anti-Dsg3 antibodies had the potential to deplete epidermal stem cells residing in the basal layer of the epidermis. Primarily the colony-forming efficiency of primary keratinocytes *in vitro* was significantly reduced after PV IgG application into neonatal mice. Concomitantly, we monitored the expression of stem cell and transit amplifying cell markers in the skin by immunofluorescence and flow cytometry. To assess the label-retaining capacity of keratinocytes, we further determined BrdU retention relative to the proliferation marker Ki67 within a 30-day chase period. While upregulating c-Myc, application of a mouse monoclonal anti-Dsg3 antibody (AK23) reduced the number of BrdU bright cells and the number of BrdU/Ki67 double positive cells in the mouse epidermis already one-day after the chase. Our results suggest that the epidermal stem cell pool is quantitatively and qualitatively affected in the PV mouse model and signaling through c-Myc could potentially affect the regenerative capacity of the epidermis in PV.

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IVIg causes a selective decrease in pemphigus autoantibodies

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A major problem with all current therapies of pemphigus is that they target all antibodies rather than only those that cause the disease. Promising pilot observations suggest IVIg can selectively lower serum levels of only pemphigus antibodies. The following study was conducted to confirm this critical observation. Twelve patients with pemphigus who failed, or had a contraindication to conventional therapy were treated with one to three cycles of IVIg. Each cycle consisted of 400 mg/kg/Ig given IV daily for 5 days. The cycles were repeated every 2 weeks. Serum levels of anti-dsg 3 and anti-dsg 1 were measured by ELISA immediately prior to IVIg treatment and a median of 2 weeks following the last cycle. As controls, serum levels of normal antibodies against HSV 1 & 2, mumps and varicella were measured concurrently. Within a median of 2 wks following the last IVIg cycle (median of 2 cycles, range 1-3) serum anti-dsg3 declined in 100% of 9 pts that were positive at baseline and in 8 of 10 (80%) pts that were positive for anti-dsg 1. Anti-dsg 3 decreased by an average of 45% and anti-dsg 1 by 32%. By contrast, serum levels of the 4 normal antibodies increased in almost all (92%) of patients, by an average of 408% ($p<0.001$). These results indicate IVIg treatment selectively removes from the circulation only the abnormal antibodies that cause pemphigus – thus achieving the ideal goal of treatment in autoantibody-mediated diseases, the selective removal of pathogenic autoantibodies. The mechanism by which we believe this occurs will be discussed.

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Autoantibody against one of the antioxidant repair enzymes, methionine sulfoxide reductase A (MSRA), in systemic sclerosis: association with pulmonary fibrosis and vascular damage

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Oxidative stresses followed by ischemic-reperfusion injury are important factors that contribute to tissue damage in systemic sclerosis (SSc). For protecting oxidative injuries, cell contains multiple antioxidant mechanisms. Methionine sulfoxide reductase A (MSRA) is one of the antioxidant enzyme peptides, which repairs oxidized methionine, methionine sulfoxide, into methionine. Serum anti-MSRA autoantibody (Ab) levels were examined in 70 patients with SSc by enzyme-linked immunosorbent assay. Serum anti-MSRA Ab levels were significantly elevated in SSc patients compared to normal controls ($p<0.01$). Anti-MSRA Ab levels were similar between patients with diffuse cutaneous SSc (n=40) and those with limited cutaneous SSc (n=30). When values higher than the mean + 2SD of the control serum samples were considered to be elevated, anti-MSRA Ab was detected in 33% of total SSc patients. Concerning clinical correlation, anti-MSRA Ab levels significantly increased in SSc patients with pulmonary fibrosis ($p<0.05$), cardiac involvement ($p<0.05$), or decreased total antioxidant power ($p<0.05$). Furthermore, anti-MSRA Ab levels correlated negatively with %VC ($r=-0.37$, $p<0.05$) and %DLco ($r=-0.26$, $p<0.05$), and positively with the PI value ($r=0.31$, $p<0.05$). Anti-MSRA Ab levels correlated positively with serum levels of 8-isoprostane ($r=0.52$, $p<0.01$), a marker for oxidative stress, and those of Hsp70, a cellular stress marker ($r=0.25$, $p<0.05$). In addition, anti-MSRA Ab levels increased in deceased SSc patients than in those alive. Finally, MSRA activity was significantly inhibited by anti-MSRA Ab in the serum of SSc patients. These results suggest that anti-MSRA Ab contributes to the development of the disease severity and activity in SSc by inhibiting one of the antioxidant repair enzymes, MSRA.

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High titer of anti-phosphatidylserine-prothrombin complex antibodies in patients with cutaneous polyarteritis nodosa

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To investigate the possible role of antiphospholipid antibodies (aPL) in cutaneous polyarteritis nodosa (CPN), we measured serum lupus anticoagulant (LAC), IgG and IgM anti-cardiolipin (aCL) and anti-phosphatidylserine-prothrombin complex (aPS/PT) antibodies, and anti-β2-glycoprotein I-dependent cardiolipin (aβ2GPI/CL) antibodies in the 16 CPN patients, 8 microscopic polyangiitis (MPA) patients, 33 systemic lupus erythematosus (SLE) patients, and 23 heathy controls. LAC was determined according to the Subcommittee on Lupus Anticoagulant/Phospholipid Dependent Antibody guidelines. aPS/PT, aCL, and aβ2GPI/CL antibodies were measured by enzyme-linked immunosorbent assay. aPS/PT antibodies and/or LAC were detected in all CPN patients, but not in any controls. Serum IgM aPS/PT antibody was found in 13 (81.3%) CPN patients. The mean ± SD serum aPS/PT IgM level (19.9 ± 12.4 units/ml) in CPN patients was significantly elevated compared with SLE patients (5.7 ± 5.9 units/ml). IgG aPS/PT antibody was detected in 5 (31.3%) CPN patients, but not in any controls. The IgG aPS/PT antibody titers were similar in CPN patients (12.3 ± 12.0 units/ml) and SLE patients (13.8 ± 14.3 units/ml). Three (18.8%) CPN patients were positive for IgG aCL antibody and 2 (12.5%) for IgM aCL antibody. No MPA patients had aPL. CPN skin manifestations included livedo reticularis (14 [87.5%]). Direct immunofluorescence revealed C3 within the affected vessels in 7 (77.8%) of 9 CPN patients. Our study demonstrated that presence of aPS/PT antibodies and/or LAC could serve as markers in CPN patients. CPN could be dependently associated with the presence of aPS/PT antibody. Clinicians need to recognize these titers to permit early accurate diagnosis and treatment. We believe that aPS/PT antibodies will become widely recognized as a new factor when diagnosing CPN.

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Clinical and therapeutic approaches to CCCA:a North American practice survey

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Central centrifugal cicatricial alopecia (CCCA) is a primary lymphocytic cicatricial alopecia that mainly affects women of African descent. It is characterized by chronic, progressive alopecia on the vertex scalp that gradually expands centrifugally. Although women frequently seek medical care for this condition and patients experience considerable psychological distress, few studies have investigated clinical characteristics and management strategies for this disorder. Much of what is known about the etiology and treatment of CCCA is based on anecdotal evidence, and to date, clinical trials investigating therapies for CCCA are lacking. To elucidate common diagnostic and treatment approaches for CCCA practiced by North American dermatologists a survey was sent to dermatologists with an expertise in either hair disorders or skin of color. 2188 surveys were sent to members of the North American Hair Research Society and the American Academy of Dermatology who designated a specialty in hair disorders and/or skin of color. Responders were asked to indicate the number of patients diagnosed in a typical month, average age of patients at presentation and onset, diagnostic tests, treatments, clinical endpoints for treatment, and response rates to treatment. 125 surveys were completed. The number of patients diagnosed per month ranged from 0 to 50, with a mean±SD of 5.23±8.34 (N=115). The mean ages±SD at onset and presentation were 34.97±7.76 years (N=91) and 43.72±7.43 years (N=102), respectively. Diagnostic tests included scalp biopsy, cultures, blood tests, trichogram, hair pull test, and dermoscopy. However, 7 responders reported that they base diagnosis on clinical exam alone and the majority of responders who perform biopsies noted that they diagnose CCCA if history and clinical findings are consistent even if the biopsy is non-diagnostic. Myriad treatments (including oral, intralesional, and topical therapies) were reported, with topical corticosteroids being the most common. Lastly, clinical endpoints for treatment varied and included inhibition of disease progression, improvement of symptoms, and/or regrowth.

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Skin cells, but not CD4+ and CD8+ T cells, are resistant to indoleamine 2, 3-dioxygenase (IDO) induced low tryptophan environment

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We have previously demonstrated that IDO expressed by dermal fibroblasts generates a tryptophan deficient environment in which immune cell lines such as THP-1 and Jurkat cells, but not skin cells, undergo apoptosis. However, it is not clear whether the primary immune cells such as CD4+ and CD8+ responsible for graft rejection would do so under similar experimental condition. Further, the mechanism by which primary skin cells such as fibroblasts and keratinocytes are resistant to this culture environment is not known. To address these issues, primary immune cells were co-cultured with IDO expressing fibroblasts and their rate of proliferation and General Control Nonderepressing-2 (GCN2) kinase pathway activity in response to allogenic antigens were evaluated by cell proliferation assay and western blot analysis, respectively. The result showed that co-culturing IDO expressing primary human fibroblasts with CD4+ and CD8+ cells significantly suppressed their immune reaction to allogeneic pieces of either epidermis or full skin relative to those co-cultured with non-IDO expressing fibroblasts. This effect was restored by using an IDO inhibitor, 1-methyl-D-tryptophan. However, under similar experimental condition, primary fibroblasts were resistant to IDO induced low tryptophan environment. To understand this differential mechanism, we then co-cultured IDO-expressing fibroblasts with bystander CD4+, CD8+ T cells, keratinocytes or fibroblast for 3 days. The results showed a significant activation of apoptotic pathway as analyzed by caspase-3 induction as well as the expression of CHOP, a downstream effector of GCN2 kinase pathway in both T cell subsets, but not in skin cells. In conclusion, the lack of caspases-3 and GCN-2 pathway activation in primary fibroblasts and keratinocytes in response to IDO induced low tryptophan environment differentiates them from CD4+ and CD8+ cells which are very sensitive to the same experimental environment..

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Evidence-based review of pregnancy outcomes after maternal use of topical steroids

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We present an evidence-based review of pregnancy outcomes after maternal use of topical steroids. We searched the current best evidence from 11 databases and identified six relevant cohort and case-control studies. Meta-analysis was inappropriate and not performed. Czeizel showed an association between oral cleft and topical steroids use throughout pregnancy and in the 1st month of gestation, but not in the 2nd and 3rd months, the organogenesis period, in a population case-control study. Mygind found there was no increase in adverse outcomes in women receiving weak/medium potency topical steroids [odds ratios (ORs) with 95% confidence interval (95% CI) for low birth weight (LBW), birth defects, and preterm births being 0.7 (0.17-2.85), 0.93 (0.23-3.80) and 1.04 (0.56-1.92), respectively] and those receiving strong/very strong topical steroids [ORs being 1.23 (0.45-3.37), 0.56 (0.14-2.28) and 0.99 (0.54-1.84), respectively] in a population cohort study. Edwards described a link between 1st-trimester use of topical steroids and oral cleft [OR 18.6 (95% CI 1.29-270)] in a hospital-based case-control study. Källén found no relation between maternal 1st-trimester use of topical steroids and oral cleft [adjusted risk ratio 2.01 (95% CI 0.55-5.15)] in a national register study. Pradat also found no relation between 1st-trimester use of topical steroids and oral cleft in a multi-country case-control study. Mahé showed a link between highly potent steroids and a lower plasma cortisol level, a smaller placenta as well as LBW infants ($p < 0.05$) in a hospital cohort study. The current data suggest a lack of association between topical steroids and adverse pregnancy outcomes, but also imply a link between highly potent topical steroids and LBW. There is not a large enough body of evidence for robust conclusions, which calls for large-scale cohort studies.

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Clinical evaluation of 2% analogue of green tea extract in a hydrophilic cream for treating seborrheic dermatitis. A placebo-controlled, double-blind study

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Objective: To evaluate the clinical efficacy, tolerability and safety of 2% analogue of green tea extract (EGCg)in a hydrophilic cream to treat seborrheic dermatitis. Methods: Subjects n=100 (61M/39F)aged 18-65 years with clinically diagnosed seborrheic dermatitis were sequentially randomized into 2 parallel groups. Each patient was allocated with an identical precoded tube containing 50-g (either active or placebo)with instructions on how to topically use the study cream 2 times a day for 6 weeks. Complete absence of clinical signs of treated inflammation, scaling and itching was considered as cured. Dermatologic and photographic methods were used both at the baseline and on weekly scheduled visits. Results: Marked clinically beneficial improvement was observed in both the groups at the end of the study period. Code disclosure revealed that 2% analogue of green tea extract (EGCg)hydrophilic cream yielded statistically significantly higher reduction in mean inflammatory lesion count than placebo. Following the investigator's global assessment, therapeutic success in terms of a clearing scalings,inflammation and itching was recorded in 76% of the active group and 10% in placebo subjects, $p<0.0001$. Conclusion: The study states that 2% analogue of green tea extract (EGCg) in a hydrophilic cream is safe, tolerable and clinically more efficacious than placebo in reducing the severity of seborrheic dermatitis.

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Identification of a novel dendritic cell subset that could drive Th17 polarization and inflammation in psoriasis vulgaris

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Inflammatory myeloid dermal dendritic cells (DCs) accumulate in chronically inflamed tissues, but little is known about their function. The importance of these cells for psoriasis pathogenesis is suggested by comparative T cell and DC cell counts, where DCs outnumber T cells in many psoriasis cases. We previously identified $CD11c^+BDCA\text{-}1^+$ cells as the main resident dermal DC population found in normal skin. We now show that $BDCA\text{-}1^+$ resident DCs are not increased in psoriasis compared to normal skin, but rather $CD11c^+BDCA\text{-}1^-$ inflammatory DCs are increased 50-fold. We isolated these $BDCA\text{-}1^-$ DCs by FACS sorting psoriatic dermal émigrés, and used them as stimulators in alloMLRs where the output was either T cell proliferation (using CFSE labeling), or polarization of responder T cells to produce IL-17 (Th17) and/or IFNγ (Th1) cytokines. $BDCA\text{-}1^-$ cells were immunostimulatory (64% proliferation, 1:50 stimulator:responder, day 8) and induced three T cell populations: $IL\text{-}17^+$, $IFN\gamma^+$, or $IL\text{-}17^+$ $IFN\gamma^+$ (16.6%, 3.6%, and 1.4% respectively). Unsorted bulk psoriatic émigrés polarized 12% of allogeneic T cells to produce both IL-17 and IFNγ – a 60-fold increase compared to induction from normal skin émigrés. As psoriasis is believed to be a mixed Th17/Th1 disease, it is likely that induction of $IL\text{-}17^+IFN\gamma^+$ cells is pathogenic. We further characterized these sorted $BDCA\text{-}1^-$ DCs using gene arrays and determined high expression of TLR1, TLR2, and CD209/DC-SIGN compared to the resident $BDCA\text{-}1^+$ DC population. TLR1 and TLR2, which are known to form heterodimers that recognize mycobacterial lipoprotein and triacylated lipopeptides, activate NFκB and downstream TNFα production. As this pathway is inhibited by TNFα-blocking drug etanercept, an effective treatment for psoriasis, we propose that psoriasis pathogenesis may be amplified by TLR1 and TLR2 signaling on $CD11c^+BDCA\text{-}1^-$ inflammatory DCs. Overall, data suggest TNFα-dependent maturation of $BDCA\text{-}1^-$ DCs in psoriasis which polarize Th17/Th1 T cells to produce IL-17 and IFNγ.

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Hypnotic trance induced relaxation and self-guided imagery for dermatologic procedures.

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The purpose of this randomized pilot study is to determine whether rapid induction of hypnosis followed by deepening and subsequent self-guided imagery is effective in alleviating anxiety and discomfort associated with dermatologic procedures. In this ongoing randomized study, live induction versus audio-recorded induction is compared with controls. Exclusion criteria include age less than 18 years; Mini Mental State Exam score less than 25; hearing impaired; and not being fluent in English. The same script is used for live and audio-recorded inductions. Zero to ten range subjective unit scales are used for rating patient expectancy and motivation prior to the procedure. Zero to ten range subjective unit scales are also used for rating patient anxiety and pain prior to the procedure and at ten minute intervals during the procedure. Blood pressure and pulse rate measurements are also obtained. The dermatologic procedures are performed by dermatology residents under direct supervision. Local anesthesia is employed but conscious sedation is not used. A Hypnotic Induction Profile is performed on each patient after the procedure is completed. Thus far advantages of live induction have been the ability to pace the induction and tailor it in response to patient responses. Disadvantages of live induction have been the higher level of training required and the extra person or extra attention required. Advantages of the audiotape induction have been the consistency obtained and the lower levels of training and attention required. Disadvantages of the audiotape induction have been the inability to match pacing to the patient's responses and to tailor the process to the timing involved in the individual procedure.

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Severe and prolonged irritant contact dermatitis in zinc deficient mouse

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Acrodermatitis enteropathica (AE) is caused by zinc deficiency. However, the pathogenesis of AE is unknown. Although numerous studies have shown that zinc deficiency diminishes cellular and humoral immunity, AE patients present the immunostimulating inflammation on acral region. Since AE are typically seen on areas subject to repeated contact, we examined allergic contact dermatitis (ACD) and irritant contact dermatitis (ICD) in zinc deficient mice. Balb/c mice were fed by zinc deficient diet (ZD) or zinc adequate diet (ZA). After 3 wks, ACD to DNFB and ICD to croton oil were examined. ACD in ZD mice was significantly decreased, when compared to that in ZA mice. Surprisingly, ICD response in ZD mice was significantly increased and prolonged, when compared to that in ZA mice. Interestingly, histological examinations of ICD lesions in ZD mice demonstrated subcorneal vacuolization and epidermal pallor, which are the histological features of AE. Although clinical, histological and immunological features of ACD and ICD generally share common characteristics, recent study revealed that inflammatory chemokines are suitable markers for their differentiation. To explore how the increased ICD occur in zinc deficiency, we next analyzed gene expression profile during ICD by microarray analysis. Epidermal sheets were obtained from ZD and ZA mice at 24 hrs after vehicle or croton oil exposure. Among 44 chemokines, CXCL1 and CCL20 were markedly induced in ICD. Moreover, these chemokines ranked among the 10 most differentially regulated chemokines discriminating between ZD and ZA mice. The difference was also confirmed by quantitative RT-PCR. In addition, when the zinc-chelating reagent TPEN was added to the culture of Pam212 keratinocytes in vitro, it significantly increased the production of these ckemokines in response to croton oil. Thus, we for the first time describes that ZD mice show severe ICD, presumably due to aberrant chemokine production, providing a biologic basis for understanding the pathogenesis of AE.

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Effect of pimecrolimus and triamcinolone acetonide on epidermal functions and their penetration profiles: A randomized, observer-blinded in vivo study in patients with atopic dermatitis and healthy human volunteers

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Long-term topical glucocorticosteroids (GC) as a standard treatment of atopic dermatitis (AD) are able to induce changes in skin structure. Even short-term topical application of GC can have profound negative effects on epidermal lipid synthesis. The topical immunomodulator, pimecrolimus, provides an alternative to GC by inhibiting calcineurin to suppress T-cell activation and anti-inflammatory activity. The objective of the present study was to assess the effect of pimecrolimus 1.0% (P) and triamcinolone acetonide 0.1 % (TA) (both cream formulations) on epidermal functions and to determine penetration profiles of both drugs. Clinical assessment and measurement of transepidermal water loss, stratum corneum hydration and skin color were performed. Water-, NMF-, lipid- and pH-profile and the drug penetration profile of P and TA were measured with an in-vivo Raman spectroscope (Skin Composition Analyzer). The treatment was performed over 14 days (2x per day) with 7 days follow-up on 10 healthy volunteers and 20 AD patients. We were able to demonstrate improved epidermal functions in both groups. No negative impact of P treatment was observed during the 14 days treatment period. The drug profile showed a deeper penetration during the initial phase of both TA and P compared to the later phase of the treatment. It was concluded that (i) skin physiology (including barrier function, stratum corneum hydration and irritation parameters) is improved in AD patients treated with pimecrolimus. (ii) Drug penetration profiling was possible especially in the initial phase of the treatment. (iii) The interaction of topically applied drugs with the target compartment (stratum corneum) can be monitored in vivo regarding lipid-, NMF- and water profiles.

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Compensatory Regulation of Granulocyte Colony-Stimulating Factor for Hematopoietic Stem Cells Results in Neutrophilic Dermatosis Associated with Myelodysplastic Syndrome

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Recalcitrant neutrophilic dermatoses (ND) or pyoderma are often associated with subclinical myelodysplastic syndrome (MDS). To assess the etiologic role of hematopoietic growth factors (HGF) in the development of MDS-associated ND, we performed karyotypic analysis of blood cells and evaluated HGF levels in patients with recalcitrant ND. Karyotypic analysis, using G-banding method, was carried out in both bone marrow cells and peripheral blood lymphocytes obtained from seven patients with recurrent ND. Serum levels of granulocyte colony-stimulating factor (G-CSF) were measured. In results, six patients had chromosomal abnormalities in the marrow cells, including del (20)(q11; q13.3) in 2 patients, trisomy 8 in 2 patients, t (11; 22)(q23; q11) in 1 patient, and der (1: 15)(q10; q10) in 1 patient. Three patients without morphological findings suggestive of MDS were diagnosed as having refractory anemia (RA). Except for patients with RA, one female had RA with ringed sideroblasts (RARS), and one male had chronic myelomonocytic leukemia (CMMoL). Two patients with RA revealed a normal karyotype in peripheral blood lymphocytes. Marked peripheral cytopenia, suggestive of a certain hematological disorder, was observed only in 1 patient with RARS. Four patients with elevated serum levels of G-CSF had more active or widespread cutaneous diseases. In conclusion, karyotypic analysis of the marrow cells is essentially important in proving a diagnosis of MDS-associated ND, even though the patients had no overt hematological abnormalities. The overexpression of G-CSF appears to be a key cytokine leading to recalcitrant ND.

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Innate immunity signals increase kallikrein expression in rosacea

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The epidermis of patients with rosacea has increased and abnormally processed cathelicidin antimicrobial peptides that are generated by serine proteases such as kallikrein 5 (KLK5). These peptides lead to an inflammatory and vascular reaction in mice that resembles the characteristic histopathological changes in rosacea (Nature Medicine 2007). Here we sought to explain the abnormal cathelicidin and kallikrein expression in rosacea. We hypothesized that innate immune pattern recognition events would be involved since microbial involvement in rosacea pathogenesis has been frequently described and cathelicidin is inducible by infection. We first examined expression of toll-like receptors (TLRs) in rosacea skin. Quantitative PCR showed higher TLR1 and TLR2 expression in rosacea skin than normal skin. TNF mRNA, which can be induced by TLRs signaling, is elevated in rosacea skin along with TLR1/2, suggesting TLR signaling is altered in rosacea skin. To test if microbes could induce KLK5, keratinocytes were treated with live and killed bacteria. Propionium acne and Staphylococcus epidermidis (10^6 CFU/ml), but not Staphylococcus aureus, induced KLK5 as high as 9-times and 3-times basal expression, respectively. Cathelicidin gene (CAMP) was induced by S. aureus and epidermidis, but not by P. acne. Among a panel of specific ligands for TLRs, TLR8 ligand (ssPolyU) and TLR9 ligand (CpG) increased KLK5, and imuquimod (TLR7 ligand) and ssPolyU increased CAMP expression. Interestingly, the combination of S. aureus and the TLR1/2 ligand Pam3CSK4 induced KLK5 although neither induced KLK5 by themselves. Moreover, type I IFN, which is inducible by TLR7, 8 and 9, enhanced Pam3CSK4-dependent CAMP expression. These data show altered TLRs expression in patients with rosacea and suggest that bacteria can modify the kallikrein expression. These also suggest that several combinations of factors in innate immunity can explain aberrant expression of cathelicidin and kallikrein in the pathogenesis of rosacea.

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Pityriasis versicolor in children: a retrospective study of 483 cases

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Pityriasis versicolor is a superficial mycosis uncommonly reported in children. The objective of this study is to evaluate various clinical characteristics of pityriasis versicolor in children and their significance as well as compare with a control group of adults in our region. A retrospective analysis was conducted in patients meeting the following inclusion criteria over a 5-year period: age<14 years, clinical presentation compatible with pityriasis versicolor, a positive patch-test. Age, sex, clinical features, family history and favoring factors were recorded for all patients. The control is a group of adults with pityriasis versicolor (aged over 18 years). A total of 483 children with clinical diagnosis of pityriasis versicolor were evaluated. Males constituted 59.2% (286) of the group and females 40.8% (197), with a male predominance. Age varied from 1 month to 12 years. Facial lesions were the most frequent (n=207, 42.9%), preferentially on the forehead (n=143; 69.1%). Hyperhidrosis was found in 405 (83.9%). A family history of pityriasis versicolor was present in 38 (7.9%) patients. All the patients instructed to apply 1% bifonazole cream on lesions for more than 2 weeks. 447 of 483 patients (92.5%) had both visual healing and negative mycological evaluation. On the whole, clinical characteristics have some difference in pityriasis versicolor between childhood and adults. Childhood cases suggests the pathogenic factors involved in childhood pityriasis versicolor should be reconsidered. Pityriasis versicolor should be included in the differential diagnosis of childhood hypopigmentation of the face.

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Clinical case of chlamydial urethroprostatitis

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One of the most prevalent STD nowadays is urogenital chlamydiosis. According to statistics it is the most widely spread sexually transmitted bacterial infection in many countries. In Ukraine, 78.1 cases of urogenital chlamydiosis were registered per 100000 of people in 2006. In spite of management with effective antichlamydial drugs, failures in treatment of urogenital chlamydiosis after their administration range from 2 to 50% and higher, that is why a search for new approaches to treatment of urogenital chlamydiosis continues. A 34-year patient with urogenital tract disease needed medical consultation. He had been treated for chlamydial urethroprostatitis for three years. The patient complained of heartburn and white discharge in the morning. Perinuclear eosinophil inclusions were detected in urethral smear and prostate secretion. Presence of Ch. trachomatis was confirmed with PCR and DIF methods. The patient was administered a complex therapy: beginning with the 1st day - amino acid tryptophan during 6 days, from the 3 day - basic therapy with antibiotic azithromycin. The patient also received immunomodulator, hepatoprotector and vitamins. The patient was observed during the 2nd, the 6th and the 12th months with DIF and PCR methods, the reactions were negative. Clinical recovery. Increase in treatment efficiency may be accounted for the fact that interferon-gamma inhibits development of chlamydial infection, promoting expression of the enzyme indolamine-2,3-dioxygenase (IDO), that leads to disruption of the cyclic tryptophan molecule, which is necessary for Chlamydia multiplication. IDO, decreasing concentration of tryptophan, activates the genes, responsible for Chlamydia persistence, that causes ineffective treatment with antibiotics of the patients with urogenital chlamydiosis. Addition of excessive amino acid tryptophan to the usual scheme of treatment leads to reactivation (transfer) of persistent forms C. trachomatis, which cause urogenital chlamydiosis, to metabolically active forms. Thus, it helps antibiotics to destroy these active forms of Chlamydia.

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High levels of CCL26 in sera and skin of patients with bullous pemphigoid

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Bullous pemphigoid (BP) is an autoimmune subepidermal blistering skin disease and often provokes blood eosinophilia and lesional eosinophil infiltration. CC chemokine ligand 26 (CCL26) is one of CC chemokines, signals exclusively via the CC chemokine receptor 3 (CCR3) and has eosinophil-selective chemoattractant activity, as does CCL11 and CCL24. In this report, we investigated the participation of CCL26 in BP. We measured the blister fluid CCL26 levels of BP patients and compared them with those of burn patients. The fluid CCL26 levels were significantly higher than those of burn patients. Additionally, we measured the serum levels of CCL26 in BP patients and healthy controls. The serum levels of CCL26 in BP patients were significantly higher than those in healthy controls. The serum CCL26 levels in patients with BP significantly decreased after oral corticosteroid treatment. Furthermore, the serum CCL26 levels were significantly correlated with the eosinophil numbers in peripheral blood and the serum CCL17 (TARC; thymus and activation-regulated chemokine) levels. By immunohistochemistry, CCL26 was strongly expressed in epidermal keratinocytes and was weakly expressed in vascular endothelial cells in the lesional skin. Our study strongly suggests that CCL26 has an important role in the pathogenesis of BP and that blocking of CCL26 would be a possible target for the treatment.

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Histo-blood group A type 1, 2 and 3 antigens are associated with poor prognosis of extramammary Paget's disease

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The aim of the present study was to immunohistochemically evaluate the expression of histo-blood group A type 1, 2 and 3 antigens in normal skin and extramammary Paget's disease (EMPD). We used monoclonal antibodies (mAbs) Bioclone-A (BA) and AR-1, which react with histo-blood groupA type 1 and 2, and type 3 antigens, respectively. We found that both BA and AR-1 uniformly stained the stratum spinosum and the stratum granulosum of normal epidermis, indicating expression of histo-blood group A type 1, 2 and 3 antigens. We also found that the duct cells in the eccrine gland were positively stained by both mAbs whether the skin was taken from the secretor or the non-secretor individuals. In contrast, the dark cells in the inner layer of the eccrine gland were positively stained by both mAbs only when the skin was taken from the secretor individuals. Considering that type 1 antigens are expressed only in the secretor individuals and type 2 antigens are expressed both in the secretor and non-secretor individuals, we can surmise that the dark cells express histo-blood group A type 1 and type 3 antigens and that the duct cells express histo-blood group A type 2 and type 3 antigens. Normal apocrine glands, hair follicles and sebaceous glands were unlabelled by any mAb. Among 16 EMPD cases, 7 cases expressed both of these antigens. Interestingly, 6 out of 7 histo-blood group A antigen-positive cases were accompanied by dermal invasion of the tumor cells, while 5 out of 6 cases without dermal invasion did not express any histo-blood group A antigens. In conclusion, these findings suggest that the expression of histo-blood group A antigens in EMPD, which are normally expressed in eccrine gland cells, is associated with a poor prognosis of the disease.

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A novel immunotherapeutic approach to cancer-bearing host with protein-transduction domain-containing immunogenic foreign antigens

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The active immunotherapy is accomplished by two critical factors; 1) induction of strong antigen-specific immune responses including CD8+, and CD4+ T cells with adjuvants, 2) abundant expression of antigen-epitopes on MHC class I of target cells. However, defined tumor-associated antigens (TAAs) are not highly immunogenic since most TAAs are autoantigens, and cancer cells may escape immune surveillance by down-regulating the TAA. Previously, we have shown that nona-arginine protein-transduction domain (R9-PTD) induced efficient protein-antigen transduction to a variety of cell types *in vitro* and *in vivo*. In addition to their protein transduction capability, arginine/arginine-rich cationic polypeptides have other biological functions, such as acting as chemoattractants. We also have demonstrated that intradermal (i.d.) injections of R9-PTD-containing immunogenic foreign Ags (rR9-OVA) induced dual immunological effects: the induction of OVA-specific Tc1- and Th1-dominant immune responses, and the induction of CTL-mediated inflammatory immune responses at the injection area by expressing OVA-epitopes onto MHC class I molecules as targets. Based on these results, we investigated the antitumor effects by multiple intratumoral (i.t.) injections of rR9-OVA into B16-melanoma mass that do not express OVA. Our results clearly demonstrated that rR9-OVA-treated B16 cells *in vitro* were lysed by SIINFEKL-specific CTL. I.t. injections of rR9-OVA, but not rR9-irrelevant protein (rR9-GFP), elicited strong antitumor effects to B16 melanoma-bearing mice, resulted in tumor regression in some animals. These effects were completely abrogated by depletion of CD8+ cells. These antitumor effects by i.t. injections of rR9-OVA were superior than those by rR9-containing melanoma-specific TAAs (autoantigens), or by physical distruction such as cryotherapy. This simple approach may lead to unique immunotherapies that provide important clinical benefits to patients.

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Cannabinoid and vanilloid agonists as novel therapeutics for Darier's disease

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Darier's disease shows autosomal dominance and is characterized by keratoderma caused by a haplo-insufficiency of the ATP2A2 gene product, SERCA2 Ca channel. Due to the decrease in the active SERCA2 protein, the affected keratinocyte cannot conduct normal Ca metabolism leading to the dyskeratosis. Based on this pathogenesis, we had screened several hundreds of bioactive reagents to identify potential agents that could activate the transcription of ATP2A2, and, thus, could be considered as candidate therapeutics for Darier's disease. Consequently, we found that drugs belonging to the classes of cannabinoid and vanilloid agonists significantly increase the ATP2A2/Serca2 expression at both mRNA and protein levels in cultured normal keratinocytes as well as in Darier's affected ones. Skin grafted on nude mice and the three-dimensional reconstituted model of keratinocytes from patients were used to confirm the effects on these therapeutics on phenotypic expression of Darier's disease. Following the application of selected agonists, the normal keratinizing process was restored without producing dyskeratotic cells and blisters, while the dyskeratotic cells and acantholytic lacunae still remained in the control treatment of DMSO. The use of siRNA and selective agonists confirmed that these pharmacological effects are produced through the activation of CB2, a cannabinoid receptor, and TRPV 3/4, a kind of thermal receptor sensing mid-range temperatures. We also noted that human body temperature tends to be somewhat higher at the seborrheic area compared with the nearby areas when measured by thermography. Based on these findings, we can expect to identify novel therapeutics for Darier's disease and, moreover, we can understand why Darier's disease worsens at seborrheic areas during summer. The temperature of lesions appears to be slightly higher than the sensing range of the TRPV receptor in order to trigger the induction of Serca2 mRNA transcription. Thus, Darier's patients suffer from severe skin lesions at the seborrheic area in the summer season.

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Alterations of serum Th1 and Th2 chemokines by combination therapy of Interferon-γ and narrowband UVB in patients with mycosis fungoides

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Mycosis fungoides (MF) is a T cell neoplasm with elevation of serum Th2 chemokines. Although interferon-γ (IFN-γ) administration and narrowband-UVB (NB-UVB) phototherapy are used for the treatment of MF, a combination therapy of these two modalities is not fully established. To define whether the combination of IFN-γ and NB-UVB affects the balance of serum levels of Th1 and Th2 chemokines in patients with MF. Twelve patients with MF received intravenous or intramuscular injections of recombinant IFN-γ (rIFN-γ) or natural IFN-γ (nIFN-γ) in combination with NB-UVB phototherapy. As control, three MF patients were treated with NB-UVB monotherapy. At the beginning and cessation of therapy, the concentrations of serum Th2 chemokines, TARC/CCL17 and MDC/CCL22, and Th1 chemokines, IP-10/CXCL10 and MIG/CXCL9 were measured by ELISA. Before treatment, not only Th2 chemokines but also Th1 chemokines were elevated in the patients. Whereas no significant changes were observed in the levels of TARC and MDC, IP-10 and MIG were further elevated by the combination of IFN-γ and NB-UVB. On the other hand, NB-UVB monotherapy did not change the level of either Th1 or Th2 chemokine. The combination of IFN-γ and NB-UVB elevated serum Th1 chemokines but unaffected Th2 chemokines. Since NB-UVB monotherapy could not affect the chemokine levels, the effect of the combination therapy is attributable to IFN-γ. Given the role of Th1 chemokines for tumor-attacking T cell recruitment at the early stage of MF, the therapy may exert a beneficial effect for early MF.

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Improvement of skin barrier by a cream containing an ethanol extract of Maitake mushroom on normal and xerosis skin

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Xerosis (dry skin) is defined as dehydration of stratum corneum and characterized by scales, fissures, erosion, and calluses on the skin, which thereby impair the skin barrier function. In addition, most xerosis patients exhibit uncomfortable itching feelings or pain. Thus, structural and functional improvement of the impaired skin barrier may facilitate a treatment for xerosis and add to the quality of life of patients. On the other hand, sebum secreted from sebaceous glands plays an important role for maintaining physiological functions by forming a biological barrier on the skin. Since we found novel evidence that an ethanol extract of *Grifola frondosa* (Maitake mushroom) fruiting body augments the sebum production in hamster sebocytes *in vivo* and *in vitro*, the Maitake extract might be an effective means of treating xerosis. In the present study, we performed a clinical trial investigation using a cream containing 0.2% Maitake ethanol extract (Maitake cream) on the posterior antebrachial and crural skin of 60 patients with moderate to severe xerosis, and on the posterior antebrachial skin of healthy volunteers. For most xerosis patients, treatment with the Maitake cream for five weeks (once a day) reduced desquamation in the posterior antebrachial and crural skin. When the Maitake cream was topically applied to the posterior antebrachial skin of 15 healthy volunteers (three times a day), the augmentation of sebum and water levels on the skin was observed in <80% of volunteers. In addition, the augmented effect of Maitake cream on sebum and water levels was sustained during our four-week trial. Therefore, these results suggest that the Maitake cream is useful for not only remission and/or cure of xerosis but also enhancement of moisturization in healthy skin by the mechanism whereby the Maitake extract augments sebum production to restore the cutaneous barrier function.

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A topical physiologic lipid-based, barrier repair formulation (Epiceram™ cream) is highly-effective monotherapy for moderate-to-severe pediatric atopic dermatitis: a multicenter, investigator-blinded trial comparing a barrier repair formulation, Epiceram™, to fluticasone proprionate (Cutivate®) cream

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Atopic dermatitis (AD) is a chronic, pruritic dermatitis that affects up to 20% of children. Since current therapy with topical glucocorticoids or immunomodulators is not always effective, and can be accompanied by potentially unacceptable side effects, an effective alternative with an improved safety profile would be highly desirable. Because inherited defects in permeability barrier structure and function underlie AD, we explored here whether Epiceram™ cream, a ceramide (Cer)-dominant, triple-lipid mixture in an optimized ratio, designed to correct the lipid biochemical abnormality in AD, offers an effective therapeutic alternative. In this study, the efficacy of Epiceram™ cream was compared to fluticasone (Cutivate®) cream in a multicenter, investigator-blinded trial in 113 children with moderate-to-severe AD. Epiceram™ was highly effective as monotherapy, reducing clinical disease severity, assessed by changes in SCORAD scores, decreasing pruritus, and improving sleep habits both 14 and 28 days after therapy was initiated. While SCORAD scores of Epiceram™ did not differ significantly from fluticasone at 28 days, the fluticasone-treated group showed significantly greater improvement than did Epiceram™-treated patients at 14 days. These results show that Epiceram™ alone comprises effective, stand-alone therapy for moderate-to-severe pediatric AD, suggesting a new paradigm for the treatment of AD that could markedly decrease the requirements for topical glucocorticoid or immunomodulator therapy.

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NF-κB decoy oligodeoxynucleotides induce regulatory T cells by selectively suppressing the NF-κB subset in dendritic cells

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We previously demonstrated that NF-κB decoy oligodeoxynucleotides (ODN) induce antigen-specific peripheral tolerance in delayed-type hypersensitivity. Here, we investigated the underlying mechanisms of peripheral tolerance in dendritic cells (DC) and induced T cells. NF-κB signaling is dissected into canonical and alternative pathways. The canonical pathway is mediated by phosphorylated and degraded IκB and regulates nuclear localization of p50 and p65. The alternative pathway is mediated by NF-κB inducing kinase and IKK1, which initiates partial proteolytic degradation of p100 to p52, which, together with RelB, transactivates the target genes. To assess whether NF-κB decoy ODN inhibits NF-κB activation in bone marrow-derived DC (BM-DC), BM-DC were preincubated with NF-κB decoy ODN for 24 h and subsequently activated with lipolysaccharides. NF-κB decoy ODN treatment reduced the nuclear localization of p65 and p50 by 60%, that of p52 and RelB by 30%, and c-Rel by 20%, indicating that NF-κB decoy ODN inhibited mainly the canonical pathway. NF-κB decoy ODN did not suppress the MAPK signaling pathway. This selective suppression might delay DC migration from the skin to the lymph nodes and modify the surface molecules on DC by upregulating the inhibitory molecules, which has been reported previously. The NF-κB decoy ODN-modified DC did not produce interleukin (IL)-10. To identify the phenotype of the T cells that induce peripheral tolerance, $CD4^+$, $CD4^-$, or $CD8^+$ T cells from tolerant mice were transferred to naïve mice. Tolerance was observed only in the mice receiving $CD4^+$ T cells, not $CD4^-$ and $CD8^+$ T cells. IL-10 and FoxP3 expression was significantly higher in $CD4^+$ T cells from lymph nodes of tolerant mice, whereas $CD4^-$ and $CD8^+$ T cells expressed only low levels of IL-10 and FoxP3. These data indicate that NF-κB decoy ODN induce IL-10-expressing $CD4^+FoxP3^+$ regulatory T cells by selectively suppressing the canonical pathway of NF-κB in DC.

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Etanercept pharmacokinetics in patients with psoriasis is similar to that in healthy volunteers and patients with rheumatoid arthritis or ankylosing spondylitis

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Etanercept, a fully human tumor necrosis factor (TNF) soluble receptor, is indicated for patients with rheumatoid arthritis (RA), ankylosing spondylitis (AS) and psoriasis. Etanercept pharmacokinetics (PK) in patients with RA, AS and psoriasis has been assessed separately; distinct models were reported using population PK methods of limited precision. The different model structure and associated significant covariates identified by estimation methods of limited precision make it difficult to compare etanercept PK among disease groups. In this integrated analysis, cumulative rich and sparse etanercept concentration data from 53 healthy volunteers, 212 patients with RA (77 with JRA), 303 patients with AS and 1135 patients with psoriasis were examined and compared using nonlinear mixed effect methodology implemented in a NONMEM VI software package. A more precise estimation method (First Order Conditional Estimate with interaction) was employed in population PK model building and evaluation. The following potential covariates were evaluated: age, gender, body weight, ethnicity, disease type. The integrated analysis showed that an optimal population PK model adequately characterized etanercept PK in all subject groups. Gender, health status or disease type did not significantly affect etanercept PK. In pediatric patients with body weight less than 60 kg and age less than 16 years old, etanercept clearance increased with age and body weight. The model-predicted clearance for the youngest, smallest patient was 0.04L/hr compared with 0.074L/hr for patients with median age (45 yr) and weight (76 kg). In adult patients, ≥ 16 yr, ≥ 60 kg, with RA, AS and psoriasis, age and body weight did not significantly affect etanercept PK. Thus, a flat dose of either 25 mg BIW or 50 mg QW, as currently recommended in the product label, is appropriate to treat patients with RA, AS or psoriasis without dose adjustment for different disease type.

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PASI75 response in patients with psoriasis is predicted by the cumulative etanercept serum exposure using a simple Emax model via logistic regression

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Etanercept is a fully human tumor necrosis factor (TNF) soluble receptor that is effective and well tolerated in patients with psoriasis. Results from clinical studies have indicated that etanercept dose and treatment duration positively correlate with the percentage of subjects achieving PASI75. To improve compliance, a 50 mg QW regimen was studied for 12 weeks against placebo in a phase III study. Because there was no other comparator in this study, an integrated population PK/PD analysis was performed to assess the correlation between etanercept serum exposure and PASI75 response and compare the PASI75 response of 50 mg QW regimen with previously tested regimens. In this integrated population PK/PD analysis, 8850 PASI 75 observations from 1433 patients who received placebo, 25 mg QW, 25 mg BIW, 50 mg QW or 50 BIW doses in 5 separate clinical studies were examined and modeled. The combined data was divided into a model development dataset and model validation dataset. The population PK/PD analysis was conducted in NONMEM VI software package. A logistic regression model linking cumulative etanercept area under the curve(AUC) exposure via a simple Emax model to percentage of patients achieving PASI75 was identified based on PASI 75 observations from 25 mg QW, 25 mg BIW and 50 mg BIW in the model development dataset. The model adequately captured the PASI75 responses (>98%) from all doses in the model development dataset. In addition, prediction based on the model tracked closely with actual PASI75 observations from 50 mg QW in model validation dataset independent of model building leading to the final model. PK simulations based on the PK/PD model showed significant overlap between the respective PK profiles and identical steady-state AUC exposures with the 50 mg QW and the 25 mg BIW dose regimens. PASI75 simulations based on the PK/PD model showed similar PASI75 responses for the 50 mg QW and 25 mg BIW dose regimens, indicating interchangeability of the two regimens for the treatment of psoriasis.

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Large facial hemangiomas: what is the risk of PHACE(S)?

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Facial hemangiomas are associated with PHACES (OMIM #606519). A multicenter, prospective study is ongoing to systematically evaluate patients with large hemangioma for PHACES association and determine if hemangioma distribution predicts CNS involvement. Methods: Fifty-eight patients with large facial hemangiomas (>22 cm2) were evaluated for PHACES association by physical examination, MRI and MRA imaging of the head and neck, echocardiography and ophthalmological examination. PHACES was diagnosed when there was at least one cardiac, posterior fossa, cerebrovascular, ocular, or midline sternal or supraumbilical defect in addition to presence of hemangioma. Results: Sixty-five percent(38/58) of patients with large facial hemangiomas had PHACES. 22/38 (58%) had CNS anomalies, 27/38 (71%) had cardiac anomalies, and 10 (26%) had other defects. The risk of PHACES varied with hemangioma distribution (12.5-100%). PHACES, and specifically, cerebrovascular and posterior fossae abnormalities were more common in patients with frontotemporal segment (Seg 1) involvement alone or in combination with other segments. With S1 involvement 15/17 (88%) had PHACES, and 9/17 (53%) had CNS abnormalities. In contrast, those patients with Seg 2 in combination with Seg 3 and sparing of Seg 1, had a lower incidence of PHACES (7/16, 44%) with only 4/16 (25%) demonstrating CNS abnormalities. Conclusions: While the study is ongoing, preliminary analysis demonstrates that 65% of patients with large facial segmental hemangiomas have PHACES. Involvement of Seg 1, alone or combined with other segments, portends a higher risk of CNS abnormalities. Seg 3 involvement is associated with cardiac abnormalities, and hemangiomas confined to Segment 2 appear to be at the lowest risk for PHACES.

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Particle size and bilayer rigidity effects on skin diffusion of nanosomes

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Diffusion of micron size liposomes through skin depends on their size, the lipid bilayer deformability and their charge. In this study we report the effect of liposome size at the nanoscale and deformability on diffusion of neutral charge liposomes through Mattek skin equivalents. Liposomes were prepared by the reverse phase evaporation method followed by extrusion through polycarbonate membranes of various pore sizes. Particle size was determined by particle size analysis and revealed a narrow distribution around the mean. The deformability of these liposomes was determined by selecting the phase transition temperature of the constituent lipids. DPPC [1,2-Dipalmitoyl-sn-Glycero-3-Phosphocholine, phase transition temperature: 40C] and DOPC [1,2-Dioleoyl-sn-Glycero-3-Phosphocholine, phase transition temperature: -20C] mixed with fluorescent lipid with NBD [1-Oleoyl-2-[12-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]dodecanoyl]-sn-Glycero-3-Phosphocholine] in 1000:1 mole ratio were used to prepare the liposomes used in this study. Diffusion experiments were carried out at 37C in a Franz diffusion cell. Concentration of liposomes in the receptor compartment at various time points was determined by measuring the fluorescence intensity from the solution in the receptor compartment. Our results indicate that the diffusion of particles between 50 and 70 nm is independent of the rigidity of the lipid bilayer, while diffusion of particles larger than 100 nm is mainly dictated by the lipid bilayer rigidity. These results could have a significant impact on selection of liposomes for transdermal delivery and on the impact of skin exposure to nanoparticles.

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Phase I/II trail of cultured autologous melanocytes and a new transplantation method for vitiligo treatment

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Conventionally, vitiligo can be treated non-invasively with topical medicaiton and phototherapy. In recalcitrant cases, cultured autologous melanocyte transplantation has been used. However, there has been no report of standardized method for melanocyte isolation and expansion conforming to current good tissue practice for human cell, tissue, and cellular and tissue-based product establishments. We established a standardized procedure for epidermal skin graft procurement and melanocyte expansion conforming to current good tissue practice. For skin graft procurement, a continuous negative pressure for about 1 hour could yield epidermal blisters. For the transplantation, we developed artificial sunburn pretreatment for the recipient site preparation. The results show that epidermal graft can be procured with minimal pain in the patients. The culture medium of ITRI-M developed by ITRI, Taiwan, can selectively expand epidermal melanocytes from the epidermal graft within a reasonable time period. The artificial sunburn prior to the transplantation yields clean and bloodless recipient site for easier cell transplantation. Repigmentation for up to more than 95 percent of the treated area after one single transplantation is observed.

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Polymorphisms predictive of outcomes of methotrexate therapy in rheumatoid arthritis are not transferable to patients with psoriasis

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Methotrexate (MTX) is a first-line systemic therapy for moderate-to-severe psoriasis and rheumatoid arthritis (RA) yet its use is limited by unpredictable efficacy and toxicity. We have previously performed a systematic screen across 9 genes involved in MTX metabolism demonstrating that genetic variation determining response to MTX may be different in psoriasis and RA patients. In an extension of our earlier work we have now proceeded to analyse a further 10 SNPs, previously cited as important in predicting response to MTX in RA patients, in our well characterised cohort of psoriasis patients. DNA was collected from 378 psoriasis patients who had been treated with MTX. Phenotype data was available as to efficacy, with polarisation into two groups "responders" and "non-responders". Liver and gastrointestinal toxicities were recorded also. Genotyping was undertaken using a mass spectrometric method and analysis performed with the statistical software package STATA version 8.2. Ten SNPs from 6 genes coding for key enzymes within the folate and purine pathways were analysed. In contrast to the findings in RA patients, no significant associations were found between these SNPs and either MTX efficacy or toxicity, including both hepatotoxicty and gastrointestinal toxicity, in psoriasis patients treated with this drug. This adds to the evidence that predictors of response to MTX are different in these two diseases. We have now analysed a total of 138 SNPs in 13 genes for response to MTX in 378 psoriasis patients. Interestingly, the key associations detected in psoriasis patients treated with MTX are in genes which influence the pharmacodynamic factors of MTX in contrast to RA patients where genes which influence pharmacokinetic factors appear more important.

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Bath-PUVA therapy restores regulatory T cells in psoriasis patients

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Photochemotherapy with psoralen and UVA is used to treat psoriasis. Bath water delivery of 8-methoxypsoralen and subsequent UVA-irradiation (bath-PUVA therapy) for the treatment of psoriasis is an effective alternative to systemic application. Circulating CD4+CD25+ regulatory T cells (Treg) in the peripheral blood maintain peripheral immune tolerance against self and foreign antigens. UVB radiation of the skin induces tolerance via Treg, therefore we hypothesized that PUVA might also induce an increase in Treg. We examined whether bath-PUVA affects circulating Treg in the peripheral blood of psoriasis patients. We analyzed CD4+CD25+Foxp3+ Treg in the peripheral blood of psoriasis patients before and after bath-PUVA therapy. Peripheral blood mononuclear cells were obtained by density gradient centrifugation and stained with fluorescence conjugated antibodies against human CD4, CD25 and FoxP3, followed by fluorescence–activated cell sorting analysis. To evaluate Treg, we calculated the percentages of CD4(+)CD25(+)FoxP3(+)cells in CD4(+)cells. CD4(+)CD25(+)FoxP3(+)Treg in peripheral blood mononuclear cells isolated from psoriasis patients before bath-PUVA therapy were decreased (Treg/CD4; 1.90 ±0.59%) compared to those of healthy volunteers (Treg/CD4; 3.58 ± 0.60 %). In contrast, the Treg number increased to normal levels after bath-PUVA therapy in these patients (Treg/CD4; 4.40 ± 0.20%). Furthermore, the Psoriasis Area and Severity Index scores concomitantly improved in all patients after bath-PUVA therapy. These findings indicate that bath-PUVA restores Treg in psoriasis patients, suggesting that the clinical efficacy of bath-PUVA therapy in psoriatic patients may be due to the induction of CD4+CD25+FoxP3+ Treg. The function and behavior of Treg in the peripheral blood after bath-PUVA remain to be determined.

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Adenoviral gene transfer of human interferon-γ gene is safe and efficacious for patients with primary cutaneous lymphomas

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Immunotherapy using gene delivery of cytokine genes has shown beneficial effects in modifying anti-tumor response. In contrast to systemic application of recombinant cytokines, intratumoral cytokine gene transfer is supposed to provide locally sustained cytokine expression levels with less systemic side effects. Cutaneous lymphomas have been successfully treated with interferons (IFNs), such as IFN-α and γ. Intratumoral injection of TG1042, a non-replicating recombinant adenovirus with a human IFN-γ cDNA insert, allows high local levels of IFN-γ without severe systemic toxicity. We undertook open-label, multicenter, dose-escalation phase I/II trial of repeated, intratumoral injection of TG1042 in 39 patients with advanced primary cutaneous T-cell (CTCL) and multilesional B-cell (CBCL) lymphomas. Local clinical response was observed in 19 (57%) out of 33 evaluable patients, of which 9 were complete responses (CR) and 10 were partial responses (PR). Fourteen (44%) global responses (including treated and non-treated lesions), of which 7 CR and 7 PR, were seen in 33 evaluable patients. Local response rate in CTCL was 50% (14 patients), whereas all evaluable CBCL were responders (5 patients). Treatment with TG1042 was well tolerated up to the highest dose level ($3x10^{11}$ viral particles). Most common side effects were irritation at the injection site (corresponding to inflammatory reaction), flu-like syndrome and fatigue, and were typically of mild and transient nature. TG1042-derived IFN-γ mRNA could be detected in treated lesions confirming successful gene transfer. Our promising results implicate that TG1042 may be advantageous for the treatment of different cutaneous lymphoma subtypes.

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Depletion of syndecan-4$^+$ T cells by toxin-bearing DC-HIL blocks elicitation of contact hypersensitivity: A new model for treating T cell-driven disease

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We discovered a unique T cell inhibitory pathway in mice and humans consisting of DC-HIL on antigen presenting cells and its exclusive ligand syndecan-4 (SD-4) on activated (not resting) T cells. It shares features with other APC/T cell pairs of inhibitory molecules (e.g., PD-L/PD-1) but is distinguished by a capacity to deactivate effector T cells (SD-4$^+$) while sparing regulatory T cells (SD-4$^-$). To determine whether the DC-HIL/SD-4 pathway can be targeted to treat T cell-driven diseases, we conjugated DC-HIL with the ribosome-inactivating toxin saporin (DC-HIL-SAP) and examined its effects. *In vitro* DC-HIL-SAP bound to activated (not resting) SD-4$^+$ T cells, became internalized, and then cytotoxic. *In vivo* DC-HIL-SAP (40 nM) injected into tail veins 3 hr before challenging oxazolone (Ox)-sensitized mice suppressed ear-swelling responses to almost baseline levels. By contrast, injection of control SAP had no effect. Mice survived without visible adverse effects and displayed suppressed contact hypersensitivity responses to additional Ox challenges up to 6 wks. We next examined the phenotype of inflammatory cells in draining lymph nodes (DLN) and Ox-challenged skin of sensitized mice infused with DC-HIL-SAP (vs. control-SAP). To begin with, SD-4 in these hapten-challenged mice was expressed by only a minor fraction (20%) of CD69$^+$ activated cells in DLN, but present in all activated cells in hapten-painted skin. The effect on DLN of infused DC-HIL-SAP (vs. control SAP) was to reduce CD69$^+$ cells by 50% and SD-4+ cells by 30%, while producing no difference in the number of CD4$^+$, CD8$^+$, or B220$^+$ cells. The most dramatic change by infused DC-HIL-SAP (vs. control SAP) was in hapten-painted skin in which there was almost complete disappearance of SD-4$^+$ cells. Our results provide the rationale for exploring use of toxin-conjugated DC-HIL to treat SD-4$^+$ T cell-driven diseases especially involving skin.

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Morphea subtypes are distinct autoimmune syndromes

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Morphea (localized scleroderma) manifested by thickening and hardening of the skin, is frequently thought to be an isolated cutaneous process. Morphea is commonly subdivided into 5 subtypes based solely on the cutaneous morphology of the lesions. This classification is incomplete. Prior studies hint that morphea is associated with an increased prevalence of autoimmunity and systemic disease, but the nature of these associations remain undefined. Further, the immunogenetics, specifically HLA types, associated with morphea subtypes are unknown. We hypothesize that morphea subtypes represent distinct syndromes with different and distinct immunologic and immunogenetic profiles. To test this hypothesis, a prospective, nested case control study of 75 morphea subjects compared with age, sex, and race matched healthy and scleroderma (systemic sclerosis) controls was undertaken. Demographic and clinical data were collected from all subjects. All sera were tested for ANA, ENA, anti-RNP, anti-sDNA, anti-dsDNA, anti-Sc170, anticentromere, antihistone, and antiphospholipid antibodies. Selection of these autoantibodies was based on previous reports in the literature. HLA class I and II allelotyping was performed in all subjects. All determinations of autoantibody profile and HLA type were done using previously published protocols in the same laboratory with uniform criteria for analysis. Our results indicate certain subtypes of morphea, particularly generalized, represent a distinct syndrome with increased risk for autoimmune disorders, systemic disease, and a specific immunologic profile.

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Evaluation of B-cell clonality using the BIOMED-2 PCR method effectively distinguishes cutaneous B-cell lymphoma from benign lymphoid infiltrates

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Clonally rearranged immunoglobulin (Ig) genes are a key feature of B-cell malignancies. However, previous investigations of Ig clonality in primary cutaneous B-cell lymphoma (CBCL) have not used standardized methods, leading to inconsistent results and questioning the utility of clonality evaluation in the diagnosis of CBCL. We hypothesized that clonality detection in CBCL with the BIOMED-2 method could effectively distinguish cutaneous malignant B-cell from benign lymphoid infiltrates. 46 CBCL and 26 benign formalin-fixed tissue samples were analyzed for Ig clonality (targeting *IGH*, *IGK* and *IGKde*) using standardized BIOMED-2 PCR protocols. The (14;18) translocation was also assessed in CBCL as an alternative marker for clonality. Among the CBCL group, 1-4 samples were analyzed per patient (total 26 patients: 15 marginal zone lymphoma (MZL), 11 follicle center lymphoma (FCL)). 1 sample per patient was analyzed in the benign lymphoid group (16 dermatitis NOS, 5 tumid lupus erythematosus, 2 arthropod reaction NOS, 1 erythema migrans, 1 syphilis, and 1 morphea). A clone was detected in 34/46 (74%) CBCL biopsy samples (17/25, 68% MZL; 17/21, 81% FCL) and in 1/26 (4%) benign samples. None of the samples in the benign lymphoid group revealed a positive clone except for one patient with morphea. Overall, a clone was detected in 23/26 (88%) patients with CBCL (13/15, 87% MZL; 10/11, 91% FCL) and 1/26 (4%) with benign diagnoses. t(14;18) was present in 4/26 (15%) patients with CBCL (1/15, 7% MZL; 3/11, 27% FCL). Our preliminary data demonstrates that Ig clonality can be detected in formalin-fixed samples of CBCL with meaningful sensitivity (74%) and high specificity (96%) using the BIOMED-2 method. t(14;18) is uncommon in CBCL and may not provide additional information for diagnosis. This study forms the basis for further investigating the role of Ig clonality in reliably distinguishing CBCL from benign atypical lymphoid proliferations that may pose a challenge in clinical diagnosis.

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Oncolytic virotherapy for malignant melanoma with herpes simplex virus type 1 mutant HF10

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Replication-competent herpes simplex viruses (HSVs) have shown promise as anti-tumor agent for cancer therapy. We have reported that a replication-competent, spontaneous HSV-1 variant, HF10 had the ability of HF10 to infect and lyse human and murine malignant melanoma cells in vitro and was effective in treating intraperitoneal and subucutaneous malignant melanoma in immunocompetent mice. In this study, we investigated the efficacy of HF10 for inducing systemic antitumor immunity in immunocompetent mouse models of malignant melanoma. First, DBA/2 mice with resolved intraperitoneal clone M3 tumors after intraperitoneal HF10 inoculations were rechallenged with a subcutaneous injection of clone M3. Five of six mice were free from forming subcutaneous tumors. On the other hand, when HF10 treated mice were rechallenged with mastocytoma cells, subcutaneous tumors were appeared on all mice, as well as on naive-control mice. Next, mice were injected subcutaneously with clone M3 cells in their bilateral flanks and underwent intratumoral inoculation of HF10 into the right-frank tumor only. Unirateral intratumoral inoculation with HF10 resulted in a significant reduction in tumor growth of both the inoculated and noninoculated contralateral. This antitumor response on contralateral tumors was not observed in athymic mice model. Adoptive transfer of splenocytes obtained from mice treated subcutaneous tumor with HF10 into synergic mice bearing subcutaneous melanomas caused significant growth suppression of these subcutaneous tumors. Cytokine release and cytotoxic T lymphocyte activity in response to M3 target cells were also confirmed by splenocytes from HF10-treated animals. These data demonstrated that intratumoral HF10 inoculation is able to elicit a systemic anti-tumor immune responses in malignant melanoma models in mice.

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Serum levels of IL-17 and IL-22 are reduced by etanercept, but not by acitretin, in patients with psoriasis: a randomized-controlled trial

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Although etanercept and acitretin are widely-used drugs for the treatment of psoriasis, there are no controlled trials comparing their clinical efficacy and their therapeutic mechanisms in such a disease, and in particular on T helper 17 cells, a recently recognized subset of T cells that may play an important role in the pathogenesis of psoriasis. In the present study, we enrolled consecutively patients with moderate-to-severe, plaque-type psoriasis without joint involvement, who were required to have a baseline Psoriasis Area and Severity Index (PASI) of ≥ 10 and at least 10% of total body surface area affected by psoriasis. 15 patients were given etanercep 50 mg twice weekly and 15 patients acitretin 0,4 mg/kg per day, both for 12 weeks. Patients were randomly assigned to one of the two groups. At the baseline and at the end of the treatment a clinical assessment by calculating PASI was made, and blood samples were taken to evaluate IL-17, IL-22 and IL-23 levels by enzyme-linked immunosorbent assay. As controls, 10 healthy donors were included in the study. After 12 weeks, PASI was significantly lower than at the baseline for both groups. However, etanercept-treated patients showed lower PASI values than acitretin-treated ones (5.06 ± 3.23, and 9.28 ± 5.20, respectively). Psoriasis patients showed higher IL-17 and IL-22 levels than controls, while no detectable IL-23 was found in any serum. Neverthless, only etanercept was able to reduce IL-17 and IL-22 levels in the patients. Our findings suggest that etanercept is more effective than acitretin in the treatment of psoriasis, and that it is able to affect Th17 system, while acitretin is not.

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Autologous fat transplantation for depressed linear scleroderma-induced facial atrophic scars

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Facial linear scleroderma results in depressed atrophic scars. Autologous fat transplantation has been widely used, and fat appears to be an ideal material for filling depressed atrophic scars and contour deformities. However, long-term results for autologous fat transplantation are controversial. The short- and long-term results of 20 patients who underwent multiple autologous fat transplantations for depressed atrophic scar correction were reviewed. Twenty patients with clinically inactive facial linear scleroderma were included. They received at least two transplantations and had 12-month follow-up evaluation. On the forehead, 51-75% improvement (average grading scale: 2.4) was achieved when observed after at least 12 months from the last treatment. For chin, correction was poor (average grading scale: 0.7) with less than 25% improvement. The infraorbital area showed fair correction but the nose showed poor correction. Two of three patients with scalp reduction surgery showed excellent results, only showing slight scar widening. Autologous fat transplantation is an effective method for long-term correction of depressed atrophic scars left by linear scleroderma on the forehead. However, it was less effective for corrections on the nose, infraorbital area, and chin.

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Cucurbitacin I inhibits Stat3 and induces apoptosis in Sézary cells

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Sézary Syndrome (Sz) is an aggressive cutaneous CD4+ T-cell lymphoma with tumor cells (Sz cells) localized in the skin, lymph nodes and peripheral blood. Using western blotting we demonstrate the expression of phosphorylated (P)-Stat3 in the Sz derived cell line Seax, and in freshly isolated tumor cells from Sz patients (n=6). In vitro overnight culture without exogenous cytokines results in decreased expression of P-Stat3 (n=3). Incubation of the Seax cell line with the Jak/Stat3 inhibitor cucurbitacin I resulted in a time and concentration dependent decrease of P-Stat3 and Stat3. In freshly isolated Sz cells (n=3) cucurbitacin I induced a concentration dependent decrease in Stat3 expression whereas P-Stat3 was undetectable. Finally, incubation of freshly isolated Sz cells (n=4) with 30 μM cucurbitacin I for 6 hours induced apoptosis in the large majority (73-91%) of tumor cells. These data strengthen the notion that activation of Stat3 plays an essential part in the malignant transformation of Sz and provide further rationale for the therapeutic targeting of Stat3 in Sz.

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Combining etanercept and acitretin in the therapy of chronic plaque psoriasis: a 24-week, randomized, controlled, investigator-blinded trial

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Background: Combination treatments may increase efficacy while reducing dosages and side effects of individual agents. No randomized controlled trials have been published combining biologics with conventional agents for psoriasis. Objective: To investigate the efficacy and safety of the association of acitretin and etanercept in the treatment of moderate to severe chronic plaque psoriasis. Methods: A 24-week, randomized, controlled, investigator-blinded trial was conducted. Sixty adult patients with moderate to severe chronic plaque psoriasis were randomized into three groups to receive etanercept 25 mg twice weekly subcutaneously, acitretin 0.4 mg/Kg/day orally or etanercept 25 mg once weekly plus acitretin 0.4 mg/Kg/day. The primary end point was a 75% or greater PASI improvement from baseline (PASI 75) at week 24. Results: At week 24, PASI 75 response was achieved by 45% of patients in the etanercept group (n=22), 28% of the acitretin group (n=20) and 45.9% of patients (n=18) treated with etanercept plus acitretin ($p = 0.001$ for both etanercept groups compared with acitretin alone). PASI 50 response was achieved by 69.5%, 50.2% and 69.5% of patients, respectively ($p = 0.001$). The safety profiles of the three groups were similar. Conclusions: Combined therapeutic regimen of etanercept 25 mg once weekly and acitretin 0.4 mg/kg/day is as effective as etanercept 25 mg twice weekly, and more effective than acitretin alone. Although larger studies are needed to confirm these results, the etanercept/acitretin association could offer several advantages in the therapy of moderate-to-severe chronic plaque psoriasis.

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Chronic plaque psoriasis is associated to an increased arterial stiffness: an hospital based case-control study

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Background: Severe chronic plaque psoriasis is associated with an increased overall mortality risk and severe psoriasis is an independent risk factors for myocardial infarction. Patients with psoriasis frequently show several comorbidities, which may favor atherosclerosis. Objectives: To investigate whether psoriasis is associated with increased arterial stiffness and to study the association between arterial stiffness and psoriasis severity. Methods: A hospital based case-control study on 20 adults patients (age mean ± SD 54.3 ± 13.2) with severe chronic plaque psoriasis and 20 patients with skin diseases other than psoriasis (controls) matched for age, gender and cardiovascular risk factors, including smoking, dyslipidemia, hypertension and hyperhomocysteinemia. Arterial stiffness was assessed by carotid-femoral and carotid-radial pulse wave velocity (PWVcf, PWVcr). Results: PWVcf in patients with psoriasis was significant higher than in controls (8.64 ± 1.93 m/s vs 7.37 ± 1.27 m/s; p<0.001), whilst PWVcr was not. This difference was still significant after adjustment for age, gender, smoking status and hypertension (7.45 ± 0.27 m/s vs 8.57 ± 0.27 m/s; p=0.007). In the psoriasis group there was a significant association between PWVcf and years of psoriasis duration (r=0.51; p=0.001), but not with psoriasis severity. Conclusions: Severe chronic plaque psoriasis is associated with higher PWVcf independently by age, gender, smoking and hypertension. A reduction of arterial compliance can be a marker of accelerated atherosclerosis in psoriatic patients.

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Novel role of HMG-CoA reductase inhibitor in keratinocyte proliferation and migration

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LDL receptors (LDLR) are equally distributed on the cell surface and within the cell of the basal layer, whereas in the suprabasal layer, they are almost all intracellular. In the upper layers hardly any LDLR molecules were detected. Thus it reflects the proliferative potential and differentiation status of keratinocytes and it was expected in psoriasis, that LDLR were highly expressed in upper layers of psoriatic epidermis. LDLR expression is regulated at different levels, and is affected by several factors. We have investigated LDLR expression in healthy skin, atopic dermatitis and psoriasis and the effect of statins on LDLR expression and on keratinocyte migration. We have found that LDLR are highly expressed in psoriasis, moderately expressed in normal controls and poorly expressed in atopic dermatitis. Topical application of HMG-CoA reductase inhibitors (pitavastatin), which is known as LDLR up-regulator, to the mouse back skin revealed increased number of Ki-67 positive keratinocytes. *In vitro* assays have shown that pitavastatin, in a certain concentration, have up regulated LDLR expression and they have increased keratinocyte migration in a dose dependent manner. These results indicated that pitavastatin may influence the keratinocytes behavior via up-regulation of LDLR.

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Trace metal homeostasis as biomarker in psoriasis

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There is no systematic approach to understand the role of trace metal homeostasis in different stages of Psoriasis. And also to interlink the changes in redox metal pool (Iron, Cobalt, Copper, Zinc) to the pathology of Psoriasis. Till date no attempt has been made in this direction. The present study generated ample data on trace metal levels, homeostasis, redox ratio and oxidative stress parameters like lipid peroxidation products in serum samples of two stages of Psoriasis (mild and severe). Mild and severe stages of Psoriasis was classified using PASI score. Serum samples were collected from 22 and 30 patients of mild and severe cases. Trace metals like Sodium (Na), Potassium (K), Copper (Cu), Zinc (Zn), Cobalt (Co), Iron (Fe), Selenium (Se), Magnesium (Mg), Calcium (Ca), and Silicon (Si) were analyzed using Inductively Coupled Plasma Atomic Emission Spectrometer. Homeostasis was calculated using mole percentage. Redox ratio was calculated based on valency and redox constant of the metals. The data was analyzed by ANOVA for calculating significance of the results. There are no significant changes in the levels of Na and K between control and Psoriasis stages. Zn significantly elevated with progression of the disease. The levels of Ca, Mg, Co, Fe, Si elevated equally in mild and severe stages. Se is elevated in mild stage and significantly (p value 0.001) elevated severe stage.. Homeostasis studies showed that Cu. Fe, Zn, Se, Si pool play significant role in the pathogenesis and therapeutic intervention. Redox ratio is significantly elevated in both Psoriasis stages compared to control. There are very significant changes in the trace metals like Ca, Mg, Co, Fe, Si, Zn in different Psoriasis stages. Redox ratio is high, there by contributing to increased oxidative products in both the stages. Trace metal homeostasis concept between two stages of Psoriasis can be used as a biochemical tool in diagnostic and therapeutic application.

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Skin barrier ameliorement: Comparison between different products with respect to humidity and transepidermal water loss

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Historically skin lubrication has been provided for by home products and by the cosmetic industry. However these products are rarely evaluated especially in third world countries. The objective of this study was to evaluate the efficiency of fourteen commercial moisturizer products by measuring the transepidermal water loss (TEWL) and the humidity index (HI). Sixteen male volunteers between 20-28 years of age participated. Basal TEWL and HI were measured on the volar forearm after which 8 grs of each of 14 commercial products was applied to a 2 cm2 area and was measured again after one, two and three hours. During this period the individuals were kept at a temperature of 25.2°C and a relative humidity of 60% and were prevented from engaging in any physical activity to avoid perspiration. Linear mixed effects models were used to analyze the repeated measures data with R version 2.6.0 at a 95% confidence level. Significant differences between the products with respect to TEWL as well as HI were found. Time was not significant for TEWL. Except for the basal values, time was not significant for HI. The HI had a 70.7% of the response of on the variable response is explicated by the different products. The present trial provides an example of the information that consumers need to rationally evaluate and select from the multitude of products available in the market. Ideally this information should be provided by an independent consumer organization or by a federal agency. It is important to mention that the best product is not always the most expensive.

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Age-related NADH oxidase (arNOX) acitivity of epidermal punch biopsies correlate with subject age and arNOX activities of serum and saliva

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Punch biopsies comparing dermis and epidermis from either sun-protected or sun-exposed skin areas plus sera and saliva from 16 female subjects, ages 28 to 72 years, were analyzed for arNOX (a cell surface-located hydroquinone oxidase capable of superoxide generation) activities based on the rate of superoxide generation determined by reduction of ferricytochrome c measured spectrophotometrically. The 3mm, full-thickness punch biopsy material was hand split into epidermal and dermal portions. Each portion was exhaustively homogenized prior to assay and centrifuged briefly to remove insoluble materials. For all six tissue samples arNOX activity and subject age were positively correlated, with arNOX activity exceeding blank (background) rates beginning at about age 30 (by extrapolation) and reaching a maximum between ages 55 and 65. For sun-exposed epidermis and both sun-exposed dermis and sun-protected dermis, arNOX activity values reached a plateau or declined between ages 55 and 72. However, for serum and saliva, activity increased with increasing age for all patients within the same study population, again beginning at about age 30. Activity normalized from the line slope was 24% greater for dermis than epidermis at age 55. Sun-exposed epidermis exhibited somewhat higher activities than sun-protected epidermis even though the explants were 25 to 30% smaller but the differences were not significant. Because of decreasing arNOX levels in the oldest subjects, the data suggest that arNOX inhibitors may be of cutaneous value in persons between ages 45 and 65.

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Vorinostat and bexarotene exert synergistic apoptotic effects in cutaneous T-cell lymphoma cells: a supporting evidence for the ongoing clinical trial

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Vorinostat (suberoylanilide hydroxamic acid, SAHA) is a pan-histone deacetylase (HDAC) inhibitor that has demonstrated an overall response rate of approximately 24-30% in two phase II studies of cutaneous T cell lymphoma (CTCL) patients [Duvic et al, Blood, 2007; Olsen et al, J Clin Oncol, 2007]. Bexarotene is an RXR-selective retinoid with an overall response rate of 45-67% in patients with CTCL [Duvic et al, J Clin Oncol, 2001; Duvic et al, Arch Dermatol, 2001]. Both are FDA approved for CTCL and selectively induce apoptosis in CTCL cell lines and patients' Sézary cells [Zhang et al, Clin Cancer Res, 2002; Zhang et al, J Invest Dermatol, 2005]. There is an ongoing, multi-center phase II clinical trial of vorinostat in combination with bexarotene in patients with CTCL. The purpose of this study was to investigate whether there is a synergistic anti-tumor effect of vorinostat and bexarotene in the vorinostat-resistant (MJ) compared to a sensitive (HH) CTCL cell line. Cells were treated at clinically achievable concentrations of either vorinostat (1 μM), bexarotene (1 and 10 μM), or the combinations for 96 hrs. Apoptosis was measured by flow cytometry analysis of annexin V/PI binding populations and confirmed by sub-G1 arrest. Vorinostat alone induced 0.1% and 6.8% apoptosis, bexarotene at 10 μM induced 4% and 11.1% apoptosis and the combination induced 24.6% and 50.9% apoptosis in resistant MJ versus sensitive HH cell lines, respectively compared to baseline vehicle controls. Bexarotene at 1 μM did not increase apoptosis but sensitized vorinostat-induced apoptosis in both MJ and HH cell lines. The Sub-G1 populations of HH and MJ cells were similarly increased with the combination. In conclusion, these data suggest that vorinostat plus bexarotene, at clinically relevant concentrations, are synergistic for inducing apoptosis in both vorinostat-sensitive and -resistant CTCL cell lines. These translational results provide a rationale for the ongoing clinical trial of oral vorinostat and bexarotene in patients with CTCL.

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How the crosstalk between effector t cells and regulatory t cells regulates tumor immunity in a patient with metastatic malignant melanoma

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The host immune response to tumor is one of the major factors that determine the growth and spread of malignant melanoma. It is believed that effective immune responses to tumors can let tumors be in occult status or even induce spontaneous regression of tumors, whereas ineffective immune responses against tumor cells lead to progressive tumor growth. T cells are a major component of the immune system to regulate anti-tumor immunity and promote elimination of tumor cells. On the other hand, accumulating amount of evidence suggests that regulatory T cells (Tregs), a specific subpopulation of T lymphocytes suppressing immune reaction, contribute to inhibit anti-tumoral immunity, thus promoting tumor progression. However, it remains obscure how Tregs and effector T cells crosstalk each other in regulating anti-tumor immunity and what are more accurate and practical methods to evaluate the host immune responses to tumor cells of malignant melanoma from this point of view. Here we report results of thorough immunological profilings of a melanoma patient to reveal the crosstalk between effector T cells and Tregs in anti-tumor immune responses. Flowcytometric analysis showed that FOXP3+ Tregs were more frequent and activated in a metastatic tumor than in a draining lymph node and peripheral blood. Immunohistochmistry revealed that advanced lesions had higher numbers of FOXP3+ Tregs than the primary legion of the same patient, which is confirmed by realtime PCR analysis of RNA extracted from paraffin embedded tissues. Furthermore, profiling of cytokine expressions in the tumor microenvironment suggested that the crosstalk between effector T cells and Tregs regulated activation and distribution of these two T cell populations. These results demonstrate the interaction of Tregs and effector T cells in anti-tumor immunity and provide efficient methods for evaluating anti-tumor immunity of melanoma patients.

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Human skin irritant response to betaine analogs

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Betaine analogs such as cocamidopropyl betaine are surfactants frequency used in cosmetic products, but it is known that they often cause irritant contact dermatitis. We carried out the occlusive patch tests using the following betaine analogs: lauryl betaine, stearyl betaine, sodium cocoamphoacetate, lauramidopropyl betaine, cocamidopropyl betaine and lauryl hydroxysultaine. We calculated the skin irritation index from the results of these tests to evaluate their irritant capacity to the skin. Subjects were 150 patients with or without skin diseases. Each betaine analog except stearyl betaine was diluted in white petrolatum, purified water, and glycerin for use in the patch tests. Stearyl bataine was diluted in only glycerin. The analog/vehicle formulations were applied to the upper back for 48 hours. Vehicles alone were also applied as controls. Patch tests readings were carried out at 48 hours and 72 hours after application. All betaine analogs produced high skin irritation indexes as compared with controls. Furthermore, we compared the skin irritation indexes among different seasons, ages, sex and skin diseases for each betaine analog. These results indicate that betaine analogs cause similar irritant responses to the skin under the different conditions.

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Asian eyelid rejuvenation using monopolar radiofrequency and botulinum toxin chemodenervation

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Although dramatic improvement can be achieved with surgical blepharoplasty, There is lengthy postoperative recovery and considerable inherent risk. The 0.25 cm2 monopolar radiofrequency (RF) ST tip (Thermage, Inc., Hayward, USA) has been shown to be safe and effective in noninvasive eyelid tightening. However, the clinical efficacy of this novel ST tip has not been addressed in Asian eyelids. The objective of this study is to compare the safety and efficacy of 0.25 cm2 monopolar RF tip versus botulinum toxin A (BTX-A) chemodenervation, and combination of both for Asian eyelid rejuvenation. This was a prospective randomized study of 30 Asian patients with periorbital rhytides. Ten subjects underwent single treatment of RF using the ST Thermage tip. Ten received RF followed by immediate BTX-A injection into the crow's feet and lower eyelids. Ten received BTX-A alone. Standardized photographs were taken by the Canfield VISIA CR system at baseline, and then serially for 6 months. At 3 months, 40% of the subjects achieved objective reduction in periocular static wrinkles following RF treatment. BTX-A injection led to a remarkable reduction in dynamic periocular wrinkles, with widening of palpable aperture in all subjects. Patients' subjective assessments were more favorable. Combination treatment achieved the highest patients' overall satisfaction rate. There was no serious complication. This represents the first prospective study of monopolar RF in Asian eyelid tightening, showing that it produces mild improvement in periorbital rhytides. Combined use of BTX-A produces synergistic effect in improvement in dynamic periorbital wrinkles and widening of palpebral apertures. However, further studies are necessary to optimize treatment parameters.

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MDA/GSH as a biomarker for vitiligo activity

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Dopamine (DA), monoamine neurotransmitter, is well known neurotoxin and plays an etiologic role in neurodegenerative disorders. Vitiligo, a depigmentary disorder of the skin characterized by selective destruction of melanocytes, has been reported to show increased levels of DA with onset and progression of the disease. The purpose of this study was to investigate the toxic effects of DA and the molecular mechanism of DA-induced apoptosis in human melanocytes. In addition, protective effects by various antioxidants were investigated. Results showed that the cytotoxic effect by DA was partly abolished by N-acetylcysteine (NAC) and glutathione (GSH). Then, molecular mechanism of cell death was investigated. DA-induced apoptosis was accompanied by activation of c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK). Furthermore, NAC or GSH abolished the DA-induced activation of JNK and p38 MAPK. In conclusion, we hypothesize that free radical stress to melanocytes can be a causative factor in vitiligo and try to find a biomarker that can indicate the activity of vitiligo progression. Then, we collected the blood samples from vitiligo patients and checked the levels of the malondialdehyde (MDA) and GSH. Lipid peroxidation is a well-established mechanism of cellular injury and is used as an indicator of oxidative stress in cells and tissues. Measurement of MDA has been used as an indicator of lipid peroxidation. GSH is the predominant non-protein thiol and functions as a redox buffer. In the first part of study, GSH was found to be most effective against DA-induced cell apoptosis. In order to find a biomarker for disease activity of vitiligo, MDA/GSH ratio was calculated.

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Effect of an active ingredient biomimetic to royal jelly on body odor regulation. *In vivo* study

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Body odor regulation has been an important area of research in personal care for a long time. Many products are able to mask the odor of sweat, but do not impact the cutaneous microflora, the (have any effect on the cutaneous bacterial floor, which is a) primary cause of body odor. To address this problem, we developed an active ingredient biomimetic to royal jelly and containing 10-hydroxydecanoic acid, with seboregulating, keratolytic and antibacterial properties, and evaluated its effect on body odor in this double blind *in vivo* study. 10 healthy volunteers participated in the study. With a slightly nutritive solution, we collected bacteria from the armpits. Then, using a randomized list, we applied 1% of the active ingredient in a gel cream, or the placebo, on the armpits. 6 hours after the applications, we collected bacteria a second time. In parallel, a clinical evaluation of armpit body odor was performed. Serial dilutions of the bacterial samples were performed and then samples were cultured in a Trycase Soy Agar for 24 hours, and total bacterial numbers were evaluated. After 6 hours of application, assessment of viable germ count under the armpits revealed a greater decrease in the number of bacteria in the active zone compared to the placebo zone. This difference was statistically significant (Wilcoxon, p=0.02345) with a decrease of -154.3% in the total number of bacteria on the active ingredient-treated side compared to the placebo side. This result was confirmed by olfactorial clinical evaluation which revealed a lower body odor on the active ingredient-treated side in 60% of volunteers. These results demonstrate that 1% of an active ingredient biomimetic to royal jelly and containing 10-hydroxydecanoic acid, efficiently decreases bacterial numbers, bacterial growth, and sweat odor, and thus can be of great use in deodorant products.

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Renin in Pityriasis rubra pilaris: a new target for therapy?

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Pityriasis rubra pilaris (PRP) is a papulosquamous disorder which has been studied clinically but not molecularly. We have previously reported the results of a gene expression microarray analysis on three patients with adult type PRP. In particular, we have identified upregulation of renin and the renin angiotensin pathway as a potential mediator of disease activity. In this current analysis, we have included the microarray data of six patients with adult type PRP. Again, we have identified members of the KEGG-defined renin angiotensin pathway significantly differentially expressed between involved and uninvolved samples, i.e. renin, angiotensin II receptor type 1 and PI3K. Renin was up regulated in PRP but not in a publicly available psoriasis data set using identical Affymetrix chip technology. Microarray data was cross-validated using real time PCR on both PRP and psoriasis specimens as well as immunohistochemistry. The renin-angiotensin pathway has been shown to have pro-inflammatory activities in renal and cardiovascular disease as well as being involved in wound healing. We conclude that upregulation of renin might contribute to the pathogenesis and pro-inflammatory features of PRP. Interestingly, specific renin inhibitors are actively being developed and have reached the clinical market that could potentially ameliorate PRP.

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Activation of tumor and nodal lymphatic vessels promotes metastasis of extramammary Paget's disease undergoing epithelial-mesenchymal transition

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Extramammary Paget's disease (EMPD), a cutaneous neoplasm of epithelial origin, often shows slow growth within the epidermis. However, adoption of an invasive phenotype by Paget cells often leads to lymphatic and distant organ metastasis, resulting in poor prognosis. However, little is known about the mechanisms how Paget cells metastasize to the lymph nodes, critical sites for metastatic spread of EMPD. To investigate the potential role of tumor angiogenesis and lymphangiogenesis in EMPD, we studied 115 cases by immunohistochemical analyses for blood vascular and lymphatic specific markers. Tumor angiogenesis and lymphangiogenesis were strongly induced already in the early stages of EMPD, as compared to other premalignant conditions such as Bowen's disease or senile keratosis. Furthermore, 14 advanced cases of EMPD, which developed regional lymph node metastases, demonstrated epithelial-mesenchymal transition (EMT) during tumor progression. Importantly, expression of the EMT markers N-cadherin and vimentin was significantly correlated with poor survival of the patients. These advanced cases also revealed strong CXCR4 expression by invasive Paget cells in close association with EMT markers. We found that tumor-associated lymphatic vessels, but not lymphatic vessels in normal skin, strongly expressed stromal cell-derived factor-1 (SDF-1), a specific ligand for CXCR4. Moreover, immunohistochemical analyses identified that the lymph node marginal sinuses, the primary sites for lymph node metastasis, abundantly produced SDF-1, indicating a pre-metastatic niche which attracts metastatic tumor cells to the lymphatic system. Most surprisingly, we found, for the first time, that metastatic Paget cells strongly induce lymph node lymphangiogenesis. This newly identified mechanism of intranodal lymphangiogenesis likely contributes to lymphatic metastasis and represents a new therapeutic target for the prevention or treatment of invasive EMPD.

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Establishment of suction blister roof grafting by injection of local anesthesia beneath the epidermis: less painful and more rapid formation of blisters

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Suction blisters generated using simple devices consisting of syringes and three-way stopcocks are used for transplantation to stabilize vitiligo lesions and for treating skin ulcers to heal wounds. However, major problems exist; the pain accompanying the negative pressure and the relatively long time required to make blisters. Objectives are to reduce the pain and time required to generate blisters. We injected lidocaine without epinephrine as an anesthetic into the sub-epidermis of the donor skin before making blisters using 5 ml, 10 ml or 20 ml syringes. We compared groups with local anesthesia (n=15) or without anesthesia (n=36) in terms of suction blister formation time (SBFT) and induced pain. We also investigated possible correlations between SBFT/cm2 and age of patients or size of syringes. We succeeded both in eliminating the pain ($p=0.001$) and in shortening the SBFT ($p<0.0001$ for all three syringes) significantly in the group with anesthesia (17 min for 5 ml, 26 min for 10 ml, and 43 min for 20 ml) compared to the group without anesthesia (65 min for 5 ml, 99 min for 10 ml, and 129 min for 20 ml). The SBFT/cm2 correlated inversely with age ($p<0.0001$; $r2=0.456$) only in the group without anesthetic. Values of SBFT/cm2 were similar among the different sized syringes used in both groups. We show that injecting local anesthetic into the sub-epidermis (just beneath the epidermis) lightens the burden imposed on patients in terms both of the pain and of the time required to produce suction blisters.

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Autoantibody against activating transcription factor-2 in patients with systemic sclerosis

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Our objective was to determine the prevalence and clinical correlation of autoantibody to activating transcription factor (ATF)-2, a transcription factor of ATF/CREB family, in patients with systemic sclerosis (SSc). Anti-ATF-2 Ab was examined by ELISA and immunoblotting using human recombinant ATF-2. ATF-2 activity to bind target DNA was evaluated by ELISA using a plate coated with oligonucleotide containing the consensus binding site for ATF-2. Our results showed that IgG anti-ATF-2 Ab levels in SSc patients (n=69) were significantly higher than those in normal controls (n=26). SSc patients positive for IgG anti-ATF-2 Ab had significantly longer disease duration, more frequent presence of decreased %VC and %DLco, and elevated levels of serum IgG, serum IgA, and erythrocyte sedimentation rates than those negative. Moreover, IgG anti-ATF-2 Ab levels correlated inversely with %VC or %DLco. The presence of anti-ATF-2 Ab in SSc patients was confirmed by immunoblotting analysis. IgG isolated from serum samples of SSc patients positive for IgG anti-ATF-2 Ab by ELISA slightly but significantly inhibited ATF-2 activity compared with normal controls. In conclusion, these results suggest that anti-ATF-2 Ab is a new autoantibody in SSc and that it serves as a novel serological marker for inflammation and severity of lung fibrosis in SSc.

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Treatment of inflammatory facial acne with 1,450-nm diode laser in type IV to V Asian skin by optimal combination of laser parameters

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The 1,450-nm diode laser with has been shown to be effective for the treatment of inflammatory acne but there is a significant risk of postlaser hyperpigmentation (PIH) (up to 39%) in Asian skin. This study is to determine if lower fleunce and shorter cooling duration of the 1450-nm diode laser with dynamic cooling device (DCD) can improve acne effectively with minimal PIH in darker Asian skin. Twenty-six subjects (skin phototypes IV-V) with mild to moderate inflammatory acne received 4 treatments using the 1,450-nm diode laser with 6mm spot size 3-4 weeks apart. We used 3 passes with a fluence of 8 J/cm2 with DCD spray duration of 25 ms which was predetermined in phase 1 forearm study to minimize PIH. Serial blinded assessment of acne lesion counts and objective sebum measurement were evaluated before and up to 6 months after treatment. Reduction of mean acne lesions was 29% ($p<0.01$) and 40% ($p<0.03$) from baseline 4 weeks and 6 months after the last treatment in the group with moderate acne, respectively. Improvements in sebum production on forehead and nasolabial folds by 17-21% were noted as earlier as after the first laser treatment and there were further reductions up to 6 months after the last treatment by 31-35%. 4 episodes of temporary PIH (3.8%) were observed out of all treatment sessions. The mean pain rating was 4.0 on a 0 to 10 scale. It has been postulated that excessive cooling using DCD leads to the high risk of PIH in Asian. We found that use of multiple passes of 1,450-nm diode laser with lower fluence and shorter DCD retains its therapeutic efficacy with substantial reduction of PIH for moderate acne.

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Responses to oral forodesine in refractory cutaneous T-cell lymphoma (CTCL): final results of a phase I/II study

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Forodesine is a potent inhibitor of purine nucleoside phosphorylase (PNP) that leads to T-cell selective intracellular accumulation of dGTP, resulting in apoptosis. An open-label dose escalation study of oral forodesine (40-320 mg/m^2 daily) for 4 wk was performed to determine the maximum tolerated and/or optimal biologic dose (OBD). Additional subjects were accrued at the OBD (80 mg/m^2) to further assess safety and clinical efficacy. Patients with refractory CTCL, stages IB-IV were eligible. The primary efficacy endpoint (objective response rate [ORR]) was defined as $\geq$ 50% improvement by a severity-weighted assessment tool (mSWAT). 36 subjects treated at the OBD of 80 mg/m^2 were the focus of the efficacy results. Median age was 62 yr (range 28-81) and 67% were males. Median number of prior systemic therapies was 3 (range 0-8). The ORR was 39% (14/36, 2 CR) with median response duration of 127+ d. Response by stage was: IB 3/8, IIA 1/1, IIB 3/5, III 4/12, IVA 2/6, and IVB 1/4. For responders, median time to response was 42 d (range 29-58). Median time on treatment was 124 d (range 1-479) with 3 remaining on treatment. For subjects with erythroderma (n=20), the ORR was 40% by mSWAT and 65% by erythroderma score; 13/19 with pruritus at baseline had improvement ($\geq$ 50%). Greater than 50% reduction in Sézary cells (by flow cytometry) was observed in 3/8 (38%) subjects with > 1000 Sézary cells/mm^3. Subjects experienced limited Gr 3-4 adverse events and DLT was not observed. Gr 3 or higher lymphopenia and low CD4 counts were observed in 70% and 32% of subjects. Median baseline, nadir, and last visit CD4 counts (1000/mm^3) were 0.9 (95%CI: .098, 47), 0.3 (95% CI: .009, 27), and 0.6 (95% CI: .018, 29), respectively. No opportunistic infection was reported. These data support that oral forodesine at the optimal biologic dose was well-tolerated and demonstrated promising clinical activity in patients with refractory CTCL.

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Development of spectral analysis for applications in dermatology

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Vibrational microspectroscopies (mid-infrared absorption and Raman scattering) are well adapted for the characterization of biological samples. They are based on the non-destructive light-matter interaction and provide molecular and structural characteristics of a sample. Skin is the subject of much research activity within our laboratory, for the analysis of cutaneous tumors or the assessment of the cutaneous penetration of active principles. Infrared micro-imaging, coupled with chemometrics and a multivariate statistical analysis of the data can characterize the tissular structure of the skin and localize tumor areas in non-melanoma skin cancers, for example. Recent developments in spectral analysis allow exploring biopsy sections directly, whatever their conditioning (freezing, fixation, paraffin-embedding) and without any previous preparation. Raman spectroscopy can also be applied to *ex vivo* (whole biopsies) or *in vivo* experiments and in a hydrated environment. Depending on the applications, the coupling of a Raman spectrometer and a confocal microscope allow measuring the penetration of molecules (active principle involved in rosacea treatment) through the cutaneous structures from a viable sample of whole skin. Mathematical corrections were applied, leading to a depth-precision of a few microns. Investigations on the single living cell can also be developed with this instrumentation. Recent technological advances have led to the set-up of Raman devices designed for intravital analyses. This instrumentation results from the coupling of a portable Raman spectrometer and a dedicated biomedical probe: spectra can be recorded directly on patients in a clinical environment. Current applications are based on the *in vivo* characterization of cutaneous cancerous lesions. These techniques are developed in close collaboration with clinical departments and pharmaceutical industries (Galderma R&D) for medical applications mostly.

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The effect of stress on heart rate variability and autonomic function in atopic dermatitis patients

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Stress has been known to aggravate atopic dermatitis and damage the skin barrier function, but there are few studies assessing the role of the nervous system and stress in atopic dermatitis. Furthermore no studies evaluated the autonomic nervous system (ANS) function. To elucidate the function of ANS physiology during stress we evaluated the heart rate variability (HRV) in patients with atopic dermatitis suffering from moderate to severe disease versus healthy controls during periods of acute stress. 15 atopic dermatitis patients with moderate to severe eczema and 15 healthy controls participated in the study. Baseline HRV measurements were performed followed by measurements taken after three main stress tests, histamine iontophoresis, artificial scratching and a psychological validated stress test the Trier Social Stress Test (TSST). Skin hydration and transepidermal water loss (TEWL) were measured after each HRV session. At baseline, atopic dermatitis patients had lower overall heart rate variability and higher heart rate than controls. AD patients also showed less overall ANS reactivity than controls when presented with the three stress tests. However, increased ANS activity associated with cardiorespiratory coherence was noted in the atopic dermatitis group during histamine iontophoresis. Finally, measurements taken on the forearm showed lower skin hydration values and higher TEWL values in AD patients across all experimental groups. These preliminary data shows that atopic dermatitis patients have lower heart ANS activity at baseline and less ANS reactivity than controls when presented with acute physiological and psychological stress. Chronic stress associated with itch may dampen autonomic nervous system response during periods of acute stress

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The spectrum of pegylated liposomal doxorubicin induced skin problems-more than palmoplantar erythrodysesthesia syndrome

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Liposomal pegylated doxorubicin is an encapsulation form of doxorubicin, with an improved pharmacokinetic profile and the ability to selectively accumulate into tumor tissue. As a result the tolerated dose can be increased with reduced neutropenia and cardiotoxicity, compared to doxorubicin. A common dose-schedule limiting adverse effect is palmoplantar erythrodysesthesia syndrome.In this retrospective study we included six patients hospitalised to the University Hospital of Zurich within the last 2 years, due to side effects by pegylated liposomal doxorubicin. The patients received this chemotherapeutic agent, due to different malignancies such as breast cancer, ovarian cancer, mycosis fungoides and cutaneous B-cell lymphoma. 3 out of 6 patients in this study developed classical palmoplantar erythrodysesthesia, one palmoplantar erythrodysesthesia associated with extensive bullous disease, one eruption of lymphocyte recovery syndrome and one intertrigo like dermatitis with stomatitis. Pegylated liposomal doxorubicin induces various skin reactions including palmoplantar erythrodysesthesia syndrome. The clinical presentation of it might depend on pre-existing skin diseases.

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The effect of Rituximab on pemphigus vulgaris via eliminating specific IgG4 isotype against desmoglein 3

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Rituximab is a monoclonal antibody directed against the CD20 molecule of B cells. It has been applied for the treatment of B cell lymphomas and refractory pemphigus vulgaris (PV). Here, we shared our experience on the application of rituximab for a 38-year-old patient with sustained oral lesions. The female patient was initially presented to our hospital with painful ulcerations and erosions on the mucosa of soft palate, gum, and buccal area. No cutaneous lesions were visible. The diagnosis of PV was confirmed by histopathology, indirect immunofluorescence and ELISA for specific anti-desmoglein 3 (Dsg3) antibody. The patient then received prednisone (initially 30 mg daily) and high-dose intravenous immunoglobulin (IVIG). No any response was obtained and the symptoms were getting worse. We then started the treatment with 6 weeks of rituximab (375mg/m2 body surface area), once a week (500mg per week). Five months after the rituximab treatment, the patient showed remarkable improvement. After additional three months, the symptoms were largely resolved. During this 8-month period prednisone was tapered from 50mg/day to 5mg/day. The Dsg3 antibody and IgG1, IgG4 isotypes were measured every two months with ELISA. We found that the anti-Dsg3 antibody titer was correlated with disease severity with a correlation coefficient index of 0.9. Interestingly, IgG1 and IgG4 showed different expression patterns. The amount of IgG 1 dropped dramatically right after the treatment, but IgG4 isotype remained unchanged until the clinical remission. The finding of this case suggests that rituximab may be useful in PV treatment. We speculate that the treatment effect may be due to the elimination of Dsg3 B-cell precursors, especially those of IgG4 isotypes, although more studies are needed. On the other hand, IgG1 could be a monitoring marker for this setting.

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Talarozole for moderate to severe plaque psoriasis – results of a dose-finding study

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Talarozole (RambazoleTM) increases intracellular levels of endogenous retinoic acid by selectively inhibiting its CYP450-dependent catabolism (CYP26). In this study, conducted in accordance with the Declaration of Helsinki, we evaluated the efficacy and safety of an OD intake of 0.5mg (n=45), 1mg (n=45) and 2mg (n=45) talarozole or placebo (n=41) for 12 weeks (W) in 176 patients with moderate to severe plaque psoriasis (PASI range: 10-42). Patients were evaluated on efficacy (PASI reduction, Investigator Global Assessment (IGA), clinical signs of psoriasis) and safety (AEs, clinical laboratory parameters, ECG, ophthalmologic and auditory testing, psychiatric evaluation of depression) at baseline, at W1, W4, W8 and W12 of treatment and at follow-up (W16 and W20). At W20 a statistically significant dose response trend in the number of patients reaching PASI75 favouring the 2mg dose was observed. In this group 37.1% (13/45; p=0.003 vs. baseline) achieved PASI75 vs. 29.4% (10/45) in the 1mg group, 18.4% (7/45) in the 0.5mg group and 17.1% (6/41) in the placebo group. The improvement in IGA scores and in clinical signs of psoriasis (erythema, scaling and plaque elevation) was greatest in the 2mg group. The majority of the AE's reported were mild to moderate and transient. No clinically significant trends were observed in the ECG readings, laboratory findings (with exception of blood triglycerides) and ophthalmologic, auditory and psychiatric evaluations. In conclusion, talarozole was well tolerated at all dose levels. The PASI75 analysis at W20 favoured the dose regimen of 2mg OD for 12 weeks. The results showed that the PASI continues to reduce after treatment; further investigation is required to evaluate the duration of this effect. * for the talarozole phase IIb investigator team

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Altered expression of retinoid-regulated genes during oral liarozole treatment of lamellar ichthyosis

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Lamellar ichthyosis (LI) is commonly treated with systemic retinoids, a therapy limited by numerous side effects. Liarozole (LIA) is an imidazole drug that has been developed to mimic the positive effects of retinoids by blocking cytochrome P450 (CYP26)-mediated catabolism of endogenous all-trans retinoic acid in the skin. Its effects on the expression of retinoid-related genes in epidermis is however scarsely known. As part of a placebo-controlled trial of two different doses of oral LIA (75 or 150mg per day), 10 patients with LI were studied with respect to mRNA expression of retinoid biomarkers (e.g., CRABPII, keratins 2 and 4, CYP26A1 and B1) and markers of inflammation (IL-1a and TNFa) in epidermal biopsies obtained before and after 4 weeks of therapy. The histometric analysis showed a tendency to reduced stratum corneum thickness during therapy consistent with a clinical improvement of LI. A significant decrease in the mRNA levels of keratin 2 and TNFa and trends toward increased expression of keratin 4 and CYP26A1 were observed in LIA treated patients (n=9), consistent with an increased retinoid stimulation in epidermis. However there was no clear dose-response in the mRNA results and the immunostaining of some of the epidermal gene products failed to confirm the observed mRNA changes. The combined results suggest that LIA exerts a therapeutic effect in LI by mildly affecting the expression of several retinoid-regulated genes in epidermis.

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Liarozole effective in treatment of lamellar ichthyosis

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Lamellar ichthyosis (LI) is a debilitating congenital disease, for which very few treatments have been approved. Liarozole (LIA) is a retinoic acid metabolism blocking agent (RAMBA) that is being studied for the oral treatment of LI. This double blind, placebo controlled phase II/III study included 64 patients (age range: 18- 65 yrs; 16 sites) with moderate to severe LI and was performed in accordance to the Declaration of Helsinki. The patients were treated for 12 weeks with LIA 75mg a day (n=27), LIA 150mg a day (n=28) or placebo (n=9)and evaluated on a 5-point 'Investigator Global Assessment' (IGA)-scale taking into account the scaling and erythema. To be considered a responder, the patient's IGA-score had to decrease with 2 points by week 12. Quality of Life assessments were also made (SF36 and DLQI). Results indicate that both dosages of LIA markedly reduced signs and symptoms as early as week 4; after 8 and 12 weeks of treatment both dosages of LIA scored significantly better than placebo for scaling (75mg: p=0.0496 and 150mg: p=0.0099). At week 12, 11/27 (44%) patients in the 75mg group (p=0.2193) and 14/28 (50%) in the 150mg group (p=0.0557) were responders vs only one of 9 (11%) in the placebo group. Within 4 weeks after ending treatment, most patients' condition reversed to baseline values. Treatment was well tolerated. Adverse events were mild and transient. The laboratory safety results, ECG and ophthalmologic exams did not show any relevant abnormality. Some minor, transient changes in bone markers were observed. The SF36 did not show significantly difference between the treatment groups, but the DLQI improved in favor of the liarozole groups. Liarozole is an effective and well tolerated treatment for LI. As the response between the 75mg and 150mg group do not substantially differ, clinicians might start the treatment with 75mg a day and increase the dose to 150 mg to reach the desired effect. * for the LILI-study investigator group.

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Effects of PRK 124 (0.125%) lotion in acne rosacea

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The primary objective of this study was to determine the efficacy and tolerability of a lotion containing PRK 124 (furfuryl tetrahydropyranyladenine), a plant cytokinin, for improving the clinical signs and symptoms of mild-to-moderate inflammatory facial acne rosacea and signs of photoaging on the face. Twenty-three healthy male and female subjects (age>21 years old) with mild-to-moderate facial acne rosacea participated in this single center, open-label study. The subjects applied PRK 124 (0.125%) lotion to the entire face twice daily (once in the morning and evening) for 12 weeks. A sunscreen with SPF 30 was applied daily after the morning application of the PRK 124 lotion. Subjects were evaluated at 4, 8 and 12 weeks compared to baseline. Evaluations included physician assessments of inflammatory lesion count, severity of erythema and telangiectasia, photodamage, and skin tolerance, and patient assessments of signs and symptoms of rosacea. Investigator's global assessment, investigator's assessment of overall improvement, and patient assessments of tolerability were also rated at each follow-up. In addition, transepidermal water loss (TEWL) measurements and facial photography were done at each visit. Eighteen subjects out of the 23 enrolled have completed 12 weeks of treatment. After 12 weeks of treatment, there was mild to moderate overall clinical improvement with reduction of erythema and papules. TEWL measurements showed a decrease in water loss which supports an improvement in skin barrier function. Subjects also had improvement in their overall appearance including pigmentation and rhytides. Subject self assessments were consistent with these results and treatment with PRK 124 lotion was well tolerated. In conclusion, the results of this study suggest PRK 124 (0.125%) lotion is a well-tolerated moisturizing lotion option for reducing the signs and symptoms of mild-to-moderate facial acne rosacea. In view of the promising results, the current study has been extended to 48 weeks to investigate its long-term safety and efficacy.

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Development of a hyaluronan pro-drug for the treatment of melanoma

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Chemotherapeutic drugs can cause debilitating side effects due to their systemic toxicities. The undesirable toxicities of these drugs are because they are not specific for tumor cells but rather, they are taken-up by both malignant and normal cells. In order to reduce bystander toxicity, tumor targeting strategies have been proposed. Their premise is as follows: The drug is chemically linked to a targeting molecule that binds strongly to tumor cells but weakly to normal cells. The tumor cells take-up the drug linked to the targeting molecule (termed the "pro-drug") and the functional drug is released inside of the tumor cells. Because many tumor cells, including malignant melanomas over express receptors (e.g., CD44) for hyaluronan (HA), we hypothesized that HA could target drugs to melanoma tumors for treatment. HA is a glycosaminoglycan composed of repeating glucuronic acid and N-acetylglucosamine subunits. To test our concept, we chemically conjugated doxorubicin (DOX) to HA and tested it on B16-F10 melanoma cells in vitro and in vivo. Because DOX is fluorescent we compared the uptake of DOX-HA with free DOX using FACS. Both DOX and DOX-HA showed dose and time dependent uptake. Analyses by confocal laser scanning microscopy showed that DOX and DOX-HA exhibited similar intracellular distribution patterns. Finally, both DOX and DOX-HA potently inhibited the proliferation of B16-F10 melanoma cells ($IC50 < 0.1$ μM). Next, we tested the impact of DOX-HA on the local growth of B16-F10 tumors in living animals. We found that injections of DOX or DOX-HA at 1 mg/kg significantly reduced tumor growth rates compared to animals treated with PBS alone ($p < 0.05$). Importantly, DOX-HA reduced the growth rates of tumors to an even greater extent than mice treated with free DOX ($p < 0.05$). Based on these results we conclude that HA may be a new targeting molecule for pro-drug development. Moreover, our findings provide the technical and conceptual foundations for the further development of HA conjugates for the treatment of malignant melanomas.

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Oral curcumin in the treatment of moderate to severe psoriasis vulgaris: a prospective clinical trial

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The purpose was to determine the safety and efficacy of oral curcumin (the active compound in the spice Turmeric) in patients with moderate to severe psoriasis. A 16-week, phase II, open-label, Simon's two-stage trial was conducted in subjects with chronic plaque psoriasis. Subjects took 4.5 grams per day of curcumin for 12 weeks and were assessed at week 16 for relapse. Endpoints included Physician's Global Assessment (PGA), Psoriasis Area and Severity Index (PASI), Skindex-29, safety endpoints, and compliance. The primary endpoint was a response rate based on the number of subjects achieving good (50-74% improvement), excellent (75-99% improvement) or cleared (100% improvement) score on the PGA compared to baseline. Twelve subjects were enrolled, 8 completed the trial and 2 responded. The intention to treat analysis response rate was 16.7% (95% CI: 2%, 48%.) Both responders achieved at least a PASI 75 which was maintained at week 16. The as-treated response rate was 25% (95% CI: 3, 65%.) Among those who completed the trial, no significant difference in Skindex scores were found. However the PASI scores at week 12 were significantly lower than at week 0 ($p=0.0418$) by Wilcoxon signed-rank test. All subjects who completed the trial were at least 85% compliant with the study drug. There were no study-related adverse events that necessitated subject withdrawal. In one subject, mild elevation in liver enzymes were found at week 12 (AST 54, ALT 59.) Oral curcumin in doses of 4.5 grams daily is safe in patients with psoriasis. Considering the low response rate and the small sample size, larger placebo-controlled trials are necessary to estimate the true efficacy.

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BH3 mimetic ABT-737 potentiates Noxa-mediated cell death in human melanoma cells when combined with a proteasome inhibitor

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The expression of Bcl-2 family proteins plays an important role in modulating sensitivity to anti-cancer drugs in many tumors, including melanomas. The BH3 mimetic ABT-737 is a potent small molecule inhibitor of the anti-apoptotic proteins Bcl-2, Bcl-XL, and Bcl-w. In this report, we examined whether ABT-737 is effective in killing three human melanoma cell lines by itself or in combination with the proteasome inhibitor MG132, and we further evaluated the mechanisms of action. MTS viability analyses, changes in cell morphology, and Annexin V apoptosis assays showed that ABT-737 alone induced little cytotoxicity, yet it displayed strong synergistic lethality (mainly apoptosis) when combined with MG-132. In addition, mechanistic analysis showed cleavage of Caspase 3 and PARP, as well as Bax/Bak activation in cells treated with the combination of ABT-737 and MG-132, indicating the induction of mitochondria-mediated apoptosis. Furthermore, Western blot analyses demonstrated that the combination treatment induced expression of the pro-apoptotic protein Noxa and caspase-dependent degradation of the anti-apoptotic protein, Mcl-1. Finally, siRNA-mediated inhibition of Noxa expression protected the cells from cytotoxicity induced by the combination treatment. These findings suggest that the combination of ABT-737 and MG-132 not only neutralizes the anti-apoptotic Bcl-2/Bcl-XL/Bcl-w/Mcl-1 proteins, but it also cleaves Mcl-1, creating positive feedback for synergistic and accelerated killing. In summary, this study indicates the promising therapeutic potential of ABT-737 in treating melanoma, and it validates rational molecular approaches to targeting anti-apoptotic defenses in developing cancer treatments. (Acknowledgement: ABT-737 was kindly provided by Abbott Laboratories.)

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The isomorphic response in sclerotic-type chronic graft-versus-host disease

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The isomorphic response of Koebner, the development of skin disease in normal appearing skin following trauma, is a phenomenon reported in the context of several skin disorders. Herein we report its occurrence in the setting of chronic graft-versus-host disease (cGvHD) following allogeneic hematopoietic stem cell transplantation. We observed that patients with morphea-like cGvHD preferentially develop lesions at body sites that are subject to chronic friction, trauma, or pressure, consistent with an isomorphic response. A retrospective chart review was performed on 110 consecutive patients with cGvHD of any organ system that were evaluated in a cross-sectional cGvHD study at the NIH. All patients received a full dermatological examination with clinical photography. Eighty-one had evidence of cutaneous cGvHD. Of this subset, 58 patients (72%) exhibited evidence of dermal sclerosis, subcutaneous sclerosis, and/or fasciitis as classified according to the NIH Consensus Criteria. Eleven of these 58 patients (19%) exhibited morphea-like cGvHD at the waistband area, often in a striking linear distribution. Lesional skin biopsies were performed in six of these patients and all were consistent with sclerotic-type cGvHD. Six out of 11 patients with waistband involvement were female and 3 exhibited similar sclerotic morphea-like cGvHD in the brassiere-band area. This study demonstrates a significant incidence of morphea-like sclerotic cGvHD lesions at friction-prone regions of trunk skin consistent with an isomorphic response. Careful evaluation of these high-risk sites may allow for early diagnosis of this potentially debilitating manifestation of cutaneous cGvHD. Because it is not possible to predict which patients may develop morphea-like cGvHD, all patients at risk for cGvHD should be educated regarding the potential for minor skin injury to induce cGvHD. Proper fitting undergarments and avoidance of restrictive clothing should also be recommended.

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Distinct retinoid acid receptor isoform mRNA expression in basal cell carcinoma and squamous cell carcinoma of the skin

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Background: Retinoids have a chemopreventive effect on squamous cell carcinoma of the skin (SCC), but not on basal cell carcinoma (BCC). Retinoic Acid Receptor (RAR) 1 beta prime transfection in Retinoic acid-resistant Lung Cancer Cells mediates growth suppression. A possible difference in RAR isoform expression between SCC and BCC may explain the different response to retinoids, prompting us to analyse SCC and BCC. Material and Methods: We extracted RNA from fresh biopsy material of clinically indicated excisions in BCC (n=28) and SCC (n=23) and assessed expression of RARα, RARβ, RARβ1, RARβ1', RARβ2, RARβ4 and RARγ using RT-real-time PCR. Results: Both mRNA of RARα ($p<0.001$) and RARγ ($p=0.012$) isoforms are more abundantly expressed in BCC. No significant mRNA expression difference was seen for RARβ, RARβ1, RARβ1', RARβ2 and RARβ4 isoforms. RARβ1' cDNA could be amplified by RT-real-time-PCR only in two SCC samples. Conclusions: RARα and RARγ RNA expression differs significantly between BCC and SCC, correlating with the different clinical reaction of these tumors to retinoid chemoprevention. Further analysis of RAR isoform protein and RBP and RXR mRNA and protein expression will yield further insight how the chemopreventive effect of retinoids is mediated in BCC and SCC.

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AN2728, a novel oxaborole in development for treatment of psoriasis, demonstrates significant activity in a micro plaque study

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AN2728 is novel boron-containing anti-inflammatory drugs which inhibits the release of TNF-α. The purpose of this study was to evaluate the antipsoriatic activity of AN2728. This single-center, randomized, vehicle-controlled, observer-blind study enrolled 12 subjects with psoriasis. Six test fields per subject were treated (two active formulations: AN2728 Ointment, 5%, AN2728 Cream, 5%, two vehicles, and two comparators). All fields were treated 10 times over 12 days. Experimental measurements included sonography, photo documentation and clinical assessments with intra individual comparison of the treatments. The infiltrate thickness showed that treatment with AN2728 led to a relevant and clear improvement. The mean percent reduction in infiltrate thickness for both AN2728-formulations was 54% vs. no changes in the mean infiltrate thickness for the vehicles. The statistical comparisons showed that AN2728, 5% demonstrated a significantly higher reduction ($p<0.025$) in infiltrate thickness than the corresponding vehicles. For the comparator Protopic™ Ointment 0.1% the mean percent reduction in infiltrate thickness (48%) was lower. A higher reduction in the mean infiltrate thickness (72%) was noted for the comparator Betnesol™-V Creme, 0.1%. No significant differences were found between the AN2728 Ointment, 5 % and the Betnesol™-V Creme 0.1% on study day 12. In all other comparisons Betnesol™-V Creme 0.1% demonstrated a significantly higher reduction in infiltrate thickness. The clinical assessments and other secondary endpoints paralleled the findings of the sonographic measurements. The two AN2728-formulations were well tolerated. Given that the application of test articles was done in drug excess and under occlusion these results are encouraging and represent the first step in the clinical proof of concept for this novel anti-inflammatory drug. Ongoing trials are evaluating this product under conditions of routine use.

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Rescue of diminished cathelicidin antimicrobial peptide expression in subjects with atopic dermatitis by administration of oral vitamin D

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The expression of antimicrobial peptides such as cathelicidin are abnormally low in inflammatory lesions of patients with atopic dermatitis (AD). The primary objective of our study was to determine if this local dysregulation of cathelicidin in atopic subjects could be corrected with oral Vitamin D. This was a single-center, controlled study of 7 subjects with AD, and 13 normal controls. 2mm punch biopsies of uninvolved skin were collected from all subjects, and AD subjects also received 2mm punch biopsies of lesional skin. Baseline calcium and vitamin D levels were obtained. Supplementation with oral Vitamin D (cholecalciferol) at 4000 iu per day was given for 21 days. At 21 days, all subjects returned for repeat 2 mm skin biopsies of uninvolved skin, repeat serum calcium and Vitamin D levels. Atopic subjects also received a 2mm punch of involved skin. Analysis of cathelicidin expression in skin was performed by qRT-PCR and normalized to GADPH. After supplementation with oral Vitamin D for 21 days, AD lesional skin as analyzed by one way ANOVA showed a statistically significant increase in cathelicidin expression (mean difference of 76.68, $p<0.01$) as compared with normal skin post supplementation (mean difference of 2.1, $p>0.05$), and AD non-lesional skin (mean difference of 18.82, $p>0.05$) suggesting that supplementation with oral Vitamin D can induce cathelicidin production only in AD lesional skin. Recent in-vitro work has shown that a VDRE is present in the human cathelicidin gene promoter (CAMP), and activation of this VDRE by 1,25-dihydroxy-vitamin D induces cathelicidin expression in keratinocytes and myeloid cells. Our in vivo study confirms this in vitro data, and supports the feasibility of skin-specific modulation of antimicrobial peptide expression in atopic subjects with oral vitamin D. Further work is still necessary to see if this change in cathelicidin expression can alter the susceptibility of atopic subjects to cutaneous infection.

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New biomarker proteins of the horny layer for evaluation of the skin condition of patients with atopic dermatitis

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Background: Evaluation of the severity of skin inflammation in atopic dermatitis (AD) still remains difficult. Objective:To develop new biomarkers to determine the status of AD, we investigated proteins expressed in the stratum corneum (SC) of AD patients and evaluated the relationship between quantity of the proteins and clinical features or laboratory findings. Methods: Thirty-four patients with AD (22 males and 12 females, average 45.2 years) were recruited in this study. SC samples from affected and unaffected skin of the forearm of these patients were obtained by the tape stripping. The expression levels of 6 proteins (epidermal fatty acid-binding protein(E-FABP), squamous cell carcinoma antigens 2(SCCA2), α-enolase, annexin II, apolipoprotein A-I, and albumin) in SC were measured by ELISA and the relationship between these proteins and clinical symptoms, blood laboratory data and skin barrier conditions (moisture and transepidermal water loss [TEWL]) was evaluated. Results: E-FABP showed correlation with both clinical features and laboratory data, such as severity score of the skin lesions, scores for erythema and lichenification, and total serum IgE level. Albumin and apolipoprotein A-I showed correlation with TEWL in the affected skin of AD. α-enolase showed correlation with the scores for both lesion severity and papule. Annexin II showed correlation with the score for lichenification and total serum IgE level. SCCA2, which showed correlation with the number of eosinophils, total serum IgE level and serum LDH level, was suggested to be the most effective biomarker for reflection of a Th2-type immune response from the laboratory data. Conclusion: E-FABP is suggested to be the most useful biomarker to evaluate the status of AD because it showed correlation with both clinical features and laboratory data.

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Clinical course and management of annular erythema associatewd with Sjögren syndrome

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Annular erythema has been recognized to be a specific, cutaneous manifestation associated with SS. Considering the literatures reported till 2007, this type of erythema preferentially occurs in Oriental but not Occidental populations. At present, 3 clinical types are kown; isolated donut-ring like erythem with an elevated border (type I), SCLE-like marginally scaled polycyclic erythema (type II) and papular insect bite-like erythema (type III) all of those show common histological characteristics described above and meet the diagnostic criteria of SS respectively. However, precise clinical course and standard regimen for the management of AESS have remained obscure, because its rare occurrence in Occidental populations and most of the Oriental cases are isolated reports. In this study, AESS of 28 our own cases and 92 cases from literatures are enrolled to evaluate clinical characteristics and most desirable management of this unrecognized skin manifestations in SS. Clinical characterists of 120 cases are as follows:, male/female ratio; 20/100,, age 7-78(average),type 1;101, type 2; 9, type 3; 6, mixed cases of each type;4. Positive anti-SSA/SSB antibodies:(+)/(+);93,(+)/(-); 17, (-)/(+);2,(-)(-);5,unknown;3 . Presence time of AESS before visiting dermatology clinic: <1M;11, 1-2M;19, 2-3M;18, 3-4M;9, >4M;3, unknown;60. Therapeutic options in each case: oral steroid only;34, oral steroid and other medicine; 19, topical steroid;33, others; 9, no medication;15. unknown;10. Effective dose of oral steroid to control AESS was 10-20 mg oral prednisolone while AESS recurred in nearly 50 % of cases applied more than 20 mg which implies several subsets might exist in AESS. In addition to oral steroid, several anti-malaria drugs are reported to be effective for AESS. In conclusion, AESS are distinct clinical entities and small dose of prednisolone around 10 mg are enough to control diseases activity except some cases with systemic manifestations.

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Withdrawn

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Clinical study to determine the efficacy and safety of a low power radiofrequency and infrared light device (home use device) for personal photorejuvenation

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Radiofrequency and infrared light sources are commonly used in laser clinics and medical spas for photorejuvenation. An innovative device (Home Use Device) utilising lower energy radiofrequency and infrared light source is being developed for personal home use by patients. We aim to assess the efficacy, safety and adverse effects of this new device. 19 patients self-treated using the Home Use Device, for management of wrinkles, pore size, skin texture, tone and laxity. The patients self-administered 3 treatments a week for 6 weeks. Pre- and post-treatment clinical photographs were taken. Patients were followed up for 3 months. Two independent clinicians assessed the photographs using the Fitzpatrick's classification for rhytidoses. The patients were also given a questionnaire to assess their subjective improvement and satisfaction level with use of the Home Device. Our results indicated that clinical assessment did not show a statistically significant improvement in wrinkles 3 months after treatment. However, 73% of patients reported improvement in skin smoothness and skin tightness, 69% reported improvement in skin tone, and 47% reported improvement in wrinkles and pore size after completing all treatments. No major adverse effects were noted. 95% of patients reported being satisfied with the post-treatment results and the majority would buy a similar device. In conclusion, the Home Use Device is a new and innovative device that allows photorejuvenation of patients in the privacy and comfort of their own homes. It appears to be effective and has a good safety profile.

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An inhibitior of Stat3 activation can be a novel drug for anti-psoriatic strategy

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Our previous study elucidated that epidermal keratinocytes in psoriatic lesions exhibited Stat3 activation, and which is required for generation of psoriatic change using a mouse model. In the mouse system, topical treatment with Stat3-decoy oligonucleotides restrained the development of psoriatic change, and also ameliorated epidermal hyperplasia of preexisting lesions (Nat Med, 11; 43, 2005). Because the decoy oligonulceotides are too large in molecular weight (>10Kd) to be absorbed unless the skin barrier function is disrupted, small chemical compounds with anti-Stat3 activity has been sought for topical use. It was recently reported that ochromycinone (STA21), which is a known antibiotic with a small formula size (MW=306), was asigned as a candidate for the Stat3-inhibitor by virtual database screening, and also validated its inhibiting function through the cell-based assays (Proc Natl Acad Sci USA, 102: 4700, 2005). Therefore, we examined whether STA21 was effective on psoriatic lesions in the mouse model and patients. Topical treatment with ointment containing 0.2% STA21 onto the skin of K5.Stat3C mice, in which epidermal keratinocytes expressed transgenic 'activated' Stat3, prevented otherwise tape-stripping-dependent generation of psoriatic lesions. Histologically, the skin of STA21 treated mice developed less severe psoriatic changes including acanthosis, vasculogenesis, and inflammatory cell infiltraton. Finally, upon approval of the ethics board for clinical trial, we treated five patients with psoriasis with 0.2% STA21 ointment. After two weeks application, four out of five patients showed significantly favorable response to STA21 compared with vehicle controls. Thus, these results suggest that topical STA21 application may be a novel anti-psoriasis drug through inhibiting Stat3 activation.

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Twenty-six year experience with total skin electron beam therapy (TSEBT) in management of T2 and T3 mycosis fungoides (MF): impact of adjuvant oral bexarotene

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This is a retrospective study of Stanford's long-term experience with TSEBT in T2 (generalized patch/plaque) and T3 (tumor) MF and the role of adjuvant oral bexarotene. 195 pts with MF(105 T2, 90 T3) were treated between 1970 and 2006 with an initial course of TSEBT ≥ 30 Gy. Actuarial survival curves were generated using the Kaplan-Meir method and differences determined by log-rank test. Multivariate analysis was conducted using Cox proportional hazards model. A subgroup of 9 pts who received adjuvant oral bexarotene(375-600mg/d for 7-14 mo)after TSEBT was analyzed and compared to a matched control group without bexarotene. Clinical responses were assessed using physician's global assessment. All pts responded (>50% improvement) to TSEBT with 60% achieving a complete clinical response (CCR). 74% of T2 and 43% of T3 pts achieved a CCR. In CCR group relapse-free interval (RFI) was significantly longer for T2 than T3 pts (median RFI 38 vs 10 mo, respectively, p=0.003). The 2-, 5- and 10-yr overall survivals (OS) of the entire cohort were 78%, 59% and 40%, respectively. Median OS for T2= 127 mo and T3= 42 mo (p <0.001). The median progression free survival (PFS) for the entire cohort was 21 mo (27 vs 18 mo for T2 and T3, respectively, p=0.04). No significant differences in OS, PFS and cause-specific survival between the adjuvant bexarotene subgroup and matched controls were observed. Median time to progression was 18 mo in the bexarotene group and 19 mo among the controls. Acute dermatitis, alopecia and nail loss were common radiation-induced toxicities. Oral bexartoene was generally well-tolerated with mild fatigue, hypertriglyceridemia, and leukopenia being the most common toxicities. These data support that TSEBT ≥ 30 Gy is highly effective and well-tolerated in T2 and T3 MF. Clinical outcomes are significantly better in T2 than T3 pts. There was no clinical advantage to using adjuvant bexarotene but larger numbers need to be analyzed before any firm conclusions can be made.

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Sensentive enzyme-linked immunosorbent assay for detection of circulating anti-peri and envo autoantibodies in patients with paraneoplastic pemphigus

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Periplakin and envoplakin are components of desmosomes which were most frequently recognized by sera from paraneplastic pemphigus with immunoblot. We now developed a sensitive ELISA to detect the autoantibodies from paraneoplastic pemphigus sera. The antigen is a recombinant forms of periplakin and envoplakin linker domain and expressed in mammanin cell line. 16 of 17 PNP patients in our department are positive and 20 of normal control,20 of PV,10 of PF and 20 of BP patients are all negative,thus providing a highly sensentive methods for diagnosis in PNP.

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Efficacy of a product associating retinaldehyde and hyaluronic acid fragments on photo-induced aging in Japanese patients

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Retinaldehyde (RAL), a natural precursor of tretinoin has been shown to be effective against photo-induced aging. Hyaluronic acid (HA), the main glycosaminoglycan in extracellular matrixes is present in the dermis and epidermis where it contributes to hydration, organization and elasticity. Its decrease with age explains its use in the correction of certain signs of aging. Several studies have suggested that HA could exert different biological effects depending on the length of the chain via the interaction of HA with its CD44 receptor on the surface of keratinocytes and fibroblasts. Indeed, CD44 is induced by retinoids. Our aim is to evaluate the effects of the application of 0.05% RAL in association with 0.5% HA fragments (HAF) on the signs of skin aging on the face and hands in Japanese women. We conducted an open study including 50 Japanese women aged between 50 and 65. Application of a standardized amount of product once a day on the face and back of one hand following randomization. Duration: 180 days. Main criteria: skin replica taken on crow's feet on day 1 and on day 180. Secondary criteria: evaluation of the physico-chemical properties of the barrier function and chromametry on pigmented and non-pigmented lesions on the face and hands. Non-invasive measurements of the skin including barrier function, hydration of the stratum corneum and skin color were performed on the face and hands at days 1, 30, 90 and 180. This *in vivo* study shows the efficacy and scientific rationale behind the association of RAL/HAF in the treatment of skin aging and the good tolerance of the product in a Japanese study population.

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Single nucleotide polymorphisms in the VEGFA and VEGFB genes are associated with atopic dermatitis in Koreans

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Erythema on the skin and dilated, tortuous vessels within the papillary dermis, along with perivascular edema, are frequently observed on the histology of atopic dermatitis (AD) patients. We genotyped 14 SNPs from four VEGF family genes for 1,120 case-control samples (646 AD patients and 474 normal controls). We measured the serum VEGFA concentration in 66 individuals (42 AD and 24 controls) via ELISA, and we compared the results among the three different genotypes. The rs2010963 (-634G/C) SNP in the VEGFA gene was significantly associated with the AD phenotype. The serum VEGFA concentration was higher in the CC genotype of AD patients. The rs12366035 SNP in the VEGFB gene showed weak association with the severity of AD. However, we could not detect any relationship between the SNPs in the VEGFC and FIGF genes and AD. We found that the rs2010963 SNP (-634C/G) in the VEGFA gene and the rs12366035 polymorphism in the VEGFB gene were associated with the susceptibility to AD. Although we conducted a gene-gene interaction analysis, we did not detect significant synergistic effect between the VEGFA and VEGFB polymorphisms. These results suggest us that VEGFA has the main effect to increase the dermal microvessels in AD patients; however, VEGFB does not augment the actions of VEGFA.

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Serum IL-6 level reflects fair indicator of treatment and management of various kinds of intractable skin ulcer

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The wound healing process is known to be divided into 3 phases consisting of inflammatory phase, proliferation phase, and remodeling phase. On inflammatory phase, many kinds of cells required for tissue repair are recruited to skin lesion via inflammatory cytokines secretion derived from skin lesion, and ordinarily terminates within a few days. Although recent reports showed that the inflammatory phase on the intractable skin ulcers could be prolonged by the insufficiency of inflammatory cell growing, it is not unveiled which secreted factors have a big effect for this clinical outcome. To reveal the reason for prolongation of the inflammatory phase, patients with intractable skin ulcer (n=36) were entree in this study including two groups as collagen diseases (n=20) and non-collagen diseases (n=16). We evaluated the ulcer reduction ratio and inflammatory cytokines levels in the serum before and after 2 weeks of therapeutic intervention. Twenty-eight out of 36 patients were treated with lipo-prostaglandin (Lipo-PGE1) (10 μg/2ml/day) via intravenous bolus injection for 2 weeks. As for a local treatment, either silicone gauze or petrolatum gauze were applied to protect the wound. As a result, the reduction ratio of ulcer area 2 weeks after administration of Lipo-PGE1 was significantly improved regardless of collagen disease. Serum CRP and IL-6 levels were significantly decreased after treatment, while higher than normal level in the patients with intractable skin ulcer. Significant elevation of serum VEGF and sICAM1 level was also observed in the group with collagen disease, and the trends to decrease in the VEGF and sICAM1 level were observed after Lipo-PGE1 administration. These results suggests that the inflammatory cytokines, especially IL-6, may reflect the therapeutic efficacy of intractable skin ulcers, and inflammation may contribute to prolongation of wound healing. Moreover, Lipo-PGE1 can control and normalize the production of cytokines related to the extravagation of intractable skin ulcers and collagen vascular diseases.

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Activation of STAT3 in epidermal keratinocytes of thymoma-associated cutaneous graft-versus-host-like disease patients

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Thymoma-associated graft-versus-host like disease is a rare autoimmune disease occurring colitis, liver dysfunction, and graft-versus-host disease (GVHD)-like skin lesion. Although sporadic case reports revealed its clinical features, little is known about the pathomechanism. Signal transducers and activators of transcription 3 (STAT3) is a latent cytoplasmic protein and its activation in the keratinocytes contributes skin wound healing and keratinocyte migration. Recently, STAT3 activation has been found to be seen in both benign and neoplastic thymic tissue and be associated with the persistence of thymic tissue and the presence of myasthenia gravis. In the present study, we evaluated the activation of STAT3 in epidermal keratinocytes of thymoma-associated graft-versus-host like disease. 4 cases with recurrent thymoma associated with other autoimmune diseases such as myasthenia gravis, hypogammaglobulinemia and SLE were analyzed. We performed staining of CD1a and STAT3 for Langerhans cells and keratinocytes, respectively. In histological examination, 3 cases showed an acute GVHD-like reaction and one case showed a lichen planus-like reaction. Langerhans cells disappeared in all 3 cases that revealed acute GVHD-like reaction, but did not in the lichen planus case. 3 cases of thymoma-associated cutaneous graft-versus-host-like disease were positive for STAT3 in the keratinocytes, but the rest of one case did not. Although the role of STAT3 activation in epidermal keratinocytes of a cuatneous thymoma-associated graft-versus-host-like disease is still obscure, STAT3 might be involved in pathogenesis of this disease.

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Single nucleotide polymorphisms and haplotypes in the VEGF receptor 3 gene and the haplotype GC in the VEGFA gene are associated with psoriasis in Koreans

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Angiogenesis plays an important role in the pathogenesis of several diseases including psoriasis, cancer and chronic inflammatory skin diseases. Inflammatory skin lesions in VEGFA transgenic mice closely resembled human psoriasis with epidermal hyperplasia, impaired epidermal differentiation, and accumulation of T lymphocyte, and human psoriatic lesional skin was characterized by enlarged and tortuous blood vessels in the dermal papillae and by marked enlarged lymphatic vessels through immunofluorescence analysis. These findings prompted us to search for the relationship between psoriasis and VEGFs and VEGFR 3. By genotyping 31 SNPs from 4 genes (VEGFA, VEGFC, VEGFD and FLT4) in 717 case-control samples, we found that two SNPs (rs3776413 and rs307823) and haplotypes in FLT4 gene and haplotype GC in VEGFA gene are strongly associated with the susceptibility to psoriasis, and the serum FLT4 concentrations were higher in the psoriasis subjects than those in the normal control without any correlation with the FLT4 polymorphism. It is very hard to explain the connection between VEGFA and FLT4 in psoriasis. Each SNP and haplotypes (GCAA, ACGA) in FLT4 showed protective and risk effect in developing psoriasis. However, we could not find any SNPs associated with psoriasis in VEGFC and VEGFD genes. From our data and previous reports, it is quite inferable either that lymphatic vessels are not only required to drain inflammation-associated tissue edema, but also may participate in the maintenance of chronic inflammatory diseases, or that VEGFA indirectly attracts the inflammatory cells producing VEGFC and VEGFD, which bind to FLT4. Further studies are needed to verify the relationship between VEGFA and FLT4 in the future.

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Computational prediction of the candidate proteins associated with atopic dermatitis by PPI mapping: proteomic applications and interactomic analyses

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Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disease typically characterized by a distribution of eczematous skin lesions with lichenification, pruritic excoriations, and dry skin with wide varieties of pathophysiologic aspects. We summarized alterations of protein expressions in the primary cultured AD cells from the patients' biopsy samples that were mostly analyzed by the current proteomic techniques. On the basis of this information, we further conducted the protein-protein interaction (PPI) mapping according to the obtained candidate proteins. The PPI prediction used employs most of the major types of PPI algorithms available to date. They are Protein Structural Interactome MAP (PSIMAP), a method that uses the protein structural domain of the SCOP (Structural Classification of Proteins) database and Protein Experimental Interactome MAP (PEIMAP), a common method that uses public resources of experimental protein interaction information such as HPRD, BIND, DIP, MINT, IntAct, and BioGrid. As a result, we found that several hub proteins which could be importantly involved with the pathogenesis of AD. Since the mechanism of AD disease has been shown to complex, our results may provide new clues to aid understanding of AD.

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Gene-gene interaction in atopic dermatitis: IL-9 and IL-9 receptor in Korean population with atopic dermatitis

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The skin lesions of AD patients are infiltrated by activated T cells, eosinophils and antigen-presenting Langerhans cells that bind and present IgE complexed allergens on their surface. In addition, the activation of peripheral blood T cells that preferentially secrete T helper (Th) 2 cytokines and that help B cells produce IgE has been reported to be associated with AD. Th2 cells produce IgE synthesis, including IL-4 and 13, eosinophilia promoting IL-5 and 9. IL-9 also enhances IgE production and stimulates T cell and mast cell growth. Atopy was shown to be associated with increased levels of IL-4, 5 and 13, accompanied by decreased, similar or increased levels of INF-gamma in allergen stimulated bulk culture with PBMC. Although IL-9 and IL-9R genes have been reported to be the candidate genes for atopy, there has been no report for the association of these genes and atopy till now. We genotyped 5 SNPs from IL-9 and IL-9R genes for 1,089 case-control samples (631 AD patients and 458 normal controls). As a result, the rs31563 (-4091G/A) SNP in the IL-9 gene showed a significant difference for the allelic and genotypic distributions between the AD and normal groups. Three SNPs were genotyped from the IL-9R gene and the rs3093467 SNP showed no significant association with AD on the allelic and genotype test. However, the rs3093467 SNP showed significant association with ADi subtype in the allelic test. To investigate whether the interaction between the rs31563 SNP in the IL-9 gene and the rs3093467 SNP in the IL-9R gene, we found that GG/C- genotype showed the most significantly increased the risk of AD in our study.

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Comparison of the change in the transepidermal water loss and the amount of antimicrobial peptide after topical application of tacrolimus and ceramide-dominant emollient in patients with atopic dermatitis

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Atopic dermatitis (AD) is a chronic, relapsing skin disorder characterized by dryness of skin, pruritus, increased susceptibility to allergy. Increased transepidermal water loss (TEWL) and down-regulated antimicrobial peptide (AMP) are observed in patients with AD. Tacrolimus and emollient are effective in the treatment of AD by preventing the production of inflammatory cytokines and by improving skin barrier dysfunction each other. The aim of the study was to investigate the different effects between tacrolimus and emollient containing ceramide on the TEWL and AMP of patients with AD. Three patients with atopic dermatitis were treated with tacrolimus in one lesion and emollient containing ceramide in another one for six weeks. Follow-up evaluations were done for the change of TEWL and AMP. TEWL was significantly decreased on the both lesions applied with tacrolimus and emollient. In contrast, expression of AMP was increased on the both lesions. No significant difference between both lesions was observed. Tacrolimus and emollient containing ceramide influence on both TEWL and expression of AMP in patients with AD, namely they have similar effect on both of two. So we suggest that the immunologic action in atopic dermatitis is related with the skin barrier function. Antimicrobial barrier and permeability barrier maybe seem to be co-localized.

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Single nucleotide polymorphisms and haplotypes in the DEFB1 gene are associated with psoriasis in Koreans

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Atopic dermatitis (AD) patients have an increased susceptibility to cutaneous colonization and infection with bacteria, fungus and viruses, and that can be associated with decreased expression of innate immunity such as defensin (DEF) and cathelicidin. We genotyped 26 SNPs from the four DEF genes-DEFA4, DEFA5, DEFA6, and DEFB1- for the 1,089 subjects. We could not find any association between AD and DEFA genes. Five SNPs were genotyped from the DEFB1 gene. As a result, the rs5743399 (-2266T/C) SNP and rs5743409 (-1241T/G) in the DEFB1 gene showed a significant difference for the allelic and genotypic distributions between the AD and normal groups. For the DEFB1 gene, the rs5743399 and rs5743409 SNPs constitute an LD block by Gabriel's method and the rs2738182, rs2980923 and rs2977776 SNPs constitute another LD block. The haplotypes in the formal LD block showed significant association with AD and this is the consistent result with the single marker analysis of the two SNPs. CT haplotypes showed odds ratio of 1.425 (95% CI: 1.059 – 1.918) comparing to TG haplotype with significant p-value =0.019. This haplotype resulted in greater OR 1.611 (95% CI: 1.168 – 2.223) in the ADe subtype analysis.

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Neuronal expression of tachykinins and their receptors in chronic plaque psoriasis

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Psoriasis is a chronic inflammatory disease, in which pruritus is a common symptom. The tachykinin family might have a role for the inflammatory process per se and also for the pruritus. Aims: The aim of this study was to investigate the expression of members of tachykinin family, substance P, neurokinin A (NKA), NKB and their receptors(R) neurokinin-1(NK-1), NK-2 and NK-3, in lesional psoriatic skin with pruritus, non-lesional psoriatic skin and in normal healthy control skin. Methods: Staining was performed by a biotinylated streptavidin technique using Lanas (formalin and picric acid) fixed sections of biopsies from psoriasis lesions (n=9), non-lesional psoriasis (n=8) and healthy control skin (n=9). Severity of psoriasis was evaluated by psoriasis area severity index (PASI). The pruritus intensity was measured by a visual analogue scale (VAS), 1-10 cm. Results: The mean (± SD) values of PASI and pruritus intensity were 19.3 ± 7.7 and 4.6 ± 1.8, respectively. The numbers of substance P positive nerve fibers were higher in lesional skin (4.7 ± 1.2 fibers per section) than in non-lesional (3.0 ± 1.4) or normal healthy skin (1.0 ± 0.4). Similarly, NKA positive nerve fibers were higher in number in lesional skin (3.6±3.0) than in non-involved (0.9±0.9) or healthy skin (0.4 ± 0.3). There were also higher numbers of nerve fibers stained for NKB in lesional (3.5 ± 1.4) compared to non-lesional (0.8 ± 0.8) or healthy skin (0.8± 0.5). Neither NK-1R nor NK-3R positive nerves could be detected in lesional, non-lesional or healthy skin. In contrast, there were NK-2R immunoreactive nerves, more in lesional skin (4.2± 1.9) than in non-lesional skin (0.2 ± 0.5) and healthy control (0). Conclusion: These results suggest a role for the tachykinin family in psoriasis, possibly also for the pruritus.

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Withdrawn

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Differential effects of corticosteroids and the immunomodulator pimecrolimus on the infant skin immune system

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Topical application of corticosteroids and calcineurin inhibitors such as pimecrolimus is a common practice during early childhood as therapy for inflammatory skin diseases, even though very little is known about their specific effects on the developing skin immune system. Therefore, we directly compared the in vitro effects of these compounds on the fate and phenotype of keratinocytes and Langerhans cells (LC) isolated from infant skin. Epidermal cells from non-inflamed infant foreskin (≤ 2 years of age) were cultured for 48 hours with vehicle, beta-methasone-17-valerate (BMV), or pimecrolimus, and then analyzed for viability, phenotype and cytokine secretion. The percentage of viable epidermal cells significantly decreased in BMV but not pimecrolimus cultures. Surprisingly, the BMV-induced reduction of epidermal cells was neither attributable to LC nor basal keratinocytes, even though both populations express the glucocorticoid receptor. Exposure of infant LC to BMV caused inhibition of the upregulation of HLA-DR (30%), CD83 (27%) and CD86 (38%; $p<0.001$), compared with controls. These effects, which were not seen with pimecrolimus, suggest that the glucocorticoid receptor is functional in LC. Consistent with this explanation is our finding that the release of MCP-1 and TNF-alpha by CD1c- epidermal cells, cultured for 24 hours in the presence of BMV, was reduced by 20-42% compared with controls. In comparison, the release of all cytokines tested was not suppressed by pimecrolimus. Highly enriched LC cultured without stimulation demonstrated constitutive expression of RANTES, MCP-1, TNF-alpha, IL-6, and IL-8. BMV but not pimecrolimus marginally inhibited the production of TNF- alpha and IL-6. In conclusion, the data suggest that corticosteroids may impair LC maturation, viability and cytokine secretion of epidermal cells in infants in vivo – effects not seen with pimecrolimus.

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Possibilities of valproic acid as a therapeutic tool for natural killer-cell neoplasms

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Histone deacetylase inhibitors (HDACi) are novel anticancer drugs against a number of different malignancies including cutaneous T-cell lymphoma. Cutaneous lymphomas expressing natural killer (NK) phenotype are characterised in most cases by a highly aggressive clinical course and difficult to manage. Despite significant advances in the treatment of cancer, NK-cell neoplasms remain still incurable to date. It is thus necessary to seek new therapeutic tools for NK-cell neoplasms. Valproic acid (VPA) is a widely prescribed drug for seizures and bipolar disorder for decades, has been shown to be an HDACi. Although it has been reported to inhibit tumor growth and induce apoptosis in several lymphoid cell lines, its effects in NK-cells are still unclear. If VPA can control proliferation ability of NK-cell lines in vitro, it may become a novel treatment tool for NK-cell neoplasms. In this study, we have observed the growth inhibitory and apoptogenic potential of VPA on two NK cell lines (KAI3 and KHYG-1). Anti-proliferative activity was measured by XTT assay and apoptosis was determined by flow cytometry analysis of annexin V binding population. Treatment with VPA resulted in a dose- and time-dependent inhibition of cell growth in both cell lines. VPA at 1-2.5 uM for 24 h induced apoptosis in these cell lines: KAI3 (2%-8%) and KHYG-1 (4%-24%). VPA also downregulated Bcl-2 transcript expression even in therapeutic concentration (1mM). These results indicate the possibility that VPA may be a novel and effective treatment tool in management of NK-cell neoplasms.

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Testing the functional integrity of cutaneous autonomous vascular regulation in diabetic foot with laser Doppler technique

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Proper microcirculatory skin blood supply is maintained by various local and systemic regulatory mechanisms. Failure of skin autonomous vascular regulation poses severe problems in wound healing in diabetic foot ulcers. Determination of the integrity of cutaneous autonomous regulation of blood supply could be an important prognostic factor in wound healing of diabetic foot ulcers. We aimed to develop a non-invasive method for monitoring the integrity of skin autonomous vascular regulation in diabetic foot. Changes in skin blood flow were initiated using the Valsalva maneuver in healthy and diabetic patients alike. We mimicked diabetic neuropathy by using bretylium locally to neutralize sympathetic activity by presynaptically blocking noradrenalin release. Servo-photopletysmography was used to monitor haemodynamic changes, while laser Doppler technique was used for measurement of skin blood flow and calculation of cutaneous vascular resistance. Without bretylium treatment the cutaneous vascular resistance showed a significant increase during the Valsalva maneuver in healthy individuals. Bretylium treatment completely inhibited this increase. In diabetic neuropathy we observed the same type of change as with the use of Bretylium in healthy individuals. Our data indicate that Valsalva maneuver results in vasoconstriction of skin arterioles and this vasoconstriction is the result of sympathetic efferent activity. We propose that monitoring of cutaneous vascular resistance over bretylium treated and non treated skin areas is a convenient and non-invasive method to determine the functional integrity of skin autonomous vascular regulation in diabetic foot. Changes in the functional integrity of microcirculatory skin blood supply due to diabetic neuropathy should be observed as the first signs of impaired wound healing in diabetic foot.

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Long term efficacy and safety of the combination therapy with 5% sodium L-ascorbyl-2-phosphate lotion plus 0.1%adapalene gel for moderate to severe acne in comparison with monotherapy

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We have reported the efficacy of sodium 5% L-ascorbyl-2-phosphate lotion(5% APL) in the treatment of acne achieved by an open comparative study at 2002, 2003 and 2006 SID meeting. APL shows the excellent effect for the prevention of oxidation of squalene, which was reported as a possible role of acne etiology and as a trigger of microcomedo. This time, we studied the long term efficacy and safety of the combination therapy with 5% APL plus 0.1% adapalene gel(ADG) for moderate to severe acne in comparison with monotherapy. In a randomized, multicenter, open label, parallel study, ninety patients of moderate to severe acne were enrolled. They were assigned to receive 5% APL plus 0.1%ADG(n =30), 5%APL(n =30) only, and 0.1%ADG(n =30) only topically twice a day. Efficacy was assessed in 5 grades at the beginning and at 1, 3, 6 and 9 months of treatment based on digital CCD microscopic device, which can capture clinical digital photographs and microscopic images. Patients were instructed to refrain from receiving any other treatment for 4 weeks prior to enrolment in the study. The number of patients who complied with the treatment protocol was 26 on the combination group, 27 on the APL group, and 23 on the ADG group. The number of patients who were evaluated as good and excellent at 9 months of treatment was 21 on the combination group, 17 on the APL group, and 12 on the ADG group. The combined therapy of APL plus ADG were well tolerable and showed no remarkable adverse effects. Efficacy rate for the combination group was 80.8%, APL 63.0%, ADG 52.2%. The efficacy of the combination therapy was superior to the monotherapy of 5%APL and 0.1%ADG. This data supports the hypothesis that the combination therapy of 5% APL plus 0.1%ADG showed the excellent long term efficacy and safety in the treatment of moderate to severe acne.

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In vitro skin penetration/permeation of pimecrolimus and topical corticosteroids from their marketed formulations

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For treatment of skin diseases with topical drugs, sufficient penetration of the agents into the relevant skin layers is required. Permeation through the skin should ideally, however, be minimal in order to avoid the risk of systemic side effects. The marketed formulations of pimecrolimus (Elidel®, 1% cream) and of two selected topical corticosteroids, namely mometason furoate (Elocon®, 0.1% ointment) and triamcinolon acetonide (Volon A®, 0.1% ointment) were applied to human cadaver skin and the penetration/permeation of the active compounds was studied *in vitro*. The skin concentration of the two drugs were similar for Elidel 1% and Elocon 0.1% (2.1+/-1.6 and 3.4+/-0.7 µg/g, respectively), while Volon A 0.1% yielded lower levels (0.44+/-0.12 µg/g). The permeation rate of pimecrolimus for the 1% cream was about 72-fold lower than for the 0.1% mometason ointment (0.04+/-0.02 vs. 2.9+/-0.87 ng/ml/hr); also, the rate was 37-fold lower when comparing Elidel 1% with the triamcinolon ointment (1.5+/-0.4 ng/ml/hr). To conclude, permeation of pimecrolimus from the 1% cream formulation through human skin *in vitro* is considerably less than permeation of the tested corticosteroids from 0.1% ointments. Thus, the lower permeation of pimecrolimus compared to steroids observed in previous studies in which a 1% solution of each substance was tested, is maintained even when marketed corticosteroid formulations, with 10-times lower drug concentrations than those previously tested, are used. This finding is noteworthy due to the link between low permeation *in vitro* and systemic exposure in patients after topical application.

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Therapeutic potential of varicella vaccine for cutaneous type and smouldering type Adult T-cell leukemia/lymphoma (ATLL)

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Adult T-cell leukemia/lymphoma (ATLL) is a malignant lymphoproliferative disorder caused by human T-cell lymphotropic virus type I (HTLV-I). There are several subtypes of HTLV-I-induced ATLL: acute, lymphoma, chronic, smouldering, and cutaneous. Chronic and smouldering type ATLL have a relatively good prognosis, even without treatment. These types, however, can evolve to acute type ATLL, which has a poor prognosis: the median survival time after diagnosis is only 6 months. ATLL commonly involves the skin in up to 50% of patients. Previously we reported that varicella-zoster virus could be capable of activating the immune state by the manifestation of active inflammation. Therefore we postulated that varicella vaccine could be a novel immunotherapeutic agent against ATLL. According to the original protocol, patients received a biweekly administration of varicella vaccine for about one year. We examined peripheral blood cell counts, the value of lactate dehydrogenase (LDH), serum soluble interleukin 2 receptors (sIL-2R) levels and HTLV-I proviral DNA loads in 2 smouldering type ATLL patients and 11 cutaneous type ATLL patients during the immunotherapy, and analyzed the associations between each parameter and the clinical characteristics among the ATLL patients. No adverse events observed during the immunotherapy. One patient eventually died from progressive disease, but the remaining patient is still alive. On the other hand, spontaneous total regression of cutaneous involvements had occurred in two of them. Most importantly, stable disease and stable laboratory findings were maintained for as long as one year in almost patient who had previously required conventional therapy for the skin involvements of ATLL. In conclusion, although this study was performed in only a limited number of patients, it does provide important information about benefit from varicella vaccine immunotherapy. Smouldering type ATLL with the skin lesions and cutaneous type ATLL are promising candidates for this immunotherapy.

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First identification of hydroxylated polychlorinated dibenzodioxins as metabolites of TCDD in human

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a highly toxic compound that may cause severe cutaneous and systemic symptoms in humans. Following its specific binding to the aryl hydrocarbon receptor (AhR), TCDD induces the expression of various phase I and phase II enzymes, in particular the monooxygenases CYP1A1 and CYP1A2. TCDD is a lipophilic compound that accumulates in body lipids, and is eliminated very slowly as unmodified TCDD, therefore being unaffected by the monooxygenases it induces. Thus TCDD metabolites have not yet been reported in humans. We have performed various prospective investigations to follow-up the routes of TCDD elimination in a patient suffering from severe TCDD poisoning. Based on TCDD analyses of the known routes of elimination (faeces, skin lesions and sweat), only 40% of the amount estimated from the decrease of TCDD in the fat compartment over a period of one year could be recovered mainly in the faeces. In order to identify possible TCDD metabolites which may account for this difference, faeces and urine were analyzed using gas chromatography high resolution mass spectrometry (GC/HRMS). Thus we identified a monohydroxy-tetrachlorodibenzo-p-dioxin (OH-TCDD) in faeces and in urine (where TCDD was likewise undetectable), and a monohydroxy-trichlorodibenzo-p-dioxin (OH-TriCDD) only in faeces. The total of the faecal concentrations of these two metabolites was similar to the concentration of unmetabolized TCDD representing thus another 40% of the total TCDD elimination from the body. This is the first identification of TCDD metabolites in humans. If humans are indeed able to hydroxylate TCDD any intervention supporting such a pathway could decrease the long TCDD half-life, which represents the major problem in the treatment of patients intoxicated with TCDD.

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Genomic profiling of mycosis fungoides reveals major differences with Sézary syndrome

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Mycosis fungoides (MF) and its leukemic variant Sézary syndrome (Sz) are the most common group of cutaneous T-cell lymphomas (CTCLs). MF and Sz are both clonal proliferations of T cells with cerebriform nuclei and a CD4+, CD45RO+, CLA+ immunophenotype. Recently we have applied array-based CGH (aCGH) to map numerical chromosomal alterations in malignant T cells of Sz patients and found that Sz is characterized by several highly recurrent copy number alterations (CNAs). The first aim of this study was to evaluate the similarity of MF and Sz by comparing the pattern of chromosomal alterations characteristic of MF with Sz. Secondly, we sought to identify candidate oncogenes and tumor suppressor genes residing within minimal common regions with highly recurrent CNA in MF by integrating aCGH and gene expression data. DNA and RNA were isolated from skin biopsy specimens of 22 patients with tumor-stage MF. Chromosomal alterations were mapped using CGH arrays containing approx. 3500 BACs and gene expression was analyzed using Affymetrix microarrays. The expression levels of genes in samples with CNA was compared to those without CNA and subsequently confirmed using quantitative PCR.Highly recurrent, focal CNAs in MF include gains of 7q36, 7q21-22 and losses of 5q13 and 9p21. By contrast, Sz is characterized by frequent gains of 17q23-25, 8q24.2, and loss of 17p13.1 and 10q25. Integration with gene expression data from MF samples yielded a refined list of candidate genes. We confirmed that the FASTK and SCAP1 oncogenes and Rb and DLEU1 tumor suppressor genes, residing in regions with frequent CNA in MF, demonstrate dysregulated expression. Our results demonstrate that the pattern of chromosomal alterations characteristic of MF markedly differs from that of Sz, which may suggest that the pathogenesis of these CTCLs is distinct.

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Silicon phthalocyanine photodynamic therapy for treatment of cutaneous neoplasms

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Silicon phthalocyanine Pc 4 is an investigational second-generation photosensitizer for photodynamic therapy (PDT). A Phase I dose-escalation trial on pre-malignant and malignant skin lesions was performed using topical Pc 4 solution plus irradiation with 675nm red light. Clinical and histologic evaluation was done. Photosensitivity was monitored via MED testing. Plasma pharmacokinetic studies were done via HPLC. A unique reflectance spectroscopic system was employed to monitor the amount of Pc 4 in the skin lesion during light exposure. 42 patients (33 patients-mycosis fungoides/3 patient- basal cell carcinomas/2 patient- squamous cell carcinomas/4 actinic keratoses) have completed Pc 4-PDT. Partial responses have been observed in 16 patients. Immunohistochemistry of post-PDT biopsies showed more upregulation of caspase-3 and HIF-1a in responders relative to non-responders, and untreated controls. No significant adverse events were observed and no systemic Pc 4 absorption was detected. Pc 4-PDT is a promising non-invasive novel therapy for premalignant and malignant skin conditions with an excellent safety profile. The use of a real-time in vivo spectroscopic system aids in the establishment of optimal treatment parameters.

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Higher affinity of pimecrolimus to soluble skin proteins: an explanation for its lower skin permeation as compared to tacrolimus

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Pimecrolimus (Elidel®, 1% cream) and tacrolimus (Protopic®, 0.1% and 0.03% ointments) are calcineurin inhibitors used for the topical treatment of atopic eczema. They are structurally similar, but display specific differences including the higher lipophilicity and the significantly lower permeation of pimecrolimus through skin, while concentrations of both drugs reached within human skin are similar. The present study aimed at understanding the reason for the lower permeation of pimecrolimus through skin, which translates to low systemic exposure in patients treated with Elidel. Firstly, we found that permeation of pimecrolimus and tacrolimus through a silicon elastomer membrane was nearly identical, both when comparing solutions and the marketed formulations. This suggests that the comparatively low permeation of pimecrolimus through skin is not caused by a significantly slower release from the cream formulation, nor by a limited solubility of pimecrolimus in the receptor fluid. The data rather point to a higher affinity of pimecrolimus to components of the skin. To test this hypothesis, we investigated binding of pimecrolimus and tacrolimus to a preparation of soluble human skin proteins. We identified one specific binding protein with a molecular weight of approx. 15 kDa, which displayed a similar binding capacity for pimecrolimus and tacrolimus, likely corresponding to FKBP12. However, less specific, non-saturating binding to other proteins was approximately three-fold higher for pimecrolimus than for tacrolimus. Due to the high local drug concentration following topical administration, the less specific, high-capacity binding is likely to be the predominant element in overall binding in skin. These data offer an explanation of why permeation through human skin is lower for pimecrolimus than for tacrolimus.

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Improving patients' adherence to topical dermatologic treatments

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Adherence to treatment is a major barrier to successful dermatologic outcomes. We investigated dermatologic patients' adherence behavior across a variety of conditions and treatments including: 1) atopic dermatitis patients treated with topical triamcinolone; 2) atopic dermatitis patients treated with topical desonide hydrogel; 3) acne patients treated with topical adapalene; and 4) actinic keratoses patients treated with topical 5-fluorouracil. Electronic medication event monitors (MEMS) were placed in medication caps as an accurate measure of adherence. With the exception of patients using 5-fluorouracil, adherence to topical treatments was generally poor. In atopic dermatitis patients, mean adherence to twice daily 0.1% triamcinolone ointment dropped by about 60% over the first few days. Better adherence was seen with desonide hydrogel and more frequent follow up, with initial adherence of 75% declining to 45% at 4 weeks. Average adherence to once daily 0.1% adapalene gel was increased by having patients return for more frequent office visits and reduced by having parents remind their teens to use the medication. Judicious use of follow up visits and less messy, patient-preferred vehicles improve patients' adherence behavior.

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Effects of pimecrolimus vs triamcinilone on skin Langerhans cells after simulated solar irradiation

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Pimecrolimus is a topical immunomodulator used for mild to moderate atopic dermatitis. Concerns regarding pimecrolimus and malignancy resulted in a black box warning for this drug in 2006. Langerhans cells (LC) are essential in immunosurveillance against development of skin malignancies. We performed a double blinded RCT to investigate the effects of pimecrolimus 1% cream vs triamcinolone 0.1% cream in combination with 1 MED exposure to SSR on epidermal LC in vivo. 20 healthy FST I-III volunteers were randomized into 2 groups. Subjects were given both creams and asked to return after 7 days of at home, twice daily application. Subjects received 1 MED of SSR to pretreated areas. 48 hours later, punch biopsies were taken from the following skin sites. Group 1; untreated-unirradiated; pimecrolimus plus SSR; triamcinolone plus SSR; SSR alone. Group 2; pimecrolimus only; triamcinolone only; pimecrolimus plus SSR; triamcinolone plus SSR. Immunohistochemical staining using antibodies to CD1a, HLADR and CD83 was performed. Results showed that pretreatment with triamcinolone plus SSR caused more depletion in CD1a+ LC relative to pimecrolimus treatment plus SSR (p=0.044). Using HLADR as a pan-marker for APCs, we found no significant difference between pimecrolimus plus SSR and triamcinolone plus SSR. Using anti-CD83 as a maturation marker, we found that skin treated with pimecrolimus and SSR showed more mature APCs, than skin treated with triamcinolone and SSR (p=0.008). Our data confirm that UV induced changes in LC are affected by both pimecrolimus and triamcinolone, although there may be more preservation of LC number and function in pimecrolimus treated skin.

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Modulation of Aβ nerve fibers perception by a daily use of a skin moisturizer

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For the present study, our objective was to investigate whether a method commonly used in the detection of early steps of neuropathic disorders allows the evaluation of skin perceptions in women with healthy skin. The modulation of skin sensations by a daily topical application of a moisturizer was documented by measuring the current perception thresholds (CPT) of cutaneous nerve fibers Aδ, Aβ, and C. Forty Caucasian women, with healthy skin, aged 40 to 45 were recruited. Test group and control group were randomly allocated: 30 women were instructed to daily apply a skin moisturizer on the whole face during 30 days (test group), and 10 women maintained their daily cosmetic habits during the whole study period (control group). CPT of sensory Aδ, Aβ and C nerve fibres were selectively measured using the Neurometer® (Neurotron, USA) and skin hydration level was measured by means of a Corneometer® (Courage & Khasaka GmbH, Germany). All measurements were performed on the right cheek of both groups at baseline and after 30 days. In the test group, the capacitance was significantly increased after 30 days of a daily application indicating an improvement of facial skin hydration. In parallel, a significant decrease in CPT of Aβ fibers was observed, whereas no effect was found on CPT of Aδ and C fibers. In the control group, neither capacitance nor the CPT was changed. Our results suggest that a daily application of a moisturizer increases sensitivity of Aβ fibers, which are known to play a role in the transmission of tactile sensations to the central nervous system. These findings complete those made by other authors who have shown that skin hydration improves tactile discrimination by altering the transfer of the stimulus toward the sensory receptors. In addition, our results demonstrate that a method commonly used for the diagnosis of peripheral neuropathies can also be used to investigate changes of sensations in healthy skin.

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Clinic, pathogenesis and therapy of cutaneous side-effects induced by inhibitors of the epidermal growth factor receptor

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Here we demonstrate the broad spectrum of cutaneous side-effects induced by epidermal growth factor receptor (EGFR) inhibitors (EGFRI), present new insights into their pathogenesis and finally suggest an algorithm for the management of these novel skin toxicities. Our study includes a large set of clinical images before and after treatment as well as <i/>in vitro *and <i/>in vivo* analysis of chemokines and cytokines applying quantitative real-time-PCR, immunohistochemistry and ELISA. EGFRI like erlotinib, gefitinib or cetuximab have been established successfully as so called targeted cancer drugs in the treatment of various solid cancers. Most frequent and severe side-effects of this class of drugs are cutaneous toxicities, including disseminated inflammatory, papulopustular eruptions, paronychias, xerosis cutis, itch, as well as a progressive atrophy of the skin, occurring in far more than 50 percent of the patients. Clinical observations assume, that the EGFR critically regulates cutaneous inflammation. Indeed, we here demonstrate, that the inhibition of the EGFR induces the overexpression of proinflammatory chemokines and cytokines in primary human keratinocytes. These <i/>in vitro *data are confirmed by <i/>in vivo* expression analyzes of chemokines in skin samples of patients treated with erlotinib. EGFRI-related cutaneous side-effects represent a severe threat for patient compliance and their management is challenging. Nevertheless, most side-effects are reversible when EGFRI therapy is stopped or doses are reduced. However, own clinical experiences have shown, that side-effects can be managed effectively by applying combinations of anti-inflammatory and anti-microbial agents according to a specific management algorithm.

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Effects on blood cells after zinc therapy for dermatological disorders

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Background: Zinc gluconate is often used as monotherapy or in conjunction with other agents in the treatment of dermatological disorders. Long term administration may result in hematological abnormalities. Aim: To evaluate whether zinc gluconate 200mg/day equivalent to 32 mg zinc (a dose routinely used in dermatological disorders) for 2 months can result in blood cell abnormalities. Patients and Methods: Fifty-two patients with acne and alopecia areata, age 22±2.3 (29 male and 23 female) without any systemic disorder were prescribed to receive 200mg zinc gluconate per day for 2 months as monotherapy. Appropriate counseling was given to patient for dietary habits that might influence zinc absorbtion. Copper and zinc levels, haemoglobin, hematocrite, white blood cell count, neutrophil leucocytes, monocytes and eosinophils, reticulocyte count, seruloplasmin, iron and TIBC in the patients serum was evaluated at baseline and 2 and 4 months afterwards. Results: Eleven patients exhibited an increase beyond normal limits in zinc levels (from 91.2±13.4μgr/dl to 169.4±24.7μgr/dl) and decrease in copper levels at the second visit that resolved at the final evaluation. These patients also exhibited mild anemia (hemoglobin from 16.2±3.4g/dl to 10.4±1.8g/dl), neutropenia (from 3100±420 to 1100±140) and leucopenia (WBC from 5.1±0.43K/uL to 3.5±0.23) in the 2 months visit that also revolved at the final evaluation. Reticulocyte count was raised from a median 0.6% to 1% at the second visit in these patients. Conclusion: Phycisians should be aware that zinc gluconate administration in doses of 200mg daily for 2 months might result in blood cell abnormalities that revert to normal after zinc uptake is discontinued. A complete blood count during treatment should be encouraged for patients receiving zinc for long periods.

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Reduced expression of the IL-23/IL-17 axis in atopic dermatitis skin may impair innate immunity

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Atopic dermatitis (AD) and psoriasis have previously been defined by polar Th2 and Th1 T cell phenotypes, but this paradigm has recently been challenged with discovery of Th17 T cells. While it has becoming evident that many inflammatory & autoimmune diseases, including psoriasis, have a strong IL-17 signal, the importance of Th17 T cells in AD is still unclear. By analysis of expressed genes using Affymetrix U95 arrays, we found low expression of lipocalin 2 (LCN2) in AD skin lesions compared to psoriasis. As LCN2 expression in osteoblasts was previously traced to IL-17, we hypothesized that low expression of LCN2, as well as other innate defense genes, might be due to a relative absence of IL-23 and Th17 T-cells (which synthesize IL-17 & IL-22) in AD. To test this hypothesis, we 1) measured IL-23 and IL-17 expression in AD vs. psoriasis using real-time RT-PCR and 2) measured genes activated in human keratinocytes after treatment with IL-17, IL-22, or interferon-γ by gene arrays and RT-PCR. Genomic expression of IL-23 and IL-17 was reduced significantly in AD compared with psoriasis, as were LCN2, hBD2, LL37, elafin, and S100A7 mRNAs. Conversely, IL-17 but not interferon-γ strongly coordinately induced mRNAs for these innate defense in keratinocytes. "En bloc" absence of these innate defense products in epidermal keratinocytes of AD lesions was then confirmed by IHC analysis. Hence, we propose that the IL-23/Th17 axis is largely absent AD skin lesions and that hypo-expression of IL-17 accounts for a relative absence of innate defense genes, perhaps accounting for recurrent skin infections in this disease.

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Risk of progression in stage I mycosis fungoides: are calcineurin inhibitors implicated?

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Two studies reported Stage IA Mycosis fungoides (MF) patients have similar overall survival to healthy controls, however, a subset progress. Anecdotal reports of lymphomas developing after calcineurin inhibitors use exist. We retrospectively reviewed histories and pathology diagnoses in 413 Stage I MF patients diagnosed from 2001-2007 to determine factors associated with time to progression (TTP). We conducted univariate and multivariate analyses evaluating demographic risk factors (age, sex, race); topical therapies prior to diagnosis (pimecrolimus (P), tacrolimus (T), or topical steroids); pathologic features; (CD30+, large cell transformation defined as > 30% LC in biopsy, T-cell receptor clonality) and labs (LDH, B2MG, CBC with differential). Of 413 MF patients, 68 were misdiagnosed with eczema and 26/413 (6.3%) progressed beyond their initial skin T1-4 stage within 6 years. Three of 20 (15%) used P, 1/10 (10%) used T, and 17/249 (6.8%) used steroids prior to diagnosis. Factors associated with decreased TTP in the multivariate analysis were prior P use (p=0.003, OR 7.3 CI 1.99-27.23), log LDH (p=0.0006, OR 20.56), and LCT in biopsy (p=0.04, OR 2.94, CI 1.08-8.04). In patients with LCT at diagnosis, 8/27 (29.6%) progressed compared to 13/233 (5.5%) without LCT over 6 years. Only 3 patients reporting P use and discontinuation prior to diagnosis subsequently progressed. All 3 had folliculotropic MF without LCT at diagnosis; 2 later developed LCT. Duration of use was 1 mo-3 yrs. Tumors developed in areas where P had been applied: head (2 pts) and antecubital fossa (1 pt) . In summary, high serum LDH, prior use of P, and LCT were associated with decreased TTP among a cohort of 413 stage I MF patients studied retrospectively. This study was limited by the small number of patients with self-reported data on prior calcineurin inhibitor use. Since eczema and early MF are often indistinguishable, consider a skin biopsy before prescribing calcineurin inhibitors.

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The effect of tyrosine kinase inhibitors whi-p131, rapamycin and imatinib on mast cell histamine release

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Mastocytosis is a disorder characterized by the abnormal accumulation of tissue mast cells which may result from auto-activating KIT mutations. We have shown that the tyrosine kinase inhibitors (whi-p131, rapamycin and imatinib) are effective in the inhibition of mastocytosis mast cell growth <i/>in vitro, *and thus may prove useful for the treatment of this disease. However, use of these growth inhibitors could cause extensive mast cell death resulting in massive mast cell mediator release and anaphylaxis. Thus, we examined the effect of whi-P131, rapamycin and imatinib on mastocytosis mast cell histamine release. Human mastocytosis mast cell lines HMC-1 (expressing auto-activated KIT V560G) and HMC-1.2 (expressing auto-activating KIT V560G and D816V) were treated with whi-P131 (20 μM), rapamycin (100 nM) and imatinib (10 μM) from 20 min to 3 days, and histamine release was determined. No histamine release above controls was observed from either cell line when incubated for up to 80 min. Treatment of HMC-1 with whi-p131 and imatinib for 2 days resulted in increased histamine release of 61% and 56%, respectively above medium controls. HMC-1.2 cells treated with rapamycin for 2 days had histamine release of 55% above controls. The increased histamine release from these cell lines is correlated with the growth inhibition HMC-1 to whi-p131 and imatinib, and HMC-1.2 to rapamycin. The results of this study demonstrated that while these tyrosine kinase inhibitors do not cause immediate mast cell histamine release, they have the potential to cause extensive mast cell death and thus massive histamine release within days. While mastocytosis patients have increased histamine metabolizing capabilities, treatment of these patients with these tyrosine kinase inhibitors should include the use of at least H1 and H2 antihistamines.*

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Use of NAC to protect nevi against UV-induced oxidative stress/damage: towards a novel paradigm for melanoma chemoprevention

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We have shown previously that the antioxidant and glutathione (GSH) prodrug N-Acetyl Cysteine (NAC) can protect melanocytes from UV-induced oxidative stress/damage and delay the onset of UV-induced melanoma in mice, suggesting its potential utility as a chemopreventive agent for human melanoma. Conventional chemoprevention strategies, however, may be problematic as large numbers of patients need to be monitored for many years to ascertain benefit, and there may be unknown toxicities associated with chronic ingestion of any agent. We propose using NAC in a novel paradigm for melanoma chemoprevention, in which patients would take NAC in anticipation of acute UV exposure. This approach avoids potential toxicities of chronic antioxidant use, and a presumed benefit would be afforded by reduction in (potentially carcinogenic) oxidative damage in melanocytes/nevi over the course of many UV exposures. Towards this end, we established an ex-vivo system for analyzing UV-induced oxidative responses in biopsied nevi and developed HPLC-based quantitative analyses of oxidative biomarkers. Fragments of individual nevi were unirradiated (control) or UV-irradiated (4000 J/m^2). After 24 h, there was a 50% increase in reactive oxygen species and a 30-50% reduction in GSH levels (n=7), and increased formation of 8-oxoguanine (8-OG) was detected. Twelve patients with nevi were given a single oral dose of NAC (1200 mg, no adverse events reported), and separate nevi were removed before and 3-24 h after taking NAC. Levels of both cysteine (NAC metabolite) and GSH increased in nevi by 30-50% 3 h following NAC ingestion, indicating a favorable oxidative shift and drug delivery to nevi. We have recruited an additional 12 patients to assess whether NAC taken under these conditions can protect nevi from UV-induced GSH depletion and 8-OG formation. Use of oral NAC to protect nevi against UV-induced oxidative stress/damage may be an effective adjuvant to sunscreen in patients with nevi to reduce their long-term melanoma risk.

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The significance of t-cell clonality analysis for possible lymphomatoid papulosis

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Lymphomatoid papulosis (LYP) is a CD30 positive lymphoproliferative disorder according to WHO-EORTC classification. LYP can be clonal and may include a spectrum of benign to neoplastic lesions. The differences of clinical behavior between clonal-LYP and non-clonal-LYP have not been fully investigated. The purpose of this study is to determine if T-cell clonality in LYP could predict clinical behavior & improve management. We reviewed clinical history, microscopic/immunologic feature, & T-cell clonality for 21 patients(41 biopsies) with LYP and associated anaplastic large cell lymphoma (ALCL) or mycosis fungoides(MF) prior to, concurrent with, or subsequent to the LYP. 11 patients (30 cases) had a clonal T-cell population identified in at least one of the biopsies. 10 patients (11 cases) were non-clonal. Among clonal-LYP, all 5 patients with follow-up biopsies had associated MF or ALCL. For clonal-LYP patients with one biopsy, 3 were re-classified as MF and 1 as ALCL based on abnormal immunophenotype, pattern of lymphocytic infiltrate & clinical history; 2 had histologic/immunologic features of LYP with short clinical presentation (skin lesions of 2 and 5 months). For non-clonal-LYP, none of the cases showed epidermotropism or abnormal immunophenotype. With additional clinical history, non-clonal LYP cases were reclassified as follows: 3 folliculitis, 1 scabies, 1 radiation site, 1 drug eruption, 2 reactive dermatitis(drug eruption?), and 2 atypical lymphoid infiltrate(MF?). In summary, our study shows that about 80% of clonal-LYP patients presented with lymphoma during the course of disease; and about 80% of non-clonal-LYP had reactive/inflammatory condition. Furthermore, epidermotropism and abnormal immunophenotype were more often identified in clonal-LYP cases. Our study results suggest that T-cell clonality testing for LYP is a valuable tool in addition to clinical history, histologic examination, and immunophenotyping. It may help delineate reactive conditions from lymphoma.

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Optimal levels of thioguanine nucleotides in patients with immunobullous diseases: an adjunctive measure to thiopurine methyl transferase monitoring of patients on azathioprine

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Background: Azathioprine is a pro-drug metabolized by the enzyme thiopurine methyltransferase (TPMT) which exhibits genetic polymorphism. Recent interest in pharmacokinetic monitoring has revolved around measuring its active cytotoxic metabolites, the 6-thioguanine nucleotides (TGN). Optimal levels of TGN above 235 pmol/8 × 108 RBC are suggested in the inflammatory bowel disease (IBD) literature and between 100-200 pmol/8 × 108 RBC for kidney transplant patients. Objective: to prospectively determine the optimal TGN level for disease remission in patients with immunobullous disease while on treatment with azathioprine. Methods: A total of 27 patients were enrolled in the study over a 2 year period. Blood levels of TGN, 6-methyl mercaptopurine (MMP), the inactive metabolite of azathioprine, TPMT by PCR and enzyme activity were measured longitudinally. The optimal level of TGN is defined as the level at disease remission and prednisone dose below 15 mg per day. Results: Twelve patients were evaluable for TGN and MMP and 25 for TPMT. The level of TGN for disease remission was in a range of 48 to 457 pmol/8 × 108 RBCs with optimal levels at 179 pmol/8 × 108 RBCs for pemphigus and 206 pmol/8 × 108 RBCs for pemphigoid. Patients with MMP above 5700 pmol/8 X 108 RBC were more likely to have higher liver ALT enzyme (mean 48.5 vs. 31.1, p=0.029). Longitudinal variability (CV>20%) in TPMT was noted in 21% of patients, including one heterozygous patient. Conclusions: Optimal levels of TGN metabolites in dermatology patients are lower than those reported for IBD. They can serve as an adjunctive monitoring tool in addition to the one time pre-treatment measurement of TPMT activity. Several pharmacokinetic parameters will be discussed including the significance of monitoring TPMT longitudinally, and preferential pathways to the inactive metabolite MMP.

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Inverse relation between serum vitamin D level and severity of psoriasis

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Psoriasis is a chronic relapsing immune-mediated skin disease characterized by rapid turnover of the epidermal keratinocytes. In addition to the known effect of vitamin D on controlling calcium and bone metabolism, it inhibits proliferation and induces terminal differentiation of human keratinocytes, and can also modulate the immune system in a variety of ways. The objective of the present study was to determine the relationship between the level of vitamin D in the serum and the clinical severity in patients with psoriasis. A total of 23 psoriasis patients seen in the Department of Dermatology, Ajou University School of Medicine, South Korea between April 2007 and August 2007 were enrolled in this study. The clinical severity of psoriasis was graded according to the psoriasis area and severity index (PASI) scoring system. Serum concentrations of 25(OH)D3 were assayed with a radioimmunoassay kit (Dia-Sorin, Stillwater, MN). The level of vitamin D in the serum of patients was lower than that in the normal control group but statistically not significant. The serum vitamin D level showed a negative correlation with the clinical severity but it showed no correlation with disease duration of psoriasis. There was no difference in serum vitamin D level in the patient with or without facial involvement. The present results suggest that the decreased level of vitamin D in the serum contributes to the clinical severity of psoriasis. But serum vitamin D level may be not related with disease duration and facial involvement.

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Quantitative comparative assessment of topical aluminum salt solutions for treatment of hyperhidrosis: a simulation using normal subjects

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Our purpose was to: (1) objectively and quantitatively compare the efficacy of two aluminum salt solutions for the reduction of hyperhidrosis; and (2) to compare the obtained objective results to patient self-report of relative efficacy. We used a double-blind randomized controlled trial of 19 healthy subjects (13 females), ages 21-30. Topical agent A (20% aluminum chloride hexahydrate -Drysol®) was randomized to either palm and corresponding axilla, and topical agent B (1% aluminum acetate- Drioff®) assigned to the contralateral side. A sauna suit induced sweating during three 30-minute intervals: (a) with no study agents (pre); (b) with both study agents, one to each side; and (c) after the agents were washed off (post). Sweat levels without the suit were measured immediately before the pre- and post suit phases. Pre- and post-treatment sweat levels were measured by securing Watman® filter paper and noting changes in paper weight. Subjects recorded their perception of sweat levels on a 10 point scale. Analysis was by two-tailed paired t-tests. The results were that sweat levels after application of topical agent A decreased nominally in the axilla ($p=.066$) and significantly in the palm ($p=.0004$); topical agent B was not associated with sweat level decrease. After adjusting for change in baseline sweat levels induced by the sauna suit, topical agent A had a significantly greater effect at reducing axillary sweating than B ($p=0.045$), but changes in palmar sweat for A versus B were not significantly different ($p=0.39$). Subjects reported both A and B significantly reduced both axillary and palmar sweat ($p<0.05$), but there was no efficacy difference noted between the two topicals at either axilla ($p=0.43$) or palm ($p=0.54$). In conclusion, in a simulation of hyperhidrosis, 20% aluminum chloride hexahydrate quantitatively and objectively appeared to reduce sweat more effectively than 1% aluminum acetate. Patients noted efficacy for both topicals.

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Creams containing nanoemulsion can control release of tretinoin

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In treatment of Acne,topical retinoids such as tretinoin have been prescribed for several years. Unfortunately, tretinoin is not used easily and comfortably because it has irritation and stinging on user's skin.Novel drug delivery systems have attempt to reduce these adverse reactions.In this study we used nanoemulsion for formulation and evaluation of percutaneous absrption. Nanoemulsions consist of fine O/W or W/O dispersions, having the size range between 50-100nm. Improved absorption, controlled release and adequate stability have been achieved by making different nanoemulsions. In this study high energy emulsification method was used to prepare O/W nanoemulsion. For oil phase, Miglyol 812 and for reducing surface tension Tegocare 450 as well as nonionic surfactants were selected. Particle size measurement, thermodynamic tests and centrifugation tests for stability study were done. Tretinoin was added in 0.05% concentration to emulsion. For in vitro release study Franz diffusion cell with PBS for receiver phase was used. The membrane was Rat skin and the cell was placed in 37°C.The release of tretinoin from two bases: simple emulsion and nanoemulsion were studied. The results showed the release of drug from nanoemulsion base is slower and sustained. It seems slower release may reduce irritation and stinging of tretinoin.

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Mechanisms and preclinical efficacy and safety of herbal medicine, solanum incanum, in the treatment of squamous cell carcinoma

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SR-T100® is a new patented product extracted from the herbal medicine, Solanum incanum, and is currently executing phase II clinical trial in Taiwan for actinic keratosis and Bowen's disease. Its mainly active compound is solamargine alkaloid. The purpose of the study was to investigate the pathways leading to cell death of SCC cell lines, and also the efficacy of topical SR-T100® gel in the treatment of UVB-induced hairless mouse SCC. The cancer cell death was evaluate by morphological observation, cell cycle analysis, change of gene expression by RT-PCR and immunoblot, fluorescent immunocytochemistry, and DNA electrophoresis for DNA fragmentation. For mouse SCC, we performed TUNEL and active caspase-3 staining before and after topical SR-T100® gel therapy. The results demonstrated chromatin condensation, DNA fragmentation, and sub-G1 peak in a DNA histogram of human epithelium carcinoma (A431), human SCC4, SCC9 and SCC25, and human kerationcytes (HaCaT) cells, and the action is dose- and time-dependent. SR-T100 increases the binding activities of FasL and TNF-alpha to the caner cells, and up-regulates Fas and TNFRs expressions and their subsequent FADD/TRADD signal cascades. In addition, SR-T100 triggers mitochondria-mediated pathways, release of cytochrome c, down-expression of Bcl-xL, up-regulation of Bax, and increase of caspase-3 activity. Furthermore, 35 of 40 UVB-induced hairless mouse SCCs disappeared within 10 weeks after 10 weeks of once-daily application of topical SR-T100® gel without obvious side effects. Our observations suggest that SR-T100 induce apoptosis of SCC via death receptor and mitochondria pathways. The SR-T100® gel may become a possible candidate as a potential alternative therapeutic for SCC, and its precursors, actinic keratosis and Bowen's disease in the future.

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Determination of skin and blood levels of benzalkonium chloride (BAC) topically applied in rat skin

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INTRODUCTION: Benzalkonium chloride (BAC) is widely used for the disinfection of skin at 0.01% in hospitals. In Japan, Osvan® commercially available as over-the-counter drug contains about 10% BAC and causes skin damage when this is used as undiluted solution. There have been arguments over the period whether the application of disinfectant on wounds has merit or not. Though BAC is frequently used on skin, no report has been found in the literature concerning dermal absorption of BAC. The purpose of this study is to investigate the degree of penetration or absorption of BAC topically applied on wound of rats. METHOD: Eighteen male Sprague-Dawley rats were divided into three groups. A piece of cotton soaked with Osvan® was applied on abdominal clipped skin for 2 (12 rats) or 6 hours (6 rats). Half of 2-hour group was treated by scissors for making skin wound reaching subcutaneous tissue in the depth. The BAC levels in the blood and the exposed skin were analyzed with HPLC method after extraction. RESULTS AND DISCUSSION: The BAC levels in the wounded skin were significantly higher than those in the normal skin, whereas the blood levels were similar in both groups. The BAC levels in the blood increased when exposed up to 6 hours, whereas the skin levels remained at constant both groups. The absorption of BAC into the blood circulation was dependent on the duration of topical application, whereas the retention of BAC in the skin was dependent on wound on skin. There result suggests that the systematic toxic effect is negligible since the retention of BAC in blood was extremely low, but the retention of BAC in the skin wound is significantly high causing unfavorable effect.

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Efficacy of tacrolimus ointment and its effect on improvement of quality of life in patient with atopic dermatitis from Asia

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Patients with atopic dermatitis (AD) have an impaired QOL such as sleep disorder affected by itch. The aims of this study are to evaluate the efficacy and safety of tacrolimus ointment and to investigate the correlation between improvement of itch and QOL in patients with AD. This pooled analysis included a total of 860 patients treated with tacrolimus ointment from the individual studies in Asia. Efficacy assessments included Physician's Global Evaluation of Clinical Response, Patient's Assessment of Itch (Itch), Patient's Assessment of Overall Response, Dermatology Life Quality Index (DLQI) and children's DLQI (CDLQI). The improvements in all efficacy parameters were demonstrated by the treatment with tacrolimus ointment. The success rate based on physician's global evaluation of clinical response was 83.6% at the end of treatment. Tacrolimus ointment improved Itch rapidly in the first week, and maintained improving throughout the treatment period. Tacrolimus therapy also improved the DLQI and CDLQI at the end of treatment. 'Symptoms and feelings'; one of the DLQI assessments, was improved markedly. Treatment with tacrolimus ointment showed the marked improvement in 'Sleep' that was one of the CDLQI assessments. The improvements in Itch during treatment with tacrolimus ointment correlated with the improvement in 'Sleep'. These correlations increased with treatment time. These results suggest that tacrolimus ointment is effective and well tolerated in the treatment of patients with AD in Asia. The improvement in itch by tacrolimus therapy improved the sleep disorder associated with AD.

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The relation between high sensitivity-CRP and body mass index in patients with psoriasis

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Background Psoriasis is a disease presenting cutaneous, immunological and vascular abnormalities. CRP has been used for the evaluation of inflammation in inflammatory diseases. Recently, high sensitivity-CRP has been shown to reflect mild and/or moderate inflammation for the prognosis of ischemic heart disease. Objective To measure high sensitivity-CRP for the evaluation of mild and/or moderate inflammation in psoriasis. Subjects and methods 52 patients with psoriasis (male: female = 32: 20, age 11-80 years, mean 54.4 years) and 147 normal controls (male: female = 91: 56, age 53.9+13.6 years-old) were studied. High sensitivity-CRP was measured with a nephelometric assay. The detection limit was 0.0036 mg/dl, and showed linearity among 0.015-1.5 mg/dl. The relation between high sensitivity-CRP and clinical and laboratory findings was also studied. Results The distribution of high sensitivity-CRP in psoriasis showed 17 low cases (32.7%), 10 mild cases (19.2%), 9 moderate cases (17.3%), 9 high cases (17.3%) and 7 highest cases (13.5%). The distribution was categorized in low-moderate group (36/52, 69.2%) and high-highest group (16/52, 30.8%). The occurrence rates of maximum body mass index (11/16, 68.8%) and elevated serum triglyceride (8/16, 50.0%) in high-highest high sensitivity-CRP patients were significantly elevated compared with those of low-moderate patients (10/36, 27.8%; 11/36, 30.6%) ($P<0.01$, $P<0.02$), respectively. Conclusion These results showed close relationship between mild/moderate inflammation and obesity-related metabolic abnormality in patients with psoriasis.

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Radiation therapy is an effective treatment for refractory Woringer-Kolopp disease

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Woringer-Kolopp disease, also known as pagetoid reticulosis, is an extremely rare variant of mycosis fungoides. Accurate diagnosis and effective treatment is essential to prevent progression to debilitating disease. In order to identify effective treatment strategies for Woringer-Kolopp disease, we reviewed the Stanford Cutaneous Lymphoma Program's clinical and pathologic databases of greater than 1500 cutaneous lymphoma patients and identified seven patients with Woringer-Kolopp disease managed at Stanford. In each case, the diagnosis of Woringer-Kolopp disease was confirmed based on review of the clinical presentation, histopathology, and immunohistochemical and molecular studies where appropriate. Of the seven patients five were female. Median age at presentation was 49 years with range of 24 - 70 years. Median time of follow-up was eleven years with a range of 2 to 23 years. Skin lesions varied from localized papules and plaques to ulcerating tumor nodules. Histopathologic features of diagnostic skin biopsies demonstrated an epidermotropic infiltrate of atypical lymphocytes. Of six patients in which immunostaining was performed, four had predominantly CD8 positive, and two CD4 positive neoplastic cells. Initial treatments included topical nitrogen mustard (mechlorethamine hydrochloride), topical steroid, or surgical excision. In five of the seven patients, the disease was refractory to these treatment modalities. In one of these patients, PUVA as salvage therapy resulted in only partial response. In the four remaining patients, radiation therapy with doses ranging from 6 to 41 Gy resulted in rapid and complete responses (CR). The median duration of CR was twelve years with a range of 2 to 23 years. The radiation therapy was well tolerated with limited skin toxicities. These data support that the most effective and appropriate treatment for recalcitrant or severe Woringer-Kolopp disease is localized radiation therapy.

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Cw6 allele confers sensitivity to efalizumab treatment in psoriasis

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Treatment with biologic drugs has proofed to be effective for moderate to severe psoriasis. Both the anti-CD11a monoclonal antibody efalizumab and the soluble TNFRII-IgG fusion protein etanercept have been shown to induce the clearing of psoriasis plaques in a high proportion of patients. However, clinical response to these drugs is often slow and cannot be predicted. We have performed HLA-Cw6 haplotyping in 82 patients affected by moderate to severe plaque psoriasis treated with either efalizumab (34 patients) or etanercept (48 patients) and found that the response to efalizumab but not to etanercept is strictly linked to the presence of the HLA-Cw6 allele. Cw6 positive patients have an high response rate to efalizumab while response to this drug is very low in Cw6 negative patients. In the group of Efalizumab patients, at week 12 PASI50 was reached by 81.8% (18/22) of Cw6+ patients and by 58.3 % (7/12) of Cw6- patients. The PASI 75 was achieved in 34.7% (8/22) of Cw6+ patients, and only in 2/12 Cw6- patients. In the group of patients treated with etanercept at week 12, PASI50 were reached by 83.3% (25/30) of Cw6+ patients and by 94.4 % (17/18) of Cw6- patients. The PASI75 were reached by 16/30 (53.5%) of Cw6+ patients and by 15/18 (63.3%) of Cw6- patients. After 24 weeks, 72.3% (16/22) of Cw6+ patients treated with efalizumab show PASI50, while 41.6% (5/12) of Cw6- patients have reached PASI 50.The PASI75 was achieved by 63.6% (14/22) of Cw6+ patients show and 25% of Cw6- patients. In the group of Cw6+ patients treated with etanercept, PASI50 and PASI75 were reached in 80% (23/30) and 76.7%, respectively, while in the subset of Cw6- patients the corresponding endpoints. The differences in response rate between Cw6+ and Cw6- patients resulted to be statistically significant in the efalizuamb group ($p<0.01$) but not in etanercept group. Although preliminary and based on a small number of patients, our data indicate Cw6 as a potential predictive marker for response to Efalizumab treatment.

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Anti-wrinkle activity of retinol is enhanced by a chromone derivative

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Retinoids are natural compounds that have been shown to improve the signs of photo-damage on skin by topical application. However the topical use of retinoids has been limited by their potential to induce intolerance reaction on the skin such as burning, redness, itching, stinging and redness. One strategy to improve the tolerance of retinoid products is to lower retinoid concentration in our topical products by enhancing the performance of retinoids in the skin. We have evaluated compounds for their ability to increase retinoid activity in the skin, using an <i/>ex-vivo *human skin explant model in which retinol activity is assessed by measuring the expression level of genes specific for retinoid activity such as heparin-binding-epidermal growth factor (HB-EGF), cellular retinoic acid binding protein II (CRABPII). We found that a chromone derivative (5,7-Dihydroxy-2-Methyl Chromone) was able to enhance the expression of both genes in our <i/>ex-vivo* model. Moreover, clinical study showed that topical application of a formulated product containing retinol and this chromone derivative is inducing the same anti wrinkle activity than a formulation containing twice more retinol and that this product displays also a better tolerance. Therefore, 5,7-dihydroxy-2-methyl chromone can be seen as a new and promising enhancer of retinoid activity that can be successfully used in anti-aging products.

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Clinical study to evaluate the efficacy and safety of fractional carbon dioxide laser (active FX) for treatment of acne scar in Asian skin

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Background and Objectives: CO2 laser has been the standard laser treatment for acne scarring. However, the treatment is relatively invasive and the down time is long. There is also a risk of post-inflammatory hyperpigmentation in darker skin types. Various treatment modalities have been investigated in order to decrease the down time to make the treatment more acceptable. The objective of the study is to evaluate the efficacy and risk of post-inflammatory hyperpigmentation (PIH) of fractional CO2 laser for treatment of acne scar in Asian skin. Design/Material and Methods: 10 Chinese subjects with skin type III-IV with acne scarring were recruited in this prospective study. Each subject has undergone 1 treatment. Each visit involves single pass treatment over the area with acne scarring. Energy level used was 100mJ, 50Hz. Setting was pattern 3, size 5, density 2. Subjects are required to start a one week course of antibiotics and antiviral 2 days before treatment. The subject is followed up up to 3 months post-treatment. Any adverse effects were recorded. Subjects were evaluated by means of standardized photos using the Canfield Visia CR System ® and patient questionnaires. Results: 10 subjects have completed treatment and some are still being followed up. Our data suggests that there is significant improvement in acne scarring (p-value 0.039). Few mild PIH were noted. 70% reported moderate to significant degree of satisfaction. Conclusion: Our evaluation indicated that active Fx improves acne scarring and the main adverse effect was mild PIH.

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Evaluation of T-cell clonality in archival skin biopsy samples of cutaneous T-cell lymphomas using the Biomed-2 PCR of T-cell receptor gene rearrangements

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An expanded clonal TCR gene rearrangement indicates a neoplastic T-cell proliferation, thus its detection by molecular investigations is widely used in the diagnosis of the various T-cell lymphomas (TCL). Recently, several European centres of lymphoma diagnosis and research jointly developed protocols for PCR-based clonality analysis in suspect T- and B-cell proliferations [BIOMED-2 (B2) Concerted Action]. Here we applied the B2 TCRγ- and β-PCR as well as our previous TCRγ-assay to a collection of archival samples from 66 cutaneous TCL (CTCL), 4 systemic TCL and 19 controls. The quality of each DNA prepared was evaluated by the B2 control PCR. 89 samples, all providing a 300 bp (or larger) amplicon were used. For the analysis of the TCR-PCR products, fluorescence fragment analysis was applied. The rate of successful TCRγ- and β VJ-PCR was comparable. Both TCRγ methods showed a similar frequency of clonality in CTCL: appr. 76% by the B2- and 80% by our PCR, respectively. The TCRβ VJ-PCR also revealed a comparable rate of clonality (76.3%). In a subset of 12 CTCL samples neither of both TCRγ assays detected clonal rearrangements. Of note, 8/16 CTCL samples which were polyclonal by the B2 γPCR still exhibited clonal rearrangements in the TCRβ VJ-PCR. The TCRβ DJ-PCR revealed clonality in only one additional CTCL specimen. If combining all B2 assays one or more clonal rearrangements were revealed in 89.4% of CTCL samples (MF 40/45, Lyp 11/13, ALCL 6/6) and in all systemic TCL, whereas only one control (pseudolymphoma) exhibited a weak clonal peak in the B2 γPCR but not in any other assay. In conclusion, the γ-methods compared here had nearly the same diagnostic sensitivity. However, in order to obtain a standardization of clonality testing the B2 method is now used in our routine analysis. Since the B2 β VJ-PCR still detects clonality in CTCL being polyclonal in TCRγ-assays, it is additionally applied to supplement the γPCR in corresponding cases.

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Incubation time of topical 5-aminolevulinic acid in the photodynamic therapy for acne vulgaris patients

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Photodynamic therapy (PDT) with topical 5-aminolevulinic acid (ALA) has been used for effective treatment of facial acne vulgaris. However, the proper incubation time of topical 5-ALA on the acne treatment has not been determined yet. The object of this study was to evaluate outcomes and side effects of PDT on acne vulgaris lesions and changes of sebum secretion between PDT with short incubation (30 minutes) and long incubation (3 hours) times of 5-ALA. Thirteen Korean subjects with varying degrees of acne were enrolled after a 4-week washout period. Twenty percent ALA hydrochloride was applied to the split face of the patients and was divided into two groups: short incubation group (N=7) and long incubation group (N=6). After each incubation periods, the whole face was exposed to intense pulsed light using a 590 nm cut-off filter at a fluence of 12-15 J/cm2. The pulse duration was 30ms with 20ms delay. The subjects were treated twice at a 4-weeks interval and the patients were clinically evaluated on 4 weeks after second treatment. All patients had no apparent side effects during study period. The mean reduction of the inflammatory lesion count was 8.83 ± 6.03 in the long incubation group, 6.71 ± 3.77 in the short incubation group and 6.07 ± 3.95 in the split faces treated with IPL alone. There were no significant changes in the non-inflammatory lesion count. The mean reduction of sebum secretion was 56.27 ± 44.84 in the long incubation group, 37.64 ± 33.42 in the short incubation group and 25.17 ± 37.35 in IPL alone. PDT on the acne vulgaris lesions showed improvements in the aspects of objective clinical assessment and subjective satisfaction, irrespective of incubation time. In conclusion, the PDT with long incubation time was more effective in reduction of inflammatory lesions and the sebum without any complications, comparing to the PDT with short incubation and IPL only. Further study for long term follow-up of PDT with variable incubation times should be performed.

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Clinical study to evaluate the efficacy and safety of plasmakinetic skin rejuvenation system for treatment of acne scar in Asian skin

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Background and Objectives: Plasmakinetic resurfacing (PSR) emits millisecond pulses of nitrogen plasma energy which induces skin regeneration. The objective of the study is to evaluate the efficacy and risk of post-inflammatory hyperpigmentation (PIH) of PSR for treatment of acne scar in Asian skin. Design/Material and Methods: 8 Chinese subjects with skin type III-IV with acne scarring were recruited in this prospective study. Each subject has undergone 1 treatment so far. Each visit involves single pass treatment with the standard nozzle over the area with acne scarring. Different energy settings were used, ranging from 1.0 to 4.0J/cm2. Subjects are required to start a one week course of antibiotics and antiviral 2 days before treatment. The subject is followed up up to 3 months post-treatment. Any adverse effects were recorded. Subjects were evaluated by means of standardized photos using the Canfield Visia CR System ® and patient questionnaires. Results: There was a statistically significant improvement in acne scars (p-value 0.025).37.5% subjects developed mild PIH and 12.5% subjects developed mild hypopigmentation. All subjects reported moderate to significant degrees of satisfaction. Minimal pain was experienced during treatment. Conclusion: Our evaluation indicates that PSR improves acne scarring with pigmentary disturbance being the main adverse effect.

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Apoptotic markers in ALA-PDT treated superficial basal cell carcinomas using a single and a two fold illumination scheme

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ALA-PDT is an established therapy modality for sBCC. The mechanism of cell death in PDT is partly due to apoptotic processes. We have shown that fractionated ALA-PDT leads to higher complete response rates in superficial BCC's, AK and Bowens disease. In search to enlighten this increased response as well as to elucidate the PDT mechanism we investigated some apoptotic markers. 43 histological proven sBCC's were included: 7 control lesions, 10 lesions treated with a single dose of 75 Jcm-2 and 25 lesions treated with a fractionated regime (20 + 80 Jcm-2 separated by a two hour dark interval) One lesion in one patient was treated with the fractionated regime and serial biopsied, 6, 12 and 24 hours after the completed treatment In the control lesions a biopsy was taken after 6 hours of ALA application. In the single illuminated lesions a biopsy was taken after 2 hours after 75 Jcm-2 .In the fractionated treated lesions a biopsy was taken after 2 dark hours after an illumination of 20 Jcm-2. In all treated lesions ALA was applied for 4 hours, a fluence rate of 50mWcm-2 and a diode laser system as light source was used. Immunohistochemical staining for Bcl-2, Bax, caspase-3 were done in frozen section. In almost all biopsie positive stained tumour cells for Bcl2 were found. In the staining for Bax in control lesions some positive tumour cells were found. In the majority of biopsies after 20 J cm-2 positive cells for Bax were shown. After 75 J cm -2 also positive cells for bax staining were found. In all illuminated lesions an upregulated expression of caspase 3 was shown. In conclusion, early, after two hours, after a 75 J cm -2 and after a small dose of 20 J cm -2 apoptotic markers show altered expression indicating apoptosis is occurring. Apoptosis induced by the mitochondrial pathway plays a role in cell death in ALA-PDT in sBCC.

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Clinical study to evaluate the efficacy and safety of short cut off filter IPL (starlux white) for treatment of lentigines in Asian skin

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Background and Objectives: Intense Pulse Light (IPL) has been studied and was used to treat vascular and pigmented lesions comprising photorejuvenation and is used in photodynamictherapy. The objective of the study is to investigate the effectiveness of short cut off filter IPL (Starlux-white) handpiece for the treatment of pigmented lesions in Asians. Design/Material and Methods: 18 Chinese female subjects with skin type III-IV with freckles were recruited in this prospective study. Each subject has undergone 1 to 7 treatments with approximately 1 month interval between each treatment so far. Each visit involves single pass of Starlux-white handpiece treatment. The wavelength of this handpiece is 400-1400nm. Spot size 12x28mm with maximum fluence 100J/cm2. Subjects were evaluated at baseline and 1 month after last treatment by means of standardized photos using the Canfield Visia CR System ® and patient questionnaires. 2 independent observers assessed the photos with the visual analog scale. Results:There was a statistically significant improvement in pigmented lesions (p-value 0.034). In terms of treatment sessions, 21.6% PIH was noted. One case of hypopigmentation was reported. 47.1% subjects was moderately to significantly satisfied. Conclusion: Our data indicated that the Starlux white handpiece is effective for treating lentigines in Asians. There is a risk of PIH and hypopigmentation. Further study with the smart device is required to generate desirable parameters in order to minimize the risks.

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Skin ageing in chinese women correlated to photoexposure

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Few data exist correlating the morphological changes of ageing skin between photoexposed and non-exposed skin of the same patient, even less knowledge in the Asian population. In this study, we designed a clinical study to compare sun-exposed skin and unexposed skin in Chinese women from different age groups. The volunteers were recruited in the southern part of China with signed confidential disclosure agreements. A total of 30 healthy Chinese women, aged between 22 and 63 years were divided into five groups (5 volunteers for each age group: 20-29 years, 30-39 years, 40-49 years, 50-59 years and 60-69 years). Skin punch biopsies (3 mm) were taken from the anterior surface of the upper arm (protected area) and the posterior surface of the forearm (exposed area). The samples were fixed in formaldehyde and embedded in paraffin for further analysis. The epidermal and stratum corneum thickness is increased with photoexposure (-38% and -36%). The stratum corneum thickness increases with age and peak the highest between 40-60 and decrease afterwards. As expected, the sun-exposed skin expressed significantly more melanin than the non-exposed skin areas (+67%). In addition, further investigation was conducted on caveolin-1, principle structural component on caveolae. In epidermis, the caveolin-1 expression is increased after photoexposure (+44%), but decreased with age only in the photoexposed skin (-29%). In dermis, no significant changes in photoprotected zone were observed. However, in photoexposed zone, the caveolin-1 expression decreased with age (-51%). Therefore Caveolin-1 expression could be an indicator for UV-induced senescence in skin. The expression of different opioid receptors in skin was measured and showed interesting correlation to the above-mentioned results. The opioid receptor expression is changed with age, but not with photoexposure.

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Induction of antimelanoma immunity by adoptive transfer of tumor-reactive MelanA-specific CTL clones in melanoma patients

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During the last decade, new insights in cellular and molecular biology have enabled the molecular identification of numerous melanoma-associated antigens recognized by T cells. These results have lead to the development of adoptive immunotherapy protocols. The aim of this prospective phase II study was to evaluate efficacy, tolerance and antitumor response of 16 patients with metastatic melanoma treated by infusion of Melan-A-specific clones. The main objectives were to evaluate 1/ clinical response according to RECIST criteria 2/ clinical tolerance according to CTC criteria 3/ immunological response before infusion, at day 7 and finally 1 and 2 months after infusion 4/ migration of transferred T cells into tumor sites. Melan-A-specific clones were generated from PBMC in Unit of Cellular and Gene Therapy, CHU Nantes, France and injected 3-4 months later. Following T cell clone injection (day 0), patients received 9 MU IL-2 from days 1 to 5, and 9 MU IFN-γ for 1 month three times/week. 14 out of 16 patients received their clones. 6 patients experienced an objective response (46%): 1 partial response (RP) for 18 months followed by a complete response lasting 10 months, 1 stabilization since 02/2002, 4 RP lasting 4, 5, 6 and 8 months. Immunological analysis in the first 10 patients revealed that transferred clones persisted in the blood for only 3 patients at day 7, 1 and 2 months. Among these 3 patients, 2 experienced a P and 1 a RP. For 2/5 patients, clones were detected in cutaneous metastasis. Frequencies of Melan-A/A2-specific lymphocytes, measured by tetramer labeling, increased after treatment in most patients. In one patient, who showed a complete response, this increase corresponded to the expansion of new clonotypes of higher avidity than those detected before treatment. Together, our results suggest that infused CTL clones may have initiated an antitumor response that may have resulted in the expansion of a Melan-A-specific CTL repertoire.

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UVB-LED phototherapy for psoriasis

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One of the major breakthroughs in phototherapy in the last 10 years is that of targeted UVB phototherapy. These innovative medical devices can selectively deliver high-intensity coherent (laser) or incoherent UVB light through a flexible fiberoptic light guide onto the diseased skin. As light emitting diodes (LED) might serve as a more convenient alternative for delivering light, we investigated the efficacy of a new, UVB-LED phototherapeutic device (Resolve™, Allux Medical, USA) in psoriasis. A prospective, right-left comparative, open study was performed to assess the efficacy of UVB-LED phototherapy in psoriasis. Ten patients with stable plaque type psoriasis were enrolled into the study. Symmetrical lesions located on extremities or trunk were chosen, and one lesion was treated with the Resolve™ device, whereas the other lesion served as untreated control. This UVB-LED device is a narrow-band UVB device with the peak intensity at 311 nm + 3 nm. The minimum erythema dose (MED) was measured on each patient, and treatment was started with 1xMED, then the dose was increased by 50% compared to the last treatment. A physical mask prevented exposure of the non-lesional skin surrounding the psoriatic plaques. Four treatments per week were given. All patients responded rapidly to the UVB-LED therapy; complete clinical and histological clearing of the lesions were achieved as soon as after 8-10 treatments. No severe side-effects occurred. These results suggest that this new innovative UVB-LED device is effective in the treatment of localized psoriasis and may be useful in other different UV responsive skin diseases. UVB-LED devices may represent an alternative for home-based phototherapy.

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Molecular markers of photo-aging in Indian skin

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Ageing is a complex process influenced by many environmental and genetic factors. This study investigates the influence of age and sun exposure on skin molecular markers of 30 Indian healthy women between 18 and 67 years. Skin biopsies were taken from the anterior surface of the upper arm (protected area) and the posterior surface of the forearm (exposed area). Samples were fixed in formalin and embedded in paraffin. 5 µm sections were cut and studied for melanin (Fontana Masson Staining), elastic fibres evaluation (Orcein staining) and expression of fibronectin (immunostaining). Quantifications were obtained by image analysis. The results on pigmentation showed a significant increase of melanin in the photoexposed zone, in the basal layer (+ 26%) and in the spinous layers (+67%). No significant modification according to the age was observed. Elastic fibers are bundles of proteins found in connective tissue. A significant decline of the average length of oxytalans fibers in the photoexposed zone was observed (-45%) associated with a significant variation of the length of these fibers with age. For the global elastic fibers analysis, only the aged patients (60-70 years), present a significant increase of the dermal elastic fibers quantity, due to the increase of the solar elastosis. The results on fibronectin expression, which is constitutive of the extracellular matrix of the dermis, showed in photoexposed skin a significant increase in the papillary dermis (+144%) and in the total dermis (+92%). No significant modification according to the age is observed. In conclusion, we showed that Indian skin presents the same markers of the cutaneous photoageing that the Caucasian or Chinese skins, i.e shortening of oxytalans fibers, increase of the solar elastosis, the melanin and fibronectin contents. On the other hand, we did not observed the classical variations of skin structure according to the age (thinning down of the skin, for example). An influence of the quality in food with high vegetable and low meat intake could partially explain these observations.

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First results of effectiveness of 12 week training program ('OnderHUIDs') for patients with selected dermatoses

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'OnderHUIDs' is a complementary education program to help skin disease patients better cope with their disease, better follow treatment schedules, resulting in better quality of life, better clinical outcomes and cost savings. Patients with chronic skin diseases were presented to enter the program. In the 12 weeks program – next to a classical medical treatment – an educational session by a dermatologist and 2 feedback consultations– psychoeducation and philosophy sessions– mindfulness meditation– physical training and yoga– dietary, sleep and anti-addiction advice and specific skin care workshops were given twice weekly. In 3 programs that have been completed now a total of 35 patients participated. 5 (= 14 %) dropped out (lack of time, psychiatric problems). Included diagnoses were: psoriasis (46%); atopic dermatitis (AD)(34%); prurigo (6%); chronic urticaria (3%); morphea (3%); vitiligo (3%); pemphigus foliaceus (3%); hidradenitis suppurativa (3%). Of 27 patients a Dermatology Life Quality Index (DLQI) score was obtained before and after the program. Of 18 patients a Skindex-29 score was obtained. Extra, a Psoriasis Disability Index before and after was obtained from 6 psoriasis patients. QoLIAD score before and after was obtained from 9 AD patients. Overall, DLQI improved by 4.6 points (p=0.004), and Skindex-29 by 23.0 points (p=0.000). In psoriasis patients DLQI improved by 3.3 points (p= 0.119), Skindex-29 by 26.6 points (p=0.034), and PDI by 9.5 points (p= 0.059). In AD patients DLQI improved by 5.3 points (p=0.102), Skindex-29 by 20.3 points (p=0.008), and QoLIAD by 5.4 points (p=0.038)(Wilcoxon rank test). This initiative for patients with chronic dermatoses was experienced as a valuable addition to classical treatment. Quality of life with regard to the skin problem was improved. These positive initial results are stimulating to set up a single center, randomized parallel group study investigating the impact on quality of life, the clinical efficacy and the cost-effectiveness of the 'OnderHUIDs' program.

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Existence of numerous CD1a- and RANKL- positive dendritic cells in the papillary dermis of psoriatic plaques

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Peri-lesional epidermis of psoriatic plaques contain numerous CD1a-poitive Langerhans cells (LCs), some of which expressed HLA-DR, CD83, CD80 and CD86, at the same time expressing Langerin. There are numerous CD83-positive, CD11c-positive, Langerin-negative dendritic cells (DCs) in the epidermal-dermal junction of peri-lesional skin, which were considered to be dermal dendritic cells (DDCs). Receptor activator of NF kappa B ligand (RANKL) is a member of TNF superfamily, and its receptor is RANK, which has recently been demonstrated to induce regulatory function in dendritic cells. We suspected that perilesional DCs are regulatory DCs that prevent enlargement of psoriatic lesions. In order to characterize the nature of dendritic cells (DCs), we investigated immunohistochemically, with a panel of antibodies for DCs and antibodies for RANK, RANKL, CD40 and CD40L, the normal appearing skin adjacent to the plaques (peri-lesional skin), lesional skin, and distant uninvolved skin from psoriasis patients. RANKL expression was weak in the epidermis of peri-lesional skin, while focal strong expression was observed in the lesional epidermis. Numerous and highly dendritic, CD1a-positive Langerhans cells in the peri-lesional epidermis were negative for RANKL, while many of the CD1a-positive dendritic cells in the papillary dermis of lesional skin were strongly positive for RANKL. These cells were weakly positive for RANK. CD40 and CD40L expression were subtle in the epidermis or dermis of lesional and peri-lesional skin. Those CD1a- and RANKL- positive DCs were HLA-DR-positive but Langerin-negative, of which distribution was completely different from that of TNF and iNOS producing DCs. These RANKL-positive DCs may be derived from CD1a-positive epidermal Langerhans cells which bound RANKL, or they may constitute a distinct population of dermal dendritic cells. These results suggest that psoriatic lesion is not only the source of inflammatory molecules, but also generate regulatory compartment to suppress inflammatory reactions.

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Study on the effects of hormones related with menstrual cycle on sebocyte, blood and acne lesion using SEB-1 cell line and volunteers

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Acne is a multifactorial disease, developing in the pilosebaceous unit. Interplay of follicular hyperkeratinization, colonization with Propionibacterium acnes, increased sebum production and inflammation is mainstay of pathogenesis of acne. However, menstrual flaring of acne is not explainable with these main pathogeneses of acne. Many patients are suffering from premenstrual worsening of acne, which occurs in approximately 70% of female acne patients. Sebaceous gland is the metabolic center of hormones in skin. Hence, pilosebaceous unit is very susceptible to hormonal changes. Sebaceous gland can be influenced by female hormonal changes. Furthermore, menstrual flare-up may be associated with hormonal sensitivity of skin. For investigating the mechanism of menstrual aggravation of acne, we compared the estrogen and progesteron level between female patients with menstrual acne flaring and those without menstrual acne flaring. In addition, skin biopsies were done before menstruation and before ovulation. With skin tissue, expression of estrogen, progesterone and androgen receptors in acne lesion were evaluated and compared between two time points. Female hormonal effect on sebocyte was investigated with SEB-1. We found significant difference in progesterone ratio of two time points between patients with premenstrual acne flaring and patients without premenstrual acne flaring. Expression of estrogen-α and androgen receptor shows significant difference between two groups. In addition, lipid production of SEB-1 cell was increased after simultaneous treatment of estrogen and progesterone. Western blot analysis of hormone receptors and apoptosis showed changes according to different hormone treatment schedule. This indicates integrated interaction of androgen and female hormones is essential in menstrual acne flaring.

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Influence of foods on the aggravation of acne and its relation with hormones of fuel metabolism

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Although pathogenesis of acne is not fully elucidated, acne is considered as a multifactorial disease. It has been suggested that hormones, genetic factors, and stress can bring on the aggravation of acne. There have been so many controversies about the relationship of acne and food. In the past, general consensus was that acne was not affected by foods. However, causative relationship of acne and foods has been suggested from some epidemiologic studies. Food changes, one of the most important thing of westernization, has been suspected as main influencing factor of acne. In addition, some recent studies showed positive correlation between the ingestion of low glycemic diet and the improvement of acne. In this study, we investigated the influence of foods on the aggravation of acne and its mechanism. In total, 1,261 subjects participated in the study, of which 502 were not acne patients, whereas 759 were acne patients. Data were collected with questionnaire survey. Acne was diagnosed by dermatologists. In addition, insulin-like growth factor-1(IGF-1), insulin-like growth factor binding protein-3(IGFBP-3) and lipid profiles were measured by blood tests. Skin tissues were obtained by skin biopsy. With tissue sample, immunohistochemical staining of insulin receptor and glucagon receptor were done. In acne patients, 402(53%) were aggravated by foods. One of the most common cause of the aggravation of acne was greasy food such as fried food, oily meat or pizza. There was a tendency that IGF-1 was significantly high and IGFBP-3 was low in patients with acne flaring by food. However, insulin level was not different between the patients with acne flaring by greasy food and the patients without it. In tissue investigation, strong expression of insulin receptor was observed in patients with acne flaring by food. These results indicate that the ingestion of greasy food can cause the increase of free IGF-1, which may bring about the aggravation of acne.

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Rituximab-induced elevation of BAFF exerts differential effects on humoral immune responses in pemphigus vulgaris

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Pemphigus vulgaris (PV) represents a severe blistering disease affecting the skin and mucous membranes. Although PV is considered a B cell mediated autoimmune disease, overexpression of BAFF (B cell activating factor) or APRIL (a proliferation inducing ligand), two factors which promote B cell generation and survival, has not been described, yet. In the current study, we investigated serum BAFF and APRIL concentrations in 5 PV patients who were treated with immunosuppressive drugs and 6 PV patients treated with systemic immunosuppression combined with immunoadsorption by ELISA. 11 PV patients who were resistant to conventional immunosuppressive medication were treated with the monoclonal anti-CD20 antibody, rituximab. Compared to healthy controls, serum BAFF and APRL levels were neither altered by immunosuppressive therapy nor by immunoadsorption over the entire observation period of 6 and 3 months, respectively. In contrast, rituximab-induced B cell depletion lead to a strong and significant elevation of BAFF, but not APRIL, in the serum of the PV patients. There was thus a significant inverse correlation of BAFF and peripheral B cell numbers in PV patients after rituximab therapy. Moreover, rituximab induced an increase of anti-Varicella-Zoster-Virus (VZV)-IgG titers in the sera of the PV patients, whereas anti-desmoglein3 specific autoantibodies were significantly decreased, accompanied by a good clinical response. Apparently, increased BAFF serum levels following rituximab-induced B cell depletion exhibit different effects on autoreactive versus pathogen-specific serum IgG concentrations, since serum BAFF and VZV-IgG show a significant correlation, while BAFF and anti-dsg3-IgG are not interdependent. Finally, our present findings suggest a novel therapeutic strategy by combining rituximab with BAFF antagonists to prolong B cell depletion and to improve the clinical response in severe cases of PV.

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Role of calcineurin in HaCaT keratinocytes migration

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Human keratinocytes migration plays a critical role in a variety of biological and pathological process. In this study, we investigated the role played by the Ca2+/CaM phosphatase calcineurin in the regulation of the keratinocyte cell line HaCaT motility. To our knowledge, after tissue injury, epithelial cell migration is rapidly initiated in order to re-establish tissue integrity. The signaling pathway involved in this process is not completely understood. Recently it has been demonstrated that induction of keratinocytes migration is conveyed through EGFR, promoted by endogenous HB-EGF and requires the activity of GSK-3. The latter is a kinase that counteracts the activation and consequent nuclear localization of nuclear factors for activated T-cells (NFATs) usually performed by calcineurin. We analysed HaCaT migration by the Boyden's Chamber assay after treatment with calcineurin inhibitors. Our data show that cell migration is significantly increased after incubation with FK506. The effect is time-dependent, as it is greater after 48 hours of treatment compared to 24 hours. Therefore, the calcineurin/NFAT pathway appears to inhibit a transcription program promoting HaCaT mobility and the use of inhibitors unmasks the promigratory pathway. To conclude, our results suggest a new mechanism of action of calcineurin inhibitors that could be responsible of wound healing and that might contribute to the normalization of cutaneous inflammatory conditions such as atopic dermatitis. This would confirm that also keratinocytes and not only lymphocytes, as previously thought, could be a therapeutic target of calcineurin inhibitors such as tacrolimus and pimecrolimus.

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Total skin electron irradiation and doxorubicin for classic Kaposi sarcoma

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Classic Kaposi sarcoma (CKS) is a rare neoplasma and the best therapeutic method has not been determined to date. A 66-year-old male in good health presented with asymptomatic skin lesions that had started to develop in June 2006. Physical examination revealed numerous painless, bluish-red papules and nodules that coalesced in deep-infiltrated lesions affecting all his body. The most largest lesions were localized on his hands, chest, legs, soles of feet and between fingers, reaching the size from 1 to 3 cm. The separate bluish macula was also presents on mucosa in hard palate. The diagnosis of Kaposi sarcoma was established by histopathological and immunohistochemical examinations. HIV infection and dissemination of the disease to other organs were excluded. In February 2007 the treatment started with the total skin electron irradiation (TSEI), rotary – dual technique and 6 MeV electron beam, on the linear accelerator. The patient was administered 1,5 Gy per day for the whole skin, four times weekly to the total dose of 34,5 Gy. Apart of that boosts with 2 Gy per day were added on thighs, feet and hands up to 10 Gy, 20 Gy, 8 Gy respectively. During the therapy lesions gradually regressed. The radiotherapy was completed in March 2007. At the end lesions on upper extremities and trunk disappeared, nodules on the legs were flatten. The dry desquamation affecting lower extremities was observed as a side effect. The radiation changes were most intensified on hands, axillary and inguinal regions. The edema of legs persisted as well as the single macula on the mucosa. As the next step the chemotherapy with doxorubicin was given intravenously in 3 courses every three weeks. After chemotherapy the mucosa lesion turned pale and the edema of legs got smaller. Six months after the therapy the patient is in a healthy good conditions with remission of CKS. The presented combined treatment regiment with TSEI and chemotherapy with doxorubicin proved to be effective and well tolerated method of CKS therapy.

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A new combination therapy delays formation of metastases in a mouse melanoma xenograft model

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We have recently used human M24met melanoma cells to establish a spontaneously metastasizing mouse melanoma model for demonstrating inhibitory effects of dimethylfumarate (DMF) on melanoma growth and lymph node metastasis. We have now used this model to test different therapeutic strategies on their potency to influence melanoma metastases. DMF and an antibody blocking VEGF-A were compared in its therapeutic efficacy to dacarbazine (DTIC). Combination therapies using these substances have also been used. Effects on the growth of primary melanoma and on the inhibition of the development of lymph node and pulmonary metastases were evaluated. All treatment conditions reduced volumes of primary tumors significantly. Regarding the growth of lymph node and lung metastases as analyzed by immunohistochemistry, combination of DMF with DTIC was the most effective treatment regimen, followed by the combination of DMF with VEGF-A blockade. The amount of pulmonary metastases did not significantly differ between individual treatment groups but combinations were slightly more efficient than single therapies. These results could also be correlated with reduced lymph vessel angiogenesis in primary and metastatic tumor sites. These results together indicate that DMF might be an appropriate new adjuvant in the treatment of advanced stage melanoma in humans.

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Daily skin care application promotes a regular physical activity and has a positive effect on physical and mental well being

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Physical exercise has been shown to maintain and even improve health status particularly amongst elderly and middle-aged men and women. The objective of the present study was to investigate physical and psychological effects of a daily application of a skin care product over the entire body. For this purpose, 40 Caucasian women, aged 40 to 45, and with healthy skin were recruited. No treatment with anti-inflammatory drugs nor face or body massage was allowed during the study. Thirty of these women were instructed to apply a skin care product over the entire body daily during 30 days with a standardized motion (test group) whereas 10 women maintained their daily skin care habits during the same period (control group). The lumbar and the upper body flexibility were measured using the "fingertip-to-floor method" and the "Back scratch test", respectively. Self-esteem and anxiety were investigated using the Self Esteem Inventory (SEI, Coopersmith) and the State-Trait Anxiety Inventory (STAI, Spielberger), respectively. Women were examined at the same time of the day at baseline and 30 days after. A significant improvement was found in the fingertip-to-floor distance of the test group (3.5 cm, $p<0.0001$), whereas no difference in the control group was detected. Similarly an improvement of the back flexibility was observed in the test group with a significant decrease of 2.4 cm of the distance measured with left arm above the head. Moreover an improvement of the social factor of the SEI score was found, whereas no difference was found in the STAI score. Our results demonstrate that the regular motion required to apply skin care products unto the entire body, can have a significant effect on joint flexibility and self esteem.

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Confocal laserscanning guided surgery in the therapy of dermatofibrosarcoma protuberans

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The danger of recurrence in incompletely excised dermatofibrosarcoma protuberans (DFPS) is apparent. No golden standard in the surgical approach is available so far. Neither excisions with wide margins nor modified techniques in Mohs surgery can guarantee tumour free surgical margins primarily. Therefore we took a 4 mm punch biopsy from the center of an excised DFPS to define laserscan microscopic criteria for DFPS. We divided the tissue into two parts: providing one part for routine histopathology and one for confocal laserscan microscopy (CLSM) examination. The laser scanning examination corresponds well with the histological examination. In the originally excised DFSP the CLSM imaging presented a diffuse proliferation of spindle cell shaped fibroblasts with monomorph elongated uniform nuclei. Clinically inconspicuous skin surrounding the DFPS was divided into eight sectors, from each sector a punch biopsy was taken and an examination with laserscan microscopy was performed. The sectoral punch biopsies from the wound margins were washed with 5% acetic acid for 30 seconds, placed on a glass slide and then imaged with a commercially available near-infrared reflectance confocal laser scan microscope. Lateral of those punch biopsies which were free of DFSP tissue we performed another excision for conventional histological examination according to the rules of the 3 dimensional Tübingen technique to confirm the result of the CLSM image. One punch biopsy from one sector of the lateral margin contained DFSP tissue. In this sector a re-excision was performed. This was subsequently confirmed by routine histopathology. In conclusion the use of CLSM provides a secure and easily performed technique to identify tumour tissue during surgery and therefore may provide the possibility for complete excision of DFPS in one session.

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Neural correlates for itch processing: a functional magnetic resonance imaging study

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Itch is known to be the most prevalent symptom of skin disease, however, its' neurobiology is poorly understood. Very few functional magnetic resonance imaging (fMRI) studies have investigated this. Prefrontal motor cortex, cingulate cortex, temporal pole, cerebellum and insula have been implicated predominantly in the cerebral itch matrix. We investigated cerebral itch processing using a placebo-controlled fMRI experimental design and a novel time-series analysis technique. Twelve right-handed, non-atopic, healthy males (mean age: 23.5, range: 20-29yrs) were recruited. Each was scanned (3 Tesla Philips MR) twice for 20 minutes (≥48 hours between scans). Three minutes into the scan, a skin prick with either histamine (10mg/ml) or saline was administered to the volar left forearm. Images were analysed using a random effects ANOVA in Statistical Parametric Mapping (SPM5) at a threshold of $p_{unc}<0.001$ and, by convention, $Z\geq3.09$. Time-series analysis evaluated temporal brain activation at 1 minute intervals. To assess the histamine effect on brain activation, the saline scan was subtracted from the histamine scan. During the histamine scan, all volunteers perceived itch and developed a wheal and flare reaction. In contrast, itch was not induced by the saline control and wheal and flare reactions were not observed. Bilateral brain activation was observed after the histamine skin prick in the following areas: (i) postcentral gyrus ($Z=4.26$); (ii) cerebellum ($Z=4.22$); (iii) superior/mid temporal gyri ($Z=3.87$); (iv) prefrontal motor cortex (superior/mid frontal gyri; $Z=3.75$); and (v) anterior insula ($Z=3.09$). In contrast to other studies, which did not account for placebo effect in the analysis or used a box-car design, these data are less multifocal in terms of involved brain regions. Our findings highlight the need for a reproducible fMRI experimental itch model and lend further support to the importance of the brain-skin connection.

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Response to adenoviral vector participates in the regression of cutaneous lymphoma lesions following adenovirus-mediated interferon-γ gene transfer

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Adenoviral vectors are commonly used in gene therapy since they provide transient expression of the delivered gene, which is often exploited in cytokine gene transfer. The use of these vectors is associated with immune activation and the production of proinflammatory cytokines, including type I interferons (IFNs). Type I IFNs, with the most prominent member IFN-α, affect different aspects of human immunity, which are believed to be crucial for inducing efficient antitumor immunity. We performed transcriptional profiling to characterize <i/>in vivo *response of cutaneous lymphomas to intralesional adenovirus-mediated IFN-γ (Ad-IFN-γ) gene transfer. For this purpose we analyzed gene expression profiles of skin lesions obtained from 19 cutaneous lymphoma patients before and after treatment with Ad-IFN-γ. We observed a distinct gene signature in treated lesions consisting of IFN-γ (type II IFN) as well as numerous IFN-α (type I IFN)-inducible genes. Additional <i/>in vitro* transfection experiments using an empty adenoviral vector (Ad-null), in addition to Ad-IFN-γ, revealed that the observed type I IFN-signature was vector dependent and that its magnitude and complexity, in terms of immune activation, was potentiated by the IFN-γ gene insert. Furthermore, patients whose lesions regressed during the treatment with Ad-IFN-γ displayed combined type I/II IFN response in treated cutaneous lymphoma lesions, reflecting the importance of the vector and the delivered gene in mounting of anti-tumor response. Biological effects of type I IFNs seem to enhance those set in motion by the transgene, in our case IFN-γ. This combination may prove to be of therapeutic importance in cytokine gene transfer using adenoviruses for cutaneous lymphomas.

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The skin tumor chemotherapeutic agent PEP005 is a substrate for the multidrug resistance protein MDR1 and damages tumor vascularity

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PEP005, extracted from *Euphorbia peplus*, is currently in phase 2 clinic trials for eradicating basal cell carcinoma, actinic keratosis and squamous cell carcinoma *in situ* by topical application. The molecular pathways that mediate PEP005's anti-cancer activity have been elucidated using an *in vivo* mouse xenograft model. Structurally related to phorbol esters, PEP005 activates PKC *in vitro* and *in vivo*. When applied topically, PEP005, not Phorbol 12-Myristate 13-Acetate (PMA), eliminated 75% of tumors derived from PAM212 xenografts. PEP005 is more effective in treating subcutaneously injected tumors than topically grafted tumors, suggesting that the responses from the mouse skin actively participate in the process. PEP005-induced an acute inflammatory response on mouse skin detected within a few hours, including infiltration of neutrophils and extravasation of red blood cells (RBC). In contrast, PMA induced inflammation without RBC extravasation. Electron microscopy revealed that PEP005-treatment damaged blood vessels, including disruption of basement membrane, activation and necrosis of endothelial cells, hemolysis of RBC intra- and extravessel, and altered collagen matrix. However, when tested *in vitro*, both PEP005 and PMA treatment disrupted preformed endothelial tubes in the endothelial cell tube formation assay, indicating that active participation of whole skin facilitated the damage on blood vessels caused by PEP005 *in vivo*. Pretreatment with topical cyclosporin A prevented PEP005-induced RBC extravasation but not PEP005-induced neutrophil infiltration. Blocking hemorrhage by cyclosporine A partially abolished PEP005's anti-cancer activity *in vivo*. In contrast, eliminating neutrophil infiltration by dexamethasone had no effect on PEP005-induced tumor ablation. PEP005, not PMA, blocked [^{125}I]-IAAP's binding to MDR1 in an *in vitro* photo-labeling assay. Verapamil, like cyclosporine A, is a commonly used MDR1 substrate/inhibitor, also blocked PEP005-induced hemorrhage. Taken together, our results suggested that MDR1 contributes to PEP005-induced tumor death *in vivo*.

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Successful treatment of chronic actinic dermatitis with infliximab

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Chronic actinic dermatitis (CAD) is a rare sun light-evoked persistent eczema of uncovered skin affecting mostly older men during the summer. Treatment is notoriously difficult. We describe a 69-year-old patient with a history of CAD for 7 years. He had been treated with topical corticosteroids and PUVA, which had only slightly ameliorated his condition. Although cyclosporine did improve the clinical picture it had to be discontinued due to nephrotoxicity. In addition, this patient also had also suffered from mild Crohn's disease for 12 years and was treated with 16 mg/d methylprednisone intermittently. This, however, failed to improve his CAD. We then began anti-TNF treatment with 5 mg/kg infliximab (IFX) intravenously at weeks 0, 2, 6 and 10. CAD improved markedly, at week 18 affected ankles, hands, neck and face completely cleared; the methylprednisone dose was tapered to 4 mg/d. One year later the CAD relapsed, which we treated with only one infusion of IFX leading to complete clearing at week 8. Topical treatment consisted of emollients and sun blockers only. A skin biopsy from the lateral aspect of the upper neck at week 0 during the first course of IFX treatment exhibited spongiotic dermatitis with psoriasiform acanthosis and papillomatosis; there were dense dermal lymphocytic infiltrates consisting of CD3+ and CD8+ cells with very few CD4+ or CD19+ or CD68+ cells. MIB-1 (Ki67) staining showed an increased labeling of epidermal cells in the basal layer; at week 18 all these changes tended to normalize. We conclude that the prominent dermal CD8+ lymphocytic infiltrates and the increased epidermal cell proliferation in CAD are normalized by successful anti-TNF treatment with infliximab. This suggests that TNF is important in pathogenesis of CAD.

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Initial pharmacogenomic assessment of TNF-alpha and TRAF1 polymorphisms in psoriasis: response to TNF blockers and other systemic therapies

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Polymorphisms in the TNF signaling pathway have been shown to influence production of TNF and response to treatment with TNF antagonists in rheumatoid arthritis and Crohn disease. However, the influence of these polymorphisms on treatment response in psoriasis remains poorly defined. To address this question, we studied 683 patients who received TNF blockers and/or systemic agents (360 M, 373 F, mean age 54 ± 14 years). Personal history of inflammatory bowel disease (IBD), or of autoimmune disease generally, was elicited in 4.8% and 6.7% of these patients, respectively, whereas family history was positive for psoriasis in 54.7% and for IBD in 11.7%. Treatment response was self-evaluated using a 0-5 visual analogue scale. Responses were characterized as good vs. poor (3-5 and 0-2, respectively). The ratios of good vs. poor responders were as follows: acetretin 61/28, adalimumab 21/8, alefacept 11/16, cyclosprine 30/18, efalizumab 17/14, etanercept 176/47, infliximab 34/11, methotrexate 201/71, PUVA 155/47, and UVB 324/79. Data were analyzed for linear trend of association between drug response and genotypes for seven SNPs—four in the promoter of the TNF-alpha gene (-238G/A, -308G/A, -857C/T and -1031T/C) and three in the gene for TNF receptor associated factor 1 (TRAF1, rs10818488, rs2416808 and rs3761847). A moderate association was observed among adalimumab responders with TNF-1031 genotypes (OR=7.83, p=0.036) and acetretin responders with TNF-857 genotypes (OR=3.20, p=0.028). Weak trends toward association were observed among etanercept responders with genotypes of TNF-238 (OR=1.56, p=0.24) and TNF-308 (OR=1.45, p=0.29), and among infliximab responders with genotypes of TNF-308 (OR=4.17, p=0.19). The other treatments manifested no TNF associations, and no treatment was associated with TRAF1. While these results are exploratory, they suggest TNF polymorphisms may affect the response to treatment with TNF blockers and acitretin, but not with other commonly-used systemic treatments (UVB, PUVA, and methotrexate).

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Nanoscalic silver – a new silver composition with broad spectrum antimicrobial activity and less toxicological side effects than silver sulfadiazine

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The rate of fungal infections in the general population has been increasing worldwide for several years. Even though there are several classes of antifungal substances available, resistances to these drugs rise constantly. Due to its well known broad spectrum antibacterial activities silver has been used successfully for decades and has become a standard treatment for burns and bacterial skin infections. Silver-containing creams, particularly silver sulfadiazine (AgSD), possess effective activities not only against bacteria but also against fungi, and resistances to these drugs are rarely reported. However, there is serious concern that silver ions applied to inflamed and/ or denuded skin might be absorbed in significant amounts, thus introducing the risk of silver deposition, potentially leading to internal organ injury. In view of these facts we compared the percutaneous absorption and the antimicrobial potency of AgSD with a new composition where nanoscalic silver is linked to an inorganic carrier material (NSAg) leading to a highly stable complex that is less likely to be absorbed via the skin. The backs of hairless mice (SKH-hr1) were treated on five consecutive days with AgSD 1%, NSAg 1%, and NSAg 0.1%, respectively. While both AgSD and NSAg showed strong antimicrobial activities against different bacterial strains, yeast species, and dermatophytes, quantification of silver ions in organs, blood and faeces revealed significantly lower absorption rates in the NSAg-treated animals than in the AgSD group. These results suggest that NSAg-containing creams are well suited for the prevention and treatment of skin infections, even when applied to large surfaces.

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Romidepsin has activity in refractory ctcl: an international multicenter study

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Romidepsin is a bicyclic peptide that inhibits Class I and II HDACs with reported activity in CTCL. To confirm, a single arm, open-label phase II study enrolled CTCL (Stages 1B-1VA) patients (pts) from 36 international sites. Pts with CTCL who failed ≥1 prior systemic therapy received romidepsin at 14 mg/m2 on Days 1, 8, and 15 q 28 days. Exclusions included significant CV abnormality or treatment with QTc-prolonging or CYP3A4-inhibiting drugs. The primary endpoint is response rate measured by a combination of imaging, circulating malignant T-cell counts and a scoring instrument to determine skin involvement. 96 pts were eligible with 73 evaluable for efficacy per protocol. Responses in evaluable pts are 4 CCRs, 21 PRs, 42 SD and 6 PD for an ORR of 34% (duration 2+-21 months). The median time to response is 6 weeks. Responses by stage at entry in evaluable pts are: Stage IB-IIA 7/24 (29%) and Stage IIB-IVA 20/49 (41%). In pruritus pts, relief was seen in 23/61 pts (38%) and in 13/35 (37%) with severe pruritus. The most frequent AEs were nausea (51%), fatigue (27%) and vomiting (22%). Serious AEs considered possibly, probably or likely related to treatment were reported in 14 pts but there were no deaths attributed to romidepsin, although 4 pts died of progressive disease and 1 from right ventricular failure. Asymptomatic ECG abnormalities were noted in 6% of the pts. This study confirms the clinical efficacy of romidepsin in refractory CTCL, including relief of pruritus and a manageable safety profile.

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Novel drug treatment for diabetic foot ulcers – results of a phase I clinical study

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Non-healing ulcers associated with severe diabetes result in high morbidity and mortality rates. Protein kinase C (PKC), a family of serine-threonine kinases is involved in skin physiology and pathology. HealOr has developed a novel drug, HO/03/03, consisting of a PKCδ activator and a PKCα inhibitor that augments all essential wound healing stages and overcomes the impairment of diabetic wounds. Successful completion of pre-clinical and toxicology studies led HealOr to conduct a phase I clinical trial in non-healing DFU patients in three medical centers in Israel. The primary objectives of this trial were safety and assessment of healing efficacy of HO/03/03 on Diabetic Foot Ulcers. Secondary objectives were time to closure and rate of healing. 19 patients underwent a screening period of 2 weeks for eligibility, where patients were treated by standardized wound care and conformed to weight off-load regimen. 4 patients who showed decrease in wound size of ≥30% were excluded from the trial while 15 patients were treated topically with HO/03/03 once daily. No toxicity or drug-related adverse events were reported. All treated wounds demonstrated a positive healing response with re-epithelialization, granulation tissue formation and dermal regeneration. 70% of the patients had their wounds closed within 14 weeks with a mean closure time of 6.6 weeks, with a range of 3 to 14 weeks. We have analyzed the data of wound closure and time to heal utilizing two methods: Visitrack® acetate tracing system and digital photo documentation utilizing Pictzar® wound management software. Both methods have demonstrated similar accuracy in wound size measurement. In confirmation of previous studies, 9/10 wounds which closed, demonstrated a decrease in wound size of more than 50% at 4 weeks suggesting the 4 weeks time point as a predictor of wound healing. No wound recurrence was observed with a follow-up of up to 6 months. In conclusion, trial final results suggest HO/03/03 as a future novel drug for Diabetic Foot Ulcers.

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Results of a multicenter phase II trial of temozolomide in patients with advanced MF/SS

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Temozolomide (TMZ) is an oral imidazotetrazine that induces DNA damage by cross-linking similar to other alkylating agents. Resistance has been associated with high levels of O6 alkylguanine DNA alkyltransferase (AGT), a DNA repair enzyme that targets cells from alkylating agents that frequently target the O6-position of guanine. Patients with MF/SS have been shown to have low levels of AGT and may be particularly sensitive to this alkylator. The efficacy of TMZ was evaluated in a multicenter phase II trial of patients with advanced stages of MF/SS. TMZ was given orally at daily doses of 200 mg/m2 for 5 days every 28 days. Results: Twenty-six patients were evaluable for response. Median follow-up time was 29 months (range 1 - 95). The overall response was 27% with 2 CRs (8%) and 5 PRs (19%). Four patients had SD (15%). Median disease-free survival was 4 months (range, 2 to 17 months). The median overall survival of all patients was 24 months. The most frequent toxicities included constitutional symptoms, gastrointestinal symptoms, and hematologic toxicities. Treatment was discontinued in 3 patients following Grade 3 thrombocytopenia, lymphopenia, and skin rash. The relationship between pretreatment AGT and MLH1/MSH2 mismatch repair protein expression levels in skin biopsies of cutaneous lesions and clinical response to TMZ was evaluated. Pretreatment levels of AGT and MLH1/MSH2 protein levels are not predictive of response to TMZ in MF/SS.

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Temporal-spatial control of tissue contouring with extracellular pH-modulated rHuCathepsin-L

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Enzymatic subcision of the extracellular matrix is a potential solution for surgically impractical dermatologic procedures. For example, cellulite is exacerbated by multiple collagenous fibrous septae that anchor the dermis to the deep underlying fascia when fat lobules in the hypodermis protrude into the overlying dermis. While enzymatic subcision of fibrous septae could theoretically be utilized to modulate surface contour, temporal-spatial control of proteinases such as collagenase are not possible since persistent degradation of surrounding tissue may create safety concerns or require the use of subpotent dosing regimens. Human Cathepsin L is a member of the lysosomal thiol proteinase family capable of degrading insoluble collagen in vitro at pH 5-6 but catalytically inactive at physiologic pH. By intradermal injection of pH indicators in increasing buffer strengths (10-100 mM MES pH 5.0), it was established that an acidic temporal-spatial extracellular environment from (1-20 minutes/100mm2 injection) could be obtained. The efficacy of recombinant human Cathepsin-L (rHuCATL) on fibrous septae in rodent and porcine models was assessed by hydroxyl-proline release and histologic evaluation compared with bacterial collagenase. 25-100 ug/mL rHuCATL in 20 mM MES pH 5 was injected into the interstitium. Control experiments included rHuCATL injection at pH 7.4, pH 5.0 buffer alone and bacterial collagenase at pH 7.4. rHuCATL administration resulted in significant hydroxyproline release at pH 5 compared to bacterial collagenase, but not at pH 7.4, consistent with histologic analysis of fibrous septae. Importantly, skin interstitial pH returned to neutrality within 20 minutes of test article administration. By temporal-spatial modulation of the extracellular environment, rHuCATL may provide a novel mechanism for tightly controlled pharmacologic subcision of collagenous interstitial matrix.

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Werner protein-binding small molecules with anti-cancer effects

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DNA fragments homologous to the telomere G-rich strand (T-oligos) induce DNA damage responses in cancer cells leading to p53 and histone H2AX phosphorylation (activation) followed by apoptosis and/or senescence. T-oligo effects are transduced by Werner protein (WRN), a DNA helicase and endonuclease that is mutated in the cancer-prone Werner syndrome. To identify small non-DNA molecules that like T-oligo bind and activate WRN, a high throughput affinity screen was performed on >11,000 compounds. One of 30 WRN-interacting spirooxindole (SPOX) molecules (0.6-1kD) and its enantiomer significantly decreased cell yields of melanoma (AN), lung carcinoma (H460), and breast carcinoma (MCF-7) cells by 44±2%, 38±1% and 80±0.5%, ($p<0.004$, $p=0.05$ and $p<0.001$), respectively at 40-80uM, within 96 hours. Furthermore, like T-oligo these SPOX compounds induced apoptosis as determined by caspase-3 activation (PARP cleavage) in MCF-7 cells or by increase in sub G_0/G_1 in AN cells (45±5% SPOX vs 10±1% diluent, $p<0.02$). Also like T-oligo, SPOX activated (phosphorylated) ATM (ataxia telangiectasia mutated) kinase and induced H2AX phosphorylation. Furthermore, in all 3 cell lines, within 48 hours SPOX decreased the level of the inhibitor of apoptosis protein, survivin, to non detectable levels. To determine WRN role in SPOX effect, osteosarcoma cells were treated with WRN siRNA to knock down WRN (WRN k.d.), decreasing WRN levels to ~50% of control cells. In WRN expressing cells, T-oligo induced H2AX and p53 phosphorylation >2000% and >600% respectively, above baseline, but only 700% and 350% respectively in WRN k.d. cells. Similarly, SPOX induced H2AX and p53 phosphorylation >400% and >250% respectively, above baseline in WRN expressing cells but only180% and 120%, respectively, in WRN k.d. cells. A control molecule that does not bind WRN had no effect on p53 and H2AX phosphorylation. These data validate WRN role in therapeutic DNA damage-like responses in cancer cells induced by T-oligos or SPOX.

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Comparison of reliability and validity between two outcome instruments for pemphigus

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Our scientific understanding of the pathogenesis of pemphigus has greatly improved in recent years. However, as we try to evaluate therapeutic options to develop treatment paradigms for pemphigus, a major obstacle in performing large, multicenter controlled trials is the lack of a validated scoring system to rate disease activity. Here we evaluate a new measurement instrument, the pemphigus disease area index (PDAI), which was developed by the International Pemphigus Committee. We compared this tool to the recently described ABSIS (autoimmune bullous skin disorder intensity score) instrument. A group of 10 experienced dermatologists scored 15 patients with mild to moderate pemphigus disease activity to estimate inter- and intra-rater reliability in a single session. To estimate validity, the PDAI and the ABSIS were also compared to the Physician's Global Assessment (PGA). PDAI total score was normally distributed with total scores ranging from 0-44 (mean 13.1±9.01) out of a possible 263 maximum score for the instrument. The interquartile range for the PDAI total score was 6.3-18.9. ABSIS total scores ranged from 0-38 (mean 6.91±7.52) out of a possible 206 maximum score for the instrument, with an interquartile range of 2-8.5. Reliability studies demonstrated an intra-class correlation coefficient (ICC) for inter-rater reliability of 0.75 [95% confidence interval (CI) = 0.60-0.90] for the PDAI and 0.77 [0.63-0.91] for the ABSIS. Intra-rater test-retest reliability demonstrated an ICC of 0.98 [0.96-1.0] for the PDAI and 0.80 [0.65-0.96] for the ABSIS. Moreover, the distribution shows that for this population of mild to moderate disease activity, the PDAI better captures variability in cutaneous disease activity. The PDAI correlated more closely with the PGA, with an rsp of 0.60 [0.49-0.71] compared to the ABSIS correlation of 0.43 [0.30-0.55]. We conclude that the PDAI is more reproducible and correlates better with overall physician impression of the patient.

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High-resolution cutaneous ultrasonography in the differential diagnosis of lymphodema and lipoedema

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Lipoedema is an accumulation of fat abnormally distributed in the lower limbs. The main differential diagnosis is lymphoedema. High-resolution ultrasound operating at 20 MHz makes it possible to show dermal oedema. We thus put forth the assumption of different dermal echogenicity in lipoedema and lymphoedema. 8 patients with lipoedema (16 legs) and 17 patients with lymphoedema (23 legs) were included. The diagnoses were established with the assistances of the Wold criteria. All ultrasound examinations were carried out with the same equipment, and by the same operator on 3 different sites for each leg: medium of thigh, medium of leg and internal malleolus. The images were then anonymized and examined by an independent dermatologist who was blind from clinical diagnosis. We observed dermal hypoechogenicity suggestive of oedema in 15 thighs (65%) and 21 legs and malleolus (91.3%) of lower limbs with lymphoedema. Ultrasonographic examination of thighs and legs with lipoedema was normal in all cases, except 2 (12.5%) with dermal hypoechogenicity on the malleolar site only. Average dermal thickness were: 2.1 mm (SD=0.54) in thighs, 2.64 (SD=0.57) mm in legs and 2.66 mm (SD=0.66) in malleolus in lymphoedema; and 1.51 mm (SD=0.31) in thighs, 1.59 mm (SD=0.27) in legs, and 1.54 mm (SD=0.29) in malleolus in lipoedema. High-resolution cutaneous ultrasonography makes it possible to differentiate lymphoedema from lipoedema. A significant increase in thickness of the dermis and a marked hypoechogenicity of the dermis were characteristic of lymphoedema. In lipoedema, echogenicity and thickness of the dermis were nearly always normal. Infraclinic venous insufficiency or physiological nychthemeral variations of dermal fluid may explain the 2 cases with slight malleolar oedema and lipoedema. Legs seems to be the most useful site to differentiate the two pathologies. Objective assessment of dermal echogenicity and thickness is a useful and non-invasive tool to help with the diagnosis of lipoedema. This will make it possible to avoid useless lymphatic drainages and to provide adequate counselling and diet.

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Availability of antimycotic agents into the nail plate after repeated application of nail lacquers to healthy human fingernails.

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A novel 8% ciclopirox (CPX) water soluble nail lacquer (P-3051) containing hydroxypropyl-chitosan as film-forming agent previously showed superior in vitro nail penetration of the active compared to water insoluble CPX or amorolfine (MRF) nail lacquers. In this experiment, we compared the penetration of CPX from P-3051 in human fingernails of 24 healthy male volunteers with that of a commercial 5% MRF nail lacquer. Test and reference products were randomly self-applied to all fingernails of either hand for a period of 28 days. P-3051 was applied once daily by a brush; MRF was applied twice weekly as per the approved labelling, by using a spatula. Both test and reference products were applied in the evening. Before each test application the subjects were instructed to wash their hands with water and soap and dry accurately; before each reference application the subjects had to remove the previous product layer with an isopropyl alcohol swab. After 15 and 25 days the free edge of the nails was cut and collected for analysis after nail cleaning as per the same procedure as above. CPX and MRF concentrations in the cut fingernails were measured by validated LC-MS-MS methods. Efficiency coefficients were calculated for different fungi as ratio of nail concentrations/MICs. Nail concentrations at t15 were 2.82 ± SD 2.85 µg/mg nail for CPX and 0.64 ± 0.56 for MRF. At t25 measured concentrations were 1.85 ± 1.52 for CPX and 0.13 ± 0.15 for MRF. Efficiency coefficients were > 5000 x MIC in 54% at t15 and 46% at t25 for CPX and 56 and 4% for MRF for *T. rubrum* ; 21% at t15 and 21% at t25 for CPX, 0% at t15 and t25 for MRF for *C. parapsilosis*. In conclusion, P-3051 exhibited a good penetration and efficiency coefficient after in vivo application to human fingernails; amorolfine, despite higher in vitro potency on dermatophyte fungi, was poorly absorbed into the nail plate and exhibited a lower efficiency coefficient on onychomycosis pathogens. Our in vivo data fully comply with previous data on in vitro nail permeation of actives from both products.

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New scale to measure pruritus: the 5-D

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The subjective nature of pruritus makes its quantification difficult, but testing of new therapies requires a tool to assess change over time. Based on clinical experience with pruritic pts, we modified the Total Peripheral Neuropathy Scale to create the 5-D Itch Scale. The 5-D measures 5 domains of pruritus: Duration (hrs/day), Degree (severity), Direction (improvement or worsening), Disability (impact on daily activities), and Distribution (body parts affected). The 5-D is a quick, 1-page questionnaire using 5-pt Likert responses and simple checkboxes to quantify & describe pruritus. This study aimed to validate the 5-D in pts with common causes of pruritus. To assess convergent validity, pts completed the 5-D as well as the current gold standard for pruritus (VAS) & a pruritus quality of life scale (PBC-40 Itch domain). At enrollment, 233 pts with pruritus completed the 5-D, VAS, & PBC-40. Of these pts, 55 had dermatologic disorders, 51 had burns, 36 had chronic kidney disease, 63 had liver disease, & 28 had HIV. 5-D scores correlated strongly with both VAS (r=.733, p<.0001) & PBC-40 (r=.719, p<.0001). A subset of pts repeated the 5-D at 3 days, revealing high test-retest reliability (r=.984, p<.0001). All individual domains of the 5-D correlated with both VAS & PBC-40. Not surprisingly, the VAS correlated most strongly with the degree domain (r=.728, p<.0001), as both measure pruritus severity. Similarly, the PBC-40 correlated most strongly with the disability domain (r=.690, p<.0001), as both measure quality of life issues. The direction domain showed the lowest correlation with VAS (r=.353, p<.0001) & PBC-40 (r=.164, p<.03), suggesting previous measures may neglect to identify pts' perceived improvement or worsening of pruritus. Strong correlation between change in 5-D score and pts' perceived degree of improvement at 6 weeks (r=.781, p<.0001) suggests the 5-D's responsiveness to change over time. Based on these results, the 5-D is a valid measure to easily quantify and describe pruritus in multiple diseases. The 5-D can assess change in pruritus over time, and therefore may be an effective outcome measure to evaluate the effectiveness of new therapies for pruritus.

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Multi-country characterization of Asian facial hyperpigmentation

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A series of Institutional Review Board-approved multi-site, facial hyperpigmentation characterization studies was conducted in multiple countries across Asia. Approximately 100 female subjects per cell (over 400 total), ages 18-65 yr were recruited with a moderate concern of facial hyperpigmentation and a willingness to seek topical treatment solutions. Clinical evaluation was performed by a dermatologist of similar ethnic origin from each country to diagnose facial hyperpigmentation across multiple attributes in China, India, Thailand, and Japan. Additional measures included color and melanin spectrophotometric measurements and digital facial photography to document and quantify subsequent treatment effects on facial skin condition. Several significant differences in hyperpigmentation conditions existed between Asian populations. For example, periocular and periorbital hyperpigmentation were more prevalent in Indian subjects, but were virtually absent in other populations. Indian and Thai subjects showed an increased prevalence of post-inflammatory hyperpigmentation (PIH) due to acne or other skin conditions, and despite an age-dependent decrease in PIH due to acne, significant numbers of these women presented with PIH even into their 40s. There was an age-dependent increase in solar lentigines in Chinese, Japanese, and to a lesser extent Thai populations, where by age 50 almost all subjects presented with solar-induced hyperpigmentation. In general, subjects with lighter overall facial skin color showed a higher prevalence of solar-induced hyperpigmentation disorders. Many Chinese and Japanese women in their 20s-30s had a significant accumulation of solar lentigines, in contrast to the delayed onset with Thai and Indian subjects.

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Aberrant promoter hypermethylation of candidate tumor suppressor genes in cutaneous squamous cell carcinoma

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Cutaneous squamous cell carcinoma (cSCC) is the second most common type of nonmelanoma skin cancer in the United States. Advanced or metastatic cSCC is associated with high mortality rate. Given the high prevalence and mortality rate of cSCC, identification of potential molecular markers is valuable as a result of their diagnostic and prognostic relevance. Previous studies on SCC in other organ systems have implicated promoter hypermethylation is one of the mechanism of tumor suppressor gene (TSG) inactivation. The aim of our study is to establish an extensive panel of TSG as molecular markers for cSCC. We have examined the promoter methylation of 7 candidate genes in 12 normal control tissues and 48 primary cutaneous skin lesions, including 39 aggressive cSCC entities and 9 carcinoma in situ samples. Quantitative methylation-specific PCR (QMSP) was applied to determine the methylation status of each gene in all tissue samples. The frequency of promoter methylation of the 7 TSG was significantly higher among skin tumor samples than normal skin samples. The most frequently methylated gene was CDH1 (33.3%), followed by DAPK, MGMT, and TIMP3 (27.1%, respectively), APC (22.9%), p16 (20.8%), and TGFBR2 (8.3%). The data also demonstrated the value of multi-gene approach in detection of aberrant methylation in cSCC. The sensitivity of marker panel is higher than single marker. Our data suggest that candidate gene approach has identified a panel of TSG that are hypermethylated in cSCC. Detection of cSCC using a panel of markers has higher sensitivity than single gene approach. Early detection of potential aggressive behavior of premalignant skin lesions based on a panel of markers is valuable for minimizing morbidity and mortality, especially for immunocompromised patients such as patients with organ transplants and other cancer types or medical conditions.

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A histopathological examination for skin atrophy with long-term treatment using triple combination cream

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A stable combination drug agent combines hydroquinone 4% with tretinoin 0.05% and fluocinolone acetonide 0.01% and is FDA approved for the treatment of melasma. Although the steroid component of this combination is a very low potency steroid, concern among dermatologists remains regarding the potential for skin atrophy with the long-term treatment that pigmentary disorders often require. However, not including the steroid component in the treatment approach sacrifices efficacy. Several long-term studies with treatment periods up to 52 weeks long have been conducted and clinical assessments of skin atrophy have been made with few reported incidences. In this study of 70 patients, clinical assessments were followed by a histological examination in patients that received treatment for a period up to 24 weeks in duration. 2 mm skin biopsies were obtained from involved and normal skin at baseline, and in involved at week 12 and week 24. Tissues samples were fixed and photographed and a standardized method was applied to measure the epidermal and dermal thickness of each sample by a blinded evaluator. Clinical assessments resulted in no reported instances of atrophy. Histologically, there were no significant differences found in measured epidermal thickness throughout the study. Furthermore, there were also no significant differences found in the measured dermal thickness. An increase in the mean number of blood vessels per mm2 of skin was found in patients at 12 and 24 weeks. Clinical assessment of telangiectasias found that the mean severity score for these was between none and mild throughout the study period. In conclusion, we have shown that skin atrophy should not be a specific concern with treatment periods up to 24 weeks in duration, but that the incidence and severity of telangiectasias should be monitored with the specific treatment goals for the individual patient in mind. Study and poster support provided by Galderma Laboratories, L.P.

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Trafficking of 8-MOP treated leukocytes after photopheresis in humans

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Photopheresis is an established treatment for cutaneous T-cell lymphoma, systemic sclerosis, graft-vs-host-disease and other autoimmune diseases. During photopheresis a leukocyte fraction (buffy coat) of peripheral blood is extracorporeally exposed to 8-methoxypsoralen and UVA before reinfusion into the patient. The precise mechanisms of photophesesis are not yet known. The aim of the present study was to investigate the fate of UVA irradiated cells after reinfusion. For this purpose a fraction of UVA-irradiated buffy coat was sampled prior to reinfusion, separated into lymphocytes (LC) and polymorphonuclear leukocytes (PMN), and labelled with Indium-111 before being reinjected into the patient. Scans were acquired at 10min, 3h and 24h after injection. Regions of interest were drawn over liver, spleen, spine, right lung and heart. Pixel-normalised ratios of organ to background were calculated with correction for physical decay. Each patient was investigated separately for migration of labelled LC and PMN with an interval of 4 weeks. Seven patients have been included in the study. Using trypan blue we found that viability of 8-MOP/UVA treated LC and PMN was only minimally affected by labelling and that our technique results in a sufficiently strong signal to follow the labelled cells for 24h. The most prominent difference between migration patterns of LC and PMN observed in all patients is retention of LC but not of PMN in the lungs immediately after injection within first 10min after injection. LC were rapidly cleared from blood pool while PMN showed blood pool activity over first 3h. These results show for the first time that specific radiolabelling of blood cells after photopheresis in humans is feasible with a high yield and low cell damage and that 8-MOP/UVA treated LC and PMN have different and specific migration patterns.

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Modulation of tumor responsiveness to photodynamic therapy using differentiation-promoting agents in a mouse model of skin cancer

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Photodynamic therapy (PDT) is an emerging treatment for non-melanoma skin cancers (NMSC). In aminolevulinic acid (ALA)-mediated PDT (ALA-PDT), ALA is given as a prodrug which selectively accumulates and is metabolized in target cells to form protoporphyrin IX (PpIX); targeted local irradiation with light induces cell death and provides dual selectivity. To make ALA-PDT more effective for treatment of deep skin tumors, we are developing a new approach which combines differentiation-inducing agents with ALA-PDT to improve the clinical response. Here, we have tested the new combination paradigm in three models: 1) human primary keratinocytes (NHEK) and carcinoma cells (SCC13 and HEK1 lines) in monolayer cultures; 2) a 3-D organotypic culture model of epidermis; and 3) tumors generated in mice by topical application of a chemical carcinogen. To achieve a differentiated state of target cells and tissues, pretreatment using a wide (1000-fold) concentration range of methotrexate (MTX) or vitamin D (Vit D) was administered for 72 h prior to ALA-PDT. Confocal images of cells and histological sections were captured and analyzed by image processing to determine relative PpIX levels. PpIX in the tumors was also monitored by real-time in vivo fluorescence dosimetry. Cell death following PDT in monolayer cultures was measured by Live/Dead (Fluorescein/Ethidium) assay. In all three models, a significant increase in levels of PpIX was observed following pretreatment with MTX or Vit D, as compared to no-pretreatment controls. This enhancing effect was observed at very low non-cytotoxic concentrations (nM range), and was highly specific to cancer cells as compared to normal cells. These results suggest that use of differentiating agents such as MTX or Vit D, as a short-term combination therapy given prior to ALA-PDT, can increase the production of PpIX photosensitizer and enhance the therapeutic response of skin cancers.

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Curcumin administration after, not before, radiation exposure effectively reduces radiation dermatitis

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Radiation dermatitis (RD) occurs in approximately 95% of cancer patients receiving radiation therapy. Despite advances in medical technology, there are no useful treatments for these skin reactions. Furthermore, based on recent world events suggesting the potential for "dirty bombs" and radiation poisoning, it is recognized that knowledge and management of radiation skin injury is truly needed. Our group's recent work with C3H mice suggests that curcumin therapy may be a candidate treatment. Curcumin, a non-toxic food ingredient found in turmeric and curry powders, is a naturally potent anti-inflammatory and antioxidant. In this study, we examined the efficacy of curcumin to prevent RD in hairless SKH-1 mice. Mice were orally administered curcumin (200mg/kg.day) or corn oil for 4 days before (pre-treated) or after radiation (post-treated). The left hind leg of each mouse was irradiated with 30 Gy. RD was monitored for 21 days. RD was significantly reduced in post-treated curcumin mice, but not pre-treated curcumin mice. At day 10, fine and moist desquamation was observed in pre-treated curcumin, pre-treated corn oil, and post-treated corn oil mice. Interestingly, RD was not visible in any mice at day 21. Histological analyses were performed on skin specimens harvested at day 21. Post-treated curcumin mice exhibited reduced epidermal hypertrophy, hyperkeratosis, and inflammation. In contrast, pre-treated curcumin mice showed increased epidermal hypertrophy, hyperkeratosis, and inflammation compared to all other mice. Additionally, immunohistochemical staining revealed radiation-induced epidermal and dermal expression of the inflammatory mediator, 5-lipoxygenase, in post-treated corn oil mice, but not in post-treated curcumin mice. Our results demonstrate that curcumin administration after, not before, radiation effectively reduces RD and is a promising non-toxic intervention for radiation skin injury.

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Microdermabrasion with a coarse grit diamond-studded hand piece induces a wound healing response and dermal extracellular matrix remodeling in human skin *in vivo*

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Microdermabrasion is a non-ablative resurfacing procedure reported to improve fine wrinkles in photodamaged skin. It is presumed that sub-clinical epidermal wounding results in a wound healing cascade that culminates in dermal collagen remodeling. We investigated whether a single microdermabrasion treatment elicits key molecular components involved in normal wound healing and collagen remodeling. A single, standard microdermabrasion procedure using a crystal-free system, with a coarse grit (125 micron), diamond-studded hand piece was performed at 15 in Hg for three passes on photodamaged forearm skin of volunteers. Skin samples were obtained from 4 hours to 16 days post treatment and genes of interest were analyzed for alterations of mRNA and protein expression by reverse-transcriptase real-time PCR, and immunohistochemistry, respectively. Sub-clinical epidermal wounding was heralded by gene expression of cytokeratin 16 (11-fold) and regulatory transcription factor, jun B (3-fold) within 6 hours after treatment ($p< 0.05$, n=20). Induction of primary cytokine IL-1β (10-fold) was also demonstrated 6 hours after treatment ($p<0.05$, n=20). IL-1β induces matrix-degrading metalloproteinases (MMPs). MMP-1 (collagenase), MMP-3 (stromelysin), and MMP-9 (92 kDa gelatinase) were maximally elevated 333-fold, 345-fold, and 27-fold, respectively, at 6 hours after treatment ($p<0.05$, n=20). Matrix metalloproteinases degrade damaged collagen causing collagen remodeling. A four-fold induction of type 1 collagen and a 3-fold induction of type 3 collagen gene expression and a 6-fold induction of type 1 collagen protein were observed at 14 days after treatment ($p<0.05$, n=20). In summary, sub-clinical epidermal disruption caused by crystal-free microdermabrasion, with a diamond-studded hand piece, stimulates a wound healing cascade that culminates in dermal collagen production in photodamaged forearm skin.

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Withdrawn

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Comparison of the effects of different pulse durations when using 585 nm pulsed dye laser in the treatment of new surgical scar

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Prior studies have shown that the pulsed dye laser (PDL) is effective in improving the quality and appearance of the scar when using the 585 nm PDL immediately after the removal of sutures. Most published studies used a pulse duration of 450 μsec (short-pulse), which along with the other studies parameters, have led to overall improvement of the scars. However, a pulse duration of 1.5 msec (long-pulse) is also available when using PDL and it should theoretically cause fewer side effects. To our knowledge, there are no other studies comparing the effectiveness of different pulse durations in the treatment on surgical scars starting on suture removal day. In this study we compared the effect of different pulse durations of 585 nm PDL in 15 patients with new postsurgical linear scars in a randomized, double-blind controlled trial design. Immediately after suture removal, scars were randomly divided into 3 equal sections. The different fields were randomly chosen to receive short-pulse, long-pulse or no treatment (as a control). The two fields chosen to be treated received treatment with the 585 PDL using 7 mm spot size at 4.0 j. The three sections were mapped and recorded. The patients received treatment immediately after the sutures were removed and then monthly for total 3 sessions. Evaluations were performed by Vancouver Scar Assessment Scale (VSS) before each treatment and one month after the last treatment. At one month after the last treatment, the short-pulse and long-pulse 585nm PDL treated sections demonstrated an overall average improvement of the VSS of 82% and 87% respectively, compared to 50% for the control. This difference was found to be statistically significant ($p < 0.05$) between treated sites and control but no significant difference between the short-pulse and long-pulse PDL treated sites. In conclusion, PDL is effective in improving the quality of new surgical scars but no difference between the two pulse durations.

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Epiceram for the treatment of mild to moderate atopic dermatitis- a pilot study

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Epiceram is an FDA-approved therapeutic skin barrier emulsion that contains a mixture of the three stratum corneum lipids with a synthetic pseudoceramide as the dominant ceramide-equivalent lipid species. This emulsion was designed to correct the epidermal barrier dysfunction found in various inflammatory dermatoses. We have performed a prospective, randomized, investigator-blinded multi-center pilot study exploring the safety and efficacy of Epiceram in the treatment of mild-to-moderate atopic dermatitis (AD) in children. Topical pimecrolimus was used as a control/comparator group. Thirty-eight subjects age 2-12 with mild-moderate AD were enrolled and treated for 4 weeks. Five subjects withdrew from the study all assigned to the Epiceram group due to worsening of their disease or noncompliance with the protocol. The primary outcome was change in Eczema and Area Severity (EASI) score from baseline as measured at Week 4. Median baseline EASI scores were 3.1 for the pimecrolimus group and 5.2 for the Epiceram group. The median reduction in EASI scores as measured at Week 4 were 2.0 for the pimecrolimus group and 1.2 for the Epiceram group, ($P=0.32$ using the Wilcoxon Rank-Sum test). The percent achieving an Investigator's Global Assessment of "clear" or "almost clear" at Week 4 was 44.4% in the pimecrolimus group and 23.5% in the Epiceram group ($P=0.17$). The most common adverse event was transient burning or stinging experienced in 4 subjects in the Epiceram group and 3 subjects in the pimecrolimus group, all rated as mild in severity. In conclusion, Epiceram appears to be safe and effective in pediatric patients with mild-moderate AD. These pilot data enable the design of larger studies that evaluate Epiceram's relative efficacy compared to traditional topical agents. Epiceram offers a novel approach to the treatment of AD with a mechanism of action distinct from traditional topical anti-inflammatory therapies.

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Procedure for evaluating change in eyebrow position induced by ultrasound skin tightening

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Our purpose was to develop an algorithm acceptable to regulatory agencies for detecting clinically significant change in eyebrow position. This was applied in the context of evaluating an ultrasound device for tightening skin. A process was developed in cooperation with regulatory agencies and then applied to assess data associated with ultrasound skin effect on brow position. The primary tool was masked clinician assessment of eyebrow position: (1) 2 experienced clinicians (EC:board certified in plastic surgery or dermatology & specializing in aesthetic facial care) review pre- and post- photographs, frontal and 45 degree (PrePostP) of 25 subjects unblinded to reach consensus on position as "change" or "no change"; (2) results of part (1) are reference standard for clinical assessment; (3) 3 masked, EC each independently evaluate randomized PrePostP of 25 subjects side by side to categorize each photograph as pre- or post-treatment based on perceived change or not in brow position; (4) change in brow position between PrePostP identified by these EC is defined as clinically significant; (5) the masked assessment method is validated by comparing results (using kappa statistic) from the 2 unmasked EC to the 3 masked EC; (6) to determine clinical efficacy, 3 masked EC evaluate paired PrePostP to determine if a discernible improvement is noted or not. Apart from the masked assessment process, a patient satisfaction tool is used to assess brow lift, skin appearance, forehead functionality, and forehead skin sensation. In summary, the 3 blinded reviewers noted that 85.7% of the 35 subjects were "improved." Of the 24 subjects completing the patient satisfaction measure at 8-10 months post-treatment, 75% were satisfied or very satisfied with the improvement in brow position. We concluded that validated masked assessment of standardized photographs by experienced clinicians, plus patient satisfaction measures, may satisfy regulatory requirements for demonstrating post-procedure change in eyebrow position.

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Photoprotective effects of bucillamine against UV-induced damage in a SKH-1 hairless mouse model

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UVB exposure of skin results in various biological responses either through direct or indirect damage to DNA and non-DNA cellular targets via the formation of free radicals, reactive oxygen species and inflammation. Bucillamine [N-(2-mercapto-2-methylpropionyl)-l-cysteine] is a cysteine-derived compound that can replenish endogenous glutathione due to its two donatable thiol groups, and functions as an antioxidant. In this study, we investigated the effects of bucillamine on UVB-induced photodamage using the SKH-1 hairless mouse model. We have demonstrated that UVB exposure (2 consecutive doses, 230 mJ/cm2) on the dorsal skin of SKH-1 mice induced inflammatory responses (edema, erythema, dermal infiltration of leukocytes, dilated blood vessels) and p53 activation as early as 6 hours after the last UVB exposure. Bucillamine pre-treatment (20 mg/kg of body weight, administered subcutaneously) markedly attenuated the UVB-mediated inflammatory responses and p53 activation. We have also demonstrated that the stabilization and upregulation of p53 by UVB correlated with phosphorylation of Ser-15 and Ser-20 residues of p53 protein and that bucillamine pre-treatment attenuated this effect. We propose that bucillamine has potential to be effective as a photoprotective agent for the management of pathological conditions elicited by UV exposure.

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Spectrum of sorafenib-induced dermatologic adverse effects

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Cancer patients treated with small molecule inhibitors experience cutaneous toxicities that can result in termination of therapy. We hypothesized that select subsets of dermatologic toxicity were drug-limiting. We examined the prevalence and characterization of the dermatologic toxicities associated with sorafenib therapy (inhibition of VEGFR-2, VEGFR-3, PDGFR-β, b-Raf, Flt3, c-KIT, RET) in a single institution (National Cancer Institute). Oncology patients referred to Dermatology (Aug 2005 - Dec 2007) were systematically evaluated for cutaneous toxicities (CT) possibly or probably associated with sorafenib. Ninety-eight patients were enrolled in NCI intramural protocols during this period: a) sorafenib monotherapy in 41 with prostate cancer and 13 with non-small cell lung cancer; b) sorafenib + bevacizumab in 42 with refractory solid tumors; and c) standard-of-care sorafenib monotherapy in 2 with renal cell carcinoma. Sixty-five patients were evaluated in Dermatology: 24 unselected patients evaluated prospectively at baseline and on-treatment and 41 evaluated in consultation after developing cutaneous side effects. CT were similar for both groups. For all 65 patients, CT were acral erythema/hand foot syndrome or HFS (75%); facial and/or scalp erythema/dysesthesias (66%); keratosis pilaris-like lesions (35%); subungal hemorrhages (32%); alopecia (32%); mucositis (20%); epidermal cysts (20%); generalized papular drug reaction (14%); inflamed benign keratoses (11%); de novo actinic keratoses (6%); keratoacanthoma (6%); and leukocytoclastic vasculitis (2%). The number of CT affecting each patient ranged from 0 to 7 (mean 3.3). Sorafenib dosage was reduced in 27 patients secondary to CT and discontinued in 2 patients secondary to HFS. The increasing use of small molecule kinase inhibitors in oncologic therapy warrants further study to elucidate the role of inhibition of individual kinases in the pathogenesis of these cutaneous toxicities and to develop measures to alleviate associated morbidity that may limit their use.

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An animal model for evaluating intra-lesional injections designed to eliminate sub-cutaneous fat

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Phosphatidylcholine (PC) injections have been widely used to reduce localized fat deposits for cosmetic purposes and to destroy lipomas. Our previous study showed that sodium deoxycholate (DC) is the active component of the PC formulation causing fat dissolution. In order to image fat viability in living animals, we developed a mouse model that emits light exclusively from living adipocytes. These Fat-luc mice express firefly luciferase driven by an adipocyte specific promoter. We imaged viable fat by measuring luciferase catalyzed light emission using a deeply cooled CCD camera. Because luciferase requires ATP as a cofactor, only living adipocytes emit light and thus the number of viable adipocytes can be semi-quantitatively imaged by the intensity of light emitted from the animal. Deoxycholate injections (1%) reduced fat viability within one hour after injection. Early histological changes associated with DC mediated fat killing included extracellular fat globules and infiltration of the fat lobules by mononuclear cells. Three weeks following treatment, fat was partially replaced by fibrosis and granulation tissue.

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Acute febrile neutrophilic dermatosis mimicking necrotizing fasciitis

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A 61-year-old man with chronic lymphocytic leukemia (CLL) presented to the Emergency Department with typhlitis. Intravenous line placement in the right antecubital fossa required seven sticks and the patient was started on intravenous antibiotics for his typhlitis and Granulocyte Colony-Stimulating Factor (G-CSF) in light of his neutropenia. Within two days, the patient developed a large, tender, erythematous to violaceous, indurated plaque over the right antecubital fossa. A clinical diagnosis of necrotizing fasciitis was made. After multiple extensive debridements, lack of improvement with broad-spectrum antibiotics and failure to isolate a causative organism, dermatology was consulted and the patient was suspected to have a pathergic response at sites of IV placement and blood draws. Skin biopsy was consistent with neutrophilic dermatosis and the diagnosis of acute febrile neutrophilic dermatosis was made (AFND). Over the next twenty-four hours, the patient responded rapidly to high-dose prednisone, with resolution of his fevers, improvement of his mental status and ultimate resolution of his skin lesions. AFND is an uncommon neutrophilic dermatosis most often associated with hematologic and solid organ malignancies, but also seen with medications such as G-CSF, gastrointestinal and respiratory infections, and HIV. Its association with fever and leukocytosis can create a septic appearance. AFND, especially when associated with hematological malignancies, can present a panniculitis-like picture and can cause myonecrosis. AFND is an important and often unrecognized entity that can cause a significant degree of morbidity, especially when it mimics a process such as necrotizing fasciitis. We will discuss the role of cytokines, leukotaxis, and immune complexes in the pathogenesis of AFND as well as the features of malignancy and chemotherapeutic agents that contribute to this clinical picture. In addition, the underlying mechanism and differential diagnosis of pathergy will be reviewed.

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C2 and C7 dermatome current perception threshold abnormalities in alopecia areata

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Skin is one of the most densely innervated mammalian organs and the hair follicle is one of its most densely innervated structures. The hypothesis that "nerves" are involved in the pathogenesis of alopecia areata (AA) has been suggested for decades. In a recently completed study we asked if patients with extensive, long-standing AA had normal peripheral nerve function. Using an established electrodiagnostic test, the Neurometer CPT, an instrument which measures current perception threshold (CPT) we studied scalp nerve function by examining CPTs in unmyelinated C fibers (5 Hz), small myelinated A-delta fibers (250 Hz) and large myelinated A-beta fibers (2000 Hz) in dermatomes C2, C7 and Trigeminal V1. Thirty-nine control subjects (17 males 22 females)and 20 patients (9 males, 11 females), ages 24-65, participated in this protocol. No significant differences in the V1 dermatome were identified but peripheral nerve function was found to be altered in AA patients in both the C2 and C7 dermatomes. In a second study, we evaluated 7 AA patients and looked for CPT differences between affected and non-affected scalp and the V1, C2 and C7 dermatomes. Data were analyzed using 2-sided paired t-tests. There were no significant differences of CPTs between affected and non-affected scalp nor V1 versus C2. However, (i) abnormal nerve function in alopecia areata patients was observed with CPT analysis of V1 vs C7 at 2 kHz ($p<.01$) and (ii) significant differences were observed between CPTs ($p<.01$) of all three nerve fibers tested - C, A-delta, and A-beta - when C2 and C7 dermatomes were evaluated. These results confirm a hyperaesthesia in the scalp of AA patients. Targeting this dysfunction may prove to be useful in the management of this complex immune mediated disease.

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Cutaneous metal sensitivity as a potential risk factor for intra-coronary stent re-stenosis

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Coronary in stent restenosis (ISR) is a major health concern with approximately 120,000 cases reported annually in the United States. Metal sensitivity as a risk factor for ISR has been proposed and studied, but still remains controversial. We have investigated the association between metal sensitivity and angiographic measures of restenosis after stent implantation. Patients were recruited from those who presented to the Cleveland Clinic cardiac catheterization laboratory for clinically driven angiography following a prior percutaneous coronary intervention with placement of one or more non-drug eluting stainless steel stents. Epicutaneous patch testing was performed on 78 patients (with 116 stents) with available cineangiograms for quantitative coronary angiography. The primary angiographic endpoint included lumen loss (LL) in the stented segment, which is the difference between the minimum lumen diameter of the stented segment at baseline and follow-up angiography. The median time between baseline and follow-up angiograms was nine months. All patients underwent 48 hour closed patch testing with one or two concentrations of nickel, molybdenum, chromium, manganese and cobalt salts. Dermatologists blinded to the cardiac findings assessed the patch test results once between 72-120 hours. Wilcoxon rank sum tests, chi-square tests and multivariable linear regression analysis were used to analyze data which was adjusted for known risk factors of ISR. Results revealed that patients with positive reactions to nickel 2.5% in petrolatum had modestly higher lumen loss compared to patients with negative patch tests to nickel with a mean of 1.1 mm [SD 0.91] vs. 0.69 mm [SD 0.79] (median 1.2 mm vs. 0.58 mm) (p=0.09). No statistically significant trends were observed between angiographic endpoints and positive reactions to other tested metals. These findings suggest a potential association between contact sensitivity to nickel and ISR in stainless steel stents, however further investigation is warranted.

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Validation of a questionnaire related to pruritus in those undergoing hemodialysis

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Itching is a troublesome and still inadequately treated symptom in those in renal failure on hemodialysis. In order measure the effect of a given therapy for this condition, a simple and reliable instrument is needed. A questionnaire was constructed based on previously validated questionnaires relating to the presence, distribution, and discomfort related to itching. These 55 questions were asked of 50 dialysis patients who also completed a visual analogue scales (VAS) measuring the level of worst itching over the same two week period. 34 (70%) individuals were found to suffer from some degree of itch while 26 (52%) were bothered by the itching. We looked at specific questions relating to the effect of the itch on the quality of life, including on the level of personal anger, depression, embarrassment, frustration, ability to concentrate, to fall and to stay asleep, as well as the fear that the itch would last for ever. In each case using the answers divided into those who had the problem "never or rarely" or "sometimes, often, all the time", or were "not bothered/a little bothered or "somewhat/very/severely bothered" comparing with the medians of the relevant VAS scores using the Wilcoxon rank sum test, a high VAS was significantly associated with the frequency of or severe bother related to the specific symptom. It may be that simple VAS measurement is a sufficiently reliable instrument for the measurement of the therapy for hemodialysis related pruritus

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First-in-skin siRNA clinical trial for pachyonychia congenita

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A phase Ib clinical trial, utilizing the first short interfering RNA (siRNA)-based therapeutic approved for skin, has been initiated for treatment of pachyonychia congenita (PC). PC is an autosomal dominant disorder resulting from mutations in keratins 6a, 6b, 16, or 17. PC presents with thickened nails, an often disabling palmoplantar keratoderma, and other cutaneous manifestations. The siRNA, TD101, specifically and potently targets the K6a N171K mutation without appreciably affecting the wild-type K6a mRNA in tissue culture and animal models. Since mutant PC keratins induce pathology by a dominant negative mechanism, selective elimination of the mutant form of K6a has the potential to improve or reverse the clinical phenotype. This 14-week prospective, double-blind, split-body, placebo-controlled, dose-escalation trial evaluates safety and efficacy of TD101. TD101 and vehicle control are administered into symmetric plantar calluses by intralesional injection. Efficacy endpoints include: assessment of mutant and wild-type keratin mRNA levels via an allele-specific quantitative RT-qPCR assay; clinical examination; photography; and subjective evaluation utilizing a daily diary and quality of life measure. In addition to being the first-in-man treatment of skin with an siRNA therapeutic, this trial also represents the first time that siRNA has been used in a clinical setting to target a mutant gene. If this proof-of-principle human trial is successful, it will represent an enormous breakthrough for PC and the technologies utilized should be readily extendable to much larger numbers of patients suffering from other untreated dominant negative genetic disorders.

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Establishing an interface between a multidisciplinary psoriasis center and community-based participatory research in psoriasis: A new paradigm

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Community-based participatory research (CBPR) has evolved as an effective new research paradigm that attempts to make research a more inclusive and democratic process to address community-relevant research priorities. We present the first reported utilization of the CPBR approach in dermatology. We will describe the structural organization of the research network and the steps involved in establishing a partnership among researchers, clinicians, and community members. Our Multidisciplinary Psoriasis Center brings together specialists from several disciplines, including dermatology, rheumatology, psychiatry, nutrition and nursing, which offers a comprehensive approach to the management of psoriasis. Researchers and educators from the center interface with community-based members to address community-relevant research priorities. They are actively involved in liaising with the participating community sites, providing education and facilitating the development and growth of support groups. IRB-approved intake questionnaires have been administered to psoriasis patients in the multidisciplinary psoriasis center and in numerous dermatology and primary care clinics in communities throughout Northeast Ohio. A well-characterized database has been created which contains key demographic and clinical data on psoriasis patients. Information gathered from this database will facilitate community relevance of basic science, translational, clinical, and outcomes research. The CBPR in psoriasis is a novel model in dermatology, which can increase the rate of knowledge translation and shorten the path between research activity, adoption and dissemination of the results.

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Development and testing of Skindex-Teen: a quality-of-life instrument for adolescents with skin disease

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Validated quality of life instruments exist for both young children and adults with skin disease, however there is no such validated instrument for affected teenagers. To measure the effects of skin disease on the quality of life of affected adolescents, we developed a prototype 22-item instrument, Skindex-Teen, based on Skindex-29 and data from patients and expert clinicians. We hypothesized that the effects on quality of life could be explained by a conceptual framework with 4 subscales: physical health, emotional health, physical functioning and social functioning. This study reports the interim analysis results of Skindex-Teen with the purpose of refining its prototype version. One hundred thirty adolescents with various skin conditions responded to the instrument. Content validity of Skindex-Teen was verified by expert and patient, and by analyzing patients' responses to open-ended questions about their skin disease. The conceptual model was evaluated by using item-scale correlation, multidimensional scaling technique, and the residual principal component factor analysis as implemented in the Rasch analsyis. Results suggested the removal of two items, yielding a 20-item refined version of Skindex-Teen with strong evidence of content and construct validity. Accordingly, the conceptual framework has been refined to better reflect quality of life for teens with skin diseases. The current model consists of two main subscales: physical symptoms and social/emotional function. To increase the statistical power and evaluating the responsiveness of Skindex-Teen, recruitment is on-going. Anticipated refinement involves further validity analyses, including test-retest reliability, concurrent validity (using the Children's Dermatology Life Quality Index), and responsiveness at 4 weeks.

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Incidence and mortality of bullous pemphigoid and pemphigus vulgaris in the UK

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Little is known about the epidemiology of bullous pemphigoid and pemphigus vulgaris; our objectives were to determine the incidence and mortality of bullous pemphigoid and pemphigus vulgaris in the United Kingdom (UK). Computerised medical records from the Health Improvement Network (THIN), a large population-based UK general practice database were used to conduct a cohort analysis. Outcome measures were incidence and mortality rates compared to an age, sex and practice matched control population by calendar period, age group, gender, geographical region and degree of social deprivation. 869 and 138 people were identified with bullous pemphigoid and pemphigus vulgaris respectively. The median (interquartile range, IQR) age at presentation for bullous pemphigoid was 80 (15) years and 534 (62%) were female. The median (IQR) age at presentation for pemphigus vulgaris was 71 (30) years and 91 (66%) were female. Incidence rates for bullous pemphigoid and pemphigus vulgaris were 4.3 (95% confidence intervals, CI 4.0-4.6) and 0.7 (95% CI 0.6-0.8) per 100,000 person-years. The incidence of bullous pemphigoid increased over time with an average yearly increase of 17% (incidence rate ratio, IRR 1.2, 95% CI 1.1-1.2). There was an average yearly increase of 11% (IRR 1.1, 95% CI 1.0-1.2) for pemphigus vulgaris incidence. The risk of death for bullous pemphigoid cases was twice as high as for controls, adjusted hazard ratio, HR 2.3 (95% CI 2.0-2.7). In pemphigus vulgaris, the risk was three times higher, adjusted HR 3.3 (95% CI 2.2-5.1). Incidence rates of bullous pemphigoid and pemphigus vulgaris are increasing. The reasons for the changes in incidence are not clearly understood but have implications for identifying aetiological factors. Both disorders are associated with high risks of death. Previous estimates may have underestimated the mortality risks of these diseases.

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Development of a quality of life measure specific for epidermolysis bullosa

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Epidermolysis Bullosa (EB) is a genetic blistering condition with no specific treatment but is at the cusp of being treated with cell, protein or gene therapy. It has significant morbidity and mortality. An important outcome measure for determining the effectiveness of therapy is patient quality of life (QOL). Generic QOL indices used for skin disorders underestimate the impact of EB, as they do not adequately address the disability in EB. The aim of this study was to develop a valid and reliable QOL questionnaire to evaluate the physical and psychosocial impact of EB for use in trials of new therapies. Non-structured face-to-face interviews were conducted with 70 individuals, 26 older EB patients, 33 family members of young EB patients and 11 experts in EB to generate initial items. Content analysis revealed 168 items which were condensed into a pilot questionnaire of 53 questions which was sent to 29 of the original EB cohort. After wording revisions and data reduction a final questionnaire of 23 questions with a total score of 0-75 was developed, named the Quality of Life in Epidermolysis Bullosa (QOLEB) Questionnaire. This EB-specific tool was validated against established generic QOL indices (Dermatology Life Quality Index, DLQI), function (the Stanford Health Assessment Questionnaire, SHAQ) and psychosocial (Hospital Anxiety and Depression Score, HADS) parameters. The QOLEB was sent to 130 EB patients, with 89 (69%) responding, consistent with previous QOL studies in EB, ranging in age from 0-99 years. The mean scores and SD for EBS were 13.5(8.6), for DDEB 18.2(10.9), for JEB 34.2(22.5) and RDEB 36.9(11.7). The questionnaire developed was valid as it covered issues raised by EB sufferers and the scores above correlated with severity of EB subtype, with Cronbach's Alpha = 0.939 and no floor or ceiling effects. Correlation with the DLQI was 0.859, HADS 0.582 overall, up to 0.7 for RDEB, and for SHAQ, 0.765. Test-retest reliability was 0.843. This questionnaire will be a useful outcome measure for trials of new interventions in EB.

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Determinants of spot and wrinkle formation in German and Japanese women

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Caucasians and Japanese markedly differ in the onset and clinical manifestations of skin aging. The reasons for this are not known to date and may involve genetic and environmental factors. In order to address this problem we recently initiated an epidemiological study (JAGE=Extrinsic skin ageing in Japanese and German women) to assess determinants of spot and wrinkle formation in women from both ethnic groups living in Düsseldorf (Germany) and Nagoya (Japan). In Düsseldorf 53 German and 18 Japanese women (age between 35 to 65 years) were enrolled. Skin aging was measured by an established clinical score which was further validated by digital imaging analysis using a Robo Skin Analyser (RSA, Inforward Inc, Tokyo). In order to determine the potential impact of particle pollution on extrinsic skin aging, smoking habits and distance of home to main traffic roads were assessed by questionnaire. Data analysis of the German study revealed a strong correlation between the score and the RSA (r=0.79). In addition, the described ethnic differences concerning skin aging were confirmed, i.e. pigment spots occured early and wrinkles late in Japanese and vice versa in German women. Interestingly, German women, who ever smoked, showed a 2.1 fold (95% confidence interval (CI):1.0-4.3) age adjusted increase in pigment spots on cheeks as compared with never smoking women. Also, there was a 1.96 fold (95% CI:0.97-3.94) increase in spots on cheeks in German women living near main roads as compared with those living farer away. This effect was stronger in Japanese women. The influence of particle pollution on wrinkle formation was less pronounced in both ethnic groups. These first data indicate that particle pollution influences skin aging and that Japanese skin may be more susceptible. This conclusion, however, has to be confirmed by the ongoing Japanese part of this study.

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Prevelence of atopic dermatitis determined by clinical examination in Japanese adults

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There have been numerous studies on the prevalence of atopic dermatitis (AD) in children and adolescents, however, there have been few studies on AD in adults. The objective of this study was to evaluate the prevalence of AD based on regular health check-ups by dermatologists in Japanese adults. The target population was stuff members visiting the Health Service Center of Tokyo University and Kinki University in 2004 and 2007, respectively. A total of 2943 (1759 men and 1184 women) stuff members were examined in this study. The average age was 40.8+-11.3 years (men: 42.4+-11.5, women: 38.4+-10.6) ranging from 20 to 69 years. The prevalence of AD was 6.3% overall, and 9.4%, 8.3%, 4.8% and 2.5%, respectively, for those in their 20s, 30s, 40s and 50s / 60s. The prevalence of 30s was significantly higher than that of 40s ($p<0.005$), and that of 40s was significantly higher than that of 50s / 60s ($p<0.05$). The prevalence of women was higher than that of men (8.4% vs. 4.8%, $p<0.0001$). Overall, 78.9%, 17.3%, 2.7% and 1.1% of those afflicted were in the mild, moderate, severe and very severe groups, respectively. This is the first study of the prevalence of AD determined by clinical examination in the general adult Japanese population. In 1999, Plunkett et al. reported the prevalence of AD based on clinical examination by dermatologists in adults in central Victoria, Australia. A total of 1457 people (670 men and 787 women) whose ages ranged from 20 to 94 years were examined in their study. Interestingly, their results and ours suggested the same tendency: (i) the overall prevalence of adult AD was about 6%, (ii) the prevalence of women was higher than that of men, (iii) the prevalence decreased with age, and (iv) about 80% of AD cases were in the mild group. Although the number and community of the subjects in this study are limited, the result suggests that AD is one of the most common skin diseases not only in children and adolescents but also in adults especially in their 20s and 30s.

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The Dermatology Life Quality Index (DLQI) is a unidimensional instrument: further data in support

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The DLQI has been extensively validated, but the reports concerning its factor structure have been conficliting[1,2]. There has been no factor analysis of the DLQI in general dermatology patients in the UK. The aim of this study was to examine the factor structure of the DLQI in a cohort of general dermatology patients. This data is part of a larger study to develop a new dermatology-specific QoL instrument[3]. Adult patients attending the dermatology out-patient clinic of a secondary referral centre were asked to complete the DLQI. It contains 10 items, each with four possible responses. The scores of individual items (0-3) are added to yield a total scale score (0-30); higher score indicates poorer QoL. Exploratory factor analysis followed by oblique rotation was conducted on the responses to the DLQI. 111 adult patients (M=43.2%, F=56.8%) suffering from different skin disease (n=42) completed the DLQI. The mean age of patients was 46.1 year (±24.1) and the mean duration of their skin disease was 8.7 years (±10.3). The mean DLQI score was 8.2 (±7.2, median=5, rage=0-29) and there was no difference in the mean DLQI scores between males and females ($p=0.83$). Factor analysis of the data using the default criterion of an eigenvalue above 1.0 for extraction resulted in a one-factor solution having an eigenvalue of 5.39 while all of the remaining factors had eigenvalues less than 1.0. The component matrix showed that all of the 10 items had loadings above 0.50. Moreover, this one-factor solution accounted for 54% of the total variance which is above the minimal requirement (i.e. 50%). In conclusion, the results of this study yield empirical support for the one-factor structure of the DLQI and hence its unidimensional nature which in turn justifies using a summary score for the measure.

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Analysis of gene-gene interaction within the filaggrin pathway

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Filaggrin is a key protein for the development of the cornified envelope. Two common null mutations in the FLG gene have been firmly established as strong risk factors for atopic eczema (AE). KLK7 encodes the protease SSCE, which has been suggested to be involved in the processing of filaggrin. In a previous UK case-control study, an insertion in the 3'UTR of the KLK7 gene was reported to be associated with AE, but this association has not been replicated so far. SPINK5 is the gene defective in Netherton syndrome and encodes the serine proteinase inhibitor LEKTI, which has been implicated in the regulation of SSCE activity. An association of a SPINK5 SNP with AE has previously been reported, but could not be confirmed so far. In the present study we aimed at clarifying the role of these genetic variants for AE. In addition, considering the potential biological interactions between filaggrin, SSCE and LEKTI, we also sought to examine potential gene-gene interactions. Association of single genes as well as gene-gene interaction was analysed in a cohort of 486 German families as well as in a pooled collection of 773 cases and 2924 supernormal controls using various statistical approaches. The strong effect of FLG polymorphisms was confirmed, while there was no association of the KLK7 insertion. For SPINK5, we observed a weak association of the rs2303067 A-allele in the family collection only with a strong maternal effect. We did not find evidence for epistatic effects between FLG, KLK7 and SPINK5 variants that significantly predict AE risk. Thus, while our data underlines the exceptional importance of filaggrin deficiency for AE risk, it does not support the idea that its effect is dependent on KLK7 or SPINK5.

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A randomized, controlled trial evaluating adherence to topical medications using electronic monitoring and text-message reminders

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When it has been measured, adherence to topical agents among patients with dermatological conditions has been poor. Few innovations exist that accurately measure topical medication adherence or remind patients to use their topical products. We designed an electronic monitoring device that could be used for topical agents that sends an SMS message to a central server in real-time each time the tube is opened. Using this technology, we conducted a randomized, controlled trial assessing the impact of an automated reminder system on adherence rates to sunscreen, powered to detect a 15% difference in daily adherence. Seventy subjects were enrolled and asked to apply sunscreen daily for 6 weeks. Their adherence was transmitted in real-time by the electronic monitoring device. Half of the subjects were randomly assigned to receiving text-messages via cellular phones, and the other half did not receive reminders. The text-message reminders contained the daily weather information and a reminder to apply sunscreen; these messages were transmitted via a secured cellular network. At the end of the study period, the subjects who did not receive reminders had a mean daily adherence rate of 35.7% (95% CI: 28.1%-45.2%). In comparison, the subjects who received daily text-message reminders had a mean daily adherence rate of 69.0% (95% CI: 59.5%-78.3%). A two-sample t-test with equal variances assessing the difference in daily adherence between these two groups yielded a t-value of 5.16 and a p-value of <0.0001. These findings revealed that, without intervention, adherence to topical agents remains low. However, intervention with electronic reminders, such as text-messaging, can be an innovative and effective way to significantly increase patient adherence to topical agents.

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Surgical monotherapy versus surgery plus adjuvant radiotherapy in high-risk cutaneous squamous cell carcinoma: A systematic review of available outcome data

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Our objective was to compare reported outcomes for patients with high-risk cutaneous squamous cell carcinoma (CSCC) treated with surgical monotherapy (SM) versus surgery plus adjuvant radiotherapy (S+ART). The Medline database was searched for reports of high-risk CSCC published between January 1980 and June 2006. Studies were included that reported outcomes of local recurrence, metastasis (regional or distant), or disease-specific death by treatment modality (SM or S+ART). Outcome data, including number of patients observed, duration of observation, and number of outcomes reported, was recorded. The final analysis included 49 reports of 2449 cases of CSCC occurring in 2,333 patients. Only 91 (3.7%) of cases were treated with S+ART. Since the study designs varied widely, statistical meta-analysis was not possible. The data are presented in the form of a systematic review without statistical collapse of data. Poor outcomes were more likely in patients treated with S+ART as compared to SM. However, since data are from uncontrolled studies, it is likely that the baseline prognosis was worse in cases referred for S+ART. Regardless of treatment, patients had significantly better outcomes with clear surgical margins as compared to unreported margins [local recurrence: 5 vs. 8% (p=0.005), regional metastasis: 5 vs. 14% (p<0.001), distant metastasis 1 vs. 7% (p<0.001), disease-specific death 1 vs. 7% (p<0.001)]. Surgical monotherapy with clear margins results in high cure rates for high-risk CSCC. Outcome data for adjuvant radiotherapy are very limited. Development of reliable prognostic models may identify patients who could benefit from ART. Controlled studies on this subset of patients are needed.

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Creation of a new psoriasis quality of life measure from five preexistent instruments using Rasch analysis: the CALIPSO questionnaire

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The aim of our study was to create a new instrument to evaluate health-related quality of life (HRQoL) in patients with psoriasis, from five preexistent instruments. Two of them were dermatology-specific [Skindex-29 and Dermatology Life Quality Index (DLQI)], and three were psoriasis-specific [Psoriasis Disability Index (PDI), Psoriasis Life Stress Inventory (PLSI), and Impact of PSOriasis questionnaire (IPSO)]. A Rasch analysis was performed on those measures in a group of 936 patients with psoriasis. The total number of items was 85. First of all, each scale was analyzed separately. The number of categories was reduced from 5 or 4 to 3 in each instrument on the basis of threshold probability curves. Only 2 items were omitted on the basis of poor fit, both of them from the IPSO questionnaire. The reliability of the instruments with the new categories did not change significantly and it improved in the DLQI and the IPSO. The number of items was reduced in order to obtain a uniform and wide spread of the items, and a balance among the different subscales. The choice of one of the items with the same meaning was based on its fit, correlation and location. We obtained a final questionnaire of 30 items, 4 of them in the symptom subscale, 9 in the emotion one, 10 in functioning, and 7 in social aspects. Fifteen items came from the Skindex-29, 8 from the PLSI, 3 from the IPSO, 2 from the DLQI, and 2 from the PDI. This new instrument was called CALIPSO (Comprehensive Appraisal of Life Impact of PSOriasis). The CALIPSO is a new psoriasis HRQoL instrument, but at the same time it is founded on already existing accurate instruments. The advantage of this new instrument is that it fits to Rasch model, and that it summarizes information from five questionnaires into 30 items, thus balancing the capability of collecting good information and the acceptability for the patient (i.e., conciseness and clearness).

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Clinical and laboratory profile of elderly patients with pruritic dermatoses

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Itch and rash in the elderly remains a challenging clinical problem. Aside from diminished barrier function and degenerative nerve changes, immune changes in aging such as Th2 predominance predispose to pruritic dermatoses. A retrospective analysis of medical records of patients 60 yrs of age or older seen at a large, academic dermatology practice from 2005 to 2007 was performed. Out of 330 patients over 60, 149(45.2%) had a pruritic rash. Complete records were available for 118 of the 149 patients and were included in the study. Demographic profile, co-morbidities, results of screening laboratory tests as well as skin biopsy results were collected and analyzed. The average age was 72.8(range 60-94) yrs. Seventy-three (61.9%) were male. The average duration of symptoms was 38(range 0.25 to 600) months and the average number of visits was 6(range 1-43). As a group, eczematous dermatoses comprised 55.9%. Eczematous dermatitis NOS was the most common diagnosis (18.7%). Twenty-eight patients (23.7%) had a history of atopy and 51 (43%) gave a history of drug allergies, but only 3 were diagnosed with a drug-related dermatitis. The most common associated condition was a history of thyroid disease (21.2%). Those with subacute prurigo were 3 times more likely to have a history of thyroid disease (p=0.003), but those with eczematous dermatitis were half as likely (p=0.04). Among those with screening labs, the most common laboratory abnormality was relative lymphopenia (34%), followed by relative and absolute eosinophilia (28%). The most common finding on biopsy was spongiotic dermatitis (42.3%),followed by urticarial hypersensitivity reaction (12.9%). Eosinophils were common on biopsy (61%). In summary, pruritic dermatoses are a common, often chronic problem among elderly patients. Association with laboratory abnormalities and co-morbidities are described and may provide further clues to etiology and directions for further research.

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Quality of life and burden of cutaneous disease in ectodermal dysplasia

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The ectodermal dysplasias (ED) are a complex, heritable group of syndromes with cutaneous manifestation including hypohidrosis, alopecia, and infections of the skin and nails. Little is known about the overall cutaneous burden of disease in ED. Our objective was to examine how quality of life (QOL) relates with degree of skin involvement in ED. From July to November 2007, children and adult patients with ED were surveyed at national and regional conferences hosted by National Foundation for Ectodermal Dyslasias. Severity of hypohidrosis, scalp alopecia and fingernail involvement were measured using validated Likert scales. QOL was measured using the Child Dermatology Quality of Life Instrument (CDLQI) in children and the Skindex-29 in adults. These scores were translated into 5 bands for clinical interpretation (band 1= no effect on patient's life, band 2= small effect, band 3= moderate effect, band 4= very large effect, band 5= extremely large effect). These bands were then compared to the three respective skin severity measurements in three separate univariate linear regression models. A p-value of <0.05 was considered statistically significant. Of the 30 subjects with ED surveyed, 43% were female, 76% were Caucasian, 66% had hypohydrotic ED, and the mean (SD) age was 23.9 (26.6) years. Oral disease was reported in 97% of our participants, while 17% reported growth development, 3% reported cognitive development, and none reported reproductive abnormalities. Our QOL results indicate that 63% fell in band 1, 13% fell in band 2, 23% fell in band 3, and 3% fell in band 4. Our linear regression demonstrated a trend for predictors of QOL, although alopecia (p=0.061) and fingernail (p=0.191) as a predictor of QOL. Hypohidrosis (p=0.398) did not show this same trend. In our small study population, alopecia seems to have the greatest cutaneous impact on quality of life as compared to fingernail involvement or hypohidrosis. Further studies with greater numbers of subjects are needed to examine these trends.

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Incidence of stroke in patients with psoriasis: a population–based study

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Chronic TH-1 inflammation is associated with cardiovascular disease. Psoriasis patients have an increased risk for myocardial infarction independent of usual cardiovascular risk factors. However, to our knowledge, no study exists on the risk of stroke among psoriasis patients. We conducted a cohort study using data collected by general practitioners in the General Practice Research Database. Patients were classified as having psoriasis if they had a diagnostic code consistent with psoriasis and were subdivided into mild and severe disease groups based on whether they never or ever received systemic therapy consistent with severe psoriasis, respectively. Controls were from the same practices and start dates as the psoriasis patients. An incident stroke was noted if participants received a code for stroke after the start date and before transferring out of the practice or dying. We performed Cox regression analyses, adjusting for the major stroke risk factors (i.e., age, sex, diabetes, smoking, hypertension, hyperlipidemia, obesity). 496,666 controls were compared to 129,143 patients with mild psoriasis; 14,330 controls were compared to 3,603 patients with severe psoriasis. Mean follow-up was 3.4-4.4 years. The adjusted relative risk of stroke in mild and severe psoriasis was 1.06 (95%CI 1.01-1.11) and 1.43 (95%CI 1.10-1.87), respectively. The attributable risk of stroke in patients with mild psoriasis was 2.43 strokes/10,000 person-years, with an excess risk of 1 stroke per 4115 cases of mild psoriasis. The attributable risk of stroke in patients with severe psoriasis was 1.89 strokes/1,000 person-years, with an excess risk of 1 stroke per 530 cases of severe psoriasis. Our results suggest that patients with severe psoriasis have a clinically significant increased risk of stroke, independent of traditional stroke risk factors.

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Prevalence of atherosclerotic diseases in patients with psoriasis: a population–based study using the General Practice Research Database

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Psoriasis is a common immune-mediated disease of the skin. Chronic inflammation, specifically TH-1-mediated inflammation, has been linked to atherosclerotic diseases (ASDs). Other studies have also suggested that certain ASDs may be more prevalent among psoriasis patients. We conducted a period prevalence study using data collected by general practitioners participating in the General Practice Research Database. Patients were classified as having psoriasis if they received a diagnostic code consistent with psoriasis, and subdivided into mild and severe groups, based on receiving systemic therapy. Controls were selected from the same practices and start dates as the psoriasis patients. Patients were classified as having ASD if they ever received a code for ischemic heart disease (IHD), cerebrovascular disease (CVD), or peripheral vascular disease (PVD). Analyses were performed using conditional logistic regression, and adjustments were made considering major risk factors for atherosclerotic diseases (smoking, BMI, diabetes, hypertension, hyperlipidemia), person-time of observation, age, and gender. We identified 129,143 mild and 3,603 severe psoriasis patients, with 496,666 and 14,330 controls, respectively. Mild psoriasis patients had a higher adjusted odds of PVD (1.11 [1.04, 1.19]) than controls, as did severe psoriasis patients (1.16 [0.82, 1.64]). Severe psoriasis patients had higher adjusted odds of IHD (1.31 [1.08, 1.60]) and CVD (1.69 [1.27, 2.02]) than controls, whereas the odds among mild psoriasis patients were 1.03 [0.99, 1.08] and 1.00 [0.95, 1.05], respectively. Our study suggests that several ASDs may be associated with psoriasis, independent of common risk factors. Strongest associations were observed in cardiovascular and cerebrovascular diseases with severe psoriasis.

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Oestrogens, oral contraceptives and hormonal replacement therapy increase the incidence of cutaneous melanoma: a population based case control study

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Multiple studies showed conflicting results on the association between oral contraceptive use and the development of cutaneous melanoma (CM). We investigated the association between oestrogen use and CM incidence. Data from PHARMO and PALGA (a pharmacy and pathology database in the Netherlands) were linked. Women, ≥ 18 years, with a pathology report of a primary CM between January 1st 1991 and December 14th 2004 and ≥ 3 years of follow up prior to CM diagnosis were elligible cases. Controls were matched for age and geographic region. Multivariate logistic regression was used to calculate adjusted OR and 95% confidence interval (CI) for the association between CM incidence and oestrogen use, oral contraceptives (OC) and hormonal replacement therapy (HRT), separately. In total, 778 cases and 4.072 controls were included. CM risk was significantly associated with oestrogen use (≥ 1/2 year; adjusted OR=1.42, 95%CI: 1.19-1.69). This effect was cumulative dose dependent (p-trend < 0.001). CM risk was also significantly associated with the use of HRT (≥ 1/2 year: OR=2.08; 95% CI: 1.37-3.14) and OC (≥ 1/2 year: OR=1.28; 95% CI: 1.06-1.54). Our study suggests a cumulative dose dependent increased risk of CM with the use of oestrogens.

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Comparison of interview based and EuroQoL derived utilities among patients with chronic pruritus

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Utilities are a health economic technique for measuring disease burden, with a lower score (possible range: 0 to 1) indicating increased burden. Utilities have been traditionally measured by face-to-face (FTF) interviews which are labor and time intensive. The health status survey, the EuroQoL, also allows for estimation of utilities based on population derived values. Our objective was to compare the EuroQoL-derived utility to the gold standard FTF derived utility in patients with chronic pain. From June to September of 2007, adult patients with chronic itch, (duration >6 weeks) were recruited from the Emory dermatology clinics. Utilities were measured with the EuroQoL as well as via FTF interviews using the time trade off (TTO) method, for the overall health state and the symptom of pruritus. A paired t-test was used to compare the FTF and EuroQoL utilities. A p-value of <0.05 was considered statistically significant. Of the 53 subjects enrolled to date, 42% were female, 72% were Caucasian, and the mean (SD) age was 56(17) years. Pruritus severity was reported by 40% as mild, 42% as moderate, and 18% as severe. Among all subjects, the mean (SD) utilities derived from the FTF interview for their overall health state and for their pruritus state were 0.883 (0.239) and 0.931 (0.188), respectively. The mean (SD) EuroQoL derived utility for pruritus was lower at 0.815 (0.155) than both the FTF interview derived utilities (p<0.001). Chronic pruritus carries a significant burden as demonstrated by utilities. The EuroQoL derived utility appears to overestimate disease burden when compared to the gold standard FTF utility in this pruritus population. Further studies are warranted to investigate this trend.

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The burden of chronic pruritus is comparable to that of chronic pain using time trade-off derived health utilities

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Utilities are measures of quality of life that represent the strength of an individual's preferences for a specific health state. Utilities, ranging from 0 to 1, are an approach to the measurement of disease burden, with a score closer to 0 signifying greater burden. Our objective was to compare the utilities for symptoms of chronic pruritus to chronic pain. From June to November of 2007, adult patients with chronic pruritus and pain, (>6 weeks) were recruited from the dermatology clinic, the Center for Pain Management, and the Spine Center at Emory University. Utilities were derived via a face-to-face interview using the time trade off method. T-test and chi-squared test were used to compare continuous and categorical variables between pruritus and pain cohorts. Linear regression was used to evaluate predictors of utility values. Of the 53 subjects with pruritus, 42% were female, 72% were Caucasian, 74% were married, and the mean age was 56(17) years. The 138 subjects with pain did not statistically significantly differ from the pruritus cohort in the above demographic factors. Subjects with chronic itch compared to pain had a higher mean (SD) utility for their symptom (0.931(0.188) vs. 0.767(0.308), p<0.01, respectively). A larger percentage of chronic pain subjects reported a symptom severity of severe compared to the chronic pruritus subjects (31% vs. 17%, p<0.05). After controlling for symptom severity (as well as demographics and duration) in a linear regression, symptom type (pain or itch) did not significantly predict symptom utility score. We have demonstrated that chronic pruritus has a significant impact on quality of life as measured by utilities and is comparable to the burden of chronic pain. Further studies are warranted to investigate utilities in patients with chronic pruritus.

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Predictors of sleep disturbance in patients with psoriasis

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Psoriasis is a cutaneous disorder manifest by scaling plaques and associated inflammatory arthritis. Research indicates that sleep disturbance has deleterious effects on health, but the impact of psoriasis on sleep is unknown. The National Psoriasis Foundation (NPF) recently reported that 49% of patients indicated that their psoriasis interfered with sleep, and 11.3% reported that psoriasis interfered with sleep greater than 15 days per month. To determine what features of psoriasis associated with sleep disorder, logistic regression analysis of NPF survey data was conducted. Participants were identified from a random sample of the NPF database of over 32,500 individuals. Sampling from a pool of 3000 telephone and 3000 email participants in the spring and fall of 2005 yielded 854 responses; after excluding questionnaires with incomplete data, analysis was performed on responses from 428 subjects. Logistic regression was used to predict those who reported sleep interference within the last month versus those who did not. Predictors included disease severity, body mass index, age of onset of psoriasis, psoriatic arthritis, income, current therapy, and 12 quality of life questions. Psoriatic arthritis was found to be the most significant predictor of sleep disturbance (odds ratio=3.21). Itch, impact on emotional well-being, and pain were also significant predictors (OR 1.26, 1.18, and 1.12, respectively). Measures of severity including body surface area, treatments, and other quality of life questions were not significant predictors of sleep disruption. We conclude that symptoms of psoriatic disease and its emotional impact may contribute more to sleep interference than other measures of severity such as body surface area. Psoriasis and sleep disorder are both linked to increased risk of numerous co-morbidities, including cardiovascular disease, diabetes, and depression. Further research on the impact of psoriatic disease on sleep and its consequences is warranted.

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The Japanese version of Cutaneous Body Image scale: Translation and validation

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Cutaneous body image, defined as the individuals' mental perception of the appearance of their skin, hair, and nails, is an important psychodermatological element in skin diseases. To measure individuals' cutaneous body image, practical and accurate instrument is necessary. In this study, we translated into Japanese, Cataneous Body Image scale (CBIS), 7-item instrument originally created by Gupta et al.(J Invest Dermatol.2004;123:405-6) using forward- and back-translation method and evaluated the reliability and validity of the instrument by psychometric tests. One hundred and sixty-five dermatology patients (56.7% atopic dermatitis/eczema, 9.8% acne, 7.5% alopecia, 6.9% psoriasis, 19.1% skin tumor/fleck/other)(30 men and 135 women, age:37.9±15.2) and 298 healthy adults (64 men and 234 women, age:28.9±9.9) responded to the Japanese CBIS. The normal distribution of the CBIS scores was suggested (Anderson & Darling test, healthy adults:0.55, patients:0.71). The internal-consistency reliability of the instrument was high (Cronbach's α, healthy adults:0.88,patients:0.84).The CBIS-measure demonstrates good test-retest reliability (healthy adults:γ=0.92, p<0.0001, patients:γ=0.79, p<0.0001). Compared to the healthy adults(4.11±1.80), the CBIS scores among dermatology patients (3.18±1.69, p=0.000) were significantly low,indicating greater cutaneous body image dissatisfaction. A stepwise multiple regression analysis revealed that an emotional aspect of quality of life was the best predictor of cutaneous body image. These results suggest that the Japanese version of CBIS is a reliable and valid measure of cutaneous body image of Japanese dermatological patients.

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Sun exposure beliefs among public school students in Central New Jersey

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The goal of this study was to establish how beliefs about sun exposure vary by gender, age, race, and ethnicity. To answer these questions, a non-randomized, non-controlled survey of 1,214 public school students was conducted in 2006 in New Jersey. Overall 55% of respondents felt that tanning made people look more attractive. This belief was held by a statistically significant higher number of males vs. females (61% vs. 39%, p<0.001). Cultural differences were also apparent, with Caucasians more likely to think that tanning made people look more attractive (64% whites vs. 50% non-whites, p<0.001, 58% non-Asians vs. 40% Asians, p=0.001, and 57% non-black vs. 38% blacks, p<0.001.) Caucasians were also statistically significantly more likely to believe incorrectly that "when using sunscreen, you can tan without hurting your skin" (62% whites vs. 54% non-whites, p<0.001, 60% non-Asians vs. 45% Asians, p=0.002, and 58% non-blacks vs. 51% blacks, p<0.001.) Despite this erroneous belief, Caucasians were more likely to correctly understand that sun protection is still needed after a sunburn (67% white vs. 53% non-white, p<0.001, 62% non-Hispanic vs. 48% Hispanic, p<0.001). In addition to cultural differences, belief differences based on age were also striking, with older adolescents more often holding statistically significant erroneous beliefs. A statistically significant number of ages 14-19 vs. ages 10-13 erroneously believed "no sun protection is needed after being sunburned" (68% vs. 29%, p<0.001), "no sun protection is needed if it is cold outside" (60% vs. 9%, p<0.001), and "you can only tan after being sunburned" (35% vs. 5%, p<0.001). There were statistically significant gender differences in false beliefs but one gender did not dominate in terms total number of incorrect beliefs. Beliefs about sun exposure do vary according to ages, genders, races, and ethnicities. These differences should be taken into account when designing skin cancer educational programs for diverse audiences.

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Nanotechnology safety

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Nanotechnology comprises the study of particles smaller than 100 nm in size. Particles in this size range possess unique properties. For example, ZnO particles, normally opaque and greasy, vanish and have an elegant feel when broken down into nanoparticles. Nanomaterials are already used in consumergoods and applications are underway for the early detection, diagnosis, and therapy of disease. As with any new technology, there are pitfalls. For dermatology and contact dermatitis, these include the development of whole new classes of irritants, allergens, haptens, cross-reactants, and unanticipated particle-particle interactions which may lead to disease. Particles 7000 nm and smaller penetrate skin damaged or frequently flexed skin. Diseases such as atopic dermatitis, contact dermatitis, acne, seborrheic dermatitis, and psoriasis can make the skin even more permeable. Once particles enter the skin, they can do harm in a variety of ways. As particle volume decreases, surface reactive groups, which may be hydrophilic, hydrophobic or catalytic, become increasingly available. Reactions involving these surface groups create singlet oxygen species which cause mitochondrial dysfunction, lipid peroxidation, protein denaturation, epitope unmasking, and DNA damage. Nanomaterial toxicity can be influenced by host and environmental factors. For example, favorable host factors might be good antioxidant production, low levels of oxidative stress, perhaps an antioxidant diet, an intact cutaneous barrier, and good hygiene. Environmental temperature, humidity, air flow patterns may all govern levels of exposure. Climate change such as global warming might lead to increased atmospheric dispersal of nanomaterials worldwide. By 2012, some 2 million US workers will be exposed to nanomaterials. Many products containing nanomaterials are marketed to women of childbearing potential. The impact of these exposures to workers, and for maternal and fetal health will need to be investigated. Studies are needed gauge the risks of nanopollution in the environment. Studies are needed to determine if and how much nanoparticles are biomagnified in the food chain.

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Lipid peroxide is involved in induction of comedogenesis and inflammation in the progression of acne vulgaris

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In this study, reduction of comedogenesis and inflammatory erythema were observed in chronic acne vulgaris patients prescribed 5% phosphoric acid vitamin C treatment. Based on these results, we predicted that lipid peroxide (LPO) is involved in the progression of acne vulgaris and investigated the effects of LPO on the progression of acne vulgaris. To assess the oxidation state of follicular infundibulum with comedo, the amount of LPO, IL1α which induces abnormal keratinization in the follicular infundibulum, NFκB and GSH were measured in the contents of comedo and facial stratum corneum. Moreover, to investigate whether LPO is involved in the progression of acne vulgaris from comedogenesis to induction of inflammation, the amount of LPO was measured in the contents of each comedo classified on a five-grade scale reflecting the progression of acne vulgaris: Inflammatory Open Comedo (IOC), Non Inflammatory Open Comedo (NIOC), Inflammatory Closed Comedo (ICC), Non Inflammatory Closed Comedo (NICC) and Micro Comedo (MC). Those findings indicated that the amounts of LPO, IL1α and NFκB were significantly large, and GSH was significantly low in the contents of comedo compared with those of facial stratum corneum, and the amounts of LPO in IOC and ICC were significantly greater compared to those in NIOC, NICC and MC. Then, the effects of LPO or IL1α on abnormal proliferation and expression of K16, which is strongly expressed in follicular infundibulum with comedo and IL8, which is involved in the induction of inflammation in acne vulgaris, were examined in HaCaT keratinocytes. Those findings indicated that LPO or IL1α induced abnormal proliferation and up-regulation of IL1α, K16 and IL8 mRNA expressions. Based on all of those findings, we concluded that LPO is involved in up-regulation of IL1α followed by induction of comedogenesis and inflammation during the progression of acne vulgaris. We proposed a new model based on the involvement of LPO in the progression of acne vulgaris.

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Quantitative analysis of cutaneous *Malassezia* species in atopic dermatitis patients

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It has been recently reported that *Malassezia* spp. in cutaneous flora has the ability of contributing to the exacerbation of atopic dermatitis (AD). Although the genus *Malassezia* consists of 11 species, few detailed quantitative analyses of the *Malassezia* species in AD patients have been done. Recent study investigates the cutaneous *Malassezia* flora in AD patients. Sixty-six AD outpatients (20 males and 46 females of age 34±13) at Fujita Health University Hospital participated in the study conducted from June to September 2007. Samples collected by swab method were obtained from two skin sites on the face (forehead or cheek); and body (chest or back). *Malassezia* species were identified using the real-time PCR technique. For the control experiment, 40 clinically healthy individuals (20 males and 20 females of age 33±7) were also analyzed. The number of total *Malassezia* spp. (the value of the logarithm indicated for each cm2) in AD males was 2.8 (comparing to 2.5 in healthy ones) on the face, and 1.6 (2.7) on the body; 1.4 (0.7) on the face and 1.3 (1.7) on the body of AD females. The predominant species were *M. restricta* and *M. globosa* on the face and *M. globosa* on the body of AD males as well as healthy individuals. On the face of AD females, *M. globosa* was predominant, although this species was hardly detected on the face of healthy individuals. Only *M. globosa* was predominant on the body of AD females, although both *M. globosa* and *M. sympodialis* were detected in healthy individuals. *M. obtusa* was only detected in severe AD patients. Our findings suggest that cutaneous *Malassezia* flora in AD patients differs from that of healthy individuals. Moreover, it was clarified that the number of total *Malassezia* spp. and the predominant *Malassezia* species in AD patients differs depending on gender, skin sites, and the severity of symptoms.

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The comprehensive analysis of stratum corneum in the gluteal area of infants

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Diaper rash is the most common dermatological problem of infancy. It is commonly believed that the skin of infants is more sensitive than that of adults. However there are not sufficient data about gluetal skin of infant. In order to further understand diaper rash, this study evaluated the physical properties of gluetal and femoral skins of infants for one year, and analyzed seasonal and site-dependent variations in comparison with those of adults. Subjects were nine infants (aged between 3 to 31 months, Japanese) and their mothers. Cutaneous functions (skin capacitance, trans-epidermal water loss [TEWL], water retention capacity) and stratum corneum components (ceramide, sebum, NMF) were measured in the gluteal and femoral areas once every month for thirteen months (August 2006-August 2007). Site-dependent variation in infants demonstrated higher skin capacitance in the gluteal area than in the femoral area. Other than this, no site-dependent variation was found. As to the seasonal variation in the gluteal area of infants, skin capacitance decreased in the winter, TEWL increased in the summer, and water retention capacity increased in the winter. The epithelial desquamation was found in half of the infants in the gluteal area in the winter but was not found in adults at all. In the gluteal area of infants, water retention capacity was lower, and TEWL was higher than observed in adults. In contrast, the skin capacitance values of infants and adults were almost the same. As to the stratum corneum components, only the sebum and ceramide level were lower in infants. These data may suggest that the gluteal skin of infants has low cutaneous barrier function, which is one of factors that induce diaper rash.

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A large cohort study of nonsteroidal anti-inflammatory drug use and melanoma incidence

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Background: Laboratory data suggest that nonsteriodal anti-inflammatory drugs (NSAIDs) may have chemopreventative as well as therapeutic efficacy against melanoma, but there have been few published epidemiologic studies examining the association between NSAID use and melanoma risk. Methods: We examined whether NSAID use was associated with melanoma risk among 63,809 men and women who were participants of the VITamins and Lifestyle (VITAL) cohort study. Participants self-reported NSAID consumption over the past 10 years and melanoma risk factors on the baseline questionnaire. 349 incident melanomas were identified through linkage to the Surveillance, Epidemiology, and End Results (SEER) cancer registry after follow-up through 2005. Results: Cox regression models were used to estimate relative risks (RR) and 95% confidence intervals (CI) for NSAID use as categorized by overall use, duration of use, and dose of use. After adjusting for melanoma risk factors and indications for NSAID use, we did not detect a significant association between NSAID use and melanoma risk. Compared with non-use, there was no melanoma risk reduction detected with use (4+ days per week on average over the past 10 years) of any NSAIDs (RR=1.12, 95% CI = 0.84-1.48); any NSAIDs excluding low-dose aspirin (RR=1.03, 95% CI= 0.74-1.43); regular or extra-strength aspirin, (RR=1.10, 95% CI = 0.76-1.58); or non-aspirin NSAIDs (RR=1.22, 95% CI= 0.75-1.99). Moreover, NSAID use was not associated with tumor invasion (p=0.38), tumor thickness, (p=0.98) or risk of metastatasis (RR= 1.09; 95% CI= 0.32 - 3.62). Conclusion: NSAIDs may not have a substantial role in the chemoprevention of melanoma.

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Melanocortin 1 receptor variants and severity of facial skin photoageing in French Caucasian women

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Photoageing is mainly induced by long-term ultraviolet exposure. Besides, melanocortin 1 receptor (MC1R) gene is known to contribute to normal variation in human skin pigmentation and skin sensitivity to the sun. The links between the severity of facial skin photoageing and MC1R variants were studied on 530 French Caucasian middle-aged women. Three standardised facial photographs and a blood sample were taken from each woman. Then, a dermatologist graded the severity of photoageing from photographs using the Larnier's scale. The effects of MC1R polymorphisms on photoageing severity were investigated by logistic regressions. Fifteen MC1R variants were identified and the most common were V60L, V92M, R151C, R160W, R163Q, R142H, D294H, D84E, and I155T. Four rare variants showed an allelic frequency < 0.5%. 185 (35%) volunteers were wild type homozygote, 261 (49%) had one common variant, 78 (15%) had two common variants, and 6 (1%) had at least one rare variant. After adjustment on possible confounders (general and phenotypic data, self reported sun exposure intensity), the presence of common variants was interestingly a risk factor for photoageing expression severity (adjusted odds ratios [95% confidence interval] for two variants: 2.61 [1.36-5.01], and for "at least one major diminished function variant": 1.82 [1.05-3.14]). In addition, three major (D84E, R142H, and R151C) and a minor (V92M) variants showed specific effects. The links found between certain MC1R variants and the photoageing severity suggest that MC1R may be one of the genes playing a major role in facial skin photoageing expression in Caucasian population. However, further investigations are needed to clarify the exact contribution of these variants.

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Sagging of facial skin is associated with polymorphisms in MC1R gene and Ser422Gly polymorphism in ELN gene

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Sagging of facial skin is related to the gravitational force but also to environmental, lifestyle and intrinsic factors. To investigate the influence of endogenous and exogenous factors on sagging of facial skin, a study was performed on 530 females living in Paris area (44-70 years). A score based on four skin features (bag under the eyes, tissue slackening, drooping eyelids and naso-labial fold assessed using ordinal scales) was built to quantify the sagging of facial skin with Principal Component Analysis method. This score was then dichotomized as follow: individual score value >=third quartile defined as "severe sagging" versus "no severe sagging". Finally, effects on "severe sagging" of age, genetic data, tobacco status, Hormonal Replacement Therapy (HRT), skin colour, self-declared sun exposure intensity and Body Mass Index (BMI), were tested using logistic regression models. The genetic data consisted of eight genes: MC1R, P53, MMP1, MMP9, ELN, COL1A1, SLC24A5 and FLG genes. After adjustment on age, "obesity or overweight" (defined as BMI>=25kg/mc^2) and no intake of HRT were associated with a significantly higher risk to have a severe sagging of facial skin. Moreover, two genes were significantly linked with sagging. Women carrying the G allele (SG or GG) of ELN gene or carrying two MC1R variants had a higher risk to have a severe sagging of facial skin. In literature, a link was found between the Ser422Gly polymorphism in exon 20 of the gene-encoding elastin and the artery wall elasticity, but no association with the skin had been reported to date. Moreover, our findings suggest that MC1R, which is well-known to play a major role in human skin pigmentation and skin sensitivity to the sun, is also linked with sagging of facial skin.

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Factors affecting sun protection and skin cancer prevention knowledge, attitudes and practices in Asian-Americans living in Northern California

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Asian-Americans are a unique, heterogeneous and understudied minority in the United States: there are few reports of factors influencing sun protection and skin cancer prevention in this group. The purpose of our study is to investigate factors which affect knowledge, attitudes and practices of sun protection and skin cancer prevention in Asian-Americans. This was a voluntary survey study of Asian-American community groups primarily in Northern California using validated acculturation scales (e.g. Suinn-Lew) as well as sun protection and skin cancer survey instruments. Results were derived from 540 respondents with a response rate >65%. Multivariate logistic regression analysis controlling for age, sex and skin type showed that Westernization (e.g. increased generation in the United States, being raised outside of Asia, or self-identification as bicultural) was significantly associated with attitudes promoting sun exposure and behaviors which increase sun exposure (e.g. increased belief that tanning is attractive, increased belief that sunscreen is too much trouble to use, increased belief that sun protective clothing is unattractive, increased weekend sun exposure, increased deliberate sun exposure to get tan and increased tanning bed use), with p all <0.05. In conclusion, our data suggests that adoption of Western culture is associated with attitudes and behaviors which lead to increased sun exposure in Asian-Americans, and which may adversely impact dermatologic health.

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The prevalence of anti-bullous pemphigoid 180 and 230 antibodies in an Olmsted county population – an epidemiological study

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Bullous pemphigoid (BP) is the most common cutaneous autoimmune bullous disorder (ABD) with an annual incidence of about 6.6 new cases per one million of the population with the maximal incidence occurring in the 7th -8th decades of life. However, the prevalence of BP180 and BP230 autoantibodies (AAB) has not been studied in a population stratified by age. The primary objective of this study was to evaluate the prevalence of BP 180 (BP 180) and 230 (BP230) AAB in a population stratified by gender and decade of life, without known BP or other ABD. Stored serum from patients who were seen at a single facility was stratified by age and gender. Data was collected for patients grouped by decade from 20-90+ years with 40 samples (20 male and 20 female subjects) collected per decade with the exception of the 90-99 group, in which 57 samples were collected (33 female and 24 male). The presence of circulating BP180 and BP230 AAB was determined by ELISA and positive cases were examined further with indirect immunofluorescence (IIF). Regression models were fit to assess the relationship between AAB levels (after logarithmic transformation) and age. Of 337 subjects examined, 25 (7.4%) had one or both AAB detected. Adjusted for age, the geometric mean of the AAB levels did not vary significantly with gender (females vs. males: BP180, 1.44 vs 1.56: BP230, 1.47 vs. 1.52 U) and remained stable over all age groups. All serum samples that were positive by ELISA tested negative with IIF. There was no correlation between the presence of BP180 or 230 AAB and other autoimmune diseases. While BP has a higher incidence in the elderly population, the prevalence of BP180 and BP230 AAB does not appear to vary by gender or to increase significantly with age in a sample of the general population. These findings suggest the presence of other factors beyond circulating AAB that may play a role in the initiation of disease within a susceptible individual.

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A clinical epidemiologic study of vitiligo in southern Taiwan

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Background: Vitiligo is an important acquired depigmented disorder in Asian people. Most epidemiological data vary from studies to studies. Patients and families are always beset by high degree of positive family history and familiar aggregation. Objective: In order to realize the epidemiology of vitiligo in Taiwan, we evaluate 264 patients with clinical and/or histopathologic diagnoses of vitiligo during 2002 to 2006. Methods: We collected all new cases of vitiligo visiting our vitiligo special clinic over a 4-year period and evaluated by questionnaires, clinical observation and basic laboratory examinations. Results: 264 patients were enrolled in this study, including 105(39.8%) males and 159(60.2%) females were enrolled in this study. Vilitigo vulgaris was the most common type, followed by segmental, acrofacial, focal, in that order. The most common site of onset was the head/neck area (79.2%), followed by the limbs (56.7%). Leukotrichia was present in 79 patients (29.9%), while Koebner phenomenon was observed in 46 patients (17.4%). Halo nevi were observed in 1 patient (0.4%). There was a positive family history of vitiligo in 19 of subjects (7.2%) with confirmation of diagnoses by dermatologists. Six patients (2.3%) were noticed to relate with malignancy after general survey. Conclusions: Our study shows that the distribution of vitiligo is similar to other reports from other parts of the world. Precise confirmation of family histories and excluding those without confirmed diagnosis of vitiligo in family members can avoid taking common hypopigmentation diseases other than vitiligo into statistical analysis, which prevents further biased results of genetic epidemiologic studies and over-anxiety of patients who may request genetic counseling. Onset of vitiligo in middle- and old-age patients with rapid progression may remind us of the possibility of internal malignancy.

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Long term use of aspirin but not statins is associated with decreased risk of cutaneous melanoma: A case-control study

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Experimental and preliminary data in humans suggest that several commonly used medications might inhibit melanoma development and progression. In part because epidemiological data are either lacking or have conflicting results, chemoprevention trials for cutaneous melanoma (CM) have not been initiated. This case control study (CCS) examined the association between use of statins and NSAIDs and the development of CM. Methods: 400 CM cases were recruited within 3 months of diagnosis. Controls included 600 individuals matched for age, gender and neighborhood. All completed a telephone questionnaire that captured demographic characteristics, CM risk factors, and detailed drug exposure history. Odds ratios were calculated with testing for statistical significance based on the Chi square with test based 95% confidence intervals calculated. Results: The study completed accrual in 07/07 with a total 400 cases (207 M/193 F) and 600 controls (299 M/301F). Mean age were 59.37 and 59.12 years respectively. Risk factors for melanoma observed in this study were comparable to those previously observed in other CCS. Duration of exposure to NSAIDs, irrespective of frequency of use, demonstrated the following: < 2 years OR 0.84 (CI:0.53-1.33); 2-5 years OR 1.03 (CI:0.74-1.45), and > 5 years OR 0.55 (CI:0.44-0.75). Subgroup analyses showed that the risk reduction observed with > than 5 years of exposure was primarily driven by ASA use (> 5 years OR 0.56 (CI:0.39-0.79). No association between statin long term use and melanoma risk was observed (OR=0.95).Conclusions: Our data suggest that extended use of NSAIDs decreases the risk of CM development. This effect appears to be primarily limited to the use of ASA, which may by explained by the low prevalence of long term regular use of other NSAIDs with resultant insufficient power. Additional studies are warranted to investigate the potential role of ASA and other NSAIDs in the prevention of CM.

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Antibiotic exposure in infancy is a risk factor for incident atopic eczema in the second year of life. A population-based cohort study

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The so-called "microflora hypothesis" of allergic disease postulates that antibiotic exposure in infancy increases the risk of allergic diseases later in life. We aimed to investigate the association between antibiotic use in infancy and incident atopic eczema in children. To address the methodological issues of selection bias and reverse causation in previous studies of antibiotic use and the development of atopic eczema we conducted a population-based cohort study using the administrative outpatient health-care and prescription databases of Saxony, Germany. The study population consisted of all children born on 01.01.2003 who were not diagnosed with eczema during their first year of life (n=370). Exposure was defined as prescription of at least one course of antibiotics during the first year of life. The outcome of interest was incident atopic eczema in the second year of life. Antibiotic exposure during the first year of life was associated with an increased risk of incident atopic eczema in the second year of life (risk ratio [RR], 1.66; 95% confidence interval [95%CI] 0.95; 2.90). There was a positive dose response relationship (p for trend = 0.04) with higher risk of children receiving two or more courses of antibiotics (RR 2.11; 95%CI 1.05; 4.22) compared to one course of antibiotics within their first year of life (RR 1.45; 95%CI 0.76; 2.75). This study confirms the hypothesis that early exposure to antibiotics is a potential risk factor for the development of atopic eczema in childhood. Therefore, caution should prevail in the prescription of antibiotics in infancy, especially in infants with a positive family history of allergic diseases.

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Changes of lip morphology with age differ in Caucasian and Asian women

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Interventions to change lip morphology by injection of fillers have become a mainstay cosmetic surgery procedure in Western countries. In Asian populations, changes affecting the lip area with age are reportedly less pronounced than in Caucasians, however, up to now, this has never been measured objectively. The present study was conducted (1) to document and quantify age-related changes of lip morphology and (2) to investigate whether these changes are similar or not in populations with different ethnic background. Digital images of the face of 120 French, 120 Korean, 120 Japanese and 120 Chinese women, aged from 20 to 80, with no history of facial surgery, were taken for this study. Using digital image analysis method, several morphological parameters of the vermilion area were measured from the images: total lip surface, lip height (maximal and minimal), indexes of vertical and horizontal symmetry, as well as a newly defined index of lip shape. In all populations, similar significant relationships were found between most parameters and age. In French Caucasian women, the lip height and the total lip surface showed a significant decrease with age, whereas the index of lip shape increased, indicating that lips become flatter with age. The index of horizontal symmetry increased with age, while no association with age was found for the index of vertical symmetry. Similar phenomena were observed in the three Asian populations, but they were found to be less pronounced in Japanese and Chinese women, than in Caucasian and Korean women. Our study shows that although similar age-related changes in lip morphology occur in both Caucasian and Asian women, the intensity of these changes differs. These differences might be considered to better understand aesthetic concerns of Asian and Western women.

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Natural history and malignant progression of actinic keratoses: the VATTC Trial

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Actinic keratoses (AKs) are associated with annual treatment costs in the United States of over $1 billion and an estimated prevalence of 40 million affected patients. They are precursors of squamous cell carcinoma (SCC), but many aspects of their natural history are surprisingly controversial for such a common lesion, including the rate of progression to SCC, previously estimated at less than 0.1% per year. We quantified the evolution of clinically defined AKs on the face and ears in a closely monitored high risk US population: 169 participants at 1 center of a randomized tretinoin chemoprevention trial who were followed clinically and photographically every six months for up to 6 years (mean 3.5 years). No treatment of AKs was performed although sunscreen was supplied and biopsies were performed on lesions that were symptomatic or suggestive of malignancy. 7784 AKs were identified on the face and ears, 25% of which were noted at the initial visit. Most (55%) were not present at one year after initial diagnosis, and that number increased over time. Biopsies were performed on 411; of these, 30% were SCC, 18% basal cell carcinoma (BCC), and 39% AK. Overall, 1.1% progressed to malignancy at 1 year and 4.1% at 4 years. 0.6% and 2.6% progressed to SCC (75% invasive and 25% in situ) at 1 and 4 years, and the progression rate was higher for AKs present at the time of recruitment than for new lesions at subsequent examinations. AKs also were noted to progress to BCC: 0.5% at one year and 1.6% at 4 years. Most AKs followed for 2 or more years were absent at one or more interim examinations. Two-thirds of SCCs and 1/3 of BCCs in these patients arose from lesions previously diagnosed as AKs. We conclude that, contrary to prior assumptions, clinically defined AKs are precursors to both SCC and BCC, that the rate of progression in high risk individuals is much greater than previous estimates, and that clinical regression is very common.

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Validity, reliability, and sensitivity-to-change properties of the psoriatic arthritis screening and evaluation (pase) questionnaire

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The goal of this study was to validate the Psoriatic Arthritis Screening and Evaluation (PASE) questionnaire, a self-administered tool to screen individuals with psoriasis for symptoms of inflammatory arthritis. After institutional review board approval, we screened a total of 183 participants with known psoriasis and/or psoriatic arthritis (PsA) using the PASE questionnaire before initiation of systemic therapy. Rheumatologists diagnosed a total of 18.6% (34/183) with PsA and 15.3% (28/183) with osteoarthritis. Participants presented with an average age of 53.7 years and 43.6% were female. PASE total scores ranged from 15 to 69 (range 15-75). The PsA group had a median total score of 53 (25th and 75th percentiles, 50 and 62 respectively), whereas the non-PsA group had a median total score of 30 (25th and 75th percentile 19 and 42) ($p<0.0001$). The median total score of individuals diagnosed with osteoarthritis was 41.5, (25th and 75th percentile 31 and 54) and was significantly different from the median PASE total score for the PsA group ($p<0.006$). Receiver operator curves showed that a PASE total score of 47 was able to distinguish PsA from non-PsA patients with 88% sensitivity and 83% specificity. PASE demonstrated significant test-retest reliability as assessed by Pearson correlation coefficient ($r>0.4$). Sensitivity-to-change analysis revealed that PsA patients scored significantly lower on PASE after systemic therapy ($p<0.008$). We conclude that dermatologists may use the PASE questionnaire productively, both to screen psoriasis patients for active PsA and to evaluate patient responsiveness to systemic therapy.

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Reliability of ectodermal dysplasia alopecia and fingernail severity instruments

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The ectodermal dysplasias (ED) are a complex, heritable group of syndromes that affect derivatives of ectoderm- sweat glands, hair, nails, and teeth. With specific regards to the cutaneous manifestation of ED syndromes, many patients exhibit hypohidrosis, alopecia, and infections of the skin and nails. To our knowledge there are no objective severity scales that measure this cutaneous burden. Our primary objective was to develop reliable instruments to measure severity of alopecia and fingernail disease. We created two independent alopecia and fingernail 5-point Likert scales. In July 2007, subjects at the National Conference hosted by the National Foundation of Ectodermal Dysplasias were surveyed and asked to rate their degree of alopecia and fingernail involvement. Two independent healthcare professionals also completed these scales. In addition to the Likert fingernail scale, subjects and raters also defined how the nail was affected. All raters were blinded to each other's results. We utilized the intra-class correlation coefficient to test the reliability of the alopecia and fingernail scales between all raters. Twenty- eight participants were surveyed in sequential order. Their mean (SD) age was 27.8 (23.4) years, 56% were female, and 60% had hypohydrotic ED. The mean (SD) alopecia score was 2.83 (1.04) and the mean fingernail score was 1.86 (1.156), as rated by the subjects. Affected fingernails were defined by the raters as "absent, brittle, fragile, thin, spooned, and mishapened." An ICC 0.91 and 0.85 was achieved for the alopecia and fingernail scale, respectively. In our cohort, we demonstrated that our alopecia and fingernail scales are reliable measures of severity across health care raters as well as across patient and health care professionals. We propose that these scales are promising and in the future may be utilized in clinical trials.

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Development and reliability of a comprehensive questionnaire on the risk factors, epidemiology and comorbidities of psoriasis

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Psoriasis affects 2% of US population but there is little known about patient reported outcomes. We developed a psoriasis questionnaire (PsorQ) that enables us to gather comprehensive epidemiologic and clinical characteristics. The PsorQ emphasizes the patient's overall health and well-being, the self-assessment of psoriasis and cardiovascular risk factors. Recent studies show that psoriasis is a multi-system disease; however, very little comprehensive psoriasis database information is available in the US. Our PsorQ was formulated using a modified Delphi method with opinions from experts in dermatology, rheumatology, psychiatry, cardiology, nutrition and nursing. Along with medical and family history of psoriasis, our questionnaire includes the SF-12, body figural image and visual analog scale to measure the subject's perception of the disease. Pilot testing was done in the first 3 months and final consensus was reached based upon subject and investigator feedback. The 19 page PsorQ, available in paper or online which takes about 30 minutes to complete, was administered in several clinical sites in Northeast Ohio. To test for reliability, patients were randomly selected to complete a short version of the PsorQ two or more weeks after completing the full PsorQ. Of the 270 patients who completed the full PsorQ, 39 completed the shortened version (21 in paper and 18 online). To maintain consistency, patients answered the shortened version in the same format as they completed the full PsorQ. Test-retest reliability for medical and psoriasis history related items ranged from substantial to almost perfect agreement (Kappa 0.67-1), while family history related items ranged from moderate to almost perfect agreement (Kappa 0.48-1). In conclusion, our comprehensive PsorQ has good test-retest reliability, adequate internal consistency and stability over time, and is able to generate large amount of data.

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An assessment of dermatology term coverage in existing general medical terminologies and a comparison of two specialist-created dermatology lexicons

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Medical terminologies (MTs) are structured collections of terms used in medicine. MTs are integral components of many medical bioinformatics tools including electronic medical records and literature search programs. In this study two popular general MTs, SNOMED and UMLS, were compared with two specialty lexicons, the Dermatology Lexicon Project (DLP) and the British Association of Dermatologists' Index (BADI) in order to assess coverage of dermatology-domain specific terms. The hypothesis was that these specialist created lexicons would have greater coverage of domain specific terms than the general lexicons. DLP and BADI coverage within SNOMED and UMLS was evaluated using a lexical algorithm. SNOMED contained 42% (4246 terms) and 59% (3555 terms) of DLP and BADI entries respectively. DLP and BADI coverage in UMLS was 51% (5232 terms) and 65% (3917 terms) respectively. Lexical matching algorithms with manual verification were used to assess differences and similarities between the DLP and BADI. This analysis showed that both specialty lexicons could benefit from integration which would expand term coverage between 12% and 16%. The results of this study demonstrate that dermatology coverage is not robust in current popular general MTs. Integration of a combination of specialist-created dermatology lexicons could improve coverage substantially.

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US dermatology resident training satisfaction is positively associated with mentoring: Survey results from a national conference in 2005 and 2006

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We evaluated US dermatology resident satisfaction with training and mentorship using a written survey distributed at the Las Vegas Dermatology Seminar in 2005 and 2006. The main outcome measures were satisfaction and importance of 26 separate components of training, overall training satisfaction, satisfaction with the availability and quality of mentors in the program, and time spent outside the clinics and classroom with mentors. Fifty percent (57 respondents) and 54% (49 respondents) of dermatology residents attending in 2005 and 2006 responded. Surveys were also mailed to seminar invitees (all US senior dermatology residents) in 2006 yielding an additional 38 respondents to comprise a total of 144 respondents for both years. More than 3 out of 4 residents (77% and 76% in 2005 and 2006 respectively) scored training at or above 7 on a 10-point rating scale. Residents were most satisfied with peer teaching, medical dermatology training, pathology slide sessions and live patient conferences, and least satisfied with business management and dermatoscopy training. Discrepancies between perceived importance and satisfaction were greatest for business management, time for independent study and responsiveness to resident input. Resident training satisfaction was consistently associated with mentoring: Residents spending 30 (the median) or more minutes per month outside of clinics and the classroom with someone they defined as a mentor reported higher training satisfaction (8.0 vs. 7.2, p=0.02). Resident perceived program mentor availability ($p<0.01$) and mentor quality ($p<0.01$) were also positively associated with increased overall training satisfaction.

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Patients with multiple solid organ transplants have a higher risk of skin cancer

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The purpose was to assess whether multiple organ transplants predipsose patients to a higher risk of cutaneous malignancy, specifically melanoma and nonmelanoma skin cancer, than single organ transplants. From January to June 2007, the Northwestern University Departments of Organ Transplantation and Dermatology cooperatively developed a database of current information about patients transplanted more than 4 years ago. Transplants included kidney, liver, heart, lung, and pancreas transplants. For 320 patients, current information from multiple sources was supplemented by a comprehensive, approximately 30-minute phone interview. During this interview, demographic data was confirmed, and a complete medical and surgical history was elicited. Interview respondents also provided dates and types of skin cancer, if any, that they had developed since their transplants. The creation of the database and the interview process were approved by the Northwestern University IRB. The number of interviewed patients with two or more solid organ transplants who reported at least one cutaneous malignancy was compared to the number reporting at least one such malignancy among those patients who received only one transplant. Fisher's exact test was used to analyze the data. Of the 23 interviewed patients receiving 2 or more transplants, 6 (26.1%) reported having at least one post-transplant skin cancer. Of the 297 interviewed patients who had only one transplant, 23 (7.7%) had at least one post-transplant skin cancer. The difference between multiple and single organ transplant recipients was statistically significant ($p=0.01$). In conclusion, recipients of multiple solid organ transplants appear to have significantly higher risk of post-transplant skin cancer than patients who have had only a single transplant. The risk ratio in our sample was 3.4.

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Immunosuppression with mycofenolatemofetil in stead of azathioprine significantly reduces the risk of skin cancer in kidney transplant recipients

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Cumulative doses of immunosuppression play an important role in the development of squamous-cell carcinoma (SCC) and basal-cell carcinoma (BCC) in kidney transplant recipients (KTR). The immunosuppressive agents, however, may also have a different carcinogenic potential. Specifically, use of azathioprine (Aza) may be associated with an increased risk of SCC since Aza acts as a prodrug of thioguanine products, causing an accumulation of 6-thioguanine in cellular DNA. The resulting selective UVA photosensitivity leads to a cascade of reactions in the skin, ranging from the induction of oxidative stress and mutagenic DNA lesions, to the development of SCC. In a large retrospective follow-up study consisting of 1111 KTR who had received their first transplant between 1986 and 2006, using Cox proportional hazard analyses, we calculated the risk of SCC and BCC in KTR who were immunosuppressed with Aza in any combination; mycofenolatemofetil (MMF) in any combination; or cyclosporine (CsA) or tacrolimus (Tac) without Aza or MMF. Until 2007, 68 (6.1%) KTR developed altogether 102 SCC and 121 BCC. The cumulative incidence of skin cancer was 3% after 5 years; 6% after 10 years and 10% after 15 years of graft survival. Immunosuppression with MMF in any combination had lead to a 6-fold decreased risk of SCC compared with immunosuppression with Aza in any combination with a hazard ratio adjusted for age and sex of 0.15 (0.04;0.59). Immunosuppression with CsA or Tac without Aza or MMF had lead to a 3-fold decreased risk of SCC with an adjusted hazard ratio of 0.35 (0.15;0.84). The risk of BCC, however, was not significantly decreased in KTR using MMF, CsA or Tac. This study showed that immunosuppression with MMF, and to a lower degree also with CsA or Tac, in stead of Aza had lead to a significantly decreased risk of SCC, but that the risk of BCC was not modulated.

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The German network for systemic scleroderma: new data on disease subsets and organ involvement from more than 2000 patients

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Systemic scleroderma (SSc) is a rare, heterogeneous disease, which affects different organs and therefore requires interdisciplinary diagnostic and therapeutic management. The German Network for Systemic Scleroderma (DNSS) was founded three years ago for basic and clinical research. Presently, it comprises dermatologists, rheumatologists, pulmonologists and nephrologists from 40 medical centers. To improve detection and follow up of patients presenting with early stages of the disease or overlap-syndromes, patients were classified in 5 subsets, i.e. limited, diffuse systemic sclerosis, overlap-syndrome, undifferentiated Scleroderma and scleroderma sine scleroderma. Recent analyses revealed that 48% of patients suffer from limited SSc (lSSc), 31% from diffuse SSc (dSSc) and 11% of patients were diagnosed with an overlap-syndrome. 8% had an undifferentiated form while Scleroderma sine scleroderma was present in 1% of patients. Pulmonary fibrosis was significantly more frequent in dSSc than in lSSc (61% vs. 24%). Accordingly, pulmonary arterial hypertension was more common in dSSc (20%) compared to lSSc (14%). Muscular involvement was typical for overlap-syndromes (69%). The onset of initial skin changes, following first attacks of RP, occurred earlier in dSSc than in lSSc. A family history of rheumatic diseases was associated with early disease onset. Follow up data revealed that the prevalence of joint contractures, hypertension and diastolic dysfunction increases significantly within one year. In this register a classification of patients with disease manifestations characteristic of systemic sclerosis in 5 groups allows to include a broader spectrum of patients with features of systemic sclerosis and to gather new insights into disease evolution.

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A global, web-based patient survey tool profiling 171 patients with pemphigus vulgaris

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Although a strong HLA class II association is established in Pemphigus vulgaris (PV), a comprehensive understanding of the genetic and environmental factors predisposing to disease remains poor. To date, studies regarding the epidemiology of PV have focused on narrowly defined demographic populations with restricted data collection. To overcome limitations in patient recruitment due to the rarity of PV, we developed an anonymous, web-based survey instrument available globally. We collected demographic and epidemiologic data pertaining to a wide range of clinical parameters including disease phase and morphology, co-morbid disease, family history of autoimmunity and possible trigger factors. From 3/07-10/07 we received responses from 137 North American PV (NAPV) and 34 non-North American PV (nonNAPV) patients. We confirm a female predominance (m:f ratio 1:2.3) and an age of onset in the fifth decade of life. While the NAPV and nonNAPV populations were generally comparable in terms of disease activity, residence in North America was positively associated with mucocutaneous disease morphology (OR, 2.25; 95%CL, 1.02-4.93). Among all PV patients, 108 had a history of mucocutaneous, 37 of mucous only and 19 of cutaneous only lesions. Among co-morbid autoimmune diseases associated with PV, thyroid conditions were most frequent (9.4%). 87 PV patients reported one or more relatives with autoimmune disease (51.1% 1st degree, 32.8% 2nd degree, and 13.8% 3rd degree). Of the 64 participants reporting a significant infection prior to disease onset, 53% indicated a history of herpes viral group disease. 105 PV participants reported subjective trigger factors including emotional trauma/stress (46.7%), medication (15.4%) and surgical/dental procedures (12.3%). Our internet survey tool facilitates future longitudinal data collection to better trace risk factors associated with disease progression.

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Psoriatic arthritis: disposing, constitutive and provoking factors in 50 patients

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Psoriatic arthritis (PsA) is a debilitating disease. We examined the records of 50 patients to find relevant factors that may prevent manifestation of latent disease or an exacerbation and also to detect possible further signs in other organs and complications of PsA. Diagnosis of PsA was based radiographically and clinically. In 50 patients we found that PsA occurred in 2 peaks in the 5th and 7th decades of life. The gender distribution was age-dependent; up to 60 years of age males prevailed, between the ages of 60 and 90 PsA was evenly distributed among males and females. 21% (n=13) of patients had a positive family history for psoriasis. In 64% (14 of 22 cases) the patients had suffered from psoriasis vulgaris for an average duration of 18 years before PsA was diagnosed. The HLA system played a dominant role among the disposing factors. HLA-B27 (RR 6,9) and HLA-B39 (relative risk, RR 7.37) occurred most often in our PsA patients, moreover, we established the disposing factor HLA-B63 (RR 5.25; control group of blood donors RR 0.19, n=295). We found the constitutive factor overweight/obesity with a body mass index of >25 in 64%, the extracutaneous manifestations cardiovascular disease in 24%, liver disease in 24%, eye conditions in 6%; furthermore hyperuricemia in 20% and hyperlipidemia in 11%. As provoking factors we found abuse of nicotine in 43% and alcohol in 27%; 75% of patients exhibited signs of inflammation including elevated ESR in 67%, low serum iron in 22%, elevated CRP in 21%, leukocytosis in 16%, elevated gamma globulin in 14% and alpha-2 globulin in 5%. Infections including dermatologic, otorhinologic, ophthalmic or urologic were found in 41%. We conclude that in disposed patients constitutive factors are less central while provoking infections play the most important role in pathogenesis of PsA.

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Skin aging in Hispanic women living in Boston

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Hispanics make up 12.6% of the US population, and that number is expected to double by 2050. They comprise a heterogeneous group of individuals of Spanish descent with diverse skin colors and phototypes, parameters known to greatly influence photoaging. To study the rate of appearance of signs of skin aging in Hispanic women living in Boston, high quality digital images were taken from 114 self-identified Hispanic women aged 20 to 80 years originally from 15 countries of Central and South America. Women were excluded if they had active skin disease or had undergone photoaging treatments within 6 months. Most women were Fitzpatrick skin type III (46%) whereas 21% were skin type II and 28% were skin type IV. An investigator blinded to subject age graded the photographs using pre-defined 0-5 reference scales. Several differences in the frequency and severity of wrinkles, age spots (lentigines) and signs related to tissue slackening were noted, compared to previously studied French Caucasian, Japanese and Chinese women. In this sample of Hispanic women, only 18% presented age spots of grade >2 on the cheek, but melasma was seen in 40% of the women. In contrast, age spots were present in more than 50% of Japanese and Chinese women and tend to aggravate with age. Crow's feet wrinkles of grade ≥ 2 were seen in 50% and nasolabial folds of grade ≥ 2 in 77% of Hispanic women aged 35-49 years. This early aggravation of expression lines is very similar to what we observed in French women whereas it occurs 15 years later in Japanese. Our data demonstrate that significant differences exist in the rate of occurrence and/or severity for several age associated skin changes in Hispanic women. The most notable differences were the earlier and more pronounced expression lines, rather than age spots, compared to Asian subjects, despite a high frequency of skin type IV in the study population. These data have implications for optimal skin care.

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The burden of auto-immune disease: A pilot comparison of prevalence ratios in patients with psoriatic arthritis and psoriasis

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Objective: To examine whether psoriatic arthritis patients carry a higher auto-immune (AI) disease burden than patients with cutaneous psoriasis. Methods: Patients > 18 years old enrolled in a health claims database were classified by two clinical subtypes: psoriatic arthritis (PsA) and psoriasis only (PsO). Control subjects were matched 3:1 to psoriasis cases on age, gender, Census region, and length of previous medical insurance coverage. Other AI disease diagnoses were identified through ICD-9 codes. The association of other AI diseases with each psoriasis cohort was assessed using the prevalence ratio (PR). Results: PsA patients (n=3,066) had significantly higher prevalence risk for both ankylosing spondylitis (AS) and rheumatoid arthritis (RA) (22.4; [CI 8.66-57.95] and 25.07 [CI 21.07-29.84], respectively) versus controls. Psoriasis (PsO) was associated with a higher PR for three gastrointestinal diseases: Crohn's disease (1.37 [CI 1.08-1.74], ulcerative colitis (1.44 [CI 1.19-1.75], and inflammatory bowel disease (1.40[CI 1.20-1.63]) but PsA was not. A pulmonary fibrosis PR of 2.00 was observed for PsA (CI 1.31-3.05), but not for PsO. Conclusions: PsA patients carry a higher overall AI disease burden when compared to PsO patients. The differences in AI disease burden of PsA and PsO patients provide insight into potential hypothesis-generating associations of shared immunopathogenesis.

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Divorce/marriage ratio in patients with psoriasis compared to patients with other chronic medical conditions

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Patients with psoriasis suffer psychological disability affecting occupational, sexual and social relations. Previous studies have demonstrated that the impact of psoriasis on patients' quality of life (QoL) is on par with other chronic diseases such as diabetes and congestive heart failure. The QoL of partners and relatives of patients with psoriasis is also negatively impacted by the disease. Using the Research Patient Database Registry (RPDR), a centralized clinical data warehouse serving the Partners Healthcare system in Boston, MA, we measured the divorce/marriage ratio among patients with psoriasis and patients suffering from other chronic medical conditions over the period of 1997-2007. The ratio of divorced/married in patients with psoriasis was 1,335/12,619, 10.6%. (95% CI ±0.54%), N=22,828. Patients with other chronic diseases had divorced/married ratios as follows: Diabetes mellitus, type II 8,479/64,318, 13.2% (95% CI ±0.26%), N=117,389; Congestive heart failure 5,916/47,032, 12.6% (95% CI ±0.30%), N=88,388; Rheumatoid arthritis 2,254/18,234, 12.4% (95% CI ±0.48%), N=32,712; Multiple sclerosis 1,011/8,585, 11.8% (95% CI ±0.68%), N=14,298; Breast cancer 4,894/43,292, 11.3% (95% CI ±0.3%), N=72,158; Crohn's disease 608/6,305, 9.6% (95% CI ±0.73%), N=11,963; The divorce/marriage ratio among all patients over 18 years of age in the RPDR was 141,660/1,639,161, 8.6% (95% CI ±0.001%), N=3,358,669. The confidence interval for psoriasis overlaps with those of multiple sclerosis, breast cancer and Crohn's disease. These findings support prior observations that psoriasis has a significant negative impact on patients' relationships on par with other chronic, debilitating conditions. This pilot study is limited by possible differences in data gathering practices between various clinical settings as well diagnostic accuracy of the claims coding. This study's strengths derive from the large numbers of patients in the dataset. These preliminary findings suggest more research in this area may be useful in quantifying the psycho-social impact of psoriasis.

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Dermatology patient information on Google: An appraisal of the top three most highly ranked websites addressing each of the top ten most common dermatologic conditions

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Patients are increasingly retrieving medical information from the Internet. The top 10 most commonly diagnosed dermatologic conditions have been previously established based on ICD-9 coding data.(1) Google search results for these 10 diagnoses were archived on December 18, 2007. Contents were independently categorized according to target audience, website sponsorship, whether the site sold a treatment, the time of last update, the number of languages on the site, and whether website content had been certified as reliable. Of the 30 websites analyzed, 90% were targeted for a lay audience while 10% primarily contained technical information for professional reference. Multiple organizations hosted the websites (30% nonprofit medical organizations, 23% public domain (Wikipedia.org), 23% medical publishers, 13% private corporations, and 10% US government). Three of the 30 websites (10%) participated in direct to consumer advertising with two of these websites (7%) selling products directly to patients for acne treatment (Acne.org Regimen and Proactiv). 77% of the websites indicated a time of last update, (median 135 days, range 1-1074 days). There was an average of 5.9 language translations available (median 2, mode 1). The seven websites hosted by Wikipedia.org had the highest average number of language options (mean 16.3, non-Wikipedia site mean 2.7). 50% of websites displayed Health on the Net Foundation certification and 7% had other forms of information reliability certification. In conclusion, the top three Google search results for the most common dermatological conditions largely retrieved websites with certified information and sites that rarely sold commercial treatments. Furthermore, Wikipedia-hosted websites were prominent among highly ranked Google search results.

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Germline mutations in the fumarate hydratase gene confer a predisposition to uterine fibroids in women with hereditary leiomyomatosis and renal cell cancer

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Hereditary leiomyomatosis and renal cell cancer (HLRCC) is the autosomal dominant predisposition to uterine and cutaneous leiomyomas and renal cell cancer. Germline mutations in the fumarate hydratase (FH) gene predisposes to HLRCC. The objective is to investigate the risk of uterine fibroids and other reproductive risk factors in women with HLRCC. We conducted a family-based case-control study including 105 women from families with HLRCC from North America. A telephone interview was administered to all participants using a standardized questionnaire. Diagnosis of uterine fibroids was confirmed by pathological diagnosis and medical report review. DNA was was screened for germline mutations in the FH gene. Of 105 women, 77 reported a history of uterine fibroids and 75 had a germline FH mutation (FHmut+). The risk of fibroids in FHmut+ women was statistically significantly increased compared with FHmut- women (OR=7.6; 95% CI: 2.9-20.0) as it was among women clinically affected with HLRCC compared to those clinically unaffected with HLRCC (OR= 8.6; 95% CI: 3.1-24.0). The median age at fibroid diagnosis for FHmut+ women (28 years) was significantly younger than that for FHmut- women (38 years) (p=0.03). Women with either a germline mutation in FH or clinically affected with HLRCC reported younger age at menarche (p<0.04) compared to women FHmut- or clinically unaffected with HLRCC. Women with HLRCC were more likely to have had treatment for fibroids (OR=4.6; 95% CI: 1.4-15.8) including having had a hysterectomy (p=0.02) at an earlier age compared with women clinically unaffected with HLRCC. This study provides the first evidence that women with germline mutations in FH have an increased risk of developing uterine fibroids. These women also have an earlier age at fibroid diagnosis, and are more likely to have treatment for fibroids at a younger age than women without HLRCC in their families.

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Cutaneous soft tissue sarcoma incidence patterns in the surveillance, epidemiology, and end results program, 1978-2004: An analysis of 12,114 cases

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Cutaneous soft tissue sarcomas (CSTS) are a heterogenous group of mesenchymal neoplasms. No prior large population-based study has focused on CSTS. We analyzed the Surveillance, Epidemiology and End Results (SEER) program incidence rates and relative survival rates of CSTS according to race, gender, and histologic type, using the 2002 criteria of the WHO classification. Among residents of the 13 SEER registries, 12,114 (incidence rate 24.4/1,000,000 person-years) cases of CSTS were diagnosed during 1992-2004. KS accounted for 71.1% of cases. Other major CSTS included dermatofibrosarcoma protuberans (DFSP) 18.4%, malignant fibrous histiocytoma (MFH) 5.3%, leiomyosarcoma (LS) 2.2%, and angiosarcoma (AS) 1.6%. Of those with known stage, more than half had localized disease at diagnosis (DFSP 55%, AS 60%, LS 75%, MFH 78%), and 7% or fewer had distant stage disease. Overall age-adjusted CSTS incidence rates were highest among blacks (30.8/1,000,000 person-years) followed by whites (25.0), and American Indians (11.2), and lowest among Asian/Pacific Islanders (7.7). DFSP rates also were highest among blacks, whereas LS and AS rates were highest among whites. The male:female rate ratio (RR) ranged from 25.5 for KS, to 4.7 for MFH, 3.7 for LS, 2.0 for AS, and 0.9 for DFSP. The 5-year relative survival rates were 99% for patients with DFSP, 89% for MFH, 92% for LS and 45% for AS. Based on data from the nine SEER registries during the last three decades, KS rates among males increased more than thirty-fold during the 1980s before peaking around 1991, and subsequently declining rapidly due to HIV-associated KS and HAART, respectively. This pattern in KS was evident not only among those age 20-59 years but also among those age 60-69 years. During 1978-2004, LS and AS rates among whites increased exponentially. CSTS rates vary markedly by race, gender, and histological type, supporting that these tumors are etiologically distinct.

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Effects of sunless tanning products on tanning behaviors

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Sunless tanning products (STP) are an emerging alternative to ultraviolet (UV) tanning. To further characterize how STP are used in relation to UV tanning methods, a total of 415 women with an average age 28±12 years were surveyed. Subjects included 223 from community locations (including gyms, pools, and university dining facilities), in addition to 192 from 3 college sororities. Tanning methods among subjects in the past year included intentional tanning in the sun (71% of subjects), STP use (48%), and tanning bed use (26%) at least once. The striking majority of subjects (93%) believed that tanned skin is more attractive than untanned skin. Furthermore, 79% of subjects reported feeling better about themselves when they have a tan. STP use was observed among all ages of subjects, with 54% of subjects ages 18-25, 41% of ages 26-40, and 40% of those over age 40 using STP at least once in the past year. Subjects' skin colors were divided into three categories: very white/freckled (17%), white/olive (68%), and brown/black (15%). Use of STP at least once in the past year was observed in 56% of subjects with very white/freckled skin and in 54% of subjects with white/olive skin, as compared to only 14% of subjects with brown/black skin. Notably, a number of STP users reported decreased time tanning in the sun (35%) and in tanning beds (25%) because of STP use. Subjects were questioned regarding reasons for using or not using STP. The most cited reasons for using STP included beliefs that STP are safer than the sun and tanning beds. Dislike of product color and unevenness were the most cited reasons for not using STP. The desire for a tanned appearance remains strong. The study suggests that STP are beginning to be used in place of UV methods, especially among women who desire a tan but are wary of the dangers of UV tanning methods. Refinement of the appearance of STP tans may allow wider acceptance by the public, and possible further decreases in UV tanning.

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Knowledge, attitudes and behaviors of elementary school students regarding sun safety after one year of a skin cancer education program

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Florida has among the most ambient sunlight in the US. As a result, Florida's school children are at increased risk of excessive ultraviolet light exposure, the most important modifiable risk factor for skin cancer. As part of NIH sponsored research, the knowledge, attitudes and behaviors of 3rd to 5th graders toward sun safety and skin cancer prevention were assessed in a randomized controlled evaluation of a skin cancer education program. A total of six control schools and ten intervention schools in Palm Beach County, Florida, with over 5000 students, participated during the first year of implementing the SunSmart America curriculum. The students were administered items tapping their knowledge of sun safety as well as inquiring about their self-reported engagement in sun-safe behaviors. Results of a one-way analysis of variance (ANOVA) on the 16 schools showed that third, fourth and fifth graders in the intervention schools had significantly higher scores on the knowledge scale after exposure to the curriculum than did students in the control schools, who had no exposure to the curriculum ($F1, 14 = 5.329$, $p = .019$, $F1, 15 = 4.744$, $p = .024$ and $F1, 15 = 9.022$, $p = .005$ respectively). Students in intervention schools also obtained a higher overall mean score on the sun-safe behaviors scale than students in the control schools, which was statistically significant (p-values between 0.025 and 0.045). These results indicate that students in all grades exposed to the SunSmart America curriculum for one year demonstrate greater knowledge of facts related to skin cancer and its prevention than do students who are not presented the curriculum. Further work will determine whether multiple years of educational intervention will alter knowledge, attitudes and behaviors in elementary school children and produce long lasting attitudinal and behavioral change.

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The superior barrier function of pigmented skin can be attributed to melanosomal effects on stratum corneum acidification

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In this study, we report that skin types V/VI individuals display superior permeability barrier function and increased stratum corneum (SC) integrity, in comparison to skin types I/II. We examined two mechanisms that could account for this difference; 1) changes in the epidermal Ca2+ gradient or 2) altered acidification of the SC extracellular spaces. The Ca2+ gradient in both groups showed no difference, excluding local variations in Ca2+ concentration. We then explored SC acidification differences between the two groups. Since barrier recovery is a pH-dependent function, variations in SC pH could account for the functional differences between pigment extremes. Decreased surface pH and pH gradient have been noted previously in skin types V/VI vs. II/III. We hypothesized that these differences, in turn, could be secondary to variations in melanosome size, shape, distribution pattern and/or persistence, which occur among skin types, or to melanocyte biology and its effects on neighboring keratinocytes. To assess the role of melanosomes in SC acidification we used C57BL6/J-Mitf mi-vit/J mice, which have patches of pigmented and nonpigmented skin that enable them to serve as their own internal control. The barrier recovery was significantly faster in pigmented vs. nonpigmented C57BL6/J-Mitf mi-vit/J skin. Also, using fluorescence lifetime imaging microscopy (FLIM), a decreased pH was found in pigmented vs. nonpigmented skin. Finally, confocal microscopy studies using the pH sensitive probe SNARF-5F showed a lower pH in melanocytic dendrites extracted from skin types V/VI vs. I/II individuals. These results confirm that pigmented skin has superior barrier function to nonpigmented skin in both humans and in our mouse model, and that differences in barrier function correlate with SC pH differences. In addition, these acidification differences reflect pigment-type dependent melanosomal pH acidification.

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Over-expression of kallikrein 5 desensitises protease activated receptor 2 in keratinocytes - a possible pathomechanism of skin barrier dysfunction of AD

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There have been reports of kallikrein5 (KLK5) abnormality in skin diseases with impaired skin barrier including atopic dermatitis (AD), due to over desquamation. We have noticed that these skin diseases share another pathological feature, hyperproliferated keratinocytes. We speculate that KLK5 may target protease activated receptor 2 (PAR2) that could influence keratinocyte maturation. Two studies were performed in this paper: i) examination of the expression of KLK5 and PAR2 in normal and AD skin from affected and unaffected areas; ii) assessment of the relation of KLK5 to PAR2 by measuring the PAR2 dependent intracellular calcium mobility following addition of a PAR2 agonist in a keratinocyte model with stable over expression of KLK5 (KLK5 cells). The expression of KLK5 and PAR2 in AD skin showed abnormal distribution with cytoplasmic staining throughout the entire epidermis. There were increased levels of KLK5 in both affected and unaffected AD skin compared to normal skin ($p<0.01$). An increased expression of PAR2 was also observed in the unaffected area of AD skin ($p<0.05$), but in the affected area, the expression level of PAR2 was the same as in normal skin. These results suggest an over-expression of KLK5 in AD epidermis with an up-regulated PAR2 level in the early stage of disease, but not in the late stage. However, functional study on PAR2 calcium mobility showed a suppressed calcium mobilisation curve over time in the KLK5 cells model ($p<0.001$). The suppressed response curve was similar to the desensitisation curve of PAR2 with repeated application of exogenous trypsin. In conclusion, the long-term over-expression of KLK5 *in vivo* may not change the expression level of PAR2 but is able to desensitise PAR2, and therefore may change the intracellular signals mediated by PAR2, further disturbing the process of keratinocyte maturation and skin barrier formation.

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Neuronal nitric oxide synthase in epidermis is involved in peripheral circulation response to external pressure

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The source of nitric oxide (NO) in the peripheral circulation remains controversial. Since neuronal NO synthase (nNOS) is expressed in epidermal keratinocytes, we hypothesized that it might generate NO in response to mechanical stress. Skin blood flow in nNOS-/- mice after application of external pressure was significantly lower than in wild-type mice. Application of pressure on a skin organ culture caused enlargement of the cutaneous lymphatic vessels, and the enlargement was significantly lower for nNOS-/- than for wild-type mice. Pressure-induced NO release was also significantly lower in skin organ culture of nNOS-/- mice, compared with wild-type mice. External pressure induced NO release even from a reconstructed skin model containing only keratinocytes and fibroblasts. NO release from the skin organ culture declined within 30 minutes after cessation of pressure application. Intercellular calcium propagation was observed in response to hydraulic pressure in cultured keratinocytes, but not in cultured fibroblasts, or vascular or lymphatic endothelial cells. These results are consistent with the idea that NO generated by epidermal nNOS plays a role in the response of the peripheral circulation to mechanical stress.

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Two distinct populations of keratinocytes exist in the basal layer of human neonatal interfollicular epidermis

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The proliferative compartment of the epidermis is a self-renewing tissue, confined mainly to the basal layer. According to the current model of epidermal homeostasis this compartment contains two distinct populations of progenitors, stem cells and their progeny, transient amplifying cells. Recently, the current model of epidermal homeostasis was challenged. Based on studies in mouse tail epidermis, it was proposed that epidermal homeostasis involves only one type of progenitor cell (Clayton et al, 2007). In this study, we characterize two cell subsets in the basal layer of human neonatal interfollicular epidermis. We have previously isolated the subsets using surface markers: α6 integrin, a basal cell marker, and β2 microglobulin (Matic 2005). Cells with the α6+/MHCI- phenotype constitute a small fraction of the basal layer (0.5-2%) and are uniformly small with low granularity. This subset has a lower proportion of cycling cells in situ than cells with the α6+/MHCI+ phenotype, as determined by Ki67 expression (0.9% vs 3.9% respectively). Keratinocytes from both subsets exhibite self renewal and extensive proliferation in culture. Microarray analyses of cells isolated directly from tissue and sorted by FACS reveal that the two cell populations are characterized by unique gene expression profiles: the α6+/MHCI- cell subset is enriched in messenger RNAs encoding surface receptors, cell adhesion molecules and extracellular matrix proteins, among others, but the cells contain fewer transcripts encoding proteins with roles in the cell cycle, ribosome biosynthesis, splicing, protein translation and degradation, DNA replication, repair and chromosomal remodeling. The gene expression signature for α6+/MHCI- cells shows high similarity with the expression profile of mouse bulge cells, indicating that the α6+/MHCI- cell subset may be enriched for keratinocyte stem cells. We conclude that two phenotypicaly distinct populations with different gene expression profiles are able both to self renew and to extensively proliferate in culture.

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Efficacy of tetraiodothyroacetic acid (Tetrac) in a humanized mouse psoriasis model

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Thyroid hormone receptors expressed in human skin are believed to be involved in the regulation of epidermal proliferation and differentiation. Ligands of these receptors might possess a potential as anti-psoriatic agents. Tetrac (3,3′,5,5′-tetraiodothyroacetic acid) is a thyroid hormone analogue with reduced hormone activity as compared to thyroid hormone. The effectiveness of topical Tetrac in the treatment of psoriasis was tested in a humanized mouse model. Skin biopsies from volunteer psoriasis patients were transplanted onto immunodeficient BNX-mice. Mice with accepted transplants were treated with either Tetrac (0.05% and 0.5% in base cream), 0.05% betamethasone, 0.005% calcipotriol or placebo, applied once daily for 3 weeks (days -1 to 21). On day 0, PBMC (0.65 x 10E6 per animal) were injected into the autologous transplants. At the end of the study epidermal thickness, expression of HLA-DR, Ki-67 and cytokeratin-16 were evaluated. Treatment with betamethasone resulted in significant reduction of all parameters. Treatment with calcipotriol significantly reduced Ki-67 expression and resulted in a downward trend for epidermal thickness, while affecting HLA-DR and CK-16 expression only slightly. Treatment with either Tetrac dose altered HLA-DR expression significantly with 0.05% Tetrac being more effective. Expression of Ki-67 was only slightly lowered after treatment with 0.5% Tetrac and unchanged after treatment with 0.05%; neither dose had a substantial effect on CK-16 expression. The epidermal thickness was slightly reduced by 0.5% Tetrac with an efficacy comparable to that of calcipotriol albeit at a 100-fold higher concentration. However, the observation that epidermal thickness was significantly increased by 0.05% Tetrac points towards a rather narrow therapeutic window. While thyroid hormone receptor ligands may bear a therapeutic potential in psoriasis, further studies and the investigation of more receptor specific ligands for further assessment of this potential are needed.

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PPAR delta plays a pivotal role in keratinocyte differentiation

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Psoriasis vulgaris is a complex inflammatory skin disease involving T cell activation as well as hyperproliferation of keratinocytes and dysregulated keratinocyte differentiation. Previously, we have shown that activation of a nuclear hormone receptor peroxisome proliferator-activated receptor delta (PPARδ) augments proliferation in human keratinocytes. Numerous studies have shown a pro-differentiation effect of PPARδ in keratinocytes. However, whether or not the differentiation – state of keratinocytes, in turn, has an effect on the activity of PPARδ has not been studied to date. Here we report, that activation of PPARδ by the specific ligand LD-160541 in primary, basal – like keratinocytes (characterized with low involucrin expression, low granularity in the FACS side scatter profile, as well as high β1-integrin (CD29) expression) decreased CD29 membrane expression and increased transglutaminase expression whereas this was not the case, or even reversed in the more differentiated cells. Next, we confirmed that maintenance of HaCaT keratinocytes in calcium-free vs. high calcium level can serve as a model for differentiation – specific effects of PPARδ activation. Taken together, these data suggest that activation of PPARδ enhanced keratinocyte differentiation in non-differenciated keratinocytes whereas it blocked differentiation in differenciated cells, consistent with abnormal distribution of differentiation states in psoriatic epidermis.

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Keratinocyte production of chemorepellent semaphorin 3A is downregulated by histamine, PAR-2 agonist, and substance P differentially from their NGF/cytokine/CD54 expression

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Upward elongation of C fiber ending into the epidermis is a promoting factor for pruritus and is controlled by nerve growth factor (NGF) and oppositely by neuron repellent element-semaphorin 3A (sema 3A), both of which are released from keratinocytes. Pruritogenic receptors, including H1 receptor (H1R), proteinase-activated receptor 2 (PAR-2), and NK1 receptor, are expressed on keratinocytes. Each receptor is activated by histamine, SLIGRL, and substance P (SP), respectively. Upon stimulation with these substances, keratinocytes produce a variety of inflammation- and pruritus-related cytokines and neural mediators, NGF and sema 3A. To elucidate the mechanism underlying epidermal sensory nerve sprouting in allergic skin disorders, we cultured normal human epidermal keratinocytes and dermal fibroblasts as control with histamine, SLIGRL, and SP under various calcium concentrations. The expressions of NGF, sema 3A, and other mediators were assessed by real-time PCR, ELISA, or beads array. Histamine and SLIGRL strongly stimulated keratinocytes to produce IL-6, IL-8, GM-CSF and to express CD54 in a dose-dependent manner, while they unaffected the levels of NGF as well as CCL5 and CCL22. Of particular interest is the observation that the expression of sema 3A was remarkably downregulated by histamine, SLIGRL, and SP as calcium concentration increased (0.15-1.8 mM). In fibroblasts, however, sema 3A was upregulated by stimulation with histamine, SLIGRL, or SP. Our study shows that: (1) sema 3A expression in keratinocytes is uniquely downregulated by the stimulants compared to NGF and other molecules; (2) the expression of sema 3A is reciprocal between keratinocytes and dermal fibroblasts; and (3) high calcium condition is a factor to depress sema 3A production. It is suggested that the nerve sprouting occurs in the epidermis at least partly by virtue of the chemorepellent suppression in response to histamine, PAR-2 agonist, and SP.

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Autophagy during keratinization in normal human and psoriatic epidermis

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Dynamic remodeling of the cells occurs during the terminal differentiation (keratinization) of the keratinocytes in the normal human epidermis including de-nucleation. However, little is known about the contribution of autophagy system in the terminal differentiation of the human epidermis. We herein investigated autophagy during keratinization in the human epidermis. The expression of LC3-I and LC3-II increased during keratinization of cultured primary human keratinocytes in a time-dependent manner, indicating that autophagy might be activated during this process. Electron microscopic analysis revealed increased number of autophagic vacuoles during the differentiation. Endogenous LC3 expression in the prickle and granular layers of normal human skin, in which keratinization begins to occur before the loss of nuclei, was observed. In the skin of patients with psoriasis, decrease of endogenous LC3 expression was found in the granular layer of the thickened epidermis. Defect in autophagy in newborn mice results in thickened epidermis with fragile cornified layer, low hematoxylin-staining in the granular layer, and increases of keratin 10 and ubiquitin in the granular layer. These results indicated that autophagy plays an important role in the normal maturation of keratinocytes in the epidermis. As far as we are aware, this is the first report of investigating autophagy in human epidermis.

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Langerhans cell (LC) migration and sensory re-innervation of psoriatic and normal epidermis

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We studied the morphology of LCs and epidermal nerve fibers (ENFs) in psoriasis and determined the spatiotemporal pattern of sensory re-innervation and LC migration in normal and psoriatic skin using the suction skin blister method. Five 2 mm suction blisters were collected from a subject with psoriasis vulgaris from each of the following locations: psoriatic plaque on the left elbow, adjacent normal skin on the left elbow and normal skin on the left thigh. The wounds were covered with hydrocolloid dressing. Two days later a 3 mm blister was made over one of the 2 mm blister wounds. The other four 2 mm blister wounds were re-blistered on days 4, 8, 11 and 14. All specimens were double-immunostained using PGP 9.5 antibody (pan-neuronal marker) and CD1a antibody (specific marker for LCs) and studied using confocal microscopy. Observations on morphology and distribution of ENFs and LCs were possible because all epidermal cells and ENFs were contained within the intact unsectioned blister roof. LC size and shape varied more in the psoriatic plaque compared to nonlesional skin. ENFs were distributed evenly in normal epidermis, whereas in psoriatic plaque ENFs were either decreased in number in some areas or very dense and complex in others. However, the pattern of re-innervation and LC migration was similar in both normal and psoriatic skin. Initially, nerve fibers from the edges of the wound elongated towards newly epithelialized skin and, in later stages, nerve fibers from the underlying dermis re-entered epidermis. Migration of LCs towards the wounded area was first observed on day 4 and was completed by day 11 on the elbow for normal and psoriatic skin and by day 14 on the thigh. This study shows that newly epithelialized normal and psoriatic skin can be re-innervated and filled with LCs following a similar spatiotemporal pattern, despite differences in LC morphology and ENF density.

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Examining the communication between UVB damaged cutaneous nerve cells and dermal keratinocytes *in vitro*

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The exposure of the skin to UVB radiation is a stressful event and the skin has multiple innate defense mechanisms to counter this threat. For instance, oxidatively damaged nerve cells will express neuroglobin, a hexa-coordinate heme protein, to scavenge free radicals such as nitric oxide (NO). Likewise, keratinocytes will express various anti-oxidant enzymes such as superoxide dismutase (SOD), which will defend the cells against oxidative threats. None-the-less, cells will still express free radicals during excessive irradiation. A fundamental question that needs to be asked is; what do these cells communicate to one another during such stressful events? This paper will present results of an *in vitro* study in which dorsal root ganglion were irradiated with UVB radiation to elicit oxidative events such as release of nitric oxide and calcitonin gene related peptide (CGRP), a potent neuropeptide. Cell survival was also examined using the standard MTT assay. A plant-based neuroglobin mimic called phytoglobin was used as a nitric oxide scavenger to see if control of nitric oxide would influence CGRP expression and cell survival. Results of this study demonstrate that control of nitric oxide expression in irradiated nerve cells can influence CGRP expression and can also increase cell survival rates. Following this preliminary study, controlled amounts of the nerve cell growth media were added to cultures of normal human keratinocytes and the keratinocytes were allowed to interact with the nerve cell media for 24 hours. Following this treatment period, human microarrays (Affymetrix, Santa Clara, CA) were run on the keratinocytes to see what genes were influenced in the keratinocytes as a result of contact with the irradiated nerve cell growth media. It was found that several critical genes expressed by the keratinocytes including nitric oxide synthase (NOS1), SOD1, Transglutaminase 1 and 3 (TGM1 and TGM3), and Filaggrin (FLG) were clearly influenced by the damaged nerve cells.

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ATP2C1 is specifically localized in the basal layer of normal epidermis and its depletion triggers differentiation of keratinocytes via manganese starvation in the Golgi apparatus

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ATP2C1 is a calcium/manganese-ATPase localized in the Golgi apparatus. We examined the immunohistochemical distribution of ATP2C1 in normal epidermis and found that ATP2C1 is localized specifically in the basal cell layer, with levels below the level of detection in the suprabasal epidermis. To explore whether ATP2C1 expression is regulated in a differentiation-dependent manner, we examined the effect of differentiation induction on ATP2C1 expression in human keratinocytes. Neither detachment of keratinocytes from the culture dish nor treatment with a high concentration of calcium suppressed ATP2C1 expression, although both induced differentiation markers, K10 keratin and involucrin. Thus differentiation states of keratinocytes per se likely do not determine ATP2C1 expression. In contrast, knockdown of ATP2C1 by RNAi induced differentiation of cultured keratinocytes with an immediate up-regulation of K10 keratin and a delayed increase of involucrin. Abrogation of manganese accumulation in the Golgi apparatus by the manganese ionophore, Br-A23187, also induced these differentiation markers whereas a Ca2+ specific ionophore, A23187, did not induce K10 keratin expression. Similar induction of keratinocyte differentiation was observed when manganese-depleted media was used for keratinocyte culture. These findings suggest that reduction of manganese in the Golgi apparatus evoked by ATP2C1 depletion stimulates keratinocyte differentiation. Taken together, our results suggest that ATP2C1 is essential to keep basal keratinocytes in the undifferentiated state and that its reduction evokes differentiation and immediate up-location of basal keratinocyes to the suprabasal layers.

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Active transport of xenobiotics and allergens in epidermal keratinocytes is mediated by multidrug resistance related proteins

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Previous studies revealed that cells of the skin express a specific pattern of several efflux proteins such as ABCC1 and ABCC3-7. In this study we provide evidence for the functional efflux activity of these transport proteins in NHEKs using an in vitro transport assay. Therefore, efflux transport of various well-characterized radiolabeled substrates including eugenol, oestrone-3-sulfate, cyclosporine and dexpanthenol was inhibited by the specific MRP inhibitor indomethacin and the intracellular accumulation of these substrates was determined. Cells not treated with the inhibitor served as control. Treatment of cells with the inhibitor indomethacin in a concentration of 200 µM and 1 mM decreased the active efflux transport of eugenol (4-allyl-2-methoxyphenol) in NHEKs by an average of 35 %. Indomethacin strongly reduced the efflux of oestrone-3-sulfate in a concentration-dependent manner since the cells accumulated this steroid hormone 1,8-fold using 200 µM inhibitor and 2-fold using 1 mM indomethacin. Pretreatment of cells with indomethacin at 1mM concentration increased intracellular concentration for cyclosporine by 44%. In contrast, treatment of cells with indomethacin had no influence on the transport of tritium labeled dexpanthenol since no alteration of intracellular accumulation could be detected compared to control cells without inhibitor treatment. In addition to previous studies revealing the expression of efflux transport-associated proteins in human epidermal keratinocytes we are now able to prove the functional activity of these transport proteins in the active efflux of xenobiotics and allergens which are relevant for skin physiology, inflammation and treatment

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Organotypic cultures reproduce the epidermal permeability barrier and epidermal ionic gradients

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We developed and characterized a flexible human-skin-equivalent model system to study the regulation of pH and calcium ion gradients in human epidermis. Our model system allows for the deletion of specific proteins and the investigation of the effects that such deletions have on the structural and functional properties of the epidermis. Organotipic, three-dimensional cell cultures were grown from human keratinocytes on devitalized human dermis (Alloderm, LifeCell, NJ). Lanthanum staining and measurement of transepithelial electrical resistence were used to demonstrate the functionality of the epidermal permeability barrier. In order to detect spatial variations in [Ca+2] and pH we used the calcium sensitive fluorescent probe Calcium Green-5N and the pH sensitive fluorescent probe BCECF respectively (Molecular Probes, OR). Two-photon excitation was used to excite both dyes, thus allowing deep sample penetration, high sectioning effect and spatial resolution, and low out of focus photodamage. Fluorescence Lifetime Imaging (FLIM) measurements at different depths in the SC stained with BCECF yield a shorter average lifetime in the extracellular spaces as compared to SC intracellular domains. This acidification was not seen in the viable epidermis, demonstrating that these cultures effectively acidify their SC. FLIM measurements of samples stained with Calcium Green 5N show a short average lifetime, which indicates a low calcium concentration, in the extracellular and intracellular regions of the SC, in agreement with previous electron microscopy or x-ray emission studies in normal human and mouse skin. Below the SC, we observed an increase in the measured average lifetime value in the extracellular spaces, indicating an increase in the calcium concentration. In conclusion, we have shown that these three-dimensional cultures effectively mimic the calcium and hydrogen ion concentration distributions observed in human epidermis, and therefore represent a powerful tool to investigate the specific role played by single proteins in regulating these ionic gradients.

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WW domain-containing oxidoreductase expression in physiological and pathological cell death of human keratinocytes

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WW domain-containing oxidoreductase (WWOX), discovered in year 2000, is a novel candidate tumor suppressor and a proapoptotic protein. Evidence demonstrated that WOX1 is abundant in skin. The aim of this study was to examine the role of WWOX in apoptosis of keratinocytes. We examined the expression of WWOX in normal epidermis and apoptosis related cutaneous disorders using immunohistochemical analysis. We also performed TUNEL staining for degraded DNA, and active caspase-3 staining for apoptosis. UVB irradiation on HaCaT cells and mouse skin were performed to induce apoptotic sunburn cells, and the expression of WWOX was examined by western blotting and immunohistochemical analysis. We observed intense nuclear WWOX expression in the granular cell layer of normal epidermis, the companion layer of anagen hair follicle, and sebocytes at the center of the sebaceous glands. TUNEL–positive cells were also observed in the same areas, indicating that the programmed cell death (PCD) of normal epidermis and appendages is closely related to the expression of WWOX protein. However, active caspase-3 is not detected in normal epidermis. Thus, the physiological cell death during cornification is a caspase-3-independent PCD. In stress-induced apoptosis of keratinocyte, including different cutaneous disorders and UVB exposure, significant increased expression of WWOX were noted, and appeared to be caspase-dependent, as active caspase-3 cleavage product is observed in the apoptotic cells of these lesional skins. Our observations suggest that WWOX play an important role in both pathological and physiological cell deaths of human keratinocytes and cutaneous appendages.

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Neutral cysteine protease bleomycin hydrolase is essential for the formation of amino acids from deiminated filaggrin as natural moisturizing factor of the stratum corneum

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Filaggrin is a specific epidermal protein which is the precursor of the free amino acids as natural moisturizing factor (NMF) in the stratum corneum. Peptidyl arginine deiminase (PAD) in the epidermis seems to be involved in the terminal modification of filaggrin that relates to the disassembly with keratin intermediate filaments and the degradation of filaggrin to free amino acids. However, the degradation process from deiminated filaggrin to amino acids still remains unknown. In the present study, we studied to identify processing enzymes of the deiminated filaggrin *in vitro*. Deimination of filaggrin by PAD was not susceptible the degradation by almost proteases, in which calpain-1 predominantly degraded the deiminated filaggrin. Then, a strong citrulline aminopeptidase activity was detected in the newborn rat epidermis extract by a double layer fluorescence zymography and it was derived from neutral cysteine protease bleomycin hydrolase (BH). BH has amino-peptidase activity towards aminoacyl-β-naphthylamide derivatives of various amino acids and short peptides, and also exhibits intrinsic exo- and endo-peptidase activities. BH released amino acids from the calpain-degraded filaggrin peptides including a citrulline in time dependent manner. BH was only present in the subcorneal layers of rat normal epidermis by immunohistochemical staining. These results suggest that both calpain-1 and BH preferentially may acts as processing proteases of deiminated filaggrin in the mammalian epidermis.

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Expression profile of a profilaggrin-like protein, hornerin, in human skin disorders and in differentiating normal human keratinocytes

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Recently we identified human hornerin gene on chromosome 1q21 by homology search using a sequence covering the EF-hand domain of mouse hornein. Human hornerin consists of an EF-hand domain at the N-terminus followed by a large repetitive domain. These structural features of human hornerin are most similar to those of human profilaggrin, an essential protein for keratinization. The hornerin protein was detected in the granular cells of regenerating skin and psoriatic skin, but not in the normal skin (J. Biol. Chem. 280: 4696-4703, 2005). In the present study, we investigated expression of human hornerin in the skin of patients with skin disorders including atopic dermatitis and some kinds of keratodermia using antibodies against the repetitive domain of hornerin. Hornerin was expressed in granular cells of the cornified epithelia in some keratodermia such as actinic keratosis and lichen planus and in chronic lesions of atopic dermatitis. The positive signals of hornerin were observed as granules, some of which were also positive for profilaggrin as revealed by a double staining method. Furthermore, we examined the expression of hornerin in differentiating epidermal human keratinocytes in culture. Differentiation was induced by maintaining cells in progressively confluent culture for up to 7days. Hornerin or profilaggrin proteins were hardly detected in the growing cells but were induced in the differentiating cells. The induced hornerin protein was shown to be associated with presumptive keratohyalin granules. These results indicate that hornerin is involved in epithelial cornification and that it may play a partly overlapped but distinct role from profilaggrin in the molecular processes of cornification.

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Anti-obesity effects of curcumin are associated with activation of AMPK signaling pathway in 3T3-L1 adipocytes

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Metabolic disorders such as obesity are major obstacles in improving the average life span. Therapeutic approach using natural compounds has been proposed as a novel strategy for preventing metabolic disorders. Curcumin is one of the natural compounds that exert anti-proliferative, antioxidant, anti-inflammatory, anti-angiogenic and anti-tumor effects. However, the anti-obesity effects of curcumin remain unclear. Here, we investigated the anti-obesity effects of curcumin using cell culture systems. Our results showed that curcumin effectively inhibits adipogenesis in cultured 3T3-L1 adipocyte cells via inhibition of PPAR-gamma. Next, to find specific target molecules based on this result, we examined whether AMPK activation is involved in the anti-obesity effect of curcumin since several published papers have indicated that AMPK signaling is involved in the regulation of metabolic disorders. Our results showed that curcumin strongly activates AMPK in 3T3-L1 adipocytes and the anti-adipogenesis effect of curcumin is inhibited by AMPK inhibitor compound C as well as AMPK specific siRNA knockdown. Moreover, our data indicated that LKB1 and cellular calcium accumulation are important upstream signals for AMPK activation in response to curcumin treatment. AMPK-induced anti-adipogenesis is mediated by phosphorylation of acetyl-CoA carboxylase or ACC and negatively regulated by mTOR activation. Taken together, our results indicate that curcumin acts via AMPK signaling pathway as a potential candidate molecule for preventing metabolic disorders such as obesity.

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SCCA contributes to inflammatory cytokine induction and accelerates keratinocyte proliferation

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Previously, we demonstrated that the serpin squamous cell carcinoma antigen 1 (SCCA1) inhibits UV-induced apoptosis in keratinocytes (J Cell Biol. 172, 983-990, 2006). We also reported that SCCA1 expression levels were correlated with cell proliferation. In the present study, we tried to elucidate molecular mechanisms of SCCA involved in these reactions. We have already established SCCA1 over-expressed cell lines in 3T3/J2 cells (1 to 2800-fold), SCCA knocked down cell lines in HaCaT cell lines (siSCCA, less than 85% expression in mRNA and protein levels) and involucrin promoter-driven SCCA-transgenic mice. First, we carried out gene expression profiling analysis among these cell lines and SCCA-Tg mice using DNA microarray. EGF families were not significantly changed in all DNA microarray analyses. Characteristic features were shown in siSCCA cells that cell cycle-related and checkpoint- related genes were remarkably altered compared with control, which include growth arrest and DNA-damage-inducible protein (GADD), p27kip1, CDC26, cyclinD1, CDK2, S-phase kinase associated protein and CDC25. To test the possible role of SCCA in cell cycle, we assessed BrdU-7AAD FACS analysis. SiSCCA cells showed S-phase arrest indicating that SCCA is associated with S-phase checkpoint signaling. In other words, SCCA regulates epithelial cell cycle via selective alteration of cell cycle-related genes. Furthermore, DNA microarray analyses suggested possible involvement of SCCA in inflammation. Expression of IFN-γ,TNF superfamily, Il-1β, IL-6, IL-18 and CXCL2 were down-regulated in siSCCA. We confirmed modulation of these genes using quantitative PCR. Interestingly SCCA1 protein levels showed strong correlation with trans-epidermal water loss(P=0.88). SCCA1 amount was highly up-regulated in sun-exposed skin (90-fold), pollen-allergic skin (232-fold) and lesional psoriatic skin (466-fold) compared to the sun-protected skin. These data reveal diversified roles of SCCA in inflammation and abnormal proliferation in the skin.

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CGI-58 plays a crucial role in skin lipid barrier formation

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CGI-58 is the causative molecule underlying the disease Dorfman-Chanarin syndrome (DCS), an autosomal recessive inherited neutral lipid storage disease with apparent clinical features of ichthyosis. CGI-58 is identical to α/β-hydrolase domain-containing protein 5 (ABHD5) and belongs to the esterase/thioesterase/lipase subfamily of proteins structurally characterized by the presence of apha/beta-hydrolase folds. The purpose of the study is to elucidate the function of CGI-58 and the pathogenic mechanisms of ichthyosis in DCS. Using an antibody we produced, CGI-58 was found to be expressed in the upper epidermis, predominantly in the granular layer cells, as well as in neurons and hepatocytes. Immunoelectron microscopy revealed that CGI-58 was localized to the lamellar granules (LGs), which are lipid transporting and secreting granules in keratinocytes. CGI-58 expression was markedly reduced in harlequin ichthyosis patient epidermis that demonstrates defective LG formation. In the CGI-58 null DCS epidermis, punctate agggregations of glucosylceramide (a major lipid component of LGs) were identified. In the developing human epidermis, CGI-58 immunostaining was observed from 49 days estimated gestational age and *CGI-58* mRNA expression was up-regulated concurrent with epidermal stratification during development. In the cultured keratinocytes, *CGI-58* expression was up-regulated under keratinizing conditions (high Ca^{2+}) at both the mRNA and protein level. CGI-58 is associated with triacylglycerol hydrolysis. Therefore, our present results indicate that CGI-58 is expressed and packed into LGs during keratinization and likely plays crucial role(s) in keratinocyte LG lipid metabolism, contributing to skin lipid barrier formation.

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Biochemical analysis of keratin in the brittle nail

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The brittle nail is a common complaint characterized by cracking, peeling or fragility of the nail. Although the decreased water content and altered composition of lipid could be a cause of the brittle nail, its precise mechanism is not understood. We performed biochemical analyses of protein components of the brittle nail as well as the normal nail to investigate whether the brittleness of the nail is accompanied by the altered composition of the nail protein keratin. In one-dimensional SDS-PAGE of the extract of the clipped normal nail, two major bands of 44~48kDa or 55~60kDa and a few minor bands of 45kDa~60kDa were identified. Analysis of each band by LC-MS/MS confirmed that the two major bands of 44~48kDa or 55~60kDa were type I and type II hair keratin respectively, and that the minor bands were type I or type II cytokeratins. When the normal nail was cut parallel to the surface of the nail into three layers, type I and type II hair keratins were found in all three layers, while cytokeratins were found in the inner and the middle layer but not in the outer layer. When the extract of the clipped brittle nail was analyzed by SDS-PAGE, type I and type II hair keratins were found as is the case for the normal nail while faint or no bands of cytokeratins were observed in most cases. Western blot analysis revealed the presence of cytokearin 16 and cytokeratin 17 in the normal nail but not in most cases of the brittle nail. Interestingly, cytokeratins were very faint in the extract of the clipped nail from children of 1~5 years old. These results suggest that the presence of cytokeratins such as cytokeratins 16 and 17 in the distal end of the nail is closely associated with the brittleness of the nail, and that the inner and the middle layers of the nail that contain these cytokeratins may play an important role in functions of the nail to resist the mechanical and chemical insults.

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Decreased ceramide transport protein (CERT) function increased ceramide in UVB-induced apoptosis

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Increased cellular ceramide (Cer) accounts, in part, for ultraviolet B (UVB)-induced apoptosis in cultured human keratinocytes (KC), with increased glucosylCer (GlcCer) but not sphingomyelin (SM) generation in these cells. Given that the conversion of Cer to non-apoptotic metabolites (i.e., SM and GlcCer) protects cells from Cer-induced apoptosis, we hypothesize that failed upregulation of SM production contributes to Cer accumulation following UVB irradiation (UVBR). Since both SM synthase and GlcCer synthase activities are decreased in UVB-irradiated-KC, we investigated whether alteration in the function of Cer transport protein (or CERT), required for SM synthesis, occur in UVB-irradiated-KC. Fluorescent-labeled-Cer relocation to Golgi was diminished after UVBR, consistent with decreased CERT function, with the CERT inhibitor, HPA-12 yielding an equivalent effect. UVBR concurrently induced the rapid formation of a stable CERT homotrimer complex in KC, evidenced by Western immunoblot and mass spectrometry analyses, a finding replicated in HeLa, HEK293T, and HaCaT, cells and in whole murine epidermis. Cer binding activity was decreased in recombinant CERT proteins containing the UVB-induced homotrimer. The middle region domain of the CERT protein is required for the homotrimer formation, while neither the PH (Golgi-binding) nor the START (Cer-binding) domains are involved. Finally, similar to that in UVB-irradiated-KC, HPA-12 blockade of CERT function increased KC apoptosis in association with increased Cer accumulation and decreased SM synthesis. Thus, UVB-induced CERT homotrimer formation accounts, at least in part, for apoptosis in UVB-irradiated-KC due to failed up-regulation of SM synthesis, revealing that formation of inactive CERT attenuates a key metabolic protective mechanism against Cer-induced apoptosis in KC.

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Skin histomorphological correlation with optical coherence tomography (OCT)

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Claim support for cosmetic products should be based on scientific evidence. Optical Coherence Tomography (OCT) is a promising non-contact, non-destructive diagnostic technique for skin imaging in vivo. The purpose of this study is to endorse OCT as a skin morphological analysis technique with the potential to substitute invasive procedures such as punch biopsies with similar accuracy as histological analysis. For this purpose, ear pigskin were sewed to copper rings and a surgical suture was used as a fixed marker. Our OCT device has a Ti:Al2O3 laser with a central wavelength of 800nm, transverse resolution of 10μm and longitudinal resolution of 10μm. We collected series of OCT images oriented at right angles to the course of the suture 1mm after the entrance of the suture. Samples were embedded in paraffin to obtain histological slices with 6μm thick and stained with hematoxylin and eosin for comparison purposes. We were able to positively identify the epidermal layers such as stratum corneum, deeper epidermal structures, dermal papillae and dermal-epidermal junctions both in OCT and in histology. The thickness of the stratum corneum and epidermis measured by OCT and histology were not statistically different, confirming thus the viability of OCT for skin analysis. Our method may provide quantitative and histological information the potential of efficacy of cosmetic and or cosmeceutical treatments on different skin layers. Thus, OCT imaging appears to be a reliable, quick and non invasive technique to analyze several skin treatments. OCT could represent a new innovative way to diagnosis and detect benefits of topical treatments.

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Mouse epidermal keratinocytes express Sema4D/CD100 and its receptor CD72 is expressed on Langerhans cells

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Semaphorins are secreted and membrane-associated proteins that are initially described to regulate development of neuronal axons. There are currently descriptions of more than 30 types of semaphorins in diverse organisms like viruses and humans. These proteins are classsified into eight classes based on their structural elements and amino-acid sequence similarity. Recently, it is becoming clear that semaphorins play important roles not only in establishing a neurological synapse, but also in organogenesis, vascular growth and neoplastic transformation. In addition, roles of semaphorins in the immune cell regulation have been identified. For example, Sema4D/CD100, which is expressed abundantly on the surface of T cells, is involved in the activation of B cells and dendritic cells (DC). Here we screened semaphorin mRNA expression in murine primary keratinocytes (KC) and murine KC cell line PAM212 by RT-PCR. Murine primary KC expressed Sema 3C, D, Sema 4A, D, Sema5A mRNA and PAM212 expressed Sema 3B, C, E, F, Sema4A, D, G, Sema 5A, Sema 6A, C mRNA. Among these, we focused on immune semaphorin, Sema4D. Murine primary KC and PAM212 expressed Sema4D in mRNA and protein levels, which were confirmed by RT-PCR and Western blot or flow cytometry, respectively. Treatment of PAM212 with TNF-α plus IFN-γ downregulated the cell surface expression of Sema4D but increased the secretion of Sema4D. EDTA or PMSF further downregulated the cell surface expression of Sema4D on TNF-α plus IFN-γ-treated PAM212. Interestingly, CD72 protein, which is the receptor of Sema4D, was expressed on the cell surface of cultured Langerhans cells (LC). Our results indicate the possible role of semaphorins in the skin immune regulation through LC-KC or T cell-KC interaction.

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ELOVL4 is required to generate epidermal-unique omega-O-(acyl)ceramides that form two structures critical for the epidermal permeability barrier

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ELOVL4 (a member of the fatty acid [FA] elongation complex) is required both for very long-chain FA (VLFA) and omega(ω)-O-acylceramide (acylCer) formation, and for normal permeability barrier function in mammalian stratum corneum (SC). Here, we investigated whether these acylCer are required for the formation of two unique VLFA-containing structures that appear critical for SC permeability barrier formation: 1) the corneocyte lipid envelope (CLE) consisting of ω-hydroxy (OH) Cer covalently bound to the outer surface of the corneocyte envelope, and thought to function as a 'scaffold' for SC extracellular lamellae; and 2) a long periodicity phase (≈13nm) repeat structure within the extracellular lamellae (evident with X-ray diffraction). Ultrastructural studies revealed not only a lack of normal lamellar membranes, but also an absence of CLE structures in homozygous mutant ELOVL4 knock-in mouse (k/i) SC, while lipid analysis confirmed the near-complete lack of bound ω-OH Cer (i.e., CLE) in k/i SC. X-ray diffraction analysis revealed an absence of long periodicity phase repeats in k/i SC. Finally, mass spectrometry structural analysis of epidermal Cer/glucosylCer species revealed diminished VLFA ω-OH FA molecular species (>C30), but identified shorter chain length (C18-26) amide-linked ω-OH FA of Cer/glucosylCer in k/i. Together, these studies show that ELOVL4-generated VLFA are required for acylCer, although shorter chain ω-OH Cer can be formed. Thus, VLFA(>C28) synthesis catalyzed by ELOVL4 is a key metabolic step in forming both the CLE and the characteristic long periodicity phase in SC extracellular lamellar membranes, both of which are critical for permeability barrier function in mammalian epidermis.

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Mice with deficiency in epidermal filaggrin display heightened susceptibility to hapten-induced atopic dermatitis.<b/>

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Humans with loss-of-function mutations in the structural skin protein filaggrin (FLG) may exhibit ichthyosis vulgaris and/or an increased propensity for atopic dermatitis (AD). To test the validity of the outside-to-inside hypothesis of AD pathogenesis, we have determined whether FLG deficiency in mice predisposes to development of an atopic response following hapten challenge. Flaky-tail (Ft) mice express a truncated form of FLG that does not undergo further processing. They exhibit generalized ichthyosis at birth that persists on the tail through adulthood without evidence of inflammation. Introduction of the Matted gene into Ft mice (MAFT) facilitates genotyping without visible alteration of the phenotype. In normal hairless mice, repeated topical challenge with the hapten oxazolone (Ox) elicits an AD-like dermatosis characterized by barrier impairment, erythema, scaling, epidermal hyperplasia, a Th2-dominant dermal lymphocytic infiltrate and increased serum IgE. In this study, a cohort of adult MAFT and C57/BL6 (Wt) mice were sensitized to Ox and then challenged every other day for 10 total challenges. Erythema, scaling and excoriations were notably increased on MAFT but not Wt skin following Ox treatment. These gross changes were associated with a CD3+, CRTh2+ lymphocytic infiltrate upon histologic examination as well as 10-fold greater serum IgE levels in MAFT versus Wt mice at endpoint. A decline in barrier function following 10 Ox challenges was demonstrated by increased transepidermal water loss ($p < 0.001$) and decreased stratum corneum hydration ($p = 0.003$) in MAFT versus Wt skin. Our observations strengthen the hypothesis that mutations in FLG predispose to atopic dermatitis through increased susceptibility to antigen ingress.

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pH-regulated mechanisms account for pigment type differences in epidermal barrier function

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Previous studies suggest that pigment type, rather than race, determine differences in epidermal function. We studied the skin surface pH, stratum corneum (SC) surface pH gradient, permeability barrier homeostasis, and SC integrity in 3 geographically-disparate populations with pigment type I-II vs. IV/V skin (Fitzpatrick scale). Darkly-pigmented subjects display a surface pH that is nearly 0.5 units lower than that of lightly-pigmented subjects, and SC integrity (mean number of tape stripping to increase TEWL by 3-fold) diverged widely in the two pigment groups (i.e. 51.7 ± 5.4 vs. 20.1 ± 0.5, $p<0.0001$), as did barrier recovery kinetics after stripping. Acidification of SC in lightly-pigmented subjects to levels similar to, or lower than darkly-pigmented subjects abolished these functional differences. We showed further that the adverse consequences of an elevated pH in lightly-pigmented subjects can be ascribed to a pH-dependent increase in serine protease activity, which accelerates desmoglein (DSG)-1 positive corneodesmosome (CD) degradation. In contrast, DSG 1-positive CD persist into the upper SC in darkly-pigmented skin. Yet, the thickness of SC does not increase in darkly-pigmented SC, due to enhanced activity of acidic-dependant proteases, such as cathepsin-D. These studies demonstrate marked differences in epidermal structure and function, which could account for disparities in the prevalence/severity of inflammatory skin disorders among pigment types, and they further point to SC hyperacidification as a potential preventive strategy for lightly-pigmented individuals.

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Efficacy of PPARα, β/δ, and LXR, but not PPARγ, activators in murine atopic dermatitis

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Atopic dermatitis (AD) is a chronic inflammatory dermatosis now increasingly linked to mutations that alter the structure and function of the stratum corneum (SC). Activators of the 'liposenser' subclass of the nuclear hormone receptors, PPARα, β/δ, and LXR, regulate protein and lipid production leading to barrier formation, and all exhibit anti-inflammatory activity in both phorbol ester-induced, irritant contact dermatitis and hapten-induced, acute allergic contact dermatitis. Because of their broad range of activities, we evaluated the efficacy of 2-3 different activators of each of the liposensors in hapten (oxazolone [Ox])-induced atopic-like dermatitis (Ox-AD) in hairless mice. Topical activators of PPARα (except clofibrate), PPARβ/δ, and LXRα (except 22-rOH-cholesterol), but not PPARγ, reversed the clinical dermatosis, and significantly improved SC structure and function, including barrier function, SC hydration, and SC pH in Ox-AD mice. Moreover, these activities also reversed the immunologic abnormalities in AD mice, including eosinophil/mast cell density, serum TARC levels, density of CTRH-positive lymphocytes (but not serum IgE levels). The rank-order efficacy of the liposensors in treating hapten-induced AD in mice was: LXR>PPARα>PPARβ/δ>>>PPARγ. These results suggest that certain activators/ligands of PPARα, β/δ, and LXR could be useful in the treatment of human AD.

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Hyperacidification prevents emergence of hapten-induced atopic dermatitis in hairless mice

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Prior studies have shown that neutralization of stratum corneum (SC) adversely impacts key epidermal functions, including permeability barrier homeostasis and SC integrity. Conversely, normalization of SC pH improves these functions in developmentally-impaired (neonatal, aged) skin, and even super-normalizes function in normal skin. Hence, we asked here whether hyperacidification also exhibits benefits in diseased skin. Applications of the polyhydroxylacid, lactobionic acid (LBA), did not normalize structure, function or immunologic parameters either in pre-established, oxazolone (Ox)-induced acute allergic contact dermatitis, or in Ox-induced atopic-like dermatitis (Ox-AD). Yet, when LBA was co-applied during the challenge phase, development of Ox-induced AD is significantly diminished. Not only SC pH, but also barrier function and SC hydration significantly improved. Moreover, acidification alone normalized both lamellar body secretion and post-secretory formation of lamellar membrane bilayers, as well as SC integrity, with normalization of corneodesmosome structure. Furthermore, acidification largely prevented the emergence of epidermal hyperplasia, while also normalizing immune parameters, such as eosinophil/mast cell density, serum TARC levels, and the density of CTRH-positive lymphocytes. Thus, maintenance of a normal SC pH prevents emergence of the structural and functional abnormalities of Ox-induced AD, while also substantially reducing epidermal hyperplasia and the downstream abnormalities in adaptive immunity. These results suggest that optimization of SC pH could comprise an effective preventive strategy for human atopic dermatitis.

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The "caveolae break hypothesis" and the epidermal barrier

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Epidermal permeability barrier is partly regulated by the polarized secretion of lamellar bodies (LB) and their fusion with the apical plasma membrane of a terminally differentiated keratinocytes at the stratum granulosum (SG)- stratum corneum (SC) junction. LB secretion creates cholesterol/glycosphingolipids rich membrane domains resembling in composition lipid rafts (LR)/caveolae. We found that the size of those domains is comparable to LR and that barrier stress modifies their dynamics and morphology. To address the function of those domains, methyl-β-cyclodextrin (MβCD)-topically treated hairless mice and/or caveolin-1 knockout mice (cav-1 -/-) were studied. MβCD delayed barrier recovery following abrogation attributable to a decreased LB secretion, whereas accelerated recovery in cav-1 -/- correlates with hyper-secretion and large raft domains formation at the SG-SC interface. Following barrier abrogation cav-1 was found to translocate from the cytoplasmic to raft domains, a key phenomenon for signaling terminal differentiation of keratinocytes. The later could be inhibited by either monensin, an inhibitor of LB secretion and consequently cav-1 translocation, and absence of cav-1. Caveolae proteins are delivered by LB trafficking and serve as a "brake" for LR formation, LB secretion arrest and terminal differentiation. Finally, persistence of rafts in cav-1 -/- increases epidermal propensity to hyperplasia. Similarly hyperproliferative psoriatic epidermis is characterized by increased LR formation.

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Depilatory cream enhances transdermal drug delivery by disrupting both intracellular and intercellular structures of stratum corneum

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The depilatory cream of thioglycolate salt has been widely used for removal of unwanted hair for decades. Interestingly, it has also been demonstrated to be a potent penetration enhancer for transdermal drug delivery. Hence, it may lead to a higher risk of increased permeation of exogenous toxic agents among depilatory cream users. Up to date, the detailed mechanism for its penetration enhancing effect is still unknown. In this work, we attempted to characterize its effect on the transepidermal drug delivery and the associated epidermal structural alterations. Human skin is pretreated by a depilatory cream and then subjected to the permeation of fluorescent model drugs. The penetration of model drugs and the structural alterations of stratum corneum are assessed by multiphoton imaging, histology, Nile red staining and electron microscopy. Our results show that pretreatment of a depilatory cream enhances the penetration of both hydrophilic and hydrophobic model drugs across stratum corneum. Disruption of cellular integrity, generation of intracellular pores, and focal detachment of corneocytes are revealed in multiphoton imaging. In addition to intracellular structural alterations, Nile red staining shows, instead of a motar-like distribution of lipid matrix around corneocytes, a homogenized lipid distribution in the stratum corneum. Ultrastructural analysis reveals disruption of intracellular keratin matrix, protein cell envelope and the regular lamellar intercellular lipid packing. Our results suggest that depilatory cream may enhance transepidermal drug penetration by reducing the resistance in both transcellular and intercellular routes of stratum corneum.

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The identification of LEKTI targets using a novel proteomic approach

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The SPINK5 gene encodes the serine protease inhibitor lympho-epithelial Kazal-type-related inhibitor (LEKTI), a protein involved in the formation and function of the skin barrier. LEKTI is synthesised in full-length form and cleaved immediately into multiple biologically active fragments that are secreted from differentiated keratinocytes. *In vitro* studies have shown both full-length and specific fragments of LEKTI to inhibit several serine proteases expressed in skin, including kallikrein 5 (KLK5), a serine protease that plays an important role in the desquamation process. However, as LEKTI is a multidomain protease inhibitor, we speculate that LEKTI may target several serine proteases in the epidermis and as a consequence be involved in multiple biological processes. In this study, a newly developed proteomic method that combines both ProteinChip Array Technology and state-of-the-art electrospray ionisation quadrupole time-of-flight mass spectrometry (ESI-Q-TOF MS) was used to identify potential targets of LEKTI. Full-length and fragments of recombinant LEKTI were bound covalently to a reactive ProteinChip Array surface, followed by incubation with protein extracts from normal skin and analysed by surface-enhanced laser desorption ionisation time-of-flight mass spectrometry (SELDI-TOF MS). Proteins bound to rLEKTI were released following ionisation in the mass spectrometer and detected according to their mass/charge ratio, appearing as peaks on the resulting mass spectra. Identical proteins from various skin samples were found to bind to different rLEKTI fragments including the full-length form. Also, several additional proteins bound only to one unique LEKTI fragment. In order to identify these targets, we extracted LEKTI targets directly from the ProteinChip Array surface, followed by in-solution digestion into a mixture of peptides. The digested peptides were then separated and sequenced using HPLC and ESI-Q-TOF. This is the first study that uses proteomic technology to identify potential targets of LEKTI in the skin.

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Human epidermal caspase-14 is increased after exposure to mild doses of UV

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This study was designed to address the role of caspase-14 in human epidermis. Caspases are aspartate proteases that are mainly found in the epidermis. Caspase-14 is not involved in apoptosis but published data from animal models suggest that epidermal differentiation, skin hydration and the UVB scavenging capacity of the stratum corneum are caspase-14 dependent. Consecutive tape strip samples were collected on the ventral forearm and analyzed for caspase-14 activity and the amount of free amino acids. Across the stratum corneum there was a gradient in caspase-14 activity with lower values towards the skin surface. There was an association between skin barrier strength, defined as the number of tape strippings required to disrupt the barrier, and the SC caspase-14 activity in the deeper layers (n=80, $p<10\text{-}6$). Further data evaluation revealed that panelists that had recently been exposed to mild outdoor sun exposure had stronger barrier ($p=0.0003$) and elevated SC caspase-14 activity ($p=0.03$). In a separate study it was shown that repetitive exposure to mild doses of UV induced an increase in SC caspase-14 activity (n=14, $p=0.0003$). These data suggest that human caspase-14 increases as part of the response to mild UV exposure. A positive association between SC caspase-14 activity and the total amount of free amino acids was additionally observed ($p=10\text{-}5$). Since filaggrin is the major source of free amino acids in the SC, it is suggested that caspase-14 is involved in the degradation of (pro)filaggrin in human skin *in vivo* and as such might contribute to the formation of the natural moisturization factor. Taken together these data suggest that human epidermal caspase-14 is upregulated as a consequence of exposure to mild doses of UV. Because of the potential role of caspase-14 on skin hydration and photoprotection it is suggested to look for alternative ways to upregulate SC caspase-14, without the damaging effects that can be expected from UV exposure.

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CD44 is not essential for cutaneous inflammations

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CD44 is a trans-membrane glycoprotein that is ubiquitously expressed in tissues including the skin and is essential for epidermal permeability barrier homeostasis and keratinocyte differentiation (JID, 126(6):1356-65, 2006). Studies have suggested that CD44 is also critical for inflammation. To further determine the role of CD44 in cutaneous inflammation, the inflammatory response was assessed in both irritant and allergic contact dermatitis in CD44 KO and wild type mice. Additionally, changes in epidermal CD44 expression were assessed in various inflammation models. Epidermal CD44 expression is stimulated by treatments that induce cutaneous inflammation including 1).acute allergic contact dermatitis induced by topical oxazolone; 2).acute irritant dermatitis induced by topical TPA treatment; 3).acute disruption of the epidermal permeability barrier, which has previously been shown to stimulate epidermal cytokine release; 4).subacute barrier disruption by repeated tape-stripping and 5).subacute dermatitis produced by repeated topical oxazolone treatment. However, the degree of inflammatory response, as measured by ear thickness in both irritant and allergic contact dermatitis, did not differ between CD44 KO and wild type mice. Furthermore, no difference in inflammatory cell infiltration was observed between CD44 KO and wild type mice. Together, these results suggest that while CD44 expression is markedly stimulated by inflammation, CD44 is not essential for the development of cutaneous inflammation.

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Bone marrow replenishes de novo keratinocytes in the regenerating hair follicles via circulating blood

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Recent study demonstrated de novo hair follicle regeneration in the severely wounded skin. The origin of the de novo follicular keratinocytes, however, is still unknown. Here, we report for the first time that bone marrow exclusively replenishes de novo keratinocytes to the regenerating hair follicles in the damaged skin via circulating blood. Newborn mouse skin with numerous developing hair follicles was excised and transplanted onto the back of the mouse with prior GFP-transgenic bone marrow cell transplantation (GFP-BMT) following lethal dose irradiation. Surprisingly, a significant number of the GFP-positive bone marrow-derived keratinocyts (BMDKs) were residing in the follicular bulge regions as well as in the epidermis of the transplanted skin even 5 months after the transplantation, suggesting that bone marrow replenishes keratinocyte stem cells in the hair follicles to regenerate severely damaged skin such as a skin graft. Cell-fusion mechanism was clearly excluded for raising BMDKs by searching fusion-dependent, Cre-recombinase-mediated GFP expression in BMDKs. We further explored BMDK-recruiting activity, designated as KOI2, in the skin extract (SE). A silicon tube inoculated with SE was subcutaneously transplanted in the GFP-BMT mouse. In 2 weeks, a significant number of GFP-positive bone marrow-derived adhesive cells (BMACs) were recovered from the tube and expandable by cell culture. BMACs contained a cell population with epithelial differentiation potential showing keratin 5 expression, suggesting that SE contains KOI2 activity. Finally, we tried to characterize KOI2 activity in SE, and chromatographically isolated a candidate 25kD protein that induces BMAC migration *in vitro* and *in vivo*. We believe that these observations will provide novel insight into the skin regeneration by recruiting circulating keratinocyte progenitors/stem cells in the hair follicles.

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A new organotypic culture system of Merkel cells using epidermal sheet

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Merkel cells exist in the basal layer of the epidermis, more especially at the bottom of the rete ridges, contacting with keratinocytes. We thus hypothesized that a direct contact between Merkel cells and keratinocytes may be relevant to the maintenance of Merkel cell survival and function. To address our hypothesis, we developed a new organotypic culture system of Merkel cells, using Merkel cell-containing epidermal sheets. This culture system maintains Merkel cell survival within epidermal rete ridges for more than 2 weeks without neurotrophic growth factors (neurotrophin-3 and nerve growth factor). Coculture of Merkel cells with nerve cells (PC12) promoted Merkel cell survival within epidermal sheets, in which keratinocytes replicated cytokeratins (CK1, CK10, CK14) and p63 expression of in vivo keratinocytes. Merkel cell proliferation was not detected in all conditions. The data suggest, firstly, that a direct contact between Merkel cells and keratinocytes is one of the critical factors in Merkel cell survival; secondly that Merkel cells may be a terminally differentiated cell type. Our culture system will open up a new way to study Merkel cell biology.

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Clinical severity is correlated with barrier defect in atopic dermatitis associated with filaggrin mutations

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Mutations of FLG encoding filaggrin, a key molecule involved in skin barrier function, were clarified as a major predisposing factor for atopic dermatitis (AD). To elucidate the relevance of FLG mutations to AD pathogenesis, we investigated stratum corneum (SC) hydration and transepidermal water loss (TEWL) as parameters of skin barrier function in 12 AD patients harboring FLG mutations (FLG-related AD) and in 12 AD patients without any FLG mutation (non-FLG-related AD). SC hydration was significantly reduced, and TEWL and SC thickness were significantly increased in both AD groups compared to healthy controls ($p<0.005$-0.05). In FLG-related AD, SC was significantly thicker than that in controls and in non-FLG-related AD ($p<0.05$). The Objective-SCORAD (OSCORAD), a disease clinical severity index, was significantly correlated with TEWL ($r=0.81$), SC hydration ($r=-0.65$) and thickness ($r=0.59$) in FLG-related AD. On the contrary, there was no correlation between these parameters and the OSCORAD in non-FLG-related AD. Interestingly, the significant correlation was obtained between the OSCORAD and specific IgE for house dust ($r=0.66$), mite allergen ($r=0.53$) and cats dander ($r=0.64$) in FLG-related AD, but not in non-FLG-related AD. All these data suggested that FLG mutation-induced skin barrier defects may play a crucial role in the clinical severity and pathomechanism in FLG-related AD.

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Leiden reconstructed human epidermal model as a tool for the evaluation of the skin corrosion and irritation potential according to the ECVAM guidelines

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Background: In the ECVAM validation studies two common skin protocols have been developed, the skin corrosion and skin irritation protocol. Both protocols include next to general and functional conditions that the skin model must meet, also the correct prediction of the activity of certain reference chemicals. For the skin corrosion protocol, the OECD TG 431 defined 12 reference chemicals that should be correctly predicted by the epidermal skin model. For skin irritation 20 test substances should meet the defined criteria. In this study we aimed to subject our Leiden human epidermal (LHE) model to both common protocols according to the ECVAM guidelines. Method: LHEs were generated by culturing Caucasian keratinocytes for 17 days at the air-liquid interface under serum free conditions. These LHE models have been fully characterized on protein level and show very high similarities with the native skin. From the ET50 value it became clear that these LHE model also contains a competent skin barrier. After minor technical changes in both protocols, the selected chemicals were applied onto the epidermal skin models according the ECVAM guidelines. Results: The corrosion classifications were obtained in concordance with those reported for the validated human skin models EPISKIN™ and EpiDerm™. The results obtained with the common skin irritation protocol were very similar to that of earlier studies with the SkinEthic, EPISKIN and EpiDerm models. In addition, IL-1α contributed to the correct prediction of the irritant potential. In conclusion, the protocols and prediction models developed during the validation studies with a specific skin model can be used with other similar skin models. This study demonstrates that reconstructed human skin equivalents have been proven to be efficient and reliable alternatives to animal testing.

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Expression of fructosamine-3-kinase and fructosamine-3-kinase-related protein in skin cells and its modulation with environmental stress and age

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Formation and accumulation of altered proteins in skin is the most common characteristic of aging. Sources of altered proteins include glycation and cross-linking of proteins by glucose. Non-enzymatic glycation of proteins by glucose (Maillard reaction) is responsible for numerous age-related protein changes in skin. Intrinsic toxicity of glycation is reduced by a cellular defense system including recently discovered protein-repair enzymes fructosamine-3-kinase (FN3K) and fructosamine-3-kinase-related protein (FN3KRP). However, the expression level and role of FN3K and FN3KRP in skin cells is not well understood. Little is known about their modulation in skin with age, cellular senescence, or environmental stress. Our aim was to analyze FN3K and FN3KRP expression in skin cells, their role in glycation-related aging of skin, and modulation with age, senescence, and environmental stress. We analyzed FN3K and FN3KRP expression in human keratinocytes (KC), fibroblasts (FB), skin, and skin equivalents (SE), and their modulation with age, cellular senescence, and UV by PCR, Western Blot, or IHC. Effect of glycation was studied in SE by IHC after methylglyoxal treatment. FN3K and FN3KRP are expressed in KC and FB. Initial studies show that FN3K expression level is similar in adult skin cells from donors of different age; and higher in neonatal compared to adult FB in early passages of replicative senescence. We also found that UVB stimulated FN3K expression in KC. FN3K silencing with siRNA and methylglyoxal treatment resulted in impaired SE formation. Our studies show that FN3K is expressed in skin cells and expression changes with UVB and cellular senescence, indicating a possible role of deglycation enzyme FN3K in protection against environmental stress and glycation-related aging of skin. Expression experiments are ongoing in human skin. Stimulating FN3K expression may represent a promising approach for preventing and treating signs of glycation-related aging of skin and for protection against environmental stress.

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Enhancement of aquaporin –3 expression in cultured human keratinocytes and *ex vivo* skin using a new active ingredient, and an *in vivo* evaluation of its benefits to the skin

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The functional significance of Aquaporins in epithelial tissues is a central area of skin research. As revealed by gene knockout studies, these water channel-forming proteins (especially aquaglyceroporins (AQP 3)) are crucial for maintaining the water gradient across the epidermis, and thus for many stratum corneum functions, including permeability barrier homeostasis. Thus, we developed a new active ingredient to stimulate aquaporins in skin, and we evaluated the active ingredient's benefits using cultured human keratinocytes, *ex vivo* human skin, and *in vivo* topical applications. Pretreatment of normal human keratinocytes (1% active, 24 hours) induced a 43% increase in AQP3, as shown by Western blot analysis. Immunofluorescence staining (IF) revealed cytoplasmic localization in the cells, and its translocation to the cell membrane following osmotic or UVB stress. At higher levels (3%), the active exerted a protective effect on cells, as was shown by the MTT test after osmotic stress. Histological *ex vivo* studies of skin under different conditions, such as dehydration and tape-stripping, indicated that increased AQP3 expression is essential for skin protection, and showed that the pattern of AQP 3 expression was more enhanced in the active ingredient-treated samples. *In vivo* studies on the volar forearm of 10 healthy human volunteers (7 day treatment; 1% active, twice a day) was followed with hydration measurements, and *in vivo* confocal microscopic imaging. 70% of volunteers showed a significant increase in hydration on the treated sites compared to placebo sites. Vivascope images showed a significant compaction of the SC, and a generally healthier appearance of skin in the treated areas. These results attest to the potential value of the active ingredient in optimizing SC hydration and the epidermal water gradient.

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The use of HepG cells to determine acute and sub-acute organ toxicity, as an alternative method to animal testing

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In order to fulfill REACH's requirements for animal test replacement, we were interested in using the viability of human hepatocytes, the HepG2 cell line, after application of an active ingredient, as a new alternative method for organ toxicity testing. Acute toxicity studies were performed by making one application of the active ingredient on cells at high concentrations, for 24 hours. As a positive control, Sodium Dodecyl Sulfate (SDS) at 0.08, 0.09, and 0.1 mg/ml was used. For sub-acute toxicity, the SDS doses used were 0.06, 0.07, and 0.08 mg/ml, and three moderate doses of the active ingredient were applied at an interval of 7 days between applications. Three products of different toxicity were tested in each protocol, and cell viability was determined by the MTT test. Acute toxicity graphic analysis of the results showed a dose-dependent decrease in viability corresponding to the toxicity of each product. For product 1, there was no loss in cell viability up to 50% of product concentration, while for product 2, cells conserved their viability only with 10% of product concentration, and only with 5% with product 3. Similar product toxicity classifications were found for sub-acute toxicity with the repeated doses, but with weaker product concentration than with acute toxicity. These results highlight the interest of performing acute toxicity and repeated-dose toxicity tests with HepG cells. The dose effect observed for each tested product indicates that the method has great sensitivity. Moreover, these alternatives to animal testing are inexpensive, fast, and reproducible. These protocols can thus be of great use as routine tests in toxicology that help evaluate the acute and sub-acute systemic organ toxicity of active ingredients.

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A (Cys-Gly)2 dimer peptide exhibits significant *in vivo* antioxidant properties for the lipids of the horny layer

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Lipids of the Stratum Corneum play crucial roles (a very important role) in different skin barrier functions. The main environmental cause of barrier function alteration is UVA and UVB, which cause oxidative stress and lipid peroxidation. Lipid degradation damages barrier function and increases cutaneous water loss, leading to skin dehydration and the formation of wrinkles. (In this domain,) We performed a double blind *in vivo* study to investigate the antioxidant effect of a (Cys-Gly)2 dimer peptide on decreasing stratum corneum lipid peroxidation, after UV exposure. The study was performed on seven healthy volunteers with skin type II. For each volunteer, two parallel zones were designated on the back. Lipids of the horny layer were extracted in absolute ethanol using a glass cylinder applied to the skin. Then, lipid peroxidation was quantified using a "LPO determiner kit" on a 96-well plate. The following day, a cream formula containing 5% of the dimer peptide was applied on one of the selected zones of the back, and the placebo on the other. Three hours later, we exposed the volunteers' backs to full spectrum sunlight for 20 minutes. Immediately after, another application of the creams was performed. Three hours after sun exposure, the lipids of the horny layers for each zone were extracted in absolute ethanol, and lipid peroxidation was quantified as described above. This study revealed a very significant decrease (Wilcoxon test, p=0.0078) of 22.17% in lipid peroxidation on the active ingredient-treated side, compared to the placebo side, in 100% of the volunteers. These results confirm the antioxidant effect of the (Cys-Gly)2 dimer peptide, and demonstrate its efficient protection of the lipids of the stratum corneum from UV-induced peroxidation. This active ingredient can thus be of great use in anti-aging and sun protecting skin care products.

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Human cornea use to determine ocular irritation potential, compared to the Het-Cam test

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At present, skin care researchers are looking to expand the limited number of existing alternative tests used for evaluating product safety. Various *in vitro* tissue substitutes have been developed for the purpose of decreasing animal testing and moving closer toward *in vivo* evaluation of the safety of products on the skin. As one of our goals is to support the replacement of animal testing, we compared the use of reconstituted human corneal epithelium (RHCE), to the recognized Hen's Egg test on Chorioallantoic Membrane (Het-Cam), which is used as an alternative to the Draize test. In our RHCE protocol, products were applied on the top of a 3D cornea model. At two time points, 1h and 24h, qualitative and quantitative determinations of corneal cell viability were performed, using the MTT test. The results were then compared to Het-Cam results. Moreover, assessment of IL-1 level in cornea was performed using ELISA. With low irritant products, the RHCE model seemed more sensitive and allowed for a distinction between two groups classified as practically non-irritant by Het-Cam: non-irritant and slightly irritant. Furthermore, IL1-α ELISA on RHCE showed a moderate increase in IL1 level, suggesting an irritation of cells from the applied product. This irritation was not revealed by visual observation of the cornea nor by Het-Cam. Similar to the Het-Cam test, RHCE helped to classify toxic products into two groups: moderately irritant and highly irritant. These results showed that, for low irritant products, the RHCE method was more sensitive than the Het-Cam test, and gave more information about toxicity intensity. They also showed that, with the RHCE method, only two application times were needed to reveal product toxicity. The human cornea model can thus be of great use in toxicological and safety evaluations.

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Purification and characterization of SERPINB12 in human skin - a new epidermal serine protease inhibitor

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Proteolytic degradation of extracellular proteins plays a crucial role in the physiological detachment of corneocytes from human stratum corneum. The presence of protease inhibitors regulates their proteolytic activity and contributes to the integrity and protective function of the skin. Their importance in diseases has been revealed impressively in principal in Netherton's disease, where the Kazal-type protease inhibitor LEKTI is absent. We speculate that decreased expression of protease inhibitors in human skin are involved in inflammatory skin diseases like eczema and that enhanced expression leads to thickening of the cornified envelope as seen in plantar callus. Therefore we analyzed human callus for the presence of protease inhibitors, which might contribute to the stratum corneum architecture. After biochemical separation by high performance liquid chromatography (HPLC) we identified by N-terminal sequencing and ESI-MS-analyses the serine protease inhibitor (SERPIN) B12 in extracts of human callus. Primary cultured keratinocytes exhibited SERPINB12 mRNA-expression by RT-PCR, which was not altered by keratinocyte differentiation induced by treatment with 1.2 mM calcium over seven days. Immunohistochemical analysis of paraffin-embedded skin samples revealed SERPINB12 expression throughout the epidermis. The expression of SERPINB12 was observed at various localizations of healthy human skin including face, trunk as well as palmar and plantar localizations. In conclusion we identify for the first time the protease inhibitor SERPINB12 in human skin. Our finding that SERPINB12 is present in the callus suggests a new physiological role for this protease inhibitor. We speculate that SERPINB12 contributes to the epidermal barrier function of healthy human skin.

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A new cytochrome c-inducing active ingredient enhances mitochondrial function and structure, and activates many key cell genes

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Cytochrome c is a crucial protein for mitochondrial function, and is a vital component of the electron transfer chain, which is responsible for cell energy production and survival. We developed a new cytochrome c-inducing active ingredient (CI), and evaluated the effect of CI on the ultrastructure and activity of mitochondria as well as gene activation in fibroblasts and keratinocytes. Immunofluorescence and Western blot studies showed a net increase in cytochrome c expression in cells treated with 1% CI for 24 hours. Image analysis of immunofluorescence, using several image processing steps followed by the generation and subsequent quantification of luminosity histograms, revealed a significant increase (80%) in cytochrome c expression in CI-treated cells, and also demonstrated that CI-treated cells exhibited more available cytochrome c despite different stresses. Further studies showed that treatment of the cells with CI enhanced cytochrome oxidase activity by 850% after 24 hours of application. Ultrastructural studies by electron microscopy showed that synthetic activity in treated-cells was obviously enhanced, as demonstrated by high levels of keratin in the cell core, and by the Golgi and ER system at the periphery of cells. These results were confirmed by microarray analysis of gene expression that revealed the upregulation of many genes important for cell activation, synthesis, and differentiation, such as various cyclins and cyclin-dependent kinase, signal transducers, ca+2 binding protein, transglutaminase 1 & 2, elastin, telomerase reverse transcriptase, and insulin-like growth factor binding protein. In summary, these studies demonstrate the significant effect of this new cytochrome c-inducing active ingredient on improving mitochondrial function and cell activation. This active ingredient can thus be of great use in skin energizing, wound healing, and skin care products.

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Enhancing cell energy and longevity by modulating SIRT3 expression in human cell mitochondria

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Activated human sirt3 is localized to mitochondria, where it plays a key role in activities such as acetyl-CoA synthesis, and the Krebs cycle. Enhancement of SIRT3 expression has various impacts on vital cellular functions, such as the protection of mitochondrial membrane potential, decrease of reactive oxygen species production, and increase of cellular respiration. In addition, a correlation has been shown between SIRT3 expression and the expression of some genes related to mitochondrial function, including cytochrome c oxidase subunits and ATP synthetase. In this domain, we developed a new active ingredient (S3) that enhances SIRT3 expression in cultured human cells and skin biopsies, and tested its effect on cell protection and function. SIRT3 immunofluorescence staining showed an increase of SIRT3 expression in cultured fibroblasts treated with 1% S3, and this effect was seen 24 and 48 hours after application. Double immunofluorescence staining of cytochrome c and SIRT3 revealed the colocalization of cytochrome c and SIRT3 within cell mitochondria. UVB studies demonstrated an increase in mitochondrial SIRT3 expression in cells two hours after irradiation, and this effect lasted for 24h. Interestingly, cells where SIRT3 was induced by S3 prior to irradiation did not express a noticeable increase in SIRT3 expression after UVB exposure. Furthermore, morphological studies demonstrated that S3-treated cells exhibited remarkably fewer UV damage signs than uninduced cells, and that they retained better morphology. This suggests that, thanks to the presence of a significant level of SIRT3, induced cells underwent less stress fighting against UV damage to protect their mitochondria. Moreover, immunostaining of human *ex vivo* skin, treated for 48 hours with 1% S3, revealed an enhancement of SIRT3 expression, compared to control skin. In conclusion, these studies demonstrate that this new active ingredient enhances SIRT3 expression, and can thus be of great use in skin energizing and skin care products.

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Enhancing aconitase expression and activity improves mitochondrial ultrastructure, mitochondrial function, and cell viability

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Aconitase plays a crucial role in mitochondrial activity and functions such as energy production and mitochondrial DNA protection. Our prior research has shown that the newly developed AC active ingredient protects mitochondrial activity from different stresses. In this study, we evaluate the effect of this active ingredient on mitochondrial structure and cell gene activation. Studies of mitochondrial and total aconitase activity were performed on cultured human fibroblasts treated with the 1% of AC for 24 hours. These studies showed that total cellular aconitase activity was significantly increased by AC treatment (+56%, p=0.0485), and that mitochondrial aconitase activity also significantly increased (+32%, p=0.0012) in AC-treated cells. These results were confirmed by cell viability studies. MTT assay revealed that cell viability was significantly enhanced (+21%) in fibroblasts treated with AC. Ultrastructural studies by electron microscopy on human fibroblasts and keratinocytes showed that AC-treated cells exhibit ultrastructural features that support increased metabolic and synthetic activities. This is reflected by the mitochondrial population, their structural attributes, as well as the abundance of Golgi elements. Moreover, microarray-based gene expression studies at different time points showed a very interesting significant upregulation of many genes that are important for cell activation, longevity, and differentiation, such as telomerase reverse transcriptase (TERT), telomerase RNA component (TERC), calcium binding protein (S100A8), cyclins, signal transducers, transcription factor AP-2, and transglutaminase (TG2). These results demonstrate the potential utility of this active ingredient for protecting mitochondria, maintaining mitochondrial activity, enhancing cell activation, and supporting cell longevity mechanisms.

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Enhancement of epidermal lipid synthesis with a newly developed active ingredient

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Extracellular lipids of the stratum corneum, composed mainly of ceramides, cholesterol, and fatty acids, are essential for skin barrier function. The present study focuses on a newly developed acive ingredient that exhibits an interesting effect on epidermal lipid synthesis. In order to evaluate lipid droplet content in cells, Nile Red, a selective fluorescent stain for intracellular neutral lipid droplets, was used. Cultured normal human keratinocytes (NHK) were treated or not with 1% of the new active ingredient for 24h and 48h. Similarly, human reconstituted epidermis (HRE) and human skin biopsies were treated with 1% of the new active ingredient for 24h and 48h, and also stained with Nile Red to visualize epidermal lipids. As lipid synthesis has been previously linked to keratinocyte differentiation, the effect of the new active ingredient on pan-keratin expression was also studied by immunofluorescence staining on NHK. Our results showed that treatment of cells with 1% of the active ingredient increased lipid droplets in NHK stained by Nile Red. The lipid droplets appeared abundant, especially in the perinuclear space, and this effect was seen as early as 24h, but greater after 48h of treatment. Moreover, Nile Red staining also revealed an increase in the lipid content of treated HRE and skin biopsies. The staining was seen mainly at the suprabasal level, as well as in the stratum corneum. Interestingly, the active ingredient increased pan-keratin expression in NHK, indicating a stimulating effect on keratinocyte differentiation and skin barrier improvement. By promoting lipid synthesis and enhancing keratinocyte differentiation, this active ingredient can be of great use in skin care products and in treatment of skin barrier alterations caused by different pathologies.

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A new active ingredient that exhibits a protective effect against irritation in normal human keratinocytes and human skin

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When skin is submitted to irritant factors that can induce barrier disruption, defense mechanisms activate, leading to restoration of normal skin homeostasis and barrier recovery. The IL-1 alpha cytokine plays a key role during skin inflammation by inducing a cascade of other inflammatory mediators. The present study focuses on a newly developed active ingredient that can exert a protective effect against irritations caused by SDS or H2O2-induced oxidative stress. In an in vitro model, normal human keratinocytes (NHK) were pretreated or not with 1% of the active ingredient for 24h, and then stressed with 15 µg/ml SDS for 24h. Following the stress experiment, we evaluated cell viability improvement using the MTT test. Our results showed that cell viability was significantly increased in keratinocytes treated with the active ingredient and stressed with SDS, compared to untreated stressed cells. Interestingly, improvement in cell viability was also observed in NHK stressed with 2mM H2O2 for 30 min, followed by a 24h re-incubation with 1% of the active ingredient. In studies on human skin biopsies, samples were treated for 24h with 1% of the active ingredient before SDS stress at 2.5% for 24h. Control skin samples received the stress without active ingredient pretreatment. Evaluation of the anti-inflammatory effect of the new active ingredient was performed using ELISA assay on extra cellular IL1-alpha cytokines in the conditioned media of skin biopsy cultures. ELISA studies on human skin biopsies stressed with SDS showed that IL1-alpha level assessed in the culture medium was significantly decreased by active ingredient treatment, compared to the untreated control. These results strongly suggest that this active ingredient possesses a significant anti-inflammatory effect, and that it can be of great use in skin care products aimed at protecting the skin against irritants and environmental stress.

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Enhancement of HMG-CoA reductase expression in normal human keratinocytes and the epidermis by a newly developed active ingredient

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Cholesterol is the main sterol of the epidermis and is produced there independently of circulating cholesterol level. After its synthesis, it is stored in epidermal lamellar bodies, which are secreted into the extracellular space during terminal differentiation to provide the epidermal permeability barrier. At the cellular level, cholesterol provides necessary fluidity to cell membranes. In the present study, we developed a new active ingredient designed to target specifically and activate (on) 3-hydroxy-3-methylglutaryl-Coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol synthesis. Expression of HMG-CoA reductase was studied by immunofluorescence (IF) staining on normal human keratinocytes (NHK), human reconstituted epidermis (HRE), and human skin biopsies treated with 1% of the active ingredient for 24h and 48h. As cholesterol synthesis has been previously linked to keratinocyte differentiation, the effect of the active ingredient on pan-keratin expression was also studied by IF-staining on NHK. We observed a cytoplasmic immunostaining pattern of HMG-CoA reductase in keratinocytes. On HRE and skin biopsies, HMG-CoA reductase immunostaining was stronger in the suprabasal layer of the epidermis. Active ingredient treatment was shown to increase HMG-CoA reductase expression on NHK, HRE, and skin biopsies. Interestingly, the active ingredient also increased pan-keratin expression in NHK, indicating a favorable effect on keratinocyte differentiation. By promoting the key enzyme of cholesterol synthesis and favoring keratinocyte differentiation, this HMG-CoA reductase-inducer is likely to improve epidermal barrier function, and can be of great use in the treatment of many skin pathologies.

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Ubiquitin-proteasome system activation in *in vitro* aged keratinocytes

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Abnormal, damaged, and senescent cell proteins are eliminated by the ubiquitin-proteasome system, an important mechanism for maintaining cellular integrity. With age, the synthesis and activity of this system decrease, leading to the accumulation of damaged or abnormal proteins that impair cells. In this domain, we developed a new active ingredient and tested its activity on the expression of ubiquitin and 20S proteasome, which represents the proteolytic core of the 26S proteasome complex. We used *in vitro* aged human keratinocytes, and cellular senescence was assessed by beta-galactosidase staining, and cellular proliferation by Ki67 immunostaining. Expression of 20S proteasome and ubiquitin were studied by immunocytochemistry and Western blotting. Proteasome enzymatic activities were determined using three specific substrates. In order to mimic the age-associated glycation process, skin biopsies were treated with methyl glyoxal for 24h in the presence of the active ingredient or not, and skin morphology was then studied by H&E staining. In our model, the expression of 20S proteasome and ubiquitin decreased during *in vitro* aging of human keratinocytes, suggesting that this model is appropriate for the study of the effect of aging on the ubiquitin-proteasome pathway. Moreover, we found that 1% of the active ingredient significantly increased the expression of 20S proteasome and ubiquitin in *in vitro* aged keratinocytes, by 34% and 22% respectively. We also found that active ingredient treatment of *in vitro* aged keratinocytes significantly increased proteasome enzymatic activities, restored the proliferation level of aged keratinocytes, and decreased cellular senescence. Under glycation stress, we observed that skin morphology was less damaged in treated biopsies compared to the untreated control. These studies strongly suggest that targeting the ubiquitin-proteasome system can be of great use in the development of anti-aging skin care products.

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The use of HaCaT cells as an additional epidermal model for the evaluation of the cytotoxicity and safety of active ingredients

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REACH legislation requires the abolition of animal testing in 2009 for all cosmetics. Accordingly, the search for new alternative *in vitro* approaches that enable toxicity testing of cosmetic products has become a central concern in skin research, and one of our principal interests. In this domain, we adapted the neutral red release method (NRR) used on rabbit cornea cells (SIRC) for use on human epidermal HaCaT cells. This method is used to determine product cytotoxicity via its IC50, the concentration that induces 50% of cell death. First, it was necessary to establish a positive control—Sodium Dodecyl Sulfate (SDS). Several concentrations were tested in order to correctly frame SDS's IC50. The selected concentrations were 0.2%, 0.4%, and 1%. Once the SDS range was determined, several products of different degrees of toxicity were tested in parallel on both cell lines. These studies showed that the products whose cytotoxicity was insignificant in SIRC cells exhibited the same results with regards to their cytotoxicity in HaCaT cells, with a similar toxicity curve profile, though with a lower percentage of cell mortality at 50% concentration in HaCaT cells. Concerning the products with moderate and high cytotoxicity in SIRC cells, the same classification was obtained in HaCaT cells; the IC50 determined for SIRC cells being, however, lower than the one determined for epidermal cells. Thus, the use of the neutral red release method on HaCaT cells seems to be a suitable approach for evaluating epidermal toxicity, even though SIRC cells are slightly more sensitive. The use of epidermal HaCaT cells presents an advantage, as skin care products and cosmetics specifically target the skin. This method thus brings an additional option to the presently unsatisfactory group of validated alternative methods to animal testing.

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Effect of a new active ingredient on the ubiquitin-proteasome system of human keratinocytes and skin biopsies submitted to UVB irradiation

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Skin is constantly exposed to environmental stresses, causing DNA alterations, as well as lipid and protein structure modifications. To maintain cellular integrity, the skin possesses several defenses including the ubiquitin–proteasome system that participates in abnormal protein elimination. The ubiquitinated molecules are recognized by proteasomes, which degrade them into small peptides. In this domain, we developed a new active ingredient and tested its ability to stimulate 20S proteasome and ubiquitin expression in normal human keratinocytes (NHK) and skin biopsies, subjected or not to UVB stress.(For testing,)Iimmunostaining for 20S proteasome and ubiquitin was performed on NHK and skin biopsies, pretreated with 1% of the active ingredient for 24h, then irradiated or not with 25 or 100 mJ/cm2 of UVB, respectively, and treated with the active ingredient for an additional 24h. Protein carbonylation, resulting from protein oxidation from UVB stress, was determined in human fibroblasts receiving a 48h treatment, before and after irradiation with 100 mJ/cm2 of UVB. Hematoxylin-eosin (H&E) staining was performed on skin biopsies maintained in culture for 7 days with or without the active ingredient and irradiated daily with 60 mJ/cm2 of UVB. The results showed a significant increase in 20S proteasome and ubiquitin synthesis in treated NHK and skin biopsies, with or without UVB-irradiation. Moreover, skin morphology seemed to improve with active ingredient treatment. Indeed, after 7 days of culture with repeated UVB irradiation, treated skin exhibited a higher integrity of epidermis with more viable cells. Moreover, these results were confirmed by a significant decrease (-34%) in UVB-induced protein carbonylation. In conclusion, this active ingredient exhibits a protective effect against UV-induced damage by its action on the ubiquitin-proteasome pathway, and can be of great use in anti-aging products.

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COL17 is a key player of epithelial-mesenchymal interaction in tooth formation

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COL17 (BP180) is an essential component of the hemidesmosome (HD), an epithelial-mesenchymal junction structure in the basement membrane zone found in a variety of tissues. Mutations in COL17 lead to junctional epidermolysis bullosa (JEB) showing amelogenesis imperfecta as well as generalized blisters. In order to elucidate the role of COL17 in tooth formation, we studied enamel formation in COL17 knockout (COL17KO) mice we recently produced (Nat Med 13(3):378-83 2007) which shows phenotype similar to JEB. In COL17KO mice, a lack of COL17 expression and malformed HD were confirmed in the basement membrane zone between ameloblasts and odontoblasts in the tooth. Reduced pigmentation and white turbidity on the enamel were apparent in COL17KO mice incisors compared to normal control. Scanning electron microscopy revealed irregular enamel prisms in the incisors and advanced occlusal wear in the molars. Elemental analysis by SEM-energy dispersive X-ray spectrometry revealed reduced iron concentration in the incisors of COL17KO mice compared with that in wild-type mice. By contact microradiography, delayed calcification was observed in the enamel of COL17KO incisors. Transmission electron microscopy showed poorly developed ameloblasts lacking Tomes' processes which are essential for secretion of various enamel proteins, resulting in hypoplasia of enamel matrix. Furthermore, in COL17KO mice, mRNA expression of several enamel proteins were reduced in the incisors *in vivo* and in dental epithelial cells isolated from the incisors *in vitro*. These findings indicate that COL17 in HD connecting ameloblasts to underlying mesenchyme regulates differentiation of ameloblasts and plays crucial roles in epithelial-mesenchymal interaction in tooth formation.

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Calcium ion gradients and dynamics in living epidermis of rat hind-paw skin slices in response to stimulation with ATP

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Ionotropic receptors, originally found in the brain, were recently also identified in epidermal keratinocytes. Moreover, concentration gradients and movement of calcium play crucial roles in epidermal homeostasis. Thus, imaging of calcium in the living epidermis is expected to provide insight into epidermal pathophysiology. Here, we describe the imaging of calcium dynamics in the living epidermis of cultured skin slices. The calcium concentration was highest in the uppermost layer of the epidermis. The increase of intracellular calcium in response to ATP varied in each layer of epidermis, and was greater at the bottom than in the uppermost layer. Further, the extent of elevation of intracellular calcium in response to ATP in cultured keratinocytes varied depending on the level of differentiation. These results suggest that keratinocytes show a different profile of response to environmental stimuli in each layer of the epidermis.

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Identification of novel ciliary proteins using microarray analyses of *in vitro* ciliogenesis

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Cilia have diverse roles in motility and sensory reception and their dysfunction contributes to cilia-related diseases. Skin fibroblasts and a part of keratinocytes have sensory cilium but their precise role in the skin morphogenesis and functions remains almost unknown. To identify novel proteins required for cilia formation, we analyzed the time-dependent gene expression profiles during ciliogenesis in the *in vitro* cultured mouse tracheal epithelial cells. We found that the expression of ciliary precursor proteins is elevated first, followed by the expression of centrosomal proteins and axonemal proteins, sequentially. Microarray analyses of time-dependent change of gene expression during ciliogenesis identified two novel proteins. The first one showed an expression profile of axonemal proteins and its expression is restricted to ciliated epithelial cells. Immunofluorescence study showed it the first protein that specifically localizes at the tip of motile cilia that we designated as "Sentan" that means "tip" in Japanese. Sentan binds to phosphatydilserine and localizes at the bridge structure between the cell membrane and axonemal microtubules. The second protein showed an expression profile of ciliary precursor proteins and its expression is restricted to ciliated cells and testis. It directly binds with PCM-1 protein with its carboxy-terminus domain and co-localizes with PCM-1 at the fibrous granules specifically appeared in the early phase of ciliogenesis that we designated as PCM-2. PCM-2 also localizes at the proximal ends of basal bodies in matured ciliated cells. PCM-2 is conserved from mammals to monocellular organisms that have flagella, which suggests this protein is indispensable to form cilia. Further characterization of these proteins is helpful not only to understand the molecular mechanism of ciliogenesis but also to unveil the sensory cilia function in the skin morphogenesis and functions.

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Immunoreactivity of rhodopsin on epidermal keratinocytes of human skin

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Our recent finding that visible radiation affects epidermal permeability barrier function indicated the existence of a photoreceptor in epidermal keratinocytes. Rhodopsin is a well-known photosensitive protein found in the retina. Here, we describe an immunochemical study using monoclonal anti-rhodopsin antibody on keratinocytes of human skin. Both mouse retina and human epidermis showed clear immunoreactivity, as did cultured human keratinocytes. Western blotting confirmed the expression of a rhodopsin-like protein in protein extracts from mouse eye and cultured human keratinocytes. These results suggest that a rhodopsin-like protein is expressed in human epidermal keratinocytes.

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Topical calcineurin inhibitors compromise stratum corneum integrity and epidermal permeability and antimicrobial barrier function

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Calcineurin is a calcium/calmodulin dependent phosphatase that activates nuclear factors activated T cells(NFAT). Topical cacineurin inhibitors(TCIs)have anti-inflammatory action through blocking this signaling. TCIs have recently been used for inflammatory disorders instead of glucocorticoids, because of comparable efficacy, but less-frequent side effects. But the effects of TCI on barrier function has not yet been reported, and viral infections such as eczema herpeticum which could indicate an impaired skin barrier, continue to occur with TCI use in atopic dermatitis. We determined here whether TCIs disrupt epidermal permeability barrier and antimicrobial function, and whether these effects can be prevented. In normal humans, TCIs delay barrier recovery without an increase in basal TEWL. In murine, TCI treatment also disrupted barrier function significantly. TCIs treated epidermis showed decreased nile red fluorescence for epidermal lipids and decreased numbers and secretion of lamellar bodies, as well as reduced activity of the lipid synthetic enzymes,implying decreased lipid synthesis. TCIs also suppressed expression of IL-1α and the antimicrobial peptides, suggesting that TCIs not only disrupt the epidermal permeability/barrier, but also antimicrobial defense. But co-application of physiologic lipid mixtures(PLM) containing of ceramides, cholesterol and free fatty acids normalized barrier homeostasis in human and murines. Additionally in murine, PLM can recover decreased AMP by TCIs. Both TCI-induced abnormalities can be overridden by topical replacement with PLM.

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Myofibroblasts differentiation is modulated by epithelial-mesenchymal interactions and conversely influences epidermogenesis in human living skin equivalents

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Myofibroblasts mainly are differentiated from fibroblasts. Myofibroblasts deposit excess ECM and exert retractile force on ECM and therefore are supposed to be responsible for scar formation and tissue fibrosis. Myofibroblasts differentiation involves the contribution of local factors and systemic factors and most studies had been focused on the dermal events. In recent years, some studies also implied the important roles of epithelial-mesenchymal interactions in scar formation. In this study, we constructed fibroblasts-populated type I collagen gel and living skin equivalents (LSE) models by seeding normal human keratinocytes on the collagen gel and investigated the influences of epithelial-mesenchymal interactions on myofibroblasts differentiation and how the presence of myofibroblasts affected the epidermogenesis. We found that the myofibroblasts/fibroblast ratio kept rising during the fibroblasts-populated collagen gel culture and the fibroblasts distributed throughout the gel. However, in LSEs, most fibroblasts were α-SMA (smooth muscle actin) -negative, except the ones along the dermal-epidermal junction. The suppression of α-SMA expression by the administration of bFGF or the application of human amnion in the dermal matrix enhanced epidermal differentiation and decreased TGF-β expression in epidermis. The inhibition of TGF-β kinase completely suppressed the α-SMA expression in dermal matrix. In conclusion, the presence of epidermis suppressed α-SMA expression in fibroblasts-rich dermal matrix except where near dermal-epidermal junction. The α-SMA-positive cells at dermal-epidermal junction contribute to the hyperproliferative phenotype of epidermis. The hyperproliferative epidermis expressed higher level of TGF-β, and may be responsible for the myofibroblasts differentiation in close proximity to epidermis.

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Epidermal tight junctions are involved in the regulation of intercellular calcium ion *in vitro*

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Epidermis has calcium gradient which is highly concentrated in stratum granulosum, considered to be responsible for keratinocyte differentiation. However, the mechanism that maintains the calcium ion (Ca^{++}) gradient through the epidermis is still unknown. Tight junctions (TJs) exist in stratum granulosum. In epithelia, TJs seal neighboring cells and control paracellular pathway of solutes and ions. We hypothesized that TJs in the epidermis might be involved in the regulation of Ca^{++} gradient, mostly in intercellular spaces. Thus, in this study, we investigated the relationship between epidermal Ca^{++} accumulation and TJ assembly. We studied Ca^{++} permeability through TJs in cultured normal human epidermal keratinocytes (NHEKs) and skin equivalent, using *in vitro* permeability assay. We measured transepithelial electrical resistance (TER) and Ca^{++} permeability through differentiated NHEKs sheet. In skin equivalent, Ca^{++} accumulation was observed by proton induced X-ray emission (PIXE) analysis and ion capture cytochemistry. To investigate TJ function, we used sodium caprate (C10, Sigma, Japan), which is an inhibitor for TJ assembly. As a result, Ca^{++} permeability decreased in differentiated NHEKs, which formed well developed TJs. With C10 treatment, TER decreased and Ca^{++} permeability increased. On the other hand, C10 releasing caused rapid recoveries of both of TER and Ca^{++} permeability as much as the control. In skin equivalent, with C10 treatment, we observed depression of TJ barrier function and acceleration of Ca^{++} permeability compared with untreated control cells. Furthermore, we found disappearance of intercellular Ca^{++} gradient using PIXE analysis and ion capture cytochemistry. In conclusion, we suggest that TJs function as a sort of ion gate or channel to form epidermal Ca^{++} gradient in intercellular spaces.

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The role of Pitx2 in human keratinocyte differentiation

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Pitx2, a bicoid-type homeodomain transcription factor, has been implicated as one of the genes responsible for Riger's syndrome in human. It has been also shown to play a central role during development. In human, there are four isoforms of Pitx transcripts. Pitx2a, Pitx2b, and Pitx2c are structurally related, differing only in their amino terminal region. Pitx2d shows somewhat different structure, and acts to repress the activity of the other Pitx2 isoforms. In the human keratinocyte, Pitx2c isoform was predominantly expressed in a differentiation-dependent manner. To investigate the role, we made a recombinant adenovirus harboring the expression cassette for Pitx2c. Overexpression of Pitx2c in keratinocyte using adenovirus led to the upregulation of the keratinocyte differentiation markers including involucrin and loricrin. Consistent with, overexpression of Pitx2c increased the promoter activities of involucrin and loricrin. In addition, Pitx2c overexpression resulted in significant up-regulation of the cell cycle-related genes including p21 and p53. Interestingly, overexpression of Pitx2c in zebrafish embryo led to the formation of thick scale structure. Together, these results implicated the potential importance of Pitx2 in skin homeostasis.

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Synergistic effect of retinoids and PPAR alpha agonist on hyaluronan expression in human keratinocyte and hairless mouse skin

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Hyaluronan (HA), a nonsulfated glycosaminoglycan, is well known to hold water, maintain the extracellular space, and facilitate the transport of ion solutes and nutrients. Epidermal structure, thickness, and keratinocyte turnover rate vary according to the developmental stage and physiological state of the tissue. HA is closely involved in keratinocyte proliferation, migration, and differentiation, and therefore participates in all these regulatory changes. The synthesis of HA in vitro has been found to be stimulated by several growth factors, retinoids, dibutyryl cyclic AMP and PPAR agonist. We examined the effects of retinaldehyde, retinyl retinoate (a novel retinol derivative) and PPAR alpha agonist on HA expression in primary human keratinocyte and hairless mouse skin. Histochemistry using HA-binding protein revealed that topical retinaldehyde, retinyl retinoate and PPAR agonist raised intensity of HA staining in murine skin. In addition, both the combination retinaldehyde and PPAR alpha agonist, and that of retinyl retinoate and PPAR alpha agonist synergistically induced HA expression. Because the Hyaluronan synthase 2 (Has2) is a major producer of HA in the epidermis, we assessed by RT-PCR the expression level of Has2 gene in primary human keratinocyte and hairless mouse skin. We found that retinaldehyde, retinyl retinoate and PPAR alpha agonist slightly upregulated Has2 mRNA. Concomitant treatment of retinaldehyde and PPAR alpha agonist, and retinyl retinoate and PPAR alpha agonist proved to have synergistic effect. Taken together, we suggested that copresence of retinyl retinoate and PPAR alpha agonist may prevent and improve the cutaneous alterations caused by the loss of HA in epidermal skin.

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The expression and the distribution of epiplakin on wound healing

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Epiplakin (EPPK) belongs to the plakin family of cytolinker proteins, and it was identified as an antigen of the autoimmne blistering disease. EPPK is mainly expressed in the outer layer of epidermis. In EPPK -/- mice, wounds on the backs closed more rapidly than those on the backs of wild type and heterozygous mice. At wound edges in wild-type mice, EPPK was expressed in proliferating keratinocytes in conjunction with keratin 6. In EPPK -/- mice, no similar proliferating keratinocytes were observed, but migrating keratinocytes weakly expressed keratin 6. We propose that EPPK might be linked functionally with keratin 6 (Goto et.al, 2006). Here we studied the relationship between the expression of EPPK, the change of shapes of the keratinocytes, and the network of the keratins on wound healing. First, we made polyclonal antibodies against keratin 5, and keratin 10. We made wounds with 6mm punch biopsy tool on the backs of the mice, and observed wounds of epidermis from day 2 to 10 with confocal laser microscope. Furthermore, we observed the colocalization of EPPK and keratins using double immunostaining technique with an electron microscopy. The wound closed completely on day 6 to 8. EPPK expressed mainly in suprabasal keratinocytes on day 4 to 6, and the expression reduced on day 8, and the staining pattern of EPPK returned to normal on day 10. EPPK did not expressed in the tip of leading edge during wound closure. The epidermis around the wound became hypertrophic as compared with the normal epidermis, and especially in the migrating tongue, keratinocytes changed in shape, size, and the arrangement of keratins. We were able to confirm that the cytoskeleton changed dynamically when the size of keratinocytes increased, and the expression and the distribution of epiplakin contributed to the change of the structure of keratin fibers in this process.

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Arginase-1 expression in human epidermal keratinocytes is regulated by differentiation status and all-trans retinoic acid

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Arginase-1 contributes to the formation of the so-called natural moisturizing factor by converting arginine, a major breakdown product of filaggrin, into ornithine and urea. Here we investigated the regulation of arginase-1 in human epidermal keratinocytes (KC) in vitro and in vivo. cDNA microarray analysis showed that the expression of the arginase-1 gene was strongly increased in differentiated as compared to proliferating KC. Quantitative real-time PCR analysis confirmed a more than hundred-fold upregulation of arginase-1 mRNA during terminal KC differentiation induced by cultivating cells for several days at confluency. Likewise the arginase-1 protein accumulated strongly in differentiated KC, as demonstrated by Western blot analysis. Treatment of KC under the same conditions with all-trans retinoic acid almost completely inhibited the increase of arginase-1 mRNA and protein expression. In contrast to a previous publication, which reported the presence of arginase-1 in the spinous layer of psoriatic epidermis and its absence in normal epidermis, we detected by immunohistochemistry arginase-1 expression in the upper granular layer and the stratum corneum of both psoriatic lesions and normal epidermis. Finally, we developed an assay for the quantification of arginase-1 activity in the stratum corneum and found high inter-individual variations. In conclusion our data suggest that arginase-1 expression in the skin is confined to the last steps of KC differentiation and that its active form is present in the stratum corneum. Our finding that retinoic acid suppresses arginase-1 expression could be relevant for the "dry skin" phenotype observed during retinoid therapy.

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Retinol stimulates epidermal cell proliferation and improves facial signs of skin aging

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Clinically, facial skin aging is associated with a variety of signs such as wrinkles, uneven pigmentation, skin roughness, and laxity. These clinical features are consecutive to structural and metabolic changes that occur during chronological aging and photo-aging. Beneficial effects of retinoids on signs of skin aging are now well documented and for example, it has been shown that retinol (ROL) alleviates some major signs of skin aging. Indeed, ROL at 1% exerts retinoid-like effects such as stimulation of collagen synthesis, reduced MMP levels, and induction of fibroblasts outgrowth. Also, long-term application of ROL at 0.4% in the upper arms reduced fine lines and provoked an accumulation of collagen in the papillary dermis. However, most studies have focused on the clinical benefits of ROL in photo-protected skin sites such as the upper arm. Moreover, the effects of ROL were studied at high doses ranging from 0.4% to 1.6%, doses at which it produces unwanted side effects such as skin dryness, irritation and itching. In the present study we explored the anti-aging action of a low concentration of retinol (0.1%) on human skin. First, we demonstrated that 0.1% retinol increases keratinocyte proliferation ex vivo as well as epidermal thickness in human skin explant culture. Second, we demonstrated that the long term topical application (up to 9 months) of retinol 0.1% on the face also stimulates epidermal cell proliferation in vivo and therefore improves the signs of aging such as fine lines, wrinkles, and tone evenness.

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A novel ingredient for skin aging

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Skin aging is characterized by wrinkles, flaky skin, xerosis and by an impaired of skin barrier repair ability. The aim of this study was to evaluate the effect of a novel bacterial extract in aqueous solution on some symptoms of skin aging. For this purpose, a topical cream containing a Bifidobacterium lysate was tested in a randomized double-blind placebo-controlled trial. Sixty six female volunteers with reactive skin where randomly given either the cream with the bacterial extract at 10% (n=33) or control cream. (N=33). The volunteers applied twice a day the cream to the face, arms and legs for two months. Skin barrier repair ability, leg dryness, facial roughness and natural moisturing factors amounts in the stratum corneum and were assessed by skin barrier recovery evaluated by measuring trans-epidermal water loss following barrier disruption induced by repeated tape-stripping, clinical assessment and biochemical measurement at day 1, d29 and d57. The results showed that the treatment led to increase skin resistance against physical and chemical aggression compared to group of volunteers who applied control cream. Noteworthy, the stripping number to obtain barrier function disruption was significantly increased for volunteers treated with the active ingredient compared to control treated group (p=0.0044) at the end of the treatment (day 57). Clinical and self-assessment revealed a significant decrease in skin dryness after 29 days for volunteers treated with the cream containing the 10% bacterial extract (p=0.028) and skin urea level for the active extract treated group increased while this natural moisturizing factor decreased in the control group. The results of this study demonstrate that this specific bacterial extract may have a beneficial effect on skin. Indeed, the findings suggest that this specific lysate may improve dryness and skin protection against environmental potentially irritating agents, external aggressions or stressed psychological conditions and prevent skin appearance associated with skin aging.

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Immunocytochemical quantitation of the desmosealin affinity to desmosomes

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Desmosealin is an epidermal chondroitin sulfate proteoglycan secreted into the extracellular spaces by keratinocytes and incorporated into the desmosome cores. We have used on-section immunogold labeling to determine the relative densities of thus detected antigen in different subcellular compartments at various differentiation stages of normal human epidermis. Quantitation of anti-desmosealin antibody binding (mouse monoclonal KM48) on Lowicryl K4M -embedded tissues revealed that the antigen was most concentrated at the keratinocyte periphery, in cell surface microvillosities (lower keratinocyte layers)and keratinosomes (differentiated keratinocytes). Once released into the intercellular spaces, desmosealin became dispersed approximately 10 fold; e.g. in the stratum spinosum, gold granule (gg) concentration within keratinosomes was 222.9/μm2 and only 24.5/μm2 in the free extracellular space. However, the quantity of this proteoglycan within the extracellular cores of desmosomes increased gradually from the basal to the upper granular layers: from 13.6 to 114.2 gg/μm2, respectively. In the spinous layer, desmosomes contained 3 times more desmosealin (80.3 gg/μm2) than the surrounding extracellular spaces and in the granular layer this ratio has increased to 4.6 times. Desmosealin persisted in the desmosomes between the first keratinized cells (106.3 gg/μm2) but was virtually no more detected in the intercorneocyte spaces filled with lamellar lipids (6 gg/μm2). There was no desmosealin labeling in the dermis or in the melanocytes. Cell nuclei and free cytoplasm showed a background level of labeling, respectively, 1.7 and 0.5 gg/μm2. Our results indicate that desmosealin demonstrates a selective affinity to the extracellular spaces of epidermal desmosomes and suggest that this affinity may be related to the differentiation-dependent composition of these junctions. The functional consequences of this peculiar proteoglycan distribution for the epidermal barrier function remain to be studied.

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An *in vitro* model for studying axolotl wound healing

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Axolotls are unique among adult vertebrates in their ability to regenerate amputated limbs. The first stage of regeneration involves the formation of a blastema at the wound site and requires the presence of fibroblasts, keratinocytes and nerve. The wound epidermis is essential for blastema formation however little is known about molecular mechanisms involved. As a step towards understanding axolotl wound healing we have developed an *in vitro* skin model to study keratinocyte migration and gene expression. The model comprises a 'donut' shaped explant formed by taking a 6mm full thickness punch biopsy of axolotl (*Ambystoma mexicanum*) limb skin and creating a 2 mm central circular wound. The 'donuts' were placed on collagen gels and incubated in 60% DMEM, supplemented with 10% FBS, ITS and gentamycin at 26°C in 5% CO2/95% air atmosphere. Wound healing was examined by video microscopy and immunocytochemistry. Keratinocyte migration usually started within 2 hrs of plating the explants and proceeded rapidly (~300μm^2/hr). The rate of cell migration was comparable to axolotl keratinocyte migration observed *in vivo* and an order of magnitude faster than mammalian keratinocyte migration. Re-epithelialization of the wound bed was normally complete within 10 hrs. Time lapse video microscopy revealed flattened lamellapodia extending from cells at the migrating wound edge. By 24 hrs the entire wound bed was covered by a multilayered epidermis. Although fibroblasts were present in the full thickness skin biopsy very few cells migrated into the collagen gel. The explants could be maintained in culture for at least 3 days. Keratinocyte migration was reversibly inhibited by matrix metalloproteinase (MMP) inhibitors and latrunculin A indicating that migration was dependent on MMP activity and actin polymerization. The 'donut' model provides simple novel system for generating wound epidermis and for studying axolotl wound healing. Understanding the mechanisms underlying wound healing in animals that regenerate is likely to lead to novel strategies for improving mammalian wound healing.

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Prevalent and rare filaggrin mutations in Japanese patients with ichthyosis vulgaris and atopic dermatitis

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Mutations in the gene encoding filaggrin (FLG) have been identified as the cause of ichthyosis vulgaris (IV) and shown to be major predisposing factors for atopic dermatitis (AD). However, these studies have been mainly carried out in European populations. In early 2007, we identified two Oriental specific FLG mutations in four Japanese families with IV and reported that the mutations were also significant predisposing factors for AD in Japan. However, the frequency of FLG mutations observed in our Japanese AD cohort (5.6%), was much lower than that seen in Europeans (up to 48%). Here we studied further ten Japanese families with IV and identified four additional nonsense mutations in FLG. We found 25% of patients in our Japanese AD case series carry FLG mutations and there is significant statistical association between the six mutations and AD. These data emphasize that skin barrier impairment due to reduced filaggrin expression plays an important role in the pathogenesis in AD and sheds further light on the genetic architecture of atopy in Japan.

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The effect of zanthoxylum bungeanum extract on peroxisome proliferator-activated receptor α and epidermal skin barrier recovery

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Peroxisome proliferator-activated receptor(PPAR) is a family of nuclear hormone receptor and plays important roles in skin barrier homeostasis by regulating epidermal cell growth, terminal keratinocyte differentiation, and inflammatory response. Among three PPAR isotypes, topically applied PPARα activators have anti-proliferative/pro-differentiating activity and anti-inflammatory activity, and accelerate skin barrier recovery which suggest that PPARα activators hold great promise for the therapy of cutaneous skin disease. To identify medicinal herbs that contain PPARα activators, fluorescence resonance energy transfer assay employing PPARα co-activator and GAL4-PPARα transactivation assay were used. It was found that the 1,3-butylenes glycol(BG) extract of Zanthoxylum bungeanum has a potent PPARα activating activity which is higher than clofibrate, a well known PPARα agonist. Expression of keratinocyte differentiation markers such as transglutaminse and involucrin were increased in keratinocytes by the BG extract. In an *in vitro* model with ultraviolet B light-irradiated human keratinocytes, the BG extract decreased the secretion of tumor necrosis factor-α, one of a major cutaneous inflammatory cytokines, in keratincytes. Its anti-inflammatory action was further demonstrated by in vivo testing in which the topically applied BG extract markedly decreased the ear thickness of the 12-O-tetradecanolyphorbol-12-acetate treated mice. Topical treatment of both flanks of hairless mice with the BG extract accelerated transepidermal water loss recovery after tape stripping. These results suggest that the BG extract of Zanthoxylum bungeanum can be developed as a potential therapeutic agent for the regulation of skin barrier homeostasis by activating PPARα.

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Imiquimod-induced interleukin-1α stimulation restores barrier abnormalities by topical calcineurin inhibitors

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Topical calcineurin inhibitors (TCIs) have anti-inflammatory and immunosuppressive effects, which have been used for dermatologic diseases including atopic dermatitis, vitiligo and psoriasis. Our previous study showed that barrier recovery was delayed after acute barrier disruption in skin treated with TCIs. In hairless mice, epidermis treated with TCIs showed a decrease in the number and secretion of lamellar bodies (LB), lipid synthesis related enzymes, antimicrobial peptides (AMP) and Interleukin 1α(IL-1α) expression. IL-1α is an important cytokine to improve barrier function, LB structure, and lipid synthesis in keratinocytes. The aim of this study was to evaluate the efficacy of imiquimod, an IL-1α inducer, in skin treated with TCIs. In hairless mice, topical tacrolimus, was applied twice a day and topical imiquimod was applied once a day for 4 days. Topical imiquimod improved epidermal permeability barrier homeostasis in comparison to a vehicle. Imiquimod-treated epidermis showed an increase in LB number and secretion and lipid synthesis related enzymes such as HMG CoA reductase, serine palmitoyl transferase and fatty acid synthases. Also Imiquimod increased the expression of AMP (CRAMP, mBD3). These results provide evidence for the role of decreased IL-1α signaling in impaired skin barrier function of skin treated with TCIs, and the possibility of cytokine augmentation to prevent barrier dysfunction in skin treated with TCIs.

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Wound healing in a model system: analysis of 4D dynamics

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Despite a large number of studies there is still debate over the cellular dynamics of wound healing. Histology, conventional imaging and electron microscopy have yielded conflicting results leading to two contradictory models; the 'leapfrog' model in which proliferating keratinocytes enter the suprabasal compartment and are pushed onto the wound bed, and the 'tractor tread' model in which basal keratinocytes migrate over the wound bed and pull the trailing epidermis. It is evident that a higher level of analysis is required to resolve such complex biological systems. We have employed an integrative approach that utilizes camera based transmission images (2D) and multiphoton microscopy to investigate the complex dynamics of wound healing in axolotl skin. Skin explants were prepared from full thickness skin excised from the forelimbs of GFP transgenic axolotls. Wounds were created by punch biopsy and re-epithelialization of the wound bed was followed using fluorescence imaging. One and 2 photon confocal microscopy were used to spatially delineate thick tissue samples in the axial direction and generate a 3D reconstruction of the cellular network. Fast data acquisition (~7mins for 3D stack of 200 μm) and optimum signal to noise ratios, make it possible to follow re-epithelialization as a function of time (4D dynamics). 3D reconstruction software and enhanced analysis algorithms based on fluctuation spectroscopy have made it possible to extract dynamic information and quantify the physical properties associated with wound healing. Specifically, it is possible to spatially resolved cellular velocities, cell size, and cell fate. Cell hypertrophy is observed with an approximate increase of 30% of cell volume during the re-epithelialization. We propose a new biological model that describes, in part, the cellular dynamics as well as the mechanics of wound healing. Evidence suggests that an essential impetus for the observed dynamics in wound closure can be attributed to cell hypertrophy where the pushing of the nearest neighbors triggers the motion.

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Histamine inhibits the recovery of cutaneous permeability barrier via its effect on IL-4 secretion followed by STAT6 activation

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The close relationship between allergic inflammation and cutaneous permeability barrier dysfunction is getting important to explain the complicated pathogenesis of allergic skin diseases, especially atopic dermatitis. Recently we found that IL-4, a prototype of Th2 cytokine, inhibited the recovery of cutaneous barrier function in vivo using STAT6 signaling pathway. Histamine, which is one of the most important mediators of allergic inflammation, is also reported to inhibit the recovery of barrier function in vivo as well as IL-4. The present study was designed to determine whether histamine inhibited the recovery of barrier function via IL-4/IL-13 and/or STAT6 signaling pathway. Cutaneous barrier functions were evaluated by transepidermal water loss that was monitored before and after disruption by tape stripping on the abdominal skin of C57BL/6 mice. Histamine and anti-histamine was applied to the skin immediately after disruption of cutaneous barrier. Intradermal injection of STAT6 decoy oligodeoxynucleotide or mixture of anti-IL-4 and anti-IL-13 neutralizing antibodies was performed 24 h or 30 min before topical application of histamine, respectively. Topical application of histamine inhibited but anti-histamine accelerated the recovery of barrier function after acute disruption. Intradermal injection of STAT6 decoy oligodeoxynucleotide and mixture of anti-IL-4 and anti-IL-13 neutralizing antibodies suppressed the inhibitory effect of histamine on the recovery of barrier function. These results indicates that histamine inhibits the recovery of barrier function via its effect on IL-4 secretion followed by activation of STAT6 signaling, while the possibility of the direct effect of histamine on the keratinocytes remains because of the accelerating effect of topical application of anti-histamine on the recovery of barrier function. In conclusion, histamine might affect the cutaneous permeability function in allergic inflammation of the skin via its effect on IL-4 secretion.

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Effect of K6EAA-L12 on the skin of aged mice

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Recently, we have reported anti-aging effects of K6PC-5 which increased sphingosine-1 phosphate (S1P) synthesis as a S1P activator. K6PC-5 induces keratinocyte differentiation and fibroblast proliferation. We performed this study to confirm anti-aging effects of new synthetic products (K6EAA series) derived from K6PC-5 by an amino group induction. Cellular responses such as differentiation, proliferation and calcium mobilization were investigated in cultured human keratinocytes and fibroblasts. Also collagen mRNA and protein expression were measured by the real time RT-PCR and ELISA. K6EAA-L12, selected by *in-vitro* screening, was evaluated for anti-aging effects on intrinsic and photo-aged model of hairless mouse. K6EAA-L12 showed anti-aging effects on intrinsic aged murine skin by collagen synthesis and eventually dermal thickening. It also increased dermal collagen density and dermal thickness in photo-aged skin. K6EAA-L12 treated photo-aged murine skin showed an increase in stratum corneum integrity by increasing corneodesmosome density and improved barrier recovery rate. However it did not change the expression epidermal differentiation maker proteins. Our results conclude that topical K6EAA-L12, a new synthetic K6PC-5 derivative, improves intrinsic and photo-aged skin by increasing collagen density and improving skin barrier function.

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Angiopoietin-like protein 4 regulates keratinocyte survival, differentiation and migration

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Epidermal homeostasis is a balance between cell proliferation and apoptosis which is regulated by numerous secreted factors. The angiopoietin-like protein 4 (ANGPTL4) is a secreted protein that regulates clearance of plasma lipoproteins. However, its role in the skin remains unknown. We investigated the function of ANGPTL4 in the epidermal wound repair using full-size excisional wounding, siRNA-mediated ANGPTL4 knockdown keratinocytes, organotypic skin coculture and in vitro scratch assays in the present study. Our results show that ANGPTL4 is upregulated along with the high expression of peroxisome proliferator-activated receptors (PPARs) during skin wound repair. Double immunofluorescence staining illustrates elevated ANGPTL4 expression in proliferating wound epidermis. We also show that ANGPTL4-deficient epidermis displays an altered epidermal architecture associated with increased apoptosis and impaired differentiation. ANGPTL4-deficient keratinocytes also displayed impaired cell migration. These results suggest that ANGPTL4, which has been implicated primarily in lipid metabolism, displays a new role as a key signal in regulating keratinocyte migration, apoptosis and differentiation important for epidermal regeneration.

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Generation of transgenic mice that inducibly express beta IG-H3 in the epidermis

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Increasing evidence suggests persistent phenotypic alterations in fibroblasts from non-healing human chronic wounds, which may result in faulty extracellular matrix deposition and keratinocyte migration. We have previously indicated that these cells are characterized by morphological changes, low proliferative potential and unresponsiveness to TGF-beta 1. Recent work from our laboratory has shown a decreased expression of beta ig-h3 in chronic wounds and their fibroblasts compared to acute wounds. Beta ig-h3 is a transforming growth factor beta (TGF-beta) induced extracellular protein involved in a number of functions essential to cell adhesion, proliferation, and tumor invasion, but little is known of the specific role of beta ig-h3 in cutaneous wound healing. To establish a viable transgenic model for studying functions of beta ig-h3 in wound healing, we have generated transgenic mice which allow focal induction of the beta ig-h3 transgene in the epidermis at different expression levels. Target transgenic mice that have a human beta ig-h3 gene fused with GAL4 binding sites upstream of the TATA promoter were generated. The transactivator mice were used that targeted the expression of GLp65 transactivators, fusion molecules containing a truncated progesterone receptor with a GAL4-DNA binding domain, with a human keratin 14 promoter. The target mice (TATA.beta ig-h3) and the transactivator mice (K14.GLp65) were mated to generate bigenic mice, and beta ig-h3 transgene expression was induced by topical application of RU 486 (mifepristone, a progesterone receptor antagonist). These inducible beta ig-h3 transgenic mice will be a novel method for better understanding of the role of beta ig-h3 in the pathogenesis of cutaneous wound healing.

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IL-4 and IL-13 inhibit the recovery of cutaneous permeability function by suppression of lamellar body secretion via STAT6 activation

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The association between Th2 pathology and cutaneous barrier functions is getting important to elucidate the pathogenesis of atopic dermatitis. Recently we found that IL-4 inhibits the recovery of cutaneous permeability barrier function after acute disruption in vivo. This study was designed to determine whether STAT6 signaling pathway mediates the inhibitory effect of Th2 cytokines and whether IL-4 affects the acceleration of formation and/or secretion of lamellar bodies, resulting in the inhibition of barrier recovery. Cutaneous barrier functions were evaluated by transepidermal water loss that was monitored before and after tape stripping on the abdominal skin of mice. Intradermal injection of IL-4 and/or IL-13 or STAT6 decoy oligodeoxynucleotide was performed immediately after or 24 h before barrier disruption, respectively. For electron microscopic analysis, the skin was resected 3 h after tape stripping from mice received an intradermal injection of IL-4 or HBSS (control). An intradermal injection of IL-13 inhibited the barrier recovery as well as IL-4. Simultaneous administration of suboptimal dose of IL-4 and IL-13 showed the significant inhibitory effect on the barrier recovery as a synergistic effect, which suggests that they use a common intracellular signaling: type II IL-4 receptor-JAK/STAT6 pathway. Direct inhibition of STAT6 function by STAT6 decoy oligodeoxynucleotide abolished the inhibitory effect of IL-4 and IL-13 on the barrier recovery. Electron microscopy suggested that IL-4 suppressed the acceleration both of formation and secretion of lamellar bodies at the stratum corneum and stratum corneum junction in mice who received an intradermal injection of IL-4 immediately after tape stripping compared with control mice. In conclusion, IL-4 and IL-13 inhibit the recovery of cutaneous permeability barrier function by suppression of lamellar body secretion via STAT6 activation.

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Targeted deletion of the murine *corneodesmosin* gene delineates its essential role in skin and hair physiology

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Controlled proteolytic degradation of specialized junctional structures, corneodesmosomes, by epidermal proteases is an essential process for physiological desquamation of the skin. Corneodesmosin (CDSN) is an extracellular component of corneodesmosomes, and although considerable debate still exists, genetic studies have suggested that the *CDSN* gene in the major psoriasis-susceptibility locus (PSORS1) may be responsible for susceptibility to psoriasis, a human skin disorder characterized by excessive growth and aberrant differentiation of keratinocytes. CDSN is also expressed in the inner root sheath of hair follicles, and a heterozygous nonsense mutation of the *CDSN* gene in humans is associated with scalp-specific hair loss of poorly defined etiology. Here, we have investigated the pathogenetic roles of CDSN loss-of-function in the development of skin diseases by generating a mouse strain with targeted deletion of the *Cdsn* gene. *Cdsn*-deficient mouse skin showed detachment of the stratum corneum from the underlying granular layer and/or detachment within the upper granular layers due to the disrupted integrity of the corneodesmosomes. When grafted onto immunodeficient mice, *Cdsn*-deficient skin showed rapid hair loss together with epidermal abnormalities resembling psoriasis. These results underscore the essential roles of CDSN in hair physiology, and suggest functional relevance of *CDSN* gene polymorphisms to psoriasis susceptibility.

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No production of pro-opiomelanocortin by keratinocytes after trichloroacetic acid peeling

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Trichloroacetic acid (TCA) is a peeling agent used to rejuvenate photoaged skin. TCA permeates the upper dermis and causes the tissue necrosis followed by wound healing. We previously reported that TCA induces production of various cytokines and growth factors, not only by a murine keratinocyte cell line but by the human epidermal tissue, before there necrosis. This effect is considered to be desirable for better wound healing, but would at the same time be undesirable if it leads to post-inflammatory pigmentation. In ultraviolet (UV)-induced suntanning, p53-dependent production of pro-opiomelanocortin (POMC), which is a precursor protein processed to act as the mature melanocyte-stimulating hormone (MSH), is reportedly responsible for pigmentation. Therefore, in this study, we have investigated whether TCA also induces p53 activation and POMC production by keratinocytes with flowcytometry (FCM) and reverse transcription-polymerase chain reaction (RT-PCR). We first studied p53 activation in murine Pam212 cells with FCM at various timepoints after various doses of UVB irradiation or various concentrations of TCA addition. P53 activation was observed 21 hours after irradiation of 45 mJ/cm2 UVB, 9 hours after irradiation of 150 and 450 mJ/cm2 UVB, but was decreased 21 hours after irradiation of 450 mJ/cm2 UVB, when the cells became dead. In case of TCA addition, p53 activation was detected only 21 hours after addition of 0.5% TCA, whereas never observed at any timepoint after addition of 1% TCA. Then, POMC production was analyzed with RT-PCR. As the result, POMC-specific band was faintly observed at 21 hours after irradiation of 45 and 150mJ/cm2 UVB and at 9 hours after 450mJ/cm2 UVB, however, no band was detected at any timepoint after addition of TCA. These results suggest that TCA does not induce p53-dependent POMC production by keratinocytes. Further analyses using human HaCaT cells and epidermal tissue are now underway to confirm this notion.

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Study of human skin-moisturizing mechanism based on the keratin structure in stratum corneum

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Stratum corneum in the outer layer of human skin is important in keeping skin moisture. Important components for moisturizing stratum corneum are generally natural moisturizing factor (NMF) and intercellular lipids. However, there are few reports about the relationship between keratin structure constituting a large part of stratum corneum and the moisture. In stratum corneum, Keratin forms fibrous substructure called keratin intermediate filament (KIF), and is seen by electron microscopy as net-like structure called "keratin pattern". It is known that the moisture decreases and keratin pattern is disappeared as NMF is extracted from stratum corneum. In this study, we investigated the relationship between keratin structure and the moisture by using X-ray diffraction. Additionally we discussed the change of moisture by the process with some moisturizers. We measured with X-ray diffraction at Spring-8 beam line (BL40B2). Samples of stratum corneum were taken from human heel and treated with water or some moisturizers. In addition, the amount of boundwater in stratum corneum was estimated by Differential Scanning Calorimeter (DSC) measurement. We found that the distance between each KIF changes according to the amount of water. In addition, it was shown that the structural change of KIF with some moisturizers is different from the one with water. Moreover, it was found that the structural change is related to the amount of boundwater in stratum corneum. We found new information about a relationship between keratin structure and the moisture of human stratum corneum from X-ray diffraction at SPring-8. These results suggest the possibility that the analysis of keratin structure leads understanding for essential skin-moisturizing mechanism.

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The functional expression of Heme Oxygenase-1 in normal human epidermis

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Hemeoxygenase-1, which catabolizes cellular heme to biliverdin, CO, and free iron, is a multifunctional protein. It provides an antioxidant defense mechanism, contributes to iron reutilization, modulates the immune response and activates several transcription factors. In most tissues, HO-1 typically occurs at low to undetectable levels under basal conditions, but its expression is rapidly activated in response to a number of stimuli. In the skin, although the over-expression of HO-1 has been reported in psoriatic lesional epidermis, its expression in normal human skin has not been precisely examined. In this study, we examined the expression of HO-1 and HO-2 in the epidermis of normal skin. In situ hybridization and immunohistochemistry revealed substantial HO-1 mRNA and protein expression in the subcorneal keratinocytes. In contrast, HO-2 was weakly but ubiquitously expressed in the entire epidermis. Next, we examined the regulation of HO-1 and HO-2 expression in the course of in vitro keratinocyte differentiation. Studies using real-time PCR and ELISA demonstrated that a significant augmentation of HO-1 mRNA and its protein along with keratinocyte differentiation was induced by high Ca medium. Again, the expression of HO-2 was not significantly modulated by keratinocyte differentiation. Interestingly, immunohistochemical studies on various inflammatory skin disorders and skin tumors revealed that HO-1 expression of subcorneal keratinocytes could only be detected in orothokeratinized epidermis, but not in the epidermis showing parakeratosis or trichilemmal keratinization. A study using anti-bilirubin antibody demonstrated the accumulation of bilirubin only in orthokeratinized straum corneum indicating that heme is actually degraded in the epidermis mainly by HO-1. These studies suggest a novel role played by HO-1 in human epidermis, where it might be involved in heme degradation, iron recusal, and the bilirubin supply to the stratum corneum for preventing lipid peroxidation.

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Comprehensive analysis of ceramides in the stratum corneum of patients with atopic dermatitis or psoriasis

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Ceramides, which are major lipids in stratum corneum, play critical roles in epidermal barrier and water holding functions. According to their structures ceramides have classified into 10 to 11 classes. Further, each class of ceramide has various species with different molecular weights. It has been presumed that the different ceramide profiles consisting of these various types of ceramide may affect the cutaneous function. To test this speculation, we analyzed ceramide profiles of stratum corneum in patients with atopic dermatitis or psoriasis, which is known as barrier disease, in comparison with healthy volunteers. Subjects were eighteen patients with mild atopic dermatitis or with psoriasis and sixteen age-matched healthy volunteers. Transepidermal water loss (TEWL) and skin capacitance were measured to evaluate their cutaneous function. Subsequently, the stratum corneum was sampled from each subject by tape striping. Ceramides in the obtained stratum corneum were analyzed by normal phase liquid chromatography electrospray ionization mass spectrometry. Eleven classes of ceramides, including a novel type of ceramide ADS (Alpha-hydroxy fatty acid amide linked with dihydrosphingosine), were identified in each subject regardless to whether or not they have a skin disorder. However ceramides with hydroxtlated sphingoid base were found to be fewer in atopic dermatitis or psoriasis patients as compared to healthy volunteers. In addition, the average molecular weights of Cer[NDS] and Cer[NS] tend to be lower in these patients compared to healthy volunteers. These characteristics were more prominent in skin regions with a high TEWL and a low capacitance. These results may suggest a close relationship between ceramide profiles and dysfunction of stratum corneum.

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Expression of the serine protease matriptase-1 is lost in psoriatic skin lesions and is down-regulated by TNF-alpha in vitro

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Matriptase-1 is a type II serine protease which plays an important role in the regular terminal differentiation of epidermal keratinocytes most likely by its involvement in the processing of filaggrin. Mice lacking matriptase-1 die within 48 hours after birth due to severe barrier defects which lead to dehydration. Skin transplanted from these mice onto SCID mice develops an ichthyosis like phenotype, displaying hyperproliferation with hyper- and parakeratosis. More importantly for the human situation, recently a mutation in the highly conserved peptidase domain of matriptase-1 has been identified and associated with the autosomal recessive ichthyosis with hypotrichosis syndrome. Here we have investigated the expression and regulation of matriptase-1 in psoriatic skin and in keratinocytes (KC) *in vitro* respectively. Activity assays and immunofluorescence staining revealed that both matriptase-1 enzymatic activity and expression was strongly reduced in psoriatic skin lesions as compared to the uninvolved adjacent skin of the same donors. Exposure of KC to TNFα, a key cytokine in the pathogenesis of psoriasis, led to a strong down-regulation of matriptase-1 mRNA and protein production in vitro. In addition, organotypic skin cultures treated with TNFα led to disturbed KC-differentiation, accompanied by complete loss of matriptase-1 expression. Furthermore, inhibition of the NFκB signaling pathway by transduction of KC with adenovirus over-expressing dominant negative IKK2, completely blocked TNFα induced down-regulation of matriptase-1. Since matriptase-1 is involved in regular terminal KC differentiation, its absence in psoriatic lesions might directly contribute to the barrier alterations in this disease. Consequently, blocking the IKK2-pathway might represent an interesting target for the treatment of psoriasis.

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TonEBP/NFAT5 is activated by epidermal barrier dysfunction and regulates keratinocyte proliferation and apoptosis in response to hypertonic stress

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TonEBP (NFAT5) is the only known transcription factor to respond to hypertonic stress and permits adaptation to a hypertonic environment. We hypothesized that keratinocytes may be exposed to hypertonic stress following epidermal barrier disruption and in dermatoses characterized by a defective epidermal barrier. Therefore, we analyzed human epidermis and cultured normal human epidermal keratinocytes (NHEKs) for the presence of the TonEBP and studied its functional role under hypertonic conditions. We showed that NFAT5 is expressed in the cytoplasm and nucleus of NHEKs. TonEBP expression, nuclear localization and transcriptional activity increased in response to hypertonicity in a time and dose-dependent manner. By immunohistology, TonEBP expression was detected at low levels in normal skin and skin equivalents, and nuclear expression was restricted to the upper granular layer of the epidermis. TonEBP cytoplasmic and nuclear expression was increased throughout the epidermis of lesional eczema (n=5) and psoriasis (n=5) and the normal gradation of TonEBP expression was lost. Barrier disruption by acetone treatment of skin equivalents and tape-stripping of normal human skin caused a significant increase in TonEBP expression. siRNA targeted against TonEBP or overexpression of TonEBP showed that TonEBP positively regulates keratinocyte proliferation/viability, and inhibits apoptosis induced by hypertonicity. These data identify for the first time a functional role for TonEBP in NHEKs of responding to hypertonic stress and protecting NHEKs from the deleterious effects of hypertonicity. Moreover, our results indicate upregulation and activation of TonEBP in dermatoses characterized by defects in epidermal barrier and in response to acute barrier disruption. Taken together, our data suggest that hyperproliferation seen in certain dermatoses and following epidermal barrier disruption may result in part from overexpression of TonEBP through an adaptive response to hypertonicity.

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The effects of pimecrolimus cream 1% on key physiology parameters in patients with atopic dermatitis

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The purpose of this study was to investigate the effects of treatment with pimecrolimus cream 1% on the physiologic al function of skin affected by atopic dermatitis.In this double-blind, within-patient vehicle-controlled, 3-week study, 20 patients (11 female, 9 male), 18 to 48 years of age (mean 25.7) with mild-to-moderate AD were randomized to apply pimecrolimus cream 1% on the volar side of one forearm and vehicle cream on the contralateral forearm. On day 0, 3, 7, 14 and 21 cutaneous blood circulation, skin hydration and transepidermal water loss (TEWL) were measured using Laser Doppler Imaging, corneometry and TEWL assessment, respectively. In addition, AD disease severity was assessed by the Atopic Dermatitis Severity Index (ADSI), the IGA and a Visual Analog Scale for pruritus. Between day 0 and 21, cutaneous blood microcirculation in lesional AD skin treated with pimecrolimus cream 1% showed a decrease from 33.90 to 15.55 au ($p<0.0001$); TEWL in pimecrolimus treated AD skin decreased from 35.30 to 21.50 g/cm2/h ($p=0.001$); and skin hydration increased from 42.86 to 52.69 au ($p=0.002$). No such significant changes were observed in the vehicle group. At Day 21, microcirculation and transepidermal water loss were significantly lower on pimecrolimus cream 1%-treated sites, whereas skin hydration was significantly higher as compared to vehicle. According to all clinical assessments, greater improvement was achieved at sites treated with pimecrolimus than on those treated with vehicle. Clinical improvement of AD was paralleled in this study by a significant decrease in cutaneous blood microcirculation probably reflecting the anti-inflammatory action of pimecrolimus. For the first time, this study showed that the anti-inflammatory and anti-pruritic effect of pimecrolimus cream is accompanied by a significant improvement of skin barrier functions resulting in an increased skin hydration and decrease of TEWL.

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Corticosteroids in contrast to pimecrolimus largely reduce the frequency of epidermal Langerhans cell precursors in topically treated newborn mice

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We have shown that the corticosteroid beta-methasone-17-valerate (BMV) but not to the calcineurin inhibitor pimecrolimus negatively affects LC maturation as well as the viability and cytokine secretion of infant EC in vitro. To investigate whether these compounds interfere with the developing skin immune system in vivo, we compared their effects on the fate, phenotype and maturation of epidermal CD45+MHC class II+/-CD205-CD207- Langerhans cell (LC) precursors in newborn mice. LC precursors start to acquire immunological important molecules, and consequently also the ability to present antigen within 3 days postpartum. Newborn BALB/c mice were topically treated twice with ethanolic solutions of the test compounds at their clinically used concentrations (1% pimecrolimus, 0.1% BMV, 0.1% clobetasol-17-propionate) on the dorsal trunk. Forty-eight hours after the last application (i.e. 3 days postpartum), we observed an increase of MHC class II+ LC precursors (6.7%) in pimecrolimus-treated animals, whereas treatment with BMV and clobetasol resulted in a decrease of these cells (2.4%, and 0.7%, respectively), when compared with vehicle-treated controls (5.6%). To evaluate whether topical application of pimecrolimus and corticosteroids has any effects on the maturation of LC precursors, 48-hour epidermal cell suspension cultures from compound-, or vehicle-treated skin were examined for phenotype. Flow cytometry analysis revealed that BMV, but not pimecrolimus partially inhibited the upregulation of CD40/CD80/CD86 costimulatory molecules on MHC class II+ leukocytes. LC precursors were abolished in cultures from clobetasol-treated skin. Taken together, our data show that corticosteroids but not pimecrolimus decrease the viability and maturation capacity of LC precursors in mice.

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Pharmacological manipulation of an oral mucosa progenitor/stem cell population

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Introduction: Progenitor/stem cells of oral mucosa reside in the small-sized cell population and will continue to differentiate, with an increase in cell size, when appropriately stimulated as opposed to large size cells which are highly differentiated. Activation of mammalian target of rapamycin (mTOR) leads to increase in cell size and keratinocyte differentiation. However, the effect of rapamycin, a specific inhibitor of mTOR, on keratinocyte proliferation has never been well characterized. Objective: To pharmacologically manipulate the small cell progenitor/stem cell population with rapamycin to stay in an undifferentiated and proliferative state for fabrication of an *ex vivo* produced oral mucosa equivalent (EVPOME) for use in transmucosal gene therapy. Methods: Primary oral keratinocytes cultured in a defined medium (EpiLife®, Cascade Biologics, Portland, OR) without serum, pituitary extract or use of a feeder layer were sorted by serial filtration into Large and Small size cell groups, and cultured with 0, 2 and 20nM rapamycin. Once they reached 70-80% confluence, cells were simultaneously counted in all 6 groups, then re-plated and continued to culture until its proliferation was exhausted. During these serial passages, Cumulative Population Doublings (CPD), Colony forming efficiency (CFE), differentiation marker expression (DME) and ability to regenerative an EVPOME were assessed. Results: Large and Small sized cell groups cultured in 2 and 20nM rapamycin retained their small-sized and proliferation ability, for up to 20 weeks with 15.7 CPD on average (N=10). In contrast, Large and Small cells without rapamycin were exhausted within 5 weeks. Higher CFE and lower DME were noted in the rapamycin treated cell groups. Both Large and Small size cells treated with rapamycin for 15 weeks in culture were still able to regenerate an EVPOME. Conclusion: The differences between the behaviors of Small versus Large size cells were eliminated when they were treated with rapamycin.

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Enrichment, culture and functional analysis of proliferating pig Merkel cells

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Introduction: Merkel cells (MC) are neuroendocrine cells of the basal layer of epidermis which produce many neuropeptides (Boulais and Misery, 2007). MC are believed to act in touch perception and skin homeostasis. However, their functions remain poorly known so far. Methods: To investigate the neuroendocrine capability of MC, we performed the first enriched culture of MC obtained from the snout of young pig after enzymatic dissociation and positive magnetic selection. The yields of the enrichments were analyzed by labeling MC against the cytokeratin 20 (CK20). The presence of MC was confirmed by electron microscopy where characteristic dense-cored granules were observed. Cultured MC were exposed to various substances. The release of the neuropeptide vasoactive intestinal peptide (VIP) was assessed by ELISA. Results: In our enriched cultures, we often reached more than 60% of MC. MC developed numerous cytoplasmic extensions, so becoming easily distinguishable. Furthermore, we could observe many cells divisions. The presence of MC undergoing mitosis was confirmed by double labeling against CK20 and the proliferating-associated antigen Ki-67. All phases of the cell cycle were observed confirming the proliferation of MC. The exposure of MC to histamine (100µM) or to a phorbol ester agonist of the transient receptor potential vanilloid 4 (TRPV4) (1µM) led to an increase in VIP released. However, the exposure to acetylcholine (Ach)(10µM) inhibited the release of VIP. Conclusion: Here we performed the first enriched cultures in MC which represent the first model to study MC. In our culture conditions, we also demonstrated for the first time that MC were capable to proliferate which was never highlighted before. The contradict effect of histamine and Ach argue for a putative role of MC in inflammation, hypersensitivity and probably atopic dermatitis. The effects of the TRPV4 activation suggest a role of MC in sensitive skin. Boulais N, Misery L (2007) Merkel cells. J Am Acad Dermatol 57:147-165

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Connexin mimetic peptide Gap27 accelerates human and porcine wound healing by enhancing proliferation and migration of fibroblasts and keratinocytes

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Connexins are transmembrane proteins that form Gap Junctions (GJ), communicating channels that allow the exchange of small molecules between adjacent cells. GJ are important for migration, differentiation and proliferation of cells. Connexin 43 (Cx43) has been shown to be ubiquitously expressed in human epidermis and to be downregulated during early wound healing at the wound margins and in regenerating epidermis. The fact that Cx43 is still present at the margins of chronic wounds implicates that the downregulation is important for an effective wound closure. Phoshorylation of Cx43 on serine368 (S368) has been shown to decrease gap junctional intercellular communication (GJIC). We could observe an even distribution of pS368 throughout the epidermal layers of native skin. After wounding, pS368 was downregulated in parallel to Cx43. To further elucidate the role of Cx43 we investigated the influence of a Cx43 mimetic peptide (Gap27) which results in a disruption of GJIC. Treatment of porcine *ex-vivo* wound healing models with Gap27 resulted in a significantly accelerated wound healing compared to the controls. The treated models showed a higher number of proliferative keratinocytes in the regenerating epidermis and at the wound margins. No difference concerning Cx43 phosphorylation (pS368) was observed. Confluent keratinocyte and fibroblast cultures that were treated with Gap27 prior to a scratch wound assay showed an enhanced migration that resulted in a faster wound closure. Interestingly, Gap27 treatment had no effect on the migration of human keratinocytes from diabetic origin. These data show the importance of Cx43 in wound healing and suggest a different influence of Gap27 on wound healing in diabetic and non-diabetic keratinocytes.

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The concentrations of amino acids are relevant indicators of stratum corneum hydration

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Raman spectroscopy has emerged as a useful, non invasive technique to study the molecular composition of the skin. Using this technique, our objectives were (1) to investigate the molecular composition of the stratum corneum (SC) in subjects with normal to very dry skin, (2) to assess molecular changes of the SC in response to treatment with a topical moisturizer. Twenty seven Caucasian women aged 35 to 45 were included in this study and grouped according to the capacitance value on their forearm: <30 arbitrary units (a.u.): group A versus ≥ 30 a.u.: group B. The concentrations of natural moisturizing factor (NMF) components including free amino acids, urocanic acid (UCA), pyrrolidone carboxylic acid (PCA), lactate and urea were measured by Raman spectroscopy (Model 3510, River Diagnostics BV, The Netherlands), on the volar forearm at baseline and one month after a standard moisturizing treatment. 1) Free amino acids histidine (His) and proline (Pro) were found in higher concentrations in group A than in group B. No other significant difference was found between the two groups. (2) After a one-month treatment with the moisturizer, group A showed a significant decrease of His and Pro contents, as well as UCA and PCA, which result from His and glutamine catabolism, respectively. Group B also demonstrated significant changes in amino acids content after treatment: serine, a major NMF component, increased and UCA decreased. Moreover, skin hydration, assessed by capacitance measurements, showed a dramatic rise in group A, whereas no significant change was observed in group B. The high concentrations of His and Pro in the group with the lower basal hydration level (A) indicate a disturbance of their metabolism during epidermal keratinisation. The decrease of His and Pro in response to twice daily application of a moisturizer might therefore be associated with a regulation of metabolic pathways within the SC, resulting in or contributing to the increase of SC hydration observed.

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Loss-of-function mutations in the filaggrin gene predicted by *in vivo* Raman spectroscopy

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Filaggrin is a key protein required for formation of the stratum corneum barrier. Filaggrin is also essential for stratum corneum hydration by acting as a source of hygroscopic amino acids and their derivatives, known as natural moisturizing factor (NMF). Recently discovered loss-of–function mutations in the gene encoding profilaggrin have been shown to strongly predispose to atopic dermatitis (AD). The aim of this study was to determine if these mutations lead to reduced levels of NMF in the SC. NMF concentration was measured *in vivo* using confocal Raman spectroscopy in 23 individuals wild type for the common filaggrin mutations (R501X, 2282del4, R2447X, S3247X) and 16 carriers of at least one of these mutations. Here we have shown for the first time that individuals who are carriers of a FLG loss-of-function mutation have significantly reduced levels of NMF ($P<0.0001$, measurement forearm: carriers relative NMF concentration 0.83±0.15 versus non-carriers 1.23±0.13) and that these changes were detectable *in vivo* even in subjects who had no history of AD, IV or other skin disease. Measurement of NMF as a marker of FLG status could enable a more targeted prevention of AD in susceptible persons, in particular infants, and is much less demanding than genotyping.

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Age-related changes in proopiomelanocortin (POMC) and related receptors gene expression in the epidermis

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Skin aging is characterized in part by the decrease of the epidermal thickness, the decrease of cell divisions from the basal layer, a lengthening of renewal time from corneal layer cells, a decrease of skin moisture, a decrease of laxity and an impaired wound healing. A decrease of innervation has been reported too. Firstly, to determine the maturation level of skin innervation, we quantified the amount of tubulin betaIII (recognized as one of the earliest immature nerve marker) and neurofilament 200 (mature neurone marker) by immunofluorescence in biopsies of women from 17 to 75 years old. Immunofluorescence revealed a decrease from 3.7 fold of tubulin betaIII and from 3.5 fold of neurofilament 200 in women after 38 years old. Many studies showed that ultraviolet radiation induced keratinocyte differentiation, and associated this effect to premature skin aging. Because ultraviolet radiation have been shown to regulate proopiomelanocortin (POMC) family level expression in the skin, we secondary investigated the changes of POMC, MC-1R, MC-2R, and mu-opioid receptor (MOR1) mRNA expression in human keratinocytes of women on the range age from 17 to 75 years old. In order to answer this question, we assessed POMC, MC-1R, MC-2R and MOR1 mRNA levels by real time PCR in the total epidermis and basal keratinocytes. It's the first study using women skin to analyse the changes of gene expression during aging. We showed that after 50 years old, MC-1R and MC-2R mRNA expression were decreased in basal keratinocytes by 4 times and 8 times respectively, whereas no gene expression changes has been found in total epidermis. Moreover, we showed that MOR1 mRNA expression was strongly decreased in basal keratinocytes (6.5 times) after 55 years old. In contrast, after 46 years old, POMC mRNA expression was increased by 5 times in basal keratinocytes. We conclude that the changes of POMC gene expression and related-receptors may be implicated in the process of chronologic skin aging.

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New way to restore the balance of POMC-related peptides and their respective receptors in the human skin

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In a previous study, we have shown that melanocortin receptor 2 (MC-2R) and mu opioid receptor (MOR1) gene expression decreased whereas POMC gene expression increased with aging in human keratinocytes. The present study concerns an in vitro method for screening active compounds capable of upregulating MC-1R, MC-2R and MOR1 gene and protein expression in order to restore the balance of POMC-related peptides and their respective receptors. *In vitro* experiments carried out on a selected list of 1050 molecules led to the discovery of 30 compounds able to induce an increase of mRNA expression specifically or not of the 3 different receptors and 3 compounds able to induce an increase of protein level. The first steps of the screening have been done by real time polymerase chain reaction (PCR). We first selected a keratinocyte donor which was able to respond to known cytokines. We tested at one concentration 350 chemical characterized molecules (0.1%), 350 molecules transformed by biotechnologies (1%) and 350 vegetables extracts (1%) on human keratinocytes by real time PCR on MC-1R, MC-2R and MOR1 mRNA expression in comparison with a selected positive control NGF (10 ng/mL). Active compounds inducing genes with a greater than two-fold difference in expression with control were considered as good inducers of the gene expression. Step one revealed a total of 30 active compounds able to induce an increase of at least 2 fold gene expression. We next analysed, by dose effect, in triplicate, the effect of the 30 active compounds resulting from step one by real time PCR. Step two revealed a total of 8 active compounds which have been finally tested by western blot. Real-time and western blot analysis revealed 2 final compounds able to induce specifically MOR1 or MC-R2 protein expression and 1 compound able to induce both MOR1 and MC-R2 protein expression. Our best compounds represent promising MCR-2 and MOR1 activators that also exhibit keratinocyte differentiation properties.

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Cloning, expression, and characterization of kallikrein-related peptidase-8 (KLK8) in Pichia pastoris

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Kallikrein-8 is a member of the kallikrein-related peptidase family (KLKs) encoded by 15 contiguous genes clustered on chromosome 19q13.4. Similar to other KLKs, KLK8 is secreted as an inactive precursor zymogen (pro-KLK8) upon removal of its N-terminal pre-peptide and is activated extracellularly by subsequent cleavage of its pro-peptide, generating the mature enzyme (mat-KLK8). Formerly, serine protease activity in human skin was solely attributed to KLK5 and KLK7, implicated in skin desquamation and antimicrobial defense. In recent years, growing evidence has shown that multiple KLKs co-localize in the upper stratum granulosum and stratum corneum (SC) of human epidermis, and in skin appendages such as hair follicle epithelia and sweat glands. A recent ELISA study has indicated that KLK8 is the most abundant epidermal trypsin-like KLK accounting for up to 40% and 60% of the total SC and sweat trypsin-like KLK concentrations, respectively. Whether the elevated levels of KLK8 reflect increased KLK8 activity remains to be determined. The function of mat-KLK8 in the skin is yet elusive, although it may also be potentially involved in skin desquamation and antimicrobial activation or in other processes such as hair maturation, lipid barrier formation, skin pigmentation, and/or tumor suppression. To better characterize KLK8, we aimed at producing active KLK8 by designing specific primers to amplify the gene region encoding mat-KLK8 using a mammalian plasmid containing full length KLK8 cDNA. The sequence of the mat-KLK8 PCR product was confirmed and subcloned into pPIC9 yeast expression vector, in frame with the α-secretion factor and the AOX1 promoter. Following restriction enzyme analysis and purification, mat-KLK8-pPIC9 was transformed into Pichia pastoris GS115 and secreted mat-KLK8 was purified by chromatography. The production of active recombinant KLK8 protein is essential for the investigation of KLK8 role in human epidermis, which may lead to novel dermatological and cosmetic applications.

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Preparation of an antibody against gamma-glutamylcyclotransferase and comparison of its reactivity between aged and young epidermis

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One of causes of senile xerosis might be decrease of natural moisturizing factors (NMF) in the stratum corneum. However though, free amino acids in the stratum conruem are well known as NMF, we found that the content of free amino acids in the surface of the stratum corneum was increased in senile xerosis than in normal aged skins or young skins (Arch Dermatol Res 295:448-452, 2004). This finding suggested that pyrrolidone carboxylic acid (PCA), which is other NMF, could be more important NMF than free amino acids in the stratum corneum. In this experiment, the amount of gamma-glutamylcyclotransferase that synthesizes PCA in vivo was compared in young and aged epidermal extracts, in order to investigate whether the synthesis of PCA is decreased in aged epidermis. An antibody against gamma-glutamylcyclotransferase was raised to immunize its peptide (A86-D101) as an antigen to rabbits. Epidermal samples were extracted using 50mM Tris-HCl buffer (pH 7.4) or lysis buffer (7M urea/2M thiourea/ 0.05M 2-ME) and they were subjected to SDS-PAGE and immunoblot analysis using anti-gamma-glutamylcyclotransferase antibody was performed. The positive protein band at 28 kDa was observed in lysis buffer extract but not in Tris-HCl buffer extract. Furthermore, the reactivity of this antibody at the 28 kDa protein was stronger in young extracts than in aged ones. These findings indicate that gamma-glutamylcyclotransferase might be membrane bound protein not water soluble one and the decrease of this enzyme would introduce the decrease of the amount of PCA and cause senile xerosis.

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Accelerated proliferation and abnormal differentiation of epidermal keratinocytes in Endo-β-galactosidase C transgenic mice

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We produced transgenic (TG) mice that systemically express Endo-β-galactosidase C (EndoGalC) to understand the roles of sugar residues in development. They had rough and flaky skin, which was detectable around 5 days post-natal and became obscure by 2 weeks after birth. Their epidermis was thickened but the dermis and hair follicles were normal in structure. EndoGalC, which removes the terminal Galα1-3Gal disaccharide (αGal epitope), was expressed in the epidermis of TG mice. GS-IB4 lectin staining showed that the αGal epitope did not exist in the epidermis in TG but wild type (WT) mice. In TG mice, N-acetylglucosamines were exposed by EndoGalC, which was detected using GS-II lectin. To understand the cause of the epidermal thickening and skin phenotype, we examined the proliferation and differentiation of kerationocytes. BrdU-pulse-labeling revealed that proliferating keratinocytes increased approximately 3-fold in TG epidermis compared to WT one. In TG epidermis, the expression domain of cytokeratin 14 increased from 1-2 layers to 4-5 layers and co-expressed with cytokeratin 6 and 10 in the upper layers. The layers expressing involucrin and loricrin also increased but those expressing filaggrin and transglutaminase looked normal. The localization of E-cadherin was similar in both TG and WT mice. Although TG mice showed delayed development of the barrier function around 8 days post-natal, they acquired the function by 12 days after birth. These results suggest that the absence of the αGal epitope or the exposed N-acetylglucosamine terminal could play a critical role in the proliferation of basal keratinocytes and differentiation of them into the spinous cells in newborn mice.

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Indigo naturalis up-regulated claudin-1 gene expression in human keratinocytes

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The use of indigo naturalis to treat psoriasis has been proved effective in our previous clinical studies and increase of claudin-1 expression in skin biopsy was also demonstrated. In this *in vitro* study, we investigated the effect of indigo naturalis on the claudin-1 gene expression in human keratinocytes. Extract of indigo naturalis was prepared from Strobilanthes formosanus Moore (Acanthaceae) and concentrations ranged from 10 to 500 μg/ml were used to treat human keratinocytes. LDH activity measurement and MTT assay were performed to determine the cytotoxicity and cell viability. RT/real-time PCR, western blotting analysis and indirect immunofluresence labeling were used to investigate the mRNA and protein expression of claudin-1. We also measured the change of transepithelial electrical resistance (TEER), an indication of barrier function, in indigo naturalis-treated keratinocytes. Results showed that indigo naturalis did not cause cell damage; however, concentration at 500 μg/ml reduced the cell number of keratinocytes by 50 %. RT/real-time PCR analysis showed dose- and time-dependently increased mRNA expression of claudin-1 in indigo naturalis-treated human keratinocytes. Similar results were obtained from western blotting analysis for the increased claudin-1 protein expression. Fluorescent immunocytochemical analysis revealed the enhanced expression of claudin-1 in keratinocytes, located especially at the cell-cell junctions. Increased TEER values were also demonstrated in indigo naturalis-treated keratinocytes. In conclusion, the action of indigo naturalis can improve skin barrier protection, at least in part, by up-regulating claudin-1 expression and therefore the tight junction function.

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Pimecrolimus but not corticosteroid improves the skin barrier in atopic dermatitis

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Genetic defects in skin barrier function were recognized as risk factors for atopic dermatitis (AD). Impaired barrier in AD allows the penetration of allergens (e.g. house dust mite or grass pollen) into the skin which results in immunological reactions and inflammation. To prevent sensitization, it is essential to understand how the epidermal barrier is influenced by drugs applied in AD. We investigated the effect of pimecrolimus and betamethasone creams on the skin barrier. In a randomized, double-blinded left-right comparison study, 15 patients with AD were treated on one upper limb with pimecrolimus and on the other with betamethasone twice daily for three weeks. Stratum corneum hydration and transepidermal water loss (TEWL), a marker of the inside-outside barrier, improved in both groups. Electron microscopic evaluation of barrier structure displayed ordered lipid layers in the stratum corneum and regular lamellar body extrusion in pimecrolimus-treated skin. Disrupted extracellular lipid bilayers, as well as reduced numbers and only partially filled lamellar bodies were noted after betamethasone treatment. This is consistent with the findings on normal human skin which showed enhanced dye penetration after betamethasone treatment, indicating a disturbed outside-inside barrier. The reduced TEWL after betamethasone treatment despite the persisting barrier defect can be explained by reduced blood flow long recognized after corticosteroid treatment. TEWL depends on the passive barrier integrity component as well as the active driving force component the blood flow. Epidermal hyperproliferation in AD was reduced by both drugs, but after betamethasone treatment the proliferation levels were below those in normal skin. These results demonstrate that betamethasone and pimecrolimus affect the epidermal barrier differently, and that pimecrolimus, in contrast to corticosteroids, improves the epidermal barrier without the risk of causing the skin atrophy.

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S100 proteins in human skin and skin cancer in the process of differentiation and proliferation

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We previously reported that calcium-binding protein S100P showed a calcium inducible expression in esophageal epithelial cells and that other S100 proteins including S100A2, S100A6 and S100A12 were expressed in the different stages in esophageal epithelium. Epidermal keratinocytes of the skin are known to express various S100 proteins during the process of differentiation. We examined the S100 proteins in human skin and found that epidermal keratinocytes expressed S100 proteins during differentiation but in a different manner. S100P was not seen in epidermal keratinocytes of human skin, S100A2 was expressed in the basal layer, and S100A6 was detected in the suprabasal layers. S100 proteins in skin cancers (squamous cell carcinoma, basal cell carcinoma) were also examined immunohistochemically and found that S100A2 and S100A6 were expressed in the skin cancers and that these S100 proteins were seen in wide range of the tumor tissue, which indicate that aberrant behavior of cancer cells might have some relation to S100 proteins. To test the S100 proteins in human keratinocyte during differentiation or proliferation, cell culture system was utilized. Human epidermal keratinocyte line and squamous cell carcinoma cell line were cultured in proper media respectively. As reagents to promote differentiation or proliferation, vitamin D (VD) or retinoid (RA) was used. As reported previously, VD enhanced differentiation step and keratinocytes grew fast by adding RA in culure media. Squamous cell carcinoma cell showed slight proliferation in RA-media and VD had almost no effect on cancer cell. Immunohistochemical study showed that S100A6 positive cells in human keratinocyte increased by exposing to RA whereas the S100A6- expression-pattern did change slightly on cancer cells. These data indicate that normal human keratinocytes have response element to VD and RA and that expression-pattern of S100 protein, especially S100A6, changed by these reagents. Squamous cell carcinoma cells have aberrant response to VD and /or RA. S100 proteins might have some link to these reagents

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Role of desmoglein 1 in homeostasis of skin barrier function

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The stratum corneum (SC) provides barrier function of the skin. Once the barrier function is disrupted, the SC becomes thickened due to increased turnover to maintain the skin barrier compensatorily. Thus, SC organization, including the control of the SC thickness and adhesive manner, is a key process in homeostasis of skin barrier. To elucidate the homeostasis of skin barrier function, we focused on the corneodesmosome (CDS), an adhesion apparatus observed in the SC. In this study, we investigated the change in distribution of desmoglein 1 (Dsg1), a presumed main component of CDS, in the process of barrier disruption and recovery. The barrier disruption was induced by stripping the SC from the inside of the upper arm of a healthy volunteer with cyanoacrylate glue and adhesive tape every day. The SC samples were stained with a monoclonal antibody against extracellular domain of Dsg1, and observed with fluorescence microscope. As the skin barrier was disrupted by stripping, confirmed by increase of transepidermal water loss (TEWL) as an index of skin barrier function, Dsg1 amount was increased in the center of the corneocytes while positive staining was observed only in the periphery of the corneocytes in initial state. Although the barrier function was recovered immediately due to quitting the skin barrier disruption, the normalization of Dsg1 staining pattern from allover to the periphery pattern was delayed for the period corresponding to the turnover of the SC. This result suggests that the increase of Dsg1 amount induced by barrier disruption contributes to the strengthening the inter-corneocyte cohesion, resulting in the compensation for the disrupted barrier function with hyperkeratosis. Furthermore, in the process of barrier disruption and recovery, the Dsg1 staining changed only in the center of the corneocyte, suggesting that the role of Dsg1 is different from that in the center and periphery. In conclusion, our study suggests the involvement of Dsg1 in the skin barrier function.

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Establishment of platform for determining dermatopharmacokinetics of Chinese herbs - Rheum palmatum L. and Rumex japonicus Houtt. as examples

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Skin is an important barrier for xenobiotics and an important portal for drug delivery for topical, regional and systemic action. Dermal and transdermal drug delivery is often limited by the poor permeability of the stratum corneum. It's an important issue to reduce the barrier resistance of stratum corneum for increasing transcutaneous permeation. Rhubarb is the dry root of Rheum palmatum L. (Polygonaceae) which containing active anthraquinones such as aloe-emodin, rhein, emodin and chrysophanol. It was commonly employed for relieving constipation by purgation, clearing away heat and toxins, purging fire, promoting blood circulation, normalizing functioning of the gallbladder and curing jaundice. Rumex japonicus Houtt. is also an herb with similar components and clinic uses as rhubarb. This study attempted to establish a screen platform for transcutaneous permeation of Chinese herbs using rhubarb and Rumex japanicus as model drugs. The concentrations of anthraquinones of these two herbs in donor and receptor compartments were determined by HPLC method in different condition to investigate the optimium condition for drug absorption. In the results, the parent and conjugated form of rhubarb anthraquinones were detected in the receptor compartment.

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Caspase-14 gene silencing in a human epidermal skin equivalent model: a proteomic analysis

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Caspase-14, a cysteine protease expressed in differentiated epidermal keratinocytes, is proteolytically active during the last phase of the keratinocyte differentiation program. Recent studies in caspase-14 knockout mice have shown that caspase-14 is involved in the processing of filaggrin, however the precise function of caspase-14 in keratinocyte terminal differentiation has not been clarified so far. To identify proteins regulated directly or indirectly by caspase-14 in human keratinocytes, we established a human skin equivalent model in which the expression of caspase-14 was silenced specifically by RNA interference and analyzed the proteome of these skin models by 2D electrophoresis and LC-ESI-MS/MS. Primary human keratinocytes were transfected with siRNA specific for caspase-14 and subsequently cultured on a fibroblast-collagen suspension for up to 7 days. Histological characterization confirmed the formation of fully differentiated epidermal layers and Western blot analysis demonstrated that the expression of caspase-14 was suppressed by more than 95%. Proteomic analysis revealed that distinct species of keratins were strongly reduced in abundance after knockdown of caspase-14 expression. In particular, the abundance of keratins 1 and 10 was strongly reduced in caspase-14-deficient skin models, which was confirmed by 2-dimensional Western Blot analysis. RT-PCR analysis showed that the reduction in the protein amounts was not caused by decrease in mRNA expression. In summary, our data suggest a role of human caspase-14 in the post-translational processing of the differentiation-specific keratins 1 and 10.

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Notch ligands, delta-like 1 and jagged 2, are expressed in suprabasal epidermal keratinocytes and HaCaT cells committed to terminal differentiation

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Mammalian developmental genes (Notch, Delta and Jagged) play a key role in cell fate determination and growth control during embryonic development. However, the role played by Notch signalling in adult tissues is also of interest. We have focused on expression of Notch 1 (N1), Notch 3 (N3) and notch ligands (jagged 2 [J2], delta-like 1 [DLL1] and delta-like 4 [DLL4]), during terminal differentiation of human keratinocytes. We have found cytoplasmic expression of J2 and DLL1 but not DLL4 in keratinocytes committed to terminal differentiation, defined by keratin 10 (K10) expression. Frozen sections of normal skin were treated with antibodies to K10, N1, N3, J1, J2, DLL1 and DLL4 and detected by immunofluorescence (IMF) microscopy. In addition, HaCaT keratinocytes were grown in low calcium (0.06 mM) medium, shifted to high calcium (1.8 mM) when 80% confluent and cultured for 1, 3, 7 or 10 days. HaCaT cells at these different stages were treated with the same antibodies as well as K14 or Ki-67 antibodies, again detected by IMF. N1 was expressed in granular cells of human epidermis while N3 was found in basal and suprabasal cells. J2 and DLL1 were expressed in all suprabasal cells. HaCaT cells in low calcium (undifferentiated) did not express K10, had prominent Ki67 and N3 nuclear staining and K14 cytoplasmic staining. Once shifted to high calcium (differentiated), HaCaT cells increased cytoplasmic N1 and N3 expression, reduced nuclear Ki67 expression and increased cytoplasmic K10 expression with time. Furthermore, all cells that expressed K10 co-expressed cytoplasmic J2 and DLL1. However, DLL4 was not detected. IMF results in HaCaT cells were confirmed by western blotting and RT-PCR. We conclude that both in vivo and in vitro, cytoplasmic expression of high levels of at least two notch ligands, J2 and DLL1, are closely linked to the commitment of keratinocytes to terminal differentiation.

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Murine TLR2, TLR4 and TLR9 are inducible by severe barrier injury

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The innate immune system supports the physical skin barrier in antimicrobial defense. Human keratinocytes constitutively express various members of the toll-like receptor (TLR) family, important for innate immunity including TLR2, TLR4 and TLR9. These TLRs recognize pathogen associated molecular pattern such as lipoteichoic acid, lipopolysaccharide and unmethylated CpG motifs, respectively. We asked whether impairment of the physical barrier stimulates the innate immune system and whether TLRs are constitutively expressed or inducible in mouse skin in vivo. Murine TLR2, TLR4 and TLR9 mRNA expression was assessed after acute barrier disruption in mouse epidermis in vivo by realtime PCR analysis and subsequent gel electrophoresis for verification. We used two methods of barrier disruption: 1. tape-stripping, repeated application of adhesive tape until a ~3-fold increase in TEWL was reached and 2. scraping of the skin surface with a scalpel blade. While tape-stripping disrupts the permeability barrier of the skin by removing the outermost stratum corneum layers, scraping results in a more severe barrier disruption by removing the entire stratum corneum and injuring the epidermis down to the upper dermis. TLR2 and 4 were constitutively expressed in mouse epidermis, whereas TLR9 was not. Barrier disruption by tape stripping did not alter TLR2, TLR4 or TLR9 expression. In contrast, severe barrier injury by scraping enhanced expression levels of TLR2 and TLR4 at 6 h following barrier disruption. In addition, severe skin barrier disruption markedly induced TLR9 expression, but this increase only occurred with a time-delay at 48 h after treatment. We conclude that epidermal TLR induction requires epithelial disruption, which may allow for bacterial invasion. Recognition of cell wall components like lipoteichoic acid and lipopolysaccharide by TLR2 and TLR4 seems to be an early event, whereas recognition of genomic CpG motifs by TLR9 seems to be a late event.

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Actin/plasma membrane dynamics regulate PAR-2-depenent permeability barrier responses to acute stress

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We have recently shown that lamellar body (LB) secretion, lipid raft/caveolae formation and granular cells transformation into corneocytes are signaled by protease activated receptor-2 (PAR-2). Knowing that PAR-2 mediates Rho-activation-dependent cytoskeletal remodeling in keratinocytes, we first addressed the cytoskeletal responses to acute barrier stress in hairless mice (SkHr-1). Under basal conditions, G- actin accumulates in the cytosol of the stratum granulosum (SG) cells, while F-actin is restricted to the lateral peri-membrane domains. Hence, barrier abrogation induces the apical movement of F-actin and the retreat of the SG-G-actin front, which parallels Rho activation and G- to F-actin severing. This phenomenon was found to be either enhanced or inhibited by SLIGRL (PAR2 agonist peptide, AP) or Cytochalasin D (CtD, inhibitor of actin polymerization) application respectively. AP known to accelerate LB secretion, increases the number of TUNEL positive cells (=cornification) at the SG-stratum corneum (SC) junction together with increased Rho-activation and F-actin formation. CtD application down-regulates cornification (assessed by caspase 14 activation and TUNEL cell staining at the SG-SC interface) and restrains LB to the cytosol while lowering F-actin staining and Rho-activation. To address the relation between the dynamics of the plasma membrane and the PAR-2 dependent plasma membrane dynamics AP was applied on both caveolin 1 null cells (cav-1 -/-) and wildtype controls (cav-1 +/+). F-actin stress fibers formation was increased in cav-1 -/- and further enhanced following AP application compared to wildtype cells. In conlcusion, PAR2- driven cytoskelletal/plamsa membrane dynmaics may regulate barrier response to acute stress and the terrminal differentiation of epidermal keratinocytes.

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The evolutionary origin of the keratinocyte-specific endonuclease DNase1L2 coincides with the appearance of an orthokeratotic stratum corneum in terrestrial amniotes

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Mammals, reptiles and birds share a multi-layered, orthokeratotic stratum corneum as a barrier against transepidermal water loss. Most likely this feature has been inherited from the common ancestor of extant amniotes that lived more than 300 million years ago. Although the acquisition of an effective epidermal barrier was one of the critical steps in our evolution, its molecular basis has remained largely elusive. Here we investigated the evolution of DNase1L2, a keratinocyte-specific endonuclease which is implicated in the degradation of nuclear DNA during human stratum corneum formation. We performed comparative genomics of a broad spectrum of vertebrates and analyzed DNase1L2 gene expression in various amniote species. In contrast to non-amniote genomes, more than 30 mammalian genomes as well as the genomes of a lizard and of the chicken contained a DNase1L2 gene. RT-PCR analysis showed that chicken DNase1L2 was expressed in the skin but not in other organs. Using polyclonal antibodies raised against recombinant human DNase1L2, we detected DNase1L2 in the outermost living layers of the chicken epidermis. Western blot analysis demonstrated a band of approximately the same size as human DNase1L2 in chicken skin lysates. During fetal chicken development the expression of DNase1L2 coincided with the formation of the stratum corneum. Our results strongly suggest that DNase1L2 originated, probably by duplication of the DNase1 gene, in a common ancestor of extant amniotes. The coincidence of DNase1L2 gene innovation with the evolution of a fully terrestrial lifestyle and the conservation of the DNase1L2 expression pattern in chicken and mammals indicates that DNase1L2 is an ancient core element of the molecular machinery of epidermal barrier formation.

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ALDEFLUOR as a new tool for analysis of epidermal stem cells

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Aldefluor is a well known marker for hematopoetic stem cells. It is a substrate for the aldehyde dehydrogenase mainly actively expressed in cells bearing stem cell potential.To our knowledge, there are no information about aldefluor dehydrogenase expression in epidermal stem cells. We analysed epidermal cell preparations from human skin biopsies by flow cytometry. We demonstrate, aldehyde dehydrogenase positive cell populations which show interestingly a co-staining with well known surface markers discussed as epidermal stem cell markers integrin beta1 and integrin alpha6. Further physiological analyses will show the stem cell activity of the aldefluor dehydrogenase/intergrin costained cells.

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Caspase-14 is a crucial protease in proper stratum corneum formation

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Caspase-14 is expressed in a tissue-restricted manner and activated during skin cornification. Caspase-14 deficient mice were generated in our lab and these mice exhibit a defect in epidermal barrier function since caspase-14 protects against epidermal water loss and UVB photodamage1. We further analyzed the defective barrier function of the caspase-14 deficient skin by electron microscopy and dye based penetration assays. We demonstrate that there is premature compensatory hypersecretion of lamellar bodies in caspase-14-/- epidermis. We show that profilaggrin is a direct substrate of caspase-14 and we identified two caspase-14 cleavage sites by means of mutational analysis. Immunoblotting reveals a differential filaggrin degradation pattern in caspase-14-/- epidermis compared to wild-type epidermis. Filaggrin is a member of the S100 fused gene-family, which comprises large polyproteins with a N-terminal S100 domain followed by tandem repeats. We identified hornerin as a second member of the S100 fused gene family which is a direct caspase-14 substrate. Immunoblotting revealed that the processing of hornerin is affected in caspase-14 deficient epidermis. The functional role of hornerin is currently not clear2. Apparently, caspase-14 is a crucial protease in the correct processing and/or degradation process of S100 fused proteins such as filaggrin and hornerin.

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Caspase-14: Natural gene knockout during the land-to-water transition of the evolutionary ancestor of dolphins and whales

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The cysteine protease caspase-14 is specifically expressed in differentiated epidermal keratinocytes. Caspase-14 knockout mice show an altered processing pattern of filaggrin and are affected by increased transepidermal water loss. Here we investigated the evolution of the caspase-14 gene by comparative genomics and comparative expression analyses. Non-mammalian species lacked a caspase-14 gene whereas representative monotremes, marsupials and placental mammals contained caspase-14, suggesting that this gene had originated in a common ancestor of all modern mammals. Among a panel of more than 30 mammalian genome sequences, caspase-14 was ubiquitously distributed, with the exception of the bottlenose dolphin. PCR analysis of genomic DNAs with degenerate caspase-14 primers revealed that caspase-14 was present in all land-living mammals investigated, whereas it was absent in cetaceans, i.e. dolphins and whales. The closest relative of cetaceans, the semi-aquatic hippopotamus, contained a caspase-14 gene and displayed an orthokeratotic stratum corneum, whereas the stratum corneum of cetaceans was parakeratotic. Anti-filaggrin Western blot analysis of dolphin and harbor porpoise skin lysates yielded a pattern of bands very similar to that reported for the caspase-14 knockout mouse. Our results suggest that the caspase-14 gene has been lost during the evolution of cetaceans and indicate that this gene loss was associated with distinct changes in the epidermal phenotype. This study demonstrates that comparative evolutionary studies can complement targeted gene knockout studies in the mouse by identifying "natural knockout" models.

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Dermal-epidermal interaction within normal and compromised human skin

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Following epidermal wounding, activated keratinocytes restore this barrier by upregulation of proliferation, differentiation and migration. The putative human epidermal stem cell compartment is located within the "bulge region" of the outer root sheath the follicular epidermis. Cells found in this region have the ability to differentiate into either follicular keratinocytes or interfollicular epidermal keratinocytes, strongly implicating them in wound healing situations. The exact mechanisms and signaling pathways involved in the initiation of stem cell activation remain unknown, but research has indicated that keratinocyte plasticity is regulated by the cells of the mesenchyme. The aim of my work is to ascertain the molecular signals that are responsible for epidermal regeneration as well as the initiation of folliculogenesis. Primary human keratinocytes were seeded on DEDs and differentiated at the medium-air interface, in presence or in absence of dermis mesenchymal cells, either fibroblasts or connective tissue sheath cells. DEDs were wax embedded and investigated immunohistochemically. Further investigation was also carried out on normal human skin as well as on suction blister biopsies of normal human skin in order to visualise differences within a wound healing situation. Preliminary results using immunohistochemistry show the transcription of genes specifically in response to the presence of either dermis fibroblasts or CTS cells, more particularly, matrix metalloproteases 12. In normal human skin sections, the expression of MMP12 is restricted to the outer root sheath cells of the hair follicle, particularly in the area of the putative "bulge region". The specific effect of different dermal mesenchymal population on keratinocytes transcriptome, i.e. keratinocytes phenotype, underlines the importance of such interactions for epidermis and appendages morphogenesis and homeostasis. Further investigations will be performed to precisely determine the function of each factor to ultimately influence healing process in patients.

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Confocal raman studies of stratum corneum biochemistry as a function of anatomical location

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Differences in physical skin properties including elasticity, friction, roughness, scaliness, and epidermal thickness have been reported across different anatomical locations. Relatively little has been reported on skin biochemistry as a function of anatomical location. To study *in vivo* differences within the stratum corneum associated with anatomical location, confocal Raman spectroscopy was used to analyze skin biochemistry at different anatomical locations. The study consisted of 53 female subjects and 5 male subjects of differing race and skin type. The subject pool contained 11 children (<3 years), 31 adults (25-35 years), and 16 elderly adults (>65 years). Three (3) anatomical test sites were evaluated on adult and elderly subjects (volar forearm, cheek, and buttock) and 2 test sites were evaluated on children (volar forearm and buttock). The following measurements were performed in the order listed: 1. Transepidermal water loss using a Tewameter® TM 300 (Courage & Khazaka Electronic GmbH) 2. Skin capacitance using a NOVA DPM 9003® (NOVA Technology Corporation) 3. Water and fingerprint profiles using a Model 3510 Skin Analyzer (River Diagnostics®). Raman measurements were repeated at a minimum of 7 different locations for both water and fingerprint profiles within each site. Similar to reports by others, the water content of the cheek was found to plateau closer to the skin surface than the water content in the buttock or forearm. Similar results were observed for natural moisturizing factor (NMF) levels. The cheek also had higher ceramide, cholesterol, and lactic acid (pH 4) values near the skin surface than the buttock and forearm. Additionally, cheek skin had a unique profile with very low concentrations of trans-urocanic acid (pH 4) at the skin surface and higher concentrations deeper into the skin. Prevalence of exposure to environmental factors may contribute to these observed differences across locations. Further studies are needed to identify the factors responsible for the differences in cheek skin relative to arm and buttock.

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Hair follicle stem cells and upper follicular keratinocytes reconstitute the epidermis after wounding in human skin *in vivo*

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Wound healing following epidermal injury is a complex process that must involve rapid regeneration of the epidermis to restore normal skin barrier structure and function. Cellular mechanisms of epidermal regeneration after wounding in humans remain to be identified. In this study, wounds were generated on forearms of healthy volunteers by CO_2 laser, which completely and reproducibly ablates the epidermis. Skin biopsies were taken 1, 2, 3, and 7 days post-wounding, and reepithelialization was studied by immunohistology. Removal of the epidermis stimulated proliferation of a subset (less than 10%) of hair follicle (HF) stem cells (SCs), identified as keratin (KRT)15+/19+/CD200+, within 24 hours after wounding. No significant increase in HF SC number was observed, suggesting that HF SC markers were quickly lost upon SC division. Intense cell proliferation was observed above the HF SC niche (outer root sheet of the HF and outer layer of the sebaceous glands) 48 hours after wounding. Two to three days post wounding, keratinocytes (KCs) migrated upward toward the skin surface. New interfollicular KC stratified above the hair follicle infundibulum, before migrating laterally on day 3. During reepithelization, only basal interfollicular KC expressed the proliferation marker Ki67, while supra-basal KC expressed KRT16, a marker of keratinocyte activation. All new KCs expressed KRT17, which is normally restricted to HF, suggesting HF origin. Reepithelization by migration of interfollicular KCs from the wound edge was not evident. KRT17 was recently shown to function as an activator of mTOR signaling, which stimulates protein synthesis and cell growth. Consistent with this function, increased activity of p70S6K1, a *bona fide* mTOR target, was observed in KRT17+ repopulating KCs. Taken together, these data demonstrate that HF SCs are transiently induced to divide during wound healing in human skin *in vivo*, and that new epidermal KCs arise primarily from upper follicular KCs, as has been reported in rodents.

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Dysregulation of channel activating protease 1 in mouse epidermis

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The aim of this study is to investigate the importance of the regulated channel activating protease 1 (CAP 1, also known as Prrs8 and Prostasin) expression in the skin by addressing CAP 1 to the stratum basale of the epidermis. CAP1 transgenic mice have been generated by micro-injection into fertilized mouse oocytes. The genotypes and phenotypes of the animals were assessed using standard molecular, cellular, histological means. The epidermal permeability barrier integrity was evaluated by measuring the trans epidermal water loss (TEWL). CAP1 transgenic mice show a skin phenotype characterized by scaly, un-elastic skin with less hair and exhibit itch. At histological level the epidermis is hyperkeratotic and thicker because of an increased cell proliferation. The histology of other organs is not affected. CAP1 transgenic mice present also lower body weight and higher TEWL and an increased postnatal mortality, suggesting skin barrier defects. Mice with an altered CAP 1 expression in the skin present impaired epidermal barrier function and skin inflammation. This study shows that regulated CAP 1 expression in the epidermis is important for the skin physiology. CAP1 transgenic mice can be used as a model for skin disorders like ichthyosis.

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SRF is an essential *in vivo* regulator of epidermal morphogenesis and barrier function.

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Serum response factor (SRF) is a ubiquitous transcription factor that regulates gene expression programs linked to growth and differentiation in many systems. However the function of SRF in skin remains elusive. To investigate the *in vivo* functions of SRF in epidermis, we generated epidermal-specific *Srf* knockout mice (SRF-EKO) using the Cre-LoxP technology. Here we report that targeted keratin 14-driven disruption of the *Srf* gene in mouse epidermis results in early postnatal lethality due to a perturbed epidermal barrier function. Moreover, mice lacking SRF in the epidermis display several morphogenetic defects, including a consistent eye-open at birth phenotype, lack of whiskers, as well as considerably shorter limbs and digits than those of normal littermates. Loss of SRF in epidermis leads to a strikingly aberrant epidermal architecture characterized by marked parakeratosis without a discernible granular epidermal layer. Immunohistochemical examination of control versus SRF-null skin revealed abnormal proliferation in the basal epidermal layer of the SRF-EKO mice as evidenced by increased number of Ki-67-positive basal keratinocytes as well as increased number of epidermal layers expressing keratin 14, a marker associated with keratinocyte hyperproliferation, in mutant mice in comparison with the wild-type mice. The normal pattern of expression of terminal epidermal differentiation markers involucrin and loricrin was completely disrupted in SRF-null epidermis with both proteins localized only in occasional cells throughout all epidermal layers in SRF-EKO mice, while expression of differentiation marker keratin 10 appeared patchy and discontinuous in the SRF-deficient epidermis. Furthermore, an extensive apoptosis was detected in the SRF-null epidermis by TUNEL assay. These data indicate that SRF is essential for epidermal development and maintenance of epidermal homeostasis.

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Functions of Rac1 in the epidermis

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The small GTP binding protein Rac1 regulates a large number of basic cellular processes. To analyze its functions in skin, we have generated different lines of genetically modified mice with disturbed Rac1 expression and activity in the epidermis. These include mice with epidermis specific deletion of Rac1 (Rac1 E-KO mice) and mice expressing dominant inhibitory (K14Rac1N17 mice) and constitutively active (K14Rac1L61 mice) mutants of Rac1 as transgenes under the control of the keratin 14 promoter. We have employed these mouse models to analyze the role of Rac1 in wound healing, tumor formation and –progression as well as hair follicle formation and morphogenesis. Here we will review the results of our studies. We can show that A) Rac1 is required for normal wound re- epithelialization by regulating proliferation and migratory activity of keratinocytes. Inhibition of Rac1 activity in primary keratinocytes causes destabilization of lamellipodia at the leading edge of the membrane resulting in impaired migration. B) Rac1 plays a role in the formation and progression of epidermal tumors: Inhibition of Rac1 activity in the epidermis of K14Rac1N17 mice prevents UV and papilloma virus induced skin tumors. Rac1 contribution to tumor progression in mice is suggested by the ability of the constitutively active mutant of Rac1, L61Rac1, expressed from a transgenic keratin 14 promoter, to convert benign sebaceous adenomas into malignant, invasive sebaceous carcinomas. C) Rac1 is required for the formation, morphogenesis and pigmentation of hair follicles and hair. Whereas hair follicle formation is a function of Rac1 expressed in basal epidermal keratinocytes and in the outer root sheath, normal hair follicle morphogenesis and pigmentation requires Rac1 expression in more differentiated parts of the hair follicle.

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Robust methods for processing confocal raman data; sources of variation and outlier cleaning

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Confocal Raman spectroscopy produces quantitative measurements for water and semi-quantitative measurements of other components of the skin. Replicate measurements within a single skin location can vary greatly due to the presence of heterogeneous skin structures and extreme values are not uncommon. The large number of data points gathered precludes manual inspection of outliers. Here we describe the application of robust statistics for the processing and cleaning of profiles that should be undertaken prior to standard analyses (e.g. test for treatment effect). The study consisted of 53 female subjects and 5 male subjects of differing race and skin type. The subject pool contained 11 children (<3 years), 31 adults (25-35 years), and 16 elderly adults (>65 years). Three anatomical test sites were evaluated on adult and elderly subjects (volar forearm, cheek, and buttock) and 2 test sites were evaluated on children (volar forearm and buttock). Prior to evaluations all subjects equilibrated for at least 15 minutes in an environmentally controlled room. Water and fingerprint profile measurements were taken on all test sites using the Raman spectrometer. Measurements were repeated at a minimum of 7 different locations for both water and fingerprint profiles within each site. Each profile contained estimates from 0 μm to a depth of up to 140 μm. Replicate Raman scans at the same location occasionally yielded data that was highly non-normal and artifact rich. To reduce the impact of outliers on the statistical inference of the underlying treatments we applied several different robust statistical methods (trimmed means, winsorizing, etc.) for estimation of the location and spread of component values. The methods presented allow for a robust way to analyze Raman scans of the skin providing sufficient power for detecting differences between treatment groups.

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Assessment of retinoid activity using *ex-vivo* human skin cultures

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The purpose of this study was to assess the effect of retinoids on human skin explant (HSE) cultures and compare it to the effect on reconstructed human epidermis (RHE) cultures from SkinEthic, which is well characterized for its response to retinoid treatment. Human skin explant cultures were established using freshly excised adult human abdominal skin, obtained from elective surgeries. The skin was dermatomed to 0.5 mm thickness, and 8 mm punch biopsies were cut and placed into transwell inserts containing growth media. The explant cultures were allowed to equilibrate at 37°C for 5 days prior to use. Cultures were dosed topically with formulations containing three different retinoids (adapalene, tretinoin, and tazarotene). Tissues were stained for filaggrin, K10, and K19 using immunohistochemical techniques. In addition, total mRNA was extracted and qPCR was performed using probes for GAPDH, filaggrin, K10, and K19. Reconstructed human epidermis (RHE) is a well characterized and commonly used model in skin research. RHE cultures were used as a reference standard for these experiments. RHEs received the same treatment as the HSE cultures. Overall, the results observed with HSE and RHE cultures were qualitatively similar. Filaggrin expression in the uppermost layer of the viable epidermis was lower in retinoid-treated skin cultures compared to untreated controls. K10 expression was decreased in the suprabasal layers of the epidermis of retinoid-treated skin cultures compared to untreated controls. K19 expression is upregulated in the epidermis of retinoid-treated skin cultures compared to untreated controls. It is difficult to compare expression levels quantitatively between HSE and RHE cultures, even with qPCR, because RHE cultures do not have a fully functional stratum corneum. Qualitatively, however, the retinoid effects on HSE cultures appear to be similar to those observed on RHE cultures.

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Epidermal normalization following treatment with infliximab in psoriatic subjects: Histology and gene expression analyses

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Objective:Psoriasis is an inflammatory skin disease marked by hyperproliferation of the epidermis.A new paradigm for the pathogenesis of psoriasis is emerging with identification of the involvement of the Th17 pathway. Cytokines including IL-20,IL-12,IL-23,and TNF-α and other proteins such as the S100 calcium binding proteins and cyclins have been implicated in the hyperproliferation of keratinocytes.The effects of infliximab (IFX) on the epidermis were studied during EXPRESS II,a phase 3 study of IFX in psoriasis. Methods:Histological response and gene expression were evaluated in a subset of subjects in this 50wk randomized,double-blind,placebo-controlled trial of 835 patients evaluating the safety and efficacy of IFX induction monotherapy(3-and 5mg/kg at wks 0,2,and 6) followed by 4 regimens of IFX maintenance monotherapy (scheduled either q8 wks or PRN at each of the 2 doses).In the subset of subjects(n=70)participating in the biopsy study,skin samples collected at baseline(BL),day 3,and wk 10 were bisected for histology(formalin-fixed)and gene expression(microarray and RT-PCR). Results:TNF blockade induced by IFX was associated with diminished cell proliferation(Ki67) and epidermal thickness by day 3,and significant improvement was seen by wk 10 in responders to IFX(PASI improvement>50%). Epidermal related genes,such as K6,K16,MKi67,and the cyclin family of genes (CCNA2,CCNB1,etc)and others,were down regulated by day 3 and continued to be suppressed at wk 10 compared with baseline.Decreases in the expression of the proliferation marker MKi67 was correlated with improvements in PASI ($p < 0.0001$).Comparisons of normal skin to IFX responders at wk 10,histologically and by gene expression,showed similar profiles. Conclusions:The decreased expression of genes related to the epidermis mirrored the histological improvements in the epidermis following IFX treatment demonstrating normalization of the epidermis of psoriatic skin.

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Epidermal regeneration: an increase in short term repopulating cells allows aged epidermis to keep up with the young

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The decreased proliferative ability of aged epidermis could be explained by alterations in epidermal stem cell homeostasis. We investigated the frequency and function of epidermal repopulating cells in aged vs. young skin. We first used an *in vivo* repopulation assay to follow individual epidermal repopulation units over time, and retrospectively classified originating cells as short-term repopulating/transit amplifying (STR) cells or as long-term repopulating/stem cells (EpiSCs). Frequency of STR cells in aged was more than twice that of young epidermis (1 in 21,000 vs. 1 in 44,000 $p = 0.04$). However, at 11 weeks the frequency of long term repopulating EpiSCs showed no significant difference (1 in 61,000 vs. 1 in 44,000) suggesting that true EpiSC frequency is not altered in aged skin. Colony forming assays confirmed that STR cells were twice as frequent in aged vs young. However, *in vitro* long-term proliferation at 11 weeks was similar. Using an *in vivo* assay we compared morphology of epidermis produced by aged and young repopulating cells *in vivo*. At 1 week, cysts from aged repopulating cells had a differentiated structure with stratified squamous epithelium and keratin production while cysts from young repopulating cells showed a ball of undifferentiated cells. In addition, cysts from aged were smaller compared to young (9.15 ± 1.1 vs. 15.9 ± 1.5 cells/cyst $p = 0.001$). Thus, STR cells are twice as frequent in aged skin as in young epidermis while the frequency of EpiSCs is equivalent. Given the less proliferative epidermis from aged repopulating cells, it may be that increases in STR cells are compensatory, and allow the aged epidermis to regenerate in response to proliferative stresses and/or wounding.

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Human skalp skin epidermis produces thyrotropin (TSH), whose production is controlled by thyroid hormones and thyrotropin-releasing hormone (TRH)

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Pituitary thyrotropin (TSH) stimulates thyroid hormone production via its cognate receptor (TSH-R). TSH secretion is up-regulated by hypothalamic thyrotropin-releasing hormone (TRH), while increased serum thyroid hormone levels send negative feedback signals. Since cultured human skin cells also transcribe elements of the central hypothalamus-pituitary-thyroid (HPT) axis, we have asked whether TSH is also translated in human epidermis in situ, and whether any intraepidemal TSH production is subject to TRH and/or thyroid hormone regulation *in situ*. Intraepidermal TSH transcription was measured by RT-PCR, TSH and TSH-R immunoreactivity (IR) were examined by immunohistology. RNA extracted from human epidermal sheets showed the presence of TSH transcripts. This corresponded to intense TSH-like IR throughout the suprabasal, differentiated layers of normal human scalp epidermis, while TSH receptor-like IR was completely absent in the epidermis, and was confined to mesenchymal compartments and nerve fibers of human skin. Full thickness human scalp skin was organ-cultured under serum-free conditions, and treated with vehicle or TRH (100 ng/ml) and TSH IR was assessed by quantitative immunohistomorphometry. TRH-treatment significantly up-regulated TSH protein expression. In contrast, when organ-cultured skin fragments were treated with thyroid hormones T3 (1 pM, 100 pM) or T4 (100 nM, 1 µM), intraepidermal TSH IR was dramatically down-regulated. In summary, we show that human epidermis expresses TSH on the gene and protein level, which may stimulate intradermal TSH receptors, and that intraepidermal TSH regulation follows rules recognized for the central HPT axis.

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Thymic stromal lymphopoetin expression is induced in human skin after experimental barrier disruption *in vivo*

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The pivotal role of thymic stromal lymphopoetin (TSLP) in the development of allergic inflammation has been well established. The mechanisms triggering TSLP expression in the human skin however have not been well characterized. In the present study, we aimed at investigating the role of the skin barrier disruption as factor for inducing TSLP expression in the epidermis by using a model of *in vivo* experimentally induced irritant contact dermatitis. The volar forearm of 8 healthy, adult, non-atopic volunteers was used as test site where a test and control field was marked. Skin barrier disruption was induced by repeated open application of 2% sodium lauryl sulfate (SLS) solution only to the test field for 96 h and monitored by bioengineering methods every 24 h up to 96 h, when biopsy from the test and control field was taken for histology and immunostaining. Compared to baseline, after 96 h there was significant increase in the visual score, skin pH, transepidermal water loss (mean±SEM 33.64±2.56 vs. 5.49±0.38 g/m2/h; p<0.0001) and erythema (chromametry a*value 12.05±1.34 vs. 5.82±0.5; p=0.0004) and significant decrease in stratum corneum hydration (37.3±1.78 vs. 19.22±2.1 AU; p<0.0001) on the SLS-treated field. Irritant contact dermatitis was confirmed by histology. The control field was unchanged. On cryosections, TSLP staining was positive in 7 out of 8 samples obtained from SLS-treated skin. The TSLP-specific staining was restricted to differentiated keratinocytes in several areas of the epidermis. No expression was found in any of the control (untreated field) samples. Our results provide evidence that the compromised barrier integrity induces TSLP expression in the human epidermis. Furthermore, this is the first report of *in vivo* inducible expression of TSLP in human skin.

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Analysis of site-dependent characteristics of skin surface ammonium-ions, transepidermal water loss and skin surface pH in smokers and non-smokers

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The aim of the present study is to analyse with respect to smoking habits site-dependent characteristics of the amount of ammonium-ions on the skin surface, transepidermal water loss (TEWL) as well as skin surface pH. Overall 40 women aged 18-60 (20 non-smokers (NSM), 20 smokers (SM)) were included at standard conditions (T: 20-22°C, relative humidity 40-60%). Ammonium-ions (Spectroquant, Ammonium-Test®, Merck, Germany), TEWL and skin surface pH (using Tewameter® TM 210 and skin-pH-meter® 900; both devices: CK electronic GmbH, Cologne, Germany) were assessed on the forearm (FA) and on the forehead (FH). Absolute values, t-test comparisons of FA with FH and correlations between FA and FH were evaluated for all volunteers together (ALL) and for NSM and SM separately. Values of p≤0,05 were considered significant. In all groups average ammonium values on the FA were higher than on the FH. Significant differences between FA and FH were found in ALL (p<0,01) and in NSM (p<0,05). Also, significant correlations between FA and FH were found in ALL (p=0,001) and NSM (p<0,001). TEWL in all groups was higher on the FH than on the FA. The differences between FA and FH were significant in all groups (all:p<0,001). Significant correlations between FA and FH were found in ALL (p=0,001) and in NSM (p=0,001). The pH-values were always lower on the FH. There were no significant differences between FA and FH. The correlations between FA and FH were significant in all groups (All:p<0,001; NSM:p<0,05; SM:p<0,01). The results obtained reveal in general very similar site-dependent characteristics of the ammonium-values and TEWL suggesting a relation between the amount of ammonium-ions on the skin surface and skin permeability. However, the absolute values suggest an inverse relationship between ammonium-ions and skin permeability. The results also indicate that smoking has an impact on the site-dependency of the ammonium ions. Further investigations are required to confirm the findings.

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PLD2 promotes primary mouse epidermal keratinocyte proliferation in a lipase activity-dependent manner

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A previous study in our laboratory has suggested the possibility that PLD2 and the glycerol channel, aquaporin 3 (AQP3) function together to produce phosphatidylglycerol (PG), a lipid second messenger thought to regulate keratinocyte proliferation and differentiation. To study the role of PLD2 overexpression alone in the effects of a moderate CaCl2 concentration on proliferation and differentiation of primary mouse epidermal keratinocytes, these cells were infected, with high efficiency (~90%), with adenovirus possessing wild-type PLD2 or lipase-dead PLD2 (PLD2-LI) and incubated in control (25µM calcium) and 125µM calcium-containing serum free keratinocyte medium (SFKM) for 24 hours. Western blotting confirmed that PLD2 and PLD2-LI were successfully overexpressed. Total PLD (PLD1/PLD2) activity was measured using a radiolabeled activity assay in the presence of ethanol to produce [3H]phosphatidylethanol (PEt), a marker of PLD activity. Initial studies showed that overexpression of PLD2 increased PEt synthesis, but overexpression of lipase-dead PLD2 did not. In control SFKM, proliferation, as measured by [3H]thymidine incorporation into DNA, was significantly enhanced by overexpression of PLD2, but this manipulation had no significant effect on the inhibition of proliferation induced by a moderate calcium concentration. In contrast, the lipase-dead PLD2 had no effect on keratinocyte proliferation under either condition. Neither PLD2 nor PLD2-LI had an effect on late differentiation, as measured by a transglutaminase activity assay. We conclude that overexpressed PLD2 alone increases primary mouse keratinocyte proliferation in a lipase activity-dependent manner, but that this stimulation can be counteracted by a moderate calcium level. However, it is as yet unclear whether PLD2 will have the same effects if overexpressed together and associated with AQP3.

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TIG3, a novel keratinocyte pro-differentiation pro-death protein, activates organelle-associated autophagy in keratinocytes

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Tazarotene-induced protein 3 (TIG3) is an important class II tumor suppressor protein. TIG3 has been implicated as a regulator of terminal differentiation in the suprabasal epidermis. However, the exact mechanism of TIG3 action remains a mystery. Previous studies from our laboratory showed that TIG3 binds to and activates transglutaminase I, a protein crosslinking enzyme that is absolutely required for cornified envelope formation. Studies with TIG3 mutants show that progressive N-terminal truncation converts TIG3 from a pro-differentiation to a pro-apoptotic regulator. Additional studies show that the c-terminal membrane anchoring domain is required for activity. We now show that TIG3 expression in human foreskin keratinocytes results in the loss of organelles including the Golgi and the endoplasmic reticulum, which coincides with a marked reduction in nuclear diameter and volume. These changes are associated with the appearance of a large intracellular beta-tubulin containing aggregate. These data provide strong evidence that TIG3 activates autophagy-related keratinocyte cell death. This may be the first evidence showing that keratinocytes can undergo autophagy, and to suggest that autophagy is a normal part of differentiation-associated keratinocyte cell death in the epidermis. It has been appreciated for several decades that keratinocytes undergo nuclear and organellar destruction during terminal differentiation. We argue that TIG3 may be an important controller of these events which we believe are mediated by lysosome-mediated autophagic processes.

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Expression of mutant keratin 10 in the nail unit results in elevated keratin 6a expression and nail dystrophy

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Epidermolytic hyperkeratosis (EHK), also called bullous congenital ichthyosiform erythroderma (BCIE), is a rare inheritable skin condition that is caused predominantly by dominant mutations in keratin 1 (KRT1) or keratin 10 (KRT10) gene. EHK patients have erythema and blisters at birth that are gradually developed into peeling and hyperkeratosis. Both generalized and mosaic forms of EHK exist. Clinical presentation varies greatly among patients. Palmoplantar hyperkeratosis and nail changes are observed in a subset of EHK patients. We have previously generated an inducible mouse model that mimicked this disease at both the phenotypic and genetic (expressing a mutant Krt10 allele) levels. Hyperkeratosis could be induced on the paws, and toe nails of the EHK mice often appear to be coarse, opaque and hypertrophic. We observed that elevated Krt6a expression in the nail bed epithelium was associated the nail phenotypes, a pathological change that was reported in the skin of EHK patients as well as in EHK mice. Since Krt6a is expressed in the nail bed epithelium, and dominant mutant form of Krt6a results in a similar form of hypertrophic nail dystrophy, pachyonychia congenita, we speculate that the compromised keratin intermediate filament network caused by mutant Krt10 gene expression in cells within the nail compartment results in such a hyperproliferative events in response to the environmental stresses on the nail unit.

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Ultrastructural morphology of the low-frequency ultrasound-induced cavities

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The effects of transdermal drug transport(TDT) using low-frequency ultrasound (LFUS) have been demonstrated and many studies about delivery of various low- as well as high-molecular weight molecules via transdermal route using LFUS have been reported. The cavitation effect is one of the important mechanisms of skin permeabilization during LFUS. Theoretically, cavitation is demonstrated to occur because of the presence of oxygen and nitrogen dissolved in the stratum corneum and the existence of lacunae between corneocytes and can be a route of drug delivery. But, morphologic studies to find and evaluate the exact cavities have not been undergone actively. In this study, we used a different type of LFUS device from that which have been used previously and examined the ultrastructural morphology of cavities with a transmission electron microscope. The device used in this study works with the ultrasound probe directly attached to the skin surface. We conducted ultrasound to normal skin, tape-stripped skin, and topical oleic acid-treated skin. After the treatment with LFUS, 1um~3um sized cavities were observed in stratum granulosum and stratum basale, especially in the intracellular spaces. After the combined use of LFUS and tape-stripping, 6um~15um sized, continuously extended lacunae were observed in some areas of the stratum corneum. Our results show that most cavities were observed in stratum granulosum and stratum basale probably due to the ultrastructural property to form the cavities easily. We suggest that these continuous formation and disappearance of cavities may have a role in the formation of a route of skin penetration.

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The epithelial sodium channel is required for the galvanotaxis of murine and human keratinocytes

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Ion channels and exchangers have been proposed to regulate cell migration by conducting ion flow at lamellipodia and anchoring actin filaments and signaling molecules. Here we report a novel function of the epithelial sodium channel, ENaC, in galvanotaxis, the directional cell migration in an electric field and one of the mechanisms facilitating wound reepithelialization. ENaC is well-studied in kidney and lung, where it regulates sodium transport and water re-absorption. It is also found in human and mouse epidermis and may mediate epidermal differentiation. Neonatal human keratinocytes were treated with phenamil, an ENaC inhibitor, and cell migration in a 100mV/mm electric field (EF) was filmed by time-lapse imaging. The phenamil-treated keratinocytes migrate at 1 μm/min, as do control cells; however, the directionality of keratinocyte migration toward the cathode in the EF decreases by 30% ($p<0.005$). Decreasing the abundance of ENaC by siRNA in cultured human keratinocytes or by gene knockout in mice (αENaC -/-) resulted in a more severe defect: an 80% decrease of directional migration in the EF ($p<0.005$). Additionally, re-epithelialization of an ex vivo human skin wound is decreased 70% when ENaC is inhibited by phenamil ($p<0.005$). To further investigate the mechanism of ENaC mediating galvanotaxis, we immunolocalized ENaC in living keratinocytes during galvanotaxis. After 60min EF exposure, we found that ENaC accumulated asymmetrically at the cathodal side of the cells. Also, in a preliminary study to visualize sodium influx with Na-green during keratinocyte migration, we found Na signals appeared as multiple patches at the lamellipodial edge of migrating keratinocytes in the absence of an EF. However, when an EF was applied, the Na signals switched to a dominant patch facing to the cathode within 5min. These results suggest that ENaC has an important function in mediating the directional migration and wound healing in keratinocytes and the EF may promote the directional migration via Na influx.

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Skin permeation of nanoparticles by magnetophoresis in hairless mouse skin

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Recently, studies about nanotechnology brought developments in biomedical field as well as industrial field vigorously. Magnetic nanoparticle that is studied by the field of this nano-engineering has been applied in medical engineering and has various advantages, and the potential is also very big. Magnetic nanoparticle has a possible composition comparatively easily and evenly to size fewer than 10nm, can attach molecules such as protein or imaging probe on the particle surface and is used by cell labeling or nanocarrier etc. In this study we investigated the magnetophoresis-induced permeation pathway in the murine skin using Fe3O4 nanoparticles as a tracer and examined with confocal microscopy and transmission electron microscopy as visualization methods. In results, tracer permeation observed in only lipid layer of SC in epidermis, and dermis observed that is permeated to subcutaneous fat layer through sebaceous glands. In interesting, permeated tracer observed definitely in sebaceous glands and surface of lipid droplet. Transfer of Fe3O4 nanoparticle has thorough lipophilic pathway, and looks that vertical permeation inclination by magnetic force strongly. We suggest that our data is thought to be applied by method to enhance selective permeation to lipophilic tissue such as sebaceous gland and subcutaneous fat layer.

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Newly synthesized molecule, K6-L19, increased antimicrobial peptides (AMPs) expression in cultured keratinocyte and murine skin

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Antimicrobial peptides (AMPs) are predominantly small cationic polypeptides and comprise the antimicrobial barrier of skin. In previous reports, significant decrease of AMPs was observed in lesional skin of atopic eczema patients, and this decrease is considered as a major factor for the increased microbial susceptibility of atopic skin. Based on these previous results, topical supplement of AMPs on skin might be a possible medication for atopic eczema. However, direct application of AMPs has a few important drawbacks, such as high price and short shelf-life. As an attempt to overcome these problems, we have screened new molecules that have a stimulatory activity on AMP synthesis in epidermis. Various molecules are treated on cultured human keratinocytes, and mRNA expression of beta-defensin 2 (hBD-2) and cathelicidin (LL-37) was evaluated using real-time reverse-transcriptase polymerase chain reaction (RT-PCR). Among the tested 35 molecules, significant increase (more than 4-fold increase than control) in hBD-2 mRNA was observed in 5 samples, and 6 samples significantly upregulated LL-37 mRNA expression. In order to verify that this increase is mediated through either proinflammatory cytokines or keratinocyte differentiation process, tumor necrosis factor-alpha (TNF-α) and involucrin mRNA expression was also measured. As a result, K6-L19 was identified as having stimulating effects on the AMPs mRNA expression in cultured human keratinocytes, without affecting the proinflammatory cytokines production. Increased protein expression by K6-L19 in cultured keratinocytes was also observed under confocal microscope. In a chronic dermatitis model induced by long-term oxazolone application, considerable increase of epidermal AMPs was observed in 1% K6-L19 applied skin. These results suggest that K6-L19 may be used as a possible medication for infectious skin diseases and atopic eczematous lesions.

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Effect of K6-L19, antimicrobial peptides (AMPs) augmenting molecule, on skin barrier function

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Previous studies suggested that AMPs are delivered to their primary site-of-action, stratum corneum (SC), via lamellar body (LB). Since the precursor molecules for the SC intercellular lipids are also transported by the LBs, these results suggested that the epidermal permeability barrier and antimicrobial barrier is structurally interrelated. Recently, we have identified a new molecule, K6-L19, which augments the antimicrobial peptides (AMPs) expression in cultured keratinocytes and hairless mice skin. In this study, we have investigated the effects of this new molecule on skin barrier function. Epidermal permeability barrier of female hairless mice flank skin was acutely disrupted by repeated tape stripping, and 1% K6-L19 in PEG/EtOH (70/30) was topically applied. Barrier function was evaluated by measuring transepidermal water loss, and statistically significant acceleration of barrier recovery rate was observed in K6-L19 applied skin site, compared to vehicle applied site, at 3 hours after barrier disruption. In a hyperplasia animal model induced by repeated barrier disruption, K6-L19 also significantly prevented the increase of epidermal thickness. In order to verify whether this barrier recovery acceleration is mediated by increased AMP expressions, effects of AMPs on barrier function was also tested. When the beta-defensin was topically applied to barrier disrupted skin, barrier recovery rate was slightly accelerated, which suggest that the effects of K6-L19 was mediated through, at least in part, the increased AMP synthesis. From these results, it might be concluded that the new chemical, K6-L19 can be used as either increasing the antimicrobial peptide production in epidermis or accelerating the recovery of damaged skin. These results also suggest that the anti-microbial barrier function and epidermal permeability barrier function are not only structurally but also functionally interrelated.

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Endogenous galectin-3 positively regulates keratinocyte migration by controlling cell surface epidermal growth factor receptor (EGFR) expression

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One crucial step in skin wound re-epithelialization is keratinocyte migration. By studying keratinocytes from mice deficient in the carbohydrate-binding protein galectin 3 (gal3-/-), we found that endogenous galectin-3 promotes keratinocyte migration in vitro. Moreover, we found that skin wound re-epithelialization was impaired in gal3-/- mice at day 2 post wound. Since the EGFR-Ras-ERK signaling pathway plays a pivotal role in keratinocyte migration, we examined EGFR phosphorylation at Y1068 and ERK phosphorylation in gal3-/- cells and found diminished responses upon EGF stimulation. Importantly, a significant decrease in cell surface EGFR expression was found in gal3-/- keratinocytes compared to wild-type as determined by flow cytometry. EGFR was instead found to be accumulated in the cytoplasm of these cells by immunofluorescence analysis. The decreased cell surface expression and cytoplasmic accumulation of EGFR were also demonstrated in vivo in the peri-wound epidermis in gal3-/- mice. Exogenously added recombinant galectin-3 did not stabilize cell surface EGFR expression in cultured human keratinocytes, but rather, induced EGFR endocytosis. The results suggest that extracellular galectin-3 does not contribute to modulation of EGFR surface expression, but intracellular galectin-3 does. Our results suggest that galectin-3 controls keratinocyte migration and reveal a critical role of galectin-3 in transport and expression of key cell surface receptors.

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Effects of K6-L12 on intrinsically aged mice skin

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A lot of molecules are currently used or under being investigated as anti-aging agents and diverse underlying mechanisms are suggested for each of these molecules. Previously, it was suggested that sphingosine kinase activator may be used for anti-aging, mainly through the sphingosine-1-phosphate involved signaling. We have recently reported that a newly synthesized molecules, K6PC-5, is a potent sphingosine kinase activator, and investigated its possible application in either intrinsically aged or UV-induced aged mice. In a continuation of the previous researches, newly synthesized molecules are tested for their activity on calcium signaling and proliferation of cultured human fibroblast. Among the tested molecules, K6-L12 showed most significant calcium signaling and also induced proliferation of fibroblast. In order to verify the anti-aging efficacy of K6-L12 on intrinsically aged skin, murine aged mice model was established and dermal changes were investigated. When 1% K6-L12 was topically applied twice a day for 2 wks in 56-wks aged mice skin, and functional measurements and histologic observation was performed. No changes in transepidermal water loss and skin capacitance was observed between vehicle treated and K6-L12 treated groups. However, dermal thickness was significantly increased in K6-L12 treated skin and, the increased number of fibroblast and collagen density was also observed. Interestingly, while in vitro experiment results suggest that K6-L12 induces increase of intracellular calcium concentration in cultured keratinocytes, immunohistochemical staining for involucirn, loricrin and filaggrin in aged mice skin did not show any significant changes. In conclusion, topical application of K6-L12 improved the intrinsic aged skin and may be used as a potent anti-aging agent.

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Mammalian TOR modulates transcription through TORRID sequences

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The epidermis, the outermost layer of the skin and the cornea are directly in contact with the outside environment, thus keratinocytes and corneal stem cells need to adapt to environmental changes constantly. Therefore, we have investigated the effect of small temperature variations on human keratinocyte stem cells. We have identified mammalian target of rapamycin (mTOR) as a major transcriptional modulator in response to physiological variations of temperature in human keratinocytes and demonstrated that the promoter regions of mTOR responsive genes share a common sequence termed TORRID that comprises two to five 20 base pairs consensus motifs. We defined a minimal DNA sequence that regulates transcription and confers temperature sensitivity. In addition, mTOR is part of a transcriptional complex that associates with TORRID sequences. This complex includes the human orthologs of the yeast TOR transcriptional complex and members of the mTORC1 and mTORC2 complexes (RAPTOR or RICTOR respectively). Altogether, our results demonstrate that mTOR transcriptional activity plays a crucial role in fine-tuning of stem cells by their microenvironment.

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The absence of urokinase type plasminogen activator receptor develops the progression of dermal fibrosis

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The fibrinolytic system is considered to play an important role in the degradation of extracellular matrices (ECM). However, the detailed mechanism how this system affects fibrosis remains unclear. Urokinase-type plasminogen activator receptor (uPAR) not only functions as a proteinase receptor, but also plays a role in cellular adhesion, differentiation, proliferation and migration through intracellular signaling. To investigate that the effect of uPAR on dermal fibrosis, we compared the skin of wild type and uPAR-deficient (uPAR-/-) mice. We found that the absence of uPAR increase dermal thickness. In addition, we showed that the absence of uPAR induce the ECM deposition. Moreover, we showed that the absence of uPAR attenuates the production and activity of matrix metalloproteinases (MMP)-9 in dermal fibroblasts. In conclusion, our study suggests that the absence of uPAR attenuates MMP-9 gene expression and activity, and results in ECM deposition. As the results, the absence of uPAR induces dermal fibrosis. Our findings provide new insight into the role of uPAR on dermal fibrosis.

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Effect of alpha2-antiplasmin on dermal fibrosis

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The transforming growth factor (TGF)-β is the major inducers of fibrosis. The fibrinolytic system is considered to play an important role in the degradation of extracellular matrices (ECM). However, the detailed mechanism how this system affects fibrosis remains unclear. The aim of this study was to examine the effect of α2-antiplasmin (α2AP) on dermal fibrosis. We examined the experimental fibrosis in mice with a deficiency of α2AP, which is a potent and specific plasmin inhibitor. We found that the absence of α2AP attenuated bleomycin-induced TGF-β synthesis and fibrosis. In addition, the production of TGF-β from the explanted fibroblasts of α2AP -/- mice decreased dramatically compared to that in WT mice. Moreover, we found that α2AP specifically induces the production of TGF-β in fibroblasts. In conclusion, the absence of α2AP attenuated TGF-β synthesis, thereby resulting in an attenuated-fibrosis. Our results provide new insight into the role of α2AP on dermal fibrosis.

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Trypsin, PAR-1 and PAR-2 mediated IL-8 releases were increased in SPINK5 knock-downed HaCaT cells

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The Netherton syndrome (NS) gene is identified as SPINK5 (Serine protease inhibitor Kazal-type 5) encoding the serine protease inhibiter, LEKT1 (lympho-epithelial Kazal-type related inhibitor). Several SPINK5 polymorphisms which are associated with AD have been reported. The pathopysiological roles of SPINK5 in atopic features of NS have been not known yet. Protease-activated receptor (PAR)-1 and PAR-2 are seven transmembrane G-protein coupled receptors which are activated by cleavage by trypsin. PAR-2 mediated inflammatory response in human keratinocyte. In this study, we investigated the immunological functions of SPINK5 in human keratinocyte cell line, HaCaT. To analyze the functions of SPINK5, we generate a SPINK5 knock-downed clonal HaCaT cell line (kdSPINK5-HaCaT) that stably express the SPINK5-specific small-interfering RNA introduced with siRNA expression vector. We treated HaCaT and kdSPINK5-HaCaT with trypsin, PAR-1 and PAR-2 agonist peptides, and measured IL-8 and MCP-1 producing from cells by ELISA. Treatment of HaCaT and kdSPINK5-HaCaT with trypsin, PAR-1 and PAR-2 agonists caused a dose-dependent increase in the IL-8 secretion and the IL-8 secretion in kdSPINK5-HaCaT was statistically higher than HaCaT. The secretion of MCP-1 from HaCaT and kdSPINK5-HaCaT which were stimulated by trypsin, PAR-1 and PAR-2 agonists were not increase. In this study, we demonstrated PAR-1 and PAR-2 mediated IL-8 releases were increased in SPINK5 knock-downed HaCaT cells. From the results, we speculated LEKT1 inhibit activation of PAR-1 and PAR-2, and these PARs mediated IL-8 releases were increased in keratinocytes of NS patients. LEKT1 inhibit protease ligands for PAR-1 and PAR-2 in human keratinocytes and may have a critical role in down-regulation of inflammatory response in epidermis.

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Mutations in ribosomal proteins cause p53-mediated dark skin

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Mutations in ribosomal proteins lead to the dominantly inherited Minute phenotype (developmental delay and thin bristles) in Drosophila, and Diamond-Blackfan anemia in humans. While a slower rate of cell division may explain developmental delay in affected individuals, it is unclear how tissue restricted phenotypes result from mutations in ubiquitously expressed gene products that are required for protein synthesis. During the course of a forward genetic screen in mice for pigmentary abnormalities, we identified missense alterations in *Ribosomal protein S19* and *Ribosomal protein 20* in two dominantly inherited, chemically-induced mutants, *Dark skin 3* and *Dark skin 4*, respectively. We show that these mutations act through a common pathophysiologic program in which p53 activation causes increased expression of Kit ligand and, consequently, proliferation of epidermal melanocytes. Activation of p53 is both necessary and sufficient for the dark skin phenotype, but occurs through a paracrine mechanism that depends on keratinocyte-melanocyte interactions. These results provide a mechanistic explanation for both the pleiotropy and tissue specificity that accompany reduced dosage of ribosomal protein genes.

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Stem cell origin and tumor microenvironment in NF1-associated neurofibroma genesis

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The tumor predisposition disorder Neurofibromatosis Type I (Nf1) is one of the most common genetic disorders of the nervous system. Nf1 patients have a wide spectrum of clinical presentations, including developmental, pigment or neoplastic aberrations of the skin, nervous system, bones, endocrine organs, blood vessels and the eyes. Neurofibromas, the most frequent tumor in Nf1, and malignant nerve tumors are serious complication of Nf1. Plexiform neurofibromas are embryonic and almost always present at birth, whereas the dermal neurofibromas occur at around puberty and are exclusively in the skin, indicating that plexiform and dermal neurofibromas originate from two different neural/Schwann progenitor cells. A recent breakthrough in our laboratory has uncovered unique paracrine interactions between the neoplastic cell (the Schwann cells) and its microenvironment (the mast cells) that are required for neurofibroma formation. The central goals of this proposal are to identify the cell of origin of Nf1-associated neurofibromas and to understand how the tumor microenvironment regulates neurofibroma development. Using inducible genetically mutant mice, we have identified a novel population of neural-crest derived neural stem cell in both human and mouse dermis as the cell of origin of Nf1-associated neurofibroma. In fact, we've discovered that these Neurofibromin-deficient neural-crest derived stem cells give rise to neurofibroma 100 % of the time only when implanted next to a nerve, indicating the essential role of neuron in the tumor microenvironment for neurofibroma genesis. These unique paracrine interactions between the neoplastic cell (the Schwann cells) and its microenvironment (the neurons and mast cells) are absolutely required for neurofibroma formation. These novel findings provide an innovative approach to gain a greater understanding of the molecular pathogenesis of neurofibroma and exploit new therapeutic approach in clinical trial to treat Nf1-associated neurofibromas.

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Molecular and clinical characterization in Japanese and Korean patients with Hailey-Hailey disease: six new mutations in the *ATP2C1* gene

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The autosomal dominant disorder Hailey-Hailey disease (HHD; MIM 169600) results from mutations in the *ATP2C1* gene, which encodes the human secretory pathway Ca^{2+}/Mn^{2+}-ATPase protein 1. To date, over 90 pathological mutations scattered throughout *ATP2C1* have been described with no indication of mutational hotspots or clustering of mutations. No paradigm for genotype-phenotype correlation has emerged. In this study we sequenced *ATP2C1* in eight unrelated Japanese and Korean patients with HHD in order to add the data to the known mutation database. We identified seven different heterozygous mutations in seven of the eight investigated patients, including three new single nucleotide deletion/duplication mutations: c.520delC; c.681dupA; c.956delC, three new donor splice site mutations: c.360+1G>C; c.899+1G>T; c.1570+2T>C, as well as a previously described nonsense mutation: p.Arg153X. RT-PCR analysis, using RNA extracted from a skin biopsy, in the mildest affected patient with a heterozygous c.360+1G>C mutation, demonstrated expression of a short in-frame mutant transcript with exon 5 skipping, which accounts for the mild phenotype. These results expand the known mutation spectrum in HHD and show the importance of RNA analysis for understanding the genotype-phenotype correlations more precisely.

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Identification of the enhancers within the first intron of *PADI1* gene in human epidermal keratinocytes

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Peptidylarginine deiminase (PAD) catalyzes the conversion of protein-bound arginine residues to citrulline residues in a Ca^{2+}-dependent manner. Three of the five known PAD isoforms are expressed in the epidermis. PAD1 is detected throughout the whole epidermis; PAD2 is located in the stratum spinosum and granulosum; PAD3 is restricted to the granular and the lower cornified layers. In order to investigate the molecular mechanisms of *PADI1-3* gene transcription in the human epidermis, we have previously characterized their minimal promoter regions. Here, in the search for other transcriptional activators, we subcloned and constructed a series of luciferase reporter plasmids containing the first intron of each of the genes *PADI1-3*, and performed luciferase assays in normal human epidermal keratinocytes. It was found that two enhancer-like regions present in the first intron of *PADI1* increases the *PADI1* promoter activity. Moreover, these two enhancer-like regions also could interfere with the core promoter area of *PADI2* and *3* to remarkably promote their activity. The potential regulatory function of the fragments were confirmed by real-time PCR DNase I hypersensitivity assay in cultured keratinocytes. Our data indicate that the interaction between the minimal promoter and some segments of the first intron of *PADI1* are important for the expression of the genes *PADI1-3* in keratinocytes, which probably helps to explain their regulation during epidermal differentiation.

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A novel *plakoglobin* mutation reveals distinct roles for plakoglobin in heart and skin

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The obligate desmosomal constituent plakoglobin (PG) is a member of the *armadillo* family of signalling and junctional proteins. To date, two mutations in *Pg* have been described and in both instances patients suffer from terminal cardiomyopathy.We have identified a homozygous nonsense mutation, S24X, in *Pg* in three non-consanguineous Argentinean patients who exhibit similar clinical features consisting of skin fragility, diffuse palmoplantar keratoderma and woolly hair. No symptoms of cardiomyopathy were observed. Immunostaining of patient skin biopsy sections showed abnormal localisation of desmoplakin and desmoglein 1 and a remarkable decrease of abnormal PG staining. RT-PCR analysis from skin RNA and subsequent sequencing revealed normal 5′ splicing of *Pg* and no loss of mutant transcript. Western blotting (WB) of skin proteins with a monoclonal antibody mapped to the C-terminus of PG revealed low levels of a truncated protein in both patient and a heterozygous family member. These results suggest that S24X mRNA is expressed at similar levels to wild type mRNA and that an alternative translation start site using an in-frame internal AUG codon downstream of the mutation results in the expression of an N-terminal truncated protein. The low levels of truncated PG seen in WB and immunostaining of patient skin could result from a reduction in either translation efficiency or in protein stability. The lack of a heart phenotype in our patients suggests that low expression of the truncated PG still facilitates functional cardiomyocyte junctions but not keratinocyte junctions. Our findings provide new insight into the distinct roles plakoglobin plays in the epidermis and heart.

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Dorfman-Chanarin syndrome: a founder mutation in Tunisians and a new mutation of *CGI-58* in an Indian family

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CGI-58 is a 349-amino acid protein coactivating a lipase, ATGL, which hydrolyses triacylglycerol. CGI-58 mutations induce a systemic accumulation of lipid droplets known as Dorfman-Chanarin syndrome (DCS). To date 16 different CGI-58 mutations have been reported. We searched for CGI-58 mutation in 3 new families with DCS, 2 from Tunisian and 1 from Indian origin. Four patients with ichthyosiform erythroderma and lipid droplets in leukocytes and six relatives were included. The 7 exons and intron/exon boundaries of *CGI-58* were sequenced. In the three Tunisian patients, a homozygous G-to-A transition at position 773 -1 was observed. This mutation was present at a heterozygous state in both parents and in a brother of one family. *In silico* splice site analysis predicted the suppression of the acceptor splice site at the intron 5/exon 6 boundary and the appearance of a cryptic splice site 95 nucleotides later. RT-PCR using the patient's mRNA revealed a shortened amplicon with decreased intensity whose sequencing showed the deletion of the first 95 nucleotides of exon 6. This shortened transcript predicted a truncated 323 amino acid protein including 65 C-ter missense amino acids. This 773-1 G>A mutation has previously been reported in two unrelated Tunisian patients and is the only mutation described in this population suggesting a founder effect. In the Indian patient, an A insertion in exon 5 after position 730 was demonstrated. It was observed at a heterozygous state in both parents but was absent in one brother. It induces a reading frame shift and a premature stop codon resulting in a 252 amino acid protein with 9 C-ter missense amino acids. In conclusion we report a 773-1 G>A mutation in 2 Tunisian families suggesting a founder effect in this population and an original 730insA mutation in an Indian family. Both mutations may result in a truncated CGI-58 protein with loss of function.

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Transglutaminase-1 gene mutations in autosomal recessive congenital ichthyosis: clinical and genetic investigations in a large cohort of 108 patients

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Autosomal recessive congenital ichthyosis (ARCI) encompasses a variety of disorders of keratinization including lamellar ichthyosis and non-bullous congenital ichthyosiform erythroderma. The purpose of this study was to characterize the Transglutaminase-1 (*TGM1*) gene mutation spectrum, and clinical features of 108 patients with ARCI ascertained through the National Registry for Ichthyosis and Related Disorders. A study questionnaire was administered to all participants, eliciting information on dermatologic features. Direct DNA sequencing was used to screen for mutations in *TGM1*. *TGM1* mutations were identified in 55% (57/104) of patients. Of the 44 germline mutations identified, 22 were missense mutations. There were 24 novel mutations: 9 missense, 5 nonsense, 7 frameshift, and 3 splice site. The most common mutations were IVS5-2 A>G (29 alleles), R142H (7 alleles), and V518M (6 alleles). Of 22 missense mutations, 15 (68%) and 6 (27%) were located in the catalytic core and the β-sandwich domain of TGM1, respectively. To evaluate the sequence conservation in TGM1, we blasted the amino acid sequences of human TGM1 and various species, and we constructed a homology model of human TGM1. Patients reported collodion membrane at birth in 61% (59/97), ectropion in 74% (80/108), eclabium in 18.5% (20/108), anhidrosis in 85% (92/108), and plate-like scales in 74% (80/108). Collodion membrane at birth was associated with ectropion (p=0.009) or eclabium (p=0.035). Ectropion was associated with eclabium (p=0.001), plate-like scales (p=0.024), and hearing problems (p=0.027). Anhidrosis was associated with palm (p=0.003) or sole involvement (p=0.016). This is the largest and most comprehensive study to date of ARCI. Germline mutations in *TGM1* account for 55% of all ARCI patients, confirming that ARCI is genetically heterogeneous. This study expands the spectrum of ARCI-causing *TGM1* mutations and the associated clinical features.

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A case of a 7-year-old boy with hidrotic ectodermal dysplasia and mutation analysis of connexin 30 gene

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The objective of this study is to investigate clinical manifestation and pathogeny of a case with Hidrotic ectodermal dysplasia (HED) (Clouston syndrome, OMIM #129500). HED is a rare autosomal dominant disorder affecting the skin and its derivatives. It is characterized by the triad of nail dystrophy, alopecia and palmoplantar hyperkeratosis. Sweat gland function in these patients is normal. It was first reported by Nicolle and Hallipre. To date, all mutations have been involving in three codons: G11R, A88V and V37E in the connexin 30 (Cx30) gene have been shown to cause this disorder. We report a 7-year-old boy with HED from Jiangsu province in China. Hyperkeratosis of the palms and soles, fissuring is sometimes troublesome. His scalp hair is severe alopecia in infancy, the eyebrows and eyelashes are completely lacking. Sweating, dentition, hearing and oral mucosa all appeared normal. The specimen obtained from the scalp region showed normal epidermis and normal distribution of eccrine glands, remnants of hair follicles with sebaceous glands attached were observed in the lower dermis. Clinical and histological characteristics supported the diagnosis of HED. None of the other family members or close relatives were affected. In order to analyze the mutations of the Cx30 gene in Chinese Han patients with HED. The whole coding region of Cx30 was amplified by polymerase chain reaction and products analyzed by direct sequencing. However, we haven't detected any Cx30 gene mutation in our case. In summary, we have reported a case of a 7-year-old boy with hidrotic ectodermal dysplasia without connexin 30 gene mutation in China and his condition may be classified as another type of hidrotic ectodermal dysplasia caused by an unknown gene.

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A novel protein-interaction on tuberous sclerosis complex

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Tuberous sclerosis complex (TSC) is an autosomal dominant disease characterized by the hamartomas. Two genes, *TSCI* and *TSCII*, which encode hamartin and tuberin respectively, have been shown to be responsible for TSC. Hamartin and tuberin associate physically in vivo, and the complex functions as a GAP for small GTPase which is called Rheb (Ras homologue enriched in brain). This complex formation provides a tentative explanation for the similar disease phenotype in either *TSC1*- or *TSCII* deficient TSC. Many functions of *TSCI* and *TSCII* are reported. However, all of the symptoms of TSC are not explained by the known functions of *TSCI* and *II*. Furthermore, linkage analysis suggests that about 20% of TSC patients did not have any mutation in either of *TSCI* and *II*. These facts suggest that another protein may contribute to TSC pathogenesis. In this study, we identified a 40KDa protein, p40, which decreased specifically in TSC and might be related to the neural symptoms of TSC. P40 is a component of 40S ribosomal subunits in mammalian cells, but it is also localized in the nucleus and mitotic chromosomes. To examine the contribution of p40 to TSC, we first analyzed the interaction of p40 with hamartin and tuberin. Co-precipitation experiments revealed that p40 was detected in the fraction immunoprecipitated with either anti-tuberin or anti-hamartin antibodies. On the contrary, hamartin was recognized in the fraction immunoprecipitated with anti-p40 antibodies. Then, gene silencing experiments using *p40*-siRNA, *TSC1*-siRNA and *TSCII*-siRNA were performed. When the expression of p40 was suppressed by *p40*-siRNA, the expression of hamartin and tuberin decreased, but rho increased. Rho is associated with hamartin and may induce metastasis of lymphangiomyomatosis (LAM), pulmonary disease in TSC. These results suggest that the p40 may stabilize both hamartin and tuberin through the direct association with hamartin and may be related to TSC pathogenesis by regulating rho function.

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Effects of a novel gene silencer pyrrole-imidazole polyamide targeting to TGF-β1 on the hypertrophic scar of skin

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Objective: TGF-β is a responsible factor in the hypertrophic scar of skin. Pyrrole-imidazole polyamides (PIP) are small synthetic molecules can bind to specific nucleotide sequences in the minor groove of double-helical DNA. We have developed PIP targeting to TGF-β1 and examined effects of a PIP targeting to TGF-β on the hypertrophic scar model in the rats. Methods: PIP targeting TGF-β was designed to the AP-1 binding site of the rat TGF-β1 promoter. The hypertrophic scar of skin was created by skin injury on the back of rats. PIP 100μg was injected in the injured area. To identify distribution of polyamide FITC-labeled PIP was also injected. Total RNA was extracted, and the expression of TGF-β, CTGF, collagen type1, and fibronectin mRNAs was determined by RT-PCR. Skin sections were stained with HE and Masson trichrome, and vimentin. Results: This PIP showed strong, fast and specific binding to the target DNA. FITC-labeled PIP was distributed in almost nucleus in the injured skin. Peak increases in expression of TGF-β1 mRNA and protein was observed 3 days after the skin injury. Expression of CTGF and extracellular matrix mRNAs was increased after the peak induction of TGF-β. PIP significantly inhibited the formation of hypertrophic scars and the increase in expression of TGF-β, CTGF and extracellular matrix mRNAs in the skin. PIP markedly inhibited fibrotic changes. Conclusion: PIP targeting TGF-β will be a feasible gene silencing agent for the hypertrophic scar on skin.

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Filaggrin null mutations in mild-moderate atopic eczema: a population-based case-control study.

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Null mutations in the filaggrin gene (FLG) are strongly associated with moderate-severe atopic eczema but their role in the mild-moderate eczema that is prevalent in the general population is unknown. 811 English children aged 7 to 9 years from an unselected birth cohort were screened for five FLG mutations. Eczema cases were defined by UK diagnostic criteria and skin examination. Association between phenotype and genotype was investigated using Fisher's exact test and logistic regression analysis. The 12-month period prevalence of atopic eczema was 24.2% (95% confidence interval 21.2-27.2%) and 96% (115/120) of cases had mild-moderate disease. The combined null genotype (one or more FLG mutations) was significantly associated with atopic eczema (p=1.2x10-4). The odds ratio for individuals having two null mutations was 26.9 (95% CI 3.3-217.1), but heterozygote carriers showed no significant increase in risk (odds ratio 1.2, 95% confidence interval 0.7-1.9). Comparison of genotype and allele-based statistical models supports a recessive pattern of inheritance. In this population cohort we were also able to estimate the true risk (penetrance) of disease. The probability of having eczema in individuals with two FLG null mutations is 88.9% (95% CI 68.4-109.4%) compared to 26.2% (17.7-34.7%) in heterozygotes and 22.9% (19.7-26.1%) with the wild-type genotype. 8/190 (4.2%) of eczema cases had two FLG null mutations and thus may be attributed to filaggrin deficiency. Our results indicate that FLG null mutations are significantly associated with mild-moderate atopic eczema in childhood but show a recessive pattern of inheritance in this phenotype.

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Mutation analyses of keratin 5 and keratin 14 in Korean patients with epidermolysis bullosa simplex

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Epidermolysis bullosa simplex (EBS) is a group of hereditary bullous disorders caused by mutations of the keratin genes KRT5 and KRT14. Patients present with widely varying severity and are classified in three main subtypes: EBS Weber–Cockayne (EBS-WC), EBS Köbner (EBS-K), and EBS Dowling–Meara (EBS-DM), based on distribution and pattern of blisters. Detailed knowledge of the spectrum of EBS mutations and their genotype-phenotype correlation is essential for accurate genetic counselling and prenatal diagnosis. Recently, about 120 mutations have been reported in EBS. Significant correlation between the position of mutations within these proteins and the clinical severity of EBS has been noted. We investigated 13 EBS patients and families of Korean origin by sequence analysis of the entire coding sequences of KRT5 and KRT14. We identified 7 novel mutations in this study, four mutations on the KRT5(p.V143F, p.R264P, p.C479X and p.Asn177del), and three on the KRT14(p.R125L, p.E392X and p.L401P), and 6 previously reported mutations within the KRT5 and KRT14 genes. These include 10 missense, 2 nonsense and 1 small deletion mutation. Three of thirteen EBS patients are EBS-DM and all mutations(p.R125H, p.C479X, p.Asn177del) located in the highly conserved ends of the alpha-helical rod domain, the helix initiation(HIP) or helix termination(HTP) peptides, respectively. In case of p.Asn177del mutant, it is a single amino acid deletion that result from c.531_533delTAA and presented with severe phenotype since birth. Seven and three mutations are identified in EBS-K and EBS-WC, respectively. The positions of mutation in both subtypes are more diffusely scattered within the rod domains and in the L12 linker domains of both keratin genes. Interestingly, mutant of p.E392X was homozygous recessive profile(c.1174G>T) and consistent with the generalized EBS-K phenotype. This study shows possible implications of the novel mutations on protein structure, keratin intermediate filament (KIF) formation and the corresponding phenotype.

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A model for non-Herlitz junctional epidermolysis bullosa in mice

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Here we describe a novel spontaneously arising mutation which results in dermal-epidermal separation that is remarkably consistent with non-Herlitz junctional epidermolysis bullosa (JEB). The mutation was positionally cloned and shown to be a hyphomorphic allele of the gene, *Lamc2*. Immunofluorescence blister mapping showed intergin alpha 6 (ITGA6) and bullous pemphigoid 180 (COL17A1) expression at the roof of the blister and type VII collagen (COL7A1) expression on the blister floor. Transmission electron micrographs showed separation at the level of the lamina lucida as well as the presence of rudimentary or absent hemidesmosomes. These results are consistent with a diagnosis of JEB. We further show that disease onset and mouse survival are dependent upon strain background thus supporting a role of modifier genes. *Lamc2*^{jeb} develop a progressive form of the disease with symptoms developing around 3 months of age, making them invaluable for understanding the pathoetiology of JEB and for developing new therapies. This new spontaneous mutation, *Lamc2*^{jeb}, is a potentially important model for the characterization and treatment of JEB.

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First successful DNA-based prenatal exclusion of harlequin ichthyosis

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Harlequin ichthyosis (HI) is the severest form of congenital ichthyosis with an autosomal recessive inheritance pattern. We recently clarified ABCA12 as the causative gene for HI (Akiyama et al, J Clin Invest 2005). Until identification of responsible gene of HI, prenatal diagnosis of HI could have been made only by elaborate electron microscopic observation of fetal skin biopsy samples, which is techinically difficult and available at limited insitutions. Here we report the first successful DNA-based prenatal exclusion of HI. The proband of the present family showed typical clinical features of HI and was a compound heterozygote for two ABCA12 mutations. He deceased three days of age and details of the proband were previously reported. Due to the parents' request, we performed prenatal diagnosis by direct sequencing and restriction enzyme digestion analysis using fetal genomic DNA from amniotic fluid cells at 16 weeks gestation. These analyses showed the fetus harbored the paternal ABCA12 mutation p.1950Arg>X, but not the maternal mutation c.3295-2A>G. The results predicted that the fetus was not affected and the pregnancy was continued. Five months later a neonate was born in good health with completely normal skin. This study demonstrates the efficacy of early DNA-based exclusion of harlequin ichthyosis, which is technically simple and reliable, and available world wide.

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Is TNF-α-targeted short hairpin RNA (shRNA) a novel potential therapeutic tool in psoriasis treatment?

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TNF-α is a well known target in psoriasis treatment and biological treatments targeting TNF-α are already clinically used against psoriasis and psoriasis arthritis. Attention is however given to a novel therapeutic tool: RNA interference that controls gene silencing. This study investigates the efficiency of targeting TNF-α with specific short hairpin RNA (shRNA) and explores its potential in treating psoriasis. ShRNAs targeting human TNF-α mRNA were generated. Their efficiency in down-regulating TNF-α protein expression was evaluated using a Renilla luciferase screening-assay and a transient co-transfection assay. The expression cassette encoding the most efficient TNF-α shRNA was inserted into a lentiviral vector, allowing proficient gene delivery. The lentiviral vector is integrated into the host genome and establishes life-long infection and persistent shRNA expression. The lentiviral vector expressing TNF-α shRNA was used to transduce HEK293 cells and verify vector-derived TNF-α knockdown *in vitro*. *In vivo*, psoriasis skin was exposed to lentiviral TNF-α shRNAs by a single intra-dermal injection. Psoriasis skin for the *in vivo* study was obtained from psoriatic plaque skin biopsies that were transplanted onto SCID mice employing the psoriasis xenograft model. Three weeks after exposure, biopsies were taken and the epidermal thickness as an endpoint for evaluating psoriasis and the levels of TNF-α mRNA were measured. The lentiviral TNF-α shRNAs down-regulated the TNF-α expression *in vitro* and *in vivo* by 50% and, most interestingly, the epidermal thickness of the psoriatic plaques was reduced. In conclusion, our results demonstrate that lentiviral TNF-α shRNAs have the potential to down-regulate TNF-α production *in vitro* and *in vivo*. The decreased epidermal thickness suggests a potential role for TNF-α shRNAs as a new therapeutic agent in psoriasis treatment.

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Altered expression of lysyl oxidase and Wnt signalling genes in Kindler syndrome skin provides new insight into mechanisms underlying skin atrophy

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Kindler syndrome (KS) is an autosomal recessive blistering and poikilodermatous disorder caused by mutations in the *KIND1* gene. *KIND1* encodes kindlin-1, an actin cytoskeleton-related component of focal contact junctions. A reduction in kindlin-1 in focal contacts at the dermo-epidermal junction provides an explanation for the skin blistering seen in KS but provides little insight into other clinical features of the disorder, such as skin atrophy, photosensitivity or increased risk of mucocutaneous malignancy. To address this, we performed whole-genome gene expression studies using Illumina Human-6 v2 beadchip arrays. We compared RNA from photoexposed skin taken from 3 patients with KS (each harbouring a different homozygous mutation in *KIND1*: p.W616X, p.R271X or p.E516X) with RNA from age and site-matched skin from 3 healthy controls. After signal averaging and data normalisation, a predefined filtering set based on two-fold change and expression difference score was used to interrogate 48688 probe sets. 445 individual genes showed differential expression ($p<0.05$), of which 116 were up-regulated and 329 down-regulated. Of interest, there was decreased mRNA expression of the lysyl oxidase gene, *LOX*. Lysyl oxidase is important in cross-linking dermal collagen and elastic tissue and may also have a direct role in tumour suppression. We also found altered expression of several Wnt signalling components in KS skin that may be relevant to tissue development (increased expression of *WNT5a* and *WISP3* but reduced expression of *SFRP2*). Differential gene expression for all the above genes was verified at mRNA level by real-time PCR and/or protein level by western blotting and immunofluorescence microscopy and replicated in 4 further KS patients with *KIND1* mutations. This gene expression microarray study thus provides further insight into molecular mechanisms that may underlie the atrophic skin phenotype of KS.

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Psoriasis is associated with increased beta-defensin genomic copy number

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Beta defensins are small antimicrobial proteins expressed in epithelia. Seven beta-defensin genes on chromosome 8p23.1 are on a large repeat unit that is variable in copy number. The pro-inflammatory nature of these beta-defensins suggested that quantitative variation in gene dosage might contribute to susceptibility to inflammatory disease. We used a multiplex amplifiable probe hybridization assay and the paralog ratio test to analyze this genomic copy number polymorphism in 179 Dutch individuals with psoriasis and 272 controls and in 319 German individuals with psoriasis and 305 controls. Comparisons in both cohorts showed a significant association between higher genomic copy number for beta-defensin genes and risk of psoriasis. There is significant support ($P = 0.005$, linear regression analysis of variance) for a linear model in which each additional copy above two copies increases the relative risk by 34 percentage points (95% confidence interval: 25–43%). Analysis at the protein level in serum of healthy controls showed a moderate but significant correlation between genomic copy number and hBD-2 protein expression. As there are several beta-defensin genes on this repeat we cannot distinguish whether one gene or a combination of genes is responsible for the gene dosage association with psoriasis. Given the cytokine-like role of hBD-2, its high expression at the protein level and its demonstrated gene dosage effect, we believe that this is the best candidate, but further work is required to test this.

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Finnish patients with systemic lupus erythematosus share predisposing genetic risk factors with other populations

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We have recruited a population-based sample of lupus erythematosus (LE) patients, comprising altogether 338 patients with systemic LE (SLE) and 177 with discoid LE, and 808 population-based controls. In this cohort, we have studied 8 published candidate susceptibility genes for SLE. After careful review of recent literature, we selected for genotyping 18 risk allele tagging SNPs in these 8 genes with the strongest evidence of susceptibility, including the most strongly replicated gene *interferon regulatory factor 5 (IRF5)*. The samples were genotyped using the Sequenom Iplex assay system with allele detection by mass spectrometry. Preliminary data analysis was performed for the families multiply affected by SLE using PDTPHASE. In accordance with previous reports, we confirmed the association of SLE to *IRF5* and *programmed cell death 1 (PDCD1)*. Preliminary analyses suggested allelic heterogeneity for *cytotoxic T-lymphocyte-associated protein 4 (CTLA4)* compared to the previous report on UK population. We conclude that LE susceptibility genes and their SNPs suggested in the recent literature are also important in the Finnish patients. We are currently performing gene-gene interaction studies to assess the risk of SLE in carriers of multiple independently confirmed susceptibility genes.

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Genetic evidence for involvement of the IL23 pathway in Thai psoriatics

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A recent genome-wide association analysis of psoriasis using gene-centric markers identified IL12B and IL23R as significantly associated with psoriasis. These associations have been replicated in additional Caucasian cohorts including our own. Together with emerging immunologic and pharmacologic data, these findings support an IL23-mediated inflammatory pathway in psoriasis pathogenesis involving IL-17-producing T-cells. Here we report association test results of a Thai cohort consisting of 206 psoriasis cases and 114 controls, collected at dermatology clinics in Bangkok. The IL23R SNPs rs7530511 (L310P) and rs11209026 (Q381R), and IL12B SNPs rs3212227 (3′ UT) and rs6887695 (60 kb 5′) were genotyped using Taqman assays. Data were analyzed for association using a logistic regression model for linear trend of association; p-values were determined by Monte Carlo simulation. One of the IL23R markers, rs7530511, had only 4 occurrences of the minor allele but the association was still marginally significant ($p=0.017$). The other IL23R marker, rs11209026, was not polymorphic. One of the IL12B markers, rs3212227, showed significant association with psoriasis (OR = 1.64, $p = 0.0058$) while the other, rs6887695, did not (OR = 1.29, $p = 0.12$). Haplotype analysis of the two IL12B SNPs yielded highly significant association ($p=0.00081$, OR=1.73), with the associated allele (AG) being the same as that found in Caucasians. These results show that IL12B is an important genetic factor in psoriasis pathogenesis in the Thai population, with an association strong enough to yield significant confirmatory evidence using a modest sample size. IL23R showed a trend in the direction of association, but will need analysis of a larger sample to be convincing. Together with previously reported evidence for IL12B association in Japanese psoriatics, our results support the hypothesis that genes encoding components of the IL23-mediated inflammatory pathway are important determinants of psoriasis across multiple racial groups.

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Effect of various stimulations on cell-surface expression of Notch ligands on cultured keratinocytes

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Notch signaling is transcriptional control system widely conserved in mammals. Notch receptors and Notch ligands are expressed on the surface of various cell types. After the binding of Notch ligands, intracellular domain of Notch receptor is recruited to nucleus and regulates the transcription of target genes by binding to *cis*-enhancing element. Recently, Notch signaling is reported to be involved in differentiation and proliferation of human keratinocytes, and in addition, there are some studies reporting the involvement of Notch signaling in skin diseases such as skin tumors and psoriasis. However, the regulation mechanism of Notch receptors and ligands expression on keratinocytes is still unclear. Therefore, we investigated regulations of expression of Notch ligands in cultured keratinocytes by stimulation with various extracellular factors, such as increase in extracellular Ca^{2+} concentration and addition of IFN-γ and TGF-β. Real time-PCR analysis showed that raised extra-cellular Ca^{2+}, IFN-γ and TGF-β increased mRNA expression level of Jagged-1 of keratinocytes. In FACS analysis using anti human Notch ligands-mouse monoclonal antibodies, the cell surface Jagged-1, which is constitutively expressed on keratinocytes, was decreased by IFN-γ. These results suggested that Notch ligand expression is regulated post-translationally. Now we are analyzing the mechanism of IFN-γ-mediated down-regulation of Jagged-1 by using various inhibitors for protease and endocytosis involved in IFN-γ pathway.

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Bone marrow transplantation restores deficient epidermal basement membrane protein and improves the clinical phenotype in epidermolysis bullosa model mice

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There are currently no effective treatments to improve the prognosis in epidermolysis bullosa (EB). Recent studies have shown that bone marrow-derived cells (BMDCs) can play a significant role in regenerative medicine by transdifferentiating into various cell types. We previously reported that 0.05% of epidermal keratinocytes at wound sites were derived from donor BMDCs in bone marrow transplantation (BMT) model mice (Inokuma et al, Stem Cells 2006). The purpose of this study was to determine whether a knocked-out protein can be re-expressed after BMT, and to explore the possibility of using this technique to treat severe forms of EB. Firstly, we investigated whether human cells could transdifferentiate into keratinocytes as demonstrated by our mouse BMT model. Human cord blood cells were transplanted into immunodeficient NOG (NOD/Shi-scid, IL-2Rγnull) mice. At the wound site we found cord blood-derived keratinocytes as well as expression of human BMZ proteins such as collagen XVII (COL17), BPAG1 and plectin. Secondly, BMT was accomplished using cells from green fluorescence protein (GFP) expressing-transgenic mice into our recently established adult COL17 knockout mice, which was used as a model for human junctional EB (Nishie et al, Nat Med 2007). Immunohistochemistry and RT-PCR analysis demonstrated the expression of COL17 beneath GFP+ epidermal cells at the wound site in BMT-treated COL17 knockout mice. More importantly, BMT-treated mice showed fewer EB-associated erosions compared to untreated mice. Furthermore, BMT-treated mice showed statistically better survival rates (73.2% versus 20.6%, 150 days after BMT, $p<0.05$). These findings indicate that conventional BMT techniques show significant potential as systemic therapeutic approaches for treatment of human severe EB.

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Evidence for an association between polymorphisms in the Toll-like receptor 4 (TLR4) gene and early-onset psoriasis

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Psoriasis is a chronic inflammatory skin disease affecting 2-3% of the Caucasian population. Toll-like receptor (TLR)2 and TLR4 are fundamental in pathogen recognition and activation of innate immunity by mediating the production of cytokines necessary for the development of effective immunity. Innate and adaptive immune events are integral to the pathogenesis of psoriasis. These events together with evidence that TLR2 and 4 are up-regulated in involved skin of patients with psoriasis indicate that these receptors present interesting, previously un-investigated candidate genes for association analysis with psoriasis. The aim of this study was to investigate whether SNPs in the TLR2 and 4 genes are associated with Type I, early-onset, psoriasis (disease onset ≤ 40 years) in a UK population. We successfully genotyped a total of 36 SNPs across the TLR2 and TLR4 regions in 664 UK Type I psoriasis patients (54.7% male; mean age of onset 19.8 years) and 566 population based controls. 3 of the 12 markers tested for TLR4 demonstrated evidence of association to Type I psoriasis ($p<0.05$ for genotypic $\chi 2$ and trend test). SNPs rs7044464 and rs752998 conferred a protective effect by carriage of two copies of the minor allele with odds ratios (OR) of 0.24 (95%CI:0.08-0.61; p=0.0013) and 0.55 (95%CI:0.33-0.92; p=0.02) respectively. rs10759932 also asserted a protective effect but by carriage of a single copy of the minor allele; OR=0.72 (95%CI:0.55-0.94; p=0.01). Interestingly rs7044464, located downstream of TLR4, is highly correlated with a 3'UTR SNP of TLR4, not tested in this study. TLR2 showed no evidence of an association to early-onset psoriasis. This study provides initial evidence that polymorphisms in the TLR4 gene region are associated with early-onset psoriasis, potentially highlighting a key role for TLR4 in its pathogenesis. Such findings should be independently replicated and explored to further investigate and confirm these novel associations.

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Identification of luca, a murine mutation in the Zdhhc13 gene producing alopecia and hair cycle abnormalities

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We have identified luca (luc), a new mouse mutation associated with alopecia and other alterations in the skin adnexa. We first determined that luc is an autosomal recessive mutation, mapped to chromosome 7. Affected luc/luc mice develop generalized hypotrichosis and multifocal patchy alopecia, starting at postnatal day 6. Hair shaft dysplasia is characterized by twisting and multifocal pigment clumping, suggesting defects in the complex differentiation program of trichocytes. At postnatal day 22, the number of anagen II hair follicles appears to be reduced in homozygous mice, whereas in wild type and heterozygous mice all hair follicles undergo their first true anagen phase. At postnatal day 30, all hair follicles in luc/luc mice have very prominent sebaceous glands, suggesting that anagen induction occurs in tylotrich hair follicles only, whereas non-tylotrich hair follicles likely remain in telogen or undergo atrophy. Multifocal alopecia and erythema in homozygous mice is also associated with inflammatory cell infiltration. No further relevant organ abnormalities were detected on histopathology of several organ systems. Genotyping of 350 F2 mice (700 meiosis) allowed us to narrow down the segment of chromosome 7 containing luc to a ~3 Mb interval between markers D7Mit185 and D7Mit93 (46.2 - 49.6 Mb), a region of homology with human chromosome 11p15.1. By sequencing several candidate genes in the ~3 Mb interval, we identified luca as a premature stop codon in the exon 4 of the palmitoyl transferase gene, Zdhhc13. This gene is involved in the post-translational addition of the fatty acid palmitate to specific argenine residues affecting trafficking and function of several substrate proteins. The mechanisms by which deficiency of this protein lead to alterations in the hair follicle is not known but it may be speculated that deficient palmitoylation may affect critical signal transduction pathways involved in the regulation of the hair follicle.

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Activation of vascular endothelial growth factor receptor II in the cellular model of loricrin keratoderma

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Loricrin is a major constituent of the epidermal cornified cell envelope. Recently, heterozygous loricrin gene mutations have been indentified in loricrin keratoderma (LK). We have previously shown that the wild loricrin construct, when transiently transfected into HaCaT cells, leads to the activation of caspases and positive TUNEL staining with features of apoptosis. The apparent transfection rate is low with loricrin construct, supporting its apoptotic role but hindering further study. To bypass this problem, we generated stable HaCaT cell lines that expressed wild and mutant loricrin using an ecdysone-inducible promoter system. The cell lines expressing mutant loricrin grew more rapidly than those expressing wild loricrin. HaCaT cells with mutant loricrin express phosphorylated forms of Akt. The confocal immunofluorescence microscopic observation reveals that phospho-Akt localizes at nucleolus. The activity of Akt kinase is about 9 times higher in HaCaT cells with mutant loricrin than those in the cell lines with mock or wild loricrin. ERK1/2, epidermal growth factor receptor, vascular endothelial cell growth factor receptor (VEGFR) II and Stat 3 are all phosphorylated in mutant loricrin cells. The docking proteins, Gab1 and c-Cbl, are also tyrosine-phosphorylated in mutant loricrin cells. Neither p38 MAP kinase nor SAPK/JNK is phosphorylated in both wild and mutant loricrin cells. Because there are reports that vascular endothelial cell growth factor (VEGF) is expressed and secreted by epidermal keratinocytes, we measured the concentration of VEGF in a culture medium. VEGF in the culture medium is about 3 times more abundant in HaCaT cells with mutant loricrin compared with wild loricrin. Furthermore, chromatin immunoprecipitation assays indicate that Stat3 protein binds to the VEGF promoter in HaCaT cells with mutant loricrin. Thus, VEGF release and the subsequent activation of the VEGFR II by an autocrine/paracrine pathway link loricrin gene mutation to rapid cell proliferation in the HaCaT LK cellular model.

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Characterization of Glycoprotein A repetition predominant protein (GARP) identified as a novel gene marker of human $CD4^+CD25^{high+}$ regulatory T cells (Treg)

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We have previously reported that GARP, a 72kD leucine rich repeat containing protein was expressed and specifically up-regulated in human Treg cells (*JID, 124(S4) 2005: A117*). Given the lack of specific knowledge regarding GARP's localization and function, we generated and expressed N- and C-terminal GFP-fusion constructs of GARP in HEK-293 cells. In keeping with the proposed transmembrane localization, expression of C-terminal GFP GARP was found to be expressed on the plasma membrane, via translocation across the endoplasmic reticulum, as visualized using confocal scanning laser microscopy. The expression of the N-terminal GFP GARP was not lipid-membrane specific; rather it exhibited a diffuse cytosolic/nuclear pattern. Anti-GFP immunoblot analysis of total lysates from C-and N-terminal expressing clones revealed a 99kD (29kD GFP+70kD GARP) and a 31kD (29kD GFP+2kD GARP) fusion protein respectively. The difference in protein size between the C- and N-terminal fusion proteins suggests a potential cleavage site in the N-terminus of the protein. Examination of the GARP protein sequence using SignalP 3.0 predicted a putative cleavage site between amino acids 17 and 18 in accordance with our findings. Thus, *in vivo*, GARP is processed from a 72kD (662aa) to a 70kD (645aa) protein during translocation. To confirm surface expression of GARP, C-and N-terminal expressing clones were surface biotinylated and then GFP immunoprecipitated fusion proteins probed for the presence of biotinylation using streptavidin-HRP. Detection of biotinylation only occurred in the C-terminal GFP-expressing clones, consistent with both surface membrane expression of GARP and intracellular N-terminal processing (removing the GFP-N-terminal peptide) prior to membrane localization. The potential utility of a new surface marker for the characterization and therapeutic manipulation of human Treg cells in health and disease would be extremely beneficial.

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Oncostatin M receptor-beta mutations underlie familial primary localized cutaneous amyloidosis

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Familial primary localized cutaneous amyloidosis (FPLCA) is an autosomal-dominant disorder associated with chronic skin itching and deposition of epidermal keratin filament-associated amyloid material in the dermis. FPLCA has been mapped to 5p13.1–q11.2, and by candidate gene analysis, we identified heterozygous missense mutations in the OSMR gene, encoding oncostatin M-specific receptor β (OSMRβ), in two multi-generational families with FPLCA. OSMRβ is a component of the oncostatin M (OSM) type II receptor and the interleukin (IL)-31 receptor and in cultured FPLCA keratinocytes we observed reduced activation of Jak/STAT, MAPK, and PI3K/Akt pathways after OSM or IL-31 cytokine stimulation but not after IL-6. The pathogenic amino acid substitutions in OSMRβ (p.G618A, P.I691T) are located within the extracellular fibronectin type III-like (FNIII) domains, regions critical for receptor dimerization and function. OSM and IL-31 signaling have been implicated in keratinocyte cell proliferation, differentiation, apoptosis, and inflammation, but our OSMRβ data in individuals with FPLCA represent the first human germline mutations in this cytokine receptor complex and provide new insight into mechanisms of skin itching.

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Hailey-Hailey and Darier's diseases as models to study tight junctions

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In normal epidermis, tight junctions are located in the granular cell layer. Aberrations in the epidermal differentiation, such as acanthosis in psoriasis and hyperproliferation of the wound edge are characterized with upregulation of tight junction proteins ZO1 and occludin. We have now investigated the localization of tight junction components claudins-1 and -4, ZO1 and occludin in frozen and paraffin-embedded skin of 6 cases Hailey-Hailey and 7 cases of Darier's diseases using indirect immunofluorescence and avidin-biotin immunolabeling. The results showed that the localization of tight junction proteins remained normal both in the lesional and perilesional epidermis in both diseases. Healing of the blisters could not be shown to induce ZO1 or occludin in the bottom of the blister. This was in contrast to previous results on normal skin in which migrating keratinocytes on the wound bed express ZO1 and occludin. Furthermore, the acanthosis of Darier's epidermis was not associated with spreading of tight junctions to the spinous cell layers. Acantholytic cells are known to show dissociation of intra- and extracellular domains of desmosomal cadherins and E-cadherin in Hailey-Hailey and Darier's disease. In contrast to desmosomal proteins, claudin-1 and occludin remained in the plasma membrane. Even the intracellular plaque protein ZO1 was present in the persisting intercellular contacts of the acantholytic cells. To conclude, our results suggest that Hailey-Hailey and Darier's diseases follow distinct regulation of tight junction components compared to acanthosis in psoriasis and healing blister of normal epidermis. These findings may be related to the inadequate response of mutated keratinocytes to the extracellular Ca2+ concentration. Claudin-1, ZO1 and occludin also have a different dynamics in acantholysis compared to desmosomal and adherens junction proteins. The results also emphasize the regulatory role of calcium in the dynamics of tight junctions.

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R501X, 2282del4 and R2447X founder mutations in the filaggrin gene in European ichthyosis vulgaris patients

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The molecular basis of ichthyosis vulgaris (IV), the most common hereditary disorder of cornification in humans, has been ascribed to loss-of-function mutations in the gene encoding the protein filaggrin (FLG), which is important for the formation of the stratum corneum during epidermal differentiation. Here we genotyped 38 European IV patients from Austria, Germany, the Netherlands, Belgium and Scotland homozygous, compound heterozygous or heterozygous for the most common European FLG mutations p.R501X, c.2282del4 and p.R2447X as well as normal controls for eight different single nucleotide polymorphisms (SNPs). We chose three SNPs mapping within a 54 kb region in exons 1 and 3 of FLG, two flanking the gene up to 16 kb in centromeric and three up to 15 kb in telomeric direction. Significant differences were evident between the genotypes of IV patients and normal controls with nearly complete association of the SNP alleles with the mutations carried on conserved haplotypes, indicating that the high frequency of these FLG mutations is due to a founder effect rather than resulting from mutational hot spots. Our results support the idea, that the high prevalence of FLG mutations in European populations could be the result of an advantage for heterozygous carriers. Together with data from FLG analyses in other geographic regions it is suggested that multiple independent founder mutations may have occurred worldwide.

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Association between polymorphisms in promoter region of the FcεRIα gene and atopic dermatitis

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FcεRI, the high affinity receptor for IgE, plays a key role in IgE-mediated allergic reaction. The α-chain, which is one of three subunits of FcεRI, is essential for expression and function of FcεRI. Recently, a single nucleotide polymorphism (SNP) in the α-chain promoter, -315C>T, has been shown by statistic studies to associate with allergic diseases, including asthma and urticaria, whereas there is no report regarding this SNP in atopic dermatitis (AD). In the present study, we analyzed the allelic frequency of -315 SNP in Japanese population and found the association between this SNP and serum IgE level in AD patients. AD patients with lower serum IgE levels exhibit higher frequency of C/C genotype in compared with AD carrying higher IgE. In addition, we confirmed the linkage between the -315 SNP and another SNP at -66 in α-chain gene, because the relationship between these two SNPs was controversial in the previous reports. This is the first report analyzing -315 SNP in AD patients and in Japanese population and showing positive relationship to serum IgE level in AD.

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A gene localized at epidermal differentiation complex (EDC) on human (1q21) and mouse chromosome

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High-throughput in situ hybridization screening of gene is a useful method for identifying genes specifically expressed in tissues. A stratified epithelium-secreted protein gene complex (SSC) was discovered by means of this method (Matsui-T et al., Genomics 84, 384-397, 2004). A skin-specific protease identified by this method was involved in wrinkle formation in mice (Matsui-T et al., J Biol Chem 281, 27512-27525, 2006). Herein, we report a gene (G3) localizing at EDC (between involucrin and sprr4) identified by this method. In situ hybridization detected expression of G3 in granular layer of mouse epidermis. A part of exon of human homolog of G3 was complementary to another gene (Unigene: Hs.87271). Mouse tissue RT-PCR showed expression of G3 in skin, but not in liver. RT-PCR in normal human cultured keratinocytes (NHEK) showed that expression was induced by elevation of extracellular calcium concentration. Exogeneous expression of tentative ORF of G3 with HA-tag showed nuclear localization in NHEK.

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Combination of short CAG and GGN repeats in the androgen receptor gene are associated with acne risk in north east China

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Acne vulgaris is one of the most common skin disorders, and androgen is known to play a key role in the development of acne. To investigate whether CAG and GGN polymorphism of the androgen receptor (AR) gene are associated with acne risk, a case-control study was conducted in 238 patients and 207 controls in North East China. The repeat lengths of the AR gene determined by GeneScan analysis, revealed that men with CAG<23 and women with CAG<24 had significant risk compared to those men with CAG≥23 (OR 2.07 at 95% CI-1.21-3.54) and women with CAG≥24 (OR 2.05 at 95% CI-1.18-3.56). In male GGN repeats, considered independently of the CAG repeat, have no significant effect on the acne risk; however, when combined with CAG repeats, the acne patients exhibited significantly higher frequency of the haplotypes CAG<23/GGN≤23 (OR 3.33 at 95% CI-1.10-10.07, $P < 0.05$) compared to the controls. To our best knowledge, this is the first study to evaluate the association between CAG/GGN repeat polymorphism and the relative risk of acne in North East China. Our results of this study strongly indicated that a shorter CAG repeat length and specific haplotypes of androgen receptor attributed to the risk of acne development and thus could serve as a susceptibility marker.

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Prediction and evaluation of protein-protein interaction during keratinocyte differentiation

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Terminal differentiation of skin keratinocytes is a vertically directed multi-step process that is tightly controlled by the sequential expression of a variety of genes. We previously investigated the gene expression profile using normalized cDNA library and found that many of differentiation-related genes expressed in a temporally-regulated manner. In this study, we attempted to find the hub-molecules and their intracellular signaling networks during keratinocyte differentiation, using in silico analysis of data obtained from normalized cDNA library. We used a protein-protein interaction network prediction software called PSIMAP, and identify several candidate hubmolecules. One of those, Shc1, was predicted to link EGFR and MAPK signal. Shc1 is expressed in various tissues except the brain or neurons. It has been known that Shc1 gene encoded two mRNA species, that is p66Shc and p46/p52Shc. Because of alternative translation start sites, p46Shc and p52Shc are derived from the same mRNA. Each Shc1 isoforms harbors three identical functional domains including N-terminal phosphotyrosine-binding domain (PTB), a central proline-rich domain (CH1), and a carboxy terminal Src homology 2 (SH2) domain. p66Shc differs from p46Shc or p52Shc by an additional N-terminal proline-rich domain (CH2). To elucidate the protein-protein interactions between p66Shc and MAPK, we overexpressed MAP2K1 in keratinocytes and performed immunoprecipitation. As anticipated Shc1 bound to the MAP2K1 in an EGF-regulated manner. These results demonstrate that in silico protein-protein interaction prediction system can be used to efficiently and cost-effectively select experimental candidates.

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Cell therapy restores skin function in an immunocompetent mouse model for dystrophic epidermolysis bullosa

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So far no therapy is available and in the severe forms scarring, mitten deformities of the extremities and skin cancer have a significant impact on the quality of life and reduce life expectancy. We recently generated an immunocompetent mouse model, the collagen VII hypomorph, which expresses 10% of normal collagen VII and displays the key symptoms of severe RDEB: mucocutaneous blistering, scarring, pseudosyndactyly and growth retardation. As there are concerns about safety and feasibility of gene therapy approaches, we investigated the efficacy of fibroblast therapy in this model. Five week-old hypomorphic mice (n = 21) were injected twice with low passage allogeneic wildtype fibroblasts (20 million cells) into a dorsal 2 x 3 cm skin area, with a 7 day interval between injections. The efficacy was evaluated after 1 - 21 days using RNA, protein and functional assays. After 7 days, both collagen VII mRNA and protein were significantly increased at the dermal-epidermal junction zone (DEJZ), as assessed by RT-PCR and semiquantitative confocal immunofluorescence microscopy, and persisted for at least 21 days. Staining of CD11b revealed a moderate inflammatory response, yet this did not impair deposition and function of collagen VII. No evidence of an antibody response to the DEJZ was found with direct / indirect immunofluorescence. Importantly, microblisters, regularly present in untreated skin of the hypomorph, were absent in the treated areas, even after mechanical stress. These observations clearly demonstrate that exogenous fibroblast-derived collagen VII is able to stabilize RDEB skin. In summary, treatment with allogeneic fibroblasts may become a clinically relevant regimen to prevent the detrimental sequence of blistering, scarring, mutilating deformities and, finally, skin cancer in RDEB.

*The first two authors contributed equally to this work Recessive dystrophic epidermolysis bullosa (RDEB) is a skin fragility disorder caused by COL7A1 mutations.

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Stimulation of DNA excision repair enzyme on Caucasian and Asian human fibroblasts studied by microarray technology.

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DNA repair capacities of cutaneous cells have been demonstrated to decrease with aging, consequently beside DNA protection, stimulation of DNA repair is a key strategy to prevent aging. Due to the lack of technology available to measure cleavage activities of DNA repair enzymes, Sauvaigo et al., has developed a specific miniaturized microarray approach, based on OLISA technology, with a set of oligonucleotides that contained different lesions. The modified oligonucleotides were hybridized onto DNA probes on a circular format within each well of a 96-wells microtiter plate to form a mini double helix. The approach was addressed to be characteristic of DNA repair pathways induced by an oxidative stress and specific DNA lesions were incorporated on double-stranded oligonucleotide probes such as ethenodeoxyadenine, 8-oxo-deoxyguanine, inosine, formylamine, uracil and hydroxymethyldeoxyuridine. In order to study the effect on DNA repair of two compounds known for their protective activities, normal cutaneous human fibroblasts from Caucasian and Asian women were treated with ascorbic acid 2-glucoside (50mM) or ergothioneine (4 µM). After a 24h treatment, we observed that all modified base excisions were induced except ethenodeoxyadenine and hydroxymethyldeoxyuridine. These results indicated the activation of different and specific DNA glycosylase repair enzymes. We observed that ascorbic acid 2-glucoside induced a homogenous enzyme stimulation profile for caucasian and asian donors and an heterogeneous but strongest effect with ergothioneine according to the cells tested. In conclusion, using a specific DNA repair biochip with caucasian and asian fibroblasts, we demonstrated for the first time that both ascorbic acid 2-glucoside and ergothioneine stimulate DNA glycosylase repair enzymes. These results indicate that these two compounds could be of interest in an anti-aging strategy based on DNA repair.

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Kindlin-1 regulates epidermal keratinocyte proliferation and migration through integrin and EGF mediated signalling pathways

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Kindlin-1 is a focal adhesion phosphoprotein mainly expressed in epidermal keratinocytes. Mutations in the kindlin-1 gene, KIND1, cause the Kindler syndrome (KS), a human disorder characterized by skin blistering, progressive poikiloderma and photosensitivity. Our previous studies had indicated that lack of kindlin-1 resulted in deficient proliferation, polarisation, adhesion, and migration of epidermal keratinocytes. Here, we extended the biochemical characterization of kindlin-1 in keratinocytes. Co-immunoprecipitation studies performed on normal human keratinocytes showed that kindlin-1 forms molecular complexes with the β1 integrin subunit, migfilin, α-actinin and focal adhesion kinase. Of interest, we demonstrated that kindlin-1 phosphorylation is induced by epidermal growth factor (EGF), but not by other growth factors, like KGF, IGF-1 or PDGF. These results suggested that kindlin-1 is involved in integrin and EGF mediated signal pathways. To assess this further, we used a genetic approach comparing the activity of Rho GTPases and MAP kinases in kindlin-1 negative KS keratinocytes and normal human keratinocytes. Kindlin-1 deficiency led to reduced activity of the Rho family GTPase, Cdc42, a key regulator of cell polarity and directional migration. Accordingly, the phosphorylation of the downstream effector PAK1 and of cofilin was reduced. This results in increased cofilin activity and enhanced activation of actin polymerisation in lamellipodia and filopodia, thus providing a further link between kindlin-1 and the actin cytoskeleton. Moreover, kindlin-1 deficiency led to reduced phosphorylation of the MAP kinases, Erk1 and Erk2, which are activated by EGF mediated signals and involved in cell proliferation and survival. Taken together, this study suggests that kindlin-1 deficiency affects important cellular processes by interference with β1 integrin and EGF signalling pathways.

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Adhesive stripping to remove epidermis in junctional epidermolysis bullosa (JEB) for revertant cell therapy

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Replacing mutant skin in epidermolysis bullosa by epithelial sheets of transduced autologous keratinocytes is the essential surgical step of *ex-vivo* gene therapy. The same applies for revertant cell therapy in which epithelial sheets of revertant autologous keratinocytes are used. Revertant cells can be found in patches of normal skin in JEB patients due to revertant mosaicism. We performed revertant cell therapy on mosaic type XVII collagen-deficient JEB patient 026-01 (AJHG 2005; 77:727-40). Skin biopsies were taken from revertant skin on the wrist (50% revertant cells). Graft production took place on 3T3-J2 feeder layer resulting in two 6x7 cm grafts. One day before procedure the acceptor site (12x7 cm, upper leg) was degreased and a polyacrylate adhesive plaster (Fixomull stretch, Beiersdorf) was placed. During transplantation ultra-superficial incisions were made around the edge of the plaster with a scalpel. The epidermis was easily lifted by removing the plaster which separated the skin at the level of the lamina lucida leaving naked, bloodless lamina densa. The grafts were placed face down, covered by a soft silicone faced polyurethane wound contact layer (Mepilex transfer, Mölnlycke) and non-adherent absorbing compresses, fixed by bandages. This procedure was well tolerated by the patient. There was no need for local or systemic anaesthesia. After 7 days dressings were removed, the graft was well attached to the acceptor site, which was still slightly erythematous; at inspection a fragile stratified epithelium without blisters was seen. After 4 months the transplantation area showed an intact epithelium with no blisters and no scarring. However blistering could be provoked in the transplantation area; functional repair was not achieved due to an unexpected low percentage of revertant cells (<3%) in the final epithelial grafts. We conclude that adhesive stripping is a simple, effective and painless procedure to remove epidermis for cell therapy in epidermolysis bullosa.

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Genetic and functional mechanisms in Harlequin Ichthyosis

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Harlequin ichthyosis (HI) is a severe and often lethal form of recessive congenital ichthyosis. HI neonates have hard, thick skin covering most of their body with impaired barrier function leading to water loss, poor temperature regulation and increased susceptibility to infection. In 2005, we and another independent study demonstrated that mutations in the ABCA12 gene were a cause of HI. We have now screened a large cohort of > 60 unrelated HI patients, all harbouring identifiable ABCA12 mutations, mainly nonsense substitution or frameshift mutations, although more complex exon deletions and duplications do occur. Strategies to identify these complex mutations will be discussed in particular with regard to prenatal diagnosis. These data firmly establishes ABCA12 as the major HI gene. ABCA12 is a member of the adenosine triphosphate (ATP)-binding cassette (ABC) superfamily of active transporters that act as lipid trafficking molecules. As predicted from our genetic studies, immunohistochemical analysis of HI skin biopsies revealed a loss of ABCA12 expression. Additional staining with antibodies raised against proteins expressed in late differentiation demonstrates that the terminal differentiation pattern in HI skin is highly dysregulated inducing premature differentiation. This could explain, in part, the abnormal lipid content of HI skin with lamellar granule formation in the lower epidermal layers being bypassed. Finally, we have generated organotypic co-cultures in which retrovirally expressed shRNA ablated the expression of ABCA12. These skin models replicate the above features of HI skin and provide a valuable model to dissect the mechanisms underlying HI including global gene expression analysis and lipid profiling.

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Reproducible and stable transgene expression towards *ex vivo* lentiviral gene therapy for Netherton Syndrome

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Netherton Syndrome (NS) is a rare but severe autosomal recessive skin disorder caused by mutations in the *SPINK5* gene, resulting in loss of activity of encoding protein Lympho-epithelial Kazal-type-related inhibitor (LEKTI). Prognosis of NS is poor with 10% mortality in the first year of life. To date there are no specific treatments and thus an urgent need to develop substantive and durable therapies. Recent reporting of successful gene therapy for Epidermolysis Bullosa has demonstrated the efficacy of using a retroviral long terminal repeat (LTR) promoter to drive prolonged, high level expression of LAM5 protein in keratinocyte cells(1). We are aiming to develop strategies for the ex vivo correction of NS based on the lentiviral delivery of *SPINK5* cDNA into primary keratinocytes. Lentiviral vector delivering *SPINK5* cDNA linked to GFP reporter gene was constructed under control of spleen focus forming virus (SFFV) LTR promoter. Long term reproducibility and stability of expression from the transduced *SPINK5* gene was assessed in keratinocytes by FACS and immunoblot. Transduced normal and NS primary keratinocytes were cultured to terminal differentiation in organotypic culture (OTC) to examine restoration of LEKTI protein. Using lentiviral vector with SFFV promoter we achieved high transduction of 100% in keratinocyte cell line and 30% in primary keratinocytes. Primary NS keratinocytes transduced with SPINK5 transgene showed increased differentiation. However, we found a declined GFP intensity of 4-6 fold over 40 days in *SPINK5*-transduced cells suggesting SFFV promoter may be prone to transgene 'silencing'. To achieve stable, long term transgene expression we used UCOE element that is able to negate transgene silencing through effects on surrounding chromatin. These studies represent the first of their kind in the treatment of NS by gene therapy. 1. Mavilio F et al. *Nature Medicine* 2006; 12; 1397-1402

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Identification of two mutations in Chinese patients with diffuse epidermolytic palmoplantar keratoderma

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Epidermolytic Palmoplantar Keratoderma (EPPK OMIM 144200) is an autosomal dominantly inhereted disease. It was first described by Vorner in 1901. It manifests as hyperkeratosis confined to the palms and soles, often presenting with a yellowish hue. There is usually a distinct erythematous margin at the border of the normal appearing skin. EPPK usually appears 3 to 12 months after birth. It was first mapped to chromosome 17q11-q23 by Reis and then many missense mutations were identified in a number of EPPK families. To date, a total of 18 kinds of mutations in kertain 9 (KRT9) have been found in EPPK patients. Most of these mutations are missense mutations occurring in the highly conserved coil 1A domain of the KRT9. A mutation in a different region of the gene has been reported in one studied case only. Here, we performed mutations detection of the KRT9 gene in two Chinese families with EPPK. A C→T mutation was found at nucleotide 484 that changes codon 162 from arginine(CGG) to tryptophane (TGG). Sequencing of PCR products from the other four affected family members showed the same mutation but not in the healthy family members from family 1. In the proband of family 2, an A→G mutation was found at nucleotide 479 that changes codon 160 from asparagine to serine. This missense mutation was confirmed in the other patients and excluded in the remaining unaffected persons in family 2.

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Mutation in sterol O-acyltransferase 1 (Soat1) results in hair medulla defects in (AKR/J) mice

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The AKR/J inbred mouse strain all have the hair interior defect mutation (hid) and adrenocortical lipid depletion (ald, Synonyms: Soat1, Acact and ACAT-1). The objectives of this project were analyzing the phenotype of hair interior defect, mapping and identifying its responsible mutation gene. We investigated the HID phenotype by SEM, TEM, and Histopathology. The medullary cells were large, elongated, and sometimes round. They had an irregular arrangement, shape, and lacked compactness with various degrees of severity compared to closely related inbred strain without this mutation. SEM revealed no abnormal changes of the cuticle or diameter along the hair fibers and the sulfur content were in the normal 2-4% range. Five crosses (AKR/J with BALB/cBy, C57Bl/6J, CAST/Ei, FVB/NJ and C3H/HeJ separately) were created. Genome wide screens of 1650 F2 hybrids narrowed the interval containing the hid locals to 0.73Mb region between 157724kb and 158446kb on chromosome 1. All 9 genes within this interval were sequenced. Soat1 had a 6.8 kb deletion in local genome which resulted in a splicing mistake missing exon 2 (118bp) in the Soat1 ORF. QPCR and Western-blot showed the Soat1 was normally expressed in the skin. How Soat1 results in hid still keeps unknown.

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Testing human alopecia areata candidate genes in the C3H/HeJ mouse model system

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Alopecia areata is an autoimmune disease affecting actively growing anagen stage hair follicles. The disease is relatively common in people and varies in severity as well as association with other autoimmune disorders. From human AA gene association studies, polymorphisms in several genes may be important for susceptibility and/or severity. In the last 5 years, 8 genes (AIRE, CRH, FCGR1, FLG, NOTCH4, POMC, PSIP1, and PTPN22) were proposed to be important in the pathogenesis of human alopecia areata. We found that only Fcgr1, Flg, and Ptpn22 were significantly dysregulated in the C3H/HeJ mouse skin graft or spontaneous alopecia areata models based on longitudinal Affymetrix gene array or large scale quantitative real time RT PCR immune response arrays. Targeted mutations for 2 of these (Airetm1.1Doi and Notch4tm1Grid) were transferred to the C3H/HeJ background (N10) thus confirming the gene expression study. Neither congenic strain had any change in frequency or severity of alopecia areata from the parental C3H/HeJ strain. Crosses with the flaky tail (ft) mutant mouse, known to have defects in filaggrin (flg) expression suspected of being associated with atopic dermatitis and severe forms of alopecia areata, are in progress. The gene expression and transferring specific mutations in individual genes onto the alopecia areata susceptible background approach indicate that some of the previously suggested human candidate genes do not increase the incidence or severity of alopecia areata in the C3H/HeJ mouse model that most closely resembles adult human alopecia areata. Other candidate genes involved in the pathogenesis of both human alopecia areata and the C3H/HeJ mouse model need to be tested.

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A murine inducible transgenic model of psoriasis

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Psoriasis is a chronic inflammatory skin disease with a significant genetic component, involving a major susceptibility locus within the MHC on chromosome 6p. Within this locus corneodesmosin (CDSN) and HLA-C are the most plausible candidate susceptibility genes for psoriasis. Although strongly associated with psoriasis, HLA-C has no known functional role in the disease. CDSN is an epidermal glycoprotein, is over-expressed in psoriasis and has a role in epidermal adhesion. The TET-ON system of doxycycline (DOX) dependant gene expression was used to overexpress transgenic CDSN in a mouse model. This system used mice carrying two transgenic constructs (bi-transgenic), a tissue specific transcription activator construct and a DOX responsive human CDSN gene construct. DOX in the mouse diet was used to induce gene expression. Bi-transgenic mice and single transgenic controls were screened for histological and phenotypic changes in response to CDSN expression by H+E staining, immunostaining and immuno EM. Altered immune responses were assessed by antigen and irritant challenge, tape stripping and TEWL measurement. Gene expression patterns were compared by microarray analysis. DOX induced mice strongly expressed transgenic CDSN in the suprabasal epidermis. Immuno EM identified CDSN localisation within keratinosomes and desmosomes, as seen for psoriasis. No specific responses to antigen, irritant, wounding or barrier disruption were observed in response to transgenic CDSN. The Zmynd19 gene was upregulated in microarrays of CDSN bi-transgenics however no differential expression of this gene was seen in psoriatic skin. Our results suggest CDSN over expression alone does not induce a psoriatic phenotype in mice. However, psoriasis is a polygenic disease with considerable evidence of a role for HLA-C. Thus a combination of CDSN, HLA-C and other genes may be involved in the pathogenesis of psoriasis. Our mouse model should provide a useful tool for future studies of psoriasis susceptibility.

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Silencing of ALOX12B by siRNA in reconstructed epidermis, mimicking recessive congenital ichthyosis, results in an altered epidermal phenotype

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Autosomal recessive congenital ichthyosis (ARCI), a group of diseases characterized by compensatory (?) hyperkeratosis and generalized scaling are caused by defects in the cornified cell envelope or by errors in the epidermal lipid metabolism. The most common etiologies are: (i) inactivating mutations in TGM1, encoding transglutaminase-1, which result in a deficient cross-linking of envelope proteins, (ii) mutations in ALOX12B and ALOXE3, encoding two lipoxygenases in the hepoxilin pathway, and (iii) mutations in ichthyin, a trans-membrane protein with yet unknown function. A straightforward method to mimic autosomal recessive congenital ichthyosis by single gene knockdown in an organotypic epidermis model is presented. Human keratinocytes were transfected with short interfering RNAs (siRNAs) specific for 12-R-lipoxygenase (ALOX12B). Twenty-four hours later the transfected cells were seeded onto cell culture inserts and allowed to build up a multilayered epidermis by culture at the air/medium interface for up to 14 days. The knock-down was confirmed by QRT-PCR and immunofluorescence staining. The mRNA expression of the targeted gene remained down-regulated by more than 50% up to 14 days after transfection. Another lipoxygenase in the same pathway, ALOXE3, was not affected. As expected, ALOX12B knockdown by siRNA altered epidermal histology and barrier formation in the organotypic skin model. It led to aberrant keratinocyte differentiation and resulted in an epidermal phenotype closely resembling that of ARCI. Our results suggest that siRNA-mediated gene silencing is highly efficient in an organotypic skin model and allows the assessment of the roles of individual genes during terminal keratinocyte differentiation.

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A novel 22-bp deletion mutation in a Chinese family with x-linked hypohidrotic ectodermal dysplasia

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The objective of this study is to identify gene mutations of ED1 gene in patients with X-linked hypohidrotic ectodermal dysplasia (XLHED). Hypohidrotic ectodermal dysplasia (HED) is found worldwide with an estimated incidence of 1 per 100,000 births. It can be inherited in autosomal dominant, autosomal recessive, or X-linked patterns. X-linked hypohidrotic ectodermal dysplasia is the most common form of over 170 different ectodermal dysplasias. It is characterized by an abnormal development of eccrine sweat glands, sparse hair, abnormal or missing teeth. The facial appearance consists of a saddle-nose, frontal bossing and thick lips. Hypohidrosis or anhidrosis may cause life threatening and brain-damaging episodes of hyperthermia. Heterozygous carriers of XLHED may show minor to moderate degrees of these typical features. About 30% do not present obvious clinical manifestations, rendering accurate diagnosis of carrier status in females difficult. The gene (ED1) for XLHED, maps to Xq12-q13, has been identified. Here, we performed mutation detection of the ED1 gene in a Chinese family with XLHED. Direct DNA sequencing showed a novel 22-bp deletion mutation (nt1117–1138) in exon 8 of ED1 resulting in a frameshift and premature stop codon (PTC + 9 amino acids). It was found in the affected members but not in the healthy individuals in this family and in 100 unrelated controls. We add new variants to the knowledge of ED1 mutations in XLHED.

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Transglutamiase-1 and bathing suit ichthyosis: molecular analysis of gene/environment interactions

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Bathing suit ichthyosis (BSI) is described as a striking and unique clinical form of autosomal recessive congenital ichthyosis (ARCI). Affected individuals are born with collodion membrane encasing the entire body and develop a dark-grey or brownish scaling restricted to the bathing suit areas, whereas extremities and central face are almost completely spared. Recently, in a group of ten individuals of independent families we identified new mutations in the TGM1 gene which differ from those which can be found in patients with generalized lamellar ichthyosis (LI). Previous clinical analysis, atomic modelling and immunohistochemical studies suggest a temperature depending activity of the identified TGM1 mutations in BSI patients. We expressed eleven different mutations in HEK 293 cells. Eight were only identified in BSI patients, three had previously been found in patients with generalized LI due to transglutaminase-1 deficiency. Wildtype TGase-1 served as a control. To analyse TGase-1 activity we performed a fluorimetric assay at different temperatures ranging from 25°C to 45°C. The data show that the recombinant proteins containing BSI-mutations were less active than the wildtype protein and showed a shift in temperature-optimum from 37°C/39°C to 31°C/33°C. We suggest that TGase-1 proteins containing BSI-mutations in comparison to the wildtype TGase-1 show less thermal stability. To further elucidate the mechanism of temperature sensitivity four selected mutations and the wildtype protein were expressed in HEK 293 cells, purified by Ni-NTA-affinity-chromatography and used for circulardicroism (CD)-spectroscopy. The data of the heating experiments confirm the supposed temperature sensitivity and show lower melting points in the cases of the BSI mutations in comparison to the wildtype.

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Connexins: non gap junction functions?

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The traditional role of connexins are to form gap junction channels, mediating cell-cell communication which plays a key role in keratinocyte biology. Mutations in four connexins are associated with a number of skin diseases / syndromes. The discovery that distinct mutations in connexin 31 (Cx31) underlie different disease phenotypes such as the skin disease erythrokeratoderma variabilis, hearing loss and/or neuropathy have allowed mechanistic insights into the function of Cx31 in keratinocytes and other cell types. Recently, we have shown a role for Cx31 in neuronal differentiation. Overexpression of (WT)Cx31 induced a differentiation phenotype characterised by increased neurite outgrowth, whereas the overexpression of the (66delD)Cx31 neuropathy mutant decreased the level of neuronal differentiation, but does not affect keratinocyte differentiation. In contrast, the skin mutants R42P and C86S induced neurite outgrowth to the same degree as (WT)Cx31, despite these mutants having impaired trafficking to the plasma membrane. In addition, the (WT)Cx31 induction of neuronal differentiation did not correlate with an increase in intercellular communication. These data reveal a non-gap junction function for Cx31. To further explore this, we have performed detailed microscopy of the intracellular localisation of these Cx31 mutants. Although all mutants are visible on punctuate structures (suggesting they remain membrane-bound), the precise sub-cellular localisation differs: the neuropathy mutant 66delD is present on small, diffuse structures throughout the cell, compared to the R42P and C86S skin mutant's localisation on large bright structures. Additionally, to understand the mechanism behind the mistrafficking of the mutants we have configured a whole-genome siRNA screen to identify proteins that mediate the delivery of Cx31 to the plasma membrane. Finally, in order to further understand the protein-protein interactions of the cytoplasmic connexin pool, we have identified a number of Cx31-interacting partner proteins using a yeast-2-hybrid screen.

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A xenograft model replicates features of skin tumors in tuberous sclerosis complex

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Patients with tuberous sclerosis complex (TSC) develop multiple skin tumors due to a germline mutation in the tumor suppressor gene *TSC1* or *TSC2*. Our goal was to develop an experimental model of TSC skin tumors that would allow studies of tumor microenvironment and potential medical therapies. Previously, we showed that fibroblast-like cells grown from angiofibromas (AF), periungual fibromas (PF), and forehead plaques (FP) from TSC patients contain "second-hit" mutations in *TSC2* that result in undetectable TSC2 protein and constitutive activation of the mTOR signaling pathway. To create an animal model of TSC skin tumors, cells from TSC tumors or paired fibroblasts from patient normal-appearing skin (NL) were incorporated into collagen gels, the surface was overlaid with normal human neonatal keratinocytes, and these constructs were grafted to the backs of nude mice. To date, 141 mice have been grafted using cells from 4 AF, 3 PF, 2 FP, and 6 NL from 7 TSC patients. To confirm persistence of human cells, sections were stained for human leukocyte antigen (HLA) using a pan-human HLA class I antibody. In our longest experiment, fibroblasts (and epidermal cells) were positive for HLA in 14/23 grafts, 16 weeks after grafting, but ungrafted mouse skin was negative. Grafts containing tumor cells showed increased activation of the mTOR signaling pathway compared to normal cells, as shown by staining for phosphorylated ribosomal protein S6. Furthermore, DNA extracted from laser-microdissected dermis of tumor cells, but not normal cells, showed "second-hit" point mutations in *TSC2*. Histologically, grafts containing tumor cells showed many features of TSC skin tumors, including increased cellularity in the dermis with stellate fibroblast-like cells, increased vessels immunoreactive with anti-CD31, and increased mononuclear phagocytes immunoreactive with anti-F4/80. This model replicates many features of TSC skin tumors and may be useful for preclinical drug testing.

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Time coursed expression studies define the progressive development of the chronic proliferative dermatitis phenotype in mice

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We recently determined that the chronic proliferative dermatitis mutation (cpdm) was due to a null mutation in the Sharpin gene. Little is known about the function of this gene in the skin or immune system. To better elucidate the pathogenesis by which the spontaneous Sharpin mutation leads to the immune system failure accompanied by skin inflammation, we analyzed time course transcriptional responses and histological changes with the whole skin of 3 female mutant (Sharpincpdm/Sharpincpdm) and 3 female control (+/+) mice at 2, 4, 6, 8, and 10 weeks of age using Affymetrix gene arrays. Multiple time-point data were analyzed together with Ingenuity® software and the potential pathways or networks were suggested. We found that marked epidermal hyperplasia developed at 4 weeks of age concurrent with increases in dermal inflammation, marked neovascularization, and keratinocyte apoptosis. These changes stabilized by 8 weeks of age. Most mice were euthanized by 10 weeks of age due to formation of cutaneous ulcers. Our cDNA microarray analyses revealed a total of 528 differentially expressed genes (>1.5-fold change, $p<0.01$). Of these genes, most of the immune-responsive genes were up-regulated in the early phase mutant mice with a concomitant up-regulation of genes involved in inflammation. We also identified some of the genes implicated in cell movement and death, tissue morphology. Our data suggest that there is a complex inter-regulatory genetic network associated with the structural changes that accompany the progression of the immune response and inflammatory reaction in chronic proliferative dermatitis mutant mice.

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Alopecia, Neurological defects and Endocrinopathy (ANE syndrome) caused by absence of RBM28, a critical constituent of the snRNP complex

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Single-gene disorders offer unique opportunities to shed light upon fundamental physiological processes in humans. We investigated a large consanguineous kindred presenting with a rare constellation of clinical signs including alopecia, progressive neurological defects and endocrinopathy (ANE syndrome). In addition, the patients displayed mental retardation of varying severity, facial dysmorphism, gynecomastia, multiple facial pigmented nevi,reticular hyper-pigmentation of the hands and lower limbs, ulnar deviation of hands and lumbar scoliosis. The syndrome was found to be inherited in an autosomal recessive fashion. Using a 250K SNP array, we identified a region of homozygosity shared by all patients on 7q32. Through microsatellite–based fine mapping and candidate gene analysis, we identified a missense mutation in RBM28, encoding a critical component of the spliceosomal snRNP complex. RBM28 protein expression was observed by immunohistochemistry in all differentiated epidermal and follicular layers. The residue affected by the causative mutation is highly conserved across all species examined. In addition, computational modeling revealed that the ANE syndrome-causing mutation is likely to destabilize the RBM28 molecule. Accordingly, the protein was undetectable by Western blotting in patient-derived fibroblast primary cell cultures; proteosome inhibition rescued the protein. In summary, through the deciphering of the genetic basis of a hitherto unreported syndromic form of alopecia, we implicate for the first time a spliceosome-associated protein in hair folliculogenesis.

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Expanded spectrum of phenotypes associated with RASA1 mutations

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Recently, a rare form of hereditary vascular malformation termed capillary malformation - arteriovenous malformation (CM-AVM), was shown to be caused by heterozygous mutations in RASA1, encoding RAS p21 protein activator 1. CM-AVM is characterized by multiple, small CMs associated with either arteriovenous malformation or arteriovenous fistula (AVF). We assessed 2 families with CM-AVM and 2 families with isolated hereditary CMs. CMs in one family were associated with limb enlargement and no evidence of AVM/AVF in the limb, suggestive of Klippel-Trenaunay syndrome (KTS), a condition previously associated with a chromosomal deletion encompassing the RASA1 locus. Isolated hereditary CM was found to map in one large kindred to the RASA1 locus. Direct sequencing of the RASA1 coding sequence revealed 4 novel heterozygous mutations segregating with CM in all families. All 4 mutations were shown to result in premature termination of translation suggesting haplo-insufficiency for RASA1, or to result in aberrant RASA1 mRNA splicing. These data indicate that the spectrum of clinical manifestations due to mutations in RASA1 is wider than previously thought and includes also hereditary CMs not associated with AVM/AVF and possibly KTS-like phenotypes.

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Phenotype of common truncating polymorphisms of profilaggrin in an unselected population

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Truncating polymorphisms of profilaggrin of the types which cause ichthyosis vulgaris and predispose to atopic eczema are common in the population. To investigate whether carriers of such polymorphisms can be identified clinically among unselected subjects, we studied patients presenting for diagnosis of unrelated skin lesions. 291 subjects in 2 age cohorts (192 aged 18-40 and 99 aged 60-75) completed a questionnaire on symptoms of dry skin and were scored for appearance and texture of skin on arms, legs & trunk. DNA samples were analysed for 4 profilaggrin polymorphisms common in this population. 15/285 (5.3%) subjects tested were heterozygous for R501X, 13/286 (4.5 %) for 2282del4, 4/292 (1.4%) for R2477X and 2/211 (0.9%) for S3247X. Compound heterozygotes R501X/R2477X (2) and R2477X/S3247X (1) were identified. In 154 women no difference was seen in reported moderately or very dry skin or use of body moisturisers twice a week or more, but in 137 men, heterozygotes were more likely to report dry skin (odds ratio 8.5; 95%CI 2.4-30), or moisturiser use (OR 12; 3.0-48) compared with men with wild type sequence at all 4 sites tested. 52% of heterozygotes in the younger age group but 100% of older carriers had some clinical evidence of dry skin. These were also present in 34% and 67% respectively of wild type. However, in both age groups, heterozygotes had visible fine scaling or rough texture significantly more often than wild type. Keratosis pilaris was commoner in young carriers but not significantly so. Palmar hyperlinearity and roughness were increased, but this was significant only in the older group. We conclude that carriage of truncating polymorphisms predisposes to signs and symptoms of dry skin, and that these increase with age, but carriage is often not detectable. When present these features are not specific to the polymorphisms studied. Other polymorphisms or factors including variation in profilaggrin expression may contribute to dry skin phenotype.

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Dyschromatosis symmetrica hereditaria: Gene and morphological analyses

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Dyschromatosis symmetrica hereditaria (DSH) is a pigmentary genodermatosis of autosomal dominant inheritance. It is characterized by hyperpigmented and hypopigmented macules on the backs of the hands and feet. We had clarified that the disease is caused by a mutation of the RNA-editing enzyme of adenosine deaminase acting on the RNA 1 gene (*ADAR1*) in 2003. In this study we reported 6 novel pathological mutations in the gene: p.F1091S, p.C893X, p.S581X, p.E498fsX517, p.F1091fsX1092, p.L855fsX856. in patients with the ordinary skin lesions and one known mutation of G1007R in a child DSH rarely associated with neurological disorders. We think that G1007R missense mutation could induce neurological disorders, since the mutation was already reported in another patient with similar neurological symptoms by Tojo et al in 2006. We further investigate histological findings of skin biopsy specimens obtained from hypo- and hyper-pigmented macules and stained with haematoxylin and eosin, Masson-Fontana silver and antibodies to S-100 protein immunohistochemically, furthermore, examined in an electron microscopy. Visual- and electron-microscopical findings are that abundant melanin pigment was deposited all over the basal layer and enlarged melaocytes with long dendrites located in the pigmented lesion have small or immatured melanosomes scattered sparsely in the cytoplasm, but in the adjacent karatinocytes many small melanosomes located in singly dispersed or aggregated. The hypoppigmented area shows little melanin deposition and reduced number of melanocytes in which a lot of degenerative cytoplasmic vacuole formation is observed electron-microscopically. Microscopical informations suggest that the clinical appearance must be developed directly by melanocyte variations which are mainly induced by *ADAR1* gene mutation.

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Clinical and genetic analysis of 4 heterozygous female patients with Fabry disease

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Fabry disease is an X-linked, inherited disorder caused by loss of activity in the lysosomal enzyme alpha-galactosidase A (GLA), which is clinically characterized by angiokeratoma corporis diffusum, and lethal renal, cardiac or cerebrovascular disease. Basically, the majority of affected patients are males, but these cardiovascular, renal and even skin abnormalities have also been observed in some heterozygous, GLA deficient females. Recently, enzyme replacement therapy using recombinant human GLA has been used to treat patients with Fabry disease. Therefore, it is important to make a precise diagnosis in the early stages of Fabry disease for affected males as well as in heterozygous female cases. We report here, the clinical and genetic analysis of 4 heterozygous females and 3 male patients from 5 unrelated Japanese families. None of the 4 heterozygous females tested here had distinctive skin or other abnormalities except for renal dysfunction. As a result, we found 3 novel and 2 previously reported GLA gene mutations. Based on the precise clinical findings and our genetic analysis, genotype-phenotype correlations were made in this group of Fabry disease patients. Consequently, we could divide them into 2 different groups. One group was associated with a mild clinical phenotype (p.R301Q and p.M296I), and the other group with more severe clinical manifestations (p.G171C and c.758delT). Interestingly, this characteristic was also observed not only in these affected female patients but also their male progeny who exhibited various abnormalities not restricted to angiokeratoma corporis diffusum. These data suggest that genotype and phenotype correlations might be found in Fabry disease.

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Congenital erythropoietic porphyria: genotype/phenotype correlation of 27 cases

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Congenital erythropoietic porphyria (CEP) is an autosomal recessive deficiency of uroporphyrinogen III synthase (UROS) enzyme due to UROS gene mutations. Due to its extreme rarity the full extent of the clinical phenotype is unclear making management decisions a challenge. Aims of the study were to comprehensively define CEP phenotypes, assess treatment outcomes and correlate these with UROS genotype. A single investigator assessed all consenting CEP patients by clinical history and examination. Biochemical, haematological parameters and UROS gene were analysed. 27 unrelated patients, aged 7.6–65 years participated (17 UK, 4 France, 4 Switzerland, 2 Germany). C73R was the commonest mutation in Caucasians (8/30 alleles) and L237P in Asians (5/18 alleles). Homozygous C73R, G188W, L237P and heterozygous G225S, T228M, S212P, L237P mutations associated with very low residual UROS enzyme activity correlated with early-onset severe photosensitivity to visible light, photophobia, photo-mutilation, corneal ulcers, blindness, severe haemolytic anaemia and osteoporosis requiring splenectomy, hyper-transfusions and/or bone marrow transplantation (BMT) in 6 cases. However, 2 with homozygous IVS9+4delA, some with homozygous T228M, L237P or heterozygous C73R, G225S, T228M associated with mutations resulting in low-moderate residual UROS enzyme activity demonstrated only mild-moderate photosensitivity and skin fragility. Not all genotypes in CEP showed consistency in phenotypic expression which may be partly explained by variation in patients' photo-protection strategies. This study confirms that all CEP patients require life-long rigorous photo-protection of skin and eyes from visible light. It is apparent that a management algorithm for CEP is now a priority to ensure that those who might benefit from BMT or future gene therapy are appropriately selected for these potentially curative therapies.

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Genetic analysis of markers on the long arm of chromosome 1 in a Chinese hans dataset reveals the association of upper respiratory tract infection triggered psoriasis in patients with IL20*-1723G allele

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Psoriasis is a multifactorial cutaneous disorder including genetic components and manifold environmental influences. In this study, we conducted a genome analysis using 20 microsatellite markers spanning the long arm of chromosome 1 (150-273cM) in 36 Chinese Han families with psoriasis. We detected evidence for linkage at 1q21 (155cM) with a non-parametric linkage score (NPL)=1.74, p=0.03 and 1q32 (220cM) with NPL=1.84, p=0.03 in all families studied. We further detected markers for HAX-1 gene (located in 1q21) and IL-20 gene (located in 1q32) in a case-control study with 247 sporadic patients and 102 healthy controls. We observed that psoriatic patients with minor allele IL20*-1723G occurred in 45.8% of patients, who have history of trigging or exacerbating disease by infection of upper respiratory tract, but only in 32.4% of controls ($X2 = 5.319$, $p = 0.019$). We speculate that trigged by infection, people with the G allele of IL20*-1723G have more susceptibility to psoriasis.

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R298Q gain-of-function mutation in the p63 gene leads to increased expression of JunB and PCNA in ADULT syndrome skin

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ADULT (acro-dermato-ungual-lacrimal-tooth) syndrome is an autosomal dominant ectodermal dysplasia disorder associated with abnormal limb development. Most cases result from missense mutations in arginine 298 of the p63 gene, a key transcription factor in epidermal development and differentiation. In contrast to other missense mutations in this region, which lead to a loss of DNA binding and result in EEC (ectrodactyly-ectodermal dysplasia-clefting) syndrome, arginine 298 substitutions have been shown to increase transactivation activity of the deltaNp63gamma isoform. Thus the downstream consequences of specific missense changes in the DNA-binding region of p63 may help explain the clinical differences between ADULT and EEC syndrome. The aim of this study was to compare expression of several recently identified p63 target genes using cDNA extracted from skin of an individual with ADULT syndrome (pathogenic mutation: p.R298Q) with that seen in the skin of 3 EEC syndrome cases (pathogenic mutations: p.R279S; p.R304Q; p.R307D) and 2 site-matched skin biopsies from control subjects. qRT-PCR was performed in triplicate with an ABI PRISM 7000 Sequence Detection System to assess gene expression for the following proteins: SUFU, Ki67, p63, K14, E-Cadherin, JunB, P-Cadherin, PCNA, Runx1, Eva1, and IKKα. In ADULT skin, we found significantly increased mRNA expression for JunB, a member of the activator protein-1 (AP-1) which antagonizes keratinocyte proliferation and PCNA (proliferating cell nuclear antigen), important for DNA synthesis and repair. There were no significant differences in EEC or control skin for either JunB or PCNA and no other differences in p63 target gene expression were noted in comparing ADULT and EEC skin. This study shows that the gain-of-function mutation p.R298Q in p63 leads to distinct changes in 2 genes important in keratinocyte proliferation and provides further insight into the molecular mechanisms involved in the pathogenesis of p63 ectodermal dysplasia syndromes.

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Severe keratin 5 and 14 gene mutations elicit the down-regulation of cell junction proteins in epidermolysis bullosa simplex cell lines

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In an earlier study we found in a severe epidermolysis bullosa simplex (EBS) patient-derived cell line expressing the K14 R125P mutation over 20 down-regulated genes encoding proteins with a function in cell adhesion. The results were confirmed both on mRNA and protein levels. Also, the use of newly engineered isogenic cell lines expressing either the wild type K14 sequence or the K14 R125P mutation helped exclude this as consequence of a particular genetic background. These results suggested that the severe K14 R125P mutation not only interferes with normal keratin intermediate filament network function, but also causes the down-regulation of a number of cell junction proteins. In the follow up to this study our aim was to test whether this is specific to the K14 R125P mutation only, or may be also true for other keratin gene mutations causative of a severe EBS phenotype. To this end we chose an EBS patient-derived cell line with a severe phenotype that is caused also by another mutant keratin, the K5 E475G mutation. In addition, control isogenic cell lines expressing either the wild type K5 sequence or the K5 E475G mutation were engineered and analyzed as well. Using semi-quantitative Real-Time PCR and Western blotting we confirmed that the K5 E475G mutant had also a number of cell junction genes and proteins down-regulated in comparison to their respective wild type control cells, such as connexin 43, desmoplakin, desmoglein 3 and plakoglobin. This indicates that severely disruptive K5 and K14 mutations may trigger signaling pathways that also regulate the expression of cell adhesion complex components. We hypothesize that the resulting altered stoichiometry of these perhaps augments the degree of cytoskeleton and cell fragility, thus contributing indirectly to the severity of the EBS patient's phenotype.

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Identification of intracellular molecules interacting with the stress-induced, psoriasis susceptibility-related non-coding RNA, PRINS

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Expression of the non-coding RNA gene PRINS (Psoriasis Susceptibility-Related RNA Gene Induced by Stress) first identified and characterized by our work group suggests a role for PRINS in psoriasis susceptibility and in cellular stress response. Our aim was to access the cellular function of PRINS by identifying intracellular molecules interacting with this stress-induced non-coding RNA. For that we performed in vitro experiments with a ribonucleoprotein (RNP) purification kit. As a target template, we used a transcript containing the 19mer sequence that has previously effectively knocked down the expression of PRINS both in HeLa and HaCaT cells. We hypothesized that this region may play an active role in binding other ribonucleic acids and/or proteins. With the help of Matrix-Assisted Laser-Desorption Ionization Time-of-Flight (MALDI-TOF) method we identified two proteins in HaCaT cell lysates that physically interact with PRINS RNA. One of the identified proteins, nucleophosmin is a ubiquitously expressed nucleolar phosphoprotein which shuttles continuously between the nucleus and the cytoplasm. The other PRINS RNA binding protein, GRP94 is a molecular chaperone heat shock protein. Immunohistochemical experiments revealed that there is no difference in the expression of GRP94 protein when compared among healthy, psoriatic uninvolved and involved skin samples, while the expression of nucleophosmin is significantly elevated in the dividing cells of the basal layer. We hypothesize that the non-coding PRINS RNA may be a structural element of a molecular complex playing a role in stress-induced cellular processes and the abnormal functioning of this complex may contribute to the pathogenesis of psoriasis.

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Pathogenesis and unique ultrastructural characteristics of ichthyosis prematurity syndrome (IPS)

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IPS is a rare form of autosomal recessive congenital ichthyosis. Key features are premature delivery (30-32 weeks) and thick caseous desquamating epidermis and respiratory symptoms at the time of birth. A striking finding in IPS is an extremely high number eosinophils and high serum-IgE levels that strongly suggest involvement of IPS gene in the development of atopic disorders. Recently, the IPS gene locus has been mapped to chromosome 9q34.11, which includes 4 candidate genes, but most of these genes have not been functionally characterized and no mutation have been identified so far. Ultrastructurally IPS is characterized by aggregations of membrane-like curved structures in the upper epidermal cells and within horny scales. Nature of these intracellular structures is not yet clear, but apparently formation of these structures in upper epidermal layers will lead to impaired barrier function of the skin. In ongoing project we want to characterize the molecular and cellular mechanisms of pathogenesis in IPS, including barrier dysfunction of the skin and regulation of eosinophil and IgE production in IPS patients. It could be speculated that the skin barrier function is responsible for the increased production of eosinophils and IgE in IPS patients, due to increased exposure of allergens to the immune system. However, the levels of blood eosinophils at birth in IPS patients are extremely high (>50%). This shows that there is reduced regulation of eosinophil production also in utero (before the body is in contact with external allergens), indicating that the protein encoded by IPS gene may have a more direct effect on eosinophil production. Based on these findings we put forward two hypotheses: 1) The IPS gene is involved in the barrier function of the skin, and the increased levels of IgE and eosinophils are secondary to the epithelial barrier defect; 2) the IPS gene is expressed in lymphoid, as well as in epithelial tissues and directly involved in the regulation of eosinophil and IgE production.

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MMP-21 expression is associated with keratinocyte differentiation

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Human matrix metalloproteinase-21 (MMP-21) is the newest member of the MMP family that has been implicated in embryogenesis, tumor progression and apoptosis, being expressed in developing skin and by tumor cells in cutaneous SCC and in melanoma. Also neutrophils, fibroblasts and macrophages can produce MMP-21 in certain skin disorders. In HaCaT cells, MMP-21 is very tightly regulated: among various cytokines, chemical agents and hormones only TGF-β1 and retinoic acid upregulate its mRNA levels in culture. Stable overexpression of MMP-21 in A431 and HEK293 cells does not induce any obvious changes in cell morphology, epithelial-to-mesenchymal transition, apoptosis or proliferation, but promotes invasion through Matrigel. In this work, we have further characterized the biology of MMP-21. In both organotypic and skin explant cultures MMP-21 was detected in superficial, differentiated epidermal layers partly colocalizing with filaggrin and involucrin, agreeing with its expression in suprabasal keratinocytes in various benign skin disorders, such as lichen planus or keratoacanthoma. Affymetrix assay performed on stably transfected A431 cells, showed that 101 genes were upregulated and 32 genes downregulated at least 2-fold. Specific proteases and keratinocyte differentiation markers were found among the induced genes while the basal cell keratin 5 belonged to the downregulated ones. MMP-21 has been proposed to belong to furin-activatable MMPs as it contains a furin cleavage site. In agreement with this, the specific furin inhibitor CMK inhibited the cleavage of secreted MMP-21 in stably transfected A431 cells. In summary, we confirm here that MMP-21 expression in keratinocytes is associated with differentiation and that it is cleaved by furin before it is secreted from the cells.

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The combined silencing of D-type cyclins results in S phase blockade and aberrant mitosis in HaCaT keratinocytes

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Previously we have shown that in HaCaT keratinocytes knocking down cyclin D1 mRNA had no effect on cell proliferation and morphology, indicating that D2 and D3 cyclins could substitute for D1. To address further function(s) and possible redundancy of D-type cyclins in HaCaT cell growth, we silenced D2 and D3 cyclins individually and in combination with each other and D1 cyclin in the cells using RNA interference. Real-time RT-PCR results revealed successful silencing for all D type cyclins. Simple D2 and D3 siRNA transfections had no effect on the morphology and the proliferation of the cells. However, those cells that were nucleofected with two or three D cyclin siRNAs at the same time, formed large multinucleated cells after silencing. The combined silencing of D-type cyclins resulted in S phase arrest and moderate growth suppression, while the expression of the proliferation related α5 integrin and the differentiation related K1/K10 proteins showed no difference compared to control cells. Our data have not revealed the specific functions for different D-type cyclins, rather it showed that in cell cycle regulation these cyclins have functional redundancy. Furthermore, D-type cyclins do not seem essential for G0/G1-S progression in HaCaT keratinocytes, but at least two of them have to function for normal G2/M transition.

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Genomic analysis of the cutaneous skin microflora

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With large-scale DNA sequencing and genomic analysis, we have comprehensively surveyed the cutaneous resident microflora of the antecubital fossa (inner elbow) in five healthy humans. The many layers and structures of the skin serve as elaborate hosts to microbes, including a diversity of commensal and pathogenic bacteria that contribute to both human health and disease. Until recently, knowledge of the skin microflora was limited to cultivation-dependent assays, although it's estimated that less then 1% of bacterial species can be cultivated. Since 16S small-subunit ribosomal (rRNA) genes are universal among prokaryotes and contain species-specific variable regions useful for inferring phylogeny, a genomic analysis of these genes surveys the complex microbial environments in a much less-biased manner. Proteobacteria (genuses Pseudomonas and Janthinobacteria) dominated the skin microflora at all depths of sampling: swab, scrape, and punch biopsy. Furthermore, intrasubject variation is approximately equivalent to intersubject variation when comparing microbial populations, suggesting that there is a baseline cutaneous microbiome. This data is a foundation for our current studies investigating alterations of skin microflora in a disease state, specifically atopic dermatitis. Our long term goal is to elucidate the contribution of the cutaneous microbiome to skin disorders and translate this into novel pharmacological treatments.

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Recessive missense mutation in plectin N-terminus causes late-onset dilating cardiomyopathy and muscular dystrophy in a patient with epidermolysis bullosa simplex

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Mutations in desmosomal proteins lead to a variety of phenotypes affecting skin, skin appendages, and heart. Plectin is a large and versatile cytolinker expressed in many different tissues and binding structures, among which desmosomes. Plectin -/- mice died shortly after birth due to cell degeneration and damage in skin, skeletal and cardiac muscle. In humans nonsense and frameshift mutations in the *PLEC1* gene are associated with loss of plectin expression and subsequent epidermolysis bullosa simplex with muscular dystrophy (EBS-MD), epidermolysis bullosa with (non)lethal pyloric atresia (EB-PA) and an early lethal multi-organ phenotype. A dominant *PLEC1* missense mutation in the rod domain has been reported in epidermolysis bullosa simplex without muscle involvement (EBS-Ogna). We report on the first recessive missense mutation in the N-terminus of plectin which is important for the mutual exclusive binding of actin and β4 integrin. In a compound heterozygous state with a nonsense mutation, this mutation leads to EBS-MD with late-onset dilating cardiomyopathy. The expression of plectin was only slightly diminished in skin, muscle and heart. Markedly disrupted binding structures (hemidesmosomes, desmosomes, intercalated discs, Z-lines) were observed in these tissues (hemidesmosomes, desmosomes, intercalated discs, Z-lines) with diminished and altered expression of plectin binding partners in all these tissues. The range of genes causing dilating cardiomyopathy can be expanded with *PLEC1* and the range of phenotypes caused by plectin mutations can be extended with EBS-MD with dilating cardiomyopathy.

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Nuclear proteins TIA and PTB regulate the alternately-spliced long transcript of lysyl hydroxylase 2 that is increased in scleroderma

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This study examines the mechanism of lysyl hydroxylase 2 (LH2) alternate splicing and its potentially therapeutic regulation in scleroderma. Lysyl hydroxylases catalyze the hydroxylation of specific lysines in collagen. These hydroxylysines are precursors for crosslink formation essential for collagen integrity. LH2 is alternatively spliced; the major form, LH2(long), includes a 63bp exon 13A that is spliced out in the short form. LH2(long) and LH2(short) appear to be specific for hydroxylating lysines in the telopeptide and helical collagen domains, respectively. Since the over-expression of LH2(long) in fibrotic cells has been correlated with increased telopeptide-based pyridinoline crosslinks in the accumulated collagen that characterizes scleroderma, our goal is to determine the factors that regulate its splicing. We report that, in multiple cell lines, the RNA binding proteins: T-cell-restricted Intracellular Antigens (TIA1 and TIAL1) and Polypyrimidine Tract Binding (PTB) proteins play important and opposing roles in the regulation of LH2(long) alternate splicing. Overexpression of TIA1 and TIAL1 led to an increase in the LH2(long):LH2(short) ratio, while the overexpression of PTB led to a decrease in this ratio. Similarly, the knockdown of the TIA proteins led to a fall in the LH2(long):LH2(short) ratio, whereas the knockdown of PTB increased this ratio. In scleroderma fibroblasts, Real Time PCR of PTB revealed a striking reduction in the PTB mRNA in these cells versus normal controls. This may, in part, explain the increased expression of LH2(long) observed in fibrosis. A knockdown of TIA proteins in scleroderma cells by specific siRNAs decreased levels of LH2(long). These results open up the exciting possibility of potential therapy against fibrotic conditions by reducing the overexpression of LH2(long) that characterizes scleroderma and offer at least two potential protein targets for therapeutic intervention in fibrosis.

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Human skin gene therapy can be used to deliver anti-hypertensive atrial natriuretic peptide (ANP) in order to prevent blood pressure elevation in high-salt diet immunocompromised mice

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Skin is a very attractive organ for targeted gene therapy to deliver therapeutic proteins systemically in order to treat cancers, skin diseases or systemic diseases. To achieve long-term stable expression of a therapeutic gene, we have developed an *in vivo* system using a bicistronic retroviral vector expressing the desired therapeutic gene linked to a multi-drug resistant gene (MDR) to create genetically modified human skin equivalents (HSE). After grafting the HSE onto immunocompromised mice, topical colchicine treatment is used to select and enrich for genetically modified keratinocyte stem cells. To validate this approach we have chosen the anti-hypertensive ANP as a therapeutic gene. *In vitro*, HSE expressing ANP/MDR in both keratinocytes (KC) and fibroblasts (FB) demonstrate normal differentiation and stratification histologically. ANP expression and secretion by bioengineered HSE was confirmed by immunohistochemistry labeling and by radio-immuno assay (RIA). RIA shows that bioengineered HSE expressing ANP from KC were able to secrete higher levels of ANP (~40 000 pg/ml/4h) than FB (~2 000 pg/ml/4h) into the culture media, and both levels can be increase by more than 2-fold after colchicine selection. Immunocompromised mice grafted with HSE expressing ANP from KC and/or FB shows significant levels of the human ANP into their bloodstream compared to the control mice. Moreover, mice expressing ANP, an inhibitor of renin-angiotensin-aldosterone pathway, have lower plasma level of renin than control mice. Telemetry monitoring of blood pressure in mice show protection against blood pressure elevation when mice grafted with ANP-expressing HSE are fed high-salt diets compared to the control mice. Taken together, those results suggest that grafting genetically modified HSE could be used to produce and deliver therapeutic molecules as an alternative approach to regular medication.

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Pseudoxanthoma elasticum/cutis laxa-like cutaneous phenotypes and coagulation factor deficiency in patients with mutations in the *GGCX* and *ABCC6* genes – evidence for digenic inheritance

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Pseudoxanthoma elasticum (PXE) and cutis laxa (CL) are clinically and genetically distinct disorders manifesting with loose and sagging skin. A characteristic feature of classic PXE, an autosomal recessive disorder caused by mutations in the *ABCC6* gene, is aberrant mineralization of connective tissues. Here, we report a family with PXE/CL-like cutaneous features in association with multiple coagulation factor deficiency, an autosomal recessive disorder associated with *GGCX* mutations. The proband and her sister, both with severe skin findings with extensive mineralization, were compound heterozygotes for missense mutations in the *GGCX* gene, which were shown to result in reduced γ-glutamyl carboxylase activity and in profound under-carboxylation of matrix gla protein, which is inactive in preventing unwanted mineralization. The proband's mother and aunt, also manifesting with PXE/CL-like changes, were heterozygous carriers of a missense mutation (p.V255M) in *GGCX* and a null mutation (p.R1141X) in the *ABCC6* gene, suggesting digenic nature of their skin findings. Thus, reduced γ-glutamyl carboxylase activity in individuals who are either compound heterozygous for missense mutations in *GGCX* or with haploinsufficiency in *GGCX* in combination with heterozygosity for *ABCC6* gene expression results in aberrant mineralization of skin leading to PXE-like phenotype.

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A novel deletional mutation of KIT in mastocytosis mast cells

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Mutations of the proto-oncogene KIT are associated with mastocytosis. KIT encodes a type III receptor tyrosine kinase that is essential for the growth and development of mast cells. KIT has 21 exons and consists of a stem cell factor (SCF) binding domain, a regulatory juxtamembrane domain (JX), a tyrosine kinase domain and a C-terminus. Previous studies have shown that point mutations in JX, such as V560G, and the kinase catalytic site, such as D816V, cause auto-activation of KIT that may lead to the development of mastocytosis. We have found a novel deletional mutation of KIT in an adult patient with mastocytosis. The patient expresses both a full length, normal KIT and a truncated KIT resulting from the deletion of exon 3 to exon 13. The truncated KIT retains a portion of the SCF binding domain as well as the entire kinase catalytic site and C-terminus. Normally, KIT is folded into an inactivated tertiary structure through the binding of its C-terminus to the JX. Activation of KIT requires the removal of an inhibitory loop located in JX through SCF binding. We speculate that the C-terminal domain of the truncated KIT may bind to the JX of a full-length KIT resulting in an unfolded, activated KIT. Alternatively, since the C-terminus contains a hydrophobic 'HDDV' binding sequence for PDZ domains, the truncated KIT may interact with G-protein coupled receptors and tyrosine kinases to trigger mast cell growth and mastocytosis. This finding may represent a novel mechanism of KIT auto-activation in mastocytosis with an emphasis on the function of the C-terminal domain. Further elucidation of the mechanism of this truncated KIT action in mast cell growth may provide insight into the pathological process of mastocytosis.

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Correlation of extracellular matrix laminin-10 and matrix metalloproteinase expression in human squamous cell carcinoma

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Squamous cell carcinoma (SCC) is the second most common and the second most aggressive type of skin cancer, and it is one of most prevalent cancers in the world. Extracellular matrix (ECM) proteins have been found to play important roles in aiding cell attachment and migration. Thus, studies focusing on manipulating these proteins and thereby halting the progression of SCC are critical. Our previous study found that the knockdown of laminin-10, a major ECM protein in the basement membrane of the skin, could block SCC cell migration and invasion. The matrix metalloproteinase (MMP) family is known to be involved in the process of matrix degradation and remodeling, and thus critically contributes to tumor motility. This study's objective was to investigate the correlation between laminin-10 activity and MMP expression in human SCC cells. We first tested the blocking effects of siRNA (small interfering RNA) on laminin-10 expression using laminin-10 alpha5 chain-specific siRNA (laminin a5 siRNA). From there, the correlated expression and activity of a panel of MMPs and their nature tissue inhibitors (TIMPs) were determined. After a 60-hour transfection with 50 nM siRNA, we achieved a laminin-10 knockdown of 47%. At the same time, we found that the expression of MMP1, MMP2, MMP9, MMP13 and MT1-MMP was decreased by 16%, 12%, 75%, 51% and 14%, respectively. Further, the data indicate an up-regulation of TIMP1 and TIMP2 activity by an average of 28% and 113%, respectively. There was no significant change in the expression of MT2-MMP. This study indicates that MMPs/TIMPs are involved in laminin-10-mediated SCC cell functions. Through MMPs' enzymatic activity and TIMPs' negative feedback, they participate in the regulation of the laminin-10 expression and SCC cell invasiveness. Our long-range goal is to develop a novel strategy targeting ECM proteins, specifically laminin-10, to increase the effectiveness of tumor therapy. This study will contribute to a further understanding of the mechanisms and to the development of novel therapies to block tumor progression.

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Molecular mechanism of Egr-1-induced premature aging in the skin of smokers

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Tobacco smoking also has deleterious effects on the skin and smoker's wrinkles are typical clinical feature of smokers. The Egr-1 gene, which is also known as G0S30, NGFI-A, zif268, krox24, and Tis 8, encodes a zinc-finger-containing transcription factor and is an immediate-early response gene that is rapidly induced by stress, injury, mitogens, and differentiation factors in diverse cell types. It have been reported that Egr-1 plays a critical role in fibrosis and matrix remodeling. Recent evidence showed that tobacco smoke extract blocks cellular responsiveness to TGFβ through the induction of a non-functional latent form of TGFβ and downregulation of the TGFβ receptor. The objective of this study is to investigate the regulatory potential of Egr-1 on the premature skin aging by smoking. We hypothesize that egr-1 may be activated during exposure to cigarette smoke extract and repress the expression of TGFβ receptor-II (TβR-II). After cigarette smoke extract (CSE) or nicotine exposure to human primary fibroblasts, we analyzed protein and mRNA expression of Egr-1, TβR-II and type I collagen with western blot and real-time PCR. To evaluate Egr-1-specific effects, we used Egr-1 small interfering (siRNA) to knock down Egr-1 expression. Through the luciferase assay, we evaluated the activity of Egr-1 promoter after CSE and nicotine. Egr-1 was markedly expressed after CSE and nicotine exposure. TβR-II and type I collagen were markedly downregulated in fibroblasts exposed to CSE and nicotine. The levels of TβR-II and type I collagen was significantly upregulated after direct inhibition of Egr-1 with Egr-1 siRNA. Our findings indicate that Egr-1 might be one of the key genetic factors that control the development of cigarette smoke-related skin aging.

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Correction of the skin defect in murine recessive dystrophic epidermolysis bullosa by SLAM family receptor enriched hematopoietic stem cells

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Recessive dystrophic epidermolysis bullosa (RDEB) is an intractable disorder of severe skin fragility and blistering caused by mutations in the type VII collagen gene (COL7A1). To assess the beneficial effects of cellular therapy for RDEB, we infused hematopoietic and nonhematopoietic stem cell populations into unconditioned RDEB (col7a1-/-) mice. Intraperitoneal injection of either multipotent adult progenitor cells, mesenchymal stem cells, epidermal stem cells, or unfractionated bone marrow (BM), was unable to prevent disease in more than 200 animals, all of which died shortly after birth. Strikingly, however, 3 of 13 (23%) recipients of SLAM family receptor positive (CD150+/CD48-) adult BM survived for several months. Surviving animals had evidence of skin engraftment of donor cells, and production of type VII collagen in the skin, with healing of skin blisters. Anchoring fibrils, which are comprised exclusively of type VII collagen, were detected at the basement membrane zone and provided evidence of functional restoration. These data demonstrate that adoptive transfer of type VII collagen producing BM cell populations is sufficient for the correction of the basement membrane zone defect in col7a1-/- murine recipients. Together, our findings provide proof-of-principle supporting the application of allogeneic hematopoietic stem cell transplantation in the treatment of human RDEB.

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Treatment of the allergic cutaneous inflammation in mouse model by STAT6 inhibition with RNA interference

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The correction of Th2 type dominant immune status may contribute to controlling the allergic inflammation. Signal transducers and activators of transcription 6 (STAT6) play a crucial role in the transactivation of IL-4 and IL-13, which are involved in the maintenance of Th2 type immune reaction. In this study, we tried to mange the allergic cutaneous inflammation by inhibiting a function of STAT6 with RNA interference techniques. In all experiment, Lipofectamine 2000 (Invitrogen) was used for the transfection of siRNA and scramble RNA was used as positive control. *In vitro* experiment, using murine skin fibroblasts, with the methods of real-time PCR and Western blot, we tried to find a sequence of siRNA (short interfering RNA) which could demonstrate most efficient RNA interference to STAT6 (STAT6 siRNA) from several synthesized candidates analyzed by BLSAT Search. *In vivo* experiment, ear swelling reaction in a hapten (TNCB; 2,4,6-trinitrochlorobenzene, DNFB; dinitrifluorobenzene) - induced murine contact hypersensitivity model was used for evaluating the effect of STAT6 siRNA. Transfer experiment was also performed. Among the several candidates, we could successfully select the STAT6 siRNA and this could effectively inhibit the expression of eotaxin in both mRNA and protein levels in murine skin fibroblasts stimulated with the combination of IL-4 and TNF alpha. Ear swelling reaction in a hapten-induced murine contact hypersensitivity model was inhibited when STAT6 siRNA (1 nmol/ear) was administered into a ear lobe at 48 hr before challenge. The ear swelling reaction was also inhibited when STAT6 siRNA-treated LNs cells were transferred and it revealed that LNs cells were one of the targets of STAT6 siRNA. Thus, in this study, the method of an inhibition of the function of STAT6 by using RNAi was established and the possibility of the control of the allergic cutaneous inflammation by using STAT6 siRNA was shown.

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Molecular analysis of mitten deformities in the collagen VII hypomorph and human RDEB reveals potential targets for therapy

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The key symptoms of recessive dystrophic epidermolysis bullosa (RDEB), a severe skin fragility disorder caused by collagen VII mutations, include skin and mucosal blistering, nail dystrophy and mitten deformities of hands and feet. To analyse molecular disease mechanisms as a prerequisite for novel therapeutic strategies, we generated a collagen VII hypomorph by intronic insertion of a PGK-Neo-cassette into the *Col7a1* gene. Homozygous mice exhibit a phenotype closely resembling human RDEB: blistering of skin and oral mucosa, nail dystrophy, pseudosyndactyly and growth retardation. The phenotypic abnormalities result from a reduction of collagen VII to about 10% of wild type levels, caused by aberrant mRNA splicing. In the extremities of homozygous animals, blistering was followed by abnormal wound healing with persistent inflammation and expression of TGF-β1 and connective tissue growth factor, CTGF. These enhanced matrix deposition and caused upregulation of α-SMA, excessive fibroblast-to-myofibroblast differentiation and strong deposition of tenascin C in the dermal matrix. The fibrotic tissue changes and contractile forces established by the myofibroblasts led to development of pseudosyndactyly, loss of digits and, in some cases, complete synechia of the paws within 8 - 12 weeks. Analysis of mitten deformities in human RDEB revealed similar molecular mechanisms. The severe dermal fibrosis as abnormal microenvironment is likely to contribute to the development of squamous cell carcinoma, a lethal complication of RDEB. The present observations explain the mechanisms of mutilating deformities in human RDEB and disclose potential targets for pharmacological intervention. Especially anti-inflammatory agents or inhibitors of TGF-β / CTGF signalling may prove valuable in the prevention of contractile fibrosis and pseudosyndactyly. The hypomorphic DEB mouse is ideal for testing such therapeutic regimens.

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A novel homozygous KIND1 mutation in a Swiss family with Kindler syndrome and recurrent actinic keratoses

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Kindler syndrome (KS) is a rare, autosomal recessive skin fragility disorder characterized by blistering in infancy, followed by photosensitivity and progressive poikiloderma. In addition, progressive skin atrophy and premature aging, severe periodontal disease, colitis and occasionally carcinogenesis may occur. KS is caused by loss-of-function mutations in the *KIND1* gene which encodes kindlin-1, an actin cytoskeleton-focal contact-associated protein predominantly expressed in keratinocytes. We report on a new Swiss family with KS. The index patient is a 62-year-old male patient with neonatal blistering, photosensitivity with poikiloderma, ectropion and conjunctivitis on both eyes, atrophic skin on hands and feet, numerous actinic precanceroses, severe periodontal disease and urethral stenosis. Recurrent precanceroses started ten years ago in sun exposed skin and were treated with cryosurgery, imiquimod and topical 5-fluorouracil. The patient has three sisters, one died soon after birth because of severe blistering disease. The second sister has also skin blistering associated with sclerodermiform lesions. The third sister is unaffected. Mutation analysis of the index patient showed a homozygous mutation in exon 3 of the *KIND1* gene in codon 110, which results in an exchange of arginine and leads to a stop codon. This finding represents a new mutation in *KIND1* which leads to a complete phenotype of KS. To the best of our knowledge, our patient is the oldest to be definitely diagnosed with KS by genetic analysis. Among the around 100 patients with known *KIND1* mutations, 4 patients were reported to have squamous cell carcinomas, or actinic keratoses. Further investigations will aim to the question why these patients develop actinic precancerous lesions and epithelial malignancy.

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Keratin 5 (KRT5) and keratin 14 (KRT14) mutation analysis by a novel approach

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Epidermolysis bullosa simplex (EBS) is a group of hereditary bullous disorders characterized by lysis of the basal keratinocytes induced by mechanical trauma. EBS patients are known to carry defects in the KRT5 and KRT14 keratin genes expressed in the basal keratinocytes. The presence of a truncated and a complete, inactive KRT14 pseudogene in the human genome greatly interferes with the mutation analysis of the functional gene, therefore extra steps are required the avoid pseudogene sequence amplification. Studying keratin mutations in Hungarian families we developed a simple allele-specific method which allows to exclude sequence contamination from KRT14 pseudogene amplification. The method was successfully applied in 9 EBS pedigrees and disclosed 6 novel and 3 previously described pathogenic mutations. Three novel missense mutations (p.L136Q, p.E411K, p.I412N) have been verified in the KRT14 and three in the KRT5 (p.I183V, p.E190D, p.R331G) genes. Five recurrent polymorphisms were also identified. Allele frequencies of the 6 novel mutations and 4 polymorphisms have been also determined in the Hungarian population (200 chromosomes/100 controls). In this study we validated a new and easy-to-do method for mutation analysis in EBS. Furthermore, the observed new mutations provide further data to EBS-associated mutation databases and can be usefully applied in genetic counseling of future EBS cases

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Epidermolysis bullosa simplex (EBS) with mottled pigmentation (EBS-MP)

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EBS-MP is a rare subtype of EBS (with about 13 published pedigrees), which can resemble all three major subtypes (EBS-Weber-Cockayne (WC), EBS-Koebner (K) or Dowling-Meara, and also displays a "mottled" brownish pigmentation on the trunk and extremities. Here we present the genetic analysis of a family with 10 affected members from four generations referred to our clinic with the diagnosis of Theresa Kindler syndrome. Symptoms of skin fragility and blistering resembling to EBS-WC were present in the early childhood while in later ages only the mottled pigmentation and an associated mild nail dystrophy were detected. The degree of pigmentation was somewhat different in the patients and the symptoms were distributed mostly on the trunk and extremities. The inheritance was clearly autosomal dominant. Within exon 1 of KRT5 all five affected members of the pedigree available for genetic study carried a heterozygous P25L mutation, the most common mutation known to be associated with the EBS-MP phenotype. Within the first exon we found two other homozygous polymorphisms H15R and R79S. Furthermore we identified G138E in all the studied affected pedigree members while G138E was absent from the non-affected individuals. Previously published EBS-MP phenotypes carrying the P25L mutations show either a mild EBS-WC or a more severe EBS-K -like disease. We speculated whether the difference could be explained with additional coding polymorphisms interacting with the keratin filament formation. In this study we could identify a mild EBS-WC-like-MP: this phenotype was associated with disease causing mutation P25L. These data suggest that the additionally altered sites in the KRT5 exon 1 like G138E and polymorphisms H15R and R79S could not be responsible an EBS-K-like-MP. However, one can not exclude that other factors beside the polymorphisms of basal keratin genes may modify the genotype-phenotype correlation in EBS-MP.

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Population-based genetic study of disease susceptibility to psoriasis (*PSORS1*)

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Psoriasis vulgaris (PV) is a complex inflammatory skin disorder caused by the concerted action of multiple genetic and environmental factors. Prevalence rate of PV in Japan is estimated to be less than 0.1%. Until now, with respect to *PSORS1* within the *HLA* region on chromosome 6p21, several genes such *HCR* (α-helix coiled-coil rod homolog), *CDSN*, *SEEK1*, as well as *HLA* itself have been candidated based on the results of analyses of family and population studies. In the present experiments, we focused on *HCR* in linkage disequilibrium with *HLA* class I alleles and killer cell immunoglobulin-like receptor genes (*KIRs*) which require *HLA* class I as ligand. Using 100 unrelated Japanese patients with PV, we performed association study on genetic polymorphisms of the three genes. *HLA-Cw*0602* (9.3% vs 0% in control: p<0.02) and *HCR-2406*G* (17.3% vs 4.0% in control: p<0.02) were associated with PV susceptibility. With respect to *KIRs*, the frequencies of activating *KIRs* including *KIR2DS1* which can activate NK-T/CD8+T lymphocytes were statistically increased. In contrast, there was no association between SNPs of *TNF-α* promoter (at-238 and at-308) and PV susceptibility. Recently it has been reported that *HCR* up-regulates expression of vitamin D receptor. It is possible that strong linkage disequilibrium within the *HLA* region has made it difficult to separate the true PV susceptibility gene. In conclusion, *HLA* class I alleles including *HLA-Cw6* seem to play a central role controlling the above genes (*HCR* and *KIRs*) via either direct or indirect effects.

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Claudin1; a p63 target gene involved in the pathogenesis of ectodermal dysplasias

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Both p63 and claudin-1 null mice die within few hours from birth due to dehydration for severe skin abnormalities. These observation suggested us the possibility that the two genes might be linked to the same regulatory pathway or belong to a common signaling cascade with p63 possibly regulating claudin-1 expression. Here we show that silencing of ΔNp63 but not TAp63 in primary mouse keratinocytes (by ΔNp63 specific siRNA), results in a severe downregulation of claudin-1 expression (- 87%). Claudin-3 and claudin-10 expression was also reduced although to a lesser extent (-55%). Moreover, ΔNp63α can activate both the endogenous claudin-1 gene and a reporter vector containing a -1400 promoter fragment of the claudin-1 gene while TAp63 isoforms acts as transcriptional repressor. Deletions of the two p53-like binding sites abolished p63 mediated transactivation of the Claudin-1 promoter. p63 mutations associated to the AEC syndrome were indeed altered in their capacity to regulate claudin-1 transcription reinforcing the hypothesys that these two genes might be linked to a common regulatory pathway; on the other hand, SHFM-IV associated p63 mutations behaved as the wild type p63 isoforms. In addition, since AEC patients display defects in the dermoepidermic junction similar to those observed in the epidermis ΔNp63 knockdown mice, our findings suggest that claudin1 may act as an important p63 target gene involved in the pathogenesis of ectodermal dysplasias.

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3D skin models and keratinisation disorders: who mimics whom?

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In the last four years several new genes for autosomal recessive congenital ichthyosis (ARCI) have been identified. Still only little is known about the pathophysiology of this heterogeneous group of severe disorders of keratinisation. So far treatment for ARCI is only symptomatic and often insufficient. In order to gain a patient independent, human model mimicking the barrier defect and hyperkeratosis typical for ARCI, we established and assessed different 3D skin models for our purpose. A major challenge for finding a 3D skin model for keratinisation disorders is the thickening of the stratum corneum typically seen over time without a genetic defect. To evaluate therapeutics with a skin model, it must develop and sustain a basal layer attached to the dermal layer and show a normal differentiation pattern. To stratify effects of inactivation of different genes involved in ARCI, we transfected primary keratinocytes from healthy volunteers with siRNA to knock down the expression of single genes prior to 3D model preparation. Structure, thickness of stratum corneum, immunohistochemical stainings, and quantitative analyses proved the efficiency of each knock-down. Keratinocytes from healthy donors were grown without a feeder layer in a serum-free system until 3D model preparation. 3D skin models differed in differentiation medium, including SKDM, SKDM with TGF-beta 1, and KCM, all with defined Ca concentrations. We found hyperproliferation and thickening of the stratum corneum as well as detachment from the dermal layer in the model w/o knock-down when cultivated with SKDM for more than 10 and up to 21 days. Added TGF-beta 1 gave less prominent thickening of the stratum corneum after 7 days and well defined basal and granular layers. 3D models with KCM showed a thin, normal stratum corneum, defined basal and granular layers and constant attachment to the dermal layer. Currently immunohistochemical stainings and ultrastructural analyses are under way. The systems will be fundamental for studies of disturbed epidermal differentiation and pharmaceutical intervention.

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Founder mutations in the DNA polymerase eta gene in Japanese patients diagnosed as xeroderma pigmentosum variant type

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Xeroderma pigmentosum variant (XP-V) patients have susceptibility to freckles and skin cancers of sun-exposed area. It is characterized by not showing severe sunburn and late onset of skin cancers, which result in failure of sun-protection during childhood. The responsible gene for XP-V, POLH encodes DNA polymerase eta, which correctly bypass thymine dimer in replication cycle. The purpose of this study is to establish the feasible method for molecular diagnosis for XP-V. We carried out molecular analysis in the POLH gene for 29 cell strains derived from Japanese patients with possible XPV. All patients except for two developed skin cancers. Most of the cell strains showed normal level of UV-induced unscheduled DNA synthesis and enhanced UV sensitivity by means of post-UV colony formation in the presence of caffeine. Immunoprecipitation (IP) using two kinds of anti-POLH antibodies revealed that cells from 26 patients did not show 83 kDa POLH band and cells from one patient had a faint band (10% signal intensity in comparison to that of normal). All of 27 cell strains without POLH band or with a faint POLH band had mutations in the POLH gene. We identified seven mutations ; 1 splicing, 3 nonsense, 1 frameshift (deletion), 2 missense mutations. All mutations involved the catalytic domain. Among them four founder mutations were noted. 18 alleles of 54 were G490T, 12 were del 1661A, 11 were G916T and 6 were C725G. These four founder mutations are responsible for 87 % of the mutations in our XP-V patients. Combination of IP analysis of POLH protein and detection of these founder mutations in POLH is a very useful method for genetic diagnosis for Japanese patients with XP-V, especially for young patients whose clinical features are not fully developed.

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Oxidative stress induced by UVA-1 and H2O2 in CSA and CSB cells induces clustering and enrichment of CSA and CSB proteins in mitochondria

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Mutations of mitochondrial DNA play a role in cancer and aging. Among a wide variety of genotoxic stressors ROS as a by-product of cellular metabolism has high mutagenic potential. Nuclear DNA can be repaired by several repair systems after oxidative insult. Nucleotide excision repair (NER) is a versatile repair system which also removes oxidative damage. Dysfunctional NER causes severe diseases like Cockayne syndrome (CS) which is caused by dysfunctional CSA and CSB proteins and is characterized by premature aging and neurodegeneration. Although there is increasing evidence that the CSB protein is especially important for removal of oxidative nuclear DNA damage, up to now mitochondria are considered to be free of NER systems. We have previously shown that CSA and CSB deficient fibroblasts exhibit a higher level of UVA induced mitochondrial DNA mutations. In mitochondria of unstressed fibroblasts we could detect low amounts of CSA and CSB by confocal microscopy. In order to further investigate the role of CSA and CSB in mitochondria we exposed fibroblasts to sublethal doses of chronic UV-A irradiation or non-toxic concentrations of H2O2 (20-40µM). Western blot analysis, and confocal fluoroescent microspcopy showed that CSA and CSB are highly enriched in mitochondria upon oxidative stress. Immunogold labelling electron microscopy confirmed these results and showed clustering of CSA and CSB proteins in mitochondria, indicating localisation at distinct sites within mitochondria. We present evidence that NER-associated CSA and CSB proteins are present in mitochondria and increase in response to different types of oxidative stress. These results strengthen a possible role of CSA and CSB in protection from neurodegeneration and aging-associated processes.

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A whole genome-wide association study of psoriasis within the Japanese population using 26,065 microsatellite markers

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Psoriasis is common, genetically determined, inflammatory and proliferative disease of the skin. The cause of psoriasis is unknown, but this disease is thought to be a multifactorial disease triggered by both genetic and environmental factors. In fact, recent genome-wide linkage studies have identified several susceptibility loci on chromosomes 6p21.3 (HLA), 17q25, 4q and many others. However, psoriasis susceptibility genes have not identified. In this study, we have systematically performed association analysis of psoriasis to define all psoriasis susceptibility loci. Independent 125 subjects with psoriasis vulgaris were pooled for the 1st, 2nd and 3rd sets. After the 1st screening, significant associations with psoriasis were recognized on 2,065 microsatellite markers out of 26,061. Similarly, after the 2nd screening, significant associations were recognized on 495 markers out of 2,065. After the 3rd screening, significant associations were recognized on 159 markers out of 495. Finally, individual typing were performed to avoid false positive associations. As the result, putative psoriasis candidate loci were narrowed down to 16 regions. We analyzed the expression of one of the putative psoriasis candidate genes on some tissues.

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Successful *ex-vivo* gene therapy of recessive DEB

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Dystrophic epidermolysis bullosa (DEB) is an inherited skin blistering disorder caused by mutations in collagen type VII, the major component of the anchoring fibrils of the dermal- epidermal junction. We have demonstrated the feasibility of an *ex-vivo* gene therapy approach for recessive DEB (RDEB) in a spontaneous RDEB dog model associated with a missense point mutation in the collagen VII gene. Primary keratinocytes obtained from skin biopsies of two RDEB dogs were transduced using a retroviral vector expressing the dog full-length collagen VII cDNA. The transduction efficiency of epidermal stem cells was 100% in dog 1 and 65% in dog 2. Autologous epidermal sheets made with the genetically engineered keratinocytes grafted on the animals regenerated a "normal" epidermis at day 8 that expressed the transgene. The "normal-looking" epidermis was maintained throughout a one-year follow-up without sign of inflammation or immune rejection. In dog 1, examination of sections of the transgenic skin showed a restoration of the epidermal adhesion, which correlated with: -i) a strong immunoreactivity of the basement membrane to anti-collagen VII antibodies; -ii) a sustained expression of the transgene; -iii) a pattern of normal skin in terms of self renewal capacity, vascularization, immunity, pigmentation and cell differentiation. Clonogenic reverted keratinocytes from the grafted area were isolated 1 year after grafting. Neither cellular (CD4, CD8, CD68, CD79a)nor humoral immune reactions were observed during the follow-up period. In dog 2, blisters within the grafted area were observed at month 5, which correlated with a decline of the transgene expression at month 2 and loss of the reverted keratinocytes. These data prove that *ex-vivo* gene therapy of RDEB is feasible, at least in subsets of RDEB patients with genetic mutations in the collagenic domains of collagen VII. The contrasting results obtained with the two transplanted RDEB dogs underscore the notion that the transduction efficiency of epidermal stem cells is crucial for successful *ex-vivo* gene therapy of EB

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Analysis of the Tetratricopeptide repeat domain 7 gene polymorphisms in Japanese psoriasis and atopic dermatitis patients

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The flaky skin (fsn) mouse develops dermatitis similar to human psoriasis, histologically characterized by hyperkeratosis with focal parakeratosis, epidermal microabscess, and dermal infiltration of mixed inflammatory cells. Therefore, the mouse has been proposed as one of the naturally-occurring mouse models for human psoriasis. In addition, the mouse has pleiotropic phenotypes including anemia, lymphadenopathy, splenomegaly, and elevation of the serum IgE level, due to dysfunctions of immunological, hematopoietic and some other systems. High level of the serum IgE rather reminds us of the similarity of this mouse to atopic dermatitis (AD). Recently, as the genetic cause for this mouse, a homozygous mutation in the Tetratricopeptide repeat domain 7 (Ttc7) gene has been reported. The mutation is caused by the transposon insertion into the intron 14 of the Ttc7 gene. Ttc7 is a polypeptide containing the tetratricopeptide repeat (TPR) domain, which is defined by the degenerative consensus sequence of 34 amino acids, whereas the function of this protein is unknown. Since there have been only few studies examining the relationship between psoriasis and the Ttc7 gene, here we investigated the Ttc7 gene polymorphisms in Japanese psoriasis and AD patients. We synthesized cDNA of the Ttc7 mRNA from the patients' peripheral blood mononuclear cells and analyzed the sequences. We successfully detected three SNPs (rs2304290, rs3739100, rs3739099), which constitute distinct haplotypes, in the coding region of the gene. However, no significant difference was detected regarding distribution of each genotype and haplotype between control, psoriasis and AD groups. At present, we are searching for other SNPs in the Ttc7 gene.

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Shared genetic determinants between psoriasis, Crohn's disease and diabetes

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The familial recurrence of psoriasis is well-established and the disease is regarded as a multifactorial trait, resulting from gene-gene and gene-environment interactions. Linkage studies have repeatedly identified a primary disease susceptibility locus, within the Major Histocompatibility Complex (MHC). A number of non-MHC loci have also been mapped, some overlapping with susceptibility intervals for Crohn's disease (CD), suggesting the existence of genetic determinants predisposing to both conditions. Association studies have demonstrated that at least two genes (IL23R and IL12B) confer susceptibility to both psoriasis and CD. To investigate this shared genetic architecture, we examined 15 CD-associated SNPs in 1,256 psoriatic cases and 2,938 unrelated controls. We observed significant P values for SNPs mapping to chromosomes 1q24 (rs12035082, P = 0.009), 6p22 (rs6908425, P = 0.00015) and 21q22 (rs2836754, P = 0.002). The marker generating the most significant association (rs6908425) maps to the CDKAL1 gene, which has been independently identified as a susceptibility gene for type 2 diabetes. To establish whether the remaining two markers were also associated with diabetes, we analyzed publicly available patient genotype data generated by the Wellcome Trust Case-Control Consortium. The frequency of SNP rs12035082 was increased in type I diabetes patients (n = 1,962), compared to controls (P = 0.025). Rs12035082 maps in a gene desert where our bioinformatic analyses have identified several non-coding conserved regions that may have a role in regulating the expression of the nearby TNFSF18 locus. In conclusion, we have identified two variants associated with psoriasis, CD and diabetes. In-depth functional analyses are now needed to elucidate the biological impact of these SNPs.

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Fine mapping of the psoriasis susceptibility locus psors1 supports hla-c as the susceptibility gene in Han Chinese population

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PSORS1 is the major susceptibility locus for psoriasis. Several fine-mapping studies have highlighted a 300-kb candidate region of PSORS1 where multiple biologically plausible candidate genes were suggested. We first performed a family-based association analysis of the PSORS1 locus by analyzing 10 microsatellite markers from the PSORS1 region as well as HLA-B, HLA-C and CDSN loci in 163 Chinese families of psoriasis. It revealed five marker loci (M6S187, C2_4_5, C2_4_4, C1_3_2 and M6S172) showing strong evidence (P<10-3) and one marker locus (C1_2_6) showing weak evidence (P=0.04) for association with psoriasis. Our subsequent haplotype cluster analysis showed that all the risk haplotypes share a 369-kb region of homologous marker alleles between HLA-B and M6S187, which carries all the risk alleles, including HLA-Cw6 and CDSN*TTC, identified in this study. We further identified the recombinant haplotypes that carry only one of the two risk alleles in 228 Chinese families of psoriasis. We found that the HLA-Cw6-/CDSN*TTC+ recombinant haplotype is clearly not associated with risk for psoriasis (T:NT = 29:57, p = 0.0025), suggesting that the CDSN*TTC allele itself does not confer risk without the presence of the HLA-Cw6 allele. Further exclusion analysis of non-risk HLA-Cw6-/CDSN*TTC+ recombinant haplotypes with common recombination breakpoints has allowed us to refine the location of PSORS1 to a small candidate region. We conclude that by performing both population- and family-based association analysis of haplotypes we were able to refine the PSORS1 gene to a small critical region where HLA-C is the only likely candidate to be the PSORS1 susceptibility gene.

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Cell-based therapy for pseudoxanthoma elasticum: reduction of connective tissue mineralization in a mouse model *(Abcc6$^{-/-}$)* by transplantation of wild-type hepatocytes

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Aberrant mineralization of connective tissues is a characteristic feature of pseudoxanthoma elasticum (PXE), a heritable disorder caused by mutations in the *ABCC6* gene expressed primarily in the liver. There is no effective treatment available so far. We have developed a mouse model for PXE by targeted ablation of the *Abcc6* gene *(Abcc6$^{-/-}$)*. In this study, we investigated the beneficial effects of cell-based therapy for PXE. Twenty-four hours after carbon tetrachloride (CCl_4) intoxication, freshly isolated wild-type mouse hepatocytes, 2-5 $\times 10^5$ with at least 95% viability, were transplanted into 4-week old *Abcc6$^{-/-}$* mouse livers by intrasplenic injection. Two months after transplantation, the mice were evaluated for Abcc6 positive cells in the livers and the PXE phenotype as determined by histopathologic examination of target tissues, including vibrissae, and biochemical assay of Ca^{2+}/P in vibrissae. Using this model, we determined that (a) CCl_4 leads to acute liver injury characterized by T-lymphocyte infiltration peaking at 24-48h, but at day 4 the livers appeared normal; (b) the transplanted cells engrafted into liver and produced Abcc6 protein as visualized by immunofluorescence; (c) the mineralization of connective tissues in transplanted mice was reduced when compared to that in untreated control mice. Specifically, the content of mineral deposits in the muzzle skin was significantly decreased (up to 90%) as determined by morphologic computerized analysis. Thus, this transplantation model suggests that cellular therapy may be valuable for treatment of PXE patients in the future.

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Keratin 17 and keratin 6b mutations in chinese patients with steatocystoma multiplex

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steatocystoma multiplex is an autosomal dominant disorder in which the main phenotype is the presence of multiple cysts,although many sporadic cases have been reported. At least one form of steatocystoma multiplex is associated with mutations in keratins K6b/K17 and as such is closely related to pachyonychia congenital type 2(PC-2).It has been demonstrated that Keratin 17 mutations could cause either steatocystoma multiplex or PC-2,and Keratin 6b mutations could lead to PC-2.However, the correlation between Keratin 6b and steatocystoma multiplex has not been identified. Here, we describe a recurrent K17 mutation in a kindred with steatocystoma multiplex and a novel K6b mutation in a sporadic case.

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Genome-wide association scan reveals novel psoriasis susceptibility loci

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To gain further insight into the genetic basis of psoriasis, we carried out a genome-wide association scan of 1,384 Caucasian psoriasis cases and 1,414 Caucasian controls on 451,724 SNPs, utilizing a Perlegen microarray. This is the largest association scan of psoriasis to date. Hundreds of highly significant signals mapped to the MHC, with the most significant signals found in the immediate vicinity of HLA-C (rs12191877, allelic OR = 2.78, p = 8.36 x 10-54). Other positive signals mapped to the vicinity of IL12B (rs2082412, OR = 1.57, p = 2.91 x 10-10) and IL23R (rs2201841, OR = 1.35, p = 2.51 x 10-7). Seventeen signals were subjected to confirmation in an independent sample of 1,612 cases and 943 Caucasian controls. Of these, seven could be confirmed ($p < 0.01$, OR = 1.21 to 2.72), of which four were novel. One of these mapped to IL23A (initial OR = 1.72, p = 9.50 x 10-6, confirmation OR = 1.45, p = 2.31 x 10-3), and another mapped to the vicinity of the tightly clustered IL4 and IL13 genes (initial OR = 1.38, p = 8.9 x 10-7, confirmation OR = 1.35, p = 3.07 x 10-5). These results demonstrate that the strongest genetic determinant of psoriasis resides in the MHC, within or near HLA-C. While causal variants remain to be identified, our studies provide significant evidence for involvement of IL23A for the first time, emphasizing the importance of IL-23 vs. IL-12. They further implicate the IL4 / IL13 gene cluster, whose products are involved in the regulation of Th2 differentiation. Taken together with emerging immunologic evidence indicating a major role for IL-23 in regulating Th17 cells, these results provide strong and unbiased genetic support for an altered balance of T-cell differentiation in psoriasis involving the Th17 - Th2 axis.

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PTPN22 R620W is not associated with pemphigus vulgaris

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Alterations in the protein tyrosine phosphatase N22 (PTPN22) gene affect the threshold required for lymphocyte activation. The PTPN22 R620W polymorphism depresses T cell receptor cascade propagation. Elevated R620W allele frequency has been correlated with several humoral autoimmune disorders. However, a recent report did not find an association between R620W and Tunisian Pemphigus vulgaris (PV) patients. R620W allele frequency is known to vary across ethnic populations. In this study, we investigated the relationship between PTPN22 R620W and PV in Caucasian and Ashkenazi Jewish populations. Participant allele status was determined in 61 PV patients and 61 healthy controls by restriction fragment length polymorphism-polymerase chain reaction genotyping. Relationships between patient and control subpopulations were calculated using right-sided Fisher's exact tests and Cochran Mantel Hanzel Chi squares. Odds-ratios (OR) and 95% confidence intervals (CI) were obtained using logistical regression analysis. Our results indicate that the PTPN22 R620W polymorphism is not associated with pemphigus vulgaris in either the Caucasian population (p=0.8413) or the Ashkenazi Jewish population (p=0.8356). Among Caucasians the association to the variant was stronger among males (p=0.2300) than females (p=0.7920) although both cases remained insignificant. Lesion history, disease duration, age of onset, history of positive anti-desmoglein 1 immunoglobulin (aDSG1 Ig) response, history of aDSG3 Ig response, family history of autoimmune disease, family history of PV, and ethnicity had no bearing on allele frequency. The variant frequency among PV patients in our study was consistent with published values for the general population.

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Prevention and reversal of aberrant mineralization in pseudoxanthoma elasticum : a mouse grafting model

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Pseudoxanthoma elasticum (PXE) is characterized by aberrant mineralization of connective tissues and caused by mutations in the *ABCC6* gene expressed primarily in the liver. The pathomechanisms of mineralization, as a result of *ABCC6* mutations, are currently unclear. To test the hypothesis that PXE is a metabolic disorder and to investigate the possibility that the mineralization process may be reversible, we examined a murine PXE model *(Abcc6$^{-/-}$)* after transplanting muzzle skin to the back skin of mice using mineralization of vibrissae as a phenotypic biomarker. Two experimental designs were utilized: a) in the *prevention study*, muzzle skin from either *Abcc6$^{+/+}$* or *Abcc6$^{-/-}$* mice at 4 weeks of age (before development of mineral deposits) was transplanted onto the back of both *Abcc6$^{-/-}$* and *Abcc6$^{+/+}$* mice; b) in the *reversal study*, muzzle skin from *Abcc6$^{-/-}$* mice at 12 weeks (after development of mineralization) was transplanted onto *Abcc6$^{+/+}$* or *Abcc6$^{-/-}$* mice. In the *prevention study*, two months post-operatively, the vibrissae in the muzzle skin of *Abcc6$^{+/+}$* mouse grafted to the back of *Abcc6$^{-/-}$* mice showed characteristic mineralization, while no mineralization was observed in the muzzle skin of *Abcc6$^{-/-}$* mouse transplanted onto the *Abcc6$^{+/+}$* mice. In the *reversal study*, the degree of mineralization of the vibrissae from *Abcc6$^{-/-}$* mouse was dramatically decreased (by ~60%) 3 months after being grafted onto the back of *Abcc6$^{+/+}$* mice compared to those grafted onto *Abcc6$^{-/-}$* mice. Collectively, these data support the notion that PXE is a systemic metabolic disorder with secondary mineralization of connective tissues and that the mineralization process can be reversed.

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Alopecia universalis is associated with PTPN22 R620W

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Alopecia areata (AA) is an autoimmune hair loss disorder that is at least in part genetically determined. Although susceptibility genes have not been established in AA, the protein tyrosine phosphatase N22 (PTPN22) R620W variant allele has been found in association with a number of human autoimmune diseases. The list includes a previously reported association with AA in European populations. The PTPN22 gene product, LYP, is an early mediator in T cell receptor signaling. The R620W variant enhances LYP and depresses the TCR activation response. In this study, we evaluated the prevalence of R620W in North American Caucasian, African and Hispanic AA populations. Participant allele status was determined in 371 AA patients, 199 related and 74 unrelated healthy control subjects via restriction fragment length polymorphism-polymerase chain reaction genotyping. Patients were compared against related and unrelated control subjects separately and a comparison between the two control groups was also performed. The analysis was further stratified by race, gender, and family history. Statistical estimates were determined via Cochran Mantel Hanzel Chi squares and logistical regression. Irrespective of control group, AA was not found to associate with R620W (related controls: p=0.6916; unrelated controls: p=0.2101). However, analysis by disease severity revealed a significant association between alopecia universalis patients and R620W (unrelated controls: p=0.0344). Additionally, control subjects with a primary family member affected by AA were significantly more likely to carry R620W than control subjects with no known family history of AA (p=0.050). We find that that ethnicity does not alter the relationship between AA and R620W. The role of the R620W variant in AA pathogenesis warrants further study.

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Transcriptional regulation of basal-specific keratin genes by direct interaction of ΔNp63 with conserved p63-response elements

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During embryogenesis, simple epithelial cells commit to stratification, a process that is marked by selective expression of basal-cell specific keratin pairs, K5 and K14. The coordinated regulation of these two genes is primarily controlled at the transcriptional level. We have previously shown that the transcription factor p63 regulates the expression of the K14 gene through a conserved enhancer segment. Here we provide evidence that p63, specifically ΔNp63, also interacts with a regulatory region of the K5 gene. This region is marked by a keratinocyte-specific DNase I hypersensitive site and is evolutionarily conserved. Biochemical studies reveal that ΔNp63 can bind to a specific DNA sequence present in the K5 enhancer and that this p63-response element is important in mediating p63 dependent transactivation. In addition, chromatin immunoprecipitation (ChIP) experiments with ΔNp63-specific antibodies demonstrate that the K5 regulatory element is occupied by ΔNp63 in cultured keratinocytes and in mouse skin epidermis. We show that ectopic expression of all ΔNp63 isoforms can induce de novo expression of not only K14 but also K5 in epithelial cells in transient transfection assays. By using ΔNp63-specific antibodies, we demonstrate that this isoform of p63 is concurrently expressed with K5 and K14 in the developing mouse epidermis. Finally, to characterize the functional properties of ΔNp63 isoforms in vivo, we have established a Tet-inducible transgenic mouse model system. Ectopic expression of ΔNp63 in lung alveolar epithelial cells of transgenic mice leads to epithelial metaplasia accompanied by induction of K14 and K5. Taken together, our studies demonstrate that contrary to previous reports, ΔNp63 isoforms are functionally competent in directly controlling the synchronized expression of K5 and K14 in a basal keratinocyte-specific manner. These observations further highlight the importance of ΔNp63 in governing epidermal development and stratification.

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The response to replicative stress at telomeres is mediated by Werner protein and induced by T-oligos

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Telomeres cap the ends of eukaryotic chromosomes and serve to protect genomic integrity in poorly understood ways. We have reported that oligonucleotides homologous to the TTAGGG telomere repeat sequence (T-oligos) provided to normal or malignant cells concentrate in the nucleus and induce DNA damage-like responses including S-phase arrest and phosphorylation of histone H2AX to form γH2AX foci at telomeres identical to those in DNA-damaged or senescent cells; and that these responses require WRN, the protein mutated in the cancer-prone progeroid Werner syndrome, known to bind and resolve G-quadruplex structures (GQS) that form in G-rich single stranded telomeric DNA and T-oligos. We now report that these T-oligo-induced γH2AX foci also contain 53BP1, Mre11, Rad51 (proteins involved in repair of damaged DNA); RPA (a co-activator of ATR); and DNA polymerase delta, known to cooperate with WRN to synthesize DNA opposite GQS. T-oligo treatment modifies PCNA, consistent with its ubiquitination, a known trigger for polymerase switching at replication blocks, and phosphorylates RPA2 and CHK-1, targets of ATR. Inhibition of WRN helicase with distamycin prevents these T-oligo responses. We propose that WRN resolves GQS when telomeres are disrupted by acute DNA damage, critical shortening during aging, or during replication, leading to ATR activation and a cascade of DNA damage-like responses that assure genomic integrity and restore proper telomere structure. T-oligos appear to enhance GQS formation at telomeres and exaggerate these responses, especially in hyperproliferative cancer cells with aberrant patterns of DNA replication.

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A long-range and evolutionarily-conserved enhancer integrates the peptidylarginine deiminase 3 gene expression to AP-1 signalling upon keratinocyte differentiation

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Peptidylarginine deiminases (PADs) catalyse the conversion of protein-bound arginine into citrulline. This post-translational modification known as deimination is though to be critical in the metabolism of the epidermal-barrier protein filaggrin. PAD3, specific for the most differentiated epidermal keratinocytes, was shown to be involved in filaggrin deimination. The mechanisms controlling the expression of its encoding gene, PADI3, are not known. We previously demonstrated that the PADI3 promoter binds NF-Y and Sp1/3 factors, like PADI4 which is not expressed in the epidermis, suggesting a higher level of transcriptional control. We here describe the first Peptidylarginine deiminase Intergenic Enhancer (PIE1). PIE1 is an evolutionary-conserved non coding segment located 80-kb from the PADI3 promoter. Luciferase and real-time PCR assays showed that it is a strong and DNase I-hypersensitive enhancer of the PADI3 promoter. Mutant plasmid screening, gel-shift assays and chromatin immunoprecipitation indicated the requirement for the AP-1 factors c-Jun and c-Fos bound to PIE1. Chromosome Conformation Capture revealed an increased physical proximity of PIE1 and the PADI3 promoter in calcium-stimulated as compared to proliferative keratinocytes in vitro. PIE1 showed no effect in HaCaT cells or carcinoma keratinocytes, or in fibroblasts. These findings disclose that the interaction of PIE1 with the promoter region of PADI3 underlies efficient transcription of this gene in differentiating keratinocytes, by the way of AP-1 factors and probably through the formation of a chromatin loop.

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Telomere homolog oligonucleotides transiently increase telomerase reverse transcriptase (hTERT) levels and elongate telomeres in normal human fibroblasts

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Telomeres, tandem repeats of TTAGGG that cap the ends of eukaryotic chromosomes, shorten with each successive round of replication and when critically short, induce cell death or replicative senescence, two presumptive anti-cancer mechanisms. In transformed cells, telomere length is maintained by expression of the enzyme complex telomerase, specifically expression of the catalytic component hTERT, immortalizing the cells. Although telomerase is considered not to be expressed in most normal cells, recent data demonstrate that it is expressed transiently at low levels in normal fibroblasts during DNA replication. However, the signal for this expression is not known. We and others have shown that DNA oligonucleotides homologous to the telomere G-rich sequence (T-oligos) act at telomeres to induce DNA damage responses. Here we report that one of the responses to T-oligos in normal human fibroblasts is transient upregulation of hTERT and telomerase activity. Following treatment with 40 μM T-oligo (GTTAGGGTTAGGGTTA), hTERT mRNA levels, as detected by real-time PCR, increased up to 8-fold during S phase compared to diluent-treated control cells, peaking at 24 hours, and subsequently decreased to control levels by 72 hours. Telomerase activity, as measured by the real time telomere repeat amplification protocol (TRAP) assay, also increased to 2.5-fold above control levels after 72 hours and declined thereafter. Fibroblasts treated with T-oligo three times over a period of 12 days showed a 16-20% increase in telomere length compared to controls. We propose that replication through difficult-to-replicate telomeric G-rich DNA leads to the transient induction of telomerase to facilitate telomere replication and prevent excessive telomere loss. The presence of T-oligos at this time exaggerates this response, favoring telomere elongation. T-oligos may provide a means to lengthen telomeres and retard the process of cell senescence without the risk of malignant progression associated with permanent hTERT upregulation.

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Unique and recurrent filaggrin mutations in Chinese ichthyosis vulgaris patients from Singapore

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Filaggrin is an abundant protein of the outer epidermis that is essential for terminal differentiation of keratinocytes and formation of an effective barrier against water loss and pathogen/allergen/irritant invasion. Recent investigations in Europe and Japan have revealed null mutations in the filaggrin gene (FLG) as the underlying cause of ichthyosis vulgaris (IV), a common skin disorder characterised by dry skin, palmar hyperlinearity and keratosis pilaris. Following the development of a strategy for the comprehensive analysis of FLG, we have identified five unique mutations and one recurrent mutation in Singaporean Chinese IV patients. Mutation 441delA is located in the profilaggrin S100 domain, representing an almost completely null allele, while two additional frameshift mutations, 1249insG and 7945delA, occur just upstream in the first partial filaggrin repeat ("repeat 0") and in filaggrin repeat 7, respectively. Both nonsense mutations Q2147X and E2422X are found in filaggrin repeat 6 whereas R4307X was found on one of the longer size variant alleles of FLG, within duplicated repeat 10.2. Mutation E2422X, previously found in a single Dutch patient, was found in one Singaporean IV patient and at a low frequency in Asian population controls. Interestingly, there was no obvious difference in the ichthyosis phenotype between patients with the 5′ mutations compared to those with more 3′ mutations, consistent with existing biochemical data showing that profilaggrin truncation leads to complete loss of processed filaggrin regardless of the position of the premature termination codon mutation. This study confirms the presence of population-specific as well as recurrent FLG mutations in Singapore.

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Bone marrow stem cells improve the survivability of RDEB mice but do not correct the fundamental type VII collagen defect

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Mesenchymal stem cells (MSC) can differentiate into a variety of cell types, offering promising cell-based and gene-based therapy for human diseases. Bone marrow derived MSC may serve as a source of skin progenitor cells and contribute to healing skin wounds. MSC are isolated from a small aspirate of bone marrow, are less immunogenic, and can be easily expanded in vitro. Recessive Dystrophic Epidermolysis Bullosa (RDEB) is caused by mutations in the type VII collagen (C7) gene. We have previously shown the feasibility of fibroblast-based cell therapy for DEB. In this study, we evaluated the feasibility of MSC-based cell therapy in a C7 null RDEB mouse which recapitulates the clinical, immunohistochemical, and ultrastructural characteristics of RDEB in humans. MSC were isolated from bone marrow of wild type litter mates and expressed C7 in culture, albeit with much lower level than fibroblasts. Interestingly, MSC-derived C7 was not secreted into the medium as an extracellular matrix. We intradermally or intravenously injected MSC daily for 6 days into the skin of 1 day old RDEB mice (N = 20) who had blisters on their paws, skin and mucosa. Skin biopsies were obtained at various times after injection and subjected to immunostaining using an antibody specific for human C7. We found that MSC-based cell therapy improved the RDEB phenotype with decreased skin fragility, a reduction in new blister formation, and markedly prolonged survival. Histology analysis in the injected skin showed intact dermal-epidermal adherence, essentially similar to normal mouse skin. 40% of the injected mice displayed an increased survival up to 4 months. However, immunostaining of the animal's skin did not reveal C7 at the basement membrane zone. Further, anti-C7 antibodies were also not found in the sera or skin of the treated mice. Our data suggest that MSC-based cell therapy improves the RDEB phenotype without correcting the fundamental C7 defect. The mechanism by which this happens is currently unknown.

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Keratin K6c mutations cause focal keratoderma

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Dominant mutations in keratins K6a, K6b, K16 or K17 cause pachyonychia congenita (PC), characterized by hypertrophic nail dystrophy and other ectodermal features, of which focal palmoplantar keratoderma (FPPK) is the most painful, debilitating aspect. Although mutations in K16 have been reported in families presenting with FPPK, either alone or with trivial nail changes, many FPPK families do not harbor mutations in any of the 4 PC-related keratins. Here, we report two families who presented with an autosomal dominant history of FPPK. Affected members of one family had minor nail changes, whereas the other family had none. The four PC-related keratin genes were excluded by DNA sequencing of all exons and splice sites. Mutation analysis of the third keratin 6 gene, KRT6C, encoding keratin K6c, revealed a three base pair heterozygous missense mutation, p.Asn172del, which fully co-segregated with FPPK in both families. Interestingly, 3/160 anonymous controls were heterozygous carriers of the mutation. We speculate that this K6c mutation may exist at a low level within the general population but does not often come to clinical attention. Thus, these K6c mutations may be a common genetic predisposing factor for plantar callus formation and a population-based prospective study is ongoing to confirm this hypothesis. The same p.Asn172del mutation has been reported in both K6a and K6b in typical PC cases having both severe FPPK and severe nail dystrophy. The expression pattern of K6c is unknown, however, the phenotype observed in our two families strongly implies overlap with K6a and K6b. The milder phenotype in the K6c FPPK families, plus the fact that some individuals in the "normal" population carry these mutations, implies that K6c is expressed at much lower levels in thick skin and nail than K6a and K6b. This data expands the genetics of keratoderma and sheds light on the function of emerging keratin genes.

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Inactivating mutations in autosomal recessive congenital ichthyosis: extended allelic heterogeneity for mutations in *ALOX12B* and recurrent mutations in *ALOXE3*

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Autosomal recessive congenital ichthyosis (ARCI) represents a group of severe keratinization disorders characterized by extended clinical and genetic heterogeneity. Besides *TGM1*, several new genes underlying ARCI were identified recently, including *ABCA12*, *ichthyin*, *CYP4F22*, and the lipoxygenase genes *ALOX12B* and *ALOXE3*. Mutations in *ALOX12B* and *ALOXE3*, coding for 12R-LOX and eLOX-3 predominantly expressed in the epidermis, are the second most common cause for this rare condition. To discover mutation distribution in ARCI, we analysed 350 unrelated patients, mostly originating from central Europe. We found mutations in *TGM1* in one third and in *ALOX12B/E3* in another 12% of the cases. In total, we identified 18 different mutations in *ALOX12B*, including 8 novel mutations. In *ALOXE3* we found 6 different mutations including 3 novel ones. None of the mutations in *ALOX12B* was seen on more than 4 alleles; in contrast, two mutations in *ALOXE3*, Arg234X and Pro630Leu, were found on 11 and 15 different alleles, respectively. For all missense mutations and one splice site mutation, we demonstrated complete loss of function of the mutant protein. Still only little is known about the pathophysiology of ARCI. The involvement of epidermal lipoxygenases, which are subsequent members of the 12-lipoxygenase pathway, in the formation of the epidermal barrier is now further analysed using expression and functional studies in patient keratinocytes, skin equivalents, and a mouse model. The characterization of allelic heterogeneity in *ALOX12B* and two mutational hotspots in *ALOXE3* is important for analysing the epidemiology of ARCI and may point to different functional domains. Moreover, it will be implemented into further automation of diagnostic procedures in ARCI and related disorders.

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Analysis of 3D skin models mimicking a syndrome of ichthyosis, follicular atrophoderma, and hypotrichosis

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Autosomal recessive congenital ichthyosis encompasses a large, heterogeneous group of disorders of cornification. Isolated forms and syndromic ichthyosis can be differentiated. We have recruited two consanguineous families with a similar phenotype of ichthyosis, follicular atrophoderma, and hypotrichosis from the United Arab Emirates and Turkey. Histopathologically, the epidermis was of regular thickness, with the stratum granulosum thinned and the stratum corneum orthohyperkeratotic. Hair follicle epithelium was thinned, hair infundibulum showed hyperkeratosis and a very thin stratum granulosum. EM analysis showed deposits of lamellar bodies in the lower parts of the stratum corneum. After genome wide linkage analysis we identified mutations in the suppression of tumorigenicity 14 gene (*ST14*). We found a homozygous splice site mutation (c.2269+1G>A) in the Emirati patients and a 1-base deletion (c.2034delG) in the Turkish patient. Furthermore, Western blot analysis showed reduced proteolytic activation of prostasin and loss of processing of profilaggrin. Since filaggrin monomers play a pivotal role in epidermal barrier formation, we suggest that matriptase acts upstream of prostasin in a zymogen activation cascade that regulates terminal epidermal differentiation. In order to investigate the role of these proteins in the pathophysiology of this syndrome, we have established 3D skin models with primary keratinocytes and fibroblasts from patients and healthy donors. The primary control keratinocytes were transfected with siRNA to knock down expression of *ST14*, *PRSS8*, and *FLG*, respectively. After seven days, the 3D skin models were harvested and the knock down efficiency was tested by quantitative RT-PCR. Samples taken from transfected keratinocytes and 3D skin models are now compared to each other and untransfected controls and further analyzed.

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A mouse model for AEC syndrome

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Dominant mutations in p63 cause Ankyloblepharon Ectodermal Dysplasia and Clefting (AEC), an ectodermal dysplasia characterized by skin fragility. To understand the molecular pathology underlying skin fragility in AEC patients, a prerequisite for the development of new therapeutic approaches that target the molecular defects in this disease, we genetically engineered a mouse model for AEC. Since ΔNp63α is the predominantly expressed p63 isoform in postnatal skin, we hypothesized that mutant ΔNp63α proteins are primarily responsible for skin fragility in AEC patients. Indeed, downregulating ΔNp63 expression in mouse epidermis resulted in skin lesions that mimicked those observed in AEC patients. Lesional skin showed defects in keratinocyte differentiation characterized by a failure of keratinocytes to withdraw from the cell cycle and to induce suprabasal differentiation markers. In addition, we observed abnormal staining for basement membrane components, indicating basement membrane abnormalities. To demonstrate that the lesions in our mouse model faithfully replicate the histopathology of AEC, we compared the histopathology of the mouse model with that of AEC patients (21 patient samples were included in this analysis). We observed suprabasal proliferation (21/21), delayed terminal differentiation (17/21), and basement membrane abnormalities (18/21) in these patients. Since the lesions that develop in our mouse model and in human AEC patients are indistinguishable on the cellular level, we conclude that our mouse model is a reliable model to further study the molecular mechanisms that cause skin fragility in AEC patients. Furthermore, this mouse model is ideally suited to develop and test new therapeutic approaches.

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A novel heat shock protein autocrine mechanism that mediates hypoxia-driven human keratinocyte migration

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Hypoxia plays a critical role in the pathogenesis of a variety of human diseases such as ischemic cardiovascular disease and cancer. On the other hand, after an acute skin injury, the microenvironment is extremely hypoxic due to the clotting of blood vessels and high oxygen consumption by cells in and around the wound site. We have shown that acute hypoxia is a potent stimulus to increase the migration of human keratinocytes (HKs). However, the mechanisms by which acute hypoxia promotes HK motility are unknown. The purpose of this study was to elucidate the mechanisms of hypoxia-driven HK migration. We found that hypoxia, via a hypoxia inducible factor 1 (HIF-1) mediated signaling, induces HKs to secrete heat shock protein 90-alpha (hsp90α) which, in turn, binds to the LRP-1/CD91 cell surface receptor in HKs and stimulates HK migration in an autocrine loop fashion. We expressed a constitutively activated form of HIF-1 in HKs by lentiviral infection and could completely mimic hypoxia-driven motility in normoxic HKs. In contrast, expression of either a dominant negative HIF-1 or siRNA down-regulation of HIF-1 completely blocked the hypoxia-driven motility. Further, the exogenous addition of recombinant hsp90α to normoxic HK motility assays fully duplicated the pro-motility effect of hypoxia. The hypoxia-HIF-1 pathway-induced hsp90α secretion required no changes in the steady-state mRNA level or in the promoter activity of hsp90α. However, inhibition of either the exosomal protein trafficking or the extracellular hsp90α blocked hypoxia-induced and HIF-1-driven HKC migration. We found that Hsp90α binds to the LRP-1/CD91 receptor in HKs and genetic silencing LRP-1/CD91 completely blocked hypoxia-triggered HKC migration. Topical application of hsp90α accelerated wound healing by increased re-epithelialization in mice. This study has unveiled a previously unrecognized mechanism of hypoxia > HIF-1 > hsp90α secretion > CD91 receptor > skin cell migration > wound healing.

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Analysis of the prodynorphin promoter polymorphism in atopic dermatitis and disease-related pruritus

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Pruritus is one of the key symptoms in atopic dermatitis (AD). The prodynorphin polypeptide is a precursor protein of pruritus-modulating opioid peptides. It is encoded by the prodynorphin gene (PDYN) on the short arm of chromosome 20. A VNTR (Variable Nucleotide Repeat) polymorphism, composed of one to four repeats of a 68 base pair (bp) element, which contains one binding site per repeat for the transcription factor AP-1, has been identified in the promoter region of the PDYN gene. To investigate associations of the PDYN promoter polymorphism with the intensity of pruritus in AD patients we genotyped 211 Austrian AD patients and 197 non-atopic controls. No statistical significant association of the PDYN promoter polymorphism with AD in general was revealed when comparing AD patients with controls. The analysis of possible associations with pruritus intensity also showed no relevant difference in the allelic distribution between patients with different pruritus-score values. These data point against an important role of the PDYN promoter polymorphism in AD in general and in the development of disease-related pruritus, although a weak effect cannot be excluded due to our small sample size. Additional studies are needed to further evaluate the PDYN polymorphism's influence in pruritus.

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Transcriptional study of p63alpha mutants found in ectodermal dysplasia syndromes

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The ectodermal dysplasia (ED) syndromes are a group of inherited autosomal dominant human diseases. The prototypic Ectrodactyly, Ectodermal dysplasia and Cleft lip/palate (EEC) syndrome is clinically characterised by ectodermal dysplasia affecting skin, hair, nails teeth and facial clefts. Heterozygous mutations in the p63 gene have been identified in EEC syndrome patient and in other ED syndromes, including Ankyloblepharon-Ectodermal Defect-Cleft Lip and/or Palate (AEC), Limb-Mammary Syndrome (LMS), Acro-Dermato-Ungual-Larimal-Tooth syndrome (ADULT), and Split hand Foot Malformation (SHFM). The majority of the p63 mutations in EEC-like syndromes involve residues present in DNA binding domain (DBD), the SAM domain (SAM) and the transactivation inhibitory domain (TID domain). Generally mutations in the DBD abolish p63 DNA binding ability. Mutations in the SAM domain are supposed to abolish protein-protein interaction, while mutations in the TID domain are predicted to impair the suppressive effect of the TID toward the TA domain, thus increasing the transactivation activity. Here, we have performed a systematic study of the transcriptional activity of different p63 mutants using different promoters: skin-specific (K14, IKKalpha, BPAG, EVPL), apoptotic (BAX) and cell cycle (p21). In addition, we have evaluated the ability of these mutants to induce cell cycle arrest and apoptosis. From the results obtained, we conclude that: i) there is a clear difference in the transcriptional activity of SAM and TID p63 mutations on skin-specific and apoptosis-related promoters; ii) the specific activity of TAp63alpha and DeltaNp63alpha on skin-specific promoters depends on an intact SAM domain; iii) the TID mutations mostly increase the activity of p63alpha on epidermal promoters. In addition, while TAp63alpha, and its active mutants, play a role in regulating cell cycle and in inducing apoptosis. DeltaNp63alpha does not affect apoptosis and cell cycle.

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Chromatin dynamics in the regulation of the CTLA-4 gene

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CTLA-4 is a co-stimulatory molecule which negatively regulates T cell activation. The gene is inducible in normal T cells and is constitutively expressed in Treg cells. We have shown that the CTLA-4 gene is upregulated in mycosis fungoides. In studying the transcriptional regulation of this gene, we have demonstrated that NFAT1 binds the CTLA-4 proximal promoter in human T cells. The dependence of NFAT1 in mediating promoter activation by remodeling chromatin is unclear. We hypothesize that NFAT1 is one of the earliest steps in activating CTLA-4 expression and binds the promoter to recruits cofactors needed for DNA and chromatin remodeling. From studies using the adenovirus E1a product, we show that NFAT is important for recruiting p300/CBP histone acetylae activity. The role of NFAT1 in remodeling the chromatin is unclear. Using DNase1 sensitivity assay in human PBMC, we find that the proximal promoter becomes accessible upon stimulation with PMA/calcium ionophore. Promoter remodeling is dependent on DNA unwinding mediated by topoisomerase activity. Using inhibitors of topoisomerase, camptothecin and etoposied, we find that activation of the CTLA-4 promoter is dependent on DNA unwinding mediated by Topoisomerase I. These findings lead to a model where the earliest step in CTLA-4 activation is dependent on NFAT activation, follwed by nuclear translocation, promoter binding, chromatin remodeling and histone modification. The elucidation of these steps in the activation of CTLA-4 transcription may lead to the development of agents that can modulate its expression specifically to alter immune response in disease.

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The genetic architecture and population genetics of filaggrin-related atopic dermatitis

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We recently showed that filaggrin null mutations cause ichthyosis vulgaris (IV) and are a strong predisposing factor for atopic dermatitis (AD). In white European populations, there are both prevalent and rare filaggrin mutations. Here we report further European mutations as well as our ongoing analysis of other ancestral groups. We screened an expanded case series of 262 Irish children with dermatologist diagnosed moderate-to-severe AD for the 5 known prevalent mutations. Comparing the combined allele frequencies for these mutations in the AD cases versus 736 ethnically matched controls gave a highly significant association (chi-square $p=5.6x10^{-63}$; heterozygote OR=8.21, 95%CI 6.61-12.03; homozygote OR=169, 95%CI 23-1243). We also selected AD patients from this cohort where features of IV were indicative of additional unknown filaggrin variants. Full sequence analysis in these cases identified 3 further mutations: S2080X and Y2092X in filaggrin repeat 5; and S3296X in repeat 9. Mutation Y2092X was recurrent in the Irish population. This brings the number of European filaggrin mutations to 16, of which 6 are prevalent and 10 are of low frequency. In Far Eastern populations, Japan, China and Singapore, we have so far identified 13 mutations, of which 5 are prevalent and 8 are uncommon. Preliminary analysis of a small number of patients has also identified 4 additional mutations: 2 in the Indian population, and 1 mutation each in Middle Eastern and African patients. With only two exceptions, both involving single cases, the 31 known filaggrin mutations are race-specific. In the Japanese population, cases:control association analysis showed Asian-specific mutations to be strongly associated with AD as well as causing IV and therefore the filaggrin-AD association replicates across races. Overall, the filaggrin gene is emerging as an important player in atopic disease worldwide.

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A genomics approach to identify cis-regulatory elements in the Epidermal Differentiation Complex (EDC)

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Combining a comparative genomics approach with cell-based reporter assays, we have identified 17 cis-regulatory elements (enhancers and repressors) in the Epidermal Differentiation Complex (EDC) locus. Located on chromosome 1q21, the EDC shares linkage to two inflammatory skin diseases, atopic dermatitis (AD) and psoriasis, and contains a majority of genes that are specifically expressed upon epidermal differentiation and barrier formation. Recently, mutations in filaggrin gene, FLG, in the EDC have been strongly associated with the atopic march of AD to asthma. Linkage studies excluding FLG mutations continue to demonstrate linkage to the EDC suggesting additional genetic variants within the EDC in AD pathogenesis. The proximity and density of genes in the EDC suggest coordinate regulation for temporal and spatial expression via cis-regulatory elements. Our goal is to identify cis-regulatory elements that control gene expression in the EDC. We hypothesize that evolutionarily conserved noncoding sequences (CNS) function as enhancers to coordinate transcriptional regulation of the EDC genes. A genomics approach using comparative multi-species sequence analysis (MultiPip) of the EDC loci from human, chimp, rhesus, mouse, rat, dog, and opossum genomes has identified 43 CNS as enhancer candidates. To date, roughly 40% of the CNS demonstrate either enhancer or repressor activity based on dual luciferase reporter assays in differentiating keratinocytes. We are currently correlating these studies with DNaseI hypersensitivity mapping in the CNS. Our results suggest that the many of the conserved noncoding sequences function as cis-regulatory elements to coordinate expression of the genes in the EDC. Taken together, our study will shed light on the genetic and epigenetic mechanisms underlying epidermal differentiation and barrier formation as well contribute to our understanding of atopic dermatitis and psoriasis.

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Studies on p63 ECC-related mutants protein stability and degradation by Itch

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The EEC-related syndromes are a group of inherited autosomal dominant human diseases. The prototypic Ectrodactyly, Ectodermal dysplasia and Cleft lip/palate (EEC) syndrome is clinically characterised by ectodermal dysplasia affecting skin, hair, nails teeth and facial clefts. Mutations in the transcrption factor p63 have been identified in EEC syndrome patients and in other ectodermal dysplasia syndromes, including Ankyloblepharon-Ectodermal Defect-Cleft Lip and/or Palate (AEC), Limb-Mammary Syndrome (LMS), Acro-Dermato-Ungual-Larimal-Tooth syndrome (ADULT), and Split hand Foot Malformation (SHFM). p63 mutations in EEC syndromes involve residues present in p63 DNA binding domain (DBD); mutations in the SAM domain (SAM) are found in AEC, while mutations in the transactivation inhibitory domain (TID domain) cause SHFM. The pattern of mutations has revealed p63 protein domains that are particularly important for skin development (the C-terminal SAM domain, see below), whereas other domains are crucially important for limb development (the DNA binding domain, TID domain, see below). We present a systematic study on p63 EEC-related protein stability and their degradation by the E3 ligase Itch. The results obtained indicate that: (i) SAM domain, TID and DBD mutations of DeltaNp63alpha are degraded normally by Itch; (ii) SAM domain and DBD mutations of DeltaNp63alpha results in increased stability that is Itch-independent; (iii) the increased stability of DeltaNp63alpha DBD mutants can be reversed by co-expression of excess wt DeltaNp63alpha; (iv) SAM domain mutations destabilize the TAp63alpha isoform by increasing interaction with Itch; (v) TAp63alpha TID mutations of increase stability by reducing Itch binding. The study on p63 mutant's stability suggest that there are at least two main pathways for p63 degradation: one that is Itch-dependent for both isoforms (TAp63 and DeltaNp63) and a novel DeltaNp63 degradation pathway dependent on DeltaNp63 transcriptional activity.

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Global gene expression analysis reveals evidence for abnormal lipid biosynthesis in uninvolved psoriatic skin

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Psoriasis is a common skin disease characterized by inflamed scaly skin lesions, nail changes and commonly arthritis. It has been well documented that the transition from normal (NN) or uninvolved (PN) into diseased lesional (PP) skin is accompanied by changes in the expression of multiple genes, but much less is known about the gene-expression status of PN as opposed to NN skin. Biochemical and morphologic changes were reported decades ago in PN skin but remain poorly understood. To globally evaluate gene expression paterns in PP vs PN vs NN skin, we extracted total RNA from punch biopsies taken from 58 psoriatic patients and 64 normal healthy controls. Samples were run on Affymetrix HU133 Plus 2.0 microarrays containing >54,000 gene probes. 793 probes were significantly (>2-fold, $p < 0.05$) up-regulated in PP vs. NN skin, while 533 were down-regulated. Sampling PN skin located at least 10 cm from any lesion, we identified 50 probes whose expression was significantly (1.5-fold, $p < 0.05$) altered in PN vs. NN skin, 49 of which were not previously known to be altered. The proteins encoded by these transcripts are involved in lipid and sterol metabolism, antimicrobial peptides, epidermal differentiation and control of cutaneous vasculature. Cluster analysis of the 50 probes detecting significantly altered expression in PN vs. NN skin identified a group of 8 genes involved in lipid and sterol metabolism ($p < 0.036$). These genes were strongly coordinately expressed in NN, PN, and PP skin, and displayed significant progressive down-regulation (NN > PN > PP) which was confirmed for ALOX15B and ELOVL3 by QRT-PCR ($p < 0.05$, $n = 25$). Taken together our results identify a "pre-psoriatic" gene expression signature, and suggest the hypothesis that the observed coordinate regulation of lipid biosynthetic genes may be related to the action of specific transcription factor(s).

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Atrichia with Papular Lesions resulting from a novel point mutation in the human hairless gene in a Korean boy

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Atrichia with papular lesions (APL) is a rare autosomal recessive disease characterized by early onset of atrichia, followed by papular eruption within the a few years of life. Mutations in the hairless(HR) gene have been implicated in the pathogenesis of this disorder. Recent studies demonstrating linkage to chromosome 8p12 and further mutation in human hairless gene have established. The proband was a 4 years old male who was born with normal scalp hairs, sparse eyebrows and eyelashes, but the scalp hair was shed soon after birth and never regrows. The scalp hair was completely shed by age 3 years. Physical examination revealed total absence of scalp hair with sparse eyelashes and eyebrows. Numerous papules were located on the face, nape, knees, and thighs. Biopsy of scalp skin failed to show normal hair follicular structures and biopsy of a papule revealed many keratinous cysts in the middermis. We collected blood samples from proband and his family to confirm the diagnosis. To differentiate with alopecia universalis (AU) and APL, gene analysis using peripheral blood samples of patient and 10 AU patients was done. To screen for a mutation in the HR gene, DNA extracted from the family's blood and then PCR amplified all exons of the human HR gene. Through DNA analysis, found it to have been to the point mutation which exon 5 of chromosome 8p12 base C was changed into to T. We also analyzed the band patterns of exon 5 (chromosome 8p 12-21) using restriction enzyme BseRI. Therefore, once confirmed again that exon 5 of chromosome 8p12 was to point mutation. And the point mutation occurred, and the 101st base of exon 5 of chromosome 8p12 should be normally composed was changed proline into leucine (P101L). Hairless gene of the all AU patients was normal. In this study, we discovered a novel missense mutations in exon 5 of the human HR gene in a Korean family affected with APL.

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Epigenetic analysis of the T-plastin gene in mycosis fungoides/cutaneous T cell lymphoma (MF/CTCL)

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Differential gene expression analysis has identified T-plastin as a gene highly expressed in malignant lymphocytes in mycosis fungoides/Sezary syndrome (MF/SS). T-plastins are proteins that have actin bundling functions with 2 major isoforms, L-plastin and T-plastin. There is tissue specific distribution of plastins with T-plastin restricted to epithelial and mesenchymal cells, whereas L-plastin is found in hematopoietic cells. This suggests the presence of cell-type specific factors that control the expression of T-plastin gene. The significance of T-plastin in MF/SS is not known, however the consistent expression in SS T cells suggest a specific mechanism important in the development of MF/SS. We have found that MF/SS PBMC can have greater than 500 fold higher expression of T-plastin than normal peripheral blood cells. In analysis of cell lines, human 293 cells and HeLa cells express T-plastin, but not in T cell derived lines such as Jurkat T cells or lymphoid CEM cells. We hypothesize that epigenetic mechanisms activate the T-plastin gene in MF/SS. In studies of histone modifications, we have found a lower level of transcriptionally active acetylated histone H3 in cells that do not express T-plastin. We believe that chromatin conformation differs between cells that express T-plastin and cells that can not express this gene. One approach to studying chromatin conformation is to determine DNaseI accessibility. Furthermore, we will analyze the role of changes in CpG methylation as a mechanism for the activation of the T-plastin gene in MF/CTCL. The results from these studies will reveal potential genetic regulatory mechanisms that play a role in the pathogenesis of MF/CTCL.

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Genome-wide linkage analysis of dermatitis herpetiformia in celiac disease

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Dermatitis herpetiformis (DH) is an intensely pruritic papulo-vesicular cutaneous eruption occurring on extensor surfaces and is precipitated by ingestion of gluten. DH is known to be associated with gluten sensitive enteropathy (GSE) but the intestinal inflammatory process in DH is generally less severe than that seen in symptomatic celiac disease(CD). In order to determine if specific susceptibility loci for families with DH and CD could be identified, we conducted a genome-wide linkage analysis in 35 CD families with at least one case of DH. As part of an ongoing genetic linkage study of CD, we have collected 180 informative families with an average of 4 cases of CD per family and genotyped them for a genome-wide panel of 418 microsattelite and/or 10,000 SNP genetic markers. Among these 180 families, there were 40 cases of DH in 35 families. 20 of these DH cases had no other clinical manifestations of CD. Because of the importance of the MHC in CD and all autoimmune diseases, we performed conditional linkage analysis, conditioning the LOD score at other regions of the genome on HLA linkage. This procedure yields an estimate of α_1, the proportion of HLA linked families linked to a given locus, and α_2, the proportion of families unlinked to HLA in the sample that are linked. Three genomic regions with evidence in favor of linkage of a CD/DH susceptibility locus of >20:1 were identified: chromosome 2p (position 22.5cm, HLOD=1.44); 2q (211 cM, 1.59); and 20q (98.2 cM, 1.97). The locus on 2p was equally implicated in HLA-linked and HLA-unlinked families while the 2q locus was estimated to be involved in 36% of HLA-unlinked families only while 100% of HLA-linked families were linked to the 20q locus. These analyses indicate possible susceptibility loci for CD that may differ from other CD loci in that they also confer susceptibility to DH. Further work is necessary to validate these loci and to identify the genes responsible.

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Development of a PCR-based quantitative clinical endpoint to determine the effectiveness of siRNA therapy in a phase 1b trial in pachyonychia congenita patients

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Pachyonychia congenita (PC) is a debilitating dominant negative skin disorder caused by mutations in the inducible keratins K6, K16 or K17. The defective genes are expressed on or near the pressure points of the foot and result in keratoderma, blistering and debilitating pain. We have recently identified a siRNA (TD101) that is capable of potent silencing of mutant K6a N171K with no effect on wildtype expression despite only a single nucleotide difference (Hickerson et al., J. Invest. Dermatol., in press). A double-blind Phase 1b clinical trial using TD101 to locally treat PC foot lesions (by intradermal injections) of all known, eligible and willing U.S. PC patients harboring the K6a N171K mutation will begin in January, 2008. This clinical trial is anticipated to be "first-in-man" for siRNA skin treatment as well as the first siRNA trial to target a mutant gene. In preparation for human clinical trials using TD101, a reverse transcriptase quantitative PCR (RT-qPCR) assay was developed that discriminates between wildtype and mutant K6a mRNAs. In addition, an efficient method to isolate RNA from PC patient and control volunteer calluses was developed. RT-qPCR analysis of RNA isolated from callus tissue collected from N171K patients revealed equal expression of wildtype and mutant K6a mRNAs, while only wildtype mRNA was detected in samples from patients with a different PC mutation or control volunteers. These results establish that RNA can be isolated from patient skin shavings and that this RNA can be effectively used in an allele-specific RT-qPCR assay. This molecular endpoint will be used for monitoring the efficacy of TD101 in the upcoming pachyonychia congenita siRNA clinical trial.

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***BHD* mutations, clinical and genotype-phenotype investigations of 351 cases with Birt-Hogg-Dubé syndrome**

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Birt-Hogg- Dubé syndrome (BHDS) (MIM 135150) is an autosomal dominant predisposition to the development of cutaneous hamartomas (fibrofolliculomas and angiofibromas), lung cysts, spontaneous pneumothorax, and kidney neoplasms. Germline mutations in the *BHD* gene are associated with the susceptibility for BHDS. To date, the NCI has reported 266 patients with BHDS from 102 families, and 36 unique *BHD* germline mutations including 21 insertion/deletions, eight putative splice site, six nonsense and one missense mutation. To date, 53 unique germline *BHD* mutations have been reported in a total of 351 BHDS patients worldwide. Sixty-eight patients with BHD germline mutation and kidney tumors have been reported in the literature. Only five BHDS patients with *BHD* mutations who died of metastatic kidney cancer have been reported to date. These individuals with metastatic kidney cancer had tumors with clear cell, tubullo-papillary and/or papillary histologic features. These histologic features are slightly different from most kidney tumors associated with BHDS. It is of interest that even though 95% of patients with BHD germline mutations and kidney tumors have been reported by the NCI, only two cases from the NCI group has developed metastic kidney disease. In contrast, only a few BHDS patients with a *BHD* germline mutation have been reported by other investigators to have kidney tumors, and most (3 of 4) of these cases had metastatic disease. These differences may be a reflection of the different ascertainment, and screening methodologies for kidney tumor used by different research groups. Overall, 77%-89% and 33-40% of patients with *BHD* germline mutations have been reported to have pulmonary cysts and history of spontaneous pneumothorax, respectively. The results of a meta analysis evaluating potential genotype-phenotype correlations including all 351 BHDS cases reported with a *BHD* germline mutation will be also presented. BHDS is characterized by a spectrum of mutations, and clinical heterogeneity both among and within families.

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Transdermal gene delivery using RTP001

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Molecular imaging with bioluminescence reporter genes requires delivery across biological barriers. Here, we assess a novel cationic peptidyl macromolecule transport systems (MTS), RTP001, to delivery noncovalently associated anionic cargo for transcutaneous gene delivery. To show in vitro transfection of a large complex, HeLa cells (n=5 per group) were treated for 48 hours with RTP001carrying an ionically associate plasmid expressing blue fluorescent protein (BFP). Efficiency was determined by the percentage of total area with positive BFP expression and toxicity was determined by dye exclusion assay. Results demonstrate up to 14.66X higher in vitro transfection efficiency with MTS treatment versus K control ($P<0.05$) and 107.82X versus superfect control ($P<0.05$). In addition, to show in vivo transcutaneous delivery, the dorsal skin of C57blk/6 mice (n=4 per group) was topically treated with RTP001 carrying plasmid expressing β-galactosidase (β-gal). The treated skin was then harvested at 24 hours. The harvested skin samples were stained for β-gal and were assessed for positive staining using a blinded image analysis of photomicrographs. Efficiency was determined by percentage of β-gal positive cells per total cells and toxicity was determined by dye exclusion assay. Results show delivery across epidermis and dermis with 500X higher in vivo transdermal gene delivery with MTS treatment versus K control ($P<0.01$) and 12.10X versus superfect control ($P<0.01$). Both experiments showed lower toxicity versus controls. We show that RTP001 mediates functional in vitro gene delivery across the cell and nuclear membranes with high efficiency and is capable of functional in vivo transdermal gene delivery across multiple layers of the dermis with high efficiency as well. These studies demonstrate the feasibility of using this system for delivery of reporter genes and other anionic cargo across biological barriers without covalent modification of cargo and combining it with other functional moieties such as targeting.

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Use of pachyonychia congenita patient-derived keratinocytes to 1) demonstrate the effectiveness of therapeutic TD101 siRNA and 2) generate a humanized mouse disease model

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A Phase 1b clinical trial for pachyonychia congenita (PC) will commence in Jan., 2008, using the therapeutic siRNA TD101 (MUT.12 in Hickerson et al., J. Inv. Derm., in press). In preparation for the clinical trial, HPV E6/E7-immortalized cell lines were prepared from keratinocytes derived from PC patients harboring the keratin 6a (K6a) N171K (PC-10) or K16 N125D (PC-2) mutations. Treatment of PC-2 cells with TD101 (targets the N171K mutation) potently and specifically inhibited expression of K6a N171K mutant mRNA with no effect on wildtype K6a expression or other related keratins including K6b, K16 or K17 as assayed by qPCR. Inhibition began within 3 h with peak inhibition at 36 h (>97%) and lasted for >72 h. Importantly, retreatment at 120 h resulted in renewed full inhibition and repeated treatment every ~5 days allowed continuous inhibition for 30 days (duration of experiment). A humanized skin PC disease model was developed, based on permanent engraftment of bioengineered skin equivalents in immunodeficient mice. In this study, keratinocytes and fibroblasts isolated from skin biopsies of PC patients carrying the K6a N171K mutation were cultured, assembled in fibrin-based bioengineered skin equivalents and grafted. While the regenerated, PC cell-derived human skin appeared normal, the use of a hyperproliferative stimulus, i.e. tape stripping, induced PC features such as acanthosis and large rete ridges. A time-course analysis of hyperproliferation-associated changes including K6 protein expression and BrdU/Ki67 incorporation clearly demonstrated that the response of engrafted PC skin was greater and more sustained than that of normal (regenerated) human skin. PC skin-engrafted mice from a single patient biopsy will enable the testing of novel pharmacological or gene-based medicines including siRNAs.

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***In vivo* delivery of functional macromolecules including siRNAs to skin by novel soluble-tip microneedle arrays**

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Despite a wealth of exciting developments in potential therapeutics for skin disorders, transfer to the clinic is hampered by difficulties in delivering these agents locally to appropriate skin cells. For example, the stratum corneum is a formidable barrier to delivery of functional short interfering RNAs (siRNAs) in particular, and to macromolecules in general. Intradermal injection is an effective method for targeted delivery of macromolecules but is unpleasant and highly localized by nature. Keen interest has been generated in the field of transdermal drug delivery by the potential use of arrays of microneedles as a relatively painless method to deliver therapeutic agents. A novel method has been developed to produce arrays of hollow microneedles. These needles dissolve upon insertion into the skin, forming a gel-like plug that subsequently releases functional macromolecules into either the dermis or epidermis, depending on the designed length of the needles. These microneedle arrays were designed to deliver fluorescently-tagged siRNA mimics (siGLO Red) *in vivo* as demonstrated by whole animal imaging as well as skin sectioning. Furthermore, firefly luciferase expression plasmids have been loaded into microneedle arrays and delivered to mouse skin and functional activity demonstrated by whole animal bioluminescence imaging following luciferin administration. In addition to nucleic acids, these microneedle arrays can be designed to deliver a large number of molecular entities including proteins, short peptides, and small molecules.

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Expression of IL-31 receptor alpha in epidermal keratinocytes depends on cell differentiation

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Recently, interleukin (IL)-31 has been identified as a 4-helix bundle cytokine that is expressed by activated T cells. While an enhanced expression of IL-31Rα has been described in inflammatory skin diseases, the influence of cell differentiation on the expression of the IL-31 receptor in skin cells was largely unknown. Therefore we analyzed the effects of the cellular differentiation state on epidermal mRNA and protein levels of IL-31Rα in culture conditions when epidermal cultures exhibit predominantly basal, spinous, and granular cell phenotypes. Interestingly, IL-31Rα mRNA completely disappeared during the process of differentiation and already at the transition from basal to spinous phenotype hardly any mRNA was detectable by quantitative RT-PCR. Expression of OSMRβ mRNA was decreased on day 5 but not significantly altered during the late phases of keratinocyte differentiation. Using an IL-31Rα specific antibody we confirmed a strong down-regulation of the receptor at the protein level during differentiation. Protein expression was not traceable in terminal differentiated keratinocytes resembling a granular cell phenotype. Additionally, we found no IL-31Rα expression in dermal fibroblasts or primary human melanocytes. Loss of receptor expression in late-stage keratinocytes consequently led to a loss of cytokine responsiveness: while proliferating keratinocytes respond to IL-31 treatment with increased tyrosine phosphorylation of STAT3, we could not detect any STAT activation in differentiated keratinocytes. In contrast, stimulation with OSM resulted in STAT3 tyrosine phosphorylation irrespective of the differentiation state which indicates that differentiated keratinocytes do respond to cytokine treatment in general. Therefore in vitro studies analyzing the effects of the novel cytokine IL-31 on epidermal keratinocytes should consider the variation of IL-31Rα expression which depends on the status of cellular differentiation.

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NFAT2 is involved in regulating keratinocyte proliferation and differentiation

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Nuclear factor of activated T cells (NFAT) proteins are calcium-responsive transcription factors thought to be implicated in regulating keratinocyte growth and/or differentiation. Immunostaining revealed nuclear localisation of NFAT2 in the basal as well as in high suprabasal keratinocytes in human epidermis. We therefore investigated if proliferation- and differentiation-inducing stimuli activate NFAT2 and if NFAT2 activation correlates with spatio-temporal characteristics of $[Ca^{2+}]_i$ flux in cultured normal human epidermal keratinocytes. $[Ca^{2+}]_i$ was assessed by measuring Fluo-4 fluorescence in real time. NFAT2 activation was studied by analyzing the nuclear/cytoplasmic ratio of GFP-NFAT2 and endogenous NFAT2 and by using luciferase assays. Lysophosphatidic acid (LPA), a phospholipid that binds to G protein-coupled receptors, is a known keratinocyte mitogen. Incubation with 10 μM LPA induced short (~100 sec) and strong (~4 fold) $[Ca^{2+}]_i$ transients in keratinocytes. Consistent with this $[Ca^{2+}]_i$ increase, early nuclear translocation of GFP-NFAT2 and endogenous NFAT2 was observed within 10 min of LPA treatment. NFAT-directed luciferase expression was significantly induced (~3 fold) after 120 min of LPA treatment. Retroviral transduction of keratinocytes with GFP-NFAT2 resulted in increased keratinocyte growth compared to GFP and also induced G_1 to S phase transition. Incubation with 1.2 mM $[Ca^{2+}]_o$, a well characterized differentiation stimulus, elicited a weak (~2 fold) and gradual (>10 min) $[Ca^{2+}]_i$ elevation. Later nuclear translocation of GFP-NFAT2 and endogenous NFAT2 was observed after 1h of increased $[Ca^{2+}]_o$. In contrast to LPA, 1.2 mM $[Ca^{2+}]_o$ induced a late ~3 fold increase in NFAT-directed luciferase expression after 18h of treatment. Taken together, our data show that NFAT2 is activated both by proliferation- and differentation-inducing stimuli. This suggests a dual role for NFAT2 in regulating both keratinocyte growth and differentiation, consistent with localization of NFAT2 in both basal and high suprabasal epidermal layers.

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Non-protein factor enhancement of keratinocyte clonal growth

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A patented protein-free serum-free basal medium (HECK109) was designed to support the serial propagation of normal human keratinocytes (NHK). It substitutes a physiological concentration of retinyl acetate for EGF (Wille, 2002). This medium is not optimal for low density growth of NHK compared with high-density culture. We report here enhancement of low density clonal growth by addition of several non-protein growth factors. Newborn NHK cells were plated at 500 cells per centimeter in serum-free HECK 109 basal medium supplemented singly with each of the following additives: dibutryl-cyclic AMP (d-camp, 1 x 10-4M), LiCl (10mM), and prostaglandin E1 (PGE1, 10 micrograms per mL)in media containing insulin(Ins) only or Ins plus EGF. All clonal growth dishes were fixed, stained and photographed ten days later. Significantly enhanced clonal growth occurred in dishes supplemneted with d-camp plus Ins, but not in dishes supplemented with d-camp plus Ins and EGF. By contrast, PGE1 significantly enhanced clonal growtrh if supplemented with either Ins only or both Ins and EGF. Finally, clonal growth was enhanced significantly by addition of Lithium ions either added with Ins alone or with Ins plus EGF. A second study examined the effect of d-camp, PGE1 and Lithium ions in HECK 109 medium containing elevated(3X) levels of six amino acids (His, Met, Phe, Tyr, and Tryp). Elevated amino acids had no discernible effect on enhanced clonal growth. The pairwise combination of Ins plus d-camp showed the best improvement in clonal growth.

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IL-24 mRNA-stability is regulated by p38 MAPK-dependent signaling

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Interleukin-24 (IL-24), a member of the IL-10 cytokine family, has been reported to selectively induce apoptosis in malignant cells, while leaving normal cells unaffected. Cellular sources of IL-24 include activated monocytes, T-cells, melanocytes and keratinocytes. The aim of the present study was to identify signaling pathways regulating IL-24 expression. IL-24 gene expression was investigated in normal human epidermal keratinocytes (NHEK) or mice skin using qRT-PCR, ELISA and Western blotting. Phosphorylation of kinases was assessed with Western blotting. Stimulation of NHEK with TPA (100 nM), IL-1β (10 ng/ml), or anisomycin (300 ng/ml) resulted in p38 MAPK activation and upregulation of IL-24 mRNA expression levels while pre-incubation with a p38 MAPK-inhibitor (SB 202190) before stimulation downregulated IL-24 mRNA expression to levels even below that observed in vehicle-treated cells. When blocking transcription (using actinomycin D) in NHEK pre-stimulated with anisomycin for 2 hours, IL-24 mRNA levels remained unaffected at a high level over the following three hours whereas treatment with SB 202190 of NHEKs pre-stimulated with anisomycin for 2 hours resulted in decreased IL-24 mRNA levels within 60 minutes. The p38 MAPK downstream kinase, MAPK-activated protein kinase 2 (MK2) expression was knocked down with siRNA in NHEK. Mean IL-24 mRNA levels in MK2-siRNA transfected, anisomycin-stimulated keratinocytes was 40% lower than that observed in keratinocytes transfected with control-siRNA and stimulated with anisomycin. Furthermore, TPA applications on the skin of mice induced IL-24 mRNA expression as well as p38 MAPK phosphorylation. It was observed that IL-24 mRNA levels in the skin of MK2 -/- mice on average were 45% lower than that observed in wild type mice. The findings of the present study suggest that p38 MAPK activation is essential for IL-24 mRNA expression and that the p38 MAPK promotes IL-24 mRNA stability through activation of MK2.

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Effect of urokinase type plasminogen activator receptor on the postburn wound inflammatory response

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Urokinase-type plasminogen activator receptor (uPAR) plays a role in cellular responses that include cellular adhesion, differentiation, proliferation and migration. The aim of this study was to examine the effect of uPAR on postburn wound inflammatory response. We examined the experimental burn in mice with a deficiency of uPAR. We found that the absence of uPAR attenuated postburn wound inflammatory response. In addition, the absence of uPAR attenuated burn induced inflammatory key regulatory genes expression. Moreover, we found that TNF-α induced inflammatory response in the explanted fibroblasts of uPAR -/- mice dramatically decreased compared to that in wild type mice. The absence of uPAR attenuated inflammatory response, thereby resulting in an attenuated-burn wound. Our results provide new insight into the role of uPAR on postburn wound inflammatory response.

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Keratinosyte-specific SOCS3 knockout mice show clinical phenotypes similar to human psoriasis

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STAT3 conveys signals to the nucleus upon stimulation with IL-6, EGF and many other growth factors. STAT3 plays critical roles in biological function such as cell proliferation, migration and so on. Suppressors of cytokine signaling (SOCS) regulate the strength of cytokine signals. SOCS3 is strongly induced by a variety of cytokines and other stimulators. The suppressive effect of SOCS3 has been shown to be relatively specific to STAT3. Therefore we investigated the role of SOCS3 in epidermal keratinocytes. Since germline targeting of the SOCS3 gene resulted in embryonic lethality, we generated keratinocyte-specific SOCS3-deficient mice (KS-SOCS3-/-mice) using Cre/loxP technology in combination with the keratin 5 promoter. KS-SOCS3-/-mice were viable and their skin appeared normal at birth. No histological alterations were found in skin of KS-SOCS3-/-mice, however, after 20 weeks, their skin was red and scaly, hyperkeratotic lesions developed in the ear, tail and the back skin in some mice. Histological analysis of skin lesions showed a marked hyperplasia, elongation of rete ridge, loss of granular layer and parakeratosis. These phenotype and histology show a strong resemblance to human psoriasis lesions. Next we utilized tape stripping which is a mild form of epidermal injury. KS-SOCS3-/-mice developed scaly lesions as early as 4days after stripping, whereas wild type mice showed a mild phenotype. These phenotype found in KS-SOCS3-/-mice prolonged until 14 days after stripping when wild type mice showed no obvious clinical phenotype. Tape stripping-induced lesions of KS-SOCS3-/-mice also showed histological feature of human psoriasis. Keratinocytes in the epidermis of KS-SOCS3-/-mice were highly positive for Ki67. Phosphorylated STAT3 staining were strong in KS-SOCS3-/-mice compared to wild type mice. In conclusion, loss of SOCS3, an endogenous inhibitor of STAT3, in epidermal keratinocytes leads to clinical phenotypes similar to human psoriasis.

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The importance of caspase-8 activation and the role of Bcl-2 in CD95 Signaling Pathway in squamous cell carcinoma cell line SCL-1

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The regulation of CD95/CD95L signaling transduction mechanism in cutaneous squamous cell carcinoma (SCC) is not fully understood. To investigate the effect of Bcl-2 and the importance of caspase-8 in the CD95/CD95L apoptotic pathway, we established the stably Bcl-2-overexpressed SCL-1 cell clone by gene transfection, and explored the proapoptotic signal transduction by C2-ceramide and anti-Fas agonistic antibody (CH-11). In Bcl-2 transfected SCL-1 cells, we found that Bcl-2 blocked C2-ceramide-induced apoptosis, in contrast, it had no protective effect from CH-11-induced apoptosis which followed by the downstream activation of caspase-8 and -3; furthermore, the caspases-8 inhibitor could effectively suppress apoptosis induced by CH-11. These results strongly suggest that apoptosis of SCL-1 cells through activation of caspase-8, -3 is almost independent on the Bcl-2-mediated mitochondrial pathway, which indicates that caspase-8 is a critical upstream caspase in CD95 apoptotic pathway. Our findings together with the others showed that after Bcl-2 blocked the mitochondrial pathway in HaCaT and SCL-1 cells, the induction of the apoptosis mainly depends on the activation of caspase-8 and its downstream transduction. Therefore, we proposed that differential CD95 signaling apoptotic pathways may be one of the biological behaviors between skin tumor derived from keratinocytes and cutaneous melanoma, and contribute to new therapeutic strategies for skin cancer.

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Psoriasis-like hyperplastic and inflammatory lesions produced by epidermis-specific, inducible activation of Raf in mice

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Raf is one of the downstream effectors of the Ras GTPases that induce epidermal hyperplasia. We characterized transgenic mice bearing the 4-hydroxytamoxifen (4OHT)-responsive mutant ER ligand binding domain-Raf fusion gene under control of the keratin 14 promoter. A single topical application of 4OHT induced the epidermis-specific expression of Phosphorylated active ERK 1/2, a downstream factor of Raf signaling, in those mice within 8-12 hrs. Massive hyperplasia with hyperkeratosis and suppressed differentiation of the epidermis developed within 7 days. Meanwhile, inflammatory cells infiltrated the skin within 24 hrs and even into the epidermis. IL-18, IL-15 and MCP-1 levels were increased in the skin lesions and IFN-γ and IL-17 were induced in T cells of the draining lymph nodes. Thus, the induction of Raf in the epidermis causes not only keratinocyte hyperproliferation but also psoriasis-like inflammatory conditions of the skin.

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Decreased expression of DKK-1 as pathogenesis of pachydermoperiostosis

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Pachydermoperiostosis (PDP) is an idiopathic disease characterized by the unique phenotype in the skin and bone manifesting as digital clubbing, pachydermia, seborrhea, dark skin, and periostosis, which occur mostly in men during adolescence. Therefore, PDP may be caused by factors common to the bone and the skin, such as bone morphogenetic proteins (BMP), transforming growth factor (TGF)-β and Wnt pathway-related proteins, which are presumably expressed in mesenchymal cells. To pursue the pathogenesis of PDP, we have focused on the role of dermal fibroblasts. The number of fibroblasts isolated from a PDP patient increased during culture much more than that from a healthy donor. Consistently, the proportion of PDP fibroblasts in cell cycle is significantly higher than that of control fibroblasts as assessed by DNA content using flowcytometry. Morphologically, phalloidin staining of PDP fibroblasts exhibited thicker actin bundles than control fibroblasts. A DNA microarray analysis of patient's and control fibroblasts was then performed. Among BMP, TGF-β, and Wnt families, only dysregulation of Wnt families was found, as the expression of mRNA for β catenin was dramatically elevated and that for dickkopf (DKK)-1 was markedly decreased. Accordingly, an immunohistochemical analysis showed enhanced β-catenin and decreased DKK-1 stainings in the skin of PDP. Moreover, the addition of recombinant human DKK-1 protein into PDP fibroblast culture decreased the frequency of fibroblasts in cycle. Considering that DKK-1 is an important Wnt-β catenin signaling antagonist and a well-known inducer of osteolysis, the decreased DKK-1 production may account for the enhanced Wnt-β catenin signaling and the resultant pachydermis and periostosis in PDP. Furthermore, the enhanced Wnt signaling is known to lead to seborrhea and hyperpigmentation. These findings suggest that the decreased expression of DKK-1 is the possible pathogenesis of PDP. No disease directly related to DKK-1 signaling has been reported. PDP could be a good disease model for evaluating the Wnt signaling in humans.

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CLIC4, in association with Schnurri-2 regulates TGF-β signaling in skin keratinocytes

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CLIC4 is a multifunctional protein that is upregulated and translocates to the nucleus under conditions of cell stress. CLIC4 induces growth arrest or cellular apoptosis upon nuclear translocation in skin keratinocytes. Since CLIC4 is excluded from the nucleus in skin cancer cell lines and mouse and human squamous tumors, the regulation of CLIC4 translocation may be an important component of skin tumor development. Although CLIC4 nuclear trafficking is tightly regulated, CLIC4 has not been shown to modify a signaling pathway. We show for the first time that CLIC4 upregulates TGF-β signaling in keratinocytes. TGF-β enhances CLIC4 expression and nuclear translocation in skin keratinocytes, and nuclear translocation of CLIC4 is essential for TGF-β signaling. To identify nuclear proteins that might interact with CLIC4, we used a yeast two-hybrid screen with CLIC4 sequences as bait. This screen revealed Schnurri-2 as a CLIC4 binding protein. Schnurri-2 is a zinc finger protein and is a gene-specific transcription factor as well as an adaptor molecule in the TGF-β signaling pathway. In keratinocytes, TGF-β increases the expression of Schnurri-2, enhances the association of CLIC4 and Schnurri-2, and causes nuclear translocation of both proteins. Knockdown studies show that Schnurri-2 is essential for CLIC4 nuclear translocation and therefore for CLIC4 enhancement of TGF-β signal transduction. These results suggest that absence of CLIC4 nuclear translocation in skin tumors may be associated with defective TGF-β signaling commonly detected during malignant transformation. Also in this light, CLIC4 may serve as a target for modifying TGF-β under conditions where this pathway is involved in pathological changes.

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Myeloid differentiation factor 88 (MyD88): an innocent bystander or an active player - pathway level analysis of expression data in psoriasis

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Gene expression studies of psoriasis have yielded several differentially expressed genes representing various functional categories ranging from epidermal differentiation and interferon responses to apoptosis and cell adhesion. However the popular statistical methods like over-representation approach (ORA) and functional class scoring (FCS) fails to merge analysis at a pathway or system level. We analysed the full psoriasis gene expression data accessed from NCBI Gene Expression Omnibus (GEO) to derive a list of differentially expressed genes with the corresponding fold changes using dChip and SAM software. This was subjected to impact analysis using Pathway-Express, which implements a deeper level of statistical analysis incorporating pathway-specific biology. We found that pathways implicated in psoriasis pathogenesis like 'Leukocyte transendothelial migration', 'Cytokine-cytokine receptor interaction' and 'cell cycle' scored impact factor over 2 along with Toll-like receptor (TLR) signaling pathway. The high impact on TLR signaling pathway can be attributed to the up-regulation of myeloid differentiation factor 88 (MyD88 - an adapter protein) and p38 (a mitogen-activated protein kinase) occupying vital positions, upstream in the pathway. This corroborates the importance of innate immunity in driving T-cell cascade leading to psoriasis and explains the triggering of psoriasis by TLR agonists like Imiquimod. MyD88 may be a potential drug target as it is involved in multiple cytokine pathways unlike therapeutic strategies focusing on inhibition of one specific cytokine or chemokine like TNF – α. These observations also suggest that the recently discovered class of anti-inflammatory agents which disrupts formation of the TRAF6-MyD88-IRAK complex without interrupting MyD88-independent TLR signaling, represented at present by the lead compound RDP58, may be useful in the treatment of psoriasis.

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Artemin; the member of glial cell line-derived neurotrophic factor is induced by substance P stimulated- fibroblasts and contributes peripheral nerve hyperplasia in the dermis of atopic dermatitis

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Itch is the major symptom of atopic dermatitis (AD), and impairs quality of life in these patients. In the recent reviews, not only histamine but also other pruritogens are involved in the mechanisms of itch and aggravate atopic dermatitis. Substance P (SP) has demonstrated various functions such as chemical mediator in the case of immunoresponse, and neurotransmitter in subjective symptoms like pain and itch. Although dermal fibroblasts may be candidate SP-responsive cells, little is known about the effect of SP. We have investigated the expression profiling of genes that are induced by substance P-treatment in normal human dermal fibroblast (NHDF) using macro-gene array. Based on this study, we focused on de novo artemin (ARTN), which is a member of the glial cell line-derived neurotrophic factor related family, gene transcription in NHDF after SP-stimulation. Besides SP-treated NHDF, RT-PCR revealed the basal level of ARTN mRNA expression in the normal human epidermal keratinocyte and Hacat cell. SP-treated NHDF-derived conditioned medium induced a proliferative response in a neuroblastoma cell line, and this phenomenon was suppressed by neutralization of ARTN. Furthermore, we verified that dermis of AD skin lesions exhibit intense immunoreactivity for ARTN, while psoriasis and healthy controls are less intense. In situ hybridization assay also revealed that expression of ARTN mRNA in dermal fibroblast was observed in AD skin lesion, but not in healthy control. Colabeling with artemin and its potent receptor GFRα3 showed massive sprouting of GFRα3-positive peripheral nerve fibers in ARTN accumulated dermal areas. SP-treated dermal fibroblast-derived artemin can be assumed to contribute to peripheral nerve sprouting. Our findings indicate novel functional aspects of SP, and may lead to a better understanding of novel mechanisms of altered skin innervation in atopic dermatitis.

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Phosphatidylinositol 3-kinase pathway suppresses tumor necrosis factor-alpha-induced matrix metalloproteinase-1 expression through inhibition of glycogen synthase kinase-3 in human skin fibroblasts

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Matrix metalloproteinase-1 (MMP-1) has been reported to be involved in the regulation of various physiological and pathological processes such as matrix remodeling. The expression of MMP-1 is regulated by a variety of extracellular stimuli. Although many studies have shown that mitogen activated protein kinase (MAPK) pathways are critical in the MMP-1 expression, other signaling pathways are also likely to play an important role in the regulation of MMP-1 expression. In this study, we investigated the role of the phosphatidylinositol 3-kinase (PI3K) signaling pathway on the expression of MMP-1 induced by tumor necrosis factor (TNF)-alpha in human skin fibroblasts. Treatment of cultured fibroblasts with a specific PI3K inhibitor, wortmannin, increased TNF-alpha-induced MMP-1 expression. Furthermore, the inhibition of Akt, a key physiological mediator of PI3K pathway, by two structurally different inhibitors, also increased TNF-alpha-induced MMP-1 expression, indicating that PI3K-Akt pathway negatively regulates TNF-alpha-induced MMP-1 expression in human skin fibroblasts. Then, we investigated whether glycogen synthase kinase-3 (GSK-3) is involved in the regulation of MMP-1 expression, since GSK-3 is known to be negatively regulated by activated Akt and to regulate the activities of a variety of transcription factors. We found that the inhibition of GSK-3 activity by three different chemical inhibitors significantly reduced TNF-alpha-induced MMP-1 expression in a dose-dependent manner, indicating that GSK-3 plays a critical role on TNF-alpha-induced MMP-1 expression in human skin fibroblasts. Taken together, our results suggest that PI3K-Akt pathway suppresses MMP-1 expression induced by TNF-alpha through inhibition of GSK-3 activity in human skin fibroblasts.

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Chemokine receptor profile on skin derived stem cells – predominant role for CCL5/ RANTES receptors

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Skin injury is associated with a sequential and differential expression of chemokines (CKs) in the wound bed. While CKs in skin wound healing were initially described to attract various leukocyte populations into the wound bed, the expression of chemokine receptors (CKRs) on skin residential cells suggests a more extensive role for CKs in wound healing. In deep skin wounds, adipose tissue is exposed in the wound bed and dermal tissue at the wound bed margins. Stem cells present in adipose tissue (ASC) and dermis (DSC) are the most likely candidates to be involved in wound repair and skin regeneration. The objective of this study was i) to characterize stem cells isolated from the adipose tissue and also the dermis of human skin and ii) to determine the chemokine receptor profile and migratory response to their corresponding ligands. DSC displayed a typical stem cell phenotype CD31-CD34+CD45-CD54+CD90+CD105+CD166+ and showed trilineage differentiation capacity, which has also been reported for ASC. Notable is that DSC represent the same cell population that is commonly referred to as dermal fibroblasts. ASC and DSC express a similar set of chemokine receptors, (CCR3, CCR4, CCR6, CCR10, CXCR1 and CXCR2). Several ligands for these receptors, with CCL5/ RANTES being the most potent, could induce migration of ASC and DSC in an in vitro wound-healing assay. Establishing optimal chemokine gradients may form a basis for future stem cell therapies focused on healing large, deep skin wounds.

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IL-8 and p53 are inversely regulated through JNK, p38 and NF-κB p65 in HepG2 cells during an inflammatory response

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Objective: It is reported that Nuclear factor-κB (NF-κB) activation is dysregulated in chronic inflammatory diseases like psoriasis, rheumatoid arthritis and cancer, resulting in an over expression of pro-inflammatory cytokines and an inhibition of apoptosis. We studied NF-κB activation and the induction of interleukin 8 (IL-8) and p53 gene expression in an interleukin 1β (IL-1β) stimulated HepG2 cell line. Methods: NF-κB induced IL-8 and p53 protein production was studied using specific siRNA, an IκB kinase 2 inhibitor, and mitogen activated protein kinase (MAPK) inhibitors. Results were analyzed by different techniques including Western blotting and ELISA. Results: IL-1β induced both the IL-8 and p53 mRNA expression and protein production of IL-8, but not p53. Knockdown of NF-κB p65 expression with siRNA strongly reduced IL-8 production and significantly induced protein levels of p53. An IκB kinase 2 inhibitor, sc514, also strongly reduced IL-8 and significantly induced p53 protein levels. Using three MAPK inhibitors we showed that p38 MAPK and JNK dependent mechanisms are involved in the regulation of the IL-8 and p53 protein expression. Conclusion: Our results indicate that IL-8 and p53 protein expression is regulated through inverse activation of the p38 MAPK and the JNK pathways and the NF-κB p65 expression.

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Novel E-3 ligase pathway in psoriasis through Tripartite motif protein Trim32 and its substrate Protein inhibitor of activated Stat Piasy

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Trim32 is an E3-Ubiquitin ligase scaffold protein genetically inactivated in human hypotrophic disorders such as limb girdle muscular dystrophy type 2H. We found elevated expression and activity of wild type Trim32 in hypertrophic skin diseases such as psoriasis and pre-neoplasia. Our laboratory provided evidence for a Trim32 role in increased cell survival in response to TNFalpha inflammatory cytokine) and UVB (reactive oxygen or environmental genotoxin) stressors. We have found that Trim32 expression is elevated in the epidermis of psoriatic lesions in humans relative to normal human skin. We previously showed that Piasy, a pro-apoptotic E3-SUMO ligase, is a Trim32 substrate. Interestingly, the Piasy gene is localized in the PSORS6 susceptibility locus on chromosome 19p13 and Piasy negatively regulates the activities of several transcription factors, NF-kappaB, STAT, and SMADs, that are implicated in the pathogenesis of psoriasis. We show that Piasy is regulated in cells by Trim32, through binding on cytoplasmic scaffolds, ubiquitylation and proteosomal degradation. Consistent with its negative regulation of Piasy, Trim32 overexpression enhanced the transcription activities of NF-kappaB and STAT. Trim32 regulation of Piasy provides a putative link between disparate factors (TNFalpha, NF-kappaB, STATs, and the TGFbeta-induced SMADs) in the etiology of psoriasis. These lines of evidence suggest that Trim32 and Piasy comprise a central mechanism for normal epidermal homeostasis that is disrupted in psoriasis. According to this model, restoring Piasy activities may offer a dual strategy in the treatment of psoriasis, by turning off keratinocyte transcription factors that fuel psoriasis and by restoring homeostasis in keratinocyte programmed cell death responses.(Supported by NIH CA98577 and OHSU Cancer Institute CA69533)

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Angiotensin II enhanced cell proliferation and EGF receptor expression levels via ROS formation in HaCaT cells

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Recent work has shown a novel function of angiotensin II (Ang II) in skin wound healing in which reactive oxygen species might be involved. As Ang II is known to increase superoxide production by activating NADPH oxidase in some nonphagocytic cells, we speculated that the produced superoxide by NADPH activation could contribute to the regulation of epidermal growth factor receptor (EGFR) in keratinocytes. In this study, we examined whether Ang II could generate superoxide and enhance EGFR expression levels in HaCaT cells. Superoxide formation was assessed by using hydroethidine. EGFR expression levels were examined by Western blotting. We have demonstrated the following results. Ang II increased the superoxide formation. Ang II resulted in a dose-dependent increase in cell proliferation in HaCaT cells. The expression levels of EGFR were increased in HaCaT cells treated with Ang II. Apocynin, a NADPH oxidase inhibitor, decreased the expression levels of EGFR. Xanthine/xanthine oxidase system, an exogenous superoxide generating system, enhanced the EGFR protein expression. Although Ang II did not affect the nitric oxide (NO) production, a NO synthase inhibitor N-nitro-L-arginine methyl ester suppressed the Ang II-induced EGFR expression levels in HaCaT cells. Thus, constitutive NO is required for the Ang II-induced EGFR expression in HaCaT cells. These results suggest that Ang II enhances the cell proliferation and EGFR expression via superoxide production under the regulation of NO in HaCaT cells, implying that Ang II may regulate the proliferation, differentiation and tumorigenesis of the epidermis by harmonizing the superoxide and NO production.

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LGR4 deletion induces eye open at birth phenotype with reduced keratinocyte motility

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We observed a consistent eye open at birth phenotype in mouse pups homozygous for a leucine–rich repeat containing G–protein coupled receptor 4 (Lgr4) allele deleting the whole transmembrane domain coding region. An in vitro wound–healing scratch assay showed increase of keratinocyte motility in the Lgr4 null mice. F–actin staining was also reduced in the eyelid epidermis. We also generated keratinocyte–specific Lgr4 deficient mice, circumventing the embryonic/neonatal lethality and kidney abnormalities. The conditional Lgr4 knockout mice showed the eye open at birth phenotype. Thus, Lgr4 might be a novel gene class regulating cell motility.

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Activation pattern of MAPK and NF-κB pathways in skin cancer and inflammation

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ERK, JNK and p38 MAPKs are the most common MAPK cascades. NF-κB is a complex formed most commonly by heterodimerization of p50 and RelA. Inflammatory stimuli that are capable of activating IKK2 what results in translocation of NF-κB to the nucleus. In addition, phosphorylation of the transactivating subunit RelA is another essential regulatory step. Both MAPK and NF-κB signal transduction pathways are involved in inflammatory processes and tumorigenesis. We were interested to what extent theses pathways are activated in the inflammatory skin diseases atopic eczema, psoriasis and lichen planus. This we compared to the pattern in spinocellular carcinoma. We can show that c-Jun is activated in samples of spinocellular carcinoma and lichen planus. In contrast, samples from atopic eczema and psoriasis revealed no significant activation. This might be a reason why lichen planus can transform. In addition, RelA was phosphorylated at Ser536 in all inflammatory skin diseases analyzed but not in spinocellular carcinoma. This went along with a strong activation of IKK2 in the respective inflammatory skin diseases. No significant difference was observed for ERK1/2 and p38 activation between the inflammatory skin diseases and spinocellular carcinoma that were both active. Staining with the proliferation marker MPM2 demonstrated no difference between the inflammatory skin diseases and spinocellular carcinoma. However, positive staining for MPM2 went along with MSK1 activation and phosphorylation of RelA at Ser276, a MSK1 target. In summary we show (1) that activation of JNK/c-Jun might be implied in the risk for transformation of lichen planus and (2) that depending of the site phosphorylated a different set of genes is regulated by RelA.

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A novel melanoma marker promotes migration and invasion by activating urokinase plasminogen activator receptor and p38 MAP kinase signaling

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The molecular mechanisms that underlie melanoma metastasis are not well understood. We have recently found that a novel GM3 derivative is strongly expressed in the highly metastatic C8161 cutaneous melanoma cell line, in comparison with the poorly invasive A375P primary melanoma cell line. We hypothesized that this neo-ganglioside promotes cell aggressiveness. Treatment of A375P cells with this GM3 derivative, purified from C8161 cells, significantly stimulated cell migration and invasion, as shown by scratch, two-layer chemotaxis, and Matrigel invasion assays. Consistently, preventing the synthesis of GM3 in C8161 cells by treatment with PPPP reversed the metastatic potential by limiting cell migration and invasion. We have previously shown that GM3 paradoxically triggers squamous carcinoma cell proliferation through urokinase plasminogen activator (uPA) receptor (uPAR)/p70S6 kinase signaling, but only if cells are grown in the presence of uPA. We then hypothesized that melanoma cell invasiveness is a function of the expression of this specific GM3 derivative and activation of uPAR signaling. Overexpression of uPAR by stable transfection of a human uPAR cDNA enhanced C8161 cell migration and invasion, while knockdown of uPAR expression by uPAR siRNA transfection eradicated cell migration and invasion. The uPAR overexpression concurrently augmented p38 MAP kinase signaling and increased the expression and activation of MMP-2, but only in the presence of this specific GM3 derivative. Blockade of p38 MAP kinase signaling with SB202190 or SB203580, or of MMP-2 activity by SB-3CT, suppressed the increase in invasiveness, but had little effect on the ability of cells to migrate. We conclude that the expression of this specific GM3 derivative stimulates melanoma cell invasiveness by activating uPAR and p38 MAPK signaling, thereby increasing MMP-2 activity. These results position this newly-described GM3 derivative as a novel therapeutic target for melanoma treatment.

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Evidence of corporation of ROCK and mDia in actin stress fiber formation of human dermal fibroblasts with SIP stimulation

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Sphingosine 1-phosphate (S1P) is a biologically active lipid mediator that has a lot of pivotal roles in the regulations of cell growth, migration, differentiation and apoptosis. However, the signal transduction promoted by S1P in human dermal fibroblasts is still unclear. We investigated the signal transduction by S1P in human fibroblasts using collagen matrices contraction. This study reveales whether or not S1P has the possibility to apply the treatment for cutaneous wound healing in the future. We found that S1P promoted floating collagen matrices contraction (FMC), which is considered as a model of initial phase of wound contraction, and some kinds of G protein, Giα, Rac 1, Rho and ROCK (Rho associated coiled-coil forming kinase) were involved in S1P promoting FMC. However, ROCK was considered to be partially involved in S1P promoting FMC. mDia as well as ROCK have been recognized to be putative downstream target molecules of Rho. In mDia-silenced cells, ROCK inhibitor suppressed the stress fiber formation regardless of the presence or absence of S1P. Our results indicate mDia as well as ROCK may be involved in the downstream of Giα, Rac1 and RhoA on developing actin stress fiber of human dermal fibroblasts with SIP stimulation.

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Acitretin induces apoptosis through CD95 signaling pathway in human cutaneous squamous cell carcinoma cell line SCL-1

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Skin Cancers are by far the most common human malignancies. Retinoids have shown promising preventive and therapeutic effects against a variety of human malignancies. The aim of this study was to investigate the apoptosis-inducing effect of acitretin on human skin squamous cell carcinoma (SCC) SCL-1 cells. We found that acitretin preferentially inhibited the growth of SCL-1 cells in a dose- and time-dependent manner, but not of nonmalignant keratinocyte HaCaT cells. This inhibition appeared to be due to induction of apoptosis as revealed by enzyme-linked immunosorbent Assay. AnnexinV/ propidium iodide (PI) assay and morphological observation confirmed the pro-apoptotic effect of acitretin on SCL-1 cells. We further demonstrated that apoptosis was induced within 1–2 days and involved activation of caspase-8, -9, -3 and poly (ADP-ribose) polymerase (PARP). Caspases-8 inhibitor effectively suppressed acitretin-induced apoptosis while caspase-9 inhibitor did not. Acitretin increased the levels of CD95 (Fas), CD95-ligand, and Fas-associated death domain. Neutralizing ZB4 anti-Fas antibody significantly inhibited the apoptosis in SCL-1 cells induced by acitretin. These results suggest that acitretin is able to induce apoptosis in skin cancer cells possibly via death receptor CD95 apoptosis pathway without affecting the viability of normal keratinocyte.

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Overexpression of Id3 enhances X-ray induced apoptosis through p53-dependent mechanism in HaCaT keratinocytes

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Inhibitor of differentiation/DNA binding (Id) proteins comprise a class of helix-loop-helix transcription factors that have various roles in proliferation, differentiation, apoptosis, and carcinogenesis. We previously showed that Id3, one of Id protein superfamily, is induced by X-ray irradiation in animal model for radiodermatitis. In this study, we investigate the functional role of Id3 in X-ray irradiated apoptosis of skin keratinocytes. To this end, we examined the expression of Id3 using RT-PCR and immunohistochemistry, and investigated the effect of Id3 on cell cycle and apoptosis using a recombinant adenovirus. X-ray irradiation markedly induced Id3 expression in the hyperplastic epidermis of mouse. Interestingly, overexpression of Id3 in HaCaT by adenoviral transduction led to the inhibition of cell proliferation in the absence and/or presence of X-ray irradiation, as compared with Ad/LacZ control group. Concomitantly, overexpression of Id3 significantly increased the X-ray-induced apoptosis in HaCaT keratinocytes. In addition, Id3 overexpression resulted in accumulation of p53 and upregulation of p21, suggesting the Id3 affects cellular apoptosis via the p53-dependent pathway. These results implicate the potential involvement of Id3 in X-ray-induced apoptosis of skin keratinocytes.

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S100A8/A9 induce reactive oxygen species and activate stress-activated protein kinase and nuclear factor-κB (NF-κB) pathways in normal human keratinocytes

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S100A8 and S100A9 are known to be up-regulated in hyperproliferative and psoriatic epidermis. Recently, we found that S100A8/A9 were secreted from normal human keratinocytes (NHK). We also showed a positive feedback loop for growth stimulation involving S100A8/A9 and inflammatory cytokines such as TNF-α and IL-8 in NHK. Here, we tried to identify signal transduction pathways acitivated by S100A8/A9 in NHK. First, using human phospho-MAPK array and Western Blot, we found that two stress-activated protein kinases (SAPKs), p38 and JNK, were activated within 30 minutes by the addition of recombinant S100A8/A9, whereas neither ERK nor Akt were affected. Second, we tested involvement of nuclear factor-κB (NF-κB) pathway by immunostaining. NF-κB component, p50, was translocated into nucleus 1 hour after S100A8/A9 stimulation. However, p65 did not show any changes in its cellular localization. Identical results were obtained for TNF-α. Since both MAPK and NF-κB pathways were activated by S100A8/A9, we focused on generation of reactive oxygen species (ROS). Using carboxy-H_2DCFDA, we detected strong up-regulation of ROS in S100A8/A9-treated NHK. This was comparable to TNF-α-treated NHK. These findings indicate that S100A8/A9 signaling pathway involves generation of ROS followed by activation of two SAPKs and NF-κB. Finally, we examined relation between S100A8/A9 and NHK proliferation using human phospho-receptor tyrosine kinase (RTK) array. Although S100A8/A9 actually increased cell number of NHK, S100A8/A9 did not induce any phosphorylation of known RTK, suggesting the presence of yet unidentified receptor for S100A8/A9. Collectively, our results revealed possible signaling events of S100A8/A9 that lead to inflammatory cytokines induction and abnormal proliferation of NHK.

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The anti-psoriatic drug anthralin inhibits calcium release from the endoplasmic reticulum, store-operated calcium entry and the translocation of stromal interacting molecule 1 (STIM1)

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Psoriasis is characterised by immune activation and epidermal hyperplasia but the aetiology is unknown. Regulation of $[Ca^{2+}]_i$ is crucial for keratinocyte growth and differentiation Moreover, several lines of evidence indicate a fundamental defect in calcium signalling in psoriatic keratinocytes (Karvonen, SJ et al, JID 2000). Anthralin (dithranol) is an effective topical treatment that induces clearance of psoriatic plaques but its effect on calcium signalling in keratinocytes has not been investigated. We examined the effect of anthralin on intracellular calcium ($[Ca^{2+}]_i$) signalling in response to calcium-mobilising agonists. Treatment with 5 μM anthralin inhibited agonist-induced store-operated calcium entry (SOCE) in HaCaT keratinocytes by 46 ± 0.61% (n=86 cells,3 exps, $P<0.001$) for UTP. Both the initial agonist-induced $[Ca^{2+}]_i$ peak and the sustained $[Ca^{2+}]_i$ stage were inhibited by anthralin pre-treatment suggesting dampening of both intracellular Ca^{2+} release and subsequent entry. In Ca^{2+}-free medium, anthralin reduced UTP-induced Ca^{2+} release from the endoplasmic reticulum (ER) by 33 ± 0.30%. Using thapsigargin to empty ER stores in Ca^{2+}-free medium, anthralin did not reduce the total capacity of the ER Ca^{2+} pool ($p=0.5626$) despite inhibiting agonist-induced Ca^{2+} release from the ER (n=90, 3 exp, $p<0.001$). Anthralin pre-treatment inhibited thapsigargin-induced Ca^{2+} entry in both HaCaT and cultured keratinocytes in the presence of extracellular Ca^{2+}, suggesting a direct effect on SOCE. We therefore examined the effect of anthralin on STIM1, a Ca^{2+} sensing protein localised to the ER. Anthralin inhibited the translocation of STIM1 to the plasma membrane, indicating a possible mechanism for the reduction in SOCE. In conclusion, anthralin inhibits calcium signalling at several sites in keratinocytes, specifically SOCE and agonist-induced calcium entry. Together these data suggest modulation of calcium flux may be involved in the therapeutic action of anthralin in psoriasis.

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Defining PKCα transcriptome in transgenic keratinocytes reveals unique signatures for downstream signaling in the AP-1 and NF-κB pathways

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Protein kinase C (PKC) isoforms are major regulators of cutaneous homeostasis and mediate inflammation in response to 12-0-tetradecanoylphorbol-13-acetate (TPA). We have previously reported that PKCα activation by TPA in transgenic mice overexpressing this isoform in the epidermis (K5-PKCα mice) leads to the formation of intraepidermal neutrophilic microabcesses, a feature also observed in human pustular psoriasis. TPA causes apoptosis in both transgenic skin and cultured keratinocytes. Our previous work indicated that NF-κB and AP-1 signaling pathways contributed to the cutaneous biological response to PKCα activation. Specifically NF-κB was required for cutaneous inflammation and AP-1 regulated the apoptotic response. In the current study we used gene expression profiling to define PKCα transcriptional targets in primary keratinocytes from K5-PKCα mice. Labeled cDNAs generated from TPA treated cells over a 6 hour time course were hybridized to NCI-printed cDNA microarray. Over five hundred genes whose expression was altered by PKCα activation were identified. Besides known TPA-regulated genes, previously unrecognized PKCα regulated genes such as c-myc or the interferon-inducible p200 (IFI-200) were identified. Looking at a subset of target genes and using siRNA targeted to PKCα, we were able to confirm the specificity of the regulation by this isoform. In order to distinguish expression changes associated with the inflammatory or the apoptotic response, K5-PKCα keratinocytes were transduced with dominant negative constructs for AP-1 and NF-κB pathways and stimulated with TPA. Our results indicate that NF-κB regulates the expression of multiple cytokines/chemokines contributing to the innate immune response initiated by PKCα in keratinocytes, while AP-1 regulates the expression of Myc and Mad genes among others. Together our data presents a unique profile of pathways regulated by a single PKC isoform and illuminates the relation of gene expression changes and biological response.

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EGFR regulates keratinocytes GM-CSF expression *in vitro* and *in vivo*: updates on the role of EGFR in skin immune regulation

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The EGFR/ERK1/2 pathway controls skin immune/inflammatory functions by modulating cytokine induced chemokine expression. In this study we focused on the role of EGFR pathway in regulating keratinocyte expression of a highly pleiotropic cytokine such as Granulocyte-Macrophage Colony-Stimulating (GM-CSF). Immunohistochemistry of the skin of patients suffering from inflammatory conditions known to have high levels of activated EGFR, like allergic contact dermatitis and psoriasis vulgaris, revealed a strong dermal and epidermal positivity for GM-CSF. Interestingly, in the papulopustolar skin rash of patients undergoing chemotherapy with a clinically approved anti EGFR antibody, we observed lower levels of GM-CSF expressed by keratinocytes and a high number of GM-CSF positive infiltrating cells that form neutrophilic abscesses. A similar staining pattern was found in the skin of mice with a skin targeted deletion of EGFR. In the skin of K5Cre mice homozygous for EGFR floxed alleles, the epidermis showed reduced signal for GM-CSF and an abundant neutrophilic infiltrate with numerous epidermal microabscesses. We then studied human and murine keratinocytes following pharmacological or genetic ablation of the EGFR pathway. In cultured keratinocytes, TNF-alpha induced GM-CSF expression was positively regulated by the activation of EGFR and negatively regulated by EGFR pharmacological or genetic blockade. EGFR regulation of GM-CSF expression was mainly exerted at the transcriptional level. These data demonstrate that EGFR activity modulates keratinocyte inflammatory responses and that a functional EGFR pathway is required for inducing GM-CSF expression after proinflammatory stimuli both *in vitro* and *in vivo*.

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The potential role of WW domain-containing oxidoreductase in keloid formation

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Keloids are abnormal scar that expand beyond the boundaries of original injury as they heal and often recur after excision. Previous studies revealed downregulation of apoptotic genes, including p53 in keloid fibroblasts (KF). WW domain-containing oxidoreductase (WWOX) is a candidate tumor suppressor protein, which plays a role in various cancers including hepatoma and prostate cancers. We sought to determine whether WWOX plays a role in keloid formation. We examined the expression of WWOX in keloids by immunohistochemistry (IHC) of keloid skin tissue and cultured fibroblasts, Western blotting (WB), RT-PCR and real-time PCR of KF with normal skin and normal fibroblasts (NF) as controls. IHC of paraffin sections revealed diffuse positive staining of dermal fibroblasts in keloid compared with normal control, and the fibroblasts were more plump and numerous in the former. The staining of cultured fibroblasts appears stronger in the areas of higher cellularity in both KF and NF; the staining was slightly stronger in KF than NF in general. WB of cultured fibroblasts revealed similar expression of WWOX proteins between KF (n=3) and NF (n=4) (50% vs 53%). In fibroblasts after 24h of serum starvation, the WWOX expression appeared slightly higher in high density dish (90% confluence) than lower density dish (30-40% confluence), suggesting that WWOX expression may be cell density dependent. RT-PCR study revealed similar expression of WWOX mRNA between KF (n=10) and NF (n=5) (45.1% vs 47.3%). By Real-time PCR, there was no difference in WWOX mRNA expression between KF (n=12) and NF (n=6). RT-PCR study on additional samples underwent serum starvation as described above also showed no obvious difference between KF and NF. In conclusion, WWOX protein in keloid tissue and cultured KF was higher than normal control by IHC, but WB, RT-PCR, and real-time PCR showed no differences. Our results do not suggest a pathogenic role of WWOX in keloid formation.

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Coordinate effects of Smad7 overexpression on blocking TGFβ signaling and activating Rac1 during cutaneous wound healing

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Smad7, an antagonist of transforming growth factorβ (TGFβ) signaling, was overexpressed during cutaneous wound healing. To assess the role of Smad7 overexpression in cutaneous wound healing, we utilized gene-switch-Smad7 transgenic mice in which the Smad7 transgene can be induced in keratinocytes during wound healing. Smad7 induction on a 6-mm excision wound resulted in accelerated re-epithelialization but reduced inflammation, angiogenesis and fibrotic response compared to wounds in control mice. In vitro migration assay revealed a positive effect of Smad7 on keratinocyte migration independent of its effect on stimulating keratinocyte proliferation. Increased Rac1 expression levels and activity were found in Smad7 wounded skin, as determined by qRT-PCR, western analysis, and Rac1-GTPase assay, which correlated with reduced Smad signaling. Knocking down Rac1 in Smad7 transgenic keratinocytes attenuated Smad7 effect on keratinocyte migration and proliferation, suggesting that Rac1 activation is necessary for mediating Smad7 effects. To further determine if Rac1 is a Smad transcriptional target, we examined Rac1 mRNA levels in keratinocytes after knocking down individual Smads. Rac1 mRNA was significantly increased after knocking down Smad2, Smad3 or Smad4, suggesting that they normally repress Rac1 expression. Additionally, chromatin immunoprecipitation from keratinocytes shows that Smad-2, -3, and -4 bound to the Smad binding element (SBE) of the Rac1 promoter 1.5kb upstream of the coding sequence. These data suggest that Smad7 activates Rac1 by abrogating the repressive effect of signaling Smads on Rac1 transcription. Notably, Smad7-mediated Rac1 activation could not be detected in unwounded skin, when Rac1 activity is at a very low level, but coordinated with Smad7 effect on blocking TGFβ1 signaling to accelerate wound healing. Thus, our study highlights the importance of studying mechanisms of Smad7 functions under different pathological conditions.

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Detection of living-cell reaction by means of ellipsometry with rotating analyzer

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It is necessary to establish new techniques to detect the activation of living cells without any labeling in real time manner. We previously reported that living cells, such as mast cell and basophile, changed the angle of resonance (AR) of surface plasmon resonance (SPR), when stimulated on the sensor chip of SPR. AR reflects refractive index (RI), which was propotional to the amount of mass distribution in evanescent light. However the inhibition of cell motility by several reagents did not affect the AR of SPR. In this study, we constructed the apparatus of ellipsometry with rotating analyzer for living cell analysis and detected the reaction of mast cells. An ellipsometry system was constructed with a prism, a rotating analyzer and a laser (535 nm) light source. Polarized beam was incomed and reflected at the sensing surface of the prism where living RBL-2H3 cells attached. The polarization state of the reflected beam was detected by the rotating analyzer. It reflects physical conditions on the prism surface including RI. The sensor clearly detected the difference of RI between methanol and water (around 0.006). It also detected a sustained change of polarization state, when RBL-2H3 cells were exposed to various stimuli on the prism. The degree of change induced by an antigen and RBL-2H3 cells was similar to that by methanol and water. Moreover, pre-treatment of mast cells with tyrosine kinase inhibitor, protein kinase inhibitor or actin polymelization inhibitor, but not that with PI3 kinase inhibitor inhibited the antigen-induced polarization states in RBL-2H3 cells. Living cells cause substantial changes of RI upon their activation and such activities were detected not only by SPR, but also by ellipsometry. The ellipsometry-based cell sensor might be useful for further elucidations of the change of RI and its mechanism in living cells.

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The effect of CTGF silencing on extracellular matrix metabolism of human dermal fibroblasts

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Systemic sclerosis (SSc) is a multisystem disease characterized by excessive fibrosis in the skin as well as various internal organs. Although the pathogenesis of SSc is still unknown, a fascinating hypothesis, a 2-step process of fibrosis, has been proposed, in which the key cytokines are transforming growth factor-β(TGF-β) that stimulates fibroblast first and connective tissue growth factor (CTGF) that maintains tissue fibrosis. Given the fact that CTGF is closely involved in the fibrosis of scleroderma, the suppression of CTGF expression could potentially result in the decrease of extracellular matrix (ECM) synthesis and may thus provide us with a novel approach for treatment of SSc. Skin fibroblasts from normal and SSc were cultured in vitro and transfected with two kinds of CTGF-specific siRNAs, respectively western blot analysis demonstrated that one CTGF-specific siRNA could suppress the synthesis of CTGF. Then, the effect of CTGF-specific siRNA on the expression and production of type 1 collagen, matrix metalloproteinase (MMP)-1,-2,-9 and tissue inhibitor of metalloproteinase (TIMP)-1 were quantified by Western blot analysis, enzyme-linked immunosorbent assay (ELISA) and real-time PCR. The production and expression of type 1 collagen were reduced by CTGF-specific siRNA in normal and SSc fibroblasts. The production and expression of MMP-1 were reduced by RNAi in normal fibroblasts, however, those were increased by RNAi in SSc fibroblasts. There were not significant changes in the production and expression of other ECM in normal and SSc fibroblasts. Our data showed that CTGF RNA interference could inhibit expression of CTGF and type 1 collagen in normal and SSc fibroblasts, and the expression and production of MMP-1 was decreased by CTGF RNA interference in normal fibroblasts while increased in SSc fibroblasts. Our findings suggest that CTGF RNA interference might be a novel therapeutic strategy for fibrosis in SSc.

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Expression of interleukin-18 binding protein (IL-18 BP) and interleukin-18 receptor alpha chain (IL-18R α) in psoriatic skin lesions

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Interleukin-18 (IL-18) is a proinflammatory cytokine that stimulates natural killer cells (NK) and T cells and enhances innate immunity as well as specific Th1 immune responses in the pathogenesis of psoriasis. IL-18 is involved in immunologically mediated tissue damage, mediates its effects by binding to a heterodimeric receptor made up of a ligand binding α subunit (IL-18R α) and a signaling β subunit (IL-18R β) but its bioactivity is regulated *in vivo* by its soluble decoy receptor, IL-18 binding protein (IL-18BP). IL-18BP is a soluble circulating protein with high affinity for IL-18 which act as inhibitors of IL-18 signaling and can neutralize its bioactivity. IL-18BP can effectively diminish IL-18–induced NF-kB activation, with subsequent synthesis of IFN-r and IL-8 *in vitro*. It is now appreciated to be an important regulator of innate and adaptive immunity and has impressive effects on type I immune responses. Recent studies demonstrating that IL-18BP derived from human keratinocytes is upregulated by IFN-γ, suggest that the activity of IL-18 is modulated by a negative feedback mechanism mediated by IFN-γ-induced IL-18BP. However, despite its important contributions to immune responses, the role of IL-18BP and IL-18R α signaling in the pathogenesis of psoriasis has not been addressed. Therefore, we attempted to clarify the possible role of IL-18BP production and IL-18R α signaling in the pathogenesis of psoriasis. In order to characterize this, we compared the level of IL-18BP and IL-18R α expression between psoriatic skin lesion and normal control using immunohistochemistry, ELISA, and Western blot analysis. As a result of this study, when it compared to the normal skin in immunohistochemistry, IL-18BP, IL-18R α expression was increased in the psoriatic skin, although normal skin has that expression in the keratinocyte, monocyte and enthothelial cell. In western blot analysis, we also found that IL-18BP, IL-18R α expression was increased in the psoriatic skin.

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Conditional ablation of Ubc13, a mediator of innate immunity, in keratinocytes induces abnormal differentiation, decreased proliferation, and apoptosis

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The Ubc13 E2 polyubiquitin-conjugating enzyme is a mediator of innate immune reaction. Ubc13 plays a key role in generating K63-linked polyubiquitin chains. In contrast to K48-linked polyubiquitin chains, K63-linked polyubiquitin chains link to biological processes, such as the stress responses, rather than to protein destruction. Polyubiquitination of TRAF6 activates TAK1, which is critical in the activation of NF-κB and MAP kinases. Moreover, TRAF6-dependent polyubiquitination of IKKγ is involved in NF-κB activation. Although Ubc13 has been shown to be critical for TLR and IL-1 receptor signaling, the function of Ubc13 in keratinocytes has not been studied. We generated keratinocyte-specific Ubc13-deficient mice by breeding Ubc13 flox/flox mice with K5-cre mice. At birth, the skin of the Ubc13-deficient mice was abnormally shiny and smooth. The Ubc13-deficient mice died by P2 without growing. IL-1 induced JNK, p38, and NF-κB activation was impaired in keratinocytes of the Ubc13-deficient mice. Histological analysis showed atrophy of the epidermis with keratinocyte apoptosis. Immunohistochemical analysis showed that the suprabasal keratinocytes of the Ubc13-deficient epidermis expressed keratin 14, which is normally confined to the basal layer. There was no loricrin expression on the epidermis of Ubc13-deficient mice. Ki67 staining showed decreased keratinocyte proliferation in the epidermis of Ubc13-deficient mice. Moreover, the removal of Ubc13 from cultured keratinocytes of Ubc13 flox/flox mice with adenovirus vector carrying Cre recombinase resulted in apoptosis, indicating that Ubc13 is essential for preventing apoptosis. These data indicate that Ubc13 regulates keratinocyte differentiation, proliferation, and apoptosis in the epidermis. In conclusion, Ubc13-mediated K63-linked polyubiquitination in keratinocytes is essential for maintaining epidermal homeostasis.

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Lipid raft disruption in epidermal keratinocytes induces the same signaling pathways as oxidative stress but the inactivation of oxidant-sensible tyrosine phosphatases is not responsible for this induction

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The disruption of lipid rafts in epidermal keratinocytes induces stress signaling pathways which are also activated by reactive oxygen species (ROS). Previous studies showed that disruption of lipid rafts, carried out with the cholesterol-sequestering molecule methyl-beta-cyclodextrin (MBCD), and oxidative stress, induced by H2O2, activate the MAPK p38 and induce the expression of Heparin-binding EGF-like growth factor (HB-EGF). Interestingly, HB-EGF expression depends on p38 activation, and both are regulated in a time-dependent manner. As same signaling pathways were observed after oxidative stress or lipid raft disruption, we hypothesized that the disruption of lipid rafts could induce a kind of oxidative stress. To address this question, we analysed the involvement of protein tyrosine phosphatases in cells subjected to lipid raft disruption or oxidative stress, because it is known that oxidative stress transiently inactivates these phosphatases. The activity of tyrosine phosphatases in lysates of control and treated cells was measured in terms of phosphate released from a synthetic tyrosine phosphopeptide. Data show that H2O2 treatments result in decreased tyrosine phosphatase activity as expected, but in MBCD-treated cells the tyrosine phosphatase activity is not different from the activity of control keratinocytes. To further analyse the involvement of ROS, we incubated cells with the antioxidant N-acetyl-cysteine (NAC). NAC effectively protects the tyrosine phosphatase activity in cells treated with ROS, and does not change the tyrosine phosphatase activity in MBCD-treated cells. These results demonstrate that the disruption of lipid rafts does not alter the activity of tyrosine phosphatases, suggesting that signaling induced by the disruption is not due to oxidative stress.

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Antagonistic effects of FK-506 on TGF-β-induced contraction of collagen gel by normal and keloid fibroblasts

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Transforming growth factor (TGF)-β induces fibroblast contraction, which is implicated in wound healing and keloid formation. FK-506 is one of the immunosuppressive regents. Our objective was to investigate the effects of FK-506 on TGF-β-induced fibroblast contraction, which is implicated in wound healing and keloid formation. We used an *in vitro* type I collagen gel contraction assay with normal or keloid dermal fibroblasts incorporated. We also examine the effect of FK-506 on α-smooth muscle actin (SMA) expression in immunofluorescence study. Our results showed that TGF-β induced contraction of collagen gels with normal dermal fibroblasts incorporated, which was efficiently suppressed by FK-506. Keloid fibroblasts showed higher basal contraction of collagen gels in the absence of TGF-β than normal fibroblasts, which was enhanced by addition of TGF-β. FK-506 suppressed both the basal and TGF-β-enhanced contraction of collagen gels by keloid fibroblasts. These inhibitory effects of FK-506 were associated with suppression of TGF-β-induced α-smooth muscle actin (α-SMA) expression in normal and keloid fibroblasts. Additionally, FK-506 enhanced Smad-7 in normal dermal fibroblasts. In conclusion, FK-506 can suppress TGF-β-induced contraction of collagen gel by normal and keloid dermal fibroblasts. Importantly, FK-506 can inhibit basal contraction of collagen gel by keloid fibroblasts. These results suggest that FK-506 may have therapeutic potential for excessive skin contraction as observed in keloid.

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Sortilin is expressed in human keratinocytes and is regulated by cutaneous neuropeptides.

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Sortilin is a member of the family of Vps10p-domain receptors has been shown to be able to bind the precursor peptide of nerve growth factor (proNGF). ProNGF interacting with sortilin and $p75^{NTR}$ receptor on the cell surface form a molecular complex that is capable to activate an apoptotic cascade. Keratinocytes can secrete proNGF and they have p75NTR on their surface. The presence of sortilin in normal human skin has not yet been investigated. The aim of the study was to demonstrate whether human keratinocytes express sortilin receptor and to investigate the effects of cutaneous neuropeptides substance P (SP), calcitonin gene-related peptide (CGRP), vasoactive intestinal polypeptide (VIP) and galanin (GAL) on the expression of sortilin in human keratinocytes. Cultures of normal human keratinocytes were treated with 10^{-8} M SP, CGRP, VIP or GAL for 30 min. After different time intervals, cells were harvested for total RNA isolation; in addition, cell lysates were collected. The effects of the neuropeptides on the mRNA expressions of sortilin were investigated by Q-RT-PCR and the protein levels were studied by means of Western blot and immunocytochemistry. We demonstrated that keratinocytes can express sortilin mRNA and the presence of sortilin protein was demonstrated on the surface of the cells. In addition, in normal human skin the sortilin receptors are found mainly in the basal layer of the epidermis. 6-12 h after neuropeptide treatment, each of the four neuropeptides caused a decrease in the sortilin mRNA expression to different extents (0.2-0.5-fold) and these decreases were reflected in sortilin protein levels 48-72 h after treatment. These results suggest that sortilin expression in keratinocytes and its regulation by neuropeptides may play an important role in skin homeostasis.

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Basic fibroblast growth factor stimulates the proliferation of human dermal fibroblast via activation of ERK1/2-dependent pathway

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Basic fibroblast growth factor (b-FGF) plays an important role in wound healing, tissue repair. b-FGF has various effects such as proliferation, angiogenesis, inflammation, and apoptosis. Particularly, it is well known to stimulate the proliferation of fibroblasts. But the mechanisms of its signaling pathways in dermal fibroblast are unknown. In this study, we investigated the signaling pathways of b-FGF in cultured human dermal fibroblasts. At first, we counted the number of dermal fibroblasts with or without b-FGF, and in the presence of various inhibitors. Next, we examined phosphorylation levels of ERK1/2 by immunoblotting. b-FGF stimulated the proliferation in 2.7-fold in dose- and time-dependent manner. b-FGF up-regulated phosphorylation levels of ERK1/2 in the cultured fibroblast, and the inhibitors of ERK1/2 (PD98059,U0126) down-regulated phosphorylation levels of ERK1/2. These results suggest that ERK1/2 is one of the signaling pathways of b-FGF in dermal fibroblasts.

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STAT5a is associated with development of psoriasis and induction of il-20 and il-24 cytokines related with keratinocytes proliferation and hyperkeratosis

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STAT (signal transducers and activators of transcription) family is involved in cytokine signal transduction pathways controlling inflammation and immune response. Among the seven members of family, STAT5 activation is involved in prolactin-dependent beta-casein production in mammary gland cells. Psoriasis is one of the most representative inflammatory skin diseases. The pathogenesis of psoriasis remains unclear although it has been suggested that deregulation of the immune system and malfunction of keratinocytes. In this study, we examined the relationship of STAT5a activation with psoriatic lesions. Immunohistochemical analyses showed that activated form of STAT5a resulted from phosphorylation and translocation to the nucleus, was found in hyperplastic epidermis, but, not in the normal epidermis. To elucidate the functional role of activated STAT5a in psoriasis, we generated HaCaT, transformed human keratinocytes, cell line expressing constitutive activated form of STAT5a. Using this cell line, we found that the expression levels of several psoriatic-related genes were elevated. Interestingly, overexpressed STAT5a induced the expression of IL-20 and IL-24 significantly. Previously, it was reported that transcripts of IL-20 were located to keratinocytes and overexpression of IL-20 in transgenic mice resulted in keratinocytes proliferation and hyperkeratosis. The upregulation of the IL-20 and IL-24 was also observed on psoriatic keratinocytes. Therefore, we postulated that the induction of Il-20 and IL-24 by activation of STAT5a might be involved in enhance of keratinocytes proliferation and pathogenesis of inflammatory skin disease psoriasis.

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The unfolded protein response is involved in epidermal keratinocyte differentiation

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When the cells are exposed to endoplasmic reticulum (ER) stress, incorrectly-folded proteins accumulate in the ER lumen. To maintain celluar homeostasis,cells cause unfolded protein response (UPR)which induces transcriptional activation of genes encoding ER-resident molecular chaperones,including BiP. The UPR is involved in the functional alteration of some cells, such as the differentiation from B cells to plasma cells. The UPR is also involved in pathological aspects of certain cells; for example, ER stress-triggered apoptosis in neuronal cells is found in neuronal degenerative diseases. The aim of this study is to determine whether the UPR is activated and plays an essential role during epidermal keratinocyte (KC) differentiation. Here, we show that the UPR-induced proteins Bip/GRP78 and HRD1 increased in cells of the supra-basal layers that contain KCs undergoing differentiation in the normal human epidermis as well as in skin-equivalent cultured KCs. Bip/GRP78 and HRD1 were immunostained poorly in the proliferating KCs of the squamous cell carcinoma tissue. Furthermore, microarray analyses and quantitative PCR revealed the altered expression of genes essential for human epidermal KC differentiation, including p21/CIP/WAF, C/EBP β, KLF4, occludin, ABCA12, PCNA, Ki-67, and integrin β4 in vitro by the well-known ER stress-inducing reagents tunicamycin, thapsigargin, and brefeldin A. Taken together, our findings strongly suggest that the UPR is critically involved in, and even facilitates, normal epidermal KC differentiation both *in vitro* and *in vivo*.

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The altered expression of syndecan 4 in the uninvolved skin of venous leg ulcer patients may predispose to venous leg ulcer

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Syndecan 4, a heparan sulphate proteoglycan and neuropilin 1, a transmembrane receptor are both involved in normal wound healing, but little is known about their possible role in venous leg ulcer pathogenesis. We aimed to investigate whether there are any expression abnormalities and/or gene polymorphisms of syndecan 4 and neuropilin 1 associated with venous leg ulcer. Syndecan 4 showed significantly lower mRNA and protein expressions in the uninvolved dermis of venous leg ulcer patients (n=15) compared to controls (n=15; p=0.0136), while neuropilin 1 showed no expression abnormalities. None of the examined syndecan 4 and neuropilin 1 polymorphisms showed difference in their allelic distribution between leg ulcer patients (n=92) and controls (n=92). We hypothethize that syndecan 4 may play an essential role not only in the inflammation and tissue formation phases of normal wound healing, but its expression abnormalities seen in the uninvolved dermis of venous leg ulcer patients may contribute to venous leg ulcer development.

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p75 neurotrophin receptor restores defective apoptosis in psoriatic keratinocytes: a role for transit amplifying cells

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p75 neurotrophin receptor (p75NTR) belongs to the TNF-receptor superfamily and signals apoptosis in many cell settings. p75NTR is detected in a subpopulation of basal keratinocytes and highly expressed in transit amplifying cells (TA), that are more susceptible to apoptosis stem keratinocytes. The aim of the present study was to evaluate whether p75NTR mediates apoptosis in human keratinocytes. We report that beta-amyloid, a ligand for p75NTR, induces apoptosis in human keratinocytes, as shown by the activation of caspase-3 only in p75NTR expressing cells. Brain-derived neurotrophic factor (BDNF) and neurotrophin-4 (NT4) which signal through p75NTR, induce a higher rate of apoptosis in HaCaT cells overexpressing p75NTR, as compared to mock cells ($p<0.01$ by MTT). On the other hand, NT4 fails to induce cell death in p75NTRsiRNA transfected HaCaT cells. Furthermore, BDNF induce JNK1 phosphorylation, which is a critical step in p75NTR signal transduction. Psoriasis is characterized by a reduced rate of apoptosis. In psoriasis, both NGF and TrkA are increased, as compared to normal skin. By contrast, p75NTR protein is absent in psoriatic lesional skin. In addition, p75NTR levels are significantly lower in psoriatic than in normal keratinocytes, as shown by flow cytometry. The rate of apoptosis in psoriatic TA cells is significantly lower as compared to normal TA cells. Indeed, p75NTR is significantly lower in TA cells from psoriatic skin than in TA from normal skin. Moreover, beta-amyloid or BDNF fail to induce apoptosis in TA from psoriatic keratinocytes. Infection of psoriatic keratinocytes with p75NTR retroviral vector restores beta amyloid-induced apoptosis. ($p<0.05$ by MTT). These results indicate that p75NTR acts as a proapoptotic receptor in human keratinocytes. Absence of p75NTR in TA cells could account for the resistance of psoriatic keratinocytes to apoptosis.

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EGFR and IL-1 synergistically promote keratinocyte antimicrobial defenses in a differentiation-dependent fashion

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Multiple ligands of the EGF and IL-1 families are overexpressed in psoriasis, and their overexpression can promote psoriasiform inflammatory hyperplasia. However, the role of these two proinflammatory signaling systems in regulating the innate immune functions of keratinocytes (KC) remains largely unexplored. We examined the responses of sub-confluent (proliferating) or 10 day post-confluent (differentiated) monolayer cultures of normal human KC (NHK) and organotypic cultures of reconstituted human epidermis (RHE) to IL-1a (10 ng/ml) and/or TGF-a (24 ng/ml). Culture supernatants were assayed for the antimicrobial peptides HBD2, S100A7 and the chemokine CCL20 by ELISA. mRNA levels were assayed using real-time quantitative RT-PCR. In proliferating NHK, TGF-a alone was ineffective in inducing either HBD2 or S100A7 transcripts, but IL-1a + TGF-a led to a 13-fold rise in HBD2 and an 8-fold rise in S100A7 relative to IL-1a alone. However, very little HBD2 protein was detected in conditioned medium, indicative of a lack of HBD2 secretion under proliferative conditions. In contrast, in differentiated NHK, TGF-a evoked a 4-fold rise in secreted HBD2 and a 3-fold rise in secreted CCL20, relative to IL-1a alone. Synergism was also apparent in RHE cultures, where we saw a 7-fold increase in HBD2 secreted into the underlying medium when IL-1a was combined with TGF-a. As assessed by pretreatment with 10 uM parthenolide, responses to IL-1a +/- TGF-a were NF-kB-dependent in proliferating cultures, but NF-kB-independent in differentiated cultures. In contrast, inhibition of EGFR tyrosine kinase activity with 1 uM PD158780 blocked the response to IL-1a +/- TGF-a in differentiated cultures, whereas parthenolide was ineffective. Based on these results, we propose that the IL-1 and EGFR signaling pathways interact at multiple levels and synergize in the production of HBD2, S100A7 and CCL20, three of the most heavily upregulated transcripts in psoriatic plaques. Moreover, we show that this synergism is highly dependent on the state of KC differentiation.

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Insulin and IGF-1 signaling are key activators of Rac in the epidermis and cooperatively determine proliferative potential

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The lifelong self-renewal of the interfollicular epidermis is driven by a progenitor cell population with high proliferative potential. To date the upstream signals that determine this potential have remained largely elusive. Potential candidates are the insulin/IGF family of growth factors because they can couple metabolic activity to renewal requirements. To examine the *in vivo* role of insulin and IGF signaling only in the epidermis we generated epidermal specific knockouts for the insulin receptor (IR), IGF-1 receptor (IGF-1R) or both (dko) in mice and found that this progressively decreased epidermal thickness without directly affecting differentiation, proliferation or apoptosis. However, clonogenic capacity was impaired in IR or IGF-1R deficient primary keratinocytes, indicating loss of proliferative potential. This coincides with reduced expression of epidermal progenitor cell markers. Moreover, IGF-1 and insulin regulate the Rac/c-Myc stem cell axis in the epidermis since loss of the receptors strongly reduced *in vivo* activity of the small GTPase Rac while upregulating c-Myc expression. Importantly, expression of dominant active Rac rescued clonogenic potential of IR/IGF-1R negative keratinocytes. We conclude that epidermal IR and IGF-1R cooperatively control epidermal morphogenesis by determining the proliferative potential of interfollicular progenitor cells via regulation of Rac. Our results thus identify insulin/IGFs as essential components of the interfollicular epidermal progenitor cell niche.

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Aryl hydrocarbon receptor is an ozone sensor in human skin

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Ozone, one of the main components of photochemical smog, represents an important source of environmental oxidative stress to which the skin is exposed, especially during smoggy and ozone alert days. Yet, only very little is known about the effects of ozone exposure to human skin. Here, we determined the effect of attainable levels of ozone exposure to normal human epidermal keratinocytes (NHEK) on cytochrome P450 (CYP) family of isoforms which plays a determinant role in biotransformation of many environmental pollutants. Ozone (0.3 ppm for 20 min) exposure to NHEK resulted in increased protein and mRNA expression of CYP1A1, CYP1A2 and CYP1B1, the CYP isoforms responsible for metabolic activation of environmental pollutant such as polycyclic aromatic hydrocarbons. Because aryl hydrocarbon receptor (AhR) induces transcription of CYP1A genes, we determined the effect of ozone exposure on AhR. Ozone exposure resulted in increased expression of AhR protein and mRNA in NHEK. Exposure of NHEK to ozone was also found to result in an increased phosphorylation of EGFR. Next we determined the effect of ozone on downstream events of EGFR and found that it resulted in an increased activation of PI3K, and phosphorylation of AKT, ERK1/2, JNK1/2 and p38. To determine whether ozone-induced CYPs expression is EGFR and/or AhR-dependent, we silenced NHEK by appropriate siRNAs for EGFR and AhR and then exposed them to ozone. We found that AhR silencing by siRNA abolished the capacity of these cells to increase the protein and mRNA expressions of CYPs upon ozone exposure. However, silencing of EGFR did not show any effect on ozone-induced protein and mRNA expression of CYPs. These data indicate that AhR signaling is an integral part of CYPs induction by ozone. These studies caution about the toxicological consequences of ozone to human skin. We suggest that supplementation of skin care products with inhibitors of AhR and/or CYPs might be beneficial to skin damages caused by ozone, especially during smoggy and ozone alert days.

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C-Jun N-terminal kinases display differential roles in human epidermal neoplasia

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The c-Jun N-terminal kinase (JNK) proteins, including JNK1, JNK2 and JNK3, comprise one family of the three major mitogen-activated protein kinases (MAPK) along with Erk and p38. Our recent studies have demonstrated that the uncontrolled epidermal proliferation due to impairment of NF-kB function is dependent on JNK and its downstream target AP1. We have also shown that JNK/AP1 induction by expression of active MKK7, the immediate upstream activator of JNK, can synergize with oncogenic Ras to transform normal human epidermal cells into malignancies resembling squamous cell carcinoma (SCC). It is not clear, however, whether different JNK proteins play differential roles in human epidermal growth and neoplasia. To address this, we first established by western blotting that JNK1 and JNK2, as well as JNK3 which was previously thought to be restricted to brain and testis, are highly expressed in human epidermal cells. In order to determine the sufficiency and requirement of each JNK in epidermal neoplasia, we applied retroviral expression systems for stable expression of active JNK proteins or shRNAs for targeted gene silencing of each JNK in human keratinocytes. Our preliminary studies revealed that active JNK2 but not JNK1 was sufficient to act in synergy with oncogenic Ras to induce epidermal malignancy. The JNK2-induced neoplasia is dependent on intact AP1 function as AP1 inhibition by dominant negative c-Jun abrogated invasive tumor growth. Gene silencing of JNK2 induced a negative effect while silencing of JNK1 has minimal effect on epidermal neoplasia induced by MKK7 and Ras, or spontaneous SCC cells both in vitro and in vivo. We are currently investigating the role of JNK3 and the molecular mechanisms that lead to the differential roles of JNK1 and JNK2 in epidermal neoplasia.

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Extracellular stratifin acts as an antifibrogenic factor in modulation of extracellular matrix proteins in dermal fibroblasts

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Epidermal-mesenchymal communication plays a key role in regulation of extracellular matrix (ECM) during wound healing. Previously, we have demonstrated that keratinocyte-releasable stratifin stimulates MMP-1 expression in fibroblasts. Current study investigates the regulatory role of stratifin in the expression of ECM proteins using a pathway-specific microarray. We also studied the interaction of exogenous stratifin at the cell surface in an attempt to identify a receptor for stratifin. ECM gene expression profile, using an ECM pathway-specific oligo-array, in fibroblasts co-cultured with keratinocytes or treated with stratifin revealed significant increase in gene expression of MMP-1, stromelysin-1 (MMP-3), stromelysin-2 (MMP-10), collagenase-2 (MMP-8), and MT5-MMP. RT-PCR and Western blot were used to validate microarray results. Expression of MMP-3 mRNA and protein by stratifin was shown to be dose- and time-dependent. To characterize interaction of stratifin with cell surface protein(s), a Receptor Binding Assay was performed by competing 125I-stratifin with excess unlabelled protein. The results revealed a classical ligand-receptor binding curve with excess unlabelled stratifin completely replacing the bound 125I-stratifin, excluding any non-specific binding. This result was further supported by Cell Binding Assay where bound biotin-stratifin was detected by immunofluorescence at the cell surface of fibroblasts and excess unlabeled stratifin competed with the bound stratifin. Furthermore, we were able to pull down exogenous stratifin by isolating plasma membrane fraction from cells incubated with biotin-stratifin for 10 minutes at 4°C. In conclusion, our results demonstrate that keratinocyte-releasable stratifin acts as a potent antifibrogenic factor by upregulating key MMPs in dermal fibroblasts and this induction appears to be initiated by interaction of exogenous stratifin with a specific cell surface protein.

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S100A11 functions as a dual mediator for growth regulation in normal human keratinocytes

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We previously demonstrated a novel signal pathway involving S100A11 for growth inhibition of normal human keratinocytes (NHK) caused by high Ca++ or TGFβ (ProNAS USA, 102:13921-13926, 2005). Exposure to either agent resulted in transfer of S100A11 to nuclei, where it induces p21WAF1. In the present study, we found an additional mechanism for S100A11-mediated growth suppression through interaction with annexin A1 (ANXA1). Binding of S100A11 to ANXA1 facilitated the latter to inhibit the activity of cPLA2, an initiator enzyme of the arachidonic acid cascade, which is essential for the growth of NHK. On exposure of NHK to EGF, ANXA1 was cleaved solely at (12)Trp, and this cleavage was executed by cathepsin D. Cleaved ANXA1 lost the binding capcity to S100A11, resulting in restoration of cPLA2 activity. In squamous cancer cells, this pathway was shown to be constitutively activated. On the other hand, S100A11 has been shown to be overexpressed in many human cancers. To address the apparent discrepancy, we analyzed possible new functions of S100A11 and provide herewith evidence that 1) S100A11 is actively secreted by NHK, 2) extracellular S100A11 acts on NHK to enhance the production of EGF family proteins, resulting in growth stimulation, 3) RAGE (receptor for advanced glycation endproducts), NFkB, Akt, and CREB are involved in the S100A11-triggered signal transduction, and 4) production and secretion of S100A11 is markedly enhanced in human squamous cancer cells. These findings indicate that S100A11 plays a dual role in growth regulation of NHK and is involved in various human skin diseases with abnormality in the growth of epidermal keratinocytes.

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Increased fibroblast proliferation under low glucose conditions is linked to changes in the TGF beta signalling pathway but not to cytotoxicity of glucose metabolites

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High concentrations of glucose, as they occur in diabetes, exert cytotoxic effects which are attributed to an increased production of protein modifying glycolytic intermediates, especially the alpha-oxoaldehyde methylglyoxal. In addition, studies on the effects of caloric restriction demonstrated the beneficial effects of lowering glucose concentration, even though the mechanisms that are discussed to induce these effects do not include a reduction of detrimental glycating agents. In this study, the effects of glucose deprivation on human dermal fibroblasts were analyzed. Reducing the concentration of glucose in the cell culture medium down to 0.3 g/l resulted in an increased vitality of fibroblasts. This was accompanied by a higher number of cell doublings and an increased population of cells in the proliferative S/G2 phase of the cell cycle. However, these changes appeared not to be caused by changes in methylglyoxal production since high and low glucose cultures of fibroblasts did not differ in the amount of secreted D-lactate, which is enzymatically produced from methylglyoxal but not from glucose. Microarray analysis of glucose deprived fibroblasts rather indicates a direct relationship between glucose concentration and the TGF beta signalling pathway which may result in the observed cell behaviour.

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Isoform-specific regulatory role of the protein kinase C system in biological processes of human sebocytes

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Isoforms of the protein kinase C (PKC) system play crucial roles in the regulation of proliferation, differentiation, and apoptosis of human epidermal keratinocytes. However, we lack information about the involvement of the PKC system in human sebaceous gland biology. Therefore, in the current study, we have investigated the functions of the PKC isoforms in human immortalized SZ95 sebocytes. As revealed by Western blotting, immunocytochemistry, and Q-PCR techniques, SZ95 sebocytes express the conventional cPKCα, β; the novel nPKCδ, ε, η; and the atypical aPKCζ. Using molecular biological approaches (e.g. RNA-interference, RNAi), and functional assays (based on a multi-well fluorescence reader, Flex Station II) to detect Nile-Red labeling (assessing lipid accumulation) or mitochondrial membrane potential suppression (measuring apoptosis), we have furthermore shown that the activation of the PKC system by the PKC activator phorbol 12-myristate 13-acetate (PMA) stimulated lipid synthesis (a hallmark of differentiation) and resulted in translocation of PKCα and δ (but did not affect cell viability, as revealed by Sytox Green staining and MTT assays). In good accord with these findings, the effect of PMA was effectively abrogated by the application of isoform-selective inhibitors and RNAi-mediated "knock-down" of PKCα and δ. Of further importance, "silencing" of PKCδ (which intervention alone resulted in a suppressed basal lipid synthesis) or application of Rottlerin (a selective blocker of PKCδ) also prevented the action of arachidonic acid to induce lipid production and apoptosis. Finally, we have also found that "knock-down" of the endogenous PKCζ activity markedly increased basal lipid synthesis and apoptosis of the cells. Collectively, our findings strongly argue for the pivotal, antagonistic, and endogenous roles of certain PKC isozymes in the regulation of biological processes of human sebaceous gland-derived sebocytes.

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Heregulin-β1 promotes reepithelialization from human skin explants in culture

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Ligand-activated epidermal growth factor receptor (EGFR) conveys strong mitogenic stimuli in epidermal keratinocytes both in vivo and in vitro. Three additional members (HER2-4) of the same receptor superfamily are recognized in human epidermis, but their function in normal and wounded skin remains unclear. HER3 can bind several endogenous ligands e.g. heregulins (HRGs), and cell signalling through this receptor is reported to positively affect wound healing. Noteworthy, HER3 lacks intrinsic kinase activity and therefore needs to heterodimerize with other HERs to elicit downstream responses. Here, we studied the contribution of HER3-4 to skin regeneration in vitro by measuring the outgrowth rates from skin explants grown in presence of exogenous HRG-β1 (HER3-4 ligand) as compared to EGF (EGFR ligand) or vehicle. We utilized a fluorescent-based imaging technique to visualize the outgrowth course of neoepidermis from punch biopsies adhered onto deepidermized dermis at the air-liquid interface. The effects of HRG-β1 on neoepidermal morphology, mitotic activity, and differentiation were also studied. HRG-β1 at 10 or 20 ng/ml had a stimulatory effect on reepithelialization corresponding to approximately 30% of that seen for equimolar levels of EGF. As expected, the outgrowth of neoepidermis was enhanced by EGF at all concentrations tested (5, 10 and 20 ng/ml). Dose-dependency was observed for HRG-β1 at 5 ng/ml vs. 10 ng/ml and for EGF at 5 ng/ml vs. 10 or 20 ng/ml. The dynamic effects were accompanied by elevated keratinocyte proliferation since the number of BrdU-positive cells increased by HRG-β1 at 20 ng/ml (+43%), EGF at 10 ng/ml (+63%) and EGF at 20 ng/ml (+85%). Immunohistochemically, the expressions of differentiation markers involucrin, loricrin and filaggrin were not affected by exposure to either ligand. The study emphasizes the prominent role of EGFR in epidermal outgrowth although signalling from HER3-4 may contribute to the proliferative component of reepithelialization.

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FGF-2 induces HGF secretion from human adipose-derived stromal cells through JNK signaling pathway and inhibits fibrogenesis after ischemia-reperfusion injury.

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It has been reported that human adipose-derived stem/stromal cells (hASCs) not only have multipotency but also secrete growth factors such as VEGF and HGF. However, regulatory mechanisms of the secretion and its relation to ischemia-reperfusion injury of the adipose tissue and wound healing have not been well studied. First, effects of wound-related growth factors (FGF-2, VEGF, HGF, and PDGF) on hASCs were evaluated *in vitro*. By supplementing each growth factor to control medium (DMEM, 10%FBS), proliferative effects in culture and secretions of the growth factors by hASCs (real-time RT-PCR and ELISA) were assessed. Using specific inhibitors, signaling pathways related to the effects were also examined. In addition, *in vivo* studies were performed using a mouse model for ischemia-reperfusion injury of the adipose tissue. FGF-2 and PDGF dose-dependently promoted the proliferation of hASCs, while VEGF and HGF had no effects. FGF-2 greatly enhanced the expression of HGF transcripts and protein secretion from hASCs. The effects of FGF-2 on proliferation and HGF secretion were significantly inhibited by a JNK inhibitor, not by ERK or p38 inhibitors. Increased phosphorylation of c-jun by FGF-2 and its inhibition by a JNK inhibitor were also observed. In animal models, FGF-2 was highly detected soon after ischemia-reperfusion injury of the adipose tissue, while HGF was up-regulated thereafter. The up-regulation of HGF was inhibited by a JNK inhibitor. In addition, inhibition of JNK signaling pathway resulted in significantly promoted fibrogenesis in the injured adipose tissue 2 weeks after injury. hASCs play an important role in the process of wound healing with increased proliferation and HGF secretion and contribute to minimize scar formation. These effects of hASCs were induced by FGF-2 released from the injured tissue mainly through JNK signaling pathway.

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Instruction of antigen-presenting cells by peptide-specific T helper 1 lymphocytes

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T helper cells control multiple functions of hematopoietic cells, especially those of MHC class II-expressing macrophages. Despite the importance of these interactions and the extensive knowledge about the effects that antigen presenting cells (APC) exert on T cell differentiation and functioning, surprisingly little is known about the reverse signalling, the education of APC by T cell signalling. To address this question, we studied the instruction of APC and dendritic cells (DC) by interferon γ (IFN-γ)-producing Th1 cells. As this situation seems to be of special relevance for the instruction of APC by tumor-specific T lymphocytes in tumor-draining lymph nodes, we used Th1 cells, specific for the tumor-associated antigen Tag and either total APC (mainly B cells and macrophages/monocytes) or DC, to analyze cell cycle associated genes and cytokines/chemokines influencing the microenvironment. We polarized naive Tag-specific CD4 T cells into interleukin-4-producing Th2 or IFN-γ-producing Th1 cells. One prominent finding was that Th1 cells potently induced anti-angiogenic chemokines, such as CXCL9 or CXCL10, while Th2 cells did not. As Th1 cells can arrest tumor growth or proliferation of intracellular pathogens, we next analyzed gene arrays focussing on pro- and anti-apoptotic, and on pro- and anti-proliferative candidate genes by reverse transcriptase polymerase chain reaction (RT-PCR) of extracts from purified APC. A cluster of pro-apoptotic genes, i.e. Bax, caspase 2 or death associated protein kinase 1, and multiple anti-proliferative genes, i.e. cyclin-dependent kinase inhibitor 2A or Reprimo, were highly (up to >20-fold) induced in APC. On the other hand, proto-tumorigenic genes, i.e. myc or early growth response 1, were down-regulated. Consistent with these results, Western blot analysis of purified APC showed a dramatic (>90%) and fast decline of c-myc phosphorylation. In conclusion, peptide-specific Th1 cells exert a strong negative feedback signalling on APC that should not only arrest APC and highly purified DC but also induce apoptosis in both populations.

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Effect of indole alkanoic acid conjugates on neurite outgrowth of primary sensory neurons *in vitro*

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Neurotrophic properties of a group of structurally related compounds, e.g. their ability to mimic the effect of growth factors essential for growth and maintenance of cutaneous nerves, were investigated in vitro. Indole alkanoic acids such as indole-3-acetic acid (IAA) or indole-3-butyric acid (IBA) are naturally occurring growth-promoting substances in plants, also called "auxins". Auxin binding to aminoacids releases auxin conjugates involved in some regulatory functions in the plant. The presence of an indole moiety confers to these auxins, and their conjugates, good free radical scavenging properties. Besides, there are some structural similarities between auxin conjugates and the neurotrophic compound ETRY 50 (J. Invest. Dermatol. (abstract), 121: 0684, 2003). These features prompted us to examine the potential of selected auxins conjugates for neuroprotection. Antioxidant properties of conjugates were quantified using regular biochemical tests. Neurotrophic properties were tested on primary rat sensory neurons obtained from dorsal root ganglia of rat embryos. Neurons were exposed to different concentrations of test compounds and NGF (nerve growth factor) was used as positive reference. Neurite length analysis was performed 72 hours post treatment, after labeling with beta-tubulin, using an IN Cell AnalyzerTM 1000 microscope. As expected, all conjugates proved to be strong antioxidants against deleterious reactive oxygen species such as hydroxyl radical or superoxide anion. Three auxin conjugates, IAA-GLU, IPA-GLU and IBA-ASP have shown significant neurotrophic effects, increasing average neurite length as compared to the control. These data support the possible use of indole alkanoic acids conjugates as treatment for some physiopathological conditions or for anti-aging.

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Impaired Akt-signalling in keratinocyte derived cell lines in response to hepatocyte growth factor stimulation

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Recently we have shown, that hepatocyte growth factor (HGF) potently protects human primary keratinocytes (KC) from UVB induced apoptosis. In contrast to KC, HGF had no protective effect on the KC-derived cell lines A431 and HaCaT. Here we investigated the molecular mechanisms underlying this phenomenon. Since both cell lines are known to bear several mutations in the p53 gene, we analyzed whether or not p53 is involved in the anti-apoptotic action of HGF. By using siRNA technology we knocked out p53 in KC and KC-derived cell lines. Twenty-four hours later the cells were exposed to UVB with or without the addition of HGF. Knock down of wildtype p53 in KC did not abrogate the anti-apoptotic effect of HGF. Interestingly, silencing of mutated p53 in KC-derived cell lines induced apoptosis by itself, which could not be inhibited by the addition of HGF. Binding of HGF to its receptor cMet leads to phosphorylation of Akt and consequently the activation of multiple downstream targets, which are involved in the anti-apoptotic machinery of KC. For full activation, Akt needs to be phosphorylated at Ser473 and Thr308. The latter is mediated by PDK1. We found that in KC HGF rapidly induced the phosphorylation of both residues. By contrast, in KC-derived cell lines the induction of phosphorylation of Thr308 was completely missing, resulting in incomplete Akt signaling. Interestingly, we could not detect any mutation in the Akt and PDK1 mRNA in the cell-lines, suggesting a defect in the activation of other modulator proteins. Our data demonstrate that the anti-apoptotic activity of HGF in KC is independent of p53 activation, and that mutant p53 in the KC-derived cell lines A431 and HaCaT is not responsible for the lack of protection by HGF. Our additional finding that signalling via cMET results in incomplete activation of the Akt pathway in these cell-lines provides the most likely explanation for the absence of the HGF-mediated anti-apoptotic effect.

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Cortisol synthesis and regulation of proliferation in keratinocytes

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Glucocorticoids (GC) have been used for over 50 years to treat psoriasis and yet the ability of normal human keratinocytes to synthesise cortisol has not been reported. In addition, one of the common side effects to chronic GC treatment is skin thinning but the mechanism behind this effect remains elusive. Using immunofluorescence histochemistry, we have shown that skin expresses steroidogenic acute regulatory protein (StAR) and metastatic lymph node 64 (MLN64), which are essential for delivering cholesterol to the inner mitochondrial membrane in other steroidogenic tissues. We find that StAR expression is restricted to the epidermal basal layer, whereas MLN64 is only found in suprabasal layers of the epidermis. Interestingly, StAR is absent in the epidermis of psoriatic skin. Using radioimmunoassay, we found that normal primary keratinocytes could synthesise pregnenolone. Treatment of keratinocytes with inhibitors of pregnenolone metabolism increased pregnenolone concentration by over three-fold compared to untreated cells. Additional analysis of steroid metabolism using thin layer chromatography demonstrated that keratinocytes metabolised pregnenolone to progesterone and progesterone to the inflammatory regulator cortisol. To examine the effects of cortisol on skin thinning, we treated primary keratinocytes with dexamethasone over a time course. We found that dexamethasone upregulated protein expression of mitogen activated protein kinase phosphatase 1 (MKP1) in keratinocytes. This pathway is common to inhibiting oesteoblast proliferation and may be a causal link between osteoporosis and skin thinning, which are both associated with chronic GC treatment. Collectively these data show that human keratinocytes are able to synthesise cortisol, that steroid synthesis could be ablated in psoriasis due a down regulation of StAR. In addition, skin thinning through chronic treatment with GC could be regulated via the activation of MKP1.

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A new pro-differentiating cotton thistle extract induces Loricrin and LEKTI expression

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Epidermal differentiation markers are the main components of skin barrier function and wound healing. Cotton thistle is a wild plant commonly known for its stimulating and cardiotonic properties as well as for its anti-rickets properties in children. This plant contains sesquiterpenic lactones and various phenolic compounds such as flavones. To address cotton thistle healing activity on cutaneous tissue, we studied immunostaining of various differentiation markers on skin biopsies. Immunostaining was carried out on skin samples 7 days after topical application of cotton thistle extract. Some skin biopsies were stripped before application of the extract. Keratinocyte proliferation and differentiation were evaluated using MTT assay and staining of epidermal markers. These studies revealed that treatment with 2% of cotton thistle extract regulated epidermal differentiation. Loricrin and LEKTI (lympho-epithelial Kazal type related inhibitor) proteins were stimulated in the treated stripped skin samples. Moreover, these experiments showed an increase in involucrin and filaggrin staining in both stripped and normal treated skins. Regeneration of the epidermis and enhanced collagen I synthesis were also demonstrated on burned and normal treated skin samples respectively. Taken together, these results point out that this new cotton thistle extract can help maintain a good skin homeostasis and barrier effect in skin care applications.

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The effect of (-)-epigallocatechin-3-gallate (EGCG) on cell cycle, apoptosis and lipid production in SEB-1 sebocytes

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Many favorable effects of green tea, such as anti-inflammatory, antibacterial, and antimutagenic activities, have been reported. The main pathogenesis of acne is interplay of an increased sebum production, inflammation, follicular hyperkeratinization, and the action of Propionibacterium acnes. Acne treatment focuses on this main pathogenesis. In our previous study, EGCG improved acne lesion by inhibition of P. acnes and it also induced the expression of transglutaminases. This study was performed to examine the effect of EGCG on immortalized sebocyte cell line (SEB-1) and to determine whether EGCG can induce apoptosis of SEB-1. To test the effect of EGCG on SEB-1 viability, cells were incubated for 24, 48, or 72 hours with 0, 5, 10, 20, and 40 μM EGCG. Viability was quantitated by MTT assay. To determine the effect of EGCG on cell cycle and apoptosis, SEB-1 sebocytes were incubated for 24, 48, and 72 hours with 0 to 40 μM EGCG, and then western blotting was performed. To determine the effect of EGCG on lipid synthesis, SEB-1 sebocytes were incubated for 72 hours with 0 to 40 μM EGCG. TLC was performed after total lipid extraction from the sebocytes and it was analyzed for individual lipid contents. Results showed that EGCG caused dose-dependent and time–dependent decrease in viable SEB-1 sebocytes. A portion of this decrease could be attributed to cell cycle alteration by decreased cyclin D1. EGCG induced apoptosis in SEB-1 sebocytes with the findings of increased cleaved caspase 3 and Bax/Bcl-2 protein ratio. EGCG inhibited lipid accumulation in SEB-1 sebocytes dose-dependently. These results demonstrate that EGCG can act directly to inhibit cell proliferation and to induce cell cycle arrest and apoptosis. These mechanisms may contribute to the reasons that EGCG can be an effective agent for the treatment of acne.

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Receptor type protein tyrosine phosphatase-kappa mediates cross-talk between transforming growth factor beta and epidermal growth factor receptor signaling pathways in human keratinocytes

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Epidermal growth factor receptor (EGFR) signaling pathways promote human keratinocyte survival and proliferation. In contrast, transforming growth factor beta (TGF-β) signaling pathways are strongly anti-proliferative. We report here that functional integration of these two opposing pathways is mediated by receptor type protein tyrosine phosphatase kappa (RPTP-κ). RPTP-κ specifically dephosphorylates and thereby blocks EGFR-dependent signaling. Over-expression of RPTP-κ inhibited keratinocyte proliferation 75% ($p<0.05$, n=6). Addition of TGF-β (2.5 ng/ml) induced RPTP-κ mRNA in a dose and time dependent manner. RPTP-κ mRNA was elevated 3-fold at 3 hours post treatment and remained elevated (8-fold) for at least 48 hours. Induction of RPTP-κ by TGF-β reduced EGF-stimulated EGFR tyrosine phosphorylation 40% ($p<0.05$, n=4). Prevention of TGF-β induction of RPTP-κ by shRNA significantly attenuated (50%, $p<0.05$, n=3) keratinocyte growth inhibition by TGF-β. Treatment of keratinocytes with TGF-β rapidly (within 30 minutes) activated Smad2, Smad3, and Smad4, and MAP kinases Erk1/2 and p38. We investigated the role of these signaling molecules in induction of RPTP-κ. Pharmacological inhibition of type I TGF-β receptor completely blocked TGF-β activation of Smads and MAP kinases, and induction of RPTP-κ. shRNA knockdown of Smads revealed that Smad3 and Smad4, but not Smad2, are involved in TGF-β induction of RPTP-κ. Pharmacological inhibition of MAP kinases demonstrated that p38 pathway, but not Erk1/2 pathway, regulates RPTP-κ expression. Regulation of RPTP-κ expression by Smad3/4 and p38 pathways was additive, accounting for 70% and 30%, respectively, of TGF-β induction. These data describe a novel mechanism of cross-talk between EGFR and TGF-β pathways, in which RPTP-κ functions to integrate growth promoting and growth inhibiting signaling pathways.

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Unravelling the RIP4 signaling pathway to NF-κB

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RIP4 belongs to a family of S/T kinases, the RIP kinases, generally involved in activation of the transcription factors NF-kB and AP-1. RIP4 consists of an N-terminal kinase domain, an intermediate domain and a C-terminus containing 11 ankyrin repeats. RIP4 deficient mice are characterized by perinatal lethality due to suffocation because all external orifices are fused. Furthermore, they are born with a strong skin phenotype that includes hyperplasia, absence of stratum corneum, parakeratosis and abnormal development of hair follicles and sebaceous glands (Holland et al., Curr. Biol. 2002). In view of these observations we are investigating which, RIP4 dependent, molecular pathway is involved. Both the NF-kB and AP-1 pathways play an important role in skin differentiation. Overexpression of different RIP4 domains show that the kinase domain is sufficient and necessary to activate NF-kB. Furthermore, the RIP4 kinase activity is required for NF-kB activation. We could demonstrate that RIP4 ubiquitination is enhanced in inflammatory conditions and that overexpression of TGF-activated kinase 1 (TAK1) enhances RIP4 ubiquitination. By co-immunoprecipitation, we showed that RIP4 is able to interact with TAK1 and that this interaction is mediated trough the intermediate domain of RIP4. As a more general approach we are performing Tandem Array Purifications (TAP) with RIP4 as a bait. Interestingly, TAK1 deficient mice are also characterized by disturbed skin homeostasis (Omori et al., J. Biol. Chem., 2006, Sayama et al., J. Biol. Chem. 2006). All together this could indicate that similarities in the skin phenotype of RIP4 and TAK1 deficient mice could originate from the same molecular pathway.

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PKCδ inactivation in mice skin alters insulin signaling similarly to diabetes

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Diabetes is associated with a variety of skin complications including impaired wound healing and the development of chronic untreatable ulcers. We have previously demonstrated that insulin plays a crucial role in skin cell physiology, regulated by PKCδ and PKCα. Therefore, we have investigated the role of PKCδ and PKCα in the insulin signaling pathway utilizing skin of streptozotocin induced diabetic mice in WT, PKCδ and PKCα null mice. Utilizing immunoblotting and immunohistochemistry we have investigated the effect of diabetes and impact of PKC deficiencies on distinct changes in expression and activation of key molecules underlying the insulin signaling pathway in skin. In diabetic skin, IR expression, and activation were significantly elevated, while IGF-1R expression remained unaffected. In addition, the diabetic state was associated with increase in the key adaptor proteins IRS1 and IRS2. Downstream to IRS proteins, Ras expression was unchanged, while both expression and serine phosphorylation of a Ras target molecule - Raf-1 were reduced, accompanied with increased accumulation of activated Erk1/2. The PI3K-Akt pathway was mostly un-affected. Similarly to the diabetic state, skin of non diabetic PKCδ null mice exhibited increased levels of IR, Raf-1 expression was reduced and Erk1/2 expression was enhanced. In contrast, in PKCα null skin, IR levels were unchanged, Raf-1 was constitutively activated while Akt activation was abrogated in both non-diabetic and diabetic animals. The changes in signaling molecules was associated with defects in skin morphology and wound healing. Diabetes skin was characterized by epidermal thickening, disruption of the dermal layer and impairment in wound healing. Similar changes were observed in skin of non diabetic PKCδ null mice but not in PKCα null mice skin. In summary, the diabetes state alters insulin signaling in skin, similarly to abrogation of PKCδ expression, associated with defects in skin physiology and wound healing impairment.

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Human keratinocytes export HSP90-alpha that drives both keratinocyte and dermal fibroblast migration through CD91/LRP-1 signaling during wound healing

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Cell migration driven by serum growth factors is critical for skin wounds to heal. For example, human keratinocytes (HKs) are induced by serum TGF-alpha to re-epithelialize and close skin wounds, a process which is blocked in chronic non-healing wounds. The purpose of this study was to identify novel downstream targets of TGF-alpha that induce HKs to migrate. We stimulated HKs with TGF-alpha and analyzed proteins secreted in the condition medium (CM) because the whole CM, when added to our migration assays, strongly stimulated the migration of BOTH naïve HKs and human dermal fibroblasts (HDFs). By standard column chromatography and performing mass spectrometry on purified CM proteins, we found that the protein responsible for the pro-motility effect of the CM was HK-derived heat shock protein 90-alpha (hsp90α). TGF-alpha causes a rapid membrane translocation and subsequent secretion of hsp90α from the HK. This secreted hsp90α then binds to the CD91/LRP cell surface receptor on BOTH HKs and HDFs and drives motility of BOTH cell types. Increased motility of HKs promotes re-epithelialization while increased motility of HDFs promotes neo-dermis formation. Further analyses of hsp90α action showed that the pro-motility domain resides in the middle domain plus the charged sequence, but did not involve the ATP-binding and ATPase domain. Intriguingly, unlike growth factors, hsp90α-driven cell migration cannot be blocked by TGF-beta family cytokines which are abundant in wounds. Thus, this study identified a novel TGF-alpha invoked autocrine loop that provides an explanation for how cells migrate during wound healing and how TGF-alpha promotes wound healing. We conclude that extracellular hsp90 α plays a central role in the healing of skin wounds.

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Sphinganine selectively activates the peroxisome proliferator activated receptor (PPAR)-β/δ in human epidermal keratinocytes

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Peroxisome proliferator activated receptors (PPARs) are members of the nuclear hormone receptor superfamily of inducible transcription factors that are activated by endogenous lipid metabolites. Both PPARα and PPARγ play important roles in lipid metabolism leading to barrier function and have anti-inflammatory properties, while PPARβ/δ has additional roles in immune function, wound healing, and keratinocyte differentiation.. Given that the synthesis of sphingolipids increases markedly during keratinocyte differentiation, we examined the role of PPARs, particularly PPARβ/δ, in the regulation of (and by) sphingolipid metabolism. Treatment of cultured human keratinocytes (CHK) with either the PPAR ligand GW 50156 (GW) or the sphingolipid sphinganine (SA) (but not other sphingolipids) activated a consensus response element (PPRE) in a dose-dependent manner. Overexpression of PPARβ/δ (but not PPARα or PPARγ) significantly enhanced the transcriptional activity of SA (6.2-fold); fumonisin B1 (FB1), which blocks ceramide synthesis and increases intracellular SA levels, also significantly increased transcriptional activity (2.5-fold). In addition, in CHK overexpressing PPARβ/δ, either GW (8μM) or SA (5μM) significantly increased the mRNA levels for sphingosine kinase 1 (SPHK1) . The mRNA levels of adipocyte differentiation related protein (ADRP), a direct PPARβ/δ-activated target gene, also increased upon treatment with either GW or SA (5.3-fold and 3.5-fold, respectively). Consistently, FB1 also increased the mRNA levels of SPHK1 and ADRP by 1.7-fold and 2.0-fold, respectively. Together, these results demonstrate selective SA activation of PPARβ/δ in keratinocytes, which in turn, regulates both the sphingolipid biosynthetic pathway and other transcriptional targets. These findings reveal a novel pathway by which (a) sphingolipid metabolite(s) can both modulate differentiation and affect sphingolipid metabolism in the epidermis.

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Phospholipase D1 regulates primary mouse epidermal keratinocyte differentiation in response to moderate calcium stimulation in a lipase activity-independent manner

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A previous study in our laboratory has suggested a role for PLD1 expression/activity during keratinocyte differentiation induced by 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$). Comparison of the time courses of the effects of $1,25(OH)_2D_3$ on PLD1 expression and various keratinocyte differentiation markers indicated that PLD1 expression correlated with the activity of transglutaminase (TGase), a late (granular) differentiation marker, suggesting the possibility that PLD1 mediates keratinocyte differentiation. To study the role of PLD1 in the proliferation and differentiation of primary mouse epidermal keratinocytes, these cells were infected with adenovirus possessing wild-type PLD1 or lipase-dead PLD1 (PLD1-LI) in control (25μM calcium) or 125μM calcium-containing serum free keratinocyte medium (SFKM) with high infection efficiency (~90%). Cell proliferation and differentiation were measured by the incorporation of [^{3}H]thymidine into DNA and a TGase activity assay, respectively. Total PLD (PLD1/PLD2) activity was monitored using a radiolabeled activity assay in the presence of ethanol to generate phosphatidylethanol (PEt), a marker of PLD activity. Western analysis indicated that both PLD1 and PLD1-LI were overexpressed. Initial studies showed that overexpression of PLD1 increased PEt synthesis, while PLD-LI did not. Upon moderate calcium stimulation TGase activity was increased 3.7- and 2.8-fold over control by overexpression of PLD1 and PLD1-LI, respectively. [^{3}H]Thymidine incorporation was inhibited to ~70% of control by a moderate calcium concentration; PLD1 and PLD1-LI decreased this value, although not significantly, to ~40%. Taken together, our results suggest that PLD1 regulates $CaCl_2$-stimulated late differentiation with minimal effects on early differentiation (growth arrest). These effects of PLD1 appear to occur independently of lipase activity, consistent with a previous report examining PLD1-regulated cell migration in HeLa cells.

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Evaluation of candidate EGFR ligand sheddases in psoriatic skin

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Epidermal growth factor receptor (EGFR) ligands including amphiregulin (AREG), HB-EGF and TGF-α are important regulators of multiple keratinocyte (KC) functions including migration, proliferation and survival, which have been implicated in various skin pathologies. EGFR ligands are synthesized as membrane-bound precursors and require proteolytic cleavage to produce the soluble, active forms. Several members of the ADAM family have been identified as candidate EGFR ligand sheddases in various cellular systems; however, little is known about the ADAMs that mediate EGFR ligand shedding in human skin. To address this, we first characterized the expression of several candidate ADAMs in psoriasis. Analyzing RNA isolated from 58 paired samples of uninvolved (PN) and lesional (PP) psoriatic skin on Affymetrix HU133 Plus 2.0 microarrays, we found that ADAMs 9, 10, 17, and 19 were significantly increased in PP vs PN skin (1.4 to 1.8-fold, $p<0.0001$). However, absolute expression levels were low. We therefore re-examined expression levels by QRT-PCR with RNA isolated from 10 paired samples of PN and PP skin. Interestingly, the highest fold-increase was observed for ADAM19 (24.6 +/- 5.0-fold, PP vs. PN, mean +/- SEM). In addition, ADAM15 (11.7 +/- 1.5-fold), ADAM9 (8.4 +/- 1.4-fold), ADAM17 (8.1 +/- 1.0-fold) and ADAM10 (2.5 +/- 0.5-fold) were all markedly and significantly increased ($p<0.01$ for all). For comparison, AREG, HB-EGF and TGF-α were overexpressed in the same samples by 7.7 +/- 4.2-fold, 27.9 +/- 9.1-fold and 25.4 +/- 5.1-fold, respectively. We next examined ADAM expression in lentivirus-infected KC overexpressing HB-EGF, because these cells shed very high levels of endogenous AREG and are highly motile (JID 126: A565). Interestingly, QRT-PCR analysis of RNA isolated from HB-EGF-infected cells revealed selective overexpression of ADAM19 (4.8 +/- 0.8-fold vs. uninfected, n=6) with no significant changes for ADAMs 9, 10, 15 and 17 (<1.5-fold). Together, our findings identify ADAM19 as a promising candidate for EGFR ligand shedding in KC and skin.

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Metalloprotease-dependent activation of the epidermal growth factor receptor following sodium lauryl sulfate exposure in an *in vitro* model of irritant contact dermatitis

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Irritant contact dermatitis (ICD) is a prevalent pathological condition induced by exposure of the skin to irritants. Despite significant research examining both in vitro and in vivo effects of a variety of irritants, early molecular events of ICD remain incompletely defined. We hypothesized that identifying early events is key to developing effective treatments for ICD. Previously, we reported de novo expression of early growth response-1 (egr-1) following treatment with sodium lauryl sulfate (SLS), a model irritant, in cultured keratinocytes or HaCaT cells. Egr-1 induction by SLS was potently inhibited by the specific epidermal growth factor receptor (EGFR) inhibitor AG1478 suggesting a key role of EGFR in irritant-induced signal transduction. We have expanded these studies and have demonstrated further evidence of EGFR activation, showing dose- and time-dependent phosphorylation of EGFR at tyrosine-1173 following SLS treatment. Furthermore, activation of EGFR by SLS appears to be linked to induction of proinflammatory cytokines since AG1478 treatment also inhibits SLS-induced expression of IL-8 and VEGF, with no effect on SLS induction of IL-6. SLS treatment may directly activate EGFR or may induce EGFR transactivation via metalloprotease-mediated cleavage of EGFR ligands. In order to test the latter hypothesis, HaCaT cells were pretreated with the metalloprotease inhibitor, TAPI-2, prior to SLS stimulation for one hour. TAPI-2 treatment inhibited egr-1, IL-8, and VEGF mRNAs 89 $\pm$ 6%, 53 $\pm$ 11%, and 72 $\pm$ 7%, respectively, but had no effect on SLS induction of IL-6 mRNA. These data suggest that induction of proinflammatory pathways by SLS occurs at least in part through metalloprotease-dependent transactivation of EGFR. These studies are the first to propose irritant activation of EGFR through a proteolytic pathway, and that activation of this pathway by irritants can potentially be modified by small molecule inhibitors.

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The novel I-BAR protein Missing in Metastasis controls guided cellular migration through spatial control of growth factor receptor signaling

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Guided cell migration plays a critical role in normal development, wound healing and in cancer progression. While growth factor signals such as EGF or PDGF are critical migratory cues and that play a well-studied role in wound healing and tumor metastasis, the cell biological mechanisms for guidance remain poorly understood. We and others have previously shown that Missing in Metastasis (MIM) is a defining member of a novel class of membrane-associated BAR domain protein (I-BAR) that regulates actin cytoskeleton and whose levels are altered in human cancer. To better understand how MIM functions in epidermal guided cell migration, we have used the Drosophila border cell migration system that allows live imaging of the cytoskeleton of defined cell clusters as the cluster moves through intact tissue. Here we provide the first genetic evidence that I-BAR proteins regulate directed cell migration. Drosophila MIM is required for guided border cell migration, with loss of MIM resulting in delocalized phosphotyrosine accumulation and decreased cell movement. Live imaging of border cell clusters demonstrates that MIM does not effect general actin polymerization or the ability to form filopodia, but the ability of the cell to send polarized cell extensions toward the guidance cue. MIM alters the temporal and spatial growth factor signaling in part through inhibition of receptor-mediated endocytosis This is accomplished in part by assembling a complex of proteins that balances the endocytosis-promoting activites of the actin regulator and src oncogene target cortactin. These studies demonstrate that the novel I-BAR family play important roles in interpreting local migratory cues to the actin cytoskeleton during cell migration.

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Keratinocyte RANK signals via AKT and ERK and regulates epidermal thickness *in vivo*

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RANK, its ligand (RANKL) and the decoy receptor osteoprotegerin (OPG) have been associated with numerous activities in several organ systems. We have previously demonstrated expression of RANK and its ligand in epidermis and shown a role for these molecules in keratinocyte proliferation. We have now explored the molecular mechanisms involved in RANKL signaling and the role of RANK signaling in epidermal homeostasis. HaCaT keratinocytes were stimulated *in vitro* with rhRANKL and analysed by fluorescent microscopy for expression of NFκB or by western blotting for activation of the AKT and ERK pathways. NFκB p65 was detected exclusively in the cytoplasm of cells under all conditions and no nuclear translocation was detected following rhRANKL stimulation. Although low levels of phosphorylated AKT (pAKT) and pERK1/2 were detected in control cultures, marked induction was detected 30 minutes following rhRANKL stimulation (100ng/ml) whereas levels of total AKT and ERK1/2 remained unchanged. To define the effects of RANKL signaling *in vivo* we compared skin of RANKL KO and wild type mice. RANKL KO epidermis was atrophic compared to WT controls (8.56 $\pm$ 0.31 μm and 12.03 $\pm$ 0.35 μm; N=8 and N=9 respectively; P<0.0001). Proliferating keratinocytes, visualized by Ki67 expression, were decreased in RANKL KO epidermis (KO: 10.65 $\pm$ 3.038 and WT: 28.14 $\pm$ 1.982 Ki67+ KC per mm of basal epidermis; P=0.0028; N=4). These data indicate that RANK signaling is mediated, at least in part, by AKT and ERK, known regulators of keratinocyte proliferation, differentiation and stratification. AKT is also involved in the resistance of KC to apoptotic stimuli and may be responsible for RANKL mediated resistance of HaCaT to TRAIL induced apoptosis. Finally, the decreased KC proliferation observed in RANKL KO mice indicate a key role for RANKL in the regulation of KC homeostasis and in maintenance of epidermal integrity in the steady state.

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The skin specific insulin receptor knockout (SIRKO) mouse: A new outlook on diabetes complications and skin cancer

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Insulin is well known for its role in glucose metabolism. However, in recent years, newly acknowledged functions have been identified. These include regulation of proliferation, differentiation, apoptosis and more. Due to these multifunctional roles of insulin, it is important to maintain a strict balance of its activation. In order to demonstrate pathological consequences of insulin dysregulation, we developed a unique *in vivo* model of skin-specific insulin receptor knockout mice (SIRKO). In this model the insulin receptor is inactivated specifically in the epidermis. Lack of insulin receptor expression in the epidermis led to a striking defect in the wound healing process. The strength of the healed wound was reduced by two folds compared to the normal littermates. On the other hand lack of insulin receptor resulted in decreased transformation rate. When skin tumors were induced by following a two-stage chemical carcinogenesis protocol, the SIRKO mice exhibited a significant decrease in skin tumor induction compared to the control group. In our process to uncover the underlying abnormality leading to the above pathologies, we carried studies aimed to identify changes in the cellular ultra structure by electron microscopy analysis. Somehow to our surprise we have found lack of insulin receptor expression was associated with abnormal structure of cellular intermediate filaments. The intermediate filaments of the SIRKO skin cells are shorter, broader and flatter compared to the control skin. Thus, closer monitoring of insulin treatment and evaluation of its local effects is required, and its role in the assembly of intermediate filaments must be further investigated. We believe that elucidating the role of insulin in cellular pathways will lead to development of innovative drugs for treatment or prevention of insulin-associated pathologies.

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Structure-function analysis of Srcasm-dependent Src-family kinase downregulation-a major role for Grb2

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Src-family tyrosine kinases (SFKs) are important regulators of cellular growth and differentiation. Srcasm is a novel Src-family tyrosine kinase substrate that has been shown to downregulate SFKs through a mechanism that requires acidic lysosomal pH. Srcasm associates with Grb2, a regulator of EGFR signaling, when phosphorylated at tyrosine 392. In this study, we characterized a mutant Srcasm Y392F that does not associate with Grb2. Although Srcasm and Srcasm Y392F can downregulate cellular Fyn levels in transfected cells, Srcasm Y392F is a more potent downregulator of SFKs, and appears to be approximately 5-fold more efficient in downregulating SFKs. During SFK downregulation, native Srcasm levels remain constant; however, the levels of Srcasm Y392F appear to be concomitantly downregulated with the Src kinase, suggesting a strong association between Srcasm Y392F and SFKs, and Grb2-Srcasm association preserves cellular Srcasm levels. Increasing cellular Grb2 levels inhibits the ability of Srcasm to downregulate SFKs. However, Srcasm, in the presence of SFKs, does not downregulate Grb2. We also tested the ability of Srcasm Y440F mutants to downregulate SFK levels, and found that this Srcasm mutant was less efficient at downregulating SFKs compared to native Srcasm. These data support the hypothesis that Srcasm is a negative regulator of activated SFKs and that Grb2-Srcasm interactions limit the potency of Srcasm in SFK-downregulation, while preserving intracellular Srcasm levels. Deficient downregulation of SFKs by Srcasm Y440F suggests that molecule(s) binding to phosphorylated tyrosine 440 may promote Srcasm-dependent SFK downregulation.

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Evidence that a large multiprotein signaling complex regulates differentiation in keratinocytes

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Our previous studies show that the mitogen-activated protein kinase (MAPK) cascades play a central and essential role in regulating keratinocyte differentiation. However, parallel evidence indicates that MAPK signaling is also a positive regulator of keratinocyte transformation and carcinogenesis. Understanding how these cascades regulate these opposing processes is a critical area of study. Previous studies show that the balance between ERK and p38delta MAPK activity is key to determining keratinocyte fate with activation of p38delta favoring cell differentiation and activation or ERK1/2 favoring survival. We have also shown that ERK1/2 and p38delta are components of a multiprotein complex in keratinocytes and that the subcellular distribution is regulated by activation of PKCdelta. However, the content of this multiprotein complex is not presently known. We now demonstrate that this complex includes p38delta, ERK1/2, MEK3, MEKK1, PKCdelta, beta-actin and beta-tubulin. In contrast, MEK6 and MEK7 are not part of the complex. As part of an effort to understand how MAPK signaling can differentially influence keratinocyte phenotype, we show that treatment with the pro-apoptotic agent hydrogen peroxide produces dissociation of part of this complex, but that this is not observed with another agent, okadaic acid. We also show that this complex is differentially distributed within the keratinocyte. We conclude that differential dissociation of this MAPK signalsome complex may underlie the differential response of keratinocyte to differentiation versus survival stimuli.

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Curcuminoids differentially regulate MAP kinases and potentiate UVB-induced signaling in human keratinocytes

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Curcuminoids are polyphenolic compounds found in tumeric that exhibit anti-proliferative effects in many cell types. However, the mechanistic effects of curcuminoids in primary human keratinocytes (PHKs) are incompletely characterized. In this study, we examined the effects of curcumin and tetrahydrocurcumin (THC), a colorless curcuminoid, on PHKs. Pretreatment of PHKs with curcumin or THC for two hours transiently activates p44/42 MAP kinases within 5-10 minutes; however, this transient activation is followed by long-term suppression of p44/42 MAP kinase activity lasting approximately 24 hours. In contrast, pre-treatment of PHKs with curcumin or THC abruptly activated p38 MAP kinases and activation of these kinases persisted for greater than 24 hours. PHKs exposed to curcumin or THC alone for two hours did not significantly activate SAPK/JNK, however, pre-treatment of PHKs with curcumin or THC potentiated the UVB-induced activation of SAPK/JNK (25 mJ/cm2). The data demonstrate that curcumin and THC differentially regulate MAP kinases in PHKs by promoting p38 and SAPK/JNK activity while suppressing p44/42 MAP kinases. In PHKs, UVB exposure promotes activation of p38 and p53; therefore, the effect of pre-treating PHKs with curcuminoids upon UVB-induced signaling was studied. Curcuminoid pre-treatment of PHKs potentiated the UVB-induced activation of p38 MAP kinases. In addition, UVB-induced p53 phosphorylation at serine 15 was promoted by curcuminoid pre-treatment. Both curcumin and THC promote G2/M block in PHKs, and pre-treatment of PHKs with these curcuminoids promotes the accumulation of PHKs in G2/M secondary to low dose UVB (25 mJ/cm2). These data support the hypothesis that curcuminoids differentially regulate MAP kinases and promote specific UVB-induced signaling events in PHKs. Given these characteristics, curcuminoids may have potential as a UVB photosensitizing agents.

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Downregulation of Src-family tyrosine kinases: a comparative analysis of Srcasm and Cbl-c

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Src-family tyrosine kinases (SFKs) are important regulators of cell growth and differentiation, and constitute a large family of evolutionarily conserved molecules. Cellular SFK activity is tightly regulated, and increased kinase activity is found in neoplasia. Although specific SFK mutants are constitutively activated and known oncogenes, these SFK mutants have been only rarely identified in human carcinomas, suggesting that additional mechanisms must account for the increased SFK activity present in the majority of human carcinomas. The Cbl-c proto-oncogene is a SFK substrate and E3 ubiquitin ligase that was previously thought to downregulate cellular SFK levels in a proteosomal-dependent manner. Srcasm (Src Activating and Signaling Molecule) is a SFK substrate that has been found to downregulate SFK levels through a mechanism requiring acidified endosomes. In this study, we compare the ability of Srcasm and Cbl-c to downregulate Fyn, Src, and activated mutants of these kinases. In transfected cells, Srcasm downregulates levels of Fyn, Src and activated mutants of Fyn and Src. Srcasm is more efficient at downregulating native Fyn and Src than the corresponding activated mutants. In parallel experiments, Cbl-c does not significantly downregulate native Fyn or Src; however, Cbl-c does downregulate activated mutant forms of Fyn and Src, more efficiently than Srcasm. The ability of Cbl-c to downregulate activated Fyn and Src may be associated with tyrosine phosphorylation of Cbl-c by the mutant kinases. Native Fyn and Src expressed at similar levels to the mutant forms do not induce significant tyrosine phosphorylation of Cbl-c. Co-transfection of Cbl-c and Srcasm with Fyn or Src inhibited the ability of Srcasm to downregulate these SFKs. These data suggest that Srcasm may efficiently downregulate native SFKs more effectively than Cbl. Since activating mutations of SFKs are rarely encountered in human tumors, Srcasm may play an important role in regulating cellular levels of native SFKs and function as an anti-oncogene.

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Novel anticarcinogenic effect of non-steroidal anti-inflammatory drugs (NSAIDs) by formation of cytoplasmic retinoid X receptor/Nur77 heterodimers that mediate COX-2-independent apoptosis

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NSAIDs block eicosanoid production by inhibition of cyclooxygenase (COX) activity and they can prevent skin carcinogenesis induced by chemicals or ultraviolet radiation. The mechanism whereby suppression of COX-2 inhibits carcinogenesis is controversial since there is accumulating evidence that COX-2 inhibition cannot account for all of the biological effects of NSAIDs. Retinoids are essential for normal skin growth, differentiation, and apoptosis, and have been used in the prevention and treatment of non-melanoma skin cancers and other skin disorders. The effects of retinoids are mediated mainly by retinoic acid receptors (RARs) and retinoid X receptors (RXRs), which act as transcription factors. Altered RXR expression is associated with multistep squamous cell carcinogenesis in human skin. In this study, we show that the COX-2 inhibitor sulindac binds to RXR at concentrations that are achievable in vivo. Sulindac binding triggers cytoplasmic translocation of RXR, and the cytoplasmic RXR targets mitochondria through its heterodimerization with the orphan nuclear receptor Nur77. Mitochondrial targeting of the RXR/Nur77 heterodimer induces Bax conformational change, resulting in extensive apoptosis. Furthermore, we observed that cytoplasmic RXR can bind to IKK, thus preventing TNFα-induced IκB degradation and activation of NF-κB. RXR is strongly expressed in the nuclei of UVB-induced SCCs in murine skin. Age-matched, non-irradiated control skin also showed positive nuclear staining in both the basal and suprabasal epidermal layers. Interestingly, RXR staining was decreased in sulindac-treated/UVB-induced tumors and, notably, RXR was cytoplasmic. Our results demonstrate that the apoptosis-inducing effects of sulindac are mediated by sulindac-induced cytoplasmic RXR/Nur 77 heterodimer formation and suggest that RXR is an important intracellular target of NSAIDs in skin that could contribute to the anticarcinogenic properties of these agents.

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Modulation of Sirtuin 1 (SIRT1) by nuclear mammalian target of rapamycin (mTOR) drives oxygen-mediated cancer cell survival and chemoresistance through anti-apoptotic Bfl-1/A1

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Reactive oxygen species (ROS) and cellular oxidant stress are implicated in skin carcinogenesis. ROS signaling may also result in cancer cell survival. Cellular senescence is a growth-arrest program that inhibits cell proliferation, and is thought to have tumor suppressive effects and to prevent the expansion of cells at risk for neoplastic transformation in vivo. Following treatment of various human squamous cell carcinoma (SCC) cell lines with naturally occurring compounds including the anthracycline antibiotic doxorubicin and the plant phytoalexin resveratrol we observed the development of apoptosis-resistant G1-arrested (senescent) cell populations. These apoptosis-resistant cells exhibit senescence-like characteristics. To probe the mechanism whereby resveratrol drives senescence we observed copper-dependent generation of ROS, followed by the induction of p21WAF1 and substantial upregulation of the anti-apoptotic Bfl-1/A1. Bfl-1/A1 induction requires RelA/p65 and p52 NF-κB. ROS-induced senescent cells are resistant to further treatment with chemotherapeutic agents, likely due to Bfl-1/A1, whereas the knockdown of Bfl-1/A1 or RelA/p65 causes senescent cells to enter apoptosis, suggesting that Bfl-1/A1 is crucial for the maintenance of the senescence-like state. In further studies we showed that resveratrol treatment drives nuclear translocation of mTOR which in turn phosphorylates SIRT1 and increases RelA/p65 acetylation, leading to Bfl-1/A1 induction. In addition, SIRT1 phosphorylation only occurs in senescent SCCs harvested from UVB-irradiated/resveratrol-treated mice. Our results indicate that ROS-induced senescence reflects a chemoresistant state and that ROS-mediated mTOR modulation of SIRT1 is crucial for cancer cell survival through Bfl-1/A1. Targeting mTOR/SIRT1 signaling may be effective in the treatment of apoptosis-resistant cancer cells.

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Differentiation of murine dermal papilla cells into skeletal muscle

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Dermal papilla (DP) plays a vital role in hair development/formation, while it contains multipotent progenitors that can differentiate into nerve, fat, bone and muscle. Previously, we reported that the CD133 (+) cells resided in DP and had the hair-inducing ability. Since the differentiation of DP into muscle has not intensely observed, we cultivated the CD133-positive (+) cells from adult mice to give rise them into skeletal muscle. The freshly isolated CD133 (+) cells neither produced muscle-cadherin, one of the markers of myoblast, nor skeletal muscle myosin, one of the differentiation markers of skeletal muscle. But after the cultivation for three weeks, DP derived cells turned to be positive for both of the markers. When the cultivated cells from Rosa26 mice were transplanted into muscle of immune-deficient mice, the Lac-z positive cells survived as skeletal muscle, one month later. We also analyzed the cultivated cells physiologically, because the DP derived round/rod shaped cells beat spontaneously (1 to 3 Herz). The analysis was performed with a calcium indicator, fluo-3 (Molecular probes). The peaks of the calcium concentration did not change by 10nM of tetrodotoxin (TTX), but dramatically declined by 10μM of TTX. The cells did not stop beating when nifedipine, a blocker of L-type calcium channel, was added at high concentration (10μM). Since the skeletal muscle is sensitive to TTX and is resistant to nifedipine, the cultivated cells were shown to have the characteristics of skeletal muscle. The cardiac muscle is resistant to TTX and sensitive to nifedipine, vice versa. Now, DP can be used as a material for skeletal muscle regeneration.

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A pure ectodermal cell line derived from human embryonic stem cells for skin and cornea cell therapy

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Embryonic stem (ES) cells represents a unique cellular model to recapitulate in vitro early steps of embryonic development, but also as an unlimited source for cell-based therapy for many diseases and targets for drug discovery and toxicology screens. Among the different cell types that can be generated from ES cells, ectodermal cells are of particular interest since they represent the precursors of many epithelial tissues and organs. While human ES cells provide a potential cellular source for tissue engineering application, a major limitation resides in the heterogeneity of the committed cell population. The absence of known epithelial-specific stem cell surface marker usually prevents cell sorting of the desired cell type, retaining thus contaminated undifferentiated huES which can still produce teratoma. Here we report the reproducible isolation of an ectodermal cell population from human ES cells (IT1). IT1 shows a stable morphological appearance, has a somatic cell cycle kinetics and does not produce teratoma in nude mice. Remarkably, like adult somatic cells, IT1 cells undergo senescence after about 60 doubling population and their karyotype remains normal. Exogenous expression of DeltaNp63 or Pax-6 efficiently induces their differentiation into K14+-keratinocytes or K12+-corneal cells, respectively. Interestingly, post confluent IT1 cells form a firm sheet that could be transplanted *in vivo* and thus may provide a ready source of ectodermal progenitor sheets for cell therapy transplantation and the development of new-drug-screening strategies. IT1 cell line could be thus a particularly useful tool to design *in vitro* models that would recapitulate the embryonic early events of epithelial lineage specification but also as a source of different cells for clinical trials of epithelial stem cell loss. Finally, it demonstrates that, contrary to what has been suggested elsewhere, huES-derived somatic cell types do not need to be immortalized for growth and expansion.

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Analysis of gene expression profiles in hair root cells and their relation to hair properties.

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It is known that the physical properties of hair, such as thickness, elasticity or luster, change with age. We assumed that these age-dependent changes are caused by metabolic changes in hair root cells. To test this assumption, we detected genes that age-dependently change their expression in hair root cells. From one hundred and thirty-two healthy Japanese women aged between 11 to 70 years, we plucked and collected approximately fifty anagen hairs per head. In order to detect genes that age-dependently change their expression, we examined transcriptional profiles of hair root cells using DNA microarray and quantitative RT-PCR. Properties of hair and scalp were then evaluated to examine their relationship to the gene expression profiles. An age-dependent change was determined in 1915 transcripts of genes expressed in hair root cells. To narrow down these candidates, we clustered them into eight groups based on the coherent expression patterns by k-means algorithm. In turn, a cluster with a strong correlation to hair elasticity was identified. We then analyzed the function of each gene in this cluster using Genomatix software and thereby identified three genes (VEGF, PPARA and CTNNB1) that play a key role in the cluster. Finally, we confirmed that expression of VEGF declines age-dependently in a manner similar to the loss of hair elasticity. These results suggest that VEGF, PPARA and CTNNB1 might be factors contributing to the maintenance of the elasticity of scalp hair.

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Influence of a topical 3-step scalp treatment regime on total hair bulb extraction during brushing *in vivo*

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Recently, we reported on an *in vivo* testing protocol for examining the effects of brushing on hair bulb extraction1. In this study, we developed a clinical method for examining and comparing the number of hairs which physically broke during brushing versus the number of hairs that were extracted from the scalp with their hair bulb intact. It was found in this study that regardless of treatment, the number of hairs that were extracted intact from the scalp was significantly greater than the number of hairs that broke at some weak point along the hair fiber. However, in this study, which also attempted to examine the influence of a topical treatment that contained a number of well-known skin care ingredients, we were unable to say with confidence that the treatment regimen influenced the ability of the hair fibers to remain embedded in the head compared to placebo treatments. Working in collaboration with Nioxin, a leading provider of advanced skin and hair care products, we expanded this testing protocol to include a complete 3-step hair care treatment regimen containing a Shampoo, a Hair Conditioner and a Leave-on Scalp Treatment. The study was conducted double blind on two groups of 12 volunteers, one group which used the 3-step regimen in which the active composition previously reported was included at 0.5 % in both the Shampoo and Hair Conditioner and 3% in the Leave-on Scalp Treatment. The other placebo group used the same treatment regimen formulated without the active ingredients. We wish to report our findings from this study which took place over a thirty day period. In addition, we will report on a method for analyzing the size of the hair bulbs using scanning electron microscopy on the hairs in which the hair bulb was extracted intact.

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Multipotent nestin-positive human hair follicle stem cells isolated from the top of bulge area can promote regeneration of peripheral-nerve injury

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We have previously shown that nestin-positive, K15-negative mouse hair follicle stem cells are pluripotent and can effect peripheral nerve regeneration (Proc. Natl. Acad. Sci. USA 102, 5530-5534, 2005; Proc. Natl. Acad. Sci. USA 102, 17734-17738, 2005; J. Invest. Dermatol. 127: 11-15, 2007). The multipotent hair follicle stem cells are located above the hair follicle bulge. We have termed this location the multipotent hair follicle stem cell area (MHFSCA). We have now isolated stem cells from the human MHFSCA. Human hair follicle stem cells can differentiate into neurons, glia, keratinocytes, smooth muscle cells, and melanocytes in vitro. The nestin-expressing human hair follicle stem cells were transplanted between severed sciatic nerve fragments of the mouse where they differentiated into glial fibrillary acidic protein (GFAP)-positive Schwann cells and promoted the recovery of pre-existing axons. These multipotent follicle stem cells can be readily isolated from the human scalp. Human hair follicle stem cells thereby provide a potential accessible, autologous source of stem cells for regeneration therapy.

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BMP signaling in dermal papilla cells is required for their hair follicle inductive properties

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Hair follicle (HF) formation is initiated when epithelial stem cells receive cues from specialized mesenchymal dermal papilla (DP) cells. In culture, DP cells lose their HF-inducing properties. During hair growth *in vivo*, they reside within the HF bulb and instruct surrounding epithelial progenitors to orchestrate the complex hair differentiation program. To gain insights into the molecular program that maintains DP cell fate, we previously purified DP cells and four neighboring populations, and defined their cell type-specific molecular signatures. Here, we exploit this information to show that the bulb microenvironment is rich in bone morphogenetic proteins (BMPs) that act on DP cells to maintain key signature features *in vitro* and hair-inducing activity *in vivo*. By employing a novel *in vitro/in vivo* hybrid knockout assay, we ablate BMP receptor 1a in purified DP cells. When DPs cannot receive BMP signals, they lose signature characteristics *in vitro* and fail to generate HFs when engrafted with epithelial stem cells *in vivo*. These results reveal that BMP signaling, in addition to its key role in epithelial stem cell maintenance and progenitor cell differentiation, is essential for DP cell function and they suggest that it is a critical feature of the complex epithelial-mesenchymal cross-talk necessary to make hair.

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Neonatal human umbilical cord epithelial cells display characteristics of epidermal keratinocytes

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Human umbilical cord epithelial cells (HUCEC) are in close proximity with the epidermis during development and both originate from the ectoderm. *In vivo*, HUCEC express keratins 14 and 10 as well as proteins related to the formation of the cornified envelope. As such, we hypothesized that HUCEC may possess features of human epidermal keratinocytes (HEK) and therefore investigated their characteristics *in vitro*. HUCEC were isolated from neonatal umbilical cords from term births of healthy pregnancies and propagated on collagen matrices in low calcium serum free media. In vitro, HUCEC were able to be propagated to at least 20 population doublings and show an increased proliferation rate on collagen matrix. They displayed polygonal epithelial morphology with expression of keratins pairs 5/14 and occasional expression of keratin 10. In the wound migration assay, HUCEC migrated to close 50% and 100% of the wound at 24 hours and 48 hours respectively, in the presence of mitomycin C. When cultured on the surface of human acellular dermis in a 3-D organotypic culture for 10 days with a period of air exposure, HUCEC formed orderly stratified epithelia with a cornified layer, resembling the epidermis. In organotypic culture, HUCEC expressed keratins 5/14 and 10 in addition to transglutaminase1, involucrin and filaggrin. Expression of desmosomal components including desmoglein 1, 3, desmocollin 1, 3 and plakophilin1 were detected. Furthermore, HUCEC expressed members of the Notch signalling pathway which is implicated in epidermal differentiation. Taken together, these results indicate HUCEC possess characteristics and behaviour of epidermal keratinocytes, affirming their close developmental relationship to HEK.

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Identification of human bulge cell counterpart in canine hair follicles: A valuable material for stem cell investigation

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Canine hair follicles (HFs) are more similar to human HFs than rodent HFs both in pathophysiology and morphology. Supposedly, homologous stem cell system may exist in canine HF. However, our understanding of canine HF stem cells is extremely limited. In this study, we attempted to identify human bulge cell counterpart in canine HFs and assess their stem cell behavior in vitro. Using dog skin xenograft, label retaining keratinocytes (LRKCs) were detected in the outermost layer of outer root sheath around the insertion point of arrector pili muscle, the area strongly positive for alpha 6 integrin and keratin 15 staining. We defined this region as human bulge counterpart in canine HFs, namely canine bulge. Next, single canine anagen HF was microdissected into several segments and KCs derived from individual segments were cultured to evaluate their colony forming efficiency. KCs either from segments containing bulge or matrix cells gave rise to colonies, however, the number of holoclone colonies, representing putative stem cell colonies, was 3-fold higher in KCs enriched for canine bulge cells ($P<0.001$). Holoclone forming KCs had undifferentiated cell structure, as shown by eloctronmicroscopy. In addition, KCs enriched for canine bulge cells were successfully passaged for more than 6 generations, while KCs obtained from other HF segments died out within 5 passages. These findings indicated that canine bulge cells shared the same anatomical location, morphological characteristics and previously reported self-renewal potency of human counterparts. Accordingly, HF stem cell system seems to be surprisingly well preserved over the species. Adequately-sized canine HFs enable practices of key techniques, such as microdissection and transplantation. Thus, canine bulge cells could provide valuable material for human HF stem cell biology and the development of methodology for human HF regeneration.

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Evaluation of radiation-induced hair follicle apoptosis in mice and the preventive effects of FGF1 therein

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Ionizing radiation at high dose causes skin damages and hair loss, and hair follicle apoptosis is one of the major prognostic factors. However, it is difficult to examine the hair loss in small animals such as mice, because total body irradiation (TBI) causes their death with a very high dose. In this study, we aimed to establish the in vivo assay system of radiation-induced apoptosis in plucking-induced anagen hair follicles and used this assay to evaluate preventive effects of FGF1. A portion of the dorsal skin of seven-week-old male BALB/c mice harboring uniform telogen phase hair follicles was depilated for induction of anagen. BrdU incorporation and PCNA staining confirmed that the follicle keratinocytes were markedly proliferating in the following anagen phase. The mice received TBI with γ-rays at doses ranging 8-16 Gy at anagen V 6 days after depilation. TUNEL assay was performed on paraffin-embedded sections of the dorsal skin to evaluate apoptosis over time after irradiation. Irradiation extremely increased TUNEL-positive cells in these follicles in a dose dependent manner 24 hours after irradiation, although they were few in the non-depilated skin in case of irradiation. FGF1 was administered intraperitoneally at a dose of 10-100 μg 24 hours before TBI. As a result, FGF1 decreased the proportion of TUNEL-positive cells in the follicles after irradiation. TBI at a dose of 8 Gy causes not only hematopoietic damage, but also intestinal damage, resulting in death of mice about a week postexposure. Therefore, the induction of anagen by plucking hair is useful to evaluate growth factors for effects on the radiation skin damages and hair loss, because it enables us to analyze the radiation-induced apoptosis 24 hours after exposure.

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Notch-deficient skin creates an environment inducing a lethal systemic B-lymphoproliferative disorder by overproducing TSLP, a sentinel for epidermal integrity.

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The Notch signaling pathway is a critical regulator of epidermal integrity. Here, we show that keratinocyte-specific deletion of total Notch signaling triggered a severe systemic B-lymphoproliferative disorder (B-LPD) causing death. To investigate how loss of Notch signaling in the skin led to systemic B-LPD, we examined the skin phenotypes of mice lacking various degree of Notch signaling. RBP-j is the DNA-binding partner of Notch but we found that both RBP-j-dependent and independent Notch signaling were necessary for proper epidermal differentiation and lipid deposition. Loss of both pathways caused a persistent defect in skin differentiation/barrier formation. In response, high levels of thymic stromal lymphopoietin (TSLP) were released into systemic circulation by Notch-deficient keratinocytes that failed to differentiate, starting in utero. Exposure to high TSLP levels during neonatal hematopoiesis resulted in drastic expansion of peripheral pre- and immature B-lymphocytes causing B-LPD associated with major organ infiltration and subsequent death, a previously unappreciated systemic effect of TSLP. These observations demonstrate that local skin perturbations can drive a lethal systemic disease and have important implications for a wide range of humoral and autoimmune diseases with skin manifestations.

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Expression of Wnt1, β-catenin, and Frizzled 3 during human hair follicle and acrosyringium development

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Wnts are potent developmental morphogens that specify different cell fates, provide positional information, organize pattern formation, and regulate cell migration, proliferation and differentiation throughout embryogenesis. Based on the study of chicken feather or murine hair follicle formation, Wnt pathway is involved in the skin appendage morphogenesis. However, their expression during human hair follicle and acrosyringium development has not yet been studied. There are at least 19 known Wnts and 10 frizzled receptors in human.Wnt-1 is reported to bind to Frizzled 3 and Frizzled 3 activates the canonical Wnt-β-catenin pathway. Here we investigate their expression during human hair follicle and acrosyringium development by immunohistochemistry. At 8 wks estimated gestational age (EGA), Wnt1 and Frizzled 3 are both homogeneously expressed in basal, intermediate and periderm cells, butβ-catenin is expressed in basal cells of epidermis only. During hair placode or hair germ formation (13-15 wks EGA), Wnt1 is equally present in the placode or inter-placode epidermis, but Frizzled 3 and β-catenin are more intensely expressed in placode or hair germ. During the bulbous hair peg stage (16-20 wks EGA), Wnt1, Frizzled 3 and β-catenin are expressed in the outer root sheath, bulge area, and sebaceous gland. Wnt1 is expressed in the inner root sheath. Frizzled 3 and β-catenin are expressed in the precortical area. Frizzled 3 is also expressed in mesenchymal cells such as in the dermal papilla and follicular sheath. In fetal palm skin, Wnt1 is expressed in the suprabasal layers, but β-catenin is expressed in the basal layer of epidermis, and Frizzled 3 is expressed in all over the epidermal layers. β-catenin and Frizzled 3 are more intensely expressed in the hemispherules of basaloid cells of eccrine units. Frizzled 3 is constitutively expressed in the eccrine glands. Wnt pathway is dynamically and differentially involved in human hair follicle and acrosyringium development.

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Role of extracellular matrix protein on self-assembling of dermal papilla cells

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Self-aggregation is a unique character of dermal papilla (DP) cells and is also associated with their hair follicle (HF) induction ability. It has been shown that DP cells have a temporal-spatial expression of a number of extracellular matrix (ECM) proteins associated with HF morphogenesis and hair cycles. However, whether the ECM proteins contribute to its self-aggregation and structural segregation from surrounding structures has not been examined. In this work, we attempted to characterize the role of ECM proteins on the self-aggregation of DP cells in an *in vitro* self-aggregation model we have previously established. Our results show that fibronectin, highly expressed in anagen DP, enhances the self-assebmling of dispersed DP cells into 3-dimentional spheroids. On the contrary, laminin, present in the dermal-epidermal junction of hair bulb, and collagen I, the major extracellular matrix protein of interfollicular dermis, inhibit the aggregation of DP cells. When preformed DP spheroids are cultured, laminin and collagen I disintegrate the spheroids into single cells while fibronectin preserves the spheroidal morphology. The effect of fibronectin, laminin and collagen I on self-aggregation is independent from their effect on cell migration and cell-substrate adhesivity. Further, the aggregated cells express higher levels of DP signature gene products as compared to that of monolayered cells. Our results suggest that the ECM proteins play an important role for the self-aggregation and segregation of DP cells from the surrounding epithelial and dermal structures. Efficient strategies for engineering DP cells into dense tissues for HF regeneration can also be developed by tailoring ECM proteins in culture conditions.

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The localization of label retaining cells in eccrine glands and nails

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Stem cells are capable of self replicating and differentiating into several lineages of the cells. They are very slow cell cycling cells and retain labels in the nucleus throughout several weeks. The purpose of this study was to examine the localization of label retaining cells in the eccrine glands and nails. BrdU pulse chase experiments in mice revealed that label retaining cells reside in the luminal cells of intraglandular eccrine duct and secretory gland. Immunohistochemical study revealed that Lhx2, a marker for stem cells in hair follicles, positive cells exist in the intraglandular duct and secretory gland. To compare the localization of label retaining cells in mouse and human eccrine glands, we transplanted human skin onto nude mice and performed BrdU pulse chase experiments. Label retaining cells exist in the human intraglandular eccrine duct and secretory gland. Moreover, immunohistochemical study showed that keratin 15 positive cells exist in the intraglandular and secretory region in human eccrine glands. In the nails, label retaining cells resided in the basal layer of nail matrix adjacent to the nail bed in mice. Lhx2 positive cells existed in the same area. To explore the molecular mechanism of onychocyte differentiation, we focused on a gene R-spondin family, member 4 (*Rspo4*), whose human homologue is responsible for an inherited anonychia. Real time RT-PCR showed that *Rspo4* mRNA levels are higher in adult mouse digits than during nail organogenesis in 18 day-old embryos. Immunohistochemistry revealed the expression of R-spondin family receptors, Fzd8 and Lrp6, in the basal layer of nail matrix, indicating dual roles of Rspo4-Fzd8-cateninβ1 signaling pathway both in nail development and differentiation. Local injections of TNF-α into the mice up-regulated the *Rspo4* expression and induced the parakeratosis in nails as seen in human psoriatic nails. Luciferase assay revealed that TNF-α induced the human *RSPO4* promoter, which contains NF-κB binding motifs. These results might lead to a therapeutic use of anti-TNF-α reagent for psoriatic nails.

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Intrafollicular distribution of cytokeratins; getting closer to the epithelial stem cell region?

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The distribution of the cytokeratin-family of intermediate filament proteins within the human hair follicle (HF) characterizes the type of epithelium, state of growth and differentiation. Several evidences in inter- and intrafollicular epithelium have shown that cytokeratins (Cks) are adopted as specific and/or complementary markers for the multiple stages of differentiating KCs. More importantly, they define the location of those KCs which still have differentiation potential and are therefore crucial in HF cycling, skin homeostasis and regeneration. With the use of a double immunofluorescent staining on human skin cryosections for FITC-Pan-cytokeratin (Pan-Ck) and Alexa 594-Cytokeratin 15 (Ck15) we could determine the precise distribution of terminally differentiated KCs (Pan-Ck+/Ck 15-), partially differentiated KCs (Pan-Ck+/Ck15+) and KCs with higher differentiation potential (Pan-Ck-/Ck15+). The used Pan-Ck antibody recognizes the new panel of cytokeratins 4, 5, 6, 8, 10, 13 and 18. Consistently with published results, KCs with an up-regulated Ck15- immunoreactivity (IR) pattern have been localized in the bulge stem cell region, although this is not an exclusive region of Ck15 expression. In addition, Ck15- IR is seen in the outer cell layer of the isthmus and distal outer root sheath (ORS) and continued to be IR in central and proximal ORS KCs of the human HF. Interestingly Ck15+/Pan-Ck+ KCs were found distributed along the ORS near to the bulge region, but were absent in isolated KCs of the bulge region and the proximal ORS. This Ck15+/Pan-Ck- cells may highlight those KCs which still have differentiation potential. The showed IR pattern for Pan-Ck/Ck15 can help to define the areas in the human HF where important KC differentiation takes place and may help to further narrow the population of multipotent epithelial stem cells.

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Effects of Bacillus *subtilis* (natto)–fermented *Astragalus mongholicus* products on collagen and hyaluronic acid production in human skin fibroblasts

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Aged skin shows decreased production of collagen, HA, and increased proteolytic activity, resulting in skin atrophy, wrinkles and dryness. Driven by rising consumer interest and demand for natural products, the potential use of traditional herbal medicines for development of skin care cosmetics has claimed serious attention recently. The purpose of this study was to investigate the effects of *Bacillus subtilis* (natto) –fermented (HQB) and nonfermented (HQNB) *Astragalus membranaceus Bge. var. mongholicus (Bge.) Hsiao* products on the production of collagen and HA in human dermal fibroblasts. The primary normal human dermal fibroblasts (age 22y and 81y) were purchased from Cascade Biologics Co. and cultured in a monolayer form in DMEM with 10% FBS. Test samples were added to the culture for indicated time and the results were compared with those of untreated fibroblasts. Type I procollagen content was determined using enzyme-linked immunosorbent assay (ELISA). Type I procollagen protein levels were assessed by western blot analysis. Real time RT-PCR was used to evaluate type I and type III procollagen gene expression. Enzyme (collagenase, elastase, gelatinase) inhibition activities were also measured. We demonstrated that HQB, but not HQNB, significantly and dose-dependently stimulated the biosynthesis of type I procollagen in both HDF cells using ELISA and Western blot analysis. Real-time RT-PCR revealed that expression of type I and type III procollagen mRNA was significantly stronger in both HQB-treated HDF cells than that in HQNB-treated and un-treated cells. As a result, there is a close correlation between the mRNA expression and protein production in the fibroblasts response to HQB stimulation. Furthermore, HQB showed much better effect on type I procollagen synthesis in aged HDF cells as compared with young HDF cells. In brief, HQB is expected to prevent the age-dependent loss of extracellular matrix proteins.

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Age-related changes in adipose tissue derived stem cells

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Previously, we focused on the stem cells existing in adipose tissue and reported that the Neurotorophic factor receptor p75 (p75NTR)-positive cells show the ability of multiple differentiation. In recent research age-related changes in multiple differentiation ability of adipose tissue derived stem cells had been examined. We detached the p75NTR-positive cells from the adipose tissue of ICR mice of the age 3 to 24 weeks and examined their proportion in the tissue on one unit of weight. The cells selected were further cultured in induction media, their coefficient of differentiation and the age-related change in the multiple differentiation ability was examined. We found that the proportion of the p75NTR-positive cells in adipose tissue on the one weight unit experiences reduction with the age. In addition, the results of differentiation-including analysis of the p75NTR-positive cells derived from the adipose tissue allowed us to confirm their preservation of multiple differentiation ability to fat, smooth muscle, osteoblast and fbroblast cells in every week of the age. We finally analyzed the coefficient of differentiation of the adipose tissue derived stem cells for each week of the age; although we could observe some numeric changes, we could not conclude the significant difference in the degree of differentiation. In conclusion, it was suggested that the proportion of adipose tissue derived stem cells on one unit of weight experience reduction with the age. There is however a possible preservation of the multiple differentiation ability of the stem cells. Currently we are conducting more precise research.

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Effect of several monoterpenes on hair growth

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Hair loss is a common and distressing phenomenon affecting the quality of life negatively. Thus it is important to develop new therapeutic materials to stop hair loss and to enhance hair growth. Monoterpenes has gained lots of interest because of their various biological activities, including antioxidant potential, antibacterial and anti-inflammatory effects. Furthermore, monoterpenes are used in topical agents as a penetration enhancer. In this study, we tested the effects of several monoterpens, including (±)-β-citronellol, (-)-β-citronellene, (+)-β-citronellene and linalool oxide, on hair growth using *in vivo* and *in vitro* test models. After topical application of (±)-β-citronellol onto the back of C57BL/6 mice, earlier conversion of telogen-to-anagen was induced. To investigate the action mechanism, we treated cultured dermal papilla cells with monoterpenes. The growth of dermal papilla cells, however, was not affected by monoterpenes. RT-PCR analysis showed that (±)-β-citronellol induced mRNA levels for keratinocyte growth factor (KGF), suggesting that the effects of (±)-β-citronellol on hair growth may be mediated through the regulation of growth factors in dermal papilla cells. We further investigate the effect of (±)-β-citronellol on the Wnt signaling pathway using adenovirus expressing TOPflash. Interestingly, (±)-β-citronellol induced the luciferase activity in a dose dependent manner, suggesting that (±)-β-citronellol may have impact on intracellular β-catenin signaling. Taken together, these results suggest that (±)-β-citronellol has hair growth promoting potential and can be used for helping hair growth.

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Bone marrow cell transfer in utero can ameliorate genetic skin abnormalities by raising bone marrow-derived fibroblasts and keratinocytes

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Recent studies have shown that skin injury generates bone marrow-derived fibroblasts (BMDFs), and possibly keratinocytes (BMDKs), in the lesion to accelerate wound healing. However, it remains uncertain whether BMDFs and BMDKs can be raised without skin injury to maintain skin homeostasis. In this study, we first investigated the appearance of BMDFs and BMDKs in normal mouse skin following embryonic bone marrow cell transplantation (E-BMT) at embryonic day 14 with GFP-transgenic bone marrow cells (GFP-BMCs) via the vitelline vein, which runs through the uterine wall to the fetal circulation. At 12 weeks after birth of the E-BMT mouse, we searched BMDFs and BMDKs in the skin, and found that not BMDKs but BMDFs, which generate matrix proteins, were abundantly residing in the dermis. BMDFs strictly collected from the skin by cell-sorting showed expression of fibroblast marker genes by RT-PCR. We then performed E-BMT via vitelline vein in dystrophic epidermolysis bullosa (DEB) model mouse lacking type VII collagen, which anchors the cutaneous basement membrane to the underlying dermal matrix. E-BMT significantly ameliorated lethal conditions for the DEB neonate mice. In the skin, type VII collagen was clearly detected in the basement membrane zone in the vicinity of the BMDFs. To access potential of E-BMT to raise BMDKs, we transplanted GFP-BMCs directly into the amniotic fluid at embryonic day 7, just before starting epidermal stratification, in utero. Surprisingly, a significant number of BMDKs expressing keratin 5 were detected in the epidermis of the amniotic E-BMT mouse even 6 weeks after birth. These data strongly suggest that E-BMT in the fetal circulation and amniotic fluid can be therapeutic options to ameliorate genetic skin diseases harboring dysfunction of fibroblast and/or keratinocytes by providing BMDFs and BMDKs, respectively.

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Generation of skin and oral mucosa from mouse embryonic stem cells under the influence of regional specific components

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This study investigated whether stratified epithelia can be generated from mouse embryonic stem cells (ESC) and whether regionally-differing epithelial phenotypes can be determined by exposure to matrix or soluble products synthesized by regionally-differing fibroblasts or keratinocytes. Feeder-independent ESC (line E14.2) were 'primed' by growth in media containing vitamin C but no LIF on acellular matrices formed by oral or skin fibroblasts and keratinocytes. Differentiation was then enhanced by growth as 3-D organotypic cultures. Mesenchymal, simple epithelial, oral mucosal, and epidermal phenotypes were distinguished by staining for vimentin and keratins 1,4,8,10,13,18 and 19. Vitamin C induced expression of epithelial markers in some ESC, but the development of stratified epithelia required matrix synthesized by fibroblasts or keratinocytes. Grown on simple collagen gels in 3-D cultures, both 'primed' and non-'primed' ESC developed two morphologically distinct cell populations: a) epithelial-like cells with dense nuclei and abundant eosinophilic cytoplasm organized in islands and, b) scattered spindle-shaped cells with elongated nuclei and little cytoplasm, suggestive of mesenchymal differentiation. However, for further differentiation into mature, regionally-relevant epithelial structures ESC required 3-D culture with vital fibroblast instruction, and only the ESC 'primed' on keratinocyte-derived matrices reached a final stage of epithelial maturation. In conclusion, our results indicate that ESC can differentiate in vitro into compound tissues but that full epithelial differentiation requires instruction from vital fibroblasts. It also suggests that the regional information generated by the matrix producing/supporting cells might be exploited in generating compound tissues with defined regional specificities. The first two authors have equally contributed to the work presented here.

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TGF-β2 is expressed in human dermal papilla and modulates folliculogenesis

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Dermal papilla cells are known to retain their hair inductive capacity even after *in vitro* culture expansion and implantation into the skin. For regenerative engineering of human hair follicles, it is indispensable to clarify the hair inducing mechanism executed by human deramal papilla cells (hDPCs). To explore the hair inducing factors from hDPCs, microarray and real-time RT-PCR analyses were performed comparing hDPCs and human dermal fibroblasts (DF) both obtained from adult scalp skin. Substances which can up-regulate hDPC-specific genes were also explored. Functional properties of the genes *in vivo* were assessed using an animal model generating rat-human chimeric hair follicles. TGF-β2, but not TGF-β1, was among genes upregulated in hDPCs, and TGF-β2 expression from hDPCs was further upregulated by a conditioned culture medium of human keratinocytes, which is known to stimulate hDPC proliferation and minimize a time-dependent loss of hair inducing capacity of hDPCs. Also found were some other substances which significantly upregulated TGF-β2 mRNA and protein secretion from DPCs *in vitro*. One of them was 1,25- dihydroxy-vitamin D3 (1,25-VD3), the active form of vitamin D. Interestingly, 1,25-VD3 also upregulated gene expression from hDPCs of Wnt10b, a prominent morphogen of hair follicles. In animal models for folliculogenesis, TGF-β2 was expressed in hDPCs of rat-human chimeric hair follicles, and a continuous administration of a selective inhibitor for TGF-β signaling suppressed the number and maturity of regenerated chimeric hair follicles. These studies suggested that TGF-β2 is highly expressed specifically in hDPCs, is upregulated by keratinocytes-derived soluble factors or Vitamin D, and acts as a potent enhancer of folliculogenesis. Vitamin D may modulate folliculogenesis by upregulating expressions of TGF-β2 and/or Wnt10b in hDPCs.

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Expression pattern of *Mpzl3*, the gene mutated in the rough coat (*rc*) mice

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We recently identified a point mutation in the natural mutant mouse strain rough coat (*rc*) by positional cloning. The *rc* mutation arose spontaneously in the C57BL/6J mouse strain, and is autosomal recessive. Homozygous *rc* pups start to show unkempt hair coat about two weeks after birth, and develop cyclic and progressive hair loss and sebaceous gland hypertrophy. In addition, more than half of the *rc*/*rc* mice over one year old develop spontaneous and persistent ulceration in the skin, suggesting a defect in epidermal wound healing. Using linkage analysis in backcross mice, we mapped the *rc* locus to a 246-kb interval, and identified a point mutation in *Mpzl3*, a novel gene with the highest levels of sequence homology to Myelin Protein Zero (*Mpz*) and Mpz-like 2 (*Mpzl2*). *Mpzl3* is conserved among all mammalian species sequenced to date, but its function is not clear. The encoded MPZL3 protein is predicted to be a single-span transmembrane protein likely involved in cell adhesion. The mutation in the *rc* mice occurred at a highly conserved residue within the conserved immunoglobulin V-type domain, thus likely altering the MPZL3 protein function. In this study, we analyzed the expression pattern of *Mpzl3* using RT-PCR, Western blot analysis, and indirect immunofluorescent staining. *Mpzl3* RNA and protein were detected in a variety of adult mouse organs, including the skin. In cultured HaCaT human keratinocytes, MPZL3 was localized to the plasma membrane, possibly in structures involved in cell adhesion. We are currently analyzing the effects of *Mpzl3* expression levels on cell adhesion.

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The role of the TAp63α in the commitment to stratification

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Mice lacking the transcription factor p63 fail to form stratified epithelia and epithelial appendages. Instead of a fully stratified epidermis, these mice are covered by a single-layered epithelium at birth, suggesting that p63 is required for the initiation of epithelial stratification. To study the role of p63 in these early stages of morphogenesis, we previously used single-layered lung epithelia as a model system. Using this system, we found that ectopically expressed TAp63α could induce epithelial stratification, whereas ectopically expressed ΔNp63α did not alter lung morphology. To further explore the potential of TAp63α in committing cells to a stratified epithelial cell fate, we generated a tetracycline inducible mouse model. In this model, TAp63α expression is targeted to the uterus by using a progesterone receptor promoter. The uterine luminal epithelium is single-layered and does not express p63. However, this luminal epithelium has the potential to stratify under pathological conditions. Furthermore, this stratification process is associated with the induction of p63 expression. Therefore, uterine luminal epithelium provides us with an ideal *in vivo* system to explore the ability of TAp63α to induce epithelial stratification. We found that ectopic TAp63α expression in the uterus resulted in severe hyperplasia of the luminal epithelium. Moreover, immunofluorescent staining using an antibody against the proliferation marker Ki67 demonstrated that this hyperplasia was the result of hyperproliferation. These data are consistent with the data obtained in lung epithelium. These data provide further support for a role of TAp63α in the commitment to stratification. Further studies using this mouse model will be performed to obtain additional insights into the role of p63 during early stages of skin morphogenesis.

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Trichothiodystrophy: a multisystem genetic disease with striking phenotypic diversity

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Trichothiodystrophy (TTD) is a rare, autosomal recessive disease characterized by defective DNA repair and sulfur-deficient, brittle hair. We performed a systematic examination of clinical features of TTD patients at the National Institutes of Health to further identify the disease spectrum. Diagnosis was confirmed by the presence of tiger tail banding (an alternating pattern of light and dark bands) of hair under polarized microscopy. A total of 21 pt were studied, ranging from 1.5 to 24 yr (median 7 yr) at time of last visit. Major features observed were: brittle hair (100%); developmental delay (100%); abnormalities of the skin (95%) and nails (71%) including photosensitivity (57%) and ichthyosis (57%); short stature (86%); abnormal birth characteristics (81%) including low birth weight (67%) and collodion presentation (38%); ocular defects (76%) mainly congenital cataracts (52%); recurrent infections (57%) and abnormal dentition (48 %). Other notable findings were an engaging personality (76%); abnormal MRI findings (48%) including diffuse dysmyelination (38%); and sensorineural hearing loss (19%). X-rays showed bone abnormalities (90%) including peripheral osteopenia (71%) and central osteosclerosis (43%). Hematologic abnormalities (90%) included iron deficiency (29%) and features of β-thalassemia minor, with low MCV (81%) and elevated HbA_2 (67%). Maternal pregnancy complications (81%) occurred including intra-uterine growth retardation (57%) and pre-term delivery (48%). Unlike xeroderma pigmentosum, there was no history of skin cancer despite mutations in XPD (8 pt), a gene involved in both DNA repair and basal transcription. TTD is a rare multi-system developmental disorder with wide phenotypic variability. While all patients exhibited hair abnormalities and developmental delay, those more severely affected had substantial neurologic, immunologic, and growth impairment.

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Ephrin B2 expression in the perinatal skin is required for maintenance of keratinocyte homeostasis

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Eph receptor and their membrane bound ephrin ligands form a complex signal network that are involved in multiple processes during embryogenesis. Among these molecules, ephrinB2 is one of most extensively studied molecule, particularly in terms of its function in angiogenesis. However, its function in the skin development has not been addressed. Here, we found ephrinB2 is expressed in hair follicle and dermal region, but its expression in dermal mesenchymal cells disappeared gradually. As ephrinB2 binding counterparts, EphA4 is expressed specifically in the basal layer of the epidermis. To investigate ephrinB2 function in keratinocytes, we mated $ephrinB2^{flox/flox}$ with a K14-Cre transgenic strain. Despite of successful deletion of ephrinB2 gene in keratinocytes, we could not detect any overt defect in the development and maintenance of the skin. Consequently, to evaluated the functional significance of ephrinB2 expression in dermal mesenchymal cells, we exploited a tamoxifen-inducible Cre-recombinase system. $EphrinB2^{flox/flox}$ was mated with a transgenic strain bearing a chicken actin promoter driven Cre-recombinase and two mutated estrogen receptors (MER). Tamoxifen was injected during neonatal 5 days, and this treatment induced apparent macroscopic phenotypes, such as growth retardation and hypercornification. Immunohistochemical analysis revealed enhanced proliferation of epidermal progenitors. To rule out the possibility that hypercornification is a result of hypoxia due to the defect of vascular morphogenesis, we compared the gene expression profile of cells harvested from skin of tamoxifen-treated and untreated mice. We could not detect increase of any molecules that have been known to be induced by hypoxia, and found a significant increase of three IL-1 family molecules that are expressed by inflammation. This work showed for the first time that ephrinB2 is expressed in dermal mesenchymal cells transiently during perinatal period and plays a role in regulating the proliferation rate of epidermal cells.

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Disruption of the P2RY5 Gene, an orphan G-protein coupled receptor, underlies autosomal recessive woolly hair

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The genetic determinants of hair texture in humans are largely unknown. Several human syndromes exist in which woolly hair comprises a part of the phenotype, however, simple autosomal recessive inheritance of isolated woolly hair (OMIM 278150) has only rarely been reported. To identify a gene involved in the control of hair texture, we performed genetic linkage analysis in 6 families of Pakistani origin with autosomal recessive woolly hair (ARWH), as well as associated features including sparse, fragile and hypopigmented hair shafts. Using the Affymetrix 10K array and subsequent narrowing of the interval with microsatellite markers, we identified a region of linkage on chromosome 13q14.2-14.3 in all families studied, with a maximum multipoint LOD score of Z=17.97. Fine mapping of critical recombination events delineated a region of 2.1 Mb, containing 10 known genes, 2 pseudogenes and 1 predicted transcript. One of the known genes, P2RY5, encodes a heptaspan transmembrane G-protein coupled receptor (GPCR) and is a nested gene residing within intron 17 of the retinoblastoma 1 (RB1) gene. Resequencing of P2RY5 revealed homozygous pathogenic mutations in all families studied, including insertions, deletions and missense mutations in evolutionarily conserved residues from human to zebrafish. P2RY5 is abundantly expressed in the cortex and cuticle of the hair shaft, as well as Henle's and Huxley's layers of the inner root sheath of the hair follicle. Our findings indicate that disruption of P2RY5 underlies autosomal recessive woolly hair, and more broadly, uncover a novel gene involved in the determination of hair texture in humans.

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Leptin is a paracrine regulator of hair cycle

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Leptin, one of adipokines secreted from adipocytes, is known to affects energy balance by interacting with hypothalamus. Recent studies have shown that non-adipose tissues also produce leptin. Leptin acts on target tissues via specific receptors (Ob-Rs), and activates STAT3. We have previously reported that the activation of STAT3 is indispensable for hair cycle progression. In this study, we examined whether leptin is physiologically involved in hair cycle progression using well-established diabetic model mice. Back skin of 5 wk old C57/BL6 mice showed late anagen phase, while the hair follicles of Ob-Rb deficient Db/Db mice were still in the first telogen phase, and the anagen entry was retarded until postnatal day 40. This indicates that the signals through Ob-Rb are required for the second hair cycle progression. Anagen was induced in the injected site 3 week after local leptin injection in 7 wk old C57/BL6 mice. Biologically active leptin fragment (22-56) also induced the anagen phase. The phosphorylation of ERK, JAK2, and STAT3 in cultured human keratinocytes were examined by western blot using specific antibodies for phosphorylation sites. All of the molecules were phosphorylated by leptin and leptin fragment treatments. Production of leptin by cultured human dermal papilla cells was stimulated by hypoxic condition, suggesting that the hypoxic condition during the catagen phase promote the production of leptin in the dermal papilla cells. These results indicate that leptin is physiologically involved in hair cycle progression, and suggest that the angen phase is induced by leptin from dermal papilla cells in a paracrine manner.

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Age-related changes in skin derived stem cells

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Skin is mainly comprised of epidermis, dermis and subcutaneous tissue. In this research we investigated the location and age-related changes in dermal stem cells and the impact of these stem cells on the dermal collagen synthesis. We extirpated the skin from the HR-1 mice of the age 1day to 48 weeks and immunohistogically examined skin derived stem cells. We then segregated possible stem cells using flow cytometer, cultured these cells in induction media causing their differentiation and analyzed their differentiation-including ability. Additionally we measured the age-related changes in elasticity, moisture and collagen synthesis of the skin. Skin stem cells were stained using an epithelial stem cell marker, Neurotorophic factor receptor (p75NTR). The immunohystological analysis showed the presence of the p75NTR-positive cells mainly in the epidermal basement membrane and dermal widely, and confirmed the differentiation of the p75NTR-positive cells in dermis to fibroblast, adipocyte and osteoblast. We observed the age-related reduction in the proportion of the p75NTR-positive cells in the tissue from the analysis flow cytometer. From the fact of existence of the age-related decrease in moisture, thickness of the epidermis and the compound amount of collagen in the dermal tissue. It was suggested that a possibility of the stem cells being involved in the skin changes with age. In conclusion, it was suggested that the p75NTR-positive cells are multipotent stem cells; their age-related reduction in the proportion it is possible to make a suggestion on their connection to the skin changes. Currently we are conducting more precise research.

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Fibroblast growth factor 18 suppresses progression of anagen hair growth

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Many of the 22 known fibroblast growth factor (FGF) family members are expressed in dermal, epidermal and hair follicular cells, where they act to regulate hair growth and skin regeneration. We reported that mRNAs encoding FGF1, -2, -5, -7, -10, -13, -18 and -22 are all strongly expressed in skin. Here we addressed the biological activity of FGF18 on hair growth. The expression pattern of FGF18 is unique; FGF18 mRNA is expressed in telogen (resting) hair follicles at very high level, and is decreased rapidly when anagen (growing) phase of hair growth cycle is initiated by mechanical depilation. Its level continues to be low throughout the anagen phase, and then increases rapidly when anagen phase is completed. Thus FGF18 expression level is reversely correlated with hair growth. We previously showed that transition from telogen to anagen phase was accelerated by single, subcutaneous injection of FGF18 into mice during telogen phase. Here we examined the effect of FGF18 injection during anagen phase. During anagen, FGF18 protein caused suppression of DNA synthesis of hair follicle cells and growth of hair follicles. Profiling the gene expression in skin revealed that FGF18 downregulated transcription of several polypeptide growth factors that are known to regulate hair follicle morphogenesis and/or growth directly or indirectly. In cultured dermal papilla cells, FGF18 suppressed transcription of several hair growth supporting factors. Conversely, expression of these factors increased when the FGF18-treated cells were deprived of FGF18. These findings indicate that FGF18 is a suppressor of anagen hair growth progression via multiple genes and suggest that both high and low expression of FGF18 during telogen and anagen, respectively, are important in regulating hair growth cycle.

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Pigmentation defect due to the deficiency of Rac1 in differentiated parts of the hair follicle

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The small GTP binding protein Rac1 is required for the formation and maintenance of hair follicles in mice. Targeted deletion of Rac1 from all epidermal keratinocytes results in alopecia. This has been attributed to the loss of hair follicle stem cells upon Rac1 deletion. We have asked whether the maintenance of the follicular stem cell population was the only function of Rac1 in hair follicle formation or whether there were other functions that could not be addressed by targeted deletion of Rac1 from all keratinocytes forming the epidermis and hair follicles. We have therefore generated transgenic mice that express a mutant, constitutively active version of Rac1, L61Rac1, only in keratinocytes of the basal epidermal layer (BEL) and of the outer root sheath (ORS) of hair follicles (K14L61Rac1TG mice). These transgenic mice show a normal phenotype. They were crossed with mice in which Rac1 had been deleted in an epidermis and hair follicle specific manner (Rac1 E-KO mice). In the resulting K14L61Rac1TG Rac1 E-KO mice (rescue mice) Rac1 activity was reconstituted in the BEL and ORS but not in more differentiated parts of the epidermis and hair follicles. Rescue mice showed, in contrast to Rac1 E-KO mice, growth of hair, indicating an essential role for Rac1 expression in the BEL/ ORS for hair follicle formation. Surprisingly, however, the hair of the rescue mice was grey instead of black in the C57Bl6 background. Although the rescue mice formed hair follicles, these did not show a normal morphology in histological sections. In particular, the pigmented parts of the medulla appeared to have a disturbed structure and showed abnormally dense clusters of melanin. We are currently analyzing the nature of these structural defects. So far we conclude that, although expression of Rac1 in the BEL/ ORS is essential for the formation of hair follicles, Rac1 expressed in more differentiated parts of the hair follicle has also functions in its morphogenesis and pigmentation.

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Identification of human sebaceous gland markers to monitor the pharmacological activity of PPARγ agonists in primary human sebocyte cultures

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Increased sebum production is involved in the aetiology of acne. To further increase the knowledge of human sebaceous gland biology, the gene expression profiles of human sebaceous glands and epidermis were compared. Cosmetic surgery provided healthy skin of the face of adult subjects. Epidermis with connected hair follicles and sebaceous glands was separated from the dermis after enzymatic digestion, and sebaceous glands were collected by dissection using binocular lenses. Gene expression profiles of dissected sebaceous glands and epidermis were analysed using Affymetrix HG-U133A arrays. Identification of well known epidermal markers demonstrated the validity of the approach. In addition, a large number of genes preferentially expressed in human sebaceous glands was identified. Gene Ontology annotation indicated a significant proportion of these genes to be involved in pathways that regulate lipid metabolism, particularly fatty acid-, steroid- and cholesterol metabolism. To confirm the above results, qRT-PCR was performed on a selected set of genes on laser micro-dissected human sebaceous glands and epidermis. Furthermore, immunohistochemistry of a set of mRNA markers demonstrated localisation of the corresponding proteins in human sebaceous glands. Finally, these markers were used to monitor the effects of the selective PPARγ agonist Rosiglitazone in primary human sebocytes compared to primary human keratinocytes. Significant induction of human sebaceous gland markers was observed in primary human sebocytes but not in primary human keratinocytes treated with Rosiglitazone. These findings improve the current knowledge of human sebaceous gland biology, and have important implications for developing treatments of Acne vulgaris.

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Microarray analysis of freshly-microdissected intact human dermal papilla identified upregulation of genes that could contribute to biological distinctiveness

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Elucidation of biological distinctiveness of human dermal papilla (DP) is indispensable for experimental regeneration of human hair follicles (HFs). Observations that cultured DP cells failed to induce HFs, suggested that expression of genes responsible for DP characteristics was lost during culture. In this study, we attempted to identify genes specifically upregulated in freshly-microdissected intact human DP by comparing global gene expression profile among intact DP, cultured human DP cells and fibroblasts. Four sets of microarrays were successfully generated from individual volunteers. Correlation coefficients between intact DP and cultured DP or fibroblast microarrays were 0.86 and 0.85 respectively, while that among intact DP arrays was 0.92. Thus, in vitro expansion remarkably altered global gene expression profile of human DP cells. Combination of two different algorithms identified 141 genes upregulated in intact DP, including LEF1, HEY1 and BMP2. Those genes were involved in cell communication, signal transduction and organ development. Of note, upregulation of 22 genes, such as WIF1, PTCH and HEY2, were also reported in mouse intact DP cells, indicating the importance of Wnt, Shh and Notch signaling in DP. However, such genes as SPRY1 or FOXO1 were over-represented only in human intact DP. Interestingly, the expression of some DP genes such as SPON1 or SNRK was recovered, when cultured human DP cells were aggregated. These findings indicated that global gene expression profile of human intact DP is distinct from human cultured or mouse intact DP cells. Accordingly, use of intact DP may be required to further investigate the factors that define biological characteristics in vivo, including hair induction capacity. In addition, expression monitoring of genes identified in this study should provide valuable tools for the optimization of culture condition for human DP.

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Description of molecular effects of PPARγ compared to PPARα, PPARδ, LXR, RXR, RAR and AR agonists in rat preputial sebocyte cultures

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Primary rat preputial sebocyte cultures are a valuable and readily available model to study sebaceous gland biology. Additive effects of androgens and PPARγ agonists on the total lipid neo-synthesis of rat preputial sebocytes have been reported in the past. To increase the current knowledge on differentiation of rat preputial sebocytes we extended the study to the analysis of sebaceous gland specific lipid classes (triglycerides, cholesterol esters, wax esters) and the expression of sebocyte differentiation markers using qRT-PCR. The inductive effects of the PPARγ agonist Rosiglitazone on lipid synthesis and sebocyte marker gene expression were inhibited in the presence of a PPARγ antagonist, indicating that these biological processes are bona fide PPARγ-mediated. To analyze in depth the mechanism of action of PPARγ agonists compared to other nuclear receptor agonists, such as PPARα, PPARδ, LXR, RXR and RAR agonists, large scale gene expression profiling using RAE230A Affymetrix arrays was performed. This analysis allowed identification of specific markers and biological processes mediated by each nuclear receptor. PPARγ activation induces the synthesis of sebocyte specific lipids and sebocyte differentiation markers, PPARδ or RXR activation augments cell proliferation and modulates expression of extra cellular matrix components, LXR activation induces triglyceride synthesis and modulates expression of extra cellular matrix components, RAR activation modulates chemokine synthesis and extracellular matrix components. Finally, the transcriptomics profile of PPARγ agonist-treated rat preputial sebocytes was compared to the transcriptomics profile of human sebaceous glands to identify the most relevant markers that can be used in combination with lipid profiling for ranking of the potency and efficacy of PPARγ agonists in this cellular model.

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Electrophysiological analysis of rat Merkel cells reveal their mechanotransduction capacity

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Epidermal Merkel cells (MC) exhibit synaptic-like connections with Aβ sensory nerve fibers which convey the mechanical stimulations. Moreover, MC are predominantly present in the tactile areas of the body. Thereby, the MC-neurite complexes are presented as mechanoreceptors with slowly adapting capacity. No evidence demonstrated that MC play a functional role in this complex so far. Thus, they are just believed to modulate the excitability of sensory neurons. To specify the mechanotransduction capacity of rat MC, we isolated and cultured epidermal cells of the rat plantar skin where MC were numerous. Cells were transfected with a βgalactosidase expression vector system under the control of the Atonal homolog 1 promoter which was demonstrated to be specifically activated in MC within epidermis. Two days after transfection, the addition of X-gal in the culture medium allowed to identify MC which were electrophysiologically analyzed. MC synthesized calcium channels involved in cell excitability. The application of suctions at 200mmHg led to increase the inward currents. Moreover, the addition of gadoliniumIII at 100μM in the medium, an inhibitor of stretch channels, inhibited the depolarization induced by suction. Furthermore, 150 stimulations of voltage-gated calcium channels were performed for 1'30 minutes. Here we could not observe a loss of excitability in the long run. In conclusion, our results argue for the mechanotransduction capacity of MC in the MC-neurite complexes. We demonstrated that MC produce slowly adapting mechanosensitive calcium channels which enable them to feel pressure and to react by depolarizations entailed in cell excitability. This capability was inhibited by the addition of GadoliniumIII that supports the presence of stretch channels.

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Selective PPARγ agonists but not antagonists decrease sebaceous gland size in the Fuzzy rats

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As one important mechanism involved in acne pathogenesis is an excessive sebum excretion, one promising therapeutic approach could be to inhibit lipid synthesis in sebaceous glands. In many tissues PPAR receptors have been demonstrated to be key regulators of lipid homeostasis, including the epidermis. Here we report that the PPARγ subtype was differentially expressed in human and rat sebaceous glands versus epidermis. Therefore we proposed this subtype plays a key role in sebaceous gland homeostasis both in humans and rodents. We subsequently decided to use the Fuzzy rat model to investigate further the effect of PPAR modulators on sebaceous gland. The Fuzzy rat is a genetic mutant expressing an androgen-dependent hyper-secretion of sebum and hypertrophic sebaceous glands. The effect on sebaceous glands was assessed by measuring the size of the glands on epidermal-split preparations using micro-video image analysis. The selective PPARγ Rosiglitazone decreased dose-dependently the sebaceous gland volume following a 4-week topical treatment. Sebaceous glands and epidermis still revealed a normal structure in histology. A similar response was obtained using a panel of chemically unrelated PPARγ agonists, both in female and male rats. A potent specific PPARγ antagonist was found to be ineffective. To test whether the sebaceous gland response depended on specific mutations in the Fuzzy strain, non-mutant rats underwent the same treatment. The sebaceous glands of OFA rats were found to shrink considerably in the presence of the same agonists. Finally, using a specific PPARγ antagonist, we demonstrated that the activity observed on the glands was PPAR-mediated. In conclusion, PPARγ agonists greatly disrupt the sebaceous gland function. The activation of PPARγ may inhibit sebum excretion in human and lead to a subsequent beneficial therapeutic effect in acne patients.

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Withdrawn

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Selective PPARγ agonists antagonize DHT-induced effect on sebaceous glands in the Fuzzy rat

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PPARs are members of the steroid/thyroid nuclear receptor super family. The γ subtype is thought to play an important role in sebaceous gland homeostasis. In previous studies we used Fuzzy rats as an *in vivo* model to study sebaceous gland function and demonstrated that selective PPARγ agonists strongly decrease sebaceous gland volume without affecting gland histological structure. We then treated female Fuzzy rats with the potent androgen DHT to investigate further the mechanism of action of PPARγ agonists. Following a 4-week treatment period, DHT strongly increased the sebaceous gland volume and the sebum excretion rate (SER). The effect on sebaceous gland was assessed by measuring the size of the glands on epidermal-split preparations. Androgenic impregnation also induced brown coloration on the dorsal skin of the animals. In a previous experiment, we had demonstrated that a potent and selective proprietary PPARγ agonist was inactive in a cellular assay toward the Androgen Receptor. In stimulated female Fuzzy rats, this compound efficiently competed with DHT in inhibiting its effect on sebum excretion rate and sebaceous gland volume. Unlike anti-androgens, no effect was shown on the skin brown coloration. A similar response was observed with 2 other chemically unrelated PPARγ agonists. To ensure that those effects were not gender-related, results were reproduced in non stimulated male Fuzzy rats. Finally, we evaluated both proliferation and apoptosis *in vivo*. We conclude that PPARγ agonists are able to counteract androgen-induced effects in female Fuzzy rats. As phenotypic changes are slightly different from those observed with an anti-androgen, the underlying mechanisms may be partially different. This remains to be investigated using a potent molecular approach such as qRT-PCR or genomic to clearly delineate genes and pathways involved in these differential responses.

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Establishing the "epidermal transcription zones" in the keratinocyte nucleus during skin development: a role for p63 in high-order chromatin remodeling and spatial arrangement of tissue-specific genes

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During development, multipotent cells establish tissue-specific programmes of gene expression that underlie a process of differentiation into specialized cell types. Increased evidence suggests that high-order chromatin organization and spatial arrangement of genes within the nuclear space play an important role in the controlling gene expression. We show here that prior to and during epidermal stratification in mouse embryo (between E11.5 and E16.5), tissue-specific gene loci (epidermal differentiation complex, keratin type I and II loci located on chromosomes 3, 11, and 15 respectively) gather in close proximity to each other in a distinct relatively small transcriptionally active nuclear compartments, "epidermal transcription zones" (ETZs). ETZs are localized at the euchromatin of the inner portion of the nucleus and are enriched in the elongating RNA Polymerase II and speckles. Formation of the ETZs in the keratinocyte nucleus during embryonic development is associated with marked increase of the transcriptional activity of tissue-specific genes and is maintained in adult epidermis. This process is significantly altered in p63 knockout mice that lack epidermis and show strongly decreased expression of key genes (Satb1, Brg1, Mi-2alpha) controlling high-order chromatin organization and remodeling. These data imply that establishment of the epidermal transcription zones in the keratinocyte nucleus is required for normal skin development and that p63 as a master regulator of epidermal morphogenesis promotes their formation via modulating high-order chromatin remodeling.

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PPARγ agonists and DHT displayed non-overlapping responses on sebaceous glands in a large scale gene expression study *in vivo*

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While most of the existing *in vitro* data tend to favor of a positive effect of PPARγ on lipid production, we recently found that treating Fuzzy rats with PPARγ agonists led to marked decrease in size of sebaceous acini. PPARγ agonists were also able to counteract partially DHT effects in female rats. To identify further the underlying pathways involved, gene expression analyses were conducted *in vivo*. Animals were treated topically with a potent PPARγ agonist or DHT and epidermal sheets were prepared. mRNA was then subjected to a large scale gene expression analysis (Affymetrix). As qRT-PCR allows the monitoring of genes expressed at a very low level, up to 400 different genes linked to lipid metabolism were also studied using Taqman® Arrays (Applied Biosystems). Using both technologies less than 30 genes were identified as selectively modulated by PPARγ in sebaceous glands of Fuzzy rats. Some were involved in lipid and steroid metabolism. According to the gene function, modulations observed were in good agreement with the effects of PPARγ agonists found in our previous studies (decrease in sebaceous gland volume and sebum excretion). Conversely, androgen treatment triggers a wide response. Only 4 genes were found to be modulated inversely by the PPARγ agonist and DHT. New pathways were identified using data mining tools. In summary, treatment with a PPARγ agonist induced a "surgical" response involving a few specific genes not regulated by androgens. These genes are key switches for sebaceous gland homeostasis. Interconnection in the PPARγ and DHT pathways are very limited. As acne coincides with an increase in endogenous androgens and the beginning of sebaceous gland hyperactivity, treatment with PPARγ agonists with or without an additional anti-androgen may be of therapeutic benefits for acne patients.

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Commercial-scale production of wnts for inducing hair follicle growth and neogenesis as a treatment for alopecias

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Three-dimensional cultures of human neonatal fibroblasts were grown under hypoxic conditions that simulate the embryonic environment (3-5% oxygen) to enhance human extracellular matrix (ECM) production and secretion of growth factors associated with the fetal environment. Under these conditions a number of growth factors key to tissue development and regeneration were significantly elevated, including several WNT proteins such as WNTs 5a, 7a, and 11. The activity of the WNT signals was confirmed by the conditioned media's ability to induce nuclear translocation of β-catenin, and thus canonical WNT signaling, in human keratinocytes *in vitro*. We tested the hypothesis that concentrated WNT and growth factor containing media could stimulate epidermal stem cells to regenerate new hair follicles in normal mouse skin. Both active and control media were injected once into C57Bl/6 mice. 17/18 overall mice in 3 different experiments injected with this product candidate, which is derived from the conditioned medium of hypoxic ECM *in vitro* (HGEN-001), showed the induction of new hair follicles after 2-4 weeks. The number and location of follicles induced to cycle being apparently proportional to the volume of material injected. None of the vehicle control mice, or those injected with medium conditioned by monolayer fibroblasts, displayed new hair growth, and 1/12 mice displayed hair growth effects when injected with adult cell conditioned medium. When actively growing anagen skin of 2 week old mice was injected with HGEN-001, conversion to catagen/telogen was blocked and the anagen phase was extended, as evidenced by darker skin color at the injection site when compared to the vehicle control and the presence of higher numbers of morphologically-identified anagen follicles by histology. In summary, completely human HGEN-001 without bovine serum has been produced in commercially-scalable fermentation style bioreactors, and we are currently preparing for GMP manufacturing of HGEN-001, prior to commencing clinical trials with volunteers with androgenic alopecia.

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Identification of new PPARγ agonists optimized for topical administration using a bioluminescent animal model

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Decreasing sebum production is an attractive way to treat acne. In a preliminary set of *in vivo* experiments, activation of PPARγ resulted in noteworthy modifications in sebaceous gland homeostasis. However, compounds of the PPARγ pharmaceutical class displayed adverse effects limiting their use by the oral route. We therefore reasoned that a topical approach with compounds sharing both optimized skin penetration and a very short half-life in plasma may alleviate these limitations. Here we described new PPARγ agonists sharing high activity together with low metabolic stability and enhanced skin penetration in rodents. Receptorial activity of all compounds was evaluated using PPARγ reporter cell lines. Metabolic stability was measured in mice and human hepatocytes. Bioavailability of the compound either by oral, intra-peritoneal or topical route was monitored *in vivo* using bioluminescent xenografts. PPARγ reporter cell lines were implanted subcutaneously in female nude mice. Animals were treated with Rosiglitazone or two proprietary compounds by different routes of administration. The signal was monitored 8 hours following treatment. Proprietary compounds strongly activate inducible reporter cells by the topical route. In comparison, Rosiglitazone was found to be preferentially available by the oral route. In conclusion, reference and proprietary compounds have been evaluated in an animal model integrating different parameters (activity, metabolic stability and bioavailability) in the same time. This allowed us to describe better *in vivo* the properties of new PPARγ agonists. Finally we found that at least one of our proprietary compounds displays a more favourable behaviour for topical administration versus reference compound.

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Human hair follicle dermal cells activate NF-κB in response to TNF-α and IL-1β but are lipopolysaccharide tolerant

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We have previously described human hair follicle dermal (DS) and dermal papilla (DP) as playing a key role in wound healing and regeneration, and provided evidence of their immune privilege (IP) *in vivo*. Although others have attributed follicle IP to low MHC I expression coupled with a local immunosuppressant milieu, our current understanding of follicle IP is limited. NF-κB activation results in the upregulation of inflammatory genes, and in comparison to detailed studies on NF-κB activation in human keratinocytes, melanocytes and fibroblasts the response of DS and DP to pro-inflammatory challenge has not been studied. Using immunofluorescent detection of NF-κB/p65, we examined DS, DP, follicular and non-follicular fibroblast cells for NF-κB/p65 responsiveness to inflammatory stimuli: TNF-α (1000units/ml for 60 min), IL-1β (2ng/ml for 60 min) or LPS (100ng/ml for 90 min). In unstimulated fibroblast cells, NF-κB/p65 was located exclusively in the cytoplasm and was absent from the nucleus, indicating inactive NF-κB/p65. In contrast, unstimulated DS and DP exhibited both cytoplasmic and homogeneous distributions of NF-κB throughout the cytoplasm and nucleus (DS=27.6±1.2% and DP=13.4±0.6% activation). Pro-inflammatory challenge with TNF-α or IL-1β induced acute NF-κB activation in all cells studied (≥80.8% and ≥98.1% activation, respectively) whilst addition of LPS induced NF-κB activation only in dermal fibroblasts (≥70% activation). It is of particular interest that DS and DP were less responsive to LPS (DS=36.9±3.7% and DP=40.6±3.2% activation). This novel finding suggests that DS and DP may possess a cytoprotective mechanism to this bacterial toxin, whilst maintaining responsiveness to TNF-α and IL-1β (which control apoptosis driven catagen in follicles) and may give an insight into therapeutic treatments where IP collapse is observed.

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Genetic interplays between Msx2 and Foxn1 are required for Notch1 expression and hair shaft differentiation

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We use compound genetic mutants to analyze two transcription factors with demonstrated functions in hair shaft differentiation, Msx2 and Foxn1. First, we show that maintenance of Notch1 expression in the hair follicle matrix requires both factors, indicating a redundant activity of these proteins. In their absence, matrix cell proliferation is compromised; medulla and inner root sheath (IRS) differentiation is affected. second, we provide molecular and transgenic data supporting a direct regulation of Notch1 by Foxn1. Whereas these aspects of the phenotype can be attributed to loss of Notch1, Msx2/Foxn1 also contribute independently of Notch1 to cortex and cuticle differentiation. Despite the presence of a normal number of hair follicles and lineage-restricted stem cells, the impact of these defects is the complete loss of all visible external hairs. Since we find that BMP4 can regulate Foxn1 expression independent of Msx2, we suggest that these two genes function in parallel pathways downstream of BMP signaling and upstream of Notch1. Our results reveal a global requirement for Msx2 and Foxn1 in the hair matrix where they maintain Notch1 expression and thus indirectly control IRS and Medulla differentiation, while directly supporting many aspects of cortical differentiation.

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Melatonin regulates expression and activity of androgen related genes in human skin cells

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It is gradually accepted that the skin can act as a neuroendocrine organ. Specifically the skin expresses functional melatoninergic system and contains the enzymatic machinery involved in the sequential metabolism of cholesterol to pregnenolone and to corticosteroids and androgens and estrogens. In order to define a role for melatonin in the cutaneous androgenic system we tested its effect on the expression of androgen receptor (AR) and genes involved in metabolism of testosterone including 5α-reductase type 1 and 2 (SDR5α1 and 2) and aromatase. The activity of androgen receptor (AR) and metabolism of testosterone was investigated in human skin cell lines. It was shown by RT-PCR, quantitative PCR (qPCR) and immunocytochemistry that human fibroblasts expressed AR, SDR5a1 and aromatase contrary to HaCaT keratinocytes where only expression of SDR5α1 and aromatase was detected. Melatonin effects on the expression of above genes were dose dependent. Low concentration (10-8 to 10-5 [M]) inhibited expression of AR and SRD5α1, while slightly stimulating expression of aromatase. High concentration of melatonin 10-4 and 10-3 [M] stimulated expression of AR and SDR5α1 and inhibited expression of aromatase. Expression of SDR5α2 was not detected in skin cell lines studied so far by RT-PCR but very low level of expression was detected by qPCR. Melatonin also inhibited activity of androgen responsive element and expression of SDR5α1 in presence of testosterone and dihydrotestosterone in human fibroblasts. Thus, melatonin can regulate expression and signaling of genes involved in the metabolism and responsiveness to sex hormones in the skin. If the current cell culture data can be confirmed in human pilosebaceous units, it deserves to be explored whether melatonin can be exploited in the treatment of androgenic alopecia or acne.

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Effect of oxygen on premature senescence of balding dermal papilla cells *in vitro* identifies oxidative stress as a potential mechanism of androgenetic alopecia

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Androgenetic alopecia (AGA) is a hereditary disorder that involves the progressive thinning of hair in a defined pattern and is driven by androgens. The hair follicle dermal papilla (DP) expresses androgen receptors (AR) and plays an important role in the control of normal hair growth. We have previously reported that balding DPC undergo premature senescence associated with up-regulation of p16INK4a/pRb decreased expression of proliferating cell nuclear antigen (PCNA) and Bmi-1; and increased nuclear expression of markers of oxidative stress and DNA damage including HSP-27, Super Oxide Dismutase, Catalase, Ataxia-telangiectasia mutated kinase (ATM) and ATM- and Rad3-related protein (ATR). We proposed that the premature senescence of balding DPC *in vitro* in association with expression of p16INK4a/pRB indicates that balding DPC are more sensitive to environmental stress. To test this hypothesis we compared the growth of balding DPC under normal atmospheric levels of oxygen (21%) and under low oxygen (2%). We found that balding DPC cultured under 2% oxygen did not senesce and grew normally whereas, balding DPC cultured under 21% oxygen senesced as previously reported by us. Balding DPC cultured in 2% oxygen did not express senescence associated beta-galactosidase (SA-β-Gal) or p16INK4a/pRb but expressed increased levels of proliferating cell nuclear antigen (PCNA) and Bmi-1. Balding DPC grown under 2% oxygen also showed decreased expression of markers of oxidative stress including HSP-27, Super Oxide Dismutase & Catalase. These data confirm our hypothesis and demonstrate that balding DPC are more sensitive to environmental stress than non-balding DPC and identifies oxidative stress as a potential mechanism of androgenetic alopecia.

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Multiple roles of Notch signaling in the regulation of epidermal development

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The development of the epidermis requires coordination of differentiation and undifferentiation programs. However, although recent studies have now identified numerous molecules that are involved in the associated pathways, the mechanisms underlying the orchestrated formation of a stratified epidermis from a single-layered ectoderm remain unknown. In our current study, we have integrated loss and gain-of-function studies of Notch receptors and the Hes1 transcription-al repressor and can now demonstrate that Notch signaling plays a central role in the coordina-tion of epidermal development. Our data show that one of the functions of Notch is to determine the spinous cell fate and induce further terminal differentiation, whilst simultaneously prevent-ing the premature terminal differentiation of the spinous cells. The mechanism underlying this role of Notch was found to be independent of Hes1, which is required to maintain spinous cells in an immature state. We further show that Notch signaling directly induces the expression of Ascl2, which promotes a terminal differentiation pathways that is blocked by Hes1. Collectively therefore, our present findings show that Notch signaling orchestrates the balance between the differentiated and immature states of the spinous cells during epidermal development.

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Hedgehog-responding stem cells in the bulge expand to regenerate the anagen follicle

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The hair follicle is a multi-lineage structure that cycles through phases of growth (anagen), apoptotic regression (catagen), and quiescence (telogen). With each cycle there is stem cell-mediated regeneration of the proximal follicle in early anagen. The Hedgehog (Hh) protein, Sonic Hedgehog (Shh) is necessary for cycle progression. Adult follicular matrix expresses Shh during anagen. Overexpression of Shh promotes proliferation in the skin. Conversely, Shh inhibition blocks the effect of growth factors and prevents anagen regeneration. Anagen regrowth is dependent on stem cells located in the follicular bulge. While Shh is required for regeneration of the proximal follicle, it is not clear if Shh signals directly to bulge stem cells, or if it drives proliferation of transient amplifying cells during regrowth. To investigate Hh signaling to hair follicle stem cells, we examined the expression of the zinc-finger transcription factors Gli1 and Gli2. In cells receiving a Hh signal, existing Gli2 is activated to induce transcription of Hh target genes including *Gli1*. Because *Gli1* transcription is dependent on Hh activity, it is a sensitive reporter of Hh signaling. Expression patterns of Gli1 and Gli2 indicated that populations of cells in the telogen bulge are Hh-responding cells. We also used Genetic Inducible Fate Mapping with an allele of *Gli1* engineered to mark Hh-responding cells at a given point in time, as well as their future progeny. By expressing an inducible Cre recombinase from *Gli1* (Gli1-CreER), and using a LacZ reporter allele (R26R), Hh-responding cells are labeled via the administration of tamoxifen. When Gli1-expressing cells were labeled in telogen and followed into anagen, the cells expanded markedly with staining seen in the entire follicle below the bulge. Thus, Hh-responding cells in the quiescent telogen bulge regenerate the proximal follicle – a function of bulge stem cells. These findings suggest that Hh-responding bulge stem cells participate in hair follicle homeostasis.

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Generation and characterization of three-dimensional human hair follicle model using hanging drop culture system

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Epithelial-mesenchymal interaction between the dermal papilla (DP) and secondary hair germ is essential for the anagen onset of new hair cycle. In this study, we attempted to generate three-dimensional hair follicle model mimicking the anagen onset. Cultured human DP cells and outer root sheath (ORS) cells were mixed, followed by cultivation using hanging drop culture system, and analyzed by immunostaining and quantitative PCR technique. Major population of DP cells positive for vimetin immunoreactivity was located at the center of the cellular masses. Alkaline phosphatase activity was also detected in the same manner. These observations imply that the autonomous aggregation and alignment was occurred among epithelial and mesenchymal cells in the cellular masses. In these cell masses, external cell budding upward was observed and this budding showed intense Ki67 immmunoreactivity with conforcal microscopy. Cell proliferation was exclusively restricted to those two areas of cell masses, at the center and budding cell branch. We then, examined the effect of Wnt pathway activation in the cellular masses by using the GSK-3 inhibitor BIO (6-bromoindirubin-3′-oxime). Expression of Ber-EP4 antigen, known as a secondary hair germ marker, was enhanced by this BIO stimulation in the epithelial portions of the cellular masses. BIO treatment also resulted in the elevation of Wnt10b expression, suggesting Wnt pathway is activated in these specific area of cell masees. We also examined the effects of cyclosporine A (CsA) on the expression level of several gene markers such as BMP4, EdaR, Shh and Wnt10b, which were involved in morphogenesis and anagen onset in mammalian hair follicles. Expression level of these markers was affected by CsA stimulation as well as elevated expression level of hair keratin acidic 1. These results support that our three-dimensional hair follicle model is suitable to examine the anagen onset and hair induction, and useful tool for the investigation of these mechanism.

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Molecular mechanism of TGF-β1 promoter activation by androgen in balding dermal papilla cells

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We have already reported that TGF-β1 is upregulated by androgen in balding dermal papilla cells (DPCs) using our coculture system of DPCs transiently transfected with androgen receptor (AR) expression vector and keratinocytes (KCs). This finding suggested that the regulation of TGF-β1 by androgen is one of the key steps in the pathogenesis of androgenetic alopecia (AGA). To examine the molecular mechanism of TGF-β1 gene expression by androgen in DPCs, we analysed TGF-β1 promoter activity of the pTGF-β1-Luc vector, which was constructed by connecting the 5′ upstream region of the TGF-β1 gene (-1362 bp upstream from the transcription start site) to the luciferase reporter gene. Reporter assays using three lines of balding and non-balding DPCs transfected with pTGF-β1-Luc and AR expression vector showed that 1 nM synthetic androgen R1881 increased luciferase activity from around 2.05, 3.17 and 10.53-fold in balding DPCs but R1881 did not influence luciferase activity in non-bald DPCs. Then, we used the balding DPC line with the highest responsibility to androgen for further study. The analysis of deleted pTGF-β1-Luc vector indicated that there are two possible regulatory regions (from -1131 to -731 and from -459 to -323) and two negative regulatory regions (from -1326 to -1127 and from -735 to -459). The region from -459 to -323 region contains two AP-1-responsive consensus regions. The reporter vector mutated in these two regions lost the androgen responsibility and showed high background activity in the ethanol mock treatment in the balding DPCs, suggesting that androgen releases the constitutive suppression of TGF-β1 promoter by AP-1 in balding DPCs. On the other hand, the reporter vectors with or without these mutations did not show any androgen induction of the luciferase activity in non-bald DPCs. Together, we conclude that the crosstalk between AR and AP-1 cell-specifically regulates the TGF-β1 promoter activation in balding DPCs.

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Differentiation of hair follicle cells studied by electron microscope

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Morphological changes of the hair cells were investigated by electron microscope. The study includes the fate of the cells belonging to medulla, hair cortex, hair cuticle, three layers of inner root sheath, companion layer, and outer root sheath from their birth to death. Skin tissues obtained from the dorsum of neonatal Sprague-Dawley rats were analyzed under conventional methods of electron microscopy. The process of a programmed death of medulla cells is shown to involve ruffling of the cell membrane, projection of the cortex, and shrinkage of the medulla cells. The keratinization of hair cuticle cells take place with a polarity from outside to inside of the hair shaft, accompanying the formation of numerous amorphous electron dense particles, which are not observed in the hair cortex as well as in the inner root sheath tissues. The ways of the keratinization of three inner root sheath tissues are similar to each other. All these tissues produce trichohyalin granules during the process of their keratinization. However they are different in some respects. For example, keratinization of the cuticle of inner root sheath occurs from inside. Other two tissues have no polarity in the keratinization. Another example is that the keratinization of Henle layer cells starts firstly in the hair follicle, and by contrast that of Huxley layer cells starts lastly and they survive all types of cells belonging to other keratinizing hair tissues. It is interesting that the companion layer cells are convex to the outer root sheath cells at the boundary between those two layers. A desmosome is often found in the inflection point. The frequency of the formation of desmosome is greatly different dependent on the combination of tissues, and is highest in Henle-Henle boundary and the second highest is companion-companion or companion-outer root sheath boundary. Some outer root sheath cells may exfoliate into the dermal matrix.

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Adenosine stimulates fibroblast growth factor-7 induction in dermal papilla cells and contributes to hair elongation.

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Adenosine is known to be an endogenous biomolecule that plays wide variety of physiological roles via its four types of receptors' mediated pathway, in various cell types including dermal papilla (DP) cells in hair follicle (1). We performed DNA microarray analysis of cultured DP cells with or without adenosine, and found that adenosine stimulates fibroblast growth factor-7 (FGF-7) mRNA expression. Quantitative analysis using RT-PCR showed that adenosine upregulates FGF-7 mRNA expression in a dose-dependent manner. Elevations of the secreted FGF-7 protein from DP cells were also observed in ELISA. These activations of FGF-7 both at mRNA and protein levels were inhibited by A2b adenosine receptor-specific antagonist, alloxazine, but not by antagonists for other subtypes (A1, A2a, A3 adenosine receptors). In addition, the intracellular cAMP levels were raised by adenosine in a dose-dependent manner in DP cells. Moreover, an increase of intracellular cAMP enhanced the FGF-7 mRNA expression. On the other hand, inhibitor of PLC activation, U-73122, did not suppress the upregulation of FGF-7 following the adenosine stimulation. Taken together, these results show that adenosine treatment of DP cells upregulates FGF-7 expression via the A2b adenosine receptor and that cAMP acts as one of the second messengers in this pathway. Furthermore, treatment with FGF-7 significantly stimulated hair fiber elongation in hair follicle organ culture. These data imply that adenosine activates hair-growth through FGF-7 upregulation in DP cells.

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The role of laminin-332 and -511 in the regulation of hair cycling and chemotherapy-induced alopecia

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Basement membrane (BM) plays important roles in hair growth. However, little is known about the role of BM components in regulating hair cycling and in inducing chemotherapy-induced alopecia (CIA). We characterize the expression and role of laminin (LN)-332 and -511 on hair cycling and assess how chemotherapy affects the expression of LNs and causes alopecia. The expressions of LN-332 and -511 with their binding partner integrins (INT) were assessed by real time quantitative RT-PCR (QRT-PCR), immunoblot (IB), *in situ* hybridization (ISH)and immunohistochemistry (IHC) using murine hair cycling and CIA model. Moreover, functional aspects were assessed using LN proteins on *in vivo* human hair culture and outer root sheath cells under the existence or absence of cyclophosphamide (CP). Our resultsindicated that 1) In the anagen, LN-332 and α6β4INT gradually downregulated and diminished after anagen VI in the BM around hair bulbs. In contrast, LN-511 and α3β1INT became constantly upregulated throughout anagen stages. The functional study suggested that LN-511 had a positive effect on the hair growth and LN-332 suppressed such an effect. 2) Addition of CP into mice, whose hairs shows anagen VI stage, caused rapid progression to CIA. In the dystrophic catagen, the expressions of LN-332 and α6β4INT were strikingly upregulated in the BM around hair bulbs, ES and CTS. In sharp contrast, the expressions of LN-511 and α3β1INT in the BM around hair follicles were rapidly depressed from the IHC and IB. The rapid disruption of the expression of LN-511 and α3β1INT was confirmed to be caused by transcriptional level from ISH and QRT-PCR. The direct effect of CP on the transcriptional expression of LN-511 was confirmed by cell culture. In conclusions, the LN-511 positively regulates hair cycle, while the LN-332 suppressed it. Rapid disruption of the transcriptional expression of LN-511 possibly induces dystrophic catagen caused by the chemotherapy.

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Hair-inducing ability of cultured human dermal papilla cells

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Dermal papilla cells lose their hair-inducing ability after long-term cultivation. We have reported that the Nissui-modified minimal essential medium (N-MEM: Nissui pharmaceutical, Tokyo, Japan) is effective for the proliferation of human dermal papilla cells (hDPCs) [Kishimoto J, et al., J Dermatol Sci 2007; 47:96]. In this study, we evaluated the hair-inducing ability of hDPCs cultured in N-MEM. Primary culture of hDPCs was done by an outgrowth method. Expression of representative hair-inducing marker genes was determined by quantitative PCR analysis. The hair-inducing ability of the cultured hDPCs with N-MEM was examined by a cellular grafting assay using silicon chambers, followed by histochemical analyses. Hair follicles were successfully reconstituted by the cellular grafting of hDPCs cultured in N-MEM but not conventional DMEM. Alu-positive hDPCs were observed below the reconstituted epidermis. Alu-positive hDPCs were localized below the bottom of hair peg-like structure. The reconstituted hair peg-like structure was positively stained for P-cadherine immunoreactivity. In the grafted skin, hair follicles were accompanied with the sebaceous gland. Interestingly, Alu-positive hDPCs were detected around the lower part of the fully formed hair follicles but not in the dermal papilla. Moreover, in the presence of 6-bromoindirubin-3′-oxime (BIO), which activates Wnt pathway through GSK-3 inhibition, gene expression of hair-inducing makers such as versican and lef-1 was maintained in hDPCs whereas in the absence of BIO, was not. In particular, the number of hair follicles was markedly increased by hDPCs treated with BIO compared to without BIO. These data suggested the possibility that N-MEM combined with BIO was efficient for the maintenance of hair-inducing activity of hDPCs. Further investigation should be necessary to verify the source of dermal papilla cells in the reconstituted hair follicles.

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PPAR binding protein as a key regulator of hair cycle in mice

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PPAR binding protein (PBP: also named as TRAP220/DRIP205/Med1/RB18A) is a coactivator of nuclear receptors, including PPARγ and vitamin D receptor (VDR). PBP also regulates cell cycle and apoptosis as a cofactor of p53. Most of cell types including keratinocytes (KC) express PBP. A recent study shows PBP is expressed and functions as a coactivator of VDR in proliferating human epidermal KC, not in differentiated KC, suggesting its role in proliferation of KC *in vitro*. However, the biological role of PBP in KC has not been fully clarified because PBP null mice are embryonic lethal. In this study, we established keratinocyte specific PBP null mice (PBPepi-/-) using K5-Cre-LoxP system to analyze its function in KC. Development of the epidermis and appendages of PBPepi-/- mice was macroscopically and microscopically normal until the second catagen. Wound healing of the PBPepi-/- is also comparable to the wild type counterparts. However PBPepi-/- mice showed rapid turning over of hair cycles after the second telogen, hair structure abnormality, dermal cyst formation, and reduced response to the percutaneous application of vitamin D. Parts of these phenotypes are shared with those of lunx1 null mice, VDR or hairless null mice, sonic hedgehog transgenic mice and noggin transgenic mice. These results suggest PBP functions as a coactivator of VDR and RXR, and possibly as an upstream regulator of IGF and BMP signaling in keratinocytes. Increased apoptotic keratinocytes after ultraviolet B irradiation on PBP null mice skin were also observed, which can be explained by its function as a regulator of p53. As PBP is a component of the large protein coactivator complexes, including TRAP and DRIP, deletion of PBP leads malfunction of several transcription factors. However the functions of any of those transcription factors are not fully inhibited, presumably because of compensation by other coactivators. One of the pivotal functions of PBP in keratinocytes is prohibition of hair follicles entering into anagen phase after the second telogen, while the mechanism should be clarified.

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L-ascorbic acid 2-phosphate magnesium salt induces secretion of IGF-1 from dermal papilla cells to promote elongation of hair shafts *in vitro*

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We have recently reported that L-ascorbic acid 2-phosphate magnesium salt (Asc 2-P), a derivative of L-ascorbic acid (Vitamin C), promotes elongation of hair shafts in isolated hair follicles *in vitro*. In this study, we examined whether IGF-1 is involved in the stimulation of human hair follicle growth in vitro by Asc 2-P. The neutralizing antibody against IGF-1 significantly suppressed the Asc2-P-mediated elongation of hair shafts in hair-follicle organ culture system. Also, the IGF-1 antibody significantly attenuated the Asc 2-P-induced growth promotion of co-cultured ORS follicular keratinocytes. Moreover, measurement of concentration of IGF-1 in the conditioned medium of dermal papilla cells showed that IGF-1 was significantly increased after treatment with Asc 2-P. Our data in this study demonstrate that Asc 2-P-inducible IGF-1 from dermal papilla cells promotes proliferation of follicular keratinocytes and stimulates hair follicle growth *in vitro*.

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Efficacy of adenosine on female pattern hair loss and male androgenetic alopecia

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Adenosine up-regulates vascular endothelial growth factor and fibroblast growth factor-7 in cultured hair dermal papilla cells. We previously showed that in Japanese men, adenosine improved androgenetic alopecia due to thickening of thin hair generated by hair follicle miniaturization for 6 months study. Our objectives were to investigate and compare the anti-hair loss efficacies of adenosine for men and women. Two sets of human test were performed. For male test, forty Japanese men with androgenetic alopecia were recruited. For female test, thirty Japanese women with a female pattern hair loss were recruited. Both tests were double-blinded, randomized, placebo-controlled study. Volunteers topically used either 0.75% adenosine lotion or placebo lotion twice daily for 12 months. Data were obtained at 0, 6 and 12 months. Efficacy was evaluated by assessing global scalp coverage by dermatologists, and from a phototrichogram. After 12 months of human test, it was shown that adenosine was significantly superior to placebo on global improvement from direct assessments by dermatologists in both male and female tests. By phototrichogram results, the average hair growth ratio increased significantly in female subjects who used adenosine including lotions for 12 months. The average anagen hair ratio in male subjects who used adenosine including lotions increased significantly. Adenosine also could show significant improvement in thick hair rates of both male and female subjects compared to placebo groups. Adenosine also showed significant improvement by self-assessment results. No side effect was encountered during the trials. In conclusion, Adenosine improved hair loss in Japanese women by stimulating hair growth and increasing thick hair. It is suggested that thickening of hair was effective in both type of hair loss. Adenosine is useful ingredients for treatments of androgenetic alopecia in men and a female pattern hair loss in women.

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Activation of transient receptor potential vanilloid-3 (TRPV3) inhibits human hair growth

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We have previously reported that transient receptor potential vanilloid-1 (TRPV1, the "capsaicin/heat receptor") plays a central role in the regulation of human and murine hair growth. Recently, another member of the thermosensitive TRPV family, i.e. TRPV3, was also implicated in the process; namely, a "gain-of-function" mutation in the trpv3 gene resulted in a hairless phenotype in mice. In the current study, we have investigated the functional involvement of TRPV3-signaling in human hair growth control using organ-cultured human scalp hair follicles (HF). The expression of TRPV3 was identified in the HF at both mRNA and protein levels (immunohistochemistry, Q-PCR). In organ culture, TRPV3 activation by plant-derived (eugenol, thymol, carvacrol) or synthetic (2-aminoethoxydiphenyl borate) agonists resulted in a dose-dependent inhibition of hair shaft elongation, suppression of proliferation (immunohistochemical identification of Ki67 proliferation marker), stimulation of apoptosis (TUNEL immunohistochemistry), and induction of premature HF regression (catagen). Furthermore, these agents also caused rapid elevation of intracellular calcium concentration (as revealed by fluorimetric Ca-imaging), suppression of cell growth, and stimulation of apoptosis (as assessed by series of fluorimetric assays) in 308 and HaCaT epidermal keratinocytes reportedly expressing TRPV3. Collectively, our findings strongly support the concept that TRPV3 signaling is a significant novel player in human hair (and possibly epidermal) growth control. Therefore, TRPV3 and the related intracellular (presumably calcium) signaling might function as a promising, novel target for pharmacological manipulations of clinically relevant epithelial growth disorders.

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Hair growth promoting effects of adipose tissue via the secretion of adiponectin

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Adipose tissues are observed to be located around hair follicles especially during anagen stage. However, any functional role of adipose tissue on hair growth was not yet clarified. The aims of this study were to evaluate the hair growth promoting effect of adiponectin, one of well known adipokines, and identify its molecular mechanisms. After treatment with adiponectin, proliferation of the outer root sheath (ORS) cells and dermal papilla (DP) cells was evaluated by MTT assays. Hair organ culture with the treatment of adiponectin was performed. RT-PCR analysis was performed to search for growth factors from DP cells and ORS cells that were affected by adiponectin. We evaluated the expression levels of IGF-1, KGF, HGF, VEGF (stimulating mediator on hair growth), and TGF-β1 (inhibitory mediator on hair growth) by adiponectin treatment . Hair follicular length was elongated with the treatment of adiponectin. Adiponectin treatment significantly increased VEGF mRNA expression in ORS cells, and IGF-1, HGF, and VEGF mRNAs expression in DP cells. We demonstrated that adiponectin-induced proliferation of hair follicular DP cells was decreased by VEGF and IGF-1 siRNA transfection. Our results suggested that adiponectin may stimulate the hair growth by increasing the secretion of IGF-1, HGF and VEGF in DP cells. Thus, adipose tissue may play a role in hair growth partially via the secretion of adiponectin.

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Evaluation of free oxygen radical and antioxidant capacity in alopecia areata

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The pathophysiology of alopecia areata (AA) has not been clearly defined. We tried to analyze the differences of free radical status and antioxidant capacity in the blood between AA patients and normal control. Sixteen patients with AA and 16 age- and sex-matched healthy controls were enrolled in this study. We analyzed serum levels of reactive oxygen metabolites (ROMs, mainly hydroperoxides, ROOH) using d-ROMs test (FRAS 4, H&D s.r.l., Italy) which represents the level of oxidative stress in the blood. BAP (biological antioxidant power) test (FRAS 4, H&D s.r.l., Italy) was also performed to assess the antioxidant power of the plasma as a measure of the ability to reduce ferric (Fe3+) ions to ferrous (Fe2+) ions. The mean levels of ROMs in serum of patients with AA (357.69±88.46 CARR U, Carratelli Units; normal value, 250-300 CARR U) were significantly higher than those of controls (287.81±20.22 CARR U). The antioxidant capacity (2063.44±132.67 μmol/l; normal value, 2200- 4000 μmol/l) in the serum of patients with AA was significantly lower than that of control (2267.44±135.18 μmol/l). In conclusion, we could find ROMs were increased and antioxidant capacity was decreased in AA patient. This could support the role of alteration of oxidant-antioxidant enzymatic system in the pathogenesis of AA.

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Site-specific regulatory function of HOX noncoding RNAs in skin fibroblasts

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Fibroblasts from unique anatomic sites in the skin have distinct gene expression profiles but the molecular basis for this site-specific specialization is not understood. The HOX genes are transcription factors that specify positional patterning of body structures including the skin and are differentially expressed in skin fibroblasts from unique anatomic sites. The HOX loci also contain extensive intergenic transcriptional units termed long noncoding RNAs (ncRNAs). The identity and function of these ncRNAs within the human HOX locus is not well known. We utilized a tiling array of all four human HOX loci to generate a high resolution map of the transcriptional activity of the HOX loci in skin fibroblasts from 11 unique anatomic sites. A majority (231/407) of the output was found to arise from intergenic regions and most (88%) were found to lack coding potential suggesting they represented ncRNAs. Similar to HOX genes, these ncRNAs showed striking site-specific expression along a developmental axis of the body. Eight of these ncRNAs were selected for further study based on their site-specific expression pattern and conservation in mammalian species. The expression for these eight ncRNAs was confirmed by qRT-PCR in cultured skin fibroblasts. To determine the function of each individual ncRNA in this panel, siRNA was utilized to deplete each of these ncRNAs in either lung (non-dermal) or foot (distal) cultured fibroblasts. Changes in gene expression were detected by interrogating oligonucleotide arrays representing 30,718 unique genes. Results revealed that a subset of this panel of ncRNAs regulate key proliferative and developmental signaling pathways including members of the canonical Wnt signaling pathway. These results expand our knowledge of a large class of regulatory RNAs within the HOX locus and suggest that these ncRNAs have unique and distinct gene regulatory function. Ongoing studies seek to clarify the mechanism(s) by which these ncRNAs regulate gene expression and their precise functional roles in skin fibroblast positional identity

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Differences of gene expression profiles related to androgenetic alopecia

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Androgens, especially dihydrotestosterone (DHT) are known as the key regulator of human hair growth and male-pattern baldness. Circulating androgens enter hair follicle dermal papilla cells (DPCs) via the capillaries located at the bottom of the follicles. Interestingly, after puberty, frontal (baldness) area is miniaturizing as androgen goes up, while occipital area is not affected. To investigate the characteristics of occipital and frontal DPCs, we we isolated RNAs from DPCs from an individual and used Human Genome U133A 2.0 gene chips (Affymetrix) to check gene expression levels. For systems biological approach to analyze them, we used "R" program and R-packages as analysis tool. We found some signaling pathway molecules are differentially expressed. This systems biological approach to androgenic alopecia would lead us to understand pathophysiologic pathway of male-pattern baldness.

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Functional overlap of ectodysplasin and troy signalling pathways in skin appendage development

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The development of skin appendages requires signalling by ectodysplasin (Eda), a tumor necrosis factor (TNF) family member, its receptor Edar, and downstream activation of the nuclear factor kappa B (NF-kappaB) transcription factor. Eda deficient mice lack primary hair placodes giving rise to guard hairs while secondary hair follicles do develop but produce abnormal hair shafts. No defects are apparent in dental or mammary placodes in Eda null embryos although abnormalities are evident at later development stages. Strikingly, mice overexpressing Eda-A1 isoform of ectodysplasin have ectopic dental and mammary placodes that develop into mature functional organs. These data suggest that Eda-A1 could play a role in the formation of tooth and mammary placodes as well, but might act redundantly with another (TNF) pathway to activate NF-kappaB. A potential candidate is Troy (or TNFRSF19 or Taj), an orphan receptor which is homologous with Edar in its ligand-binding domain, and is expressed in an overlapping pattern. We have characterized Troy null mice, and crossed them with Eda mutants. The double mutants mainly resembled Eda deficient mice with few exceptions. Surprisingly, analysis of NF-kappaB activity using a transgenic reporter in wild type and Eda deficient backgrounds suggested that the functional overlap of Troy and Eda signalling pathways is mainly mediated by NF-kappaB independent pathways.

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The dimensions and characteristics of the subepidermal nerve plexus in human healthy skin

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The subepidermal nerve plexus covers the entire body surface with a dense network of fibers. As the most distal part of the peripheral nervous system, it enables sensory perception from the environment. Yet, only scarce data on its dimensions and composing fiber qualities are available. We used dermal sheet preparations of human healthy skin of different body sites for its immunohistochemical and -fluorescent depiction. Analysis was done by light- and laser scanning microscopy and the obtained images were further evaluated with Imaris image analysis software. Analysed by light microscopy, skin of the chest contained the most dense subepidermal nerve plexus with a total Nerve Growth Factor Receptor positive fiber length of 100±18mm/mm2 (median±standard deviation) followed by skin of the abdomen (78±22mm/mm2), the shoulder (73±13mm/mm2), the upper arm (66±20mm/mm2), the inside lower arm (66±19mm/mm2), the thigh (59±16mm/mm2), the outside lower arm (53±10mm/mm2) and the lower leg (42±6mm/mm2). We therefore used chest skin for further laser scanning analysis of fiber quality. As a control and reference, total NGFr positive fiber length was measured again. Here we found a total length of 115±25mm/mm2. 49% of fibers were positive for Protein Gene Product 9.5 corresponding to a length of 57±23mm/mm2. Neurofilament expression, representing Aδ-fibers, could be detected in 14% of fibers conforming to a length of 16±10mm/mm2. Peptidergic fibers, as characterized by the expression of Calcitonin-gene-related peptide comprised 4% (4±3mm/mm2). Although PGP9.5 is widely used as a pan-nerve fiber marker, its discontinuous and speckled staining pattern results in a remarkably lower calculated total fiber length than the use of NGFr. In conclusion, our data provide the first detailed reference for the measures of the subepidermal nerve plexus in healthy human skin in situ and serve as a basis for the evaluation of pathological changes occuring for example in polyneuropathies.

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Keratin expression in the developing mouse nail unit

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During mouse limb development the nail unit develops on the dorsal side of the distal extremities. While major insights have been gained by studies of limb development in genetically engineered laboratory mice, few studies exist on the development of the nail unit. We have investigated the spatio-temporal pattern of keratin expression during morphogenesis in distal limbs of C57BL/6J mice at different timepoints between day 15.5 post conception (dpc) and day 21 post partum (dpp). We performed routine histology and immunolabeling with antibodies against keratins 1, 5, 6, 6a, 10, 14, 17 and hard keratins (detected with the antibody AE13), as well as involucrin, filaggrin and loricrin. We found that the expression of hard keratins was first detectable at 15.5 dpc in the forelimb in single cells of the dorsal digits, close to the tip. At this stage a depression above the developing distal interphalangeal joint marked the proximal border of the nail field, where the epithelium of the matrix primordium started to invaginate. At this time K1/10 was detected suprabasally in the epidermis of the whole digit. At 16.5 dpc K1/10 expression was reduced in the epithelium of the nail field to a patchy pattern, which was also observed for filaggrin. Between 16.5 dpc and 17.5 dpc a translucent nail plate became apparent in the area of the nail field. By 18.5 dpc hard keratin expression had extended to more than half of the nail field, while markers of epidermal differentiation such as K1/K10, loricrin and filaggrin had mostly disappeared from this region. K14 expression was only weakly detectable in the distal digit during early nail development. The expression pattern of K5, K17, K6 and K6a in the distal digit epithelium showed distinctive changes during development, reflecting the dynamics of regional patterning of the nail unit. These data provide a valuable reference for the study of mouse models with abnormal nail development.

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Pro-inflammatory lipid mediators in the pathogenesis of cicatricial alopecia

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We previously showed deficient PPAR gamma regulated pathways (peroxisome biogenesis and lipid metabolism) in scalp hair follicles from individuals with lichen planopilaris (LPP), a cicatricial or scarring alopecia (CA). Targeted deletion of PPAR gamma in hair follicle stem cells caused CA in a mouse model. The dual role of PPAR gamma in lipid homeostasis and inflammation raises the question whether deregulated lipid metabolism is the cause or the effect of the inflammatory response in LPP. Comparative gene expression analysis of paired unaffected and affected LPP scalp biopsies shows that lipid metabolic changes are already seen in unaffected tissue. In contrast, inflammatory changes take place only in affected tissue. This suggests that lipid metabolic changes represent early or primary events in disease pathogenesis and are not due to loss of sebaceous glands or scarring. It also suggests that deregulated lipid metabolism is the cause rather than the effect of the inflammatory response in LPP. Pathway analysis of differentially expressed genes reveals a negative feed-back regulatory loop between PPAR gamma and lipid oxidation enzymes, prostaglandin synthase 2 (COX2) and 5-lipoxygenase (5-LO). Intriguingly, both genes are significantly upregulated in LPP and in the PPAR gamma knockout mouse. Lipid analysis by gas chromatography detects increased arachidonic acid in all lipid fractions in LPP, suggesting that leukotrienes and prostaglandins play a role in the pathogenesis of LPP. Similarly, analyses of other lymphocytic and neutrophilic CA show the involvement of distinct pro-inflammatory lipid mediators in disease pathogenesis and provide a molecular basis for differences in clinical phenotypes. These data provide compelling evidence that PPAR gamma deficiency leads to the accumulation of pro-inflammatory lipid signals that trigger the pathogenesis of CA. Thus, PPAR gamma agonists or 5-LO and COX2 inhibitors may provide novel therapeutic strategies in the treatment of CA.

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Abnormal hair follicle development and follicular keratinocyte transformation in transgenic mice expressing ΔNp63

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During embryonic development, multipotent stem cells give rise to the interfollicular epidermis (IFE) and appendages such as hair follicles. The transcription factor p63 is essential for epidermal morphogenesis and the development of hair follicles, as evidenced by the skin phenotype of p63 null mice. While the analysis of p63 null mice have illustrated the critical function of p63 in the development of stratified epithelia, its role in postnatal hair follicle development remains elusive. Furthermore, all isoforms of p63 are absent in the p63 knockout mice, which has precluded dissecting out the biological role of individual p63 proteins, such as the ΔNp63 isoforms, which are highly expressed in skin epidermis. To investigate the role of ΔNp63 in hair follicle morphogenesis, we have engineered tet-inducible transgenic animals with targeted expression of ΔNp63 to the Outer Root Sheath (ORS) of the hair follicle. Interestingly, ΔNp63 transgenic animals develop severe hair growth and cycling defects as well as alterations in the hair follicle differentiation program. Ectopic expression of ΔNp63 results in a block in the differentiation of matrix cells to generate the Inner Root Sheath (IRS) and the hair shaft compartment. This phenotype is accompanied by alterations in well established signaling pathways and transcription factors known to be involved in hair follicle morphogenesis such as Wnt/β-Catenin and Gata-3, which are known targets of ΔNp63. In addition, transgenic animals display a progressive increase in hair follicle size with an expanded ORS and the appearance of epithelial buds, resembling new hair follicles developing from pre-existing ones. Finally, we demonstrate that overexpression of ΔNp63 results in a switch in cell lineage determination of hair follicle keratinocytes to adopt an IFE cell fate as evident by the appearance of specific markers. Collectively, our data provides the first documented *in vivo* evidence of a critical role for ΔNp63 as a potential transcriptional hub in regulating various facets of the hair follicle growth and differentiation program.

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$CD200^{high}$ α-6-$integrin^{high}$ keratinocytes possess characteristics of early stem cell progeny, are depleted in androgenetic alopecia, and localize to the bulge and secondary hair germ.

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Because androgenetic alopecia (AGA) is characterized by drastic miniaturization of the hair follicle, we tested the hypothesis that depletion of hair follicle stem cells is associated with this condition. Hair follicle stem cells reside in the bulge region and preferentially express CYTOKERATIN 15 (KRT15) and FOLLISTATIN (FST). We verified that cells expressing the highest levels of KRT15 or FST possess stem cell characteristics in that these cells are the smallest and most quiescent (highest percentage of cells in G_0) epithelial cells within the hair follicle and epidermis. Remarkably the $KRT15^{high}/FST^{high}$ population did not change in number in bald versus haired scalp. In contrast, we discovered a population of cells, present in haired scalp, but virtually absent in bald scalp, which were slightly larger and more proliferative than the hair follicle stem cells. These putative progenitor cells expressed the highest levels of CD200 and α-6-INTEGRIN within the cutaneous epithelium, and localized to both the bulge and secondary hair germ in mouse and man. In mouse, we showed that $CD200^{+}$α-6-$integrin^{+}$ cells within the secondary hair germ were enriched for cells in G2/M. $CD200^{+}$α-6-$integrin^{+}$ cells combined with inductive neonatal dermis reconstituted hair follicles and sebaceous glands more efficiently than $CD200^{-}$α-6-$integrin^{+}$ cells. These results suggest that the loss of a population of multipotent early stem cell progeny, defined by CD200highALPHA-6-INTEGRINhigh expression, may contribute to the development of androgenetic alopecia.

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Role of heat shock protein 70 in the development of alopecia in the C3H/HeJ mouse model

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Alopecia Areata (AA) is one of the most common dermatological autoimmune disorders, with an incidence of 1.7% in the general population. AA is characterized by the presence of CD4+ and CD8+ leukocyte infiltrates in the follicular and perifollicular area. Animal models such as the C3H/HeJ mouse model have facilitated the study of AA. C3H/HeJ mice spontaneously develop AA in a pattern similar to the human in approximately 20% of mice by 18 months of age. Nevertheless, the precise molecular mechanisms mediating the development of AA in the C3H/HeJ mice remain to be elucidated. In our previous work, we have demonstrated that expression of heat shock proteins (HSP) protects hair follicles from chemotherapy by inducing dystrophic catagen. HSPs are a group of proteins ubiquitously expressed in cells which react to physical stresses such as heat stress, radiation, hypoxia or chemical stimuli. Modulation of HSPs confers on cells increased stress resistance. We therefore hypothesized that HSPs, most specifically, HSP70 may play a role in the development of AA by affecting hair follicles. We therefore proceeded to locally administer heat in C3H/HeJ mice (48°C, for 20 minutes) daily starting at 6 months of age. At this stage, spontaneous development of AA has not reach its zenith, with barely 1-5% of the animals evidencing AA lesions on arrival to the facility. We randomized the C3H/HeJ mice into two groups: 35 mice received treatment while 40 did not. After 6 weeks, we observed a statistically significant ($p=0.000006$) increase in the incidence of AA in the group receiving heat. Immunohistological studies of lesions from mice of group A revealed an increase in the follicular and perifollicular expression of HSP70. In conclusion, our results indicate that expression of HSP70 induced by localized heat application exacerbates the development of AA in C3H/HeJ mice and may be involved in the molecular pathway of AA in humans.

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Keratinocyte mediated differentiation of MSCs

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Human bone marrow mesenchymal stem cells (MSCs) possess enormous regenerative potential and have been shown to engraft and appropriately integrate as various cell types in injured tissue. However, the cues that guide MSC differentiation in tissue remain unclear. Here we show that a single cell type, the epidermal keratinocyte, can selectively direct MSC differentiation toward either epidermal or dermal lineages based on the nature of their interaction. To do this, we set up two coculture systems, one in which the keratinocytes and MSCs were in direct contact and another in which the cells were separated by a porous membrane. In direct contact cocultures, a subpopulation of the MSCs exhibited epithelialoid morphology, and expressed the keratinocyte markers p63, K14, and β4-integrin. Using FACS and FISH, we rigorously showed that 43% of this subpopulation did so without evidence of cell fusion. In contrast, non-contact cocultured MSCs did not express keratinocytes markers but surprisingly differentiated into myofibroblastic and neural morphologies with increased expression of αSMA and TuJ1, respectively. We extended our work by delivering MSCs into ex vivo human skin wounds and found that MSCs integrating into the re-epithelializing epidermis express the keratinocyte markers β4-integrin and K14. We removed the MSCs that were not in contact with the epidermal layers by laser capture microdissection and subsequent RT-PCR analysis showed increased expression of TuJ1 and αSMA, similar to our in vitro findings. Thus, MSCs in direct cell contact with keratinocytes were induced to express epidermal lineage markers while MSCs that were non-contact cocultured with keratinocytes expressed dermal lineage markers of myofibroblast and early neural phenotypes. To our best knowledge, this is the first evidence that signals derived from a single cell type can direct alternate pathways for MSC differentiation. These results shift our understanding of how intercellular interactions guide MSC differentiation and expand our knowledge as to why and how MSCs are able to appropriately integrate into tissue.

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Hair follicle morphogenetic signaling pathways are activated in follicular basal cell hyperplasia overlying dermatofibroma

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Embryonic follicular morphogenesis requires reciprocal signaling between the surface epithelium and dermis. Hair follicles undergo postnatal cyclical growth, but do not usually form after birth. Dermatofibromas are common cutaneous lesions of mesenchymal origin that typically exhibit hyperplasia of the overlying epithelium. Interestingly, follicular basal cell hyperplasia, characterized as incomplete hair follicle differentiation, occurs in a subset of dermatofibromas. To explore the molecular mechanisms underlying this phenomenon, we asked whether expression of factors required for embryonic hair follicle morphogenesis is induced in lesional areas corresponding to follicular basal cell hyperplasia. Activation of Wnt/β-catenin signaling is the earliest known event in embryonic hair follicle induction and is characterized by nuclear localization of β-catenin protein that complexes with the transcription factor LEF1. We observed nuclear β-catenin and LEF1 staining in basal cells at the leading edges of epithelial invaginations into the dermis, and in the matrix/precortical region of small, hair follicle-like structures developing in lesional areas. Mesenchymal cell aggregates resembling dermal condensates were apparent beneath areas of basal cell invagination. These cells exhibited staining for nuclear β-catenin, and also expressed mRNAs for the Sonic hedgehog activated gene PTC1, and WNT5A, which is regulated by SHH in hair follicle cells. Strong expression of PTC1 and WNT5a, and another hair follicle-associated Wnt, WNT10B, and nuclear localization of the placode and outer root sheath marker SOX9, were also observed in invaginating epithelial cells. These data indicate that embryonic follicular morphogenetic signaling pathways are activated in adult skin overlying dermatofibroma, presumably in response to secreted signal(s) from dermatofibroma cells. Identifying these secreted factors could yield new molecular tools for therapeutic hair follicle neogenesis.

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The role of Dlx3 in hair development

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The Dlx3 homeodomain transcription factor is crucial for developmental processes including tissue differentiation and organ formation. Tricho-Dento-Osseous Syndrome (TDO) is an ectodermal dysplasia linked to a 4-bp deletion immediately downstream of the DNA-binding homeodomain in the Dlx3 gene and that is characterized by abnormalities in hair, teeth, and intramembranous and ectochondral bones. To examine Dlx3 gene expression throughout development, we generated a knockin mouse line carrying the reporter gene beta-galactosidase (lacZ) under the control of the endogenous Dlx3 promoter. Dlx3 expression is detected in tissues and organs derived from epithelial-mesenchymal interactions such as hair follicles, teeth and limbs, and in craniofacial bones and interfollicular epidermis. We have characterized Dlx3 expression through hair differentiation and growth cycle. Dlx3 expression was initially detected in the matrix of hair follicles directly adjacent to dermal papilla, and then gradually extended to inner root sheath (IRS), cortex, medulla, and cuticle of the differentiating hair. The role of Dlx3 in hair development is being investigated intensively using a Cre-mediated knockout mouse model. The most striking defect in those mice was alopecia due to a failure in hair follicle differentiation. Taken together with pathological conditions of TDO patients, our results support the hypothesis that Dlx3 is an essential regulator for development of hair follicle.

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Forced activation of β-catenin signaling in adult mice induces *de novo* tooth development

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Wnt/β-catenin signaling is required for embryonic development of ectodermal appendages including hair follicles and teeth. Forced activation of β-catenin signaling in embryos causes ectopic tooth formation, while activation of this pathway in adult skin leads to hair follicle neogenesis. To determine whether manipulation of β-catenin signaling could be used to promote *de novo* tooth development in adult life, we utilized triple transgenic *K5-rtTA tetO-Cre Ctnnb1*$^{fl(ex3)/+}$ mice in which expression of stabilized β-catenin can be induced in oral epithelia as well as epidermis by dosage with doxycycline. The oral cavities of uninduced mutant mice appeared normal. In adult mice examined after two weeks of doxycycline treatment, we observed multiple tooth-like structures with differentiated ameloblasts and odontoblasts, forming on the labial side at the base of incisor teeth. Markers of embryonic tooth bud epithelium including *Shh* and *Wnt10a* were expressed in the epithelium of the tooth-like structures, and *Msx1* was up-regulated in the mesenchyme, indicating that these structures represent *de novo* tooth development in the adult. To determine the origin of the ectopic teeth we examined mutant mice after five days of induction. At this stage, the cervical loop structure at the base of each mutant incisor tooth was expanded and out-pouched into dental mesenchyme. Proliferation of stellate reticulum cells at the center of the cervical loop, the location of epithelial stem cells, was markedly upregulated compared with controls, suggesting that teeth forming *de novo* in the adult oral cavity may be derived from incisor tooth stem cells. Our findings suggest that activation of β-catenin signaling may turn back the developmental clock of adult oral stem cells, conferring embryonic-like properties that allow them to initiate dental neogenesis. Manipulation of this pathway provides a possible approach for tooth replacement in cases of hereditary absence or loss through disease.

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***Foxn1* is a regulatory target of Hoxc13 in ectodermal development and dysplasia**

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Over the past 3 years, we have used our *Hoxc13*-overexpressing mouse model*(Tg(Hoxc13)61B1Awg)* to identify multiple *Hoxc13* regulatory target genes (*Crisp1*, *Foxq1*, *Krtap16*, etc.). More recent observation with the gene-targeted *Hoxc13*^{tm1Mrc} mice indicate that these mice share multiple phenotypic characteristics with the nude (*Foxn1*^{nu} *null*) mouse, including hairlessness and a severe nail dystrophy. This is intriguing as our previous DNA microarray and *in situ* hybridization data obtained with *Hoxc13* overexpressing mice indicated downregulation of *Foxn1* in the abnormally differentiated hair follicles of these mice. Furthermore, considering the overlap of *Foxn1* and *Hoxc13* expression domains in the precortical region of anagen hair follicles and the presence of multiple bona fide *Hoxc13* binding sites in the *Foxn1* promoter region, we hypothesize that *Foxn1* is a direct target of Hoxc13 regulation. Both genes are likely part of a regulatory network essential for both normal hair and nail plate development. We present supporting evidence for this hypothesis through a comparative analysis of the *Hoxc13* overexpressing mouse, the *Hoxc13* null mouse, and the *Foxn1*^{nu} null mouse. Data obtained by immunohistochemistry, *in-situ* hybridization, real-time PCR, and *Hoxc13*-chromatin immuno-precipitation (ChIP) assays of the *Foxn1* promoter region, suggest indeed that *Foxn1* is a direct regulatory target of Hoxc13. This is the second example of *Hox* mediated transcription of *Forkhead* transcription factors. Additionally, the evidence provided here suggests that *Hoxc13* is a key regulator of both normal hair and nail plate development and likely plays a central role in the complex regulatory networks underlying various forms of Ectodermal Dysplasia.

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Global changes in mRNA levels and failure of hair follicle stem cell maintenance in adult Dicer-deleted skin

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MicroRNAs (miRNAs) are endogenous approximately 22 nt RNAs that regulate gene expression by binding the 3′ untranslated regions of mRNAs, resulting in mRNA cleavage or inhibition of translation. Conserved vertebrate miRNAs are predicted to target more than 400 regulatory genes, suggesting broad roles in biology. Multiple miRNAs are expressed in developing and postnatal skin and hair follicles, and constitutive deletion of the miRNA processing enzyme Dicer in embryonic mouse skin causes failure of hair follicle morphogenesis and subsequent follicular degradation. To determine whether Dicer and miRNA functions are also required in established hair follicles and epidermis in the adult mouse we generated *K5-rtTA tetO-Cre Dicer*$^{fl/fl}$ mice in which the *Dicer* gene can be inducibly deleted in the basal epidermis and hair follicle outer root sheath, including hair follicle stem cells, by dosage with oral doxycycline. Inducible *Dicer* deletion from postnatal day (P) 20, when hair follicles are just entering the first postnatal growth cycle, resulted in loss of external hair starting within 10 days of doxycycline treatment. Histological analysis at P45 revealed that mutant hair follicles remained in an abnormal growth phase, while hair follicles in control littermate skin had entered telogen. Mutant follicles were not maintained, resulting in hairless skin. An early abnormality detected in induced mutant hair follicles was loss of expression of the bulge stem cell marker K15, suggesting that failure of stem cell maintenance contributes to the *Dicer* mutant phenotype. To begin to identify potential miRNA target genes in mature skin we carried out mRNA profiling experiments on control and inducible mutant skin 15 days after induction. These experiments revealed over-expression of multiple mRNAs in the mutant, including members of the *Hox* gene family that are expressed in the skin and hair follicles and are known miRNA targets.

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The human hair follicle contains two forms of ATP-sensitive potassium channels, only one of which is sensitive to minoxidil

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Hair disorders cause psychological distress but are generally poorly controlled; more effective treatments are required. Despite minoxidil's longstanding use as a topical treatment for balding, its mechanism is still unclear; suggestions include acting on vasculature or follicle cells. Some similar drugs like diazoxide also stimulate hair implicating ATP-sensitive potassium (K_{ATP}) channels. To investigate whether K_{ATP} channels are present in human follicles, we used organ culture, molecular biological and immunohistological approaches. Minoxidil and tolbutamide, a K_{ATP} channel blocker, opposed each others effects on the growing phase (anagen) of scalp follicles cultured in standard media with, and without, insulin. RT-PCR identified K_{ATP} channel component gene expression including regulatory sulfonylurea receptors (SUR) SUR1, SUR2B but not SUR2A, and pore-forming sub-units (Kir), Kir6.1 and Kir6.2. When hair bulb tissues were isolated and examined separately, the epithelial matrix expressed only SUR1 & Kir6.2, while both the dermal papilla and dermal sheath exhibited SUR2B and Kir6.1. Immunohistochemistry of frozen scalp sections demonstrated similar protein distributions. Thus, human follicles respond biologically to K_{ATP} channel regulators in organ culture and express both genes and proteins for two K_{ATP} channels: Kir6.2/SUR1 in keratinocytes & Kir6.1/SUR2B in dermal papilla and dermal sheath. Interestingly, minoxidil only stimulates SUR2 channels. This indicates that: the human follicular dermal papilla, the main regulator of the folicle, contains K_{ATP} channels which can respond to minoxidil, while matrix keratinocytes contain channels which would respond to other modulators; tolbutamide may suppress hair growth clinically with potential use for hirsutism; and novel drugs designed more specifically for these K_{ATP} channels could be developed to treat hair disorders.

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Estrogen treatment reduces progression of doxorubicin-induced catagen in human hair follicle *ex vivo*

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Chemotherapy-induced alopecia (CIA) is considered one of the major problems of clinical oncology. No effective preventive or therapeutic modality is so far available and there remains a critical need for effective new approaches to this problem. It has been shown in mice that topical 17-β-estradiol significantly alters the cycling response of murine follicles after chemotherapy. Our aim was to further explore the effect of estrogen receptor agonist (E2) and estrogen receptor antagonist (anti-E2) on the sensitivity of human hair follicles (HFs) to chemotherapy. In this study, we investigated the effect of E2 and anti-E2 in the prevention of doxorubicin-induced apoptosis in human scalp hair follicles cultured *ex vivo*. Isolated human HFs were pre-treated with either E2 or anti-E2, followed by doxorubicin treatment. HFs from each group were collected and processed for hair cycle stage analysis by histo-morphometry, for assessment of apoptosis by TUNEL and proliferation by Ki67 immunohistostaining as well as for the TaqMan analysis. Doxorubicin treatment alone resulted in a rapid progression of human anagen hair follicles into catagen stages already 24 hours after treatment and in appearance of numerous apoptotic cells in the hair follicle matrix with gradual decrease in number of proliferating cells. Pre-treatment with E2 resulted in reduction of catagen progression of the doxorubicin-treated hair follicles and in apoptosis inhibition. In contrast, anti-E2 showed potent pro-apoptotic effect and induced more rapid progression into catagen stage. Estrogen receptor signaling operates as a regulator of apoptosis in human anagen hair follicles and further exploration of the mechanism of its action may result in potential applications of estrogen agonists/antagonists for the treatment of several hair growth abnormalities seen in clinical practice, including CIA.

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Stem cell factor/c-Kit signalling in normal and androgenetic alopecia hair follicles

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Androgens stimulate many hair follicles to alter hair colour and size via the hair growth cycle; in androgenetic alopecia tiny, pale hairs gradually replace large, pigmented ones. Since stem cell factor (SCF) is important in embryonic melanocyte migration and maintaining adult rodent pigmentation, we investigated SCF/c-Kit signalling in human hair follicles to determine whether this was altered in androgenetic alopecia. Quantitative immunohistochemistry detected 3 melanocyte-lineage markers and c-Kit in 4 focus areas: the epidermis, infundibulum, hair bulb (where pigment is formed) and mid-follicle outer root sheath (ORS). Co-localisation confirmed melanocyte c-Kit expression; cultured follicular melanocytes also exhibited c-Kit. Few ORS cells expressed differentiated melanocyte markers or c-Kit, but NKI-beteb, which also recognises early melanocyte-lineage antigens, identified fourfold more cells, confirmed by co-localisation. Occasional only NKI-beteb stained cells were also seen in the hair bulb. Melanocyte distribution, concentration and c-Kit expression was unaltered in balding follicles. Androgenetic alopecia cultured dermal papilla cells secreted less SCF, measured by ELISA, than normal cells. This identifies 3 types of melanocyte-lineage cell in human follicles. c-Kit expression by dendritic, pigmenting, bulbar melanocytes and rounded, differentiated, non-pigmenting ORS melanocytes implicate SCF in maintaining pigmentation and migration into regenerating hair bulbs. Less differentiated, c-Kit-independent cells in the mid-ORS stem cell niche and occasionally in the bulb, presumably a local reserve for long scalp hair growth, implicate other factors in activating stem cells. Androgens appear to reduce alopecia hair colour by inhibiting dermal papilla SCF production, impeding bulbar melanocyte pigmentation. These results may facilitate new treatments for hair colour in hirsutism, alopecia or greying.

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17-beta estradiol and prednisolone promote "dystrophic catagen" in chemotherapy-damaged human hair follicles: Potential stimulators of normal hair re-growth?

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Chemotherapy-induced hair follicle (HF) damage and alopecia are common side-effects of cancer therapy and constitute a major burden of disease in clinical oncology. Although several treatment strategies have shown promising effects in animal models, they remain to be transferred to the human system, and to be tested clinically. Topical 17-beta estradiol (E2) and glucocorticoids potently modulate murine chemotherapy-induced alopecia (CIA) *in vivo*: they accelerate the regrowth of normally pigmented hair shafts by shifting HFs into the "dystrophic catagen" pathway, (= greater initial alopecia, but much faster HF recovery). Here, we asked whether E2 and/or prednisolone display similar activities in the human system, using a human HF dystrophy *in vitro*-model (Bodó et al., Am J Pathol 2007). E2 and/or prednisolone were pre-and co-administered with a key cyclophosphamide metabolite (4-HC). Indeed, E2 shifted 4-HC-treated human HFs into the dystrophic catagen pathway *in vitro*, and potentiated the 4-HC-induced pigmentary disturbances. 4-HC-induced hair growth inhibition was further enhanced by E2, associated with an additional reduction of proliferation and increased apoptosis of matrix keratinocytes. The observed effects tended to be enhanced by co-administration of prednisolone. These preclinical data suggest that, just as in murine CIA *in vivo*, E2 and glucocorticoids can force chemotherapy-damaged human HFs into the dystrophic catagen pathway and provide first indications that a combination of E2 with prednisolone may indeed serve as an effective clinical tool for accelerating hair re-growth in human CIA by dystrophic catagen promotion.

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Glycation level of scalp hair proteins varies by site in a subset of diffuse hair loss patients

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Rapidly proliferating cells of anagen hair follicles metabolize glucose for energy through aerobic glycolysis. Impaired glucose metabolism and subsequent energy deficit could compromise hair growth, contribute to thinning hair, and result in increased hair protein (keratin) glycation. Scalp hairs cut from the side (thinning) and back (non-thinning) regions of non-androchronogenetic alopecia (non-AGA), diffuse hair-loss patients (n=244) were assayed for glycation products (fructoselysine) based on colorimetry of 2-keto-glucose (modified method of Kobayashi, et.al., 1994). Briefly, hair is weighed, minced to 3-8mm, boiled in 4M hydrazine hydrate (pH 9.4), and color is developed with phenylhydrazine HCl. Results (µg glucose/mg hair) were used to compute side-to-back ratios (SBR) expressed here as [mean (range)]. There were 94 patients who had low [0.93 (0.03-0.1.10) SBR and 130 patients with high SBR [1.88 (1.11-19.0)]. Hair diameter was not a factor in SBR analysis; cross-sectional areas of hairs from side and back were equal in individual patients regardless of SBR. Samples (n=24) of shed, uncut hairs collected by patients and separated by length into long and short yielded a mean (sd) glucose concentration of 213 (70) µg/mg for long hairs versus 303 (107) for short hairs (p=0.002), suggesting that the duration of anagen may be affected by impaired glucose metabolism. To help identify the role of impaired glucose metabolism in hair loss, an assay is being developed to measure glucose and glycogen content per µg DNA of side and back hair follicles plucked at time zero, 30min, 1hr, 2hr and 3hr after administration of a 75g oral glucose challenge to patients with a high SBR. Blood levels of glucose and insulin are also measured. The results show that regional differences in hair protein glycation exist and that impaired local glucose tolerance could contribute to diffuse hair loss in some patients.

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CRH-mediated stress response of *in vitro* cultured human anagen hair follicles from men expressing androgenetic alopecia is modified by caffeine

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Corticotropin releasing hormone (CRH) is a known key stress mediator that inhibits human hair growth in vitro and may be involved in stress-incuded hair loss. To characterize the CRH-mediated stress-response in hair follicles from men with androgenetic alopecia (AGA), scalp skin biopsies were electively taken from the balding vertex area of men with AGA (Hamilton stage III – VI) after informed consent. Anagen stage VI hair follicles were extracted and organ-cultured over 120 hrs in serum-free medium with or without CRH. In addition, caffeine was tested for protective effects against CRH-induced hair growth-inhibitory effects. CRH (10-7 M) suppressed hair shaft elongation, induced catagen, inhibited matrix keratinocyte proliferation and induced apoptosis. Caffeine (0.001%) inhibited the CRH-induced suppression of hair shaft elongation and even stimulated growth over untreated (non-CRH-treated) control level, but had only minor influence on CRH-mediated catagen-induction. However, matrix keratinocyte proliferation was significantly stimulated and CRH-induced apoptosis down-regulated. These data provide the first indications that androgen-sensitive vertex area hair follicles from men with AGA are sensitive to CRH and that the hair growth-inhibitory effects of this major neuroendocrine stress mediator can be inhibited by caffeine. Thus, the androgen-dependent miniaturization of genetically predisposed hair follicles in AGA may be complemented by other (e.g. stress-related) hair growth-inhibitory agents such as CRH, while cell energy-promoting substances such as caffeine, may offer an effective adjuvant therapeutic principle in AGA.

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Human hair follicles as direct, non-classical targets for thyroid-stimulating hormone (TSH): Indications to novel functions for an ancient neuroendocrine signal in human skin

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Pituitary thyroid-stimulating hormone (TSH) regulates thyroid hormone synthesis via TSH receptors (TSH-R). Since the hair follicle (HF) is uniquely hormone-sensitive and since increased TSH serum levels can lead to hair loss, we asked whether human HFs are a direct target for TSH. Here, we report that normal human scalp skin and microdissected human HFs express TSH-R mRNA. TSH-R-like immunoreactivity is limited to mesenchymal skin compartments *in situ*. These receptors are functionally active since their stimulation upregulates cAMP and leads to differential gene/protein expression (see below). TSH-R activation may alter HF mesenchyme functions, since it up-regulates alpha-smooth muscle actin expression, while that of the collagen-specific molecular chaperone HSP47 is down-regulated in connective tissue sheath fibroblasts of the hair follicle. We investigated TSH-R stimulation by its natural ligand in organ culture by microarray analysis and confirmed differentially expressed genes/gene products by real-time PCR/immunhistochemistry. We observed altered gene or protein expression of several mesenchymal and epithelial factors: i.e. up-regulation of keratin associated proteins and connective tissue growth factor by TSH, and TSH increased staining intensity of immunoreactivity for mitochondrial cytochrom c oxidase 1, alpha smooth muscle actin and keratin 5 in several HF compartments. These findings document that intracutaneous TSH-R are fully functional in situ and that human HFs are direct targets for non-classical, extrathyroidal TSH bioregulation.

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Periodic patterning stem cells and induction of skin appendages: p-ERK-dependent mesenchymal condensation is coupled with Turing mechanism to convert stripes to spots

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A fundamental problem in biology is to understand the mechanisms driving periodic pattern formation of skin appendages. Recent works show that Turing model is involved in the initial induction of hair and feather primordia (Maini, PK, Baker, RE, Chuong, CM., 2006. The Turing model comes of molecular age. Science. 314:1397-1398). Reaction diffusion mechanism converts stem cells from the basal homogeneous status to chemical patterns, reflecting activator and inhibitor activities. How the Turing pattern is then translated to discrete cellular patterns remains unknown. Using the feather model, here we show ERK activity-dependent mesenchymal cell chemotaxis toward initial peaks is essential for completing pattern formation. Adding ERK inhibitors produced a spectrum of placode patterns, ranging from broad stripes (early addition) to spots (late addition). Stripe regions exhibit broadly Shh-positive epithelium and NCAM-positive mesenchyme, implying a status intermediate to the basal and feather bud states. We propose that periodic patterning occurs in a restrictive mode; first a reaction-diffusion mechanism converts the homogeneous stem cell field into patterned regions of pre-primordia. Then cells respond to FGF and other signals by moving toward pre-primordia and also shape the patterning process, until large discrete dermal condensations are established. We developed a mathematical model integrating experimental data and simulating this patterning process.

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Human hair follicles are direct targets for thyroid hormones: involvement in anagen prolongation, hair matrix proliferation, hair pigmentation and metabolism

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Both insufficient and excess levels of thyroid hormones (THs:T3, T4) can result in altered hair/skin structure and function (e.g. effluvium). However, it is still unclear whether THs exert any direct effects on human hair follicles (HFs). Studying microdissected, organ-cultured human scalp HFs, we show here that T4 up-regulates the proliferation of hair matrix keratinocytes, while their apoptosis is downregulated by THs. The latter also prolong anagen duration *in vitro* possibly due to the down-regulation of the key anagen inhibitory factor, TGFβ2. Human HFs transcribe deiodinases and should thus be capable of converting T4 to T3 and of inactivating T3. Immunoreactivity (IR) for the thyroid hormone-responsive keratins CK6 and CK14 is significantly modulated by THs (CK6: enhanced, CK14: down-regulated) and significantly stimulate intrafollicular melanin synthesis. Since THs can stimulate erythropoietin (EPO) expression and human scalp HFs are an extrarenal EPO source, we also assessed whether THs increase intrafollicular EPO expression. Both erythropoietin mRNA expression and immunreactivity were significantly increased by THs. Furthermore, THs upregulate intraepithelial HF IR for a key respiratory chain enzyme, cytochrome- c- oxidase I, suggesting the possible involvement of thyroid hormones in HF metabolism. We identify further thyroid hormone target in the HF connective tissue sheath: thyroid hormones activate (increase number and degranulation) mast cells. Thus, human HFs are direct targets of thyroid hormone, which modulate multiple different HF functions, ranging from HF cycling to pigmentation.

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Reversal of epidermal aging by NF-κB blockade

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Aging is associated with subtle yet widespread alterations in gene expression, but the mechanisms controlling these alterations are not well understood. By identifying combinations of cis-regulatory elements that are predictive of expression patterns in individuals of different ages, we predicted that the transcription factor NF-κB is a major regulator of gene expression changes associated with aging in many human and murine tissues. Inducible genetic blockade of NF-κB in the epidermis of chronologically aged mice substantially reverted the global gene expression program and tissue characteristics to those of young mice. We further show that a classic mouse mutant of premature aging is due to excessive NF-κB activation. These results suggest that NF-κB activity is required for enforcing many characteristics of aging, especially in skin.

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Wnt modulates the differentiation status of interfollicular epidermal melanocytes arising from melanocyte stem cells in the hair follicle bulge after wounding or UVB irradiation

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Melanocyte stem cells residing in the hair follicle bulge area are ultimately responsible for pigmentation of the hair. However, it is unknown whether bulge melanocytes contribute to pigmentation of the interfollicular epidermis. To study whether melanocyte stem cells located in the bulge migrate to the interfollicular epidermis after injury or UVB irradiation, we utilized Trp2-lacZ transgenic reporter mice that express LacZ in melanocytes. We found that melanocytes originally located in the hair follicle bulge migrated to the infundibulum and then epidermis where they remained for at least 4 months. The bulge- derived melanocytes in the epidermis were well-differentiated and produced melanin. Using transgenic mice (tet-O-dkk1/K5-rtTA) in which Dkk1, a potent inhibitor of Wnt signaling, was over-expressed in the basal epidermis, we found that Wnt signaling regulated melanocyte stem cell differentiation during their migration to the epidermis. Dkk1 over- expression in the epidermis during reepithelialization resulted in a dramatic down- regulation of melanocyte differentiation markers such as tyrosinase, although it did not significantly change the number of melanocytes in the wound epidermis. Cessation of Dkk1 induction after reepithelialization permitted hair follicle neogenesis and the bulge-derived melanocytes then exhibited the capacity to regenerate the de novo pigmentary unit in the new hair follicles. These findings indicate that melanocyte stem cells residing in the hair follicle bulge have the ability to give rise to durable epidermal melanocytes and that wnt signaling modulates melanocyte stem cell differentiation status.

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Laminin-511 is an early epithelial message promoting dermal papilla development and function during early hair morphogenesis

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Hair morphogenesis takes place through reciprocal epithelial and mesenchymal signaling, however the mechanisms controlling signal exchange are poorly understood. Laminins are extracellular matrix proteins that play critical roles in both adhesion and signaling. We have previously shown that laminin-511 is required for hair morphogenesis and here we elucidate the underlying mechanism. Although we showed that endogenous laminin-511 in hair morphogenesis was produced by epithelium, surprisingly we found that it was dermal papillae (DP), in the absence of laminin-511 (in lama5-/- skin), which displayed several key developmental defects by E16.5 including a failure to maintain expression of the key morphogen noggin. This maintenance was critical as it coincided with an abrupt arrest of hair follicle downgrowth ultimately resulting in complete hair involution of lama5-/- skin. Exogenous introduction of purified laminin-511, noggin or the downstream noggin effector Sonic hedgehog (Shh), was sufficient to restore hair follicle development in lama5-/- skin. Laminin-511 with a deleted heparin binding domain supported noggin production and hair follicle development after application to lama5-/- skin, however laminin-511 lacking the integrin binding site or wild type laminin-111 showed no hair promoting effects. Previous studies have shown that Shh signaling requires microtubule based signaling organelles termed primary cilia. Interestingly, mutant lama5-/- DP showed a striking decrease in primary cilia length and structure, both *in vitro* and *in vivo*. Laminin-511, but not laminin-111, restored primary cilia formation in lama5-/- DP and dramatically triggered noggin expression in E16.5 mouse dermal mesenchyme in an Shh and PDGF dependent manner. These studies show that epithelial-derived laminin-511 is a critical early signal which directs DP ciliary function and maintenance as a requirement for hair follicle development.

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Canonical Wnt signaling activity is required for Hedgehog pathway-induced epithelial bud and follicular hamartoma development

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Aberrant Hedgehog (Hh) signaling plays a critical role in the development of several undifferentiated tumors arising in skin, including basal cell carcinoma (BCC) and basaloid follicular hamartoma. Although these tumors are considered to be of follicular origin, the identity and location (follicular versus interfollicular epidermis) of tumor progenitor cells have not been firmly established. Superficial BCCs frequently exhibit buds of proliferating basal cells growing directly from the epidermis into the underlying dermis, strongly resembling embryonic hair germs which are initiated by canonical Wnt/β-catenin signaling. We investigated the potential involvement of canonical Wnt signaling in epithelial bud development in early human BCCs and a mouse model of deregulated Hh signaling, driven by an activated Hh pathway effector, SMO*. Similar to embryonic hair germs, human BCC buds exhibited elevated levels of nuclear and cytoplasmic β-catenin, a marker of canonical Wnt signaling, and expressed early hair follicle lineage markers, including Sox9, K17, and CDP. Analogous changes were detected in BCC-like epithelial buds and follicular hamartomas arising in SMO* mice, along with expression of multiple *Wnt* genes, *Tcf4*, the Wnt target gene *Axin2*, and non-phosphorylated (active) β-catenin. Furthermore, by selectively blocking canonical Wnt signaling with Dkk1, we dramatically inhibited epithelial bud and hamartoma development in SMO* mice, despite continued, uncontrolled Hh signaling. Our findings reveal an essential function for Wnt signaling downstream of pathologic Hh signaling in skin, help elucidate how 'follicular' tumors can arise from interfollicular epidermis, and provide a molecular explanation for the morphological and biochemical similarity between BCC buds and hair germs.

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Hedgehog signaling and sebaceous gland maintenance

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We previously reported that ectopic Hedgehog (Hh) signaling leads to *de novo* sebaceous glands in hairless skin and sebaceous gland hyperplasia in hairy skin, whereas inhibition of Hh signaling leads to a deficiency of sebocytes, implicating the Hh pathway in early stages of sebaceous gland development. To begin exploring whether continued Hh signaling plays a role in sebaceous gland maintenance, we examined X-gal-stained whole-mount tail preparations, and dorsal and tail skin sections, from a panel of *lacZ*-based Hh pathway reporter mice. Since Gli2 is the major transcriptional effector of Hh signaling in mice, *Gli2-lacZ* mice were used to identify β-galactosidase-positive cells potentially capable of responding to Hh ligand. Since *Ptch1* and *Gli1* are reliable Hh target genes, corresponding *lacZ* reporter mice were used to identify cells in which the Hh pathway is active. Examination of skin from *Gli1-lacZ*, *Ptch1-lacZ*, and *Gli2-lacZ* mice failed to identify β-galactosidase-positive cells in either the differentiated sebocyte compartment or in sebocyte progenitors, whereas each of these reporter mice exhibited positive cells in hair follicle epithelium and/or mesenchyme. *In situ* hybridization failed to detect Hh target genes *Gli1* and *Ptch1*, or *Indian hedgehog (Ihh)*, in sebaceous glands. This result was unanticipated since previous studies have implicated secreted Ihh, produced by differentiated sebocytes, as a paracrine stimulator of sebocyte progenitor proliferation. To further test the role of Ihh in sebaceous gland biology, we examined skin from *Ihh*-null embryos and skin grafts on immuno-deficient mice. Whole-mount analysis of Oil Red O-stained skin revealed early-stage sebaceous glands in both *Ihh*-null and control littermates. Moreover, skin grafts from control and *Ihh*-null mice produced normal-appearing hair with associated sebaceous glands. These data strongly suggest that Ihh is not required for sebaceous gland development or homeostasis, and argue against a role for Hh signaling in sebaceous gland maintenance.

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c-myb knockdown results in a cyclic alopecia

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The protooncogene, c-myb, is a transcription factor that is required for adult hematopoiesis. The expression of the c-myb gene is essential for proliferation and prohibitive of differentiation. Viral transduction of a truncated c-myb gene results in leukemia in chickens, and murine c-myb null mutation is embryonically lethal, due to the specific failure of erythropoiesis. We have previously demonstrated that c-myb expression patterns in proliferating and differentiating keratinocytes are similar to hematopoietic cell expression patterns. c-myb is induced in human keratinocytes by the release of cells from a serum deprivation-induced block in proliferation and downregulation of c-myb expression is a late marker of calcium-induced keratinocyte differentiation *in vitro*. Furthermore, we showed that c-myb is expressed in the basal and suprabasal layers of the murine epidermis and that c-myb is expressed in the murine hair follicle in a hair cycle-dependent manner, a similar pattern to that of another transcriptional regulator of the hair cycle, hairless. Because both genes play a role in development and homeostasis, we examined whether the c-myb transcription factor can regulate the expression of the hairless gene. Ectopic expression of c-myb in NIH 3T3 cells results in a 5-fold induction of activity by a full-length hairless promoter-luciferase fusion gene. To examine whether this relationship exists *in vivo*, we examined the hair cycle of a c-myb knockdown mouse, which expresses approximately 10% of normal c-myb levels. Unexpectedly, c-myb knockdown mice shed their hairs in an anterior to posterior wave between days 15 and 18, and undergo a new round of hair growth coincident with the beginning of the next anagen at around day 40. This cyclic alopecia continues in subsequent hair cycles with hair shedding at the onset of catagen, demonstrating a role for c-myb in the regulation of the mammalian hair cycle.

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Anti-apoptotic effect of *Houttuynia cordata* on cyclophosphamide-induced apoptosis process of Human hair dermal papilla cells

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Houttuynia cordata (HC) has an antibacterial, anti-inflammatory, antiviral and antioxidant properties which is extensively used as the traditional medicine for the treatment of wounds, skin diseases in China, Korea, and Japan. A unique feature of hair growth is its cycle with anagen, catagen and telegen. The hair loss is the result of premature catagen caused by apoptosis signal. The regulation in apoptosis process is controlled by the members of bcl-2 proteins family and p53. In this study, we investigated cell proliferation and gene expression of Bcl-2, Bax and p53, MTT assay in the co-treatment of ethanol extract of HC and cyclophosphamide on HHDP cells. The cell growth increased 14%, 22% at the concentration of 1ug/ml, 10ug/ml respectively after 72 hour post treatment. The expression of Bcl-2 protein increased 43% at 1ug/ml and 12% at 10 ug/ml of HC extract with cyclophosphamide compared with cyclophosphamide alone but the expression of bax and p53 protein decreased dose dependently. Our result suggest that HC have anti apoptotic effect on cyclophosphamide induced hair apoptosis process, which prolong anagen cycle in the mouse hair growth, hence it may be a new therapeutic strategy for the prevention of chemotherapy-induced hair loss.

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Effects of herbal oriental medicine on hair growth promotion in human hair dermal papilla cells

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A unique feature of hair growth is its cyclicity with anagen, catagen and telogen. Hair loss is the result of premature catagen caused by apoptosis signal. The Bcl-2 is known for preventing from catagen induction. The H0039 (serial number coded) is as herbal medicine, has potential effects such as regulating the endocrine and immune system, and anti-oxidation. The histological change of hair follicle was investigated with using extracts of H0039. For the study, H0039 extract dissolved into 70% EtOH and the depilated back-skin with wax-rosin mixture of mice and treated trypsin and cyclophosphamide. Also, H0039 were tested for proliferation of human hair dermal papilla cell (HHDPC) and induction of Bcl-2 expression from keratinocyte. Proliferation of HHDPC by the extracts increased and Bcl-2 gene expression was induced in time and dose dependent manners. Hair growth of the groups treated with the H0039 extracts was observed more 20% growth in dose dependant manners. Also, the rate of follicle number of anagen phase/total was counted more 15%. This result explains that H0039 has effect on prolong of anagen phase. Hence, H0039 might be a potential new therapeutic source for the prevention from hair loss.

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Immunosuppressants improve the effectiveness of IVIg treatment in bullous pemphigoid

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Intravenous immunoglobulin (IVIg) appears to work by rapidly lowering serum levels of pathogenic autoantibodies. Unfortunately, a physiological regulatory feedback mechanism maintains constant the level of individual antibodies in serum and triggers new synthesis of any antibody whose level is lowered. The resulting rebound in level sometimes exceeds that previously present. This feedback mechanism limits the effectiveness of any treatment that reduces serum levels of antibodies. We propose that the addition of an immunosuppressive agent will improve the ability of IVIg to lower pathogenic antibodies. The following observations support this hypothesis. A 78-year-old pt with bullous pemphigoid (BP) was treated with IVIg on four occasions – alternatively with or without an immunosuppressive agent. BP antibodies were measured by indirect immunofluorescence at baseline prior to each IVIg cycle and repeated every 1-2 months. There was a 4-16 fold decrease in serum levels of IgG and IgG4 BP antibodies on the two occasions IVIg was combined with an immunosuppressant agent. By contrast, serum level of these antibodies remained stable or increased up to 4-fold on the two occasions IVIg was used without an immunosuppressive agent. The decrease in BP antibodies was not due to the immunosuppressant agent alone, as their level did not change when the pt was treated with high doses of steroids and an immunosuppressive agent without IVIg. These results show that agents that suppress antibody synthesis can offset the rebound in antibody level that follows their depletion with IVIg, and thus imply they can improve the effectiveness of IVIg treatment of BP and other autoantibodies mediated diseases.

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Macrophage migration inhibitory factor (MIF) is important for eosinophilia in allergen-induced skin inflammation

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Macrophage migration inhibitory factor (MIF) is a pluripotent cytokine that plays an essential role in the physiopathology of experimental allergic inflammation. Other studies indicate that MIF is essential for Th2 differentiation. Recent findings suggest that MIF is involved in several allergic disorders, including atopic dermatitis (AD). In the present study, the role of MIF in allergic skin inflammation was examined using a murine model of AD elicited by epicutaneous sensitization with ovalbumin (OVA). Dermatitis was induced in MIF Tg mice or WT littermates by epicutaneous application of OVA applied in a patch to tape-stripped skin for 3 weeks; each week was separated by a 2-week resting period. Skin biopsies from sensitized skin areas were used for RNA isolation, histology, and immunohistochemical examination. In addition, the effects of MIF on the eotaxin expression in fibroblasts were also investigated. We found that the number of skin-infiltrating eosinophils significantly increased in the OVA-sensitized MIF Tg mice in comparison to the WT littermates. The mRNA expression of eotaxin and IL-5 significantly increased in the OVA-sensitized skin sites of the MIF Tg mice in comparison to the WT littermates. Interestingly, IL-4 and MIF synergistically increased the expression of eotaxin in fibroblasts. These findings indicate that MIF can induce eosinophil accumulation in the skin, and therefore the targeted inhibition of MIF is thus considered to be a promising new strategy for the future therapeutic treatment of allergic skin diseases.

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Essential Roles of SHPS-1 in induction of contact hypersensitivity of skin

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SHPS-1 is a transmembrane protein that binds the protein tyrosine phosphatases SHP-1 and SHP-2 and is abundant on the surface of CD11c+ dendritic cells (DCs). We recently showed that SHPS-1 is essential for priming by DCs of CD4+ T cells and for development of Th17 cell–mediated experimental autoimmunity. We have now further evaluated the importance of SHPS-1 and that of its ligand CD47 in contact hypersensitivity (CHS) to 2,4-dinitro-1-fluorobenzene (DNFB). Whereas the DNFB-induced CHS response was impaired in mice that express a mutant form of SHPS-1 lacking most of the cytoplasmic region, it was unaffected in CD47-deficient mice. Moreover, treatment of wild-type mice with mAbs to SHPS-1 that either block or do not block the binding of SHPS-1 to CD47 inhibited the CHS response. A mAb to CD47 had no such effect. The 2,4-dinitro-benzenesulfonic acid–induced proliferation of, and production of IFN-γ or IL-17 by, T cells from DNFB-sensitized wild-type mice were inhibited by either mAb to SHPS-1 but not by that to CD47. In contrast, the blocking mAbs to SHPS-1, but not that to CD47, inhibited an allogeneic MLR. Both mAbs to SHPS-1, but not that to CD47, also inhibited the LPS- or poly(I:C)-induced production of TNF-α by DCs. These results suggest that SHPS-1 is essential for development of CHS, likely as a result of its positive regulation of the priming by DCs of CD4+ T cells. However, such regulation by SHPS-1 does not appear to require its interaction with CD47.

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Langerhans cells are biased towards TH2 functions by exposure to calcitonin gene-related peptide

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Calcitonin gene-related peptide (CGRP) inhibits Langerhans cell (LC) antigen presenting capability in several experimental systems. We have examined the ability of CGRP to regulate chemokine expression by LCs. By RT-PCR, the murine epidermis-derived dendritic cell line XS106 (from A/J), as well as freshly-obtained LCs (fLC) (purified from BALB/c epidermis by FACS using anti-I-A^d mAb), expressed mRNA for components of the functional CGRP receptor [receptor activity modifying protein (Ramp)1, Ramp2, Ramp3, calcitonin gene-related peptide-receptor component protein (CRCP) and calcitonin receptor-like receptor (CRLR)]. CXCL9 (chemokine induced by IFNγ) and CXCL10 (IFNγ-inducible protein 10) are ligands for CXCR3, preferentially expressed on Th1 cells; CCL22 (macrophage-derived chemokine) and CCL17 (thymus and activation-regulated chemokine) act on CCR4 and CCR8, preferentially expressed on Th2 cells. XS106 cells, as well as LC enriched to ~95% homogeneity (eLC, using anti-I-A^d mAb conjugated to magnetic beads) were treated for 1 h with varying concentrations of CGRP and then cultured with or without the addition of IFNγ (in the continued presence of CGRP). Supernatants were collected at 12, 24, 48 and 72 h and chemokine concentrations assessed by ELISA. Production of CCL17 and CCL22 was enhanced in XS106 cells and cultured eLC by treatment with CGRP, while production of CXCL9 and CXCL10 induced by IFNγ in XS106 cells and eLC was suppressed in a dose-dependent manner. By Northern blotting, treatment of XS106 cells with CGRP upregulated mRNA levels for CCL17 and CCL22 but inhibited upregulation of CXCL9 and CXCL10 mRNA induced by IFNγ. Furthermore, CGRP treatment of eLC inhibited the ability of these cells to present antigen to a TH1 clone (as previously reported) but enhanced their ability to present antigen to a TH2 clone. CGRP may regulate LC function, in part, by modulating the balance of Th1/Th2 functionality, thereby directing the character of an immune response.

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Direct development of primate connective tissue-type mast cells from immature state of CD34+ hemangioblastic progenitors, independently from the development pathway of mucosal-type mast cells

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Mast cells (MCs) have heterogeneity between the sites they located. Connective tissue-type MCs (CTMCs), predominantly located in normal skin, contain tryptase and chymase, whereas mucosal-type MCs (MMCs) contain only tryptase. However, the conditions that influence and the committed progenitors that fate the selective development of CTMCs are not well understood. By co-culturing for 10 to 15 days with AGM-S1 stromal cells established from murine aorta-gonad-mesonephros, GFP-labeled monkey embryonic stem (ES) cells differentiated into CD34+ cells with cobblestone-like morphology. Interestingly these GFP+/CD34+ cells highly maintained the character of immature hemangioblastic progenitors. These CD34+ cells also displayed robust growth when replated onto AGM-S1 cells in the presence of stem cell factor, IL-6 and Flt3-ligand, and within 3 weeks generated almost 100% tryptase+/chymase+ CTMCs. Although in ever established MC culture system chymase+ cells appeared only from tryptase+ MCs after long culture period, at any time points in our present culture system the percentage of tryptase+ cells unexpectedly did not exceed that of chymase+ cells. In addition, these ES-derived MCs contained abundant basophilic granules and expressed surface Kit and the high affinity IgE receptors and they activated via IgE and by substance P to releasing histamine, indicating the phenotype of functionally mature CTMCs. On the other hand, cord blood-derived CD34+ cells, which we used as the source of more matured hematopoietic progenitors, maintained in the same culture conditions required more than 3 months until they became chymase+ cells that still could not respond to substance P. Thus, the rapid maturation of CTMCs from ES-derived immature state of hemangioblastic progenitors suggests a unique embryonic pathway in primates for early development of CTMCs that may be independent from the development of MMCs.

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Single nucleotide polymorphisms of Ficolin 2 gene in psoriasis

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Genome-wide scans for psoriasis have implicated several loci of susceptibility. However, psoriasis is not a simple hereditary disease and it is exaggerated by exogenous stimuli. Ficolin-2, derived from FCN2 gene, is pattern recognition protein acting in innate immunity and, upon recognition of pathogens, it triggers the activation of the lectin complement pathway. The aim of the study is to elucidate the contribution of FCN2 gene in the pathogenesis of psoriasis. The frequencies of genotypes and alleles of FCN2 gene single nucleotide polymorphisms (SNPs) in the promoter regions (-986, -602, -557, -64, -4) and exon 8 (+6359, +6424) were examined in 34 patients with psoriasis, including psoriasis vulgaris, psoriasis arthropathica, and generalized pustular psoriasis, and 44 healthy controls by genotyping with a DNA sequencing method. There were no significant differences in genotype and allele frequencies of FCN2 gene SNPs between psorisis patients and healthy controls. No significant differences in genotype and allele frequencies of FCN2 gene SNPs were detected among different clinical subgroups in psoriasis patients. In conclusion, we show that these FCN2 mutant alleles are not associated with psoriasis.

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Peripheral CD14+ cells from keloid patients augment fibroblast release of MCP-1 that enhances AKT-dependent fibroblast proliferation: immunological aberrations in the development of keloids

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Keloids are fibrous overgrowth induced by cutaneous injury or wound. The difficulty in keloid treatment attributes partially to the poor understanding of keloid pathogenesis. The MCP-1/CCR2 axis hyperactivation has been reported to lead to fibrosis in liver cirrhosis, atherosclerosis and lung fibrosis. Previous report showed that circulating CD14+ monocytes are the precursors of circulating fibrocytes and contribute directly to fibrogenesis and collagen synthesis by a MCP-1/CCR2-dependent amplification loop. Increased monocyte lineages have been also demonstrated in the keloid tissues. We hypothesized the peripheral CD14+ monocytes from keloid patients induce fibroblast proliferation through the MCP-1 release and may result in aberrant fibrosis in keloids. By immunohistochemistry and RT-PCR, we demonstrated that MCP-1 expression was increased in the keloid tissue. Next, coculture of CD14+ cells from keloid patients and normal fibroblasts enhanced the fibroblast proliferation up to 50%, paralleled with the increase in extracellular MCP-1. Blocking with a MCP-1 neutralizing antibody completely abrogated the enhancement of fibroblast proliferations by keloid CD14+ cells. Exogenous MCP-1 at 100 ng/ml induced up to 40% of fibroblast proliferation accompanied with activated AKT. Addition of an AKT inhibitor, wortmannin, completely obliterated the MCP-1-induced proliferation. In conclusion, CD14+ cells from keloid patients enhanced fibroblast MCP-1 release and stimulated proliferation via AKT pathway. CD14+ cells contribute to the keloid pathogenesis by interaction with fibroblasts through MCP-1 upregulation. The immunological aberrations in the keloid patient contribute to the development of keloids.

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Langerhans cells serve as immunoregulatory cells by activating NKT cells

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Exposure to UV radiation alters the morphology and function of epidermal Langerhans cells (LCs), which are believed to play a role in UV-induced immune suppression. It is generally believed that UV exposure triggers the migration of immature LCs from the skin to the draining lymph nodes (DLN), where they induce tolerance. However, because most of the previous studies employed *in vitro* irradiation of LCs, the data generated may not adequately reflect what is happening *in vivo*. In this study we used a mAb recognizing the external domain of Langerin/CD207 to sort migrating LCs from the DLN of UV-irradiated mice and study their function. We found prolonged LCs survival in the lymph nodes of UV-irradiated mice. We noted that LCs that migrated to the DLN of UV-irradiated mice were CD80+, CD86+ and CD1d+. Dermal dendritic cells (CD11+, $CD8alpha^{low}$ Langerin-) from UV-irradiated mice presented antigen to T cells, whereas LCs (CD11+, $CD8alpha^{low}$ Langerin+) did not. Moreover, co-mixing LCs with dermal dendritic cells did not depress the APC function of the dermal dendritic cell. We found that LCs co-localized with lymph node Natural Killer T (NKT) cells in UV-irradiated mice. NKT cells isolated from the lymph nodes of UV-irradiated mice secreted significantly more IL-4 than NKT cells isolated from non-irradiated controls. Transferring LCs from UV-irradiated mice into wild type recipients transferred immunosuppression, but no suppression was observed when LCs were transferred into NKT-deficient mice. Our findings indicate that LCs induce immune regulation *in vivo* by activating NKT cells.

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CRTH2-dependent, STAT6-independent induction of cedar pollen dermatitis

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Airborne contact dermatitis to cedar pollen is a disease that generally affects individuals with cedar pollinosis; it causes nasal and/or ocular symptoms, as well as atopic dermatitis in some patients. In this study, we established a mouse model of cedar pollen dermatitis by epicutaneous sensitization with Japanese cedar pollen antigen and attempted to elucidate the pathological mechanisms of the disease. Histologically, there was marked dermal cellular infiltration, including eosinophils and mast cells, with epidermal thickening. The induction of dermatitis was accompanied by production of cedar pollen-specific IgE. In the lesional skin, IL-13, IL-18, eotaxin/CCL11, RANTES/CCL5, MDC/CCL22, and TARC/CCL17, but not IL-4 and IFN-γ, were produced. Mast cell-deficient WBB6F1-W/Wv mice failed to develop cedar pollen dermatitis. Surprisingly, however, the induction of dermatitis was independent of STAT6/IgE. In contrast, mice deficient in CRTH2, a receptor for prostaglandin D2, had decreased inflammation. These data suggest that prostaglandin D2-CRTH2 signaling contributes to inflammation in cedar pollen dermatitis, and unlike in cedar pollinosis of the nasal mucosa, STAT6 is not a therapeutic target for treatment.

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Construction of novel *in vitro* epithelioid cell granuloma model from mouse macrophage cell line

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There have been several attempts to make granuloma model to clarify the mechanism of granulomatous diseases like sarcoidosis. However, a unique *in vitro* model that generates multinucleated giant cell (MGC) through epithelioid cells resembled to human granuloma, has not yet been clearly established. In this study, the generation of granuloma model that forms MGC via epithelioid cells from the mouse macrophage cell line was investigated. A RAW 264.7 mouse macrophage cell line was cultured with lipopolysaccharide (LPS) and concanavalin A (Con A) in various concentrations either alone or both. We found that separate treatment of LPS and Con A induced around 35% and 20 % MGC respectively whereas cotreatment of these chemicals drastically accelerated granuloma formation rate and it was around 80%. The highest fusion index (MGC formation rate) was observed at days 7. A gradual increase of tumor necrosis factor alpha (TNF-α) production in the culture supernatant was analyzed by enzyme-linked immunosorbent assay (ELISA). And the neutralization of the elevated level of TNF-α production by its monoclonal antibody leads to significant decrease of MGC formation. Interestingly, we found that the RAW cells were changed into spindle cells, which morphologically resembled to epithelioid cells and eventually MGC was formed from these spindle cells. Our *in vitro* granuloma model appeared to be similar with *in vivo* epithelioid cell granulomas like sarcoidosis. Thus, our model would be useful as *in vitro* epithelioid granuloma model for analyzing the mechanisms and screening the effective drugs of granulomatous diseases in future.

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Increased release of IL-8 and GM-CSF from keratinocytes stimulated with mite protease-allergens Der p 1 and Der f 1, and its inhibition by cystatin A

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Der p 1 and Der f 1 are the most immuno-dominant allergens from house dust mites, and their cysteine protease activities are supposed to be involved in the pathogenesis of allergy. However, stimulation of keratinocytes by these protease-allergens, as well as inhibitory molecules against these proteases in the skin have not been well investigated. We have identified cystatin A as the dominant skin-derived inhibitor against the proteolytic activities of the recombinant Der p 1 and Der f 1. We have also analyzed its inhibitory effect on activation of keratinocyte by Der p 1 and Der f 1. Human cultured keratinocytes and HaCaT cells (the human keratinocyte cell line) were stimulated by recombinant Der p 1 and Der f 1 in the absence or presence of cystatin A. IL-8 and GM-CSF secreted into culture supernatants were measured by ELISA. The mite protease-allergens upregulated the release of IL-8 and GM-CSF from keratinocytes and HaCaT cells, and this effect could be inhibited by the addition of cystatin A. Der p 1 and Der f 1 did not digest cystatin A. Proteolytic activity of Der f 1 was partially increased after incubation with keratinocytes even when the proteases were not pre-activated by L-cysteine. These results suggested possible contributions of mite major protease-allergens (Der p 1 and Der f 1) to stimulating the skin of atopic dermatitis, and possible inhibition by cystatin A against these proteases as likely "biochemical skin-barrier".

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Deficiency of PGD2 receptors, DP and CRTH2, enhances contact hypersensitivity

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Prostaglandin D2 (PGD2) is one of the arachidonic acid metabolites secreted by activated mast cells; it has been implicated in allergic diseases. The biological activities of PGD2 are mediated by PGD2 receptors (DP and CRTH2). However, the functional roles of DP and CRTH2, as well as the differences between them, in acute contact hypersensitivity reactions have not been elucidated. In the present study, these issues were addressed using mice deficient in DP and/or CRTH2. Compared to wild-type mice, CRTH2-deficient mice showed weaker ear swelling responses in contact hypersensitivity to TNCB. Conversely, DP-deficient mice exhibited an enhanced skin response. Unexpectedly, deficiency in the genes of both receptors resulted in significant enhancement of the ear swelling response. On histology, the ear swelling responses were characterized by increased numbers of mononuclear cells, eosinophils, and neutrophils. The IL-17 and IFN-γ levels in the lesional skin were higher in DP(-/-)/CRTH2(-/-) mice than in wild-type mice. Flow cytometric analysis of immune lymph node cells from DP(-/-)/CRTH2(-/-) mice revealed increased numbers of CD4(+)/IL-17 (+) T cells, γδ(+)/IL-17 (+) T cells, and CD8(+)/IFN-γ(+) T cells compared to wild-type mice. Deficiency of DP and CRTH2 receptors promoted Langerhans cell/dendritic cell migration to regional lymph nodes. There was no difference in the number of CD4(+)/Foxp3(+) regulatory T cells between these two mouse strains. These data suggest that simultaneous deficiency of these two PGD2 receptors results in enhanced dendritic cell migration, and in the induction of IL-17- and/or IFN-γ-producing cells, which mediate enhanced contact hypersensitivity.

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The stable form of galectin-9, a ligand for Tim-3, as a novel therapeutic tool for Th1- and/or Th17-mediated skin inflammation

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Tim-3 is a cell surface molecule preferentially expressed on Th1 and Th17 cells. It has been suggested that Tim-3 negatively regulates cell function. Recently, it was found that galectin-9 is a ligand for Tim-3 and that the binding of galectin-9 to Tim-3 induces cell apoptosis. Galectin-9 is a tandem-repeat type galectin that consists of two carbohydrate domains and a linker peptide. However, the natural form of galectin-9 is easily inactivated by proteases due to the labile nature of the linker peptide. We recently developed a stable form of galectin-9 (sGal-9) by partial deletion of the linker peptide. The aim of the present study was to determine the therapeutic effects of sGal-9 on inflammatory reactions in murine contact hypersensitivity and in IL-23-induced psoriatic mouse models. In contact hypersensitivity to DNFB of BALB/c mice, the ear swelling response was significantly suppressed by systemic administration of sGal-9. On histology, the cellular infiltrate consisting of mononuclear cells, eosinophils, and mast cells was reduced. Furthermore, sGal-9-treated mice had lower skin lesion levels of IFN-γ, IL-17, RANTES, and IP-10 compared to untreated mice. In the sGal-9-treated mice, these findings were associated with decreases in CD4(+)/IFN-γ(+), CD4 (+)/IL-17 (+), CD8(+)/IFN-γ(+), and CD8(+)/IL-17 (+) cells in the regional lymph nodes. Similarly, sGal-9 reduced cellular infiltrate levels in IL-23-induced skin inflammation (a mouse model of psoriatic inflammation), which was accompanied by decreased pSTAT3 expression by epidermal keratinocytes. Thus, sGal-9 could be a unique and useful therapeutic tool for the treatment of Th1- and/or Th17-mediated skin inflammation.

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Effective anti-tumor immunity induced by immunization with total tumor RNA

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We have previously shown that intradermal (id) administration of total cellular RNA from the S1509a spindle cell tumor (from A/J, H-2^a) immunizes CAF$_1$ (H-2$^{a/d}$) mice against growth of live S1509a tumor cells. To further characterize immunity induced in this manner, we immunized CAF$_1$ mice by id administration of 10 μg of S1509a total cellular RNA at each of 2 sites; in vitro synthesized, capped, translation-competent total RNA from the S1509a tumor or PBS. Primings were repeated twice at 7 day intervals and 1 week after the final priming mice were sacrificed and splenocytes (SC) examined for production of γIFN in an ELISPOT assay. Spots from each group were assessed after stimulation by S1509a cells or by stimulation with the irrelevant murine melanoma line B6F10. SC from mice primed with either total S1509a RNA or in vitro synthesized S1509a RNA yielded a robust response when stimulated with S1509a tumor cells but not when stimulated with B16F10 cells. SC from mice primed with PBS alone failed to yield a significant response with or without stimulation with tumor cells. An additional control group immunized with a soluble extract of the S1509a tumor yielded a high non-specific response (i.e., without stimulation) while stimulation with S1509a but not B16F10 cells yielded a response significantly higher than the non-specific background response. SC from each group were also restimulated in vitro for 5 days with mitomycin-C treated S1509a cells and examined for ability to lyse S1509a targets in a 5-hour cytotoxicity assay at an E:T ratio of 40:1. Mice immunized with total S1509a RNA, in vitro synthesized S1509a total RNA or the soluble extract of S1509a cells each yielded a higher degree of cytotoxicity than mice immunized with PBS alone. These preliminary results confirm that a naked RNA vaccine comprised of total cellular RNA from a malignant tumor can yield significant anti-tumor immunity via intradermal priming. Optimization of this technique may lead to new strategies for treatment of cancer.

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Stimulation of TSLP secretion from human primary cultured keratinocytes by Poly I:C and Th2/TNF cytokines

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Recent findings have revealed that thymic stromal lymphopoietin (TSLP) is highly expressed by epidermal keratinocytes in skin lesions of patients with atopic dermatitis (AD). However, the mechanism of TSLP induction in AD is still obscure. In this study, we have tested the activation of TSLP secretion from human primary cultured keratinocytes (PKCs) by various toll-like receptor (TLR) ligands in the presence or absence of IL-4, IL-13 and TNF-α (Th2/TNF). TSLP protein was detectable in culture media only when PKCs were stimulated by a TLR-3 ligand (Poly I:C) which mimics viral dsRNA. Upregulation of TSLP mRNA expression by Poly I: C was observed by real-time PCR. TSLP protein was secreted in dose- and time-dependent manners by Poly I:C, and this was synergistically enhanced by co-addition of Th2/TNF cytokines. Co-stimulation with type-I IFNs (IFN-α and IFN-β) enhanced the Poly I:C induced TSLP release. On the other hand, co-stimulation with IFN-γ or TGF-β inhibited the TSLP release by Poly I:C. Our findings suggested the mechanisms underlying elevated TSLP expression in AD skin, and implicated the possible contribution of viral infection to induce TSLP activation.

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CD4$^+$CD25$^+$ regulatory T cells suppressing anti-Dsg3 antibody production in pemphigus vulgaris develop in the absence of Dsg3

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The CD4$^+$CD25$^+$ regulatory T cells (Tregs) play suppressive roles in various types of autoimmunity. It has been reported that Tregs develop in the thymus after high-affinity interaction of their TCR with self-peptide/MHC ligands. However, most studies were based on TCR-transgenic system and it is unknown whether the specific antigen is required for the development of Tregs. In this study, we explored this issue by focusing on polyclonal Treg populations in pemphigus vulgaris (PV), an autoimmune blistering disease caused by anti-desmoglein 3 (Dsg3) IgG antibodies (Abs), as a model system. We generated an active mouse model for PV by the adoptive transfer of splenocytes from Dsg3$^{-/-}$ mice into Rag2$^{-/-}$ immunodeficient mice expressing Dsg3. We have shown that Tregs control anti-Dsg3 Ab production in PV model mice: adoptively transferred Tregs suppressed the Ab production and the depletion of endogenous Tregs augmented the Ab production. To examine whether the endogenous expression of Dsg3 is necessary for the generation of these PV-relevant Tregs, we compared the potential of wild-type Tregs with that of Tregs from Dsg3$^{-/-}$ mice. When the same numbers of Tregs from wild-type or Dsg3$^{-/-}$ mice were transferred into the PV model mice, the titers of anti-Dsg3 Abs were similarly suppressed in both groups. Next, we depleted endogenous Tregs from wild-type and Dsg3$^{-/-}$ splenocytes before transfer. The admixture of equal numbers of naïve splenocytes from wild-type and Dsg3$^{-/-}$ mice with or without endogenous Tregs, were transferred into Rag2$^{-/-}$ mice. To our surprise, the titers of anti-Dsg3 Abs were significantly higher when Dsg3$^{-/-}$ Tregs were depleted in comparison with the depletion of wild-type Tregs, suggesting that Tregs in Dsg3$^{-/-}$ mice are more potent than wild-type Tregs. Our observation implies that antigen specific Tregs can develop in the absence of the target antigen.

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Critical roles of FcεRI-mediated mast cell activation for development of the skin contact hypersensitivity

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Previous studies suggested that mast cell derived-TNF-α plays important roles in the development of contact hypersensitivity induced by hapten. Recently, we revealed that FcεRI β-chain controls production of proinflammatory cytokines including TNF-α in mast cells through function of three tyrosine residues (Y219/Y229/Y225) of its ITAM. In the present study, we investigated the biological functions of mast cells expressing wild-type or mutated β-chain ITAM in development of the contact hypersensitivity employing mast cell "knock-in" mice. We prepared mast cells harboring wild-type (YYY) or FcεRI β-chain ITAM mutant (FFF) by employing a retrovirus-mediated gene transfer into BMMCs derived from FcεRI β-chain -/- mice. Those mast cells were intradermally transferred into ears of mast cell deficient mice (W/Wv). The mice were pre-sensitized by application of 2% oxazolone to the shaved abdomen. On day 5, 1% oxazolone and vehicle were applied to right and left ears, respectively. Severity of the contact hypersensitivity evaluted by ear thickness. Cytokine expression in the tissue was analyzed by Real-Time PCR. As a result,oxazolone failed to cause ear swelling in W/Wv and FcεRI β-chain -/- mice. In addition, we showed that ear swelling is significantly reduced in the FFF-knock-in mice as compared with that of YYY- knock-in mice. Consistent with these results, infiltration of CD3+ T cells and eosinophils into the inflammation sites was severely reduced in FFF-knock-in mice. Furthermore, an experiment using Real-Time PCR demonstrated that expression of TNF-α mRNA is also decreased in the ear skin of FFF-knock-in mice. Taken together, these results suggest that regulation of mast cell activation through FcεRI β-chain is critical for development of contact hypersensitivity reaction following cutaneous exposure of hapten.

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Time dependant upregulation of serotonin transporter protein-immunoreactive cells in human allergic contact eczema: a kinetic study

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The aim of this study was to investigate the expression of serotonin transporter protein (SERT) in nickel-allergic patients (n= 9), using biopsies from epicutaneous patch test reactions to 5 % nickel sulphate, obtained at different time points (0, 6, 24, 48, and 72 h) after application of the antigen. The skin biopsies were fixed in a mixture of 10% formalin with 0.2% picric acid for 2 h, and rinsed in 0.1 M phosphate buffer containing 10% sucrose for at least 24 h, before being cut into 14 µm thick sections and stained using immunohistochemistry and a streptavidin-biotin technique. SERT expression was observed on dermal mononuclear cells that were seen to infiltrate the epidermis, in the biopsies obtained at the later time points (48 and 72 h), and also being visible in vesicles in the highly inflamed skin biopsies. The total (dermis plus epidermis) number of SERT positive cells tended to increase (median, 1.20 (cells/section), quartile difference, 1.65) at 6 h compared to 0.25 cells/section, quartile difference, 0.75 at 0 h. At 24 h a dramatic elevation ($p<0.01$) in the number of SERT immunoreactive cells was observed (median 13.75, quartile differnece 32.13) compared to both 0 h and 6 h (indicating a turning point) in the inflammatory sequel. Furthermore, the number of SERT positive cells then gradually increased ($p<0.001$) at 48 h (median 33, quartile difference 38,27) and reached the maximum at 72 h (43.5 cells/section, quartile difference 57.5). Moreover, SERT expression on the apical keratinocytes was stronger than the expression on the basal keratinocytes and generally this immunoreactivity gradually increased with time. Our findings suggest an involvement of SERT during the onset of the allergic contact eczematous reaction and a possibility to target this protein for therapeutics at an early time point.

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The basement membrane in skin contains pores which act as a conduit for the passage of immune cells out of the epidermis

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The basement membrane (BM) acts as a selective barrier in the skin, allowing the passage of immune cells between the epidermis and dermis. The exact mechanisms whereby Langerhans cells (LCs) and other immunocytes traverse the BM remain to be defined. The BM of other epithelial surfaces (lung, eye and gut), contains pores which allow the movement of immune cells through this structure. The purpose of the current study was to ascertain if the BM in skin has similar pores and to investigate whether LCs use these pores as conduits for migration out of the epidermis. Following biopsy, the epidermis was removed from human skin by incubation with EDTA and the dermis was stained with an anti-collagen IV monoclonal antibody to confirm a confluent intact BM over the dermis (n= 13 volunteers). Confocal microscopy demonstrated the presence of channels within the BM and scanning electron microscopy (SEM) showed pores (mean diameter 0.8 µm) within it. Similar pores were identified in murine skin (n=6), with a mean diameter of 0.59µm. In several volunteer skin samples (n=7), cells were identified within occasional pores which had a wider mean diameter of 2.41µm. To examine the role of these pores in LC migration, skin was exposed in vivo to stimuli which are known to induce LC migration (dithranol, dinitrochlorobenzene, UVB). Staining of human epidermal sheets with CD1a demonstrated a reduction in LCs and SEM of the dermal specimens showed an increase in mean pore diameter to 3.39µm (1.21-6.93µm), with multiple pores containing cells within them. A similar effect was observed following UV irradiation of murine skin in vivo. The results suggest that LCs migrate through pre-defined pores in the cutaneous BM and that the pore size increases as a result of LC passage via these structures.

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Mast cell migration from the skin to the draining lymph nodes upon UV-irradiation represents a key step in the induction of immune suppression

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The UV radiation in sunlight is the primary cause of skin cancer. UV is also immunosuppressive and previous studies demonstrated that dermal mast cells play a critical role in the process. Using mast cell deficient mice bearing GFP+ skin grafts, we found that GFP+ mast cells preferentially migrated to the B cell areas of the draining node following UV exposure. Mast cell express CXCR4+ and UV exposure up regulated the expression of its ligand CXCL12, by lymph node B cells. Treating UV-irradiated mice with a CXCR4 antagonist, blocked mast cell migration and abrogated UV-induced immunosuppression. Our findings indicate that UV-induced mast cell migration to draining lymph nodes, mediated by CXCR4 interacting with CXCL12, represents a key step in UV-induced immunosuppression.

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Modulation of inflammatory dendritic cell functions by the toll-like receptor (TLR) signaling network

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Dendritic cells (DCs) are the critical link between innate and adaptive immunity. Recent murine studies have identified a novel population of DCs that are important in clearance of bacterial infections. These cells produce tumor necrosis factor-alpha (TNF-α) and nitric oxide, and were therefore named tumor necrosis factor and iNOS producing dendritic cells (TIP-DCs). They are a non-resident population of myeloid ($CD11c^+$) inflammatory DCs, which may be pathogenic in psoriasis and autoimmunity. To better understand factors that regulate the *in vivo* activation and differentiation of human TIP-DCs (as well as the more general category of inflammatory DCs), we have performed genome-wide expression studies on distinct populations of DCs FACS-sorted from psoriasis lesions. Inflammatory DCs expressed an array of surface receptors and signaling molecules linked to TLR pathways. To test the function of individual TLRs and other surface receptors on human cells, we evaluated the effects of adding various TLR agonists to pre-DCs and immature DCs *in vitro*. Stimulating agents included HKLM and PAM3CSK (TLR1/2 agonists), LL37 (TLR9 agonist) and imiquimod (TLR7 agonist), as well as several sets of cytokines. We were able to derive conditions that altered the phenotypes and functions of DCs, including TNF and iNOS production (measured by FACS) and cytokine/chemokine production (measured by expression arrays or multiplex ELISA). Our studies thus identify specific pathways that affect differentiation and activation of inflammatory DC populations that appear in psoriasis and other inflammatory conditions, while also elucidating specific molecular pathways that can mediate the effector responses of these cells. These results have important implications for modulating DC functions in a range of human skin diseases and conditions.

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Low dose application suppresses contact hypersensitivity by abrogating endothelial ICAM-1 up-regulation at the elicitation site

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Painting subsensitizing doses of contact sensitizers induces hapten-specific tolerance (low zone tolerance, LZT), which has been experimentally demonstrated by inhibition of contact hypersensitivity (CHS). To elucidate the LZT effects on the efferent (elicitation) phase, LZT was investigated in mice lacking either L-selectin or ICAM-1, since the elicitation phase of CHS is regulated by these cell adhesion molecules. Mice were sensitized by application of the immunogen onto pre-painted subsensitizing doses of contact sensitizers of back skin and ears were challenged 5 days later. Low dose application resulted in CHS suppression in wild type mice and L-selectin-deficient mice but not in ICAM-1-deficient (ICAM-1-/-) mice. ICAM-1 deficiency did not affect the sensitization phase of CHS, since ICAM-1-/- mice were sensitized to hapten and had normal generation of hapten-specific suppressor and effector cells. This LZT was associated with a lack of up-regulation of endothelial ICAM-1 expression after Ag challenge at the elicitation sites. A blockade of IL-10, a possible mediator of LZT produced by hapten-specific suppressor cells, abrogated LZT and restored ICAM-1 up-regulation at the elicitation sites. These results indicate that low dose hapten application inhibits CHS by abrogating up-regulation of endothelial ICAM-1 expression after Ag challenge at the sites of elicitation.

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Monovalent hapten bound to nanostructured surfaces as a model for multivalent antigens

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Receptor cross linking is a prerequisite for FcεRI dependent mast cell degranulation. Monovalent haptens, representing one epitope, cannot elicit cross-linking unless they are bound to larger carrier proteins. Hence, monovalent haptens may be a suitable model to investigate spatial epitope requirements for FcεRI cross linking. We used nanolithography to create artificial full antigens by coupling haptens to glass plates in different distances on a nanoscale basis. Nanolithography allows depositing gold dots on glass providing anchor points to which haptens can bind mediated by polyethylene glycol (PEG). Glass surfaces were created with either monovalent hapten dinitrophenol (DNP) bound to gold dots with a distance of exactly 70nm between each anchor point or with DNP bound to a homogeneous gold layer without nanostructure. In addition, long chain PEG3000 (>70 monomers) and a short chain PEG6 (6 monomers) linkers were used to investigate the impact of epitope motility on the efficiency of cross linking. Mast cells were passively sensitised by anti-DNP IgE before incubation on differently coated glass surfaces or with soluble DNP bound to human serum albumin (HSA) as control. Mast cell degranulation was detected by measuring β-hexosaminidase release. Release of β-hexosaminidase in response to DNP bound to a homogeneous gold layer by PEG3000 (27±8%) and using the short PEG6 as linker (38±4%) was well detectable and comparable to soluble multivalent DNP-HSA (42±11%). Controls such as unsensitised cells on PEG3000 or PEG6 with or without DNP as well as sensitised cells on PEG3000 or PEG6 without DNP showed only background release (8-11%). The same results were obtained using nanostructured DNP with an epitope distance of 70nm coupled to PEG6. Our data demonstrate that monovalent allergens can mediate effective cross linking of FcεRI on mast cells with an epitope distance of up to 70nm. These and further analyses will allow to design also natural multivalent allergens that avoid harmful adverse events during systemic immunotherapy.

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Antihistamines are immunosuppressive, increase susceptibility to infections and impairs allergen-specific immunotherapy in mice

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Antihistamines are widely used against allergies, pruritus, nausea, cough, and as sleeping aids. Their immunological properties have been rarely studied, but some studies suggest a negative effect on immunity. This study demonstrated that mice treated with clemastine and dimetindene succumbed to bacterial and viral infections. This was associated with a disruption in morphology and function of the lymphoid organs, which allowed unrestricted growth of bacteria in monocytes, granulocytes and lymphocytes. The immune suppression was partly endorsed by impaired production of TNF-alpha and IL-6 by macrophages and, subsequently, IFN-gamma production and proliferation of lymphocytes. By the same token, human macrophages were immunologically impaired upon treatment with clemastine in vitro and in vivo. Medication with antihistamines also impaired allergen-specific immunotherapy in mice. The results demonstrated that sensitisation against bee venom was strongly enhanced during treatment with antihistamines. Clemastine increased IgE production while decreasing IgG2a production against bee venom. This Th2 shift of the humoral response appeared to be caused by reduced IFN-gamma and enhanced IL-4 secretion from allergen-specific T cells. We also found reduced TNF-alpha, IL-6 and MHC class-II expression by macrophages. In sensitised mice, the efficiency of allergen specific immunotherapy was reduced by clemastine treatment. Hence, antihistamine treatment may have strong immunosuppressive effects and may enhance allergic sensitisation and reduce the efficiency of allergen-specific immunotherapy. Future studies will need to demonstrate to what extent pre-medication with antihistamine also affects allergen-specific immunotherapy in humans.

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The role of versican in the biology of Sézary cells

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Sézary syndrome (SéS) is an aggressive variant of cutaneous T cell lymphoma defined by erythroderma, lymphadenopathy and the presence of neoplastic T lymphocytes, so called Sézary cells (SéCs) in the skin, lymph nodes and peripheral blood. Gene expression profiling has been employed on several occasions to expand the understanding of the pathogenesis of SéS by using peripheral blood mononuclear cells or bulk CD4+ T cells. To gain more insight in the biology of SéCs, we previously performed microarray analysis on CD4+ clonal T cells additionally sorted according to patient-specific TCR Vβ expression (Vβ+). The expression profile of clonal CD4+ T cells (Vβ+) from 5 patients were compared to those of non-clonal CD4+ T cells obtained from the same patients (Vβ-). Using HG-U95Av2 Affymetrix arrays, we identified versican as the highest up-regulated gene in clonal CD4+Vβ+ T cells. Versican is a large chondroitin-sulphate proteoglycan and has at least 4 isoforms (V0-V3). Because each isoform has its own expression pattern and function (e.g. lymphocyte migration, control of apoptosis), the expression of these isoforms was assayed by qPCR using isoform-specific primers. Further investigation of expression pattern revealed preferential expression of one of the V1 isoform in the clonal CD4+Vβ+ T cell population as compared to the other isoforms. Western blotting was in line with PCR data showing increased V0/V1 protein expression 5/7 clonal CD4+Vβ+ cell preparations. Currently, we are testing the effects on versican overexpression, introduced through an lentiviral vector, in lymphoma cells lines (SeAx, MyLa and Hut78) on cell proliferation, migration, cell cycle and apoptosis.

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Differentiation of dendritic epidermal T-cell subsets with distinct cytokine profiles

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Depending on the cytokine environments during antigenic stimulation or activation, naive CD4+ and CD8+ αβ T cells as well as NK cells differentiate into distinct effector (helper) or regulatory subsets characterized by their cytokine profiles and functions. The best-characterized subsets are CD4+ Th1, Th2, Th17, and iTreg cells, whose differentiation is regulated by the transcription factors, T-bet, GATA-3, RORγt, and Foxp3, respectively. In this study, we determined whether intraepithelial γδ T cells of the mouse skin, dendritic epidermal T cells (DETCs), can also differentiate into effector subsets producing distinct patterns of cytokines. Epidermal cells from C57BL/6 mice were stimulated with immobilized anti-TCRCδ mAb, and DETCs were differentiated for 14 days under neutral (IL-2 alone), Th1- (IL-2 + IL-12 + anti-IL-4), Th2- (IL-2 + IL-4 + anti-IL-12 + anti-IFN-γ), and Th17- (TGF-β + IL-6 + IL-23 + anti-IL-4 + anti-IFN-γ + anti-IL-27) priming conditions. Intracellular cytokine staining after re-stimulation revealed that Th1-, Th2-, and Th17-priming conditions led to the augmented generation of IFN-γ-, IL-13-, and IL-17-producing cells, respectively. Irrespective of the priming conditions, however, differentiated DETCs as a whole secreted both IFN-γ and IL-13. DETCs differentiated in the Th2-priming condition contained IFN-γ+/IL-13-, IFN-γ+/IL-13+, and IFN-γ-/IL-13+ subsets, whereas IFN-γ and IL-17 were produced by distinct populations in the DETCs from the Th17-promoting culture. Th17-promoting culture also generated a Foxp3+ DETC subset. Expression of T-bet, GATA-3, and RORγt mRNA was correlated with differentiation of IFN-γ-, IL-13-, and IL-17-producing cells, respectively. Like conventional CD4+ T-cell subsets, DETC subsets with distinct cytokine profiles may display different functional properties in vivo.

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Effects of toll-like receptor 2 ligand lipoteichoic acid on the development of atopic dermatitis

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Atopic dermatitis (AD) is a chronic inflammatory skin disease induced by infiltrating Th cells. While Th2 cells dominate the acute flares of AD as seen in atopy patch tests, chronic AD lesions are indistinguishable from other forms of eczema and are dominated by Th1 cells. However, the mechanism underlying this change of inflammatory pattern in AD remained elusive. Colonization with Staphylococcus aureus is seen in >90% of AD patients and innate immune signals will influence inflammation in AD. However, the influence of innate immune signals on the change of the inflammatory pattern in AD has not been investigated so far. To this end, we established a model for the initial phase of AD by adoptively transferring and activating OVA-specific Th2 cells in the skin of naive mice. The increase of ear thickness after adoptive transfer correlated with antigen-specific inflammation. While injection of Th2 or OVA alone only lead to minor alterations the transfer of Th2 plus OVA provoked an inflammatory reaction and resulted in a strong ear swelling after 24h. One important pathogen associated molecular pattern (PAMP) of Staphylococcus aureus is the cell wall component and TLR2 ligand lipoteichoic acid (LTA). Interestingly, the presence of LTA during the AD-like inflammation resulted in prolonged and increased dermatitis compared to Th2 plus OVA alone, an inflammation pattern identical to OVA-specific dermatitis observed after transfer of Th1 cells. Investigations using TLR2-deficient mice as recipients showed complete abrogation of this effect demonstrating that LTA binding of TLR2 is operative in this change of inflammation pattern. These data indicate that S. aureus derived LTA shifts transient cutaneous inflammation mediated by Th2 cells towards a chronic and persistent form of dermatitis implicating an important role of LTA in the process of AD development and chronification.

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Transcutaneous anti-influenza vaccination induces cellular immune responses in healthy volunteers– results of a pilot and a phase I clinical study

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The induction of potent T cell responses has become one of the major goals in vaccination against viral diseases, such as HIV or hepatitis C, and cancers. Recent implication of epithelial dendritic cells (DC) in CD8 cell cross-priming suggest, that transcutaneous vaccination (TC) may also be relevant in the induction of cellular immune responses. For this purpose, we developed a standard operating procedure (SOP) for TC vaccine delivery based on cyanoacrylate skin surface stripping. In a pilot study on 12 healthy volunteers and a subsequent Phase I study on 24 human volunteers vaccinated with an influenza vaccine, we found that this newly developed SOP is safe and efficient at inducing a significant increase in cellular immune responses as assessed by IFN-gamma ELISPOT assays, subtyping of CD4 and CD8 subtyping, specification of IFN-gamma and IL2 production, pentamer staining and multiparametric analysis. Frequencies and absolute numbers of IFNγ-producing CD4 cells were increased in both TC and IM vaccinated individuals. Vaccine-induced antigen-specific T cells were producing both IFNgamma and IL-2, confirming a Th1 profile. In the pilot study, IFNγ-producing CD8 cells, however, could only be detected in individuals receiving TC vaccination. The Phase I clinical trial confirmed a significantly higher induction influenza-specific CD8 cell in TC vs. IM vaccinated individuals. The results of these two clinical studies suggest, that application of vaccine preparations via the TC route might be critical for the quality of cellular immune responses.

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Glucocorticoids enhance toll-like receptor 2 expression in human keratinocytes stimulated with *Propionibacterium acnes* or proinflammatory cytokines: possible pathogenetic roles in corticosteroid-induced acne and rosacea.

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Toll-like receptors (TLRs) on keratinocytes are important cell surface receptors involved in the innate and acquired immune response to invading microorganisms. In acne vulgaris, TLR2 activation by *Propionibacterium acnes* (*P. acnes*) may induce skin inflammation via induction of various proinflammatory molecules such as cytokines, chemokines, and cell adhesion molecules. Although corticosteroids themselves exert immuno-suppressive or anti-inflammatory effects, it is well known clinically that systemic or topical glucocorticoid treatment provokes an acneiform reaction. Nevertheless, the effect of steroids on TLR2 expression in human keratinocytes remains unknown. Here, we analyzed TLR2 gene expression in cultured human keratinocytes by quantitative PCR method and found that addition of glucocorticoids such as dexamethasone and cortisol increased their TLR2 gene expression. Moreover, these glucocorticoids markedly enhanced TLR2 gene expression, which was further stimulated by *P. acnes*, TNF-α and IL-1α. Gene expression of MAPK phosphatase-1 was also increased by the addition of dexamethasone. By using several inhibitors and activators, we found that TLR2 gene induction by glucocorticoids was mediated by the suppression of p38 MAPK activity following induction of MAPK phosphatase-1. These findings strongly suggest that steroid-induced TLR2 together with *P. acnes* existing as normal resident flora plays an important role in the induction of corticosteroid-induced acne.

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Increased epidermal RANKL of grafted skin induces IL-10-producing Langerhans cells and regulatory T cells tolerogenic for contact hypersensitiviry

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RANK and its ligand (RANKL; also known as CD254 and TRANCE) are key regulators of T cell/dendritic cell communication. It has recently been reported that RANKL is expressed on keratinocytes of the inflamed skin and alters the function of epidermal dendritic cells, thereby inducing CD4+CD25+ regulatory T cells (Treg). We have shown that the development of murine contact hypersensitivity (CHS) is depressed when mice are sensitized with a hapten via the full-thickness grafted skin. Here we elucidated the mechanism underlying the hyposensitization of CHS. RANKL was expressed on keratinocytes of the grafted skin, and Langerhans cells (LCs) isolated from the grafted skin produced a high amount of IL-10. Adoptive transfer of immune lymph node cells from the tolerated mice suppressed CHS response of recipients in an antigen-specific manner. CD4+CD25+ but not CD4+CD25- T cells purified from the lymph node cells were responsible for this suppression. Finally, recombinant RANKL stimulated epidermal cell suspension(including LCs) to produce IL-10. These findings suggest that the hyposensitization of CHS via the grafted skin is mediated by RANKL, and regulatory T cells.

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Autoantibody against a protease domain of Caspase-8 in patients with systemic sclerosis

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Systemic sclerosis (SSc) is characterized by autoantibodies against various cellular components. To determine the presence or levels of antibodies (Abs) against a protease domain (PD) of caspase-8 and their clinical relevance in SSc. Anti-caspase-8 PD Ab was examined by enzyme-linked immunosorbent assay and immunoblotting using human recombinant caspase-8 PD. Caspase-8 activity was evaluated by spectrophotometric detection of cleavage from p-nitroanilide-labeled IETD, a substrate of caspase-8. IgG anti-caspase-8 PD Ab levels in patients with SSc, systemic lupus erythematosus, or dermatomyositis were higher than in normal controls (CTL). Furthermore, anti-caspase-8 PD Ab levels in limited cutaneous cutaneous SSc (lSSc) patients were elevated compared to diffuse cutameous SSc (dSSc) patients. To investigate the clinical correlation, laboratory findings were compared between SSc patients with high levels (> the mean + 2SD of CTL) of anti-caspase-8 PD Ab and those with low levels. SSc patients with high levels exhibited lower frequency of male and pulmonary hypertension and decreased C-reactive protein levels relative to those with low levels. Immunoblotting showed that anti-caspase-8 PD Ab was present in all SSc patients examined, while it was also detected in 75% of CTL. Caspase-8 activity was inhibited by IgG isolated from sera of SSc patients and CTL, although inhibitory effect was greater in SSc patients than CTL. These results suggest that immune response to caspase-8 occurs in healthy individuals, although it is greater in patients with systemic autoimmune diseases including SSc. Furthermore, high levels of anti-caspase-8 PD Ab may be a serological indicator for a milder SSc subset.

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Effects of tacrolimus, betamethason-17-valerate and fexofenadine on novel allergic dermatitis model in NCN24 (hairless NC/Nga) mice

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In order to characterize novel allergic dermatitis model in NCN24 (hairless NC/Nga) mice, the effects of tacrolimus, betamethason-17-valerate and fexofenadine, which are used for the treatment of atopic dermatitis (AD) were evaluated. 2, 4-dinitrofluorobenzene (DNFB) was repeatedly applied onto the rostral part of the back after initial sensitization by application with DNFB onto the abdomen of NCN24 mice. Dermatitis scores, skin histopathology, trans-epidermal water loss (TEWL), scratching behavior and total and dinitrophenyl (DNP)-specific IgE levels in serum were evaluated. Tacrolimus (0.1w/v%, topical application), betamethason-17-valerate (0.12w/v%, topical application) and fexofenadine (30mg/kg, p.o.) were administered once a day for 5 days/week from the first application of DNFB. Repeated DNFB challenge gradually exacerbated AD like dermatitis. Tacrolimus and betamethason-17-valerate suppressed the exacerbation of dermatitis significantly and inhibited the infiltration of inflammatory cells in the skin lesion. Moreover, both drugs significantly suppressed the elevation of TEWL. As to scratching behavior, tacrolimus significantly inhibited the increase of scratching number, whereas betamethason-17-valerate failed to have any effect. Tacrolimus inhibited the elevation of total and DNP-specific IgE levels, but betamethason-17-valerate inhibited only DNP-specific IgE levels. On the other hand, fexofenadine failed to have any effect on all parameters. These results suggest that our dermatitis model reflects the clinical therapeutic properties of these drugs. Therefore, our dermatitis model is beneficial not only for elucidating the pathogenesis of AD but also for evaluating the effects of therapeutic agents for AD.

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Establishment of novel inducible allergic dermatitis in NCN24 (hairless NC/Nga) mice as a model for atopic dermatitis

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NCN24 mice are hairless transgenic mice into the genetic background of NC/Nga, which has been established as a model for human atopic dermatitis (AD). The aim of this study was to establish novel inducible allergic dermatitis model using NCN24 mice and investigate the usefulness of this model, compared with wild-type NC/Nga mice. Mice were sensitized by an application with 2, 4-dinitrofluorobenzene (DNFB) onto the abdomen. One week after sensitization, mice were repeatedly challenged with DNFB (twice a week, 9 times in total) onto the rostral part of the back. The severity of dermatitis was evaluated macroscopically. Trans-epidermal water loss (TEWL) and scratching behavior were measured as secondary evaluation. On the day after the ninth challenge of DNFB, serum and skin specimens were obtained to evaluate total and dinitrophenyl (DNP)-specific IgE levels and histopathology. Repeated application of DNFB induced exacerbation of dermatitis symptoms (mainly hemorrhage, scarring, scaling, and dryness), elevation of TEWL, frequent scratching behavior and elevation of serum total and DNP-specific IgE levels in both NCN24 and NC/Nga mice. Histopathological analyses proved that potent inflammatory changes, such as thickening of epidermis and accumulation of inflammatory cells in the dermis, have occurred. Compared with NC/Nga mice, marked scarring and scaling were observed in NCN24 mice. These results demonstrated that this model using NCN24 mice exhibits similar feature of human AD. Due to hairless feature, NCN24 mice are advantageous to evaluate dermatitis symptoms and skin functions compared with NC/Nga mice. In conclusion, novel allergic dermatitis model using NCN 24 mice may be useful for evaluating the effects of therapeutic agents on AD.

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ProstaglandinE2-EP3 signaling suppress skin inflammation by inhibiting keratinocyte activation in mouse contact allergy

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Prostaglandin (PG)E2 is one of the lipid mediators that play various physiological roles, and exerts its effect through four G-protein coupled receptors called EP1, EP2, EP3 and EP4. PGE2 has both pro-inflammatory and anti-inflammatory effect depending on the situation. We have recently revealed that PGE2-EP3 signaling has anti-inflammatory effect in mouse allergic asthma model (Nature. Immunology, 6:524-531,2005). To investigate whether PGE2-EP3 signaling has anti-inflammatory effect in skin allergic inflammation, we examined the therapeutic effect of EP3 selective agonist, AE248, on mouse contact hypersensitivity (CHS) model, a mouse model of allergic contact dermatitis. Administration of AE248 on the elicitation phase of CHS significantly suppressed ear swelling compared with that of vehicle treated mice. Skin histopathology of AE248 treated mice showed reduced inflammatory cell infiltration compared with vehicle treated mice. Microarray analysis of hapten challenged skin revealed that administration of AE248 inhibited the chemokine gene expression of CXCL1. EP3 receptors are supposed to be expressed in keratinocytes by X-gal staining in EP3 deficient mice, and AE248 inhibited the CXCL1 production from cultured keratinocyte. EP3 receptor deficient mice showed significantly increased ear swelling compared with wild type mice in CHS of repeated hapten application.These results suggest that activation of EP3 signaling play anti-inflammatory role in mouse CHS model by inhibiting keratinocyte activation, and that endogenous PGE2 signaling also plays suppressive roles in the development of CHS.

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Id3 controls the developmental window of γδ T cells

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T cell progenitors permit active rearrangement at the TCRδ, TCRγ, and TCRβ loci prior to bifurcation of γδ and αβ T cell lineages. In postnatal thymus, majority of these bi-potential progenitor cells develop into the αβ T cell lineage whereas only a small fraction chooses to adopt the γδ fate. Expression of a fully rearranged and functional TCRβ gene defines the checkpoint for αβ lineage commitment. A substantial fraction of β rearranging cells will not develop further due to out of frame rearrangements. We show here that loss of Id3, a transcription regulator, promotes γδ T cell development from progenitor T cells which have completed TCRβ rearrangement but without producing a functional TCRβ allele. Our data suggests that Id3 may play an important role in shutting off the window of γδ development after the completion of V-DJβ rearrangement.

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The timing of dysfunction of regulatory T cells determines the pattern and outcome of T cell-mediated severe drug eruptions

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Toxic epidermal necrolysis (TEN)/Stevens-Johnson syndrome (SJS) and drug-induced hypersensitivity syndrome (DIHS) represent two ends of a spectrum of severe drug eruptions: DIHS is unique in that severe epidermal damage seen in TEN/SJS is absent; sequential reactivations of herpesviruses occur during the disease course; and autoimmunity ensues as long-term sequelae of disease. In this regard, we reasoned that a fundamental abnormality in Treg cells would contribute to this complex sequence of events. To test this, we sought to compare the frequency and function of Treg cells both during the acute stage and again during the resolution stage. Dramatic expansions of fully functional $Foxp3^+$ Treg cells were specifically found at the acute stage of DIHS, thereby allowing herpesviruses to reactivate in an uncontrolled fashion. In contrast, although Treg cells were present in normal frequency at the acute stage of TEN/SJS, their functional capacity was profoundly impaired. Skin-homing addressins, CCR4 and E-selectin ligand, were more preferentially expressed on Treg cells expanded in DIHS than those in TEN/SJS, indicating that the expanded Treg cells have the potent skin-homing capacity. Indeed, they were more abundantly present in the skin lesions of DIHS than in those of TEN/SJS. Expansions of Treg cells were experimentally induced by stimulating $CD4^+$ T cells from patients with DIHS, but not those with TEN/SJS, with the causative drug in vitro in an antigen-specific manner. Surprisingly, Treg cells contracted upon resolution of DIHS became functionally deficient, preceding clinical progression to autoimmune disease. These results indicate that their functional defects at the acute stage of TEN/SJS and at the resolution stage of DIHS might have detrimental effects on severe epidermal damage and progression to autoimmune disease, respectively.

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UV-induced regulatory T cells stimulate antigen presenting cells to further induce regulatory T cells

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UV-induced regulatory T cells (UV-Treg) inhibit sensitization in an antigen-specific fashion. There seems to be a tight cross-talk between UV-Treg and antigen-presenting cells (APC) since the migratory behavior of UV-Treg can be reprogrammed by tissue-specific APC in an antigen-specific manner. In turn, UV-Treg inhibit APC to present antigens. Subcutaenous injection of antigen-loaded APC, which have been coincubated with UV-Treg activated in an antigen-specific fashion, did not result in sensitization of the recipient mice. This inhibition appears to be antigen-independent since APC coupled with TNBS, the water soluble analogue of trinitrochlorobencene (TNCB), were not able to sensitize in vivo against TNCB upon coincubation with activated dinitrofluorobencene (DNFB)-specific UV-Treg. Upon antigen-specific activation, UV-Treg released high amounts of interleukin (IL)-10 which might be responsible for the inhibition of APC. Accordingly, addition of a neutralizing anti-IL-10 antibody prevented the inhibition of APC by UV-Treg. To determine whether APC pretreated with UV-Treg in turn are able to induce Treg, APC were coincubated with activated DNFB-specific UV-Treg. APC were isolated 48 hours later, coupled with TNBS and injected into naïve recipients. 5 days after injection splenocytes were obtained from the recipients and injected into naïve mice which were sensitized against TNCB 24 hours later. 5 days later ear challenge with TNCB was performed. Mice which had received T cells from donors treated with Treg-exposed APC were significantly suppressed in their contact hypersensitivity response against TNCB. This indicates that activated UV-Treg can alter APC in such a way that they further induce Treg when injected into naïve recipients.

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Human dendritic cells function as a productive source of PGD2 in the skin

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PGD2 is one of the arachidonic acid metabolites; it exerts a wide range of biological activities, including vasodilatation, bronchoconstriction, and inhibition of platelet aggregation. PGD2 has been also implicated in allergic diseases. A major source of PGD2 in the skin has been considered to be mast cells that express hematopoietic PGD synthase (H-PGDS). In this study, we analyzed the expression of H-PGDS in human dendritic cells (DC) and sought to identify the regulatory mechanisms of PGD2 production in DC. Immunohistochemical study revealed that H-PGDS was detected in some epidermal Langerhans cells, CD1a(+)/CD207(-) dermal DC, BDCA-2(+)/CD123(+) plasmacytoid DC (pDC), and BDCA-1(+) myeloid DC (mDC). Both pDC and mDC isolated from peripheral blood expressed H-PGDS protein and transcript as demonstrated by flow cytometric analysis and RT-PCR, respectively. DC generated from CD14(+) monocytes (MoDC) cultured with GM-CSF/IL-4 secreted PGD2 when stimulated with the calcium ionophore A23187. While LPS induced minimal PGD2 release, pre-incubation with IFN-γ followed by LPS stimulation resulted in marked enhancement of PGD2 production. A chemical hapten, TNBS, and an irritant, SDS, were also capable of inducing PGD2 release. In contrast, neither PMA nor TSLP stimulated MoDC to secrete PGD2, despite the increased H-PGDS and Cox-2 mRNA levels. Skin DC may be an important source of skin PGD2 and may mediate skin inflammation by releasing PGD2 in response to a variety of stimuli.

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Sezary tumor cells express NKG2D-ligands mediating NK cell recognition

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Sezary Syndrome (SS) is a rare lymphoma characterized by the clonal expansion in the skin and in blood of CD4+CD158k+ T-cells. NK activation against tumor in leukemia models is partly based on the recognition of the target through the NKG2D/NKG2D-ligands interactions. We analyzed ex vivo SS malignant lymphocytes for the expression of the NKG2D-ligands as the MHC class I-related molecules (MIC) A and B and the UL16-binding proteins (ULBP). The expressions of NKG2D, the natural cytotoxicity receptors (NKp30, NKp44 and NKp46) and the activating receptor DNAM-1 were simultaneously investigated on circulating patients' NK and CD8+ non malignant lymphocytes. Interestingly, while at least one of the NKG2D ligands was expressed on the circulating malignant lymphocytes of 9 out of 10 patients, NKG2D was expressed on effector lymphocytes with broad variations. We found that soluble MICA in patients' sera was increased, which may constitute a mechanism to escape the immune response. In vitro, freshly isolated SS tumor lymphocytes induced the degranulation of NKL, a NK cell line specifically expressing NKG2D as activating receptor. Altogether, these data show that SS malignant cells are sensible to lymphocyte mediated cytotoxicity and could be targeted for potential immunotherapy clinical trials.

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The decrease of cell-surface thiols in dendritic cells treated by chemicals augments their CD86 expression, a possible role in DC maturation by haptens

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Although several previous studies suggested a crucial role of p38 mitogen-activated protein kinases (MAPK) in the activation of monocyte-derived dendritic cells (MoDCs) by haptens, the upstream signals of p38 MAPK remain undetermined. We have demonstrated that an imbalanced ratio of the oxidized (GSSG) vs. the reduced (GSH) form(GSH/GSSG) of cellular glutathione plays a crucial role in triggering DC maturation by haptens, depending on the activation of p38 MAPK. Recently it has been reported that the oxidation of exofacial cell membrane thiol groups reduces the intracellular GSH content and phosphorylates p38 MAPK. In this study, we first examined whether impermeable thiol-reactive compound, o-phenanthroline copper complex (CuPhen; surface thiol oxidizer) and mersaryl acid(MA; surface thiol blocker) decrease the cell-surface thiols, phosphorylate p38 MAPK and induce MoDC maturation. When MoDCs were treated with CuPhen or MA for 15 min to 2 hr, their cell-surface thiols were significantly decreased at non-toxic concentrations by flow cytometrical analysis using an nonpermeable thiol-reactive compound, Alexa fluor 488 C5 maleimide (AFM). In parallel with the decreased cell-surface thiols, these reagents phosphorylated p38 MAPK, which was demonstrated in Western blotting, and augmented CD86 in MoDCs by flow cytometric analysis. A similar response pattern was observed in a human monocytic cell line, THP-1. Treatment with the antioxidant, N-acetyl-L-cysteine, totally abrogated the decrease in cell-surface thiols and the augmentation of CD86 expression by these reagents. Interestingly, representative sensitizers, DNCB, NiCl2, and DPCP also reduced the cell-surface thiols with augmented phosphorylation of p38 MAPK and CD86 expression. These data suggest that the decrease of the cell-surface thiol groups plays a role in DC maturation by haptens.

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TNF-α mediates adjuvant effect of lipopolysaccharide on contact hypersensitivity in mice

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Tumor necrosis factor-α (TNF) is a novel factor to facilitate dendritic cell (DC) migration. We previously reported that TNF mediates the local effect of ultraviolet (UV) to impair the induction of contact hypersensitivity (CH). This phenomenon is not observed in TLR4-deficient C3H/HeJ (HeJ), implying TLR4-induced TNF mediates impaired CH by UV. However, cutaneous injections of lipopolysaccharide (LPS) can enhance CH induction. TLR4 is a receptor for LPS, which potently stimulates DCs to undergo migration and maturation. We previously showed that the effect of LPS is dependent of TLR4 but not MyD88. We wanted to know the mechanism in which TNF controls the magnitude of CH. We first determined whether the effect of UV on CH induction was dependent of the TLR4-MyD88 pathway. The TLR4 or MyD88 gene knockout (KO) mice received 4 successive daily doses of 400 J/m2 of UV. Immediately after the last exposure, 185 μg DNFB was applied at the exposed site. Ear swelling response was measured 24 h after the challenge. TLR4-KO and MyD88-KO mice displayed intense and feeble CH, respectively. These data indicate that the deleterious effect of UV on CH is dependent of TLR4 but not MyD88. We previously reported that LPS and UV could alter the density and morphology of epidermal I-A+ cells and anti-TNF antibodies restored these effects on the skin. The others and we reported that LPS and UV could stimulate DCs to migrate to the LNs. We thus determined the effect of TNF on CH induction. TNF (200 ng) itself impairs CH induction in UV-susceptible (S), but not UV-resistant (R) mice. Surprisingly, UV-R (BALB/c) mice receiving simultaneous injections of LPS and TNF displayed much intense CH than PBS-treated mice. These data indicate that TNF can facilitate DC migration, leading to either enhanced or impaired CH. Since UV-R is TNF-dependent, we checked LPS effect in various strains of UV-S and UV-R mice. Expectedly, only UV-R strains of mice ("d" of H-2S) were LPS-resistant, indicating that the susceptible gene of both LPS and UV may be the TNF gene, although the effect upon CH is opposed.

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Induction of eosinophil-infiltrating drug photoallergy in mice

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Drug photoallergy is one of the highly incident adverse effects. Several different histological patterns have been recognized in this sensitivity. Here we established a murine model of the eosinophil-infiltrating type of drug photoallergy by using afloqualone (AQ), a representative photosensitive drug. AKR/J mice were sensitized by an intraperitoneal injection of AQ solution (2 mg/kg/mouse) and irradiation of shaved abdomen with UVA (12 J/cm^2). This sensitization procedure was repeated 2-12 times (twice a week), and 3 days after the last immunization, mice were challenged by a subcutaneous injection of AQ solution and irradiation of the same site with UVA (12 J/cm^2). Mice receiving more than 10 times of sensitization exhibited a massive infiltrate of eosinophils and lymphocytes at the challenged site. This reaction was AQ photoantigen-specific, and AKR/J mice were a high responder strain of this sensitivity. The sensitivity was successfully transferred with 5-8x10^7 immune lymph node and spleen cells into naïve mice. CD4+ T cells were responsible for this sensitivity, since 1x10^7 CD4+ cells alone induced a high level of sensitivity reaction and the addition of other lymphocyte populations did not enhance the reaction. Culture supernatants from immune lymph node cells contained a higher level of IL-4 and a lower level of interferon-g than those from naïve mice. Finally, the skin of AQ-photochallenged site exhibited high expression of CCL24/eotaxin-2, a chemokine for eosinophils. It is suggested that eosinophilic drug photoallergy is mediated by sensitized Th2 cells and locally produced eosinophil-attracting chemokines. This model is useful for exploring the mechanism of eosinophil-infiltrating photosensitivity.

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IL-10-producing B cells regulate contact dermatitis in mice

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B cells have many functions that influence immune and inflammatory responses. Using B cell depleting antibodies and B cell hyper-reactive or hypo-reactive mice, a phenotypically unique spleen B cell subset was found to inhibit T cell-dependent contact hypersensitivity (CHS) responses through IL-10 secretion. These rare IL-10-producing B cells represented 1-2% of spleen B220+ cells in wild type mice, were absent in B cell-hypo-reactive mice, yet were expanded to ~10% of B220+ cells in B cell-hyper-reactive mice. Consequently, oxazolone-induced CHS responses were reduced in mice with increased numbers of IL-10 secreting B cells. By contrast, CHS responses were exacerbated in mice with decreased numbers of IL-10 producing B cell and in wild type mice depleted of CD20+ B cells, but were normalized by the adoptive transfer of spleen IL-10 producing B cells. Thereby, we describe a novel cellular mechanism of contact dermatitis regulation that involves a specific regulatory B cell subset. It is likely that these cells are also operable in other inflammatory diseases.

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Single desmoglein 3-reactive CD4$^+$ T cell clones activate polyclonal naïve B cells and promote the production of pathogenic IgG in experimental pemphigus vulgaris

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Pemphigus vulgaris (PV) is an autoimmune bullous disease caused by anti-desmoglein 3 (Dsg3) IgG. T-B collaboration is expected to be critical for anti-Dsg3 IgG production in PV pathogenesis. We recently demonstrated pathogenic activity of Dsg3-reactive T cell clones to induce PV phenotype including oral erosions *in vivo* by their adoptive transfers with primed B cells isolated from rDsg3-immunized Dsg3$^{-/-}$ mice into Rag2$^{-/-}$ mice. In this study, we studied a potency of Dsg3-reactive T cell clones to non-primed Dsg3$^{-/-}$ B cells isolated from non-immunized Dsg3$^{-/-}$ mice and the correlation of T cell and B cell epitopes. When two T cell clones reactive to a middle extracellular domain (210 to 345 aa) of Dsg3 were co-transferred with non-primed Dsg3$^{-/-}$ B cells to Rag2$^{-/-}$ mice, the recipient mice produced anti-Dsg3 IgG and developed the PV phenotype. The epitopes of circulating anti-Dsg3 IgG were characterized by competition ELISA with four domain-swapped Dsg1/Dsg3 molecules. Anti-Dsg3 IgG reacted with the entire extracellular domains, but mainly the middle to C-terminal regions (195 to 565 aa, n = 4). In addition, when primed Dsg3$^{-/-}$ B cells were co-transferred with these T cell clones (n = 4) or another T cell clone reactive to N-terminal region (1 to 119) of Dsg3 (n = 2), the recipient mice produced anti-Dsg3 IgG antibodies recognizing different parts of Dsg3 with a similar tendency to the mice receiving non-primed B cells. Those results indicate that a single T cell clone has an ability to exert helper activity on polyclonal Dsg3-reactive B cells *in vivo* and induce pathogenic anti-Dsg3 IgG production in PV mouse model. This finding provides an important framework to elucidate immunological mechanisms in antibody-mediated autoimmune diseases.

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1α,25-dihydroxyvitamin D3, but not retinoic acid promotes apoptosis and suppresses the expression of cutaneous lymphocyte-associated antigen on human peripheral blood monocyte-derived langerhans-like cells.

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Cutaneous lymphocyte-associated antigen (CLA) is a surface glycoprotein expressed on human T cells, monocytes, granulocytes, and Langerhans cells, and has a critical role for the recruitment into skin. Langerhans cells are intraepidermal dendritic cells expressing CLA, E-cadherin, CD1a, and Langerin. Recently, we reported that 1α,25-dihydroxyvitamin D3 (VD) and retinoic acid (RA) suppress the expression levels of CLA on human cultured T-lymphocyte, and also inhibit skin infiltration of CD4+ T cell in vivo. However, the effects of VD or RA for CLA expression on Langerhans cell is obscure so far. In this study, we investigated the effects of VD and RA for CLA expression on human peripheral blood monocytes derived Langerhans-like cells (mo-LCs). To establish mo-LCs, monocytes were cultured with GM-CSF, IL-4, and TGF-β for 6days. Mo-LCs were then supplemented with VD or RA for additional 3days. The apoptosis and surface expression of CLA were analyzed by FACS. We also analyzed effects of intraperitoneally administerd VD or VA in delayed type hypersensitivity (DTH) mice model. As a result, VD treatment promoted apoptosis on mo-LCs and inhibited cell survival in a dose dependent manner. However, RA did not. CLA expressed on mo-LCs was partially suppressed after VD treatment. Additionally, VD decreased mo-LCs counts in the epidermis of a DTH model mouse. These results implicate that VD but not RA is a potent suppressor for CLA expression on mo-LCs and is an inducer for apoptosis.

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Essential roles of Runx/AML transcription factors for FoxP3$^+$ regulatory T-cell function

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FoxP3$^+$ regulatory T cells (Tregs) control various immune responses including autoimmunity and tumor immunity, and their therapeutic potential is now attracting notice. The transcription factor FoxP3 is specifically expressed in Tregs and regulates the differentiation and function of Tregs. However, little is known about how FoxP3 regulates Treg function at molecular levels. We previously reported that FoxP3 controlled Treg function by interacting with the transcription factor Runx1/AML1 and that knockdown of RUNX1 in human Tregs resulted in the loss of suppressive activity of Tregs *in vitro*. In addition, we showed that FoxP3 could interact with the other members of Runx proteins as well. These findings suggest essential roles of Runx proteins in Treg function. However, roles of Runx proteins for the differentiation and function of Tregs *in vivo* remain to be elucidated. To address this issue, we generated and analyzed mice with Treg-specific conditional deletion of CBFβ (cofactor for all the Runx proteins, stabilizing Runx-mediated transcription). We generated *FoxP3-Ires-Cre (FIC)* knockin mouse, in which the expression of Cre was synchronized with that of FoxP3. By crossing FIC mouse with CBFβ-floxed mouse, we obtained Treg-specific CBFβ conditional knockout (CBFβ CKO) mouse. CBFβ CKO mouse developed autoimmune diseases with autoantibody production and IgE hyperproduction. Interestingly, FoxP3$^+$ Tregs from CBFβ CKO mice produced effector cytokines such as IFN-γ and IL-4, which could be associated with dysfunction of CBFβ-deficient Tregs. In conclusion, Runx-mediated gene regulation is essential for the Treg function *in vivo*.

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Control of regulatory T cell function by AML1/CBFβ-dependent transcriptional programs

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Regulatory T cells (Treg) suppress activation of pathogenic T cells and thereby prevent the development of autoimmunity and allergy. The transcription factor FoxP3 regulates the phenotype and suppressive function of Treg, the molecular mechanisms of which are largely unknown. Besides, genetic studies showed that anomalies of AML1(Runx1)-mediated transcriptional regulations were associated with the susceptibility to SLE, rheumatoid arthritis and psoriasis by unknown mechanisms. Recently we reported that AML1 interacts with FoxP3 and regulates Treg function and that AML1 is required for human Treg function, which raised a possibility that AML1 regulated autoimmunity via controlling Treg. However, it is still almost unknown which genes are regulated by AML1 in Treg, and hence it is unclear how the interaction of AML1 and FoxP3 regulates Treg function and controls autoimmunity. In this meeting, we report that Treg-specific inhibition of AML1-mediated transcription by deletion of either AML1 or CBFβ (the beta subunit of AML heterodimers) results in dysregulation of Treg function and leads to the development of autoimmune diseases. Here we show the molecular mechanisms of Treg function by characterizing AML1/CBFβ-dependent transcriptional programs. Through microarray analysis of the transcriptome of CBFβ-deleted Treg and chromatin immunoprecipitation assay combined with microarray analysis (ChIP-on-chip) using natural Treg, we identify the AML1/CBFβ-mediated, key regulatory mechanisms of Treg. These results demonstrate the roles of AML1/CBFβ in regulating Treg function, and give clues to understand the molecular mechanisms of the pathogenesis of various skin diseases including autoimmunity, allergy and psoriasis.

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Chemokine receptor CCR3 may enhance mast cell homing and survival: indication for mastocytosis in neurofibroma

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Neurofibromatosis 1 is the most common neurocutaneous disorder featured by multiple neurofibromas. Characterized by cellular heterogeneity, neurofibroma is composed of several different types of cells including Schwann cells (SCs), mast cells (MCs), fibroblasts, endothelial cells, and perineural cells. While SCs have been established as the major progenitor cells for neurofibroma formation, our knowledge about how MCs become one of the constituent cells of the tumor and whether they may also contribute to neurofibroma tumor formation and progression remains limited. Recent reports have shown that chemokine receptor CCR3 plays multiple essential roles in MCs, including trafficking of MC progenitors and selective modification of MC phenotype and effector function. We therefore hypothesized that SCs may attract MCs by CCR3-mediated mechanisms. In expressional studies, CCR3 expression by mouse bone marrow-derived MCs (BMMCs) has been confirmed by quantitative PCR and flow cytometry; on the other hand, chemokine CCL7, one of the CCR3 ligands, has been shown to be expressed by mouse SC line SW10. In chemotaxis assay, BMMCs demonstrated specific responses to chemokine CCL7 and conditioned medium from SW10 cells, indicating the possible role for CCR3 to mediate migration of MCs toward neurofibroma. Furthermore, in cell survival assay, while exogenous CCL7 could not enhance BMMCs' survival, it could be significantly attenuated by CCL7-neuralizing antibody, and expressional study further demonstrated comparable level of CCL7 expression in MCs (0.4-fold versus SW10), indicating that while exogenous (paracrine) CCL7 may be important for MC chemotaxis and migration toward neurofibroma, MC survival in the tumor may depend on autocrine CCL7 secretion. Moreover, the expression of pathogenic gene NF1 in SW10 is lower in comparison to mouse fibroblast, indicating possible correlation may exist between NF1 gene deficiency (pathogenic for neurofibroma) and CCR3-mediated MC migration.

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The stromal change of syringoma on eyelids

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Syringoma are common benign cutaneous neoplasms demonstrating sweat gland differentiation, and usually found around the upper dermis as multiple cords or small ducts lined by epitheliums. Stromal fibrosis of syringoma is found uniquely unlike other appendageal tumors. The aim of this study is to investigate the stromal change of syringoma on eyelids. We reviewed the disease duration of 26 cases of syringoma and performed H & E stain, Masson trichrome stain, Victoria blue stain, and immunoperoxidase staining using monoclonal antibody to tryptase protein for these cases. Correlation analyses were performed to find relationships between disease duration and stromal fibrosis in H & E stain, and those among stromal fibrosis in H & E stain, elastic fiber loss in Victoria blue stain, and tryptase-positive mast cell density. Statistical analysis shows no correlation between the grade of stromal fibrosis and the disease duration in syringoma (p=0.317). However, the grades of stromal fibrosis and elastic fiber loss, the grade of stromal fibrosis and tryptase-positive mast cell density, and the grade of elastic fiber loss and tryptase-positive mast cell density were all positively correlated with statistical significance (p=0.001). In conclusion, it is suggested that stromal fibrosis of syringoma is related to elastic fiber loss and mast cell infiltration.

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Naringin augments LPS-induced activation of murine bone marrow-derived dendritic cells

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Naringin (NG) is one of the most abundant flavonoids in grapefruit and citrus fruits. NG has been shown to exhibit antimicrobial, antimutagenic, anticarcinogenic, anti-inflammatory, and antioxidant effects. However, the effects of NG on dendritic cells (DCs), which play central role on T cell immunity, remain still unknown. In this study, we attempted an experiment whether NG can influence surface molecule expression, IL-12 production, the capacity to induce T-cell proliferation, and the signaling pathways underlying these phenomena in the murine bone marrow-derived DCs (mBM-DCs). As a result of this study, NG moderately augmented the expression of CD80, CD86, and MHC class II in the mBM-DCs stimulated by lipopolysaccharide (LPS). NG also augmented LPS-induced IL-12 expression in the mBM-DCs. NG amplified the activation of p38 mitogen-activated protein kinase (MAPK) in the LPS-stimulated mBM-DCs. These results provide new insight into the immunopharmacological functions of NG, especially with regard to their impact on the DCs. NG may be used in the development of therapeutic adjuvants for cancers and immunodeficient diseases through the up-regulation of immunostimulatory function of DCs.

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Cutaneous IL-15 induced strong protective MHC class I-restricted antitumoral immunity in mice

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IL-15 is important for antitumoral immunity. To study the mechanisms of antitumoral immune responses induced by cutaneous IL-15 production, wildtype (wt) and K14-IL-15 transgenic (tg) mice were subcutaneously injected with TS/A mammary carcinoma cells. K14-IL-15 tg mice showed a significantly reduced tumor growth with markedly increased numbers of CD8+ effector T cells and NK cells infiltrating the TS/A tumors. Subsequently, NK cells and CD8+ effector T cells from tumor bearing tg mice were adoptively transfered into naive wt recipients that were then challenged with TS/A cells. Inoculated TS/A cells were rejected in recipients treated with CD8+ T cells from tumor-bearing K14-IL-15 tg mice suggesting strong MHC class I-restricted antitumoral immunity. Furthermore, CD8 depletion studies in tumor-bearing K14-IL-15 tg mice supported the important role of IL-15 induced antitumoral CD8+ effector T cell functions. In tumor draining lymph nodes from K14-IL-15 tg mice increased numbers of IFN-gamma+granzymeB+CD8+ T cells were detectable indicating that reduced tumor growth was associated with increased IL-15 induced cytotoxicity of CD8+ T cells. Since Qa-1/NKG2 signaling is involved in the downregulation of CTL responses the number of Qa-1+ cells in tumor draining lymph nodes was analyzed. K14-IL-15 tg mice showed significantly decreased numbers of Qa-1+ cells pointing to a role of reduced Qa-1/NKG2 signaling, which results in increased CTL function. To test this, Qa-1+ cells were isolated from tumor draining lymph nodes of wt and K14-IL-15 tg mice and adoptively transfered into naive wt recipients prior to challenge with TS/A cells. Interestingly, the transfer of Qa-1+ cells from tumor-bearing K14-IL-15 tg mice but not from wt mice resulted in the rejection of inoculated TS/A cells in recipients. Together, these results indicate that cutaneous IL-15 overexpression leads to strong protective antitumoral immunity via stimulation of MHC class I-restricted cytotoxicity. The increased cytotoxicity of tumor-specific CD8+ T cells in K14-IL-15 tg might be mediated by reduced Qa-1/NKG2 signaling.

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Alpha-Melanocyte stimulating hormone induces tolerogenic dendritic cells that generate CD4+ regulatory T cells

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The neuropeptide alpha-melanocyte-stimulating hormone (α-MSH) is a potent immunomodulator that is able to induce immunosuppression and tolerance. To elucidate the mechanisms underlying α-MSH-induced immunomodulation we investigated whether α-MSH affects dendritic cell (DC)-T cell communication since this interaction plays an important role in the induction and regulation of immune responses. In comparison to non-stimulated controls, DC stimulated with α-MSH showed an increased expression of CD205 (DEC205), a surface marker that has been previously associated with DC-mediated induction of CD4+CD25+ regulatory T cells. To investigate whether α-MSH stimulated DC were able to induce a regulatory phenotype in naive CD4+ T cells, bone marrow-derived DC were treated with α-MSH and co-cultured with CD4+ T cells. Interestingly, T cells from co-cultures with α-MSH stimulated DC were anergic, produced high levels of IL-10 and suppressed the proliferation of CD4+CD25- effector T cells in vitro. Moreover, CD4+ T cells co-cultured with α-MSH stimulated DC displayed enhanced expression of markers characteristic for regulatory T cells (Foxp3, Neuropilin-1, CTLA-4, TGF-β) suggesting that α-MSH stimulated DC are indeed able to confer a suppressor phenotype to naive CD4+ T cells. To test whether CD4+ T cells co-cultured with α-MSH treated DC were suppressive in vivo T cells from co-cultures with PBS- as well as α-MSH stimulated DC were injected into mice previously sensitized to the contact allergen DNFB. Upon ear challenge with DNFB, mice that had been treated with CD4+ T cells co-cultured with PBS stimulated DC mounted a normal contact hypersensitivity response. Interestingly, injection of CD4+ T cells from co-cultures with α-MSH treated DC inhibited contact allergy. Together, these findings indicate that α-MSH induced immunosuppression is mediated via tolerogenic DC and CD4+CD25+ T cells. Therefore, α-MSH might be successfully used to control inflammatory or autoimmune disorders with polyclonal T cell activation.

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T cell mediated immune responses depend on cell volume regulation by the taurine transporter Taut

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The β-amino acid Taurine functions as an osmolyte to regulate cell volume and its cellular uptake is accomplished by the taurine transporter Taut. Since T cell activation is followed by profound changes in cell volume we wanted to analyse whether Taut plays a role in T cell biology by examing Taut deficient mice (taut-/-). Using the established B16 melanoma model we investigated tumor rejection after cutaneous inoculation of intact melanoma cells in mice previously sensitized with irradiated B16 cells. Rejection was significantly reduced in sensitized taut-/- mice compared to wt mice indicating impaired T cell mediated immune responses. This indication was confirmed in a second model of T cell mediated immune reaction: contact hypersensitivity responses (CHS) to TNCB were significantly decreased in taut-/- mice . While no difference was detected in naïve mice, exclusively T cells were significantly reduced in skin draining lymph nodes after CHS induction in taut-/- mice. This refers to a role of Taut in rescuing T cells from apoptosis which was supported by significant higher levels of the apoptosis marker AnnexinV after in vitro activation of taut-/- T cells. In vivo relevance of Taut was proven by adoptive transfer of TNCB specific CFSE loaded T cells from taut+/+ and taut-/- mice into naïve wt recipients consecutively challenged with TNCB. Monitoring divisions of transferred cells showed a significant reduction of dividing CFSE+ taut-/- T cells compared to wt demonstrating increased apoptosis of taut-/- T cells in vivo. As apoptosis also regulates T cell memory formation, CHS mice were analyzed for the presence of central memory T cells (TCM) which were reduced by about 50% in taut-/- mice compared to wt mice. In summary, we showed Taut to be essential for T cell survival, T cell memory generation and maintenance, and T cell mediated immune reactions. These findings highlight a new level of immune regulation and describe a hitherto unknown but crucial pathway underlying functional immune reponses.

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Development of novel intravital imaging system for real-time monitoring of macromolecule endocytosis by Langerhans cells in situ

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Technological advances in confocal imaging and mouse genetic engineering have enabled direct visualization of Langerhans cells (LC) in living animals. Here we recently reported time-lapse 4D images of LC recorded in the IAβ-EGFP knock-in mice, in which LC are engineered to exhibit fluorescent signals. We have developed a novel imaging system that enables direct visualization of the dynamic process for macromolecule endocytosis by LC in normal living animals. BALB/c mice received s.c. injection of FITC-conjugated dextran (DEX) probes into the ear skin and then examined by confocal microscopy. FITC-DEX probes with 20 or 70 kD were incorporated by some epidermal cells, while the 4 kD probe failed to demarcate cell-associated fluorescent signals. In time-course experiments, fine networks of FITC+ epidermal cells with dendritic morphology became detectable 12-18 hr after injection as background fluorescent signals began to disappear. Most (>90%) of these epidermal cells were indeed LC as judged by Langerin expression. Conversely, >95% of Langerin+ cells exhibited significant FITC signals. Focal accumulation of the internalized probes was observed later (30-48 hr) primarily in the MHC II compartment. Fluorescent signals remained detectable up to 144 hr. Dual-color imaging experiments, in which a DEX probe conjugated with a red fluorescent tag (Alexa Fluor 546) was injected into the IAβ-EGFP knock-in mice, confirmed selective and progressive uptake of DEX probes by a majority of LC. In time-lapse imaging experiments, LC were found to extend and retract their dendritic processes repetitively while incorporating FITC-DEX probes. Our results demonstrate for the first time a potent capacity of LC in situ to uptake macromolecules from the extracellular space and, thus, support the concept that they play an immunosurveillance role at the environmental interface. The new intravital, real-time imaging system may enable us to visualize dynamic behaviors and endocytotic activities of LC in the skin of healthy individuals as well as patients with various skin disorders.

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Identification of immediate dendritic cell precursors in newly generated IL-1β promoter-driven DsRed transgenic mice

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Pathways for dendritic cell (DC) development still remain relatively unclear, although several candidates of DC precursors have been reported in the literature. Based on our recent finding that DC activation is accompanied by rapid expression of IL-1β mRNA, we constructed an IL-1β promoter-driven DsRed transgenic mouse line. Conventional $CD11c^+$ DC cultures (Day 6) derived from the bone marrow (BM) of the pIL1β-DsRed transgenic mice, indeed, exhibited heightened DsRed fluorescent signals in response to LPS stimulation. Surprisingly, strong DsRed signals were already detected within the $CD11b^+/CD11c^-$ fraction as early as 12 hr after culturing BM cells in the presence of GM-CSF. Time-course experiments revealed striking (>200-fold) expansion of $DsRed^+$ cells in the first 24 hr, followed by a modest increase with a peak on Day 3-4. By contrast, neither M-CSF nor Flt3-L induced robust DsRed expression. The $CD11b^+/CD11c^-/DsRed^+$ fraction purified from 24 hr BM cultures readily differentiated into bona fide DC expressing CD11c and MHC class II. The $CD11c^+$ DC population, but not the $CD11b^+/CD11c^-/DsRed^+$ fraction, exhibited the characteristic dendritic morphology and presented OVA antigen efficiently to CD4 T cells (from OT-II mice) and CD8 T cells (OT-I mice). The $CD11b^+/CD11c^-/DsRed^+$ fraction was characterized by uniform expression of Gr-1, lack of MHC class I and II, CD48, and F4/80 expression, and marked uptake of FITC-dextran. With respect to in vivo relevance, small fractions (0.2-0.7%) of BM cells and spleen cells in the pIL1β-DsRed transgenic mice expressed DsRed constitutively and this subset was, again, mostly $CD11b^+/CD11c^-$/MHC $II^-/Gr\text{-}1^+$. Moreover, clusters of $CD11b^+/DsRed^+$ cells were detected in the skin, preferentially around the hair follicles, under inflammatory conditions, i.e., 24 hr after tape stripping or topical application of haptens. Thus, we propose that the $CD11b^+/CD11c^-/DsRed^+$ cells we identified in this study represent an immediate precursor of DC, termed the DC committed population or "DC.com."

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Differential homeostasis and function of human dermal macrophages and dendritic cells: implications for the induction of graft versus host responses

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Graft versus host disease (GVHD), a potentially fatal complication of hematopoietic stem cell transplantation (HSCT), is initiated by recipient antigen presenting cells (APC) activating donor T cells. The skin, a commonly affected organ, contains diverse APC whose individual contribution to GVHD induction is unknown. We set out to define the homeostasis and function of dermal dendritic cells (DC) and macrophages in normal skin and a cohort of HSCT recipients. Flow cytometry of digested dermis show CD45+ HLA-DR+ cells can be subdivided into CD1a+ CD1c+ DC and CD14lo CD163+ fXIIIa+ macrophages. DC are CCR7+ and spontaneously migrate in vitro. Macrophages lack CCR7, migrate poorly, phagocytose FITC-coated dextran particles more efficiently, adhere to plastic and contain high amounts of ingested melanin. Upon stimulation, macrophages upregulate the co-stimulatory molecules CD80 and CD86 but to a lower extent than DC. In allogeneic transplant recipients, we find that macrophages are more resistant to conditioning and remain recipient in origin for more than 100 days. In contrast, DC are rapidly replaced by donor cells, reaching near complete donor chimaerism within 40 days. Persistence of macrophages does not correlate with local proliferative potential as previously shown for murine LC; with less than 1% in S phase in the steady state. Macrophages are immunoregulatory and upregulate IL-10 secretion after stimulation with microbial ligands. In contrast to DC, macrophages are poor initiators of alloresponses, inducing proliferative anergy and inhibition of IFNγ, TNFα, IL-4 and IL-10 production by allogeneic T cells upon restimulation. In conclusion we have identified the dermal macrophage, a major persistent recipient APC after hematopoietic transplantation and shown that it has regulatory function in alloresponses. It may serve as a critical inhibitor, rather than inducer of GVHD.

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The DC-HIL/SD-4 pathway for regulating T cell activation is operative in humans and recruits CD148 tyrosine phosphatase to mediate its inhibitory function

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Having discovered that binding of DC-HIL on antigen presenting cells to syndecan-4 (SD-4) on activated T cells potently inhibits T cell activation in mice, we questioned whether the same is true for humans. As in mice, we showed that human DC-HIL binds activated (not resting) $CD4^+$ and $CD8^+$ T cells, and this binding attenuated T cell activation triggered by anti-CD3 Ab. DC-HIL expression in human peripheral blood cells is restricted to $CD14^+$ monocytes, whose alloreactive activity (via MLR) was enhanced by antagonizing endogenous DC-HIL/SD-4 using soluble DC-HIL or by knocking-down DC-HIL expression using siRNA. Conversely, treatment of $CD14^+$ cells with TGFβ (10 ng/ml) increased DC-HIL expression 10-fold, while lowering MLR activity by 50%. Moreover, we demonstrated human SD-4 to be the T cell ligand through which DC-HIL mediates its inhibitory function. We next elucidated the intracellular signaling responsible for inhibition by DC-HIL/SD-4. Because SD-4 lacks an inhibitory motif that can bind protein tyrosine phosphatase (PTP), we considered that SD-4 may associate instead with a membrane molecule containing PTP activity. CD148 was a promising candidate since it was known to attenuate TCR signaling. We showed CD148 on T cells to be upregulated by PMA plus ionomycin, and demonstrated coculture of activated human T cells with DC-HIL (but not control) to induce tyrosine phosphorylation and CD148 PTP activity (malachite green assay). Finally, using anti-SD-4 Ab, we coprecipitated CD148 from extracts of activated human T cells treated with immobilized DC-HIL (but not control), indicating that binding of DC-HIL to SD-4 induces the latter to associate with CD148. We conclude that the DC-HIL/SD-4 pathway for inhibiting T cell activation is operative in humans and involves membrane-type PTP CD148 in its negative signaling. Manipulating this pathway=s expression/function will open new avenues for treating T cell-driven diseases.

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Langerhans cells are not required for efficient skin graft rejection

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The mechanism of skin allograft rejection has been thought to require presentation of graft antigen by resident epidermal Langerhans cells (LCs). We have previously engineered mice that have a selective and constitutive absence of epidermal LC. Using donor skin from these LC-deficient mice, we show that LC are not required for rejection of major (FVB—>B6) or minor (H-Y, male—>female on B6 background) antigen-mismatched skin grafts. On the FVB background, however, where H-Y mismatched grafts are normally maintained indefinitely, grafts lacking LC are efficiently rejected. Thus, LC in the donor graft are required for long-term skin engraftment. This finding is consistent with our previous data showing that LC deficient mice develop exaggerated contact hypersensitivity responses and supports a model in which LC are a regulatory DC subset.

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Type-I hypersensitivity response induced by adalimumab injections

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Tumor necrosis factor α (TNF-α) inhibitors such as adalimumab, etanercept and infliximab play an increasingly important role in the management of a variety of chronic inflammatory disorders in dermatology, rheumatology and gastroenterology. With their increasing use, a wide spectrum of dermatological adverse effects, including injection site reactions and the development of dermatitis, have been recognized. Previous studies have implicated the role of the delayed type hypersensitivity reaction in mediation of injection site reactions to etanercept. This study is the first to implicate the role of immediate type-I hypersensitivity response in mediation of worsening injection site reactions to adalimumab. We report two patients with a history of worsening injection site reactions to adalimumab. Skin testing in both patients were suggestive of an immediate type I hypersensitivity reaction to adalimumab. A histamine release assay was performed on peripheral blood leukocytes from both patients demonstrated significant histamine release upon exposure to adalimumab. Furthermore, passive transfer of serum from one of the above-mentioned patients to basophils from a non-atopic, healthy donor sensitized those cells to release significant amounts of histamine with exposure to adalimumab. This study demonstrates that an IgE-mediated immediate type I hypersensitivity reaction plays a role in the mediation of a worsening injection site reactions in some patients receiving adalimumab.

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Monocyte-derived interferon-alpha dendritic cells: emerging players in autoimmunity and cancer immunotherapy

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Type I interferons (IFNs) play an important role in the pathogenesis of many autoimmune disorders including systemic lupus erythematosus (SLE), type 1 diabetes, rheumatoid arthritis and psoriasis. In the presence of IFN-alpha and granulocyte/macrophage colony-stimulating factor monocytes differentiate into dendritic cells (DCs) referred to as IFN-DCs, which potentially mimic DC populations involved in autoimmunity. Recent studies showed that monocytes of SLE patients, but not those from healthy individuals, act as DCs. This activation may be driven by circulating IFN-alpha that possibly comes from plasmacytoid DCs. IFN-DCs express a wide range of toll-like receptor (TLR) subtypes including TLR7 and TLR8, which are capable of recognizing single-stranded RNA (ssRNA). Viruses containig ssRNA and complexes containing self RNA may potentially trigger these receptors. Our findings showed that incubation of IFN-DCs with ssRNA upregulated TLR7 and TLR8, significantly improved their costimulatory molecule expression, stabilized their phenotype and enhanced their capacity to stimulate naïve T cell proliferation. Additionally ssRNA stimulation led to a significant production of T-helper 1 cytokines including IL-12 and the new IL-12 family members IL-23 and IL-27. These discoveries shed light on a potential role for IFN-alpha and self/non-self TLR signals in triggering DC populations in autoimmune diseases including psoriasis. These results provide additional data to better understand human autoimmune and antiviral responses and may also have implications for strategies developing cancer immunotherapy.

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CD11b+/Gr-1+ myeloid suppressor cells increase in chronic contact dermatitis induced by repeated hapten application

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Although short-term inflammatory activity could be beneficial for the host, the persistence of inflammatory processes often results in detrimental tissue damage. Therefore, in the course of a response, inflammation is usually counteracted through the development of anti-inflammatory mechanism. Inflammatory reaction reaches its plateau after approximately 4 weeks in chronic contact dermatitis induced by repeated hapten application, suggesting some regulatory system develops during this course. CD11b+/Gr-1+ myeloid suppressor cells are known to play an important role in suppression of inflammatory reaction against tumor cells. To study by which the inflammatory reaction is regulated in chronic dermatitis, we examined the kinetics and function of CD11b+/Gr-1+ myeloid suppressor cells in lymphoid tissue of mice with repeated hapten application. Chronic contact dermatitis was developed by applying (q2d) picryl chloride on the ear skin of picryl chloride-sensitized BALB/c mice. Flow-cytometric analysis showed that CD11b+/Gr-1+ cells increased in association with the development of chronic dermatitis in the spleen and lymph nodes. These CD11b+/Gr-1+ cells shared the phenotypical characteristics with alternatively activated macrophage such as F4/80+, CD115+, and CD206+. These CD11b+/Gr-1+ cells exhibited low expression of MHC class II, CD80, and CD86, indicating their immature character. When compared with control, hapten-induced CD11b+/Gr-1+ cells significantly inhibited CD3/CD28-mediated T cell proliferation and IL-2 production. In addition, CD11b+/Gr-1+ cells showed poor antigen-presenting capacity in allogenic murine mixed lymphocyte reaction. These results suggest that myeloid suppressor cells expressing CD11b+/Gr-1+ markers in the lymphoid tissue may contribute to the regulation of inflammatory reaction, which may have clinical implications in the treatment of chronic allergic skin diseases such as occupational dermatitis and atopic dermatitis.

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Basophils involved in cutaneous allergic responses, detected by newly established mAbs specific to basophil granular enzymes mMCP-8 and mMCP-11

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The infiltration of basophils is often observed at the lesions of allergic inflammation such as atopic dermatitis in human. However, their roles in the pathogenesis of allergic disorders are poorly understood. The lack of proper animal models, including basophil-deficient mice, and the unavailability of mouse basophil-specific mAbs have been practical obstacles to clarifying localization and functions of basophils in vivo. Since mouse basophils contain much fewer basophilic granules than human ones, the standard methods for histopathological examination, such as Wright Giemsa staining, hardly detect basophils in inflamed tissues of mouse models of allergy. In the present study, to overcome this problem, we have established two mAbs specific to enzymes localized to secretory granules in mouse basophils. RT-PCR analyses revealed that mouse mast cell protease (mMCP)-8 and mMCP-11 were predominantly expressed in basophils rather than in mast cells, unlike other members of the mMCP family. Anti-mMCP-8 and anti-mMCP-11 mAbs prepared from rats immunized with recombinant proteins did not cross-react with each other or other mMCP members. They brightly stained secretory granules in fixed basophils while none of cell lineages other than basophils were stained by them, indicating the mAbs as powerful tools to specifically detect basophils in tissue sections. Identification and localization of basophils in various cutaneous allergic responses will be presented.

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A characterization of invariant Natural Killer T cells in mouse skin

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The purpose of this study was to investigate whether 'invariant' (*i*) Natural Killer T (NKT) cells are present in mouse skin. *i*NKT cells are an evolutionarily conserved T cell subset that use a single T cell receptor (TCR) alpha chain (Vα14Jα18 in mice; Vα24Jα18 in humans) combined with a limited repertoire of TCR beta chains. The highly restricted TCR repertoire of *i*NKT cells recognises glycolipid antigens presented by the antigen-presenting molecule CD1d, which immediately distinguishes them from most other αβ T cells that recognise peptides in conjunction with major histocompatibility complex (MHC) class I or II molecules. *i*NKT cells have the ability to powerfully influence immune responses due to their capacity to rapidly produce copious amounts of immunomodulatory cytokines. We used a highly specific reagent, αGalCer-loaded CD1d tetramers (which bind directly to the TCR of *i*NKT cells by antigen recognition), and 6 colour flow cytometry to detect *i*NKT cells in epidermal cell suspensions from two commonly used mouse strains: C57BL/6 and BALB/c. Here, we report for the first time the direct identification of *i*NKT cells in normal mouse skin. This observation has important implications for skin immunity – for example, a glycolipid extracted from Borrelia burgdorferi, the causative agent of Lyme disease which infects humans through deer tick bites, has recently been found to stimulate *i*NKT cells. In addition, *i*NKT cells have been implicated in the mechanism by which ultraviolet (UV) radiation from sunlight suppresses the immune system – a significant contributing factor in the development of skin cancer induced by UV radiation. Our novel findings, that *i*NKT cells are present in skin in significant numbers, indicate that these cells may represent major mediators of skin immunity.

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Histamine as a key enhancer for skin innate immunity to fungi

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Epidermal keratinocytes produce antimicrobial peptides, such as β-defensin and cathelicidin and express Toll-like receptors (TLRs) in and non-TLRs. Fungi are highly incident and intimate pathogens for epidermal milieu and recognized by TLR2, 6 and dectin-1. Although many studies have implicated that histamine and mast cells play a role for innate immunity, little is known concerning the combinational effects of fungal elements and histamine an skin innate immunity. Cultured normal human epidermal keratinocytes were stimulated with β-glucan or curdlan, and/or histamine in combination and examined (1) the expression of TLRs, dectin-1, and antimicrobial peptides by real-time PCR; (2) the expression of surface and intracellular TLR2 by flow cytometry and confocal microscopy; and (3) the enhanced production of the cytokine / chemokines by ELISA. The mRNA expression of innate immune proteins was not increased when stimulated with β-glucan alone. However, we found a synergistic effect of β-glucan and histamine on the expression of TLR2 and 6, dectin-1 and antimicrobial peptides. The cell surface expression of TLR2 was barely detectable by flow cytometry, and its surface level was not increased by β-glucan or histamine. However, when the intracellular expression of TLR2 was monitored, we found an apparent elevation of its expression. A confocal microscopic analysis also revealed the enhanced expression of TLR2 in the cytoplasm. The synergism between β-glucan and histamine was also observed in cytokine/chemokine production by keratinocytes, as assessed by ELISA analysis of the culture supernatants. We concluded that in keratinocyte operation of innate immunity, fungal elements and histamine exerts synergistic action, suggesting a defensive role of histamine.

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Functional characterization of IL-17F as a selective neutrophil attractant in psoriasis

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Background: IL-17F is known to be involved in many inflammatory diseases, but its role in skin diseases has not been examined. IL-8 plays a crucial role in many skin diseases such as psoriasis. Objective: We investigated the functional effects of IL-17F in the production of IL-8 in normal human epidermal keratinocytes (NHEKs) and the expression of IL-17F in lesional skin of psoriasis patients. Methods: IL-8 expression in NHEKs stimulated by IL-17F, TNF-α, IL17A, and control were examined using real-time PCR and ELISA. The levels of IL-8 production in the presence and absence of various kinase inhibitors were compared. Recombinant mouse IL-17F was injected intradermally into mice, and histological changes were investigated. Moreover, IL-17F expression in skin biopsy samples from psoriasis patients was examined by Western blotting. Results: IL-17F induced production of IL-8 in NHEKs in a time-dependent manner. Interestingly, the amounts of IL-8 stimulated by IL-17F were much higher than those stimulated by TNF-α or IL-17A. It was also confirmed that the selective MEK inhibitors inhibited IL-17F-induced IL-8 production significantly. Histological examination of mouse skin 48 h after injection of IL-17F revealed marked neutrophilia in dermis. Furthermore, IL-17F protein was clearly detected in lesional psoriatic skin compared with non-lesional skin. Conclusion: Our results show for the first time that IL-17F plays a crucial role in psoriasis via, in part, the induction of IL-8 in keratinocytes.

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Pivotal role for dermis derived CXCL12 in the initiation of Langerhans cell migration

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Langerhans cell (LC) migration from the epidermis to the lymph node involves migration of maturing LC and has been reported to be CCR7 dependent. However, the initial step of LC migration requires the migration into the dermis. Here, we investigated the migration of LC out of the epidermis after exposure of the skin to harmful environmental substances such as contact allergens and irritants. Ex vivo intact human skin, epidermal sheets, and LC derived from the human MUTZ-3 cell line (MUTZ-LC) were used to determine whether dermal cells, such as fibroblasts, play a role in mediating LC migration towards the dermis. Exposure of epidermal sheets or MUTZ-LC to environmental assault (e.g. allergens, irritants) resulted in LC migration towards dermal fibroblasts. For allergen-matured LC this was due to up-regulation of CXCR4 on maturing LC and secretion of CXCL12/stromal derived factor (SDF)-1 chemokine by fibroblasts. Neutralizing antibodies to either CXCL12 or CXCR4 completely blocked migration in a trans-well in vitro assay, while injection of CXCL12 neutralizing antibodies into intact human skin totally inhibited LC migration into the dermis. In contrast, injection of a CCL19/CCL21 neutralizing antibody-cocktail, both ligands for CCR7, did not inhibit LC migration into the dermis. The CXCL12/CXCR4 mediated LC migration was specific for maturing (e.g. allergen exposed) LC. A different, as yet unknown mechanism is involved in migration of immature or irritant exposed LC. In conclusion, we have identified a novel and essential role for dermis derived CXCL12 in initiating migration of maturing LC, thus permitting their further journey to the draining lymph nodes. However, the fate of irritant exposed migrated LC is still unknown. To study the effect of allergen and irritant exposed migrated LC in more detail, a reconstructed full skin equivalent model with integrated MUTZ-LC in the epidermis was recently developed.

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Human conventional dendritic cells acquire a Th1 instructive phenotype in the presence of IL-4

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Peripheral blood contains distinct subsets of myeloid human conventional dendritic cells (cDC) - MDC1, MDC2 and slanDC. In contrast to monocyte-derived DC, cDC are readily available *in vivo* and are among the first cells to initiate and direct the outcome of immune responses such as in atopic inflammation. Since an IL-4-dominated inflammatory milieu is a hallmark of atopic inflammation, the impact of interleukin (IL)-4 on the functional phenotype of cDC has been analysed. Toll-like receptor (TLR) expression of immature cDC was determined by quantitative real-time PCR as well as confocal fluorescence microscopy and cDC were activated with the respective TLR ligands. Among others, lipopolysaccharide (LPS) activation of slanDC (M-DC8^{pos}/CD11c^{pos}/CD86^{low}/CCR5^{pos}) via TLR4 led to vigorous upregulation of MHCII and CCR7, while CCR5 was no more detectable. Morphology and maturation of cDC were not altered by the combination of signals via TLRs and IL-4R. However, the presence of IL-4 markedly altered the cytokine profile of all three cDC subsets and strongly enhanced IL-12p70 production. In the absence of IL-4, high levels of anti-inflammatory IL-10 were detected, whereas the combination of TLR ligands and IL-4 vigorously upregulated IL-12p70 and almost completely abolished IL-10 production. Furthermore, TLR/IL-4R-activated cDC primed naïve autologous T helper cells solely toward an IFN-γ^{high}IL-4^{low} Th1 phenotype, whereas the IL-10^{++} phenotype of cDC activated in the absence of IL-4 allowed the development of other Th subsets such as Th 2 cells. Our findings are of general importance demonstrating the functional plasticity of human peripheral blood cDC at their final activation stage. Moreover, these data highlight a new key regulatory role of the classical Th2 cytokine IL-4, which decisively determines the phenotype of ongoing immune responses by orchestrating cDC function and consequently Th polarization.

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CCL21 acts as an important regulator of skin inflammation by induction of antigen uptake, survival and migration of skin-residing dendritic cells

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Skin inflammation is a sequence of successive events regulated by plethora of proinflammatory cytokines and their inducers. The relative contribution of distinct cytokines to this process is only partly understood. CCL21 may act as a homeostatic as well as an inflammatory chemokine. Here, we have dissected the activity of CCL21 on the key players of skin inflammation, dendritic cells. By in vivo gene transfer into the skin, ex vivo analysis of a mouse strain (plt/plt) mutant for CCL21 expression, and in vitro experiments with recombinant/transgenic CCL21 we show that CCL21 induces antigen uptake by and CD40 expression on skin-residing DCs, but not maturation as defined by morphology (granularity/size), phenotype (CD80/CD86/CCR7-expression) and function (allostimulation). In vivo, skin-expressed transgenic CCL21 induced recruitment of CD11c+ DCs and CD4+/CD62L+ naive/central memory T cells into draining lymph nodes. By regulating antigen uptake by and CD40 expression on DCs and by encounter of spatially and functionally segregated cell populations in a defined immunological compartment, CCL21 acts as an important regulator of skin inflammation. These data have important implications on the understanding of the successive events leading to skin inflammation, on the identification of critical regulators, and on the future design of immunotherapeutic strategies including DC-based vaccination.

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$CD14^+CD16^+$ monocytes are induced from fresh human monocytes by Interleukin-10/M-CSF/TGF-β and its induction is inhibited by minocycline

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Sarcoidosis is a systemic disease of unknown etiology characterized by noncaseating granulomas, consisting mainly of epithelioid cells and multinucleated giant cells derived from monocyte-macrophage lineage cells. Monocytes fall into subpopulations comprising $CD14^{++}CD16^-$, and $CD14^+CD16^+$ cells, and expansion of the later monocytes has been documented under some pathological conditions. We previously reported that the percentage of $CD14^+CD16^+$ monocytes was significantly higher in sarcoidosis patients compared with those in healthy control subjects and correlated with the serum ACE levels. Since $CD14^+CD16^+$ monocytes produce high levels of IL-1, TNF-α and IL-6 and express higher cell surface densities of MHC class II antigens and adhesion molecules, they may play a role in T cell-mediated reaction in sarcoidosis. However, the differentiation pathway of $CD14^+CD16^+$ monocytes has not been defined. We examined herein the involvement of cytokines in their induction. IL-10/M-CSF/TGF-β triggered the differentiation of $CD14^+CD16^+$ cells from fresh human monocytes. These cytokines-induced $CD14^+CD16^+$ monocytes expressed CCR5 but not CCR2, CXCR3 or CCR4. This differentiation was inhibited by minocycline, which was reported as an anti-inflammatory drug in various skin diseases including sarcoidosis. These findings suggest that minocycline is effective for sarcoidosis by acting on monocytes directly and reducing the differentiation to $CD14^+CD16^+$ cells which are origin of cytokines.

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Role of S1P-S1P1 signaling in the establishment of contact contact hypersensitivity

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An immunosuppressant drug, FTY720, inhibits lymphocyte emigration from lymphoid organs, and phosphorylated FTY720 binds one of sphingosine-1-phosphate (S1P) receptors, S1P1, and down-regulates S1P1, providing an explanation for the mechanism of FTY720-induced lymphocyte sequestration. T cell traveling between secondary lymphoid organs to survey for antigens are suggested to play an important role to establish acquired skin immune response, however, the role of FTY720 in this process remained to be elucidated. Initially we found that mouse contact hypersensitivity (CHS) response was impaired by the treatment with FTY720 during the sensitization phase, but not elicitation phase. And adoptive transfer of immunized lymph node cells from mice treated with FTY720 during sensitization phase did not induce apparent CHS response in the recipient. Sensitization phase consisted of dendritic cell (DC) capturing antigens, migration into regional lymph nodes where they present antigens to naive T cells. Since we could not detect functional defects of DC by FTY720 treatment, we focused on the role of FTY720 on T cells. FTY720 decreased the number of mouse blood CD44-naive T cells markedly and that of CD44+ memory T cells modestly. Among memory T cells, the CD62L- effector memory T cell subset was more resistant to FTY720 treatment than the CD62L+ central memory T cell subset. Accordingly, the level of S1P chemotactic response was high in naive T cells, marginal in effector memory T cells, and very low in central memory T cells. Consistently, the S1P1 mRNA level was downregulated in CD44+ memory T cell subsets compared to naive T cells. These findings establish that S1P-S1P1 signaling is essential for recirculation of naive T cells but not that of memory T cells. FTY720 seems to decrease the incidence of interaction between antigen-bearing DCs and circulating naive T cell clones in the draining lymph nodes, thereby depressing the sensitization of naive T cells in the induction phase of CHS.

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Minocycline inhibits multinucleated giant cell formation from monocytes by supernatant of concanavalin A-stimulated mononuclear cells

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Minocycline is a tetracycline derivative that exhibits significant anti-inflammatory properties in a variety of dermatological conditions. There have been reported cases showing that minocycline has beneficial effects on some patients with sarcoidosis and other granulomatous diseases. However its action mechanisms are not defined. A characteristic pair of infiltrating cells in sarcoidal granulomas is monocyte/macrophage-lineage cells and their fused multinucleated giant cells (MGCs). MGCs are classified as Langhans-type (LGC) or foreign-body type (FGC) cells based on their nucleus array. Both types of MGCs are also formed *in vitro* from peripheral blood mononuclear cells by stimulation with cytokines. Here we examined the *in vitro* effects of minocycline on the formation of MGCs from human peripheral monocytes. Supernatant of concanavalin A (Con A)-stimulated mononuclear cells induced LGC and FGC from fresh human monocytes. Addition of 25 or 50μg/ml minocycline inhibited the formation of both MGCs. Minocycline decreased the nuclei numbers in MGCs. Fluorescence-activated cell sorting analysis showed that minocycline-treated monocytes had lower expressions of intercellular adhesion molecule-1 (ICAM-1). These findings suggest that minocycline is effective for cutaneous lesions in some cases of granulomatous disorders partly through a direct effect on monocyte/macrophage-lineage cells.

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Connective tissue mast cells acquire monocyte- specific gene expressions by transcription factor PU.1 in atopic dermatitis model mice

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Transcription factor PU.1 is expressed in lymphoid cells, macrophages, dendritic cells (DC), neutrophils, and mast cells in a cell type-specific manner. We recently found that overexpression of PU.1 in BM-derived cultured mast cells (BMMC) and their progenitors induced the expression of several monocyte-specific genes and caused certain morphological changes (*J. Immunol*, 2005). These results suggest that mast cells have the ability to transform into monocytes through the mediation of PU.1 in allergic disorders. In the present study, we examined skin and peritoneal mast cells (PMC) and analyzed PU.1 expressions among infiltrated mast cells in AD model mice in order to determine whether infiltrated mast cells in atopic dermatitis model mice had a monocyte-specific gene expression. 2,4,6-trinitro-1- chlorobenzine(TNCB) was applied for 28 days to the skin of both ears of one group of BALB/c mice, and to the skin of the ears, neck, and back of the second group. Both groups were used as AD models. The immunohistochemistry of the skin of the ears indicated that about 50% of the mast cells (FcεRI+, *c-kit*+) expressed CD11c and CD11b. Moreover, PU.1 was found to have up-regulated the expression of mast cells (tryptase+) in AD. PMC was then analyzed for gene expressions using real-time PCR in AD. PU.1 and CD11b genes showed higher expression than in the control ($p<0.05$), but CD11c was expressed in neither the AD, nor in the control group. Our data indicated that the mast cells of the skin and the peritoneal cavity elicit monocyte-specific gene expression and show high expression of PU.1 in AD. Our results confirmed that mast cells acquire monocyte-specific gene expression *in vivo*.

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Aspirin regulates leukotriene synthesis in mast cells via dihydropyridine–sensitive calcium entry

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Aspirin is the most widely used anti–inflammatory drug worldwide that can potentiates some acute allergies such as food–dependent exercise–induced anaphylaxis and causes adverse immunological reactions collectively referred to as aspirin intolerance. Aspirin intolerance is accompanied by increased leukotriene (LT) synthesis, in which mast cells appear to play a role. We therefore examined whether aspirin directly regulates LT secretion in mast cells. Low concentrations (0.1~0.3 mM) of aspirin enhanced antigen-induced LTC_4 secretion and Ser–505 phosphorylation of cytosolic phospholipase A_2 ($cPLA_2$). These effects were not mediated by extracellular signal–regulated kinases (ERKs) and p38 mitogen–activated protein kinase (p38MAPK), because aspirin did not enhance antigenic activation of these kinases. Antigen–induced LTC_4 secretion was strictly dependent on extracellular Ca^{2+}. Aspirin dose–dependently reduced Ca^{2+} release from intracellular stores and Ca^{2+} influx through store–operated Ca^{2+} channels (SOCs). Antigenic stimulation evoked substantial Ca^{2+} influx in a dihydropyridine (DHP)–sensitive manner, under conditions in which SOCs are not inactive. Moreover, DHP–sensitive Ca^{2+} entry was involved in antigen–induced LTC_4 secretion and was enhanced by low concentrations of aspirin. These findings suggest that aspirin enhances antigen-induced LTC_4 secretion in mast cells by facilitating DHP–sensitive Ca^{2+} entry, a novel mechanism for LTC_4 secretion that may contribute to aspirin-induced adverse immunological reactions including aspirin intolerance.

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The serum TSLP levels are not elevated in patients with atopic dermatitis

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Thymic stromal lymphopoietin (TSLP) is a IL-7 like cytokine produced by epidermal keratinocytes (KCs) (1). TSLP induces thymus and activation regulated chemokine (TARC/ CCL17) production by dendritic cells (DCs) and it also activates epidermal Langerhans cells. A high level of immunreactivity for TSLP is detected on the lesional KCs in patients with atopic dermaitis (AD). Keratin 14 driven TSLP transgenic mice showed inflammatory eruptions showing a pathology similar to that of AD. However, little is known about the serum levels of TSLP in AD patients. We herein examined the serum levels of TSLP in 46 patients of AD (average age 26.5 years old, 19 males and 27 females) and 32 healthy controls (HC) (average age 29.7 years old, 16 males and 16 females) using an ELISA assay kit (R&D Systems, Minneapolis, MN). The serum levels of TSLP in the AD patients were 192.2 +/- 54.1 pg/ml, while those with HC were 112.6 +/- 51.6pg/ml. No significant difference in the serum TSLP levels were detected between these two groups ($p=0.139$). There were no significant differences in the serum TSLP levels among the different severities of AD patients.These data suggest that TSLP may exert its effects either directly in the inflammatory skin or in the lesional lymph nodes in patients with AD. Further examinations will be required to elucidate the function of TSLP in the pathogenesis of AD.

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Profiles of Foxp3+regulatory T-cells (Tregs) in Bowen's disease and squamous cell carcinoma

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It is well known that regulatory T cells (Tregs), identified by their expression of CD4, CD25, and Foxp3, play a crucial role in maintaining peripheral tolerance. Recently, it has been demonstrated that a Treg population resides in normal human skin. Moreover, we previously reported the profiles of Foxp3 regulatory T cells in skin inflammatory disease, eczematous dermatitis and psoriasis, and skin neoplastic disease, mycosis fungoides. These observations strongly suggest the crucial role of Tregs in various skin disorders, including skin tumors. In this study, we immunohistologically examined the presence of CD4+CD25+Foxp3+ Tregs in the lesional skin of Bowen's disease and Squamous cell carcinoma, and for the control, we compared the lesional skin of eczematous dermatitis. We investigated the cutaneous expression of Foxp3 in 17 Bowen's disease patients, 13 Squamous cell carcinoma patients, and 18 eczematous dermatitis patients by using formalin-fixed paraffin embedded sections. The double staining was performed by using the combination of murine anti-CD3 Ab, anti-CD4 Ab, anti-CD8 Ab, anti-CD25 Ab with peroxidase-conjugated anti-mouse Igs and that of anti-Foxp3 Ab with alkaline phosphatese-conjugated anti-rabbit Igs. These studies demonstrated as follows: 1) the percentages of Foxp3+ cells among CD3+, CD4+ and CD25+ cells in Bowen's disease, 15.0±1.0%, 17.2±1.5% and 22.4±1.8%, and, especially, that of Foxp3+ cells among CD3+, CD4+, and CD25+ cells in SCC, 23.9±2.4%, 25.0±2.5%, and 29.5±1.7% were significantly higher. (p=0.0018, p=0.0115, p=0.0070), 2) none of the CD8+ cells express Foxp3, 3) around the 15% of CD3+, CD4+ or CD25+ cells express Foxp3 in Bowen's disease and spongiestic dermatitis while more than about 25% of CD3+, CD4+ or CD25+ cells express Foxp3 in SCC. These findings suggested that the increased Treg frequency in the infiltrate surrounding SCC diminishes anti-tumor immune responses by hosts.

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Reactive oxygen species (ROS) protect from bacterial toxin-induced lethal shock trough induction of the negative regulating transcription factor ATF-3

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Severe, bacterial infections (e. g. erysipelas) lead to shock fragments (fever, hypotonia) through the release of bacterial toxins. Despite similar infection pathways, the clinical courses vary widely between patients. Part of these reactions are due to bacterial toxins, the best studied being lipopolisaccharides (LPS), associated with gram- bacteria. IL-1, TNF and IL-6 released from dendritic cells (DC) and macrophages are responsible for most of the acute phase reactions, such as fever, hypotonia and shock. ATF-3 (activating transcription factor 3) is a central transcription factor, activated in response to TLR4-mediated signalling; ATF-3 seems to initiate a negative feedback loop. As we found that increased oxidative stress inhibits production and release of inflammatory cytokines, namely IL-6, we analyzed the effects of ROS on the regulation of ATF-3 and their role in LPS-induced septic shock. Stimulation of bone marrow-derived DC (BMDC) and peritoneal macrophages (PM) at either high or low conditions of ROS through TLR-4 with LPS resulted in moderate induction of ATF-3 and IL-6. High ROS levels did not directly modulate ATF-3 mRNA or protein. Yet, under conditions of high ROS levels, LPS induced a three- to four-fold increased expression and production of ATF-3. This increase in ATF-3 was associated with a significant inhibition of IL-6 mRNA and protein. This inhibitory effect was AFT-3-dependent, as stimulation of BMDC or PM from ATF-3.KO mice with LPS resulted in increased IL-6 mRNA and production at conditions of low and of high ROS concentrations. Importantly, high ROS levels induced ATF-3 in vivo during toxin-induced septic shock and protected mice from lethal shock. In contrast, this protective ROS effect was abrogated in ATF-3.KO mice. Thus, regulation of ATF-3 by ROS regulates IL-6 mediated inflammatory immune responses, such as sun burn reactions, erysipelas and is critical for the survival in response to bacterial toxins.

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Maintenance of long-lived plasma cells and serological memory despite mature and memory B Cell depletion during CD20 immunotherapy in mice

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CD20 mAb-mediated B cell depletion is an effective treatment for B cell malignancies and some autoimmune diseases, including systemic lupus erythematosus and pemphigus vulgaris. However, the full effects of B cell depletion on natural, primary, and secondary antibody responses, and the maintenance of Ag-specific serum Ig levels are largely unknown. The relationship between memory B cells, long-lived plasma cells, and long-lived humoral immunity also remains controversial. To address the roles of B cell subsets in the longevity of humoral responses, mature B cells were depleted in mice using CD20 mAb. Peritoneal B cell depletion reduced natural and Ag-induced IgM responses. Otherwise, CD20+ B cell depletion prevented humoral immune responses and class switching, and depleted existing and adoptively-transferred B cell memory. Nonetheless, B cell depletion did not affect serum Ig levels, Ag-specific antibody titers, or bone marrow antibody-secreting plasma cell numbers. Co-blockade of LFA-1 and VLA-4 adhesion molecules temporarily depleted long-lived plasma cells from the bone marrow. CD20+ B cell depletion plus LFA-1/VLA-4 mAb treatment significantly prolonged Ag-specific plasma cell depletion from the bone marrow, with a significant decrease in Ag-specific serum IgG. Collectively, these results support previous claims that bone marrow plasma cells are intrinsically long-lived. Further, these studies now demonstrate that mature and memory B cells are not required for maintaining bone marrow plasma cell numbers, but are required for repopulation of plasma cell-deficient bone marrow. Thereby, depleting mature and memory B cells does not have a dramatic negative effect on pre-existing antibody levels.

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Dibutylphthalate plays a role as adjuvant in FITC-induced contact hypersensitivity by inducing thymic stromal lymphopoietin expression in mice

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Fluorescein isothiocyanate (FITC) is known as a hapten preferentially inducing type II helper T cell (Th2)-mediated immune responses in mice. Thymic stromal lymphopoietin (TSLP) is one of the most important cytokines contributing to Th2-dominant allergic pathogenesis such as asthma or atopic dermatitis (AD) and is known to indirectly differentiate naïve T cells to inflammatory Th2. Moreover, it has been reported that keratinocyte-specific expression of TSLP resulted in AD-like inflammatory symptoms in mice and that AD patients expressed increased level of TSLP protein in their keratinocytes. In the present study, we demonstrated that FITC-induced allergic delayed type hypersensitivity (DTH) might require topically applied dibutylphthalate (DBP) which induced TSLP expression at the mRNA and the protein levels. Mice were sensitized on shaved belly with FITC dissolved in acetone:DBP [1:1] (ADBP) and then were challenged on ear with FITC dissolved in ADBP. FITC-induced DTH edema in challenged ears or cytokines production from lymph node cells (LNCs) were evaluated 24 hours after the challenge. FITC induced ear swelling which was accompanied by Th2 cytokines production from LNCs. However, FITC-induced DTH response was totally abrogated, when DBP was removed from the solvent for FITC sensitization or challenge. To determine the essential effects of DBP in FITC-induced DTH, we topically stimulated ears with DBP and evaluated TSLP expression levels in the ears by quantitative polymerase chain reaction or enzyme immunoassay. The expression of TSLP was increased at the mRNA and the protein levels. Interestingly, picryl chloride, which was another hapten preferentially induced Th1 response, did not affect the expression of TSLP mRNA in painted ear. These results suggested that TSLP played an important role to induce Th2-mediated inflammatory allergic responses in skin and that probably kinds of environmental toxic material, like DBP, may affect atopic predisposition in human.

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IL-18 gene polymorphism -137 G/C is associated with susceptibility to psoriasis vulgaris and not to atopic dermatitis in Japanese patients

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BACKGROUND: It is reported that there is a significant correlation between serum interleukin (IL)-18 levels and severity of psoriasis and atopic dermatitis (AD). In keratinocytes of lesional AD or psoriasis, there is a significantly higher expression of the IL-18Ra as compared with keratinocytes from normal donors. IL-18 gene has SNP at positions –137 G/C in the promoter region. The SNP has been reported to be associated with AD in Europeans and with atopic asthma in Japanese. OBJECTIVE: To examine whether this SNP is associated with susceptibility to AD and psoriasis vulgaris (PsV) in Japanese patients. METHODS: We examined 160 AD, 153 PsV patients and 104 healthy individuals. Genotyping was performed using the polymerase chain reaction-restriction fragment length polymorphism method. RESULTS: There was significant differences in genotype and allele frequencies between PsV patients and controls (genotype: P=0.044, allele: P=0.020). No significant association between the genotypes or alleles of this SNP and susceptibility to AD was observed (genotype: P=0.080, allele: P=0.14). CONCLUSION: These results suggest that the -137 SNP of the IL-18 gene is associated with susceptibility to PsV and not to AD in Japanese population.

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Utilization of the recombinant wheat proteins for diagnosis of wheat-dependent exercise-induced anaphylaxis

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Previously we identified wheat ω-5 gliadin and high molecular weight glutenin subunit (HMW-glutenin) as major allergens for wheat-dependent exercise-induced anaphylaxis (WDEIA) and defined IgE-binding epitopes of these allergens. In this study, we produced recombinant ω-5 gliadin and HMW-glutenin, and examined the usefulness of IgE detection for these recombinant proteins for the diagnosis of WDEIA. Recombinant ω-5 gliadin and HMW-glutenin were expressed in *E.coli* by using pET system, and conjugated to ImmunoCAP. Allergen-specific IgE levels for sera were detected in the patients with WDEIA who had a positive-provocation test, healthy subjects without episodes of food allergy and patients with atopic dermatitis (AD) without episodes of wheat allergy. As a result, ω-5 gliadin specific IgE was detected in 83.6% of WDEIA patients, 6.5% of AD patients and 0% of healthy subjects. HMW-glutenin specific IgE was also examined in the patients with WDEIA. With a combination of ω-5 gliadin and HMW-glutenin, positive rate was found to be 90.9% of the patients with WDEIA. Our results suggest that recombinant wheat proteins are useful tools for the diagnosis of WDEIA.

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PXR controls T lymphocyte function in inflammatory skin disease

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Pharmacologic activation of PXR has been reported to inhibit NF-kappaB signaling. We here report that PXR activation anergizes T cells by decreasing CD69, CD25 and IFN-gamma expression. We! show that PXR is expressed in T-lymphocyte rich infiltrates in inflammatory skin disease. Whereas PXR is expressed at low levels in lymphocytes under basal conditions, its expression is markedly up-regulated upon immune activation. PXR deficiency results in T cell hyperproliferation. In vivo, the modulation of T cell function by PXR activation demonstrates anti-inflammatory effects in mouse models of irritant dermatitis and allergic contact hypersensitivity. Together, these results indicate a regulatory role of PXR in the cutaneous immune system. Supported by Austrian Science Fund grant P16990-B05.

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CD4+CD25+ regulatory T cells inhibit the priming of CD8 T cells in a murine contact hypersensitivity model by suppressing stimulatory functions of dendritic cells

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Contact hypersensitivity (CHS) is induced by sensitization of individuals against haptens. This sensitization occurs in the draining lymph nodes (dLN) where the antigen-specific priming of CD8+ T cells by dendritic cells (DCs) takes place. Our initial studies showed that CHS reactions can significantly be reduced by injection of CD4+CD25+ regulatory T cells (Treg) before sensitization. Thus, we aimed to investigate the means by which Tregs cause the reduction of the immune reaction in a murine CHS model. At first, we analyzed the influence of the LN homing, CD4+CD25+CD62L+ Treg subpopulation on the most important cells involved in the sensitization phase, namely DCs and CD8+ T cells. After injection into mice, Treg migrated to the LN and showed a high affinity for interaction with DCs after sensitization, which was demonstrated by fluorescence microscopy of cryosections of the dLN stained with fluorescence markers for Treg, and DCs or CD8+ T cells, respectively. In in vitro co-cultures of Treg and bone marrow derived DCs (BMDC) we could detect a close contact between these two cell types. This was accompanied by a reduction of the antigen-presenting capacity of the BMDCs. Likewise in vivo, functional changes of the DCs after contact with Treg became apparent when DCs were isolated from Treg injected mice and cultured together with CD8+ T cells in vitro. Here, proliferation of CD8+ T cells was significantly lower when in vivo Treg-exposed DCs were used as stimulators as compared to control DCs. On the whole our data indicate that the T cell stimulatory capacity of hapten-presenting DCs is suppressed by Treg and thus provides a means by which CD4+CD25+ regulatory T cells suppress the priming of CD8+ effector cells during the sensitization against haptens.

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IL-10 producing suppressor T cells induced by non-pathogenic gram-negative bacteria clear atopic dermatitis

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Non-pathogenic bacteria are part of the physiological flora on skin and gut epithelium without inducing inflammation. Some strains have even been used to alleviate allergic inflammation. We therefore performed a prospective, double-blind, placebo controlled, randomized clinical trial with 80 patients to investigate the potential of non-pathogenic, gram-negative, thermal water bacterium Vitreoscilla filiformis (Vf) applied to atopic patient skin. Vf mediated reduction of SCORAD and pruritus was highly significant compared to placebo. To understand the underlying mechanisms, Vf activated dendritic cells (DC) were analyzed in detail. Several Vf preparations led all to DC maturation but marked differences were seen in regard to cytokine production. Isolated Vf LPS induced high levels of IL-12p70 via a TLR4-dependent pathway while IL-10 levels remained low. Investigating the complete Vf bacterial extract revealed that the effects of LPS were only minor with IL-12 levels being low while large amounts of IL-10 were secreted. Analyzing DC from several knock-out and wildtype mice demonstrated that IL-10 production induced by Vf was almost completely dependent on TLR2 suggesting a dominant immuno-modulatory TLR2 ligand. Indeed, cocultures of Vf stimulated DC with naïve T helper cells resulted in the induction of remarkable levels of IL-10 in Th cells. To investigate functional consequences, cocultures with Th1 effector cells were carried out demonstrating strongly suppressed proliferation of T effector cells by the addition of these IL-10+ Th cells. Thus, effective treatment of atopic dermatitis by application of Vf may be the result of Vf induced, TLR2 dependent IL-10 induction in DC orchestrating the induction of IL-10+ suppressor T cells. Our data demonstrate not only a new strategy for skin diseases like atopic dermatitis, but also a possible general principle of how non-pathogenic bacteria stabilize the immune barrier of surface organs such as the skin or the gut.

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Langerhans cells induce de novo regulatory T cells by presenting epidermal self-antigen

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Langerhans cells (LCs) and dermal dendritic cells (DDCs) migrate into skin-draining lymph nodes (LNs) in steady state. Although cross-presentation of epidermis self-antigen by DCs has been well demonstrated, little is known about MHC class II presentation. Here we show epidermal self-antigen presentation by DCs on MHC class II in vitro and in vivo. K5-mOVA is a transgenic mouse expressing ovalbumin (OVA) in keratinocytes. OVA-specific CD4+ cells of OT-II cells were used as epidermal self-antigen specific T cells. When OT-II cells were incubated with CD40hi migratory DCs of skin-draining LNs from K5-mOVA, they proliferated in vitro. Adoptively transferred OT-II cells were activated and increased Foxp3+ population in K5-mOVA mice, but not in chemokine receptor CCR7-deficient background, or in LC-ablated Langerin-DTR background. Adoptively transferred naïve CD4+ T cells of OT-II x RAG-1-/- mice were converted into Foxp3+ CD25+ regulatory T cells in K5-mOVA mice. These results suggest that migratory DCs, especially LCs, transport epidermal self-antigen to skin-draining LNs, present it on MHC class II, and induce de novo regulatory T cells.

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Treatment of established RMA-Tag tumors via DC directed targeting of tumor antigens is further improved by depletion of regulatory T cells

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Tumor antigens, chemically coupled to the antiDEC205 antibody, can target dendritic cells in vivo and were successfully applied in murine preventive tumor vaccination studies. In this study our aim was to test whether antiDEC205-protein conjugates have the potential to reduce the tumor growth of already established tumors. For this end we coupled the tumor antigen Tag to antiDEC205 and treated RMA-Tag tumor-bearing mice with these conjugates. When the tumors had reached an average diameter of 5 mm the mice were injected with antiDEC205-Tag conjugate or Tag protein, plus adjuvands in 5-day intervals. Although tumor growth was initially slowed down after injection of the antiDEC205-Tag conjugates as compared to Tag-treated mice, it resumed two to three weeks after the first vaccination. Analysis of the immune response revealed initially a strong induction of tumor specific CD8+ cells in antiDEC205-Tag treated mice. However, when tumor growth revived increased numbers of CD4+CD25+FoxP3+ regulatory T cells (Tregs) in the tumor draining lymph node were noticeable. Thus, to assess the suppressive effects of Tregs during tumor therapy, anti-CD25 antibodies were used to deplete Tregs from tumor bearing animals during antitumor treatment. By concomitant injection of antiDEC205-Tag conjugates we could cure up to 40% of the tumor bearing mice and tumors were rejected. Furthermore, the survival of tumor bearing mice of the autochthonous tumor model RIP-Tag5 could be prolonged for up to ten weeks after treatment with antiDEC205-Tag conjugates compared to untreated, or Tag-treated mice respectively. In aggregate our data indicate that depletion of CD25+ Treg during cancer therapy bolsters the effectiveness of DC based anti-tumor therapies and that antiDEC205 targeted vaccination also improves treatment of autochthonous tumors.

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Immunization with tumor antigens fused to a novel single chain fragment variable for murine DEC205 suppresses melanoma growth in a B16 Tumor model

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The DEC-205 receptor is expressed specifically by DCs and greatly increases antigen presentation. Our first aim was to create a novel single chain fragment variable (ScFv) specific for DEC-205 in order to target DCs in vivo. The novel ScFv was created through RT-PCR using degenerative primers on total RNA from a hybridoma cell line (HB290) producing a monoclonal antibody for murine DEC-205. The isolated variable heavy and variable light regions were subcloned into an appropriate expression vector and then expressed as a native protein in the E. coli. To combine the ScFv for mDEC-205 with various melanoma tumor antigens, we created fusion proteins using monopeptide mGP100, polypeptide mGP100 or a combination of polypeptides from mTRP2 and mGP100. Initial immunohistochemical staining of cytospins from CD11c+ isolated cells displayed a positive staining of ScFv comparable to the monoclonal antibody. In further experiments, mice were injected with the ScFv-mGP100 fusion protein or control saline subcutaneously. Using immunohistochemistry the mice injected with ScFv had positive stained DCs in the lymph nodes, using an ScFv-specific anti c-myc antibody. We then examined the tumor suppressive effects of the ScFv-mGP100 in the B16 transplantable tumor model. When the vaccination regime was implemented after a visible tumor has formed at the site of s.c tumor cell injection, we observed suppression of tumor growth from the ScFv-mGP100 in comparison to mGP100 peptide injected, ScFv-control injected or WT mice. Furthermore the induction of GP100 specific CD8+ T cells examined via ELISPOT IFN-γ assays indicated the superior antigen targeting and presentation of the ScFv-mGP100 fusion protein. Together our data indicate the novel ScFv is a viable method of targeting various tumor antigens to dendritic cells in order to elicit an improved immune response.

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Synergistic activations of murine mast cells by monomeric IgE and Toll-like receptor ligands

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Mast cells are distributed in various tissues throughout the body,and can trigger allergic and inflammatory responses by releasing a wide variety of mediators. Recent studies have suggested the possibility that mast cells are activated by the binding of Immunoglobulin (Ig) E to the high affinity receptors for IgE (FcεRI). Mast cells are also activated by receptors other than FcεRI,such as Toll-like receptors (TLR),by which pathogen-associated molecules from various microbes are recognized. Since infections with various pathogens including bacteria and viruses are known to exaggerate allergic diseases,we examined whether the combination of monomeric IgE and TLR ligands can synergistically activate mast cells. Bone marrow-derived murine mast cells were stimulated by various concentrations of monomeric IgE and TLR2 or TLR4 ligands (peptidoglycan (PGN) and lipopolysaccharide (LPS)). The concentrations of cytokines in the culture supernatant were determined by ELISA. Activation of MAP kinase was investigated by Western blotting. Cytokine production stimulated by monomeric IgE was further enhanced by the addition of LPS or PGN. Enhancement by LPS or PGN on cytokine production was mediated by TLR4 and TLR2, respectively,since TLR4 or TLR2–deficient mast cells did not show synergistic activation by monomeric IgE and LPS/PGN. Synergistic activation of mast cells was obtained via phosphorylation of several MAPKs. These results suggested that the binding of IgE to its receptor with simultaneous TLR activation on mast cells might contribute to the exaggeration of allergic diseases.

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Decreased endothelial activation results in defective secondary T cell immunity in aging skin

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Defective immunity occurs in the old and is associated with cutaneous infection and cancer. We investigated the kinetics of a cutaneous antigen-specific CD4+ T cell response in old and young volunteers to determine whether there was a specific defect in secondary immunity during aging. We injected candida antigen into the skin of young and old subjects and biopsied or raised suction blisters over the injected site. Old subjects had significantly decreased clinical responses to candida injection ($P<0.001$). Furthermore, the peripheral blood T cell proliferative response to candida in vitro was similar in young and old indicating that the defect was specific for skin and not a global decrease in anti-candida immunity. Adhesion molecule expression by the endothelium in old skin (E-selectin,ICAM-1,VCAM-1) was significantly reduced. The expression of these molecules is controlled by TNF-α and IFN-γ and levels of these cytokines at the site of candida antigen injection($n=10$ old, 6 young) were significantly lower in old compared to young ($P<0.01$). We confirmed that CD4+ T cells from both old and young could migrate across dermal endothelial monolayers that were stimulated with TNFα and IFNγ but that the migration could be blocked by antibodies to E-selectin, ICAM-1 and VCAM-1 in vitro. Old individuals therefore develop a specific cutaneous defect that is associated with defective inflammation and endothelial activation. This indicates first, that cutaneous responses to antigen cannot be used to inform on immune status of old subjects, second increased skin infection and malignancy during aging may be linked to this problem and finally, identification of the defective inflammatory pathway is crucial for the promotion of cutaneous immunity in the old.

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Vitamin D3 derivatives, alone or in combination with glucocorticoids, suppress streptococcal pyogenic enterotoxin A-stimulated proliferation of PBMCs in psoriasis patients

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Bacterial colonization on the skin or the tonsils might influence the clinical response of psoriasis patients to immunosuppressive drugs, thus affecting their efficacy. However, to date, few studies have been carried out to investigate the effect of bacterial superantigens on the treatment of psoriasis. Recent clinical evidence has shown that psoriasis therapy combining topical glucocorticoids (GCs) and vitamin D3 is more effective than GCs or vitamin D3 monotherapy alone. We evaluated the suppressive effect of betamethasone butyrate propionate (BBP), three vitamin D3 (VD3) derivatives, cyclosporine, and a combination of BBP and VD3 on concanavalin A (conA)-or superantigen-induced proliferation of peripheral blood mononuclear cells (PBMCs) obtained from 35 psoriasis patients. *In vitro*, drug concentrations causing 50% inhibition (IC50s) of PBMC-proliferation stimulated by conA or streptococcal pyrogenic enterotoxin A (SPEA) derived from hemolytic streptococci were recorded. Concentrations of TNF-α, interferon (INF)-γ, interleukin (IL)-1b, -2, -4, -5, -6, -8, -10, or -12 in PBMC-culture media were also measured by bead-array assay. The efficacy of BBP significantly decreased when PBMCs were stimulated by SPEA, as compared to PBMCs stimulated by conA. However, the efficacy of calcipotriol and tacalcitol significantly increased when PBMCs were stimulated by SPEA, as compared to PBMCs stimulated by conA. The effects of BBP on PBMC-proliferation stimulated by SPEA significantly improved with the addition of 1-1000ng/ml calcipotriol as compared to the effects of BBP alone. BBP alone or in combination with calcipotriol suppressed cytokine production of SPEA-stimulated PBMC, as compared to calcipotriol alone. We concluded that SPEA induced by the colonization of *hemolytic streptococci* reduces the therapeutic efficacy of BBP, whereas VD3 derivatives were not affected.

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Vitreoscilla filiformis bacterial extract, to improve the efficacy of emollient used in atopic dermatitis symptoms

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Atopic dermatitis is a common chronic skin disease and a defect in the epidermal barrier function lead to increase penetration of pro-inflammatory environmental compounds. The daily use of emollients is the first line therapy of AD. Vitreoscilla filiformis is a micro-organism found in sodium rich spa waters. La Roche Posay (LRP) spa water has been used for a long time in the treatment of skin inflammatory disorders, especially AD. Because the LRP water containing microelements possess anti-pruritus and anti-inflammatory effects, we hypothesized that V.f. culture in LRP water, may result in bacterial extract which combines properties of the V.f. extract and of the LRP water. Therefore LRP Biomass may be endowed with optimal emollient activity for the management of AD abnormalities and the prevention of relapses. In a monocenter, intra-individual left-right comparison study, we show that a cream containing 5% LRP Biomass is able to improve AD symptoms to a greater extent than that of a standard cream formulated with LRP water. A standard base cream was prepared with either 75% distilled water (dw) or LRP water (LRPw). Dw cream was used during the wash out and post treatment periods. The verum (5% LRP Biomass-containing dw cream) and the control cream (LRPw cream) were applied twice daily on symmetrical AD lesions. Results show that the improvement of AD lesions for the 5% LRP Biomass was more efficient than the LRPw cream at day 21, maintained at day 28 and during the 2 weeks follow-up ($p=0.0041$). A significant reduction in itching was observed in both cream with no difference between them, which may be related to the presence of selenium and strontium from the LRP water in both emollient formulations. The results confirm our previous study and suggest that culture the microorganism in LRP water may improve the efficacy of the V.f. extract to reduce skin symptoms associated with atopic xerotic skin.

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Expression of self-antigens on bone marrow-derived dendritic cells induce peripheral tolerance to self-antigen-specific TCR-transgenic CD8$^+$ T cells

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Self-antigen presentation by thymic epithelial and dendritic cells (DCs) is a well recognized mechanism of central tolerance. Here we directly address whether self-antigen presenting extra-thymic dendritic cells alone can maintain self-tolerance. We utilized two different lines of transgenic (Tg) mice in which ovalbumin (OVA) is expressed as a membrane protein at different levels by stratified squamous epithelial cells via the keratin-14 promoter. mOVA(6) mice have a higher copy number of the transgene than mOVA(3) mice. When OVA-specific TCR-Tg CD8$^+$ T cells (OT-I cells) were adoptively transferred into mOVA(6) mice, the mice consistently developed GVHD-like skin disease. However, mOVA(3) mice were tolerant to the adoptively transferred OT-I cells and did not develop disease. This finding does not reflect differences in gene copy number or antigen expression level since mOVA(6) heterozygotes consistently developed skin disease to almost the same degree as mOVA(6) homozygotes while mOVA(6)×mOVA(3) F1 mice never developed disease after OT-I cell transfer. mOVA(6) mice chimerized with mOVA(3) bone marrow (BM) cells also did not develop disease, indicating that the tolerance observed in mOVA(3) mice could be transferred by BM-derived cells. Aberrant expression of the OVA transgene by blood cells, including DCs, of mOVA(3) mice, as evidenced by PCR, suggests a likely mechanism for tolerance transfer. mOVA(3) mice on a Rag-/- background were also tolerant to the adoptively transferred OT-I cells, which suggests that neither T nor B cells were required for tolerance to ensue, a finding consistent with those above. OT-I cells taken from tolerized mice expressed lower levels of both CD3 and TCR and higher levels of PD-1 compared to OT-I cells obtained from mice with GVHD-like skin disease. Taken together, we demonstrate that self-antigens expressed by BM-derived DCs can induce peripheral tolerance to antigen-specific CD8 T cells.

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Induction of human beta-defensin 3 in keratinocytes by a poly-G oligodeoxynucleotide

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Despite of its permanent exposure to microbes intact human skin is remarkably resistent to invasion by pathogens. Previous studies have shown that the production of antimicrobial peptides might be an important reason for this. Since it is known that DNA oligodeoxynucleotides can have anti-inflammatory effects on skin we investigated their influence on the expression of antimicrobial peptides. Surprisingly, a poly-G oligodeoxynucleotide strongly induced the human beta-defensin 3 (hBD-3) gene expression in reconstructed human epidermis as detected by real-time-PCR and western blot analysis. Moreover, we could confirm our results in the Phenion® Full Thickness Skin Model by immunostaining with a polyclonal antibody to hBD-3. To our knowledge these results show for the first time the induction of an antimicrobial peptide by DNA oligodeoxynucleotides providing new insight into the regulation of these molecules. Furthermore, our work suggests the possibility of treating infections and superinfections of the skin with poly-G oligodeoxynucleotides.

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Enhancement of DNA-vaccine induced protection against lymphoma using limiting amounts of helper plasmid-encoded antigen

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Nucleic acid-based vaccines are successful in infectious disease models, but their efficacy in targeting tumor-associated self-antigens has been limited. Administration of helper antigens with the tumor antigen may improve vaccine efficacy. We previously developed DNA-based vaccines encoding the alpha or beta chains of the T cell receptor of a model T cell lymphoma (MBL-2), and determined that they failed to protect mice against subcutaneous tumor challenge when delivered via a gene gun. A single-chain fusion construct encoding the two chains was only marginally efficacious. High levels of tumor protection were achieved with constructs encoding the beta or alpha + beta chains, however, when they were delivered with a low dose of plasmid encoding beta galactosidase as a helper antigen. Protection required co-delivery of the TCR and helper antigens on the same gold particles, suggesting a requirement that TCR and helper antigens be expressed by the same antigen presenting cells. We wondered if the helper effect of foreign antigen depended on (1) the nature of the antigen, (2) a distinct ratio between the target- and the helper-antigen doses and (3) the physical form of the two antigens (fusion vs. independent molecules). Using the single-chain TCR fusion construct and the MBL-2 tumor rejection model, we found that (1) fragment C of tetanus toxin could substitute for beta galactosidase, and (2) only low doses of fragment C were effective. No helper effect was achieved when the helper and tumor antigens were contained in a fusion protein or when helper and tumor antigens were expressed at similar levels. These findings indicate that helper antigen can be used to enhance tumor vaccine efficacy but delivery as a fusion protein is not optimal, perhaps because of immunodominance of exogenous determinants. Careful titration of helper antigen dose may establish a ratio of helper to tumor antigen that allows a significant helper effect to be achieved.

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Differential EpCAM expression and growth factor requirements reveal heterogeneity of dendritic cells in murine skin

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Normal murine skin was found to contain at least three subpopulations of dendritic cells (DC) that can be distinguished based on differential expression of the C-type lectin langerin and the adhesion molecule EpCAM. EpCAM was easily detected on DC surfaces while langerin was not. Langerhans cells (LC) in epidermis, dermis and lymph nodes all expressed abundant EpCAM and langerin. Dermal DC did not express EpCAM, but a minor subpopulation was langerin-positive, corresponding to the newly identified langerin-positive dermal DC. LC migrated from skin explants in the absence of any exogenous cytokines, while emigration of langerin-positive dermal DC was dependent on CCL21. EpCAM-negative, langerin-negative dermal DC did not migrate ex vivo. Studies of mice that had been conditionally depleted of langerin-expressing cells and bone marrow chimeras revealed that EpCAM-negative langerin-positive DC repopulated the dermis well before Ep-CAM-positive langerin-positive LC appeared in epidermis, that EpCAM-positive and EpCAM-negative DC were found exclusively in the epidermis and dermis respectively, and that DC populations in each compartment could proliferate. EpCAM-positive langerin-positive LC were not present in the epidermis, dermis or lymph nodes of Rag2 -/- TGFβ1 -/- mice, whereas EpCAM-negative langerin-positive dermal DC were frequent. Differential expression of EpCAM and langerin by DC defines three subpopulations in murine skin that are functionally distinct and that may represent different lineages. The precise roles that these three DC subpopulations play in skin-centered immune responses remain to be delineated.

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IL-15 is a critical costimulator in determining the activity of autoreactive CD8 Tcells

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We previously reported that autoreactive TCR-transgenic (Tg) CD8 T cells (OT-I cells) adoptively transferred into K14-mOVA^{high}(high transgene copy number-TCN) Tg mice induced GvHD while K14-mOVA^{low}(low TCN) Tg mice did not develop disease. Exogenous IL-15, administered in vivo or in culture with OT-I cells, rendered cells immunogenic in K14-mOVA^{low}Tg mice. Conversely, when we used αIL-2/IL-15RβmAb or αIL-15mAb to block endogenous IL-15 function, GvHD was attenuated in K14-mOVA^{high}Tg mice. Furthermore, K14-mOVA^{high}Tg mice on an IL-15KO background did not develop GvHD after OT-I cell transfer. Collectively these results identify IL-15 as a critical costimulator that determines the outcome of immune responses by affecting the checkpoint determining whether effector function or unresponsiveness ensues. To determine the precise mechanism of IL-15 as a costimulator, we established a gene microarray system to determine the checkpoint where the effect of IL-15 is critical and compared gene expression patterns of OT-I cells cultured under different conditions. When OT-I cells were cultured with different concentrations of peptide, 0.3 pg/ml of peptide did not induce proliferation of OT-I cells. In the presence of 15nM of IL-15, 0.3 pg/ml of peptide induced maximum responses of OT-I cells, while IL-15 alone induced only minimal responses. These results indicate that 0.3 pg/ml of peptide is a suboptimal dose of antigen to induce immune response and IL-15 converted OT-I cells from being unresponsive to having effector functions. We then performed microarray on OT-I cells cultured with (1) peptide (0.3 pg/ml)+IL-15(15nM), (2) peptide alone or, (3) IL-15 alone. As expected, Bcl-2, CIS, and several JAK-STAT genes were upregulated in the IL-15 treated cells. We also found that several other genes were upregulated in OT-I cells cultured with peptide and IL-15, suggesting that there are important genes involved in regulating this checkpoint. Our findings provide new evidence that cytokines may be essential costimulators that affect this critical checkpoint.

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CD8$^+$DEC205$^+$ spleen dendritic cells are specialized antigen presenting cells to differentiate Foxp3$^+$ regulatory T cells from Foxp3$^-$ precursors using endogenous TGF-β

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Foxp3$^+$CD25$^+$CD4$^+$ regulatory T cells (T reg) mediate immunological self-tolerance and are able to suppress tumor immunity, allergy, transplantation rejection and immune responses to microbes. We have previously reported that spleen dendritic cells (DCs) were 100 fold more potent than DC-depleted antigen presenting cells (APCs) for the induction of Foxp3$^+$CD25$^+$CD4$^+$ regulatory T cells from peripheral Foxp3$^-$CD4$^+$ precursors with small amounts of added TGF-β (Yamazaki et al, Blood, 2007). Here we further investigated the role of 2 major DC subsets from mouse spleen for the differentiation of Foxp3$^+$ T reg from Foxp3$^-$ DO11.10 RAG$^{-/-}$OVA transgenic CD4$^+$T cells. We find that CD8$^+$ DEC205$^+$ DCs, relative to CD8$^-$ 33D1$^+$ DCs, are specialized to induce Foxp3$^+$ T reg from Foxp3$^-$ precursors without added TGF-β, both in vitro and in vivo. In vitro, the CD8$^+$ DEC205$^+$ DC subset was able to differentiate some Foxp3$^+$ T reg from Foxp3$^-$ precursors using endogenous TGF-β, whereas the CD8$^-$ 33D1$^+$ DC subset induced more Foxp3$^+$T reg in the presence of exogenous TGF-β. In vivo, when adoptively transferred DO11.10 RAG$^{-/-}$Foxp3$^-$CD4$^+$T cells were stimulated with OVA antigen delivered within anti-DC mAb, antigen delivery to CD8$^+$ DEC205$^+$ DCs by DEC-205 mAb differentiated more Foxp3$^+$ T reg from Foxp3$^+$ precursors than antigen delivery to CD8$^-$33D1$^+$ DCs by 33D1 mAb. These results indicate that the CD8$^+$ DEC205$^+$ DC subset in spleen is specialized to differentiate peripheral Foxp3$^+$ T reg, which might be useful for a designing a mAb therapy that induces antigen specific Foxp3$^+$ T reg for autoimmune diseases, transplantation rejection or allergy.

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Suppressing IgE responses and memory B cells by a gene therapy approach using IgE specific super antigen

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IgE plays a critical role in some host defenses, but antigen binding to IgE activates mast cells and induces allergic responses. To suppress IgE we explored the effect of expressing a super antigen against IgE *in vivo* using a gene-therapy approach. Single chain fusion genes were generated from two anti-IgE hybridomas: R1E4 and EM95. R1E4 recognizes free IgE but not IgE/FceR1 complexes and is neutralizing, whereas EM95 is non neutralizing. Genes encoding chimeric single chain anti-IgE antibody were fused to mouse IgG1 Fc domain and expression plasmids generated. R1E4 plasmid transiently expressed *in vivo* led to the appearance in sera of several ug/ml soluble anti-IgE that neutralized circulating IgE completely for >3 weeks. Levels of IgE bound on mast cells and basophils also declined. Soluble anti-IgE effectively suppressed IgE levels even in mice with high initial IgE levels, such as those preimmunized with ovalbumin in alum or given anti-IgD injection. However, in the latter case qPCR data indicated that IgE mRNA was still present and FACS analysis revealed that intracellular IgE+ plasma cells were retained after treatment. Importantly, R1E4 could bind membrane IgE expressed on memory B cells and also could suppress the IgE secretion of IL4+anti-CD40 stimulated splenocytes, indicating that the mechanism of action of anti-IgE involved not only blocking of IgE binding to FceR1 but also apoptosis or suppression of IgE+ memory cells. Expression of a transmembrane form of R1E4 was less effective than the soluble form. Interestingly, expression of EM95 in vivo as a membrane bound form was rapidly extinguished in high IgE mice, indicating that IgE binding to the plasma membrane was highly immunogenic or toxic to the (liver) expressing cells. Although R1E4 is not entirely comparable to Omalizumab, the anti-human IgE, our results suggest that long term anti-IgE therapy may be beneficial because it can kill surface IgE+ B cells including developing B cells, memory B cells and pre-plasma cells. In addition, anti-IgE targeting may be useful as an adjuvant for tumor therapy, vaccination.

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Disease spectrum of late-onset lupus (≥ 50 yrs) presenting to dermatology from 1996-2006

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We sought to investigate if age at onset has a modifying effect on the clinical features of lupus erythematosus(usually thought a disease of females in fertile years),as previously reported. We recorded data by retrospective chart review of 35 consecutive patients whose first presentation of cutaneous lupus was ≥50 yrs. Results are compared with corresponding retrospective data of 60 patients diagnosed with SLE in a connective tissue clinic in the same hospital. Fisher's exact test was used in statistical analysis. Female:male ratio was 4:1 (28:7). Average age of onset was 65.2 yrs (51-80 yrs). Average time to diagnosis was 44 weeks (3 wks-5 yrs). The most common cutaneous diagnosis was subacute cutaneous lupus (23/35,66%), discoid lupus in 11/35 (31%) and lupus panniculitis in 1/35. Nine patients (25%) fulfilled ARA criteria for SLE. Pulmonary symptoms were found in 34% (10/29) and 53% (15/23) had neuropsychiatric morbidity. Thrombotic episodes were noted in 27% (7/26) and 39% (12/31) had sicca symptoms. Sixty-nine percent (18/26) of patients had hypertension. A new drug (most frequently anti-hypertensive drugs) had been commenced in 43% (12/28)within 6 months of disease onset. One patient had hypertension-related chronic renal failure. Glomerulonephritis was not identified. Disease spectrum did not differ significantly between the groups. Laboratory findings of note included; positive ANA (≥1:160) in 62% (19/30), Ro/La antibodies in 90% (29/32), RF in 53% (8/15), DNA Crithidea in 0/22, ↓C4 in 20% (4/18) and anti-phospholipid antibody in 0/17. Cutaneous disease was well controlled in most with 47% responding to photoprotection, topical steroid and/or drug avoidance alone. There were 3 deaths, 2 unrelated and 1 of unknown cause. Subacute LE is the most common lupus presentation ≥50. New drug exposure may have relevance in the pathogenesis. There is notable frequency of pulmonary, neuropsychiatric, hypertensive and thrombotic events. Whether some of these findings are related to age, independent of lupus diagnosis is deserving of further study.

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Effect of silencing of DP1 mRNA expression in the skin by administration of siRNA on the scratching behavior of NC/Nga mice

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While itching and scratching are important factors in the development of atopic dermatitis, the pathogenetic mechanisms underlying these phenomena also remain unclear. We have previously reported that topical application of prostaglandin D_2 inhibited the scratching behavior of NC/Nga mice, a model of atopic dermatitis, via their specific DP1 receptor, but the precise mechanism of that action remains unknown. In this study, we showed, using an RT-PCR method, that the DP1 receptor mRNA was expressed in the skin and dorsal root ganglia (DRG) of the NC/Nga mice. This finding prompted us to investigate if prostaglandin D_2 inhibited the scratching behavior via their specific DP1 receptor expressed in the skin cells or on the sensory nerve endings. Therefore, we examined the effect of silencing of DP1 mRNA expression in the skin by the introduction of siRNA on the scratching behavior of the NC/Nga mice. Although introduction of the siRNA into the skin inhibited the DP1 mRNA expression in the skin of the NC/Nga mice, no significant effect on the scratching behavior of the NC/Nga mice was observed. These results suggest that the inhibitory effect of Prostaglandin D_2 on the scratching behavior is not mediated by the DP1 receptor expressed by the skin cells. DP1 receptor production in the DRG cannot be inhibited by the introduction of DP1 siRNA into the skin, since we have shown that the localization of the transfected siRNA was limited to the targeted skin area and some receptor proteins existing on the sensory nerve ending was produced in the DRG. These results suggest that the inhibitory effect of Prostaglandin D_2 on the scratching behavior may be via their specific DP1 receptor expressed on the sensory nerve endings in the NC/Nga mice.

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Interleukin 15 inhibits the function of dendritic cells in T cell activation and immune responses

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Interleukin-15 (IL-15) plays important roles in the regulation of immune responses. In IL-15 (IL-15-/-) or IL-15 receptor (IL-15R-/-) knockout mice, the function of NK and CD8+ T cells are impaired whereas CD4+ T cells are less affected. The effect of IL-15 on dendritic cells (DC), however, is largely unexplored. The current study shows that IL-15 inhibits the function of DC in the activation of T cells and induction of contact hypersensitivity responses (CHS). Adoptive transfer of hapten labeled IL-15-/- or IL-15R-/- spleen or bone marrow derived DC into naïve wild type (WT) mice induced a higher level of CHS than WT DC. Further analysis showed that the draining lymph node T cells from hapten labeled IL-15-/- DC treated mice produced higher levels of IFN-γ and IL-17 than those from hapten labeled WT DC treated animals and that hapten primed WT T cells in the culture with hapten labeled IL-15-/- DC also produced higher levels of IFN-γ and IL-17 than those in culture with WT DC. In addition, IL-15-/- or IL-15R-/- DC induced a higher level of OT-I and OT-II cell proliferation than WT DC. The increased activity of IL-15-/- DC in the induction of CHS and activation of T cells was abrogated by treatment with exogenous IL-15. In attempt to define mechanisms for IL-15 mediated inhibitory effects on DC, cytokine production by spleen and bone marrow derived DC was examined. We found that IL-15-/- or IL-15R-/- DC produced a lower level of IL-12p70, IFN-γ and IL-10 than WT DC. In contrast, the gene knockout DC produced a higher level of IL-12 p40 and IL-23 than WT DC. Treatment with exogenous recombinant IL-15 reduced the production of IL-12p40 and IL-23 whereas it increased IL-12p70, IFN-γ and IL-10 by IL-15-/- but not IL-15R-/- DC. The results implicate that IL-15 mediated increase of IL-10 production may be a primary mechanism for its inhibitory effects on DC functions. This provides insights into a novel mechanism for IL-15 mediated regulation of immune responses.

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Search for biomarkers to evaluate immunotoxicity of chemicals by gene expression analysis.

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We are always exposed to enormous numbers of chemicals. It is conceivable that these chemicals impact our immune system. In such a case, dendritic cells (DC) including Langerhans cells sense these chemicals as sentinel cells and trigger or modulate the immune response. Although uncountable numbers of chemicals are always invading our body through various routes, we hypothesize that DC respond to these chemicals according to some restricted patterns. In this study, to discover the common response patterns which are shared by DC stimulated with different chemicals, we stimulated human monocyte-derived DC (MoDC) with 5 different chemicals that have been reported to affect the immune system, 2,4-dinitrochlorobenzene (DNCB), $NiCl_2$, formalin, Diesel exhaust particles (DEP), and $HgCl_2$, and analyzed their effects on mRNA expression by MoDC using a DNA microarray (Human Genome U133 Plus2.0 GeneChip) and real-time PCR. To exclude the secondary response induced by the representative danger signal released from dying cells, ATP, we selected the genes which were either up-regulated or down-regulated by each chemical treatment but were not significantly affected by ATP treatment in two independent analyses as the chemical-modified genes. The chemical-modified genes were 600 up and 300 down by DNCB, 600 up and 300 down by $NiCl_2$, 600 up and 300 down by formalin, 600 up and 300 down by DEP, and 600 up and 300 down by $HgCl_2$ on average. Among them, we found that these 5 different chemicals commonly augmented IL-1a, IL-1b, MCSF, and IL-8. Real-time PCR analyses confirmed the results obtained by DNA microarray analysis. These data suggest that several chemicals share some common mechanisms by which they affect our immune system irrespective of their chemical structure. In addition, these genes can be candidates as a biomarker to predict the immunomodulatory activity of chemicals.

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The effect of Er:YAG laser for promoting skin vaccination

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Transdermal delivery of vaccines is attractive for treatment and prevention of diseases. However, this delivery method is limited by the low permeability of the stratum corneum (SC). The objective of this study was to enhance and control skin permeation of the vaccines using an erbium:yttrium-aluminum-garnet (Er:YAG) laser. The SC layer was partly ablated by an Er:YAG laser, resulting in a greater enhancement effect on delivery. A thinned and discontinuous SC layer was observed after laser exposure. The result of confocal laser scanning microscopy (CLSM) indicated a significant increase in the skin deposition of vaccine-related peptides into laser-treated skin. In an *in vivo* study, mouse skin was treated with the laser followed by skin vaccination with a lysozyme antigen. It was demonstrated that laser treatment with no adjuvant or penetration enhancer significantly enhanced the production of antibodies in the serum by 3-fold. To reveal the mechanisms underlying these changes, a proteomic technique combined with mass spectrometry was used. Laser-controlled and -enhanced peptide/vaccine delivery opens a new perspective in the development of non-invasive immunization methods, which will greatly facilitate the administration of human and veterinary vaccines.

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A novel approach to study tumor-specific CD8+ cytotoxic T lymphocytes in cutaneous T-cell lymphoma patients treated with extracorporeal photopheresis

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Cutaneous T-cell lymphomas (CTCLs), including Mycosis Fungoides (MF) and Sézary Syndrome (SzS), are non-Hodgkin's lymphomas characterized by clonal expansion of skin-homing malignant T cells. Extracorporeal photopheresis (ECP) is an FDA-approved therapy used frequently as front-line treatment for CTCL. ECP induces apoptosis of tumor cells and other normal cells. It is hypothesized that tumor antigen-loaded dendritic cells prime tumor-specific cytotoxic T lymphocytes (CTLs). It is unclear whether or not tumor-specific CTLs are affected by ECP. A major challenge is the limited number of tumor-specific CTLs present in patients. This study was undertaken to develop a new antigen-independent methodology for expanding tumor-specific CTLs from SzS patients undergoing ECP. The results of one patient with >50% circulating tumor cells were reported. Peripheral blood mononuclear cells (PBMCs) were isolated pre- and 1 month and 3 months post-ECP. CD8+ T cells were enriched by negative selection, and expanded *ex vivo* for 2 weeks with anti-CD2, anti-CD3, and anti-CD28 antibodies and IL-2. CTL activity was assessed by IFN-γ and granzyme B Elispot assay. CD8+ T cells were enriched from 9.7% to 55.7% pre-ECP, from 11.9% to 66.8% at 1 month, and 12.6% to 84.1% at 3 months post-ECP. After 12 days, cell numbers were increased by 3.0, 3.7 2.1-fold pre-ECP, 1 and 3 months post-ECP, respectively. IFN-γ and Granzyme B-producing CTLs were detected at all time points when autologous tumor cells but not normal CD4+ T cells were used as targets. IFN-γ and Granzyme B production was partially inhibited by HLA-ABC antibodies. In summary, tumor-specific CTLs are present in SzS patients with high tumor burdens, and can be enriched and expanded *ex vivo*. The study will continue to enroll patients with longer follow-up periods to further study the effects of ECP.

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CCR7-mediated migration of dendritic cells through activation of Rho kinase is regulated by Src homology 2 domain-containing protein tyrosine phosphatase substrate 1

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We previously reported that Src homology 2 domain-containing protein tyrosine phosphatase substrate 1 (SHPS-1) regulates the migration of Langerhans cells (LCs) from epidermis into the regional lymph nodes. Here we show that Rho kinase (ROCK) plays an important role in LC migration, and that SHPS-1 regulates the migration of dendritic cells (DCs) through the modulation of ROCK activation in response to the ligands of CCR7, CCL19. LC migration was assessed by the decreased number of epidermal I-A^+cells following DNFB application on the skin and the increased population of FITC$^+$I-A^{high+}cells in the regional lymph nodes following FITC painting. XS52 cells were cultured with GM-CSF and IL-4 for 9 days, thereby they matured and expressed CCR7. Bone marrow cells were cultured with GM-CSF, then purified using MACS into CD86$^+$population of which over 95% expressed CCR7 (CD86$^+$BM-DC). The motility of mature XS52 cells and CD86$^+$BM-DCs to CCL19 was assessed by the chemotaxis assay. The activation of ROCK was evaluated by the phosphorylation of myosin-binding subunit of myosin phosphatase (MBS), a downstream effector of ROCK. We found that migration of LCs from epidermis to lymph nodes were inhibited by the *in vivo* injection of ROCK inhibitors, Y-27632 and fasudil. The ROCK inhibitors suppressed the chemotaxis and the MBS phosphorylation of mature XS52 cells. Anti-SHPS-1 mAb inhibited both the chemotaxis of mature XS52 cells to CCL19 and the CCL19-induced MBS phosphorylation. In the SHPS-1 mutant mice, CCL19-induced CD86$^+$BM-DC migration as well as the CCL19-induced MBS phosphorylation were significantly reduced as compared to wild type mice. These observations indicate that SHPS-1 regulates DC migration through, at least in part, an activation of ROCK in response to CCL19 stimulation.

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Prevention of atopic dermatitis by pretreatment of phosphodiester-CpG-ODN in mice

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Atopic Dermatitis (AD) is a chronic, pruritic inflammatory skin disorder characterized by increased total IgE level, inflammatory cell infiltration, and the elevated expression of Th2 cytokines such as IL-4. Oligodeoxynucleotides (ODN) containing CpG motifs (CpG ODN) have been brought to an attention as immunomodulators that reduce Th2-mediated responses via increasing Th1 responses. We have previously demonstrated that a phosphodiester (PO) form of CpG-ODN serves as a good immunotherapeutic tool for AD because it significantly decreased Th2-responses without much increase of Th1-responses. In this present study, we investigated the preventive effect of PO-CpG-ODN on AD using mouse model of Ovalbumin (Ova)-induced skin inflammation. Pretreatments of PO-CpG-ODN before the Ova sensitization prevented the development of Th2-mediated responses such as the immune cell infiltration, and the production of IgE and IL-4, irrespective of the number of pretreatments of PO-CpG-ODN (once, twice and four times). However, four times treatment of PO-CpG-ODN were much more effective in the prevention of AD. Taken together, our data indicate that PO-CpG-ODN may be possible candidates for the prevention of AD.

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Corticotropin releasing hormone is involved in the stress-induced exacerbation of atopic dermatitis

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Cutaneous inflammatory diseases such as atopic dermatitis and psoriasis are worsened by stress. Corticotropin releasing hormone (CRH), which activates the hypothalamic-pituitary-adrenal axis under stress, has been discovered in peripheral tissues and also has proinflammatory peripheral effects. However, there have been no reports about functional role of CRH in peripheral blood mononuclear cells (PBMCs). The purpose of this study was to investigate the expression of CRH receptors and the functional role of CRH in the T cells and PBMCs of atopic dermatitis (AD) patients and nonatopic (NA) healthy control. The exposure of PBMCs to CRH resulted in the increase of IL-18 and IL-10 in AD patients, contrary to findings of the decrease in NA control. In addition, CRH induced the increase of IL-4 in CD4 T cells of PBMCs in both AD patients and NA control. To confirm direct effect of CRH on only T cells, we investigated the expression of CRH receptor on T cells. mRNAs for CRH-R1α, 1β, as well as CRH-R1 protein was detected in T cells and CRH-R2α (but not R2β or R2γ) mRNA and CRH-R2 protein were present in T cells. Exposure of only T cells to CRH resulted in no significant changes in both atopic dermatitis (AD) patients and nonatopic (NA) control. In conclusion, CRH didn't directly alter the expressions of Th2 cytokines in only T cells in spite of discovery of CRH-R in T cells. However, CRH indirectly induced Th2 shift in AD through monocytes. Therefore, this study demonstrates that CRH could induce Th2 cytokines and aggravate atopic dermatitis under stress condition.

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Expression of IL-31RA gene and its involvement in the itchy skin developing in mice with scratching behavior

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Pruritus is an important symptom of atopic dermatitis (AD), although the major pruritogen(s) has not yet been identified. NC/Nga mice that spontaneously develop eczematous AD-like skin lesions when kept under conventional conditions, but not under specific pathogen-free (SPF) conditions, have been thought to serve as a suitable animal model of AD. SPF-NC/Nga and BALB/c mice cohabited with skin-lesioned NC/Nga mice showed aggravation of spontaneous scratching behavior. These mice showed sustained scratching behavior, which serves as a measure of the itchy sensation, in response to not only pruritogens such as histamine, serotonin and compound 48/80, but also to algogens such as acetylcholine, bradykinin and capsaicin. The itchy skin in these mice closely resembled the "alloknesis" observed in subjects of AD. We have already reported that the mRNA expression level of the itch-related cytokine, interleukin-31 (IL-31), was increased in the skin of the mice showing aggravated scratching behavior following cohabitation with the skin-lesioned NC/Nga mice. Then, to investigate the involvement of IL-31 in this model further, we examined the changes in the expression level of IL-31 receptor A (IL-31RA) in the neurons of the dorsal root ganglia (DRG) by real-time PCR. The increase in the IL-31RA mRNA expression level was observed in both NC/Nga and BALB/c mice following cohabitation with skin-lesioned NC/Nga mice. Moreover, the IL-31RA mRNA expression level was consistently higher in the NC/Nga mice than in the BALB/c mice, the former also showing higher scratching counts than the BALB/c mice, and the increase in the IL-31RA mRNA expression level was correlated with the increase in the scratching counts. These results suggest that the cohabitation induced an increase in the expression level of IL-31 in the skin and of IL-31RA in the DRG and that the IL-31-IL-31RA interaction in the sensory nerve endings might induce sensory nerve hypersensitivity and the development of itchy skin in mice.

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Cutaneous hypersensitivities to hapten are controlled by IFN-γ-upregulated keratinocyte Th1 chemokines and IFN-γ-downregulated langerhans cell Th2 chemokines

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There are three types of cutaneous hypersensitivity to exogenous agents, including immediate, late phase, and delayed-type reactions. To address the modulatory roles of IFN-γ for these reactions, we used IFN-γ-/- and wild-type B6 mice and examined the contact hypersensitivity response to picryl chloride (PCl) for assessing delayed-type reaction, and the responses to repeatedly challenged FITC for immediate and late phase reactions. The delayed-type reaction was depressed and the immediate and late phase reactions were enhanced in IFN-γ-/- mice. Since skin-infiltrating lymphocytes were scarce at the PCl-challenged site of IFN-γ-/- mice, we investigated Th1/Th2 chemokine production by keratinocytes and Langerhans cells (LCs). A real-time PCR analysis of crude, CD11c+ cell (LC)-depleted, and CD11c+ cell-purified epidermal cells from PCl- or FITC-challenged ears demonstrated that Th1 chemokines (Mig/CXCL9 and IP-10/CXCL10) were produced by keratinocytes, while Th2 chemokines (TARC/CCL17 and MDC/CCL22) were derived from LCs. Challenge with PCl or repeated FITC augmented keratinocyte expression of Th1 chemokines in the wild-type but not IFN-γ-/- mice, and repeated FITC enhanced Th2 chemokines in IFN-γ-/- but not wild-type mice. Moreover Th2 chemokine production by LCs was induced efficiently in IFN-γ-/- mice. Finally, transfer of CFSE-labeled draining lymph node cells from hapten-sensitized B6 mice or lymph node cells from sensitized GFP mice to naïve IFN-γ-/- mice revealed less infiltration of CFSE+ or GFP+ lymphocytes at the challenged site. Our study suggests that one of the crucial actions of IFN-γ is upregulation of keratinocyte production of Th1 chemokines and downregulation of LC production of Th2 chemokines.

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Effects of orally administered Actinidia arguta extract on 2-chloro-1,3,5-trinitrobenzene (TNCB)-induced atopic dermatitis-like skin lesions in NC/Nga mouse

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Background: Atopic dermatitis (AD) is characterized by a highly pruritic, chronic, and relapsing inflammatory skin lesions. Therapeutic choices are quite limited especially in long-standing cases in spite of the increasing prevalence. We need to develop effective alternative agents Objective: This study was performed to examine the clinical efficacy as well as the therapeutic mechanism of Actinidia arguta extract (AAE) in AD-like animal model. Methods: To evaluate the effects of AAE on the development of AD-like skin lesion, the measurement of clinical skin severity scoring (SCORAD), epidermal thickening, mast cell infiltration/degranulation, the levels of total serum Ig isotypes (IgE, IgG1), the mRNA expression levels of cytokines (IL-4, IL-5, IFN-γ, and TNF-α) and Toll-like receptors (TLR2, 4, and 9) were done on 2-chloro-1,3,5-trinitrobenzene (TNCB)-induced AD-like skin lesion in NC/Nga mouse. Results and Conclusions: Orally administrated AAE significantly reduced the clinical dermatitis severity, suppressed the epidermal thickness as well as the dermal infiltration/degranulation of mast cell, and decreased the levels of total serum IgE and IgG1. The suppression of total serum IgE and IgG1 was accompanied with both the decreasing IL-4 and the increasing IFN-γ expression in the skin and splenocytes. Interestingly, the expression levels of TLR2, 4, and 9 were also significantly increased by AAE-administration. In conclusion, we confirmed that the clinical efficacy and therapeutic mechanism of AAE in AD might be associated with the modulation of biphasic Th1/Th2 cytokine, the inhibition of Th2-mediated IgE overproduction, and possibly with the upregulation of TLR2, 4, and 9 expressions.

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1,24-dihydroxyvitamin D3 (tacalcitol) inhibits allergic contact dermatitis via suppression of cutaneous lymphocyte-associated antigen expression

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Cutaneous lymphocyte-associated antigen (CLA) is a surface glycoprotein expressed by skin-homing T cells, and has a critical role for T cell recruitment into skin. The active form of vitamin D3 (1,25 dihydroxyvitamin D3, abbreviated VD) has been used to treat certain T cell-mediated inflammatory skin diseases such as psoriasis. Recently, we reported that VD suppresses the expression levels of CLA on human cultured T-lymphocyte, and also inhibits skin infiltration of CD4+ T cell *in vivo*. 1,24-dihydroxyvitamin D3 (tacalcitol) is one of the analogs of active form of vitamin D3 and is used in the clinical field. In this study, we investigated the effect of tacalcitol for CLA and other lymphocyte homing receptors on human peripheral blood T cells. We also analyzed effects of intraperitoneally administerd tacalcitol in an antigen-dependent delayed type hypersensitivity (DTH) mice model. As a result, tacalcitol down-regulated the expression of CLA and in parallel functional E-selectin ligand; however, it had no effect on other homing receptors. In an *in vivo* assay, treatment with tacalcitol down-regulated the skin infiltration of effecter CD4+ T cells. These findings suggest that tacalcitol selectively down-regulates CLA expression without influencing lymphocyte migration patterns to other tissues.

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Cytokine-mediated arrest of multistage carcinogenesis by adaptive immune responses

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It is currently believed that tumor cells are primarily eliminated by cytotoxic T cells. Yet, recent evidence suggests that innate or adaptive immune responses may also arrest multistage carcinogenesis through mechanisms different from tumor cell killing. To analyze those mechanisms in more detail, we studied multistage carcinogenesis in mice that endogenously develop β-cell carcinomas due to the expression of T antigen (Tag) of the simian virus 40 under control of the insulin-promoter. Previous work revealed that IFN-γ producing Th1 cells, specific for Tag, arrest multistage carcinogenesis and tumor angiogenesis through mechanisms independent of tumor cell killing. Studying the underlying mechanisms we first found, that Tag-specific Th1 cells strongly reduce tumor cell proliferation (BrdU-labelling) *in vivo*, through IFN-γ -dependent pathways. Subsequently, we found that IFN-γ signaling preserved insulin production by tumor-prone β-cells *in vivo*. To investigate the functional responsiveness, we isolated β-cells at different stages of multi-stage carcinogenesis (8, 12 and 15 weeks after birth) and determined the glucose-sensitivity of the β-cells by a mitochondrial activity assay (XTT-test) and the expression of insulin by immune fluorescence. In the absence of IFN-γ -signaling from Tag-specific T cells, tumors rapidly lost first their responsiveness to glucose and then the capacity to release insulin. In sharp contrast, in the presence of IFN-γ-signaling by Tag-specific T cells, tumor cells largely preserved their responsiveness to glucose and kept the insulin-producing phenotype. Blocking the IFN-γ action with mAb entirely reversed the protective effect of the Tag-specific T cells and promoted multistage carcinogenesis. Thus, adaptive immune responses can induce tumor dormancy by inhibiting malignant transformation even of cells driven into multistage carcinogenesis by deregulation of the tumor suppressors p53 and Rb. Uncovering the role of adaptive immunity in arresting multistage carcinogenesis is critical for the design of tumor immune therapies.

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IgE preferentially protects female mice against hapten-induced immunopathology

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The effect of IgE normally present in animals has been ignored in most animal models of hypersensitivity reactions. In this regard, basal levels of IgE have been shown to be required for optimal sensitization to hapten. This raises the possibility that the IgE may have a strong impact on the initial event occurring upon hapten exposure. We therefore explored the impact of the IgE on the primary response induced by initial hapten exposure and the subsequent immunopathology after repeated hapten exposure. We previously demonstrated that initial hapten exposure results in the development of Toll-like receptor 2 -dependent, immediate-type hypersensitivity-like responses. In this study, we found that males were more susceptible to the development of this innate-type response. Surprisingly, basal serum IgE levels in females were 1.5~4-fold higher than those in males: there was a striking inverse relationship between the basal IgE levels and the magnitude of the innate-type response. A targeted disruption of neuromedin U (NMU), the neuropeptide released from keratinocytes and involved in the innate-type response, abolished the gender difference in the basal IgE levels and reversed the gender bias to male in the innate-type response. Prior local injection with either hapten-specific or -irrelevant IgE protected WT or$\mu^{-/-}$ mice lacking IgE against the innate-type response. This protection was successfully transferred to mast cell-deficient mice by IgE-preloaded mast cells from WT mice. Similar female preponderance in serum IgE levels was also observed after repeated hapten exposure in $NMU^{-/-}$ mice as well as WT mice. In this setting, however, female $NMU^{-/-}$ mice exhibited severe immunopathology similar to males despite their higher IgE levels, while in WT mice females were also protected against severe immunopathology. These results indicate that females, as far as NMU is retained in the epidermis, are protected against hapten-induced immunopathology through the antigen-independent effect of IgE.

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Exposure to Efalizumab down-modulates T cell responses to proliferative stimuli

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The use of the monoclonal antibody (mAb) Efalizumab (E) is a new treatment option in moderate to severe psoriasis. Its activity is mediated by binding to the α-chain (CD11a) of the lymphocyte function associated-molecule-1 (LFA-1). Although LFA-1 is ubiquitously expressed on peripheral blood leukocytes, it is generally assumed that E exerts its effects mainly on T lymphocytes by blocking their migration and by interfering with the immunological synapse. To test the validity of this assumption, we asked whether E could interfere with T cell proliferation induced by qualitatively and quantitatively different stimuli. Using peripheral blood mononuclear cells (PBMC) of both E-treated patients and, after in vitro exposure to E, healthy individuals, we observed that E, while unable to interfere with T cell proliferation under optimal stimulation conditions (plate-bound anti-CD3, PHA), does effectively block the allogeneic mixed leukocyte reaction and anti-CD3-driven T cell proliferation induced under suboptimal conditions. The further observations (i) that the expression level of LFA-1 on antigen-presenting cells (APC) compares favorably with that on T cells but (ii) that E exerts its inhibitory effect solely on the T cell side, argues against physical hindrance being the only reason for the E-induced downregulation of the T cell response. Our findings suggest that binding of E to its ligand induces events which increase the threshold of T cells to a proliferative stimulus via the T cell receptor. They also provide an explanation as to why E is highly effective in patients with stable psoriasis, but often fails to control disease flares.

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Increase of cutaneous barrier dysfunction depends on a decrease in the ability of skin prostaglandin D_2 production in aged mice

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Advanced age is associated with an increase in the risk of skin diseases, such as senile xerosis, characterized by itchiness. Therefore, we evaluated the effects of aging on the functions of the cutaneous barrier and examined their relationship with skin prostaglandin (PG) production. We carried out quantification of long-lasting scratching behavior, transepidermal water loss, melanin value, redness value and skin prostaglandin concentrations (PGD_2, PGE_2, 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ in BALB/c mice aged 6,12,25 and 50 weeks. The ability of the skin for PG production was evaluated by measuring the amounts of PGs produced in the skin after topical application of arachidonic acid. The long-lasting scratching behavior, transepidermal water loss, melanin value and redness value increased with advanced age of the animals. The long-lasting scratching behavior was significantly increased in the 25- and 50-week-old animals as compared with that in the 6-week-old animals. Transepidermal water loss, which demonstrates barrier disruption, was also significantly increased in the 25- and 50-week-old animals as compared with that in the 6-week-old animals. The amount of PGD_2 in the skin did not differ significantly among the animals of different ages, whereas those of PGE_2, 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ were higher in the 50-week-old animals as compared with those in the 6-week-old animals. On the other hand, the ability of the skin for the production of PGD_2, PGE_2, 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ was significantly decreased in the 25- and 50-week-old animals as compared with that in the 6-week-old animals. The increase of transepidermal water loss in the 50-week-old mice improved significantly following topical application of exogenous PGD_2. These results suggest that aging impairs the skin barrier functions and predisposes to pruritus and dry skin, which appear to be related to the decrease in the ability of the skin to produce prostaglandins, especially PGD_2.

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Regulatory effects of antihistamines on the responses to staphylococcal enterotoxin B of human monocyte-derived dendritic cells and CD4+ T cells

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Antihistamines are widely used for the treatment of allergic diseases, such as urticaria and allergic rhinitis. They are also effective for the treatment of diseases in which T cells are mainly involved in the pathogenesis, such as atopic dermatitis (AD) and contact dermatitis. Dendritic cells (DCs) drive polarization of naive T cells into Th1 or Th2 subsets, and are also likely to be involved in AD pathogenesis. The aim of this study was to determine the effects of antihistamines on DCs and CD4+ T cells stimulated with staphylococcal enterotoxin B. Human monocyte-derived DCs (MoDCs) and autologous CD4+ T cells were obtained from healthy subjects, and cocultured in the presence of antihistamines. MoDCs were also cultured alone. The concentrations of cytokines and chemokines in culture supernatants were measured by ELISA. Cell proliferation in the cocultures of MoDCs and CD4+ T cells (DC-T cocultures) was measured by a [3H]thymidine incorporation assay. We observed that antihistamines inhibited the production of IFN-gamma, thymus and activation regulated chemokine (TARC), macrophage-derived chemokine (MDC), IFN-gamma-inducible protein-10 (IP-10), and enhanced the production of IL-4 and monokine induced by IFN-gamma (Mig) in DC-T cocultures. Antihistamines also inhibited the production of IFN-gamma, TNF-alpha, TARC, MDC, IP-10, and Mig in MoDCs. Epinastine, one of antihistamines, inhibited the proliferation of CD4+ T cells cocultured with MoDCs. In conclusion, our findings show that antihistamines regulate immune responses by affecting the interaction between DCs and CD4+ T cells, which may partially contribute to the control of allergic diseases such as AD and contact dermatitis.

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Reduced function of the peripheral CD4+25+ fraction in silicosis patients exposed to environmental factors for SSc

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Patients with pneumoconiosis suffer from several complications, in addition to respiratory disorders.In silicosis patients (SIL), one of the most important complications is the development of autoimmune disorders such as systemic sclerosis (SSc). Our previous analyses showed (1) SIL exhibited alteration of Fas and Fas-related molecules such as higher soluble Fas, (2) detected anti-Fas-autoantibody, and (3) detected anti-topoisomerase I antibody.Thereafter,the quality and quantity of CD4+25+ regulatory T cells (Treg) in SIL were examined and compared with results for healthy donors (HD) because SIL often develop autoimmune diseases along with pulmonary disorders. Treg frequency and clinical parameters were subjected to a factor analysis. Treg and CD4+25- T cells (Tneg) from five HD and five SIL sorted by a flowcytometer were used for functional assays of Treg and the expression pattern of Treg specific genes. Although the actual frequency of Treg did not differ between SIL and HD, the age-corrected level was reduced in SIL. The factor analysis showed that Treg frequency was positively associated with the serum level of IL-2. The inhibitory effect of Treg on Tneg activation decreased when the Treg:Tneg ratio was 1:1/4 to 1/2. In addition, Treg dominancy of foxp3 and ctla-4 expression was lost in SIL and with pcd-1, a marker gene for activated T cells, overexpression in the Treg fraction was detected in SIL but not in HD. And the Fas expression level is higher in CD4+ FoxP3+ fraction from SIL than that from HD. These results indicate that the reduction in size and fraction in CD4+25+fraction in SIL due to substitution by chronically activated T cells, which may also are highly susceptible to FasL-mediated cell death, may cause the subsequent occurrence of autoimmune diseases in SIL and represent a pre-autoimmune status is SIL.

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CD4+CD25+Foxp3+ regulatory T cells induced by plasmacytoid dendritic cells show unique cytokine profile in atopic dermatitis

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Human plasmacytoid dendritic cells (pDCs) have tolerogenic capacity of inducing CD4+CD25high-Foxp3+ regulatory T cells (Tregs) from naïve CD4+ T cells. Because of qualitative and quantitative aberration of pDCs in atopic dermatitis (AD), Tregs induced by atopic pDCs might display unique immunological profiles. In the present study we characterized Tregs induced by pDCs from patients with AD in comparison with those induced by pDCs from normal individuals and patients with allergic contact dermatitis and psoriasis. Allogeneic naïve CD4+ T cells of a healthy individual differentiated to CD4+CD25high T cells upon culturing with pDCs from all the study groups. CD4+CD25high T cells contained CD4+CD25high CTLA-4+Foxp3+ CD127-Tregs, expressed skin oriented chemokine receptors, CCR4 and CCR7, and inhibited T cell proliferation. Atopic pDC-induced Tregs overproduced TNF-α, IFN-γ and IL-5 in addition to inhibitory cytokines, i.e. TGF-β and IL-10, whereas Tregs induced by pDCs from other participant categories synthesized only the inhibitory cytokines. Immunohistochemical stainig and in vitro propagation of skin infiltrating cells showed that T cells with the phenotype and cytokine profiles similar to pDC-induced Tregs resided in AD skin lesions. These data suggest that Tregs with the unique cytokine profiles aberrantly control skin inflammation in situ and thus protract the clinical course in AD.

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Antimicrobial activity of human beta-defensin-3 against metallo beta lactamase producing pseudomonas aeruginosa

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A treatment of P. aeruginosa infection is often complicated because this organism is inherently resistant to many drug classes and metallo beta lactamase (MBL) producing P.aeruginosa is especially getting serious nosocomial problem. The urgent development of new classes of antimicrobial agent is aspired. In this context, anti-microbial peptides, alone or combined with conventional antibiotics, are attractive candidates as new therapeutic agents. Defensins are a family of antimicrobial peptides and vital contributors to host immune response. Human beta-defensin (HBD) -3 has salt-insensitive broad spectrum of potent antimicrobial activity against both gram-positive and gram-negative strains, while HBD-1 and -2 show microbial activity against gram-negative bacteria. HBD-3 is expected as a powerful solution against multi-drug resistant microbes such as MBL (+) P. aeruginosa and MRSA. Its activity against MRSA has already been reported by some authors, however not yet fully investigated against MBL (+) P. aeruginosa and a combination therapy of defensin and conventional antibiotics has not been reported so far. To examine above, we cloned HBD-3 and obtained recombinant HBD-3 by pET28a expression system. Its His-Tag component was cleaved by Factor Xa and purified by RP-HPLC. The assay of antimicrobial activity of rHBD-3 alone and a combination of rHBD-3 and levofloxacin against MBL(+) P. aeruginosa clinically isolated was performed. rHBD-3 demonstrated almost the same potent activity against MBL(+) P. aeruginosa as well as P. aeruginosa without it. However, rHBD-3 did not improve the anti-MBL(+) P.aeruginosa activity of LVFX when they are combinated. HBD-3 might be a new choice against MBL(+) P. aeruginosa. Although simultaneous administration with LVFX did not function, efficacy of combination with any other classes of antibiotics has not yet been contradicted.

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Alpha4beta7 integrin is essential for contact dermatitis by regulating migration of T cells to inflammatory skin

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Beta7 integrin is a cell adhesion molecule and presents in the form of alpha4beta7 integrin or alphaEbeta7 integrin. Alpha4beta7 integrin is expressed by most leucocytes and essential for leukocyte migration to gut-associated lymphoid tissues (GALT) by interacting with its primary ligand, mucosal addressin cell adhesion molecule-1 which is preferentially expressed in GALT. Although the importance of alpha4beta7 integrin in intestinal inflammation has been established, its role in cutaneous inflammation remains to be seen. Here, we showed that beta7 integrin deficient mice, not alphaE integrin deficient mice, was defective in contact hypersensitivity responses. Beta7 integrin deficiency did not affect non-specific inflammation, and neither do the number of dendritic cells in epidermis. Moreover, in beta7 integrin deficient mice, the number of migratory dendritic cells from ears was not decreased, and lymphatic drainage was normal. We further demonstrated that antigen presentation of dendritic cells from either ears or draining lymph nodes was normal, suggesting that the process of antigen uptake and presentation was not impaired in beta7 integrin deficiency. Sensitized beta7 integrin deficient T cells were able to respond normally to antigen stimuli by proliferation assay, and induce ear swelling when injected intradermally into the ear. Finally, we evaluated migration capacities of beta7 integrin deficient T cells. When T cells from either sensitized beta7 integrin deficient or wild type mice were injected intravenously to wild type mice, ear swelling was significantly reduced in those received beta7 integrin deficient T cells after the elicitation. Moreover, when sensitized beta7 integrin deficient T cells were injected intravenously, they hardly reached the ear skin after the antigen application. These results suggest that beta7 integrin presumably with alpha4 integrin regulates T cell migration to inflammatory skin, is important in antigen specific contact dermatitis, and targeting beta7 integrin may be an option for treating inflammatory skin disorders.

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HSP70 is a target for TNBS-induced carbonylated proteins in XS-106 dendritic cells

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Reactive oxygen species (ROS) are produced in dendritic cells (DCs) during antigen presentation in contact hypersensitivity (CHS). As a result, ROS cause a number of non-enzymatic protein modifications, including carbonylation, which is the most widely used marker of oxidative stress. To study ROS-dependent protein carbonylation response to 2,4,6-trinitrobenzene sulfonic acid (TNBS), we used the well-established mouse DC line, XS-106. A hapten is a small molecule, which can elicit an immune response when attached to a larger carrier molecule. TNBS is a well-known hapten. So, we sought to study whether the XS-106 DCs would produce ROS upon TNBS stimulation and focused on the effects of ROS induced by TNBS including protein carbonylation and secretion of cytokines in XS-106 DCs. We have demonstrated TNBS-induced ROS and carbonylated proteins in XS-106 DCs. The increase of oxidative status was further confirmed by increase of oxidation-dependent protein modification, such as carbonylation. TNBS strongly induced carbonylation of heat shock protein 70 (HSP70), identified by MALDI-TOF analysis and Western blotting. Furthermore, we fused to the protein transduction domain (PTD) of HSP70, the fusion protein PTD-HSP70 successfully entered the cells in vitro and showed transient excessive effects. After transient expression of HSP70 in XS-106 DCs, PTD-HSP70 fusion protein inhibited both ROS production and subsequent carbonylations and IL-12 secretion. Our results demonstrated that TNBS-induced carbonylated protein is HSP70. Decreased HSP70 expression means compensative effect by oxidative stress. Transiently excessive expression of HSP70 in XS-106 DCs effect may contribute to the pathophysiology of CHS.

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Pro-inflammatory cytokine release is mediated by a MAPK signal-integrating kinase (Mnk1) dependent mechanism in human keratinocytes

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The mitogen-activated protein kinases (MAPKs) are known to play a key role in the regulation of inflammatory processes. Mnk1 is a kinase known to be activated through both the p38 MAPK and the extracellular signal-regulated kinase (ERK1/2) pathway. The purpose of this study was to characterize the role of the Mnk1 signalling pathway in the regulation of pro-inflammatory cytokine expression in cultured human keratinocytes. Western blotting was used to analyze specific kinases in whole cell lysate from cultured normal human keratinocytes. Chemical inhibitors were applied to cells prior to stimulation with either IL-1β or anisomycin. Cytokine release in cell supernatants was analyzed by ELISA. Mnk1 was shown to be significantly phosphorylated in a time-dependent manner in response to stimulation with either IL-1β or anisomycin with peak intensities occurring after approximately 15 and 60 minutes, respectively. By the use of chemical p38 MAPK or ERK1/2 inhibitors prior to stimulation of the keratinocytes with either IL-1β or anisomycin we identified p38 MAPK as the responsible kinase in phosphorylating Mnk1. Phosphorylation of Mnk1 was followed by phosphorylation of its downstream target eIF4E. In a concentration study we found that phosphorylation of eIF4E was inhibited in a dose-dependent manner by the Mnk inhibitor CGP57380, and that the optimal concentration for inhibiting eIF4E-phosphorylation was 50 μM. A viability study performed at this concentration for 13 hours demonstrated no insult to cell membrane integrity. Interestingly, our data showed that in cultured normal human keratinocytes pretreated with CGP57380 prior to stimulation for 12 hours with either IL-1β or anisomycin the IL-1β-induced cytokine release of TNF-α, and the anisomycin-induced release of TNF-α, IL-1β and IL-6 were significantly reduced. Our results imply that Mnk1 might play a pivotal role in the inflammatory response of keratinocytes. Therefore, Mnk1 might prove to be a possible future target in the treatment of inflammatory skin diseases.

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Langerhans cells and dermal dendritic cells as a new model for selecting immunomodulator active ingredients

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Sensitive skin is currently an extremely popular subject and a large variety of skin care products is already designed for persons claiming sensitive skin. Multi-factorial factors are involved in the sensitive skin physiopathology such as disrupted skin barrier, increased neuron-sensory activity and increased immune sensitivity. In order to regulate the hyper-reactivity of sensitive skin, we have chosen to focus on the skin immune part and more particularly on skin dendritic cells. Recently, we developed a culture model containing both Langerhans cells and dermal dendritic cells generated from blood monocytes (Bechetoille et al., 2006). After active ingredient treatment for 24 h, protein and mRNA levels of CD86 (specific activation marker) are analyzed on Langerhans cells and dermal dendritic cells by flow cytometry and qRT-PCR, respectively. We have also investigated the cell recovery after active ingredient treatment and the cell responsiveness to danger signals. For each experiment, Dexamethasone is used as the positive control. After a screening of 300 active ingredients, we selected 6 ones displaying the following abilities: 1/ no cytotoxic effect at appropriate concentrations; 2/ decreasing of CD86 expression at protein (15 to 60%) and mRNA (10 to 40%) levels on active ingredient-treated cells; 3/ recovery of CD86 basal expression at protein (78 to 100%) and mRNA (87 to 100%) levels when cells are no longer treated by active ingredients (except for one active ingredient); 4/ increasing of CD86 expression at protein (126 to 140%) and mRNA (150 to 187%) levels on active ingredient-treated cells and after LPS/TNFα challenge. Taken together, selected active ingredients are able to decrease the activation level of Langerhans cells and dermal dendritic cells in steady state. However, cells are still able to be responsive when challenged by danger signals.

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Human apolipoprotein C1 transgenic mice: a unique novel model of atopic dermatitis

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Atopic dermatitis is a chronic inflammatory skin disease characterized by clinical symptoms such as a dry and scaly skin, hyperplasia of the dermis, lichenification and pruritus. In acute lesions, inflammation in atopic dermatitis is associated with an initial increased Th2 response while Th1 cells, eosinophils and mast cells are involved in the more chronic phase. Animal models are indispensable in obtaining insight into the pathogenesis of atopic dermatitis and in the development of new therapeutic strategies. Mice with a chronic overexpression of human apolipoprotein C1 in the liver and skin develop spontaneously clinical symptoms of atopic dermatitis i.e. scaling, lichenification, papules, and excoriation from an age of 7 weeks onwards. Development of atopic dermatitis in these mice is accompanied by an increased pruritus during aging. Histological analysis shows a hyperplasia of both the epidermis and dermis and increased numbers of CD4+ T cells, eosinophils, mast cells and IgE-positive cells in the dermis. Serum levels of IgE are increased as well. Impairment of the skin barrier is most likely the underlying cause of the development of atopic dermatitis in this model since these mice show a loss of skin-barrier function as is evident from an increased trans-epidermal water loss. Development of atopic dermatitis in this model was found to be sensitive to topical treatment with triamcinolone-acetonide, fluticasone-isoproprionate and tacrolimus. Moreover, oral treatment with dexamethasone successfully inhibits several aspect of disease in this model. In conclusion, this animal model is useful for identifying new therapeutic strategies and obtaining new insight into the pathogenesis of atopic dermatitis.

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Keyhole limpet hemocyanin elicits a Th2-biased de novo immune reaction in man

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In atopic disorders, allergen-specific T helper 2 (Th2) cells play an important role in the pathogenesis of the disease. No model system exists yet in man to study the cellular and molecular requirements of this imbalanced immune response. To address this issue, we used Keyhole Limpet Hemocyanin (KLH) as a neoantigen coupled to aluminum hydroxide for the immunization of healthy and atopic individuals. The immunization protocol was well tolerated and highly specific and efficient, as shown by the KLH-specific proliferation of PBMC. The presence of KLH-specific IgG4 in sera of KLH-immunized but not of non-immunized subjects indicated a Th2-skewed immune response, as did the production of antigen-specific Th2-cytokines (mainly IL-5 and IL-13). The immediate type skin response induced by an intra-dermal KLH challenge was stronger in atopic individuals, and the late phase skin reaction was accompanied by a strong upregulation of IL-4 transcripts in biopsies taken from the site of injection. In conclusion, the immunization protocol tested induced a Th2-biased de novo immune response in humans, and will be a useful tool for investigating and monitoring immunomodulatory strategies in man.

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TLR5-mediated detection of bacterial flagellin induces the up-regulation of the antimicrobial peptides S100A7c (psoriasin) and human beta defensin 2 (hBD-2) in keratinocytes

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Antimicrobial peptides are a critical part of the innate immune system of the skin. Epidermal keratinocytes express numerous such peptides including S100A7c (psoriasin), the most efficient killer of E. coli. To investigate the responsiveness of S100A7c expression to microbial components, we stimulated epidermal keratinocytes with ligands for toll-like receptor (TLR) 1 through TLR9. Real-time PCR analysis revealed that the TLR5 ligand flagellin strongly induced the expression of S100A7c, whereas all other TLR ligands had no significant effect. S100A7a, another isoform of S100A7 with anti-bacterial activity was also induced by flagellin, however, its absolute expression level was several 100-fold lower than that of S100A7c. The antimicrobial peptide human beta defensin (hBD)-2 was up-regulated most strongly, but not exclusively, by flagellin. Supernatant from wild-type E. coli, but not from a flagellin-deficient E. coli strain (ΔFliC), strongly induced S100A7c expression in keratinocytes both at the mRNA and protein levels. siRNA-mediated knock-down of TLR5 expression suppressed the ability of keratinocytes to up-regulate S100A7c and hBD-2 expression in response to flagellin or E. coli supernatant. An essential role of the alternative flagellin receptor Ipaf (interleukin-1 beta converting enzyme protease-activating factor) could be excluded because blockade of the interleukin-1 signaling pathway, which is downstream of Ipaf, did not affect flagellin-induced S100A7c and hBD-2 up-regulation. Our data suggest that flagellin, which is absent from commensal skin microbes but present on several pathogenic bacteria, is sensed by TLR5 on epidermal keratinocytes to induce the expression of antimicrobial peptides.

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Lympho-Epithelial-Kazal-type-Inhibitor 2 (LEKTI-2), a novel epithelial peptide antibiotic

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We recently purified several human peptide antibiotics from psoriatic scale and stratum corneum (SC) extracts. Following the hypothesis that healthy skin is protected from infection by producing a panel of antimicrobial peptides, we systematically analyzed heel SC extracts for human antimicrobial peptides (AMPs). Here, we report the identification and characterization of a novel E. colicidal AMP isolated from SC. Determination of the full-length cDNA sequence revealed the corresponding gene is located on chromosome 5q33.1, telomeric to Spink5 and Spink7. The deduced protein consists of a signal peptide at the N-terminus and a typical Kazal domain highly homolog to domains 2 and 15 of the multi- "Lympho-Epithelial-Kazal-type-Inhibitor (LEKTI)". Thus, this protein was termed "LEKTI-2". Quantitative real-time PCR analyses revealed that Spink9 is weakly expressed in the skin, tonsils, adenoids, bronchial epithelial cells and cultured primary keratinocytes but highly expressed in the thymus. To get insight into the biological function of LEKTI-2, we generated recombinant LEKTI-2 in E. coli. To our surprise, this rLEKTI-2 form with 61 residues exhibits no AMP-activity. To address this observation, we re-analysed our natural LEKTI-2 preparations by additional HPLCs and found by ESI-MS-analyses that they contained several LEKTI-2 variants formed by N-terminally extension or truncation of the LEKTI-2 core peptide. Among them, the 62-residue pyroglutamyl-variant and 63-residue variant were found to be major contaminants of the dominant 61-residue LEKTI-2 form. We therefore generated further the 63-, 62-, 60-, 59-, 58- and 57-residue LEKTI-2 variants recombinantly. Antimicrobial assays revealed that the N-terminally extended 63- and 62-residue LEKTI-2 variants exhibit antimicrobial activity, whereas the other variants are not AMPs. In conclusion, LEKTI-2 represents a novel skin-derived antimicrobial peptide.

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Critical role of TLR2 and TLR4 in lpr mutation-induced murine lupus

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SLE is an autoimmune disease characterized by pathogenic antibodies directed against nuclear antigens, immune complex deposition and end organ damage. Environmental factors, in particular microbial pathogens have been thought to play a role in the onset of the disease. The recognition of pathogen-associated molecular patterns is mediated by Toll-like receptors (TLRs) and in particular by TLR2 and TLR4 which bind Gram+ and Gram- bacteria respectively and we sought to determine the role of these TLRs in SLE by creating C57BL/6-lpr/lpr-TLR2-/- and C57BL/6-lpr/lpr-TLR4-/- mice and analyzing their phenotype. TLR2 and TLR4 deficient mice develop a less severe clinical disease with few immunological alterations. Indeed, assessment of the renal disease showed that glomerular IgG deposits were less intense in these TLR deficient lupus mice and were associated with a diminution of mesangial cell proliferation. The production of autoantibodies showed significant differences compared with C57BL/6-lpr/lpr mice. Anti-dsDNA and anti-cardiolipin autoantibodies were diminished in both TLR deficient mice whereas the response against nucleosome remained unaffected in TLR4-/- mice, suggesting the role of TLR4 in the production of antibodies directed against T-independent antigens. Moreover, B cell phenotypes and cytokine profiles presented abnormalities which suggested an important role of microbial flora through TLRs in the sustained B lymphocyte activation and autoantibody production observed in SLE.

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Critical role of $CD8^+CD11c^+$ dendritic cells in tolerance to contact allergens

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Induction of tolerance is an essential process in the regulation and circumvention of allergies. In the model of low zone tolerance (LZT), induced by epicutaneous application of low doses of contact allergens, $CD8^+$ suppressor T cells inhibit the development of Tc1-mediated contact hypersensitivity (CHS). In this study, we analyzed the role of dendritic cells (DC) for tolerance induction to allergens. CD11c-DTR (diphteria toxin receptor) mice were used in which systemic administration of diphteria toxin leads to a transient and rapid decline of $CD11c^+$ DC in lymph nodes (LN) and spleen. Notably, depletion of $CD11c^+$ DC by injection of diphteria toxin during the induction of LZT abolished the development of tolerance and characteristic features of LZT, demonstrated by the absence of suppression of a CHS reaction (significant ear swelling, hapten-specific T cell proliferation and Tc1 cytokine pattern). To evaluate the role of $CD8^+CD11c^+$DC, we performed adoptive transfer experiments of $CD11c^+$ LN-derived DC obtained from tolerized WT and $CD11c^+$ DC from tolerized ICSBP-/- mice (interferon consensus sequence-binding protein) lacking relevant numbers of functional $CD8^+CD11c^+$ DC. Transfer of $CD8^+CD11c^+$ DC induced a tolerance reaction in mice shown by an inhibited inflammatory skin reaction and generation of regulatory T cells, whereas recipients of DC generated from ISCBP-/- mice developed a CHS reaction, indicating that $CD8^+CD11c^+$ DC are involved in the development of LZT. In addition, reconstitution of transgenic DC-depleted CD11c-DTR mice with $CD8^+CD11c^+$ DC before tolerance induction restored the tolerance development. Our findings identify $CD8^+CD11c^+$ DC as key APC for LZT to contact allergens that are critical for priming of regulatory T cells.

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Role of TLR9 in anti-nucleosome and anti-DNA antibody production in lpr mutation-induced murine

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Lpr/Lpr mice are widely used as an animal model for human systemic lupus (SLE). SLE is characterized by nephritis and vasculitis associated with the production of antibodies directed against nucleosome and DNA which correlate with the disease activity. Toll-like receptors 9 (TLR9) which bind DNA via unmethylated CpG sequences is thought to play a role in the production of these autoantibodies through the capacity of nuclear immunogenic particles to interact both with BCR and TLR9. We sought to determine this role by creating B6lpr/lprTLR9-/- mice and analyzing their phenotype. We show that TLR9-/- and TLR9+/+ mice presented a massive lymphadenopathy and splenomegaly, associated with the expansion of CD4-CD8- double negative T cells. Concerning lupus-like autoimmunity, the TLR9-/- seroprofile was very different compared with TLR9+/+ since anti-nucleosome antibodies remained negative until 6-month-old in TLR9-/- mice while no difference was observed in the frequency of anti-dsDNA autoantibodies whose titer was strikingly higher in TLR9-/- mice. Thus in B6lpr/lpr mice, TLR9 deficiency dissociates the anti-dsDNA from the anti-nucleosome antibody response. The assessment of the renal disease showed that glomerular IgG deposits were more intense in TLR9 deficient mice and a higher rate of mesangial proliferation was also observed. All these results clearly demonstrate that TLR9 receptors are absolutely required for the anti-nucleosome antibody response but not for anti-DNA antibody production which is involved in mesangial proliferation. Moreover, this study provide clear evidence for a protective role of TLR9 in the development of the lupus disease since TLR9-/- mice present more important renal lesions.

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IL-23 and Th17 cytokines are not involved in the pathogenesis of psoriasis-like disease in K5.hTGF-β1 transgenic mice

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T helper (Th-) 17 cells have been implicated in the pathogenesis of psoriasis in humans and secrete pro-inflammatory cytokines. Transforming growth factor (TGF)-β1, in the presence of inflammatory cytokines, induces development of Th17 cells from naïve T cells in mice. In mice and humans, interleukin (IL)-23, a heterodimeric cytokine composed of p19 and p40 subunits, stimulates the growth and expansion of Th17 cells. To determine the association between TGF-β1, the IL-23/Th17 inflammatory pathway, and the development of psoriasis, we studied psoriasis-like disease in transgenic (Tg) mice that constitutively express human TGF-β1 in basal keratinocytes (K5.hTGF-β1 Tg mice). As expected, Tg mice exhibited psoriasis-like inflammatory skin disease; these mice had high levels of human TGF-β1 protein in both skin and serum. In contrast, protein levels of Th17 cytokines were not elevated in inflamed Tg skin compared to wild-type littermate skin. In addition, p40 and p19/p40 heterodimer (IL-23) protein levels were not increased in skin or serum of these mice. IL-12 and IFN-γ, cytokines associated with Th1 immune responses, were also not elevated in inflamed skin of K5.hTGF-β1 Tg mice. Furthermore, weekly treatment of K5.hTGF-β1 Tg mice with monoclonal antibodies (mAbs) directed against p19 or p40 had no clinical impact on progression of psoriasis-like disease in these mice. The same anti-IL-23 mAbs were able to block development of murine experimental autoimmune encephalitis, a Th17 cell-mediated disease. Finally, genetic crosses of K5.hTGF-β1 Tg mice with mice lacking the p40 chain of IL-23 also indicated that the IL-23/Th17 pathway is not responsible for skin inflammation. Thus, although TGF-β1 has been linked to Th17 cell development in mice, these data indicate that IL-23 and associated Th17 cytokines are not involved in the pathogenesis of psoriasis-like disease in K5.hTGF-β1 Tg mice.

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Reversal of regulatory T cell mediated immune evasion in human squamous cell carcinomas of the skin

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We found previously that squamous cell carcinomas of the skin (SCC) exclude CLA^+ skin-homing T cells, the subset most likely to destroy SCC, and instead recruit $FOXP3^+$ regulatory T cells (Treg) that express central memory markers. This recruitment appears to be specific, as approximately 50% of the T cells infiltrating SCC were $FOXP3^+$ Treg. Imiquimod is a topical TLR7 agonist that can induce the immunologic destruction of skin cancers. SCC treated with imiquimod prior to excision reproducibly had a five-fold lower percentage of $FOXP3^+$ Treg and pretreatment of Treg in vitro with imiquimod blocked their ability to suppress T cell proliferation. We now report two separate mechanisms for the inhibition of Treg activity by TLR7 agonists. First, we found that Treg from SCC treated in vivo and human skin Treg treated in vitro with imiquimod produced significantly less IL-10 and TGF-β, and expressed lower levels of the FOXP3 protein. Treated skin Treg also expressed decreased levels of CD73 and CD39, cell surface receptors associated with contact-mediated Treg suppression in mice. Second, several groups have demonstrated that IL-6 renders effector T cells resistant to suppression by Treg. We found that 50% of effector T cells from imiquimod treated SCC produced IL-6 compared with only 6% from untreated tumors. Up to 30% of T cells from normal human skin produced IL-6 after treatment with imiquimod; this effect was indirect and required other skin cell types. In summary, we have evidence that Treg are recruited to and/or locally expanded by SCC and that TLR7 agonists act by at least two separate mechanisms to nullify the activity of tumor infiltrating Treg, thus overcoming immune paralysis. Treg have been shown to inhibit the immune response to many human cancers. Our work suggests that TLR7 agonists, by virtue of their ability to paralyze Treg function and induce IL-6 in effector T cells, may be useful adjuncts to immunotherapy for other human malignancies.

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CCR2 ligands: The foundation of immune evasion in squamous cell carcinomas

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Squamous cell carcinomas of the skin (SCC) are a leading cause of death in organ transplant recipients and develop as a result of T cell immunosuppressive medications. Most patients have no prior history of skin cancer, suggesting immune control of SCC in healthy individuals. We have found that SCC tumor vessels lack E-selectin and that tumors exclude the population of CLA^+ skin-homing T cells most capable of recognizing the tumors. We report now that SCC are infiltrated by $CD11c^+$ $iNOS^+$ TNF-α-producing DC (TIP-DC). SCC treated in vivo with the TLR7 agonist imiquimod lacked TIP-DC and had upregulation of E-selectin on tumor vessels. Nitric oxide (NO) inhibits E-selectin expression on microvascular endothelial cells and we hypothesize that NO produced by TIP-DC inhibits E-selectin expression on tumor vessels. We have also found that SCC recruit $FOXP3^+$ Treg of a subtype usually restricted to the blood or lymph nodes; these cells likely play a role in establishing a local immunosuppressive environment around the tumors. CCR2 mediates the recruitment of TIP-DC precursors in mice and recruits Treg to human glioblastomas. Immunostaining of SCC tumors confirmed that TIP-DC express CCR2. Microarray studies of $FOXP3^+$ Treg from SCC vs. Treg from normal skin demonstrated a 40-fold increase in CCR2 expression in tumor Treg. Flow cytometry studies confirmed that CCR2 is expressed by the majority of $FOXP3^+$ Treg associated with SCC compared with a minority from normal skin. There was a 6-fold enrichment for CCR2 expressing Treg in human SCC vs. normal blood, suggesting specific recruitment of $CCR2^+$ Treg to SCC. The CCR2 ligand MCP-1 is expressed by esophageal SCC and we find increased production of CCR2 ligands MCP-2 and MCP-3 in skin SCC. In summary, we find that SCC recruit $CCR2^+$ TIP-DC and $CCR2^+$ $FOXP3^+$ Treg. We hypothesize that tumor production of CCR2 ligands mediates the recruitment of both cell types, establishing an immunosuppressive environment around the tumor. Our work suggests a role for suppression of CCR2 ligand production in the treatment and prevention of SCC.

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Amphiregulin regulates keratinocyte and T-cell interactions: A key mediator in psoriasis?

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Psoriasis develops through complex interaction between the skin and immune system. Overexpression of amphiregulin (AREG) in epidermis has been shown to induce a psoriasis-like cutaneous phenotype in transgenic mouse model. Therefore, AREG may serve as a pivotal mediator that links keratinocyte dysfunction to the immunological abnormalities. We examined gene transcripts of cultured human keratinocytes exposed to recombinant human AREG and TGFα at concentrations equipotent in EGF receptor activation using Affymetrix HG-U133A 2.0 GeneChip Arrays that measures 22,215 transcripts. In addition, trancriptomes of keratinocytes co-cultured with peripheral mononuclear blood cells(PBMC) in the presence of AREG and TGFα were also profiled. Both AREG and TGFα induce significant upregulation of genes involved in epidermal differentiation and molecules associated with activation of innate immunity. However, only AREG but not TGFα was able to induce the upregulation of multiple interferon-induced proteins and receptors. When PBMC were added to the keratinocyte cultures, both AREG and TGFα groups demonstrated significant increase in interleukin 2, 7 &8, CD2,3, 48 &52, CCL5 & 20, CXCR4, defensins; selectins and ICAMs. Interestingly, there are four distinct genes, including linker for activation of T cell family (LAT2), integrin beta 3 binding protein (ITGB3BP), macrophage stimulating 1 receptor and interleukin 17 receptor C that were only upregulated in AREG-exposed human keratinocytes co-cultured with PBMC. These results suggest AREG not only induces epidermal dysfunction but also directly induces release of chemokines and cytokines from keratinocytes that are related to the activation of innate and adaptive immunity. In addition, there is clear evidence of the interplay between the keratinocytes and immunocytes facilitated by AREG. Cytokines characterized by three T helper cell subsets Th1, Th2 and TH17 were all induced differentially by AREG. Further studies are elucidating the role of AREG in mediating keratinocyte and T cell interactions and the pathogenesis of psoriasis.

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NKG2D-ligand upregulation promotes rapid reorganization of a local immune compartment with pleiotropic effects on carcinogenesis

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We present an experimental system that provides a novel framework in which to assess early events in tissue immunosurveillance. Inducible, tissue-specific (bi-transgenic, bi-Tg) mice were generated to express the NKG2D-ligand, Rae-1, independent of any other aspect of tumorigenic or inflammatory dysregulation. We revealed that epidermal up-regulation of Rae-1 induced rapid, coincident and reversible changes in the activation, morphology, and emigration of tissue-resident Vgamma5/Vdelta1+ intraepithelial T cells and Langerhans cells (LC), followed by epithelial infiltration by NKG2D+NK1.1+alpha/beta+ natural killer T (NKT) cells that bound specifically to alpha-GalCer-CD1d tetramers. This NKT increase in the induced BiTg Rae-1 mice was greater than single Tg mice ($P = 0.003$). Despite the complete reconfiguration of the epidermal immune compartment evident by 120h after induced Rae-1 expression, all changes were fully reversible within three days of halting Rae-1 expression - a rapid "resetting" of the local immune compartment upon removal of the epithelial stress ligand. Paralleling these findings, tumor-infiltrating lymphocytes (TIL) from papillomas and carcinomas induced by 2-stage chemical carcinogenesis were 10-fold-enriched in CD4(-)CD8(-) alpha/beta+ T cells, relative to systemic lymphocytes (77.68%±2.50% in TIL vs 0.70%±0.31% in PBL; $P = 0.03$). Mice selectively double-deficient in Vgamma5 and Vdelta1 showed increased tumor development (9.92±1.29 vs 4.23±0.83 tumors/mouse in controls ; $P<0.01$), whereas LC-deficient mice were conversely tumor-protected (1.00±0.47 vs 5.67±1.63 tumors/mouse in controls, $P<0.005$). In sum, the two tissue-resident immune cell types that rapidly and co-ordinately responded to acute Rae-1 upregulation had diametrically opposed effects on tumor incidence, thus establishing the pleiotropy of local immunosurveillance. The experimental system used here has promising potential to aid the design of immunotherapeutic and prophylactic protocols.

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Age-dependent decrease of allergic contact dermatitis correlates to diminished allergen-specific Th17 and Th1 responses, and enhanced immune regulation in ageing mice

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Allergic contact dermatitis (ACD) decrease with age and older individuals are less susceptible to sensitization by contact allergens. To address the mechanism responsible for the age-dependent decrease in ACD, we investigated the helper, cytotoxic and regulatory T cell responses to DNFB in young (7 - 9 weeks) and old (12 - 16 months) mice. Old mice showed reduced dermatitis upon challenge with DNFB compared to young mice. Interestingly, old and young mice showed similar activation of allergen-specific CD8+ cytotoxic T cells in the draining lymph nodes; however the number of activated allergen-specific CD4+ helper T cells was significant reduced in older mice. We compared the Th17, Th1 and regulatory T cell profile of young and old mice. As observed previously, old mice contained relative higher numbers of memory (CD44+CD62L-) and regulatory (Foxp3+CD25+) cells in the CD4+ T cell compartment. We found, that this translated into a higher absolute number of Th17 (IL-17+), Th1 (IFNγ+) and Tr1 (IL-10+) cells in old mice. Although, old mice contained more pro-inflammatory Th17 and Th1 cells the capacity of these cells to produce IL-17 and IFNγ was significant lower. On the contrary, the anti-inflammatory Tr1 cells of old mice produced more or comparable levels of IL-10 per cell compared to young mice. When analyzing the phenotype of CD4+ helper T cells in the draining lymph nodes during the elicitation phase of ACD it was found, that old mice had lower number of allergen-specific Th1 cells, but similar numbers of allergen-specific Th17 and regulatory T cells compared to young mice. These results suggest that age-dependent amelioration of ACD is caused by enhanced anti-inflammatory mechanisms leading to diminished pro-inflammatory Th17 and Th1 responses to contact allergens.

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Beta2-integrin deficiency impairs lymphocyte homing to peripheral lymph nodes and generates unconventional T cells

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Exclusively expressed on leukocytes, β2 integrins (CD11/CD18) are specifically involved in leukocyte function participating their transmigration and activation. Absence of CD18 in humans and mice leads to severe immunodeficiency as well as autoimmunity accompanied by a defective lymphocyte recirculation and an altered phenotype. CD18-/- mice yield unconventional CD3+CD4-CD8- (DN) T cells with an apparently antigen-experienced phenotype in the periphery. These unconventional DN T cells were more than 30-fold elevated in comparison to WT mice, whereas single-positive (SP) T cells were reduced. We hypothesized that during intrathymic T cell development the transition from the DN to the SP state may be disrupted. But neither was a correlating increase in DN nor a decrease in SP thymocytes detected by FACS. Thus an impaired intrathymic T cell maturation to the SP state did not account for the high prevalence of DN T cells in the periphery. Since T cells are known to expand homeostatically under lymphopenic conditions, we next hypothesized DN T cells were expanded primarily in the periphery of CD18-/- mice due to the lack of peripheral naïve SP T cells in CD18-/- mice. Indeed, when purified and subjected to CFSE proliferation assays, only peripheral DN T cells but not CD4+ or CD8+ SP T cells were highly susceptible to expansion by exogenous IL-7, a central cytokine driving homeostatic T cell expansion, *in vitro*. However, no significant proliferation was observed upon IL-15, another homeostatic key cytokine. We conclude that homeostatic expansion may be a critical mechanisms leading to the expansion of DN T cells in the lymphopenic periphery of CD18-/- mice. It may further explain the antigen experienced-like phenotype of the observed DN T cells, which may hence not be the result of true high-affinity antigen encounter. Our results may contribute to the conception of apparently opposing pathomechanisms linking immunodeficiency with autoimmunity.

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The inflammasome regulates antigen presenting cell trafficking in the skin

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We have previously shown that contact hypersensitivity due to irritant chemicals, such as dinitrochlorobenzene (DNCB), is dependent on the inflammasome. This protein complex, composed of a Nod-like receptor (NLR) and the adaptor protein ASC, regulates the activation of caspase-1 and, subsequently, of the pro-inflammatory cytokines IL-1b and IL-18. We could recently show, using ASC and IL-1 receptor deficient mice, that danger signaling acts as a major switch between tolerance and sensitization. We have now started to further explore the mechanism underlying this phenomenon by studying the impact of the inflammasome on antigen presenting cell trafficking from and into the skin. We found that Langerhans cells (LC) fail to migrate to draining lymphnodes after exposure to a contact sensitizer, if either the inflammasome or the IL-1R signaling pathway were disrupted. Surprisingly, activation of the inflammasome by SDS, in the absence of a hapten, was not sufficient to induce LC migration. Moreover, we found that LC migration and sensitization can be uncoupled, as the weak sensitizer DNTB can be turned into a strong sensitizer in the presence of danger signals yet fail to induce LC migration. Hence, the inflammasome is necessary but not sufficient to induce LC migration. Concordantly, we found TNF to be also necessary for optimal LC migration, as has been previously reported. However, how contact sensitizers activate TNF remains to be clarified. Finally, using chimeric mice, we could show that the inflammasome is crucial to the recruitment of circulating dendritic cells (DC) to the epidermis independently of the DC attractant CCL-20. Taken together, these results suggest that the inflammasome acts as a key regulator ofantigen presenting cell trafficking into and from the skin.

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During secondary cutaneous immune response regulatory T cells proliferate in parallel to effector T cell pool

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Human naturally occurring regulatory T cells (nTregs; CD4+ FoxP3+) in peripheral blood are highly proliferative and may be derived in part, from the memory T cell pool. We sought further evidence for this possibility by examining a cutaneous secondary response induced by injection of tuberculin purified protein derivative (PPD). We utilized this model to examine the kinetics at which CD4+FoxP3+ T cells accumulate in the skin after antigenic challenge, to determine the proliferative activity of the FoxP3 expressing cells and to test the hypothesis that Tregs can differentiate from a responsive antigen-specific memory T cell population at the site of inflammation *in vivo*. Both CD4+FoxP3- (memory) and CD4+FoxP3+ (putative nTreg)populations had similar kinetics of accumulation and equivalent proliferative activity that peaked 7 days after initiation of the response. The proportion of CD4+FoxP3+ T cells was fairly constant throughout the response comprising 5-20% of the total CD4+ T cell infiltrate. However, CD4+FoxP3+ T cells in skin were potentially a mixture of responsive cells that were induced to express FoxP3 transiently and regulatory cells that were either generated *in situ* or recruited from the circulation.

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Th1 chemokine induces "swarm of bees" around hair follicles in alopecia areata

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Alopecia areata (AA) is recently regarded as organ-specific autoimmune disease, and autoreactive CD8+ T cells may recognize hair follicle (HF)-autoantigen. Many inflammatory cells surround HF in the lesion of acute AA, that are constituted by 60-80% CD4+T cells, 20-40% CD8+ T cells and some NK cells. In cytokine balance, Th1 cytokine, such as IFN-γ, is dominantly detected in AA-lesions that can induce the collapse of HF-immune privilege. Although the phenomenon of cell infiltration "swarm of bees" in acute AA-lesion is well known, the mechanisms of chemotaxis around the HFs has been largely ignored. Therefore, we investigated how the cells accumulate around the HFs in AA-lesions. In normal human scalp skin, CXCR3+ and CCR4+ cells were rarely detected. In contrast, CXCR3+ cells were dominantly detected in the lesion of AA. In addition, IP-10 was very strongly expressed in and around the HFs in AA lesion compared to normal control by immunohistochemistry that was supported by the result of real-time PCR. Next, in order to investigate the lymphocytes chemotaxis functionally in AA patients, we used EZ-TAXIScan that can show real-time chemotaxis directly under a microscope. The strong tendency of chemotaxis toward IP-10 were observed in freshly isolated PBMC from AA patients and CD4+ T cells from AA lesions. These results suggest that increased production of IP-10 may induce accumulation of CXCR3+ T cells in AA lesions that result in autoimmune attack against HF-autoantigen.

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"Dermal dendritic cells" comprise two distinct populations: CD1+ dendritic cells and CD209+ macrophages

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A key cell type of the resident skin immune system is the dendritic cell, which in normal skin is located in two distinct microanatomical compartments: Langerhans cells (LC) mainly in the epidermis and dermal dendritic cells (DDC) in the dermis. Here, the lineage of dermal dendritic cells was investigated using monoclonal antibodies and immunohistology. We provide evidence that "dermal dendritic cells" comprise at least two major phenotypic populations of dendritic appearing cells: immature DC expressing CD1 and macrophages expressing CD209, CD163 and CD68. These data suggest that dermal dendritic-appearing macrophages comprise a novel part of the innate immune response in the resident skin immune system.

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CXCL13: a valuable biomarker for monitoring lupus flares?

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The lack of reliable, specific biomarkers for lupus erythematosus (LE), a complex B-cell mediated autoimmune disorder, impedes precise assessment of disease activity and prompt identification of patients at risk for flares and organ damage. Recently, it has been demonstrated that the B lymphocyte chemoattractant CXCL13 (BLC, BCA-1) produced by mature myeloid dendritic cells is highly expressed in the target organs of aged BWF1 mice, a lupus mouse model, and that B1 cells, which are considered to be involved in autoantibody production, are preferentially chemoattracted toward CXCL13. Furthermore, elevated serum levels of type I interferon (IFN-I)-regulated chemokines, such as CXCL13, could be correlated with active systemic LE (SLE) in humans. The purpose of this study was to analyze the CXCL13 levels in ANA positive sera of 28 patients with different skin manifestations of LE. The Quantikine CXCL13 enzyme-linked immunosorbent assay (R&D Systems) was performed on sera derived from 13 patients with SLE, 13 patients with subacute cutaneous LE (SCLE), and 2 patients with chronic cutaneous LE (CCLE) in comparison to 20 ANA negative controls. Additionally, the CXCL13 levels and anti-dsDNA antibody titers were correlated in these patients. The CXCL13 levels differed significantly within the subgroups of LE ($p<0.001$). In contrast to ANA negative controls (median: 39.7 [min-max: 11.9-68.0]pg/ml), the CXCL13 levels were significantly higher in patients with SLE (90.2 [39.1-253.7]), whereas no statistically significant differences could be detected for the SCLE group (43.5 [23.3-431.6]) and for the 2 patients with CCLE (CXCL13: 48.8pg/ml; 71.1pg/ml). The CXCL13 and anti-dsDNA antibody levels were found to correlate in all patients ($r=0.56$; $p=0.002$). These data suggest that the CXCL13 ELISA might become a valuable tool for monitoring lupus flares. Further studies to correlate these chemokine levels with other lupus activity parameters should be performed.

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Anti-pruritic effect of prostaglandin D2 is normalized the hypersensitivity of primary afferents in mice

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Itching is considered to be a physiological phenomenon inducing scratching to eliminate foreign matters from the skin surface. In addition, the scratching also skin-impairing behaviors. It is therefore presumed that there should be some strict regulatory mechanism to suppress excessive skin impairment and maintain cutaneous homeostasis. We investigated the relation between scratching and the cutaneous prostaglandin D2 (PGD2) production level by the mechanical-scratching test, since PGD2 is considered to be an endogenous anti-pruritic substance. Moreover, topically applied PGD2 significantly suppresses scratching in conventional NC/Nga mice, a model of atopic dermatitis, but the precise mechanicsm of that action remains unknown. On the other hand, "alloknesis" refers to the phenomenon of itching elicited by innocuous mechanical, normally not-itch-provoking stimuli in patients with atopic dermatitis. We recently reported that pruritogens (histamine, serotonin, compound 48/80) and algogens (acetylcholine, bradykinin, capsaicin) intradermal injection in BALB/c mice cohabitated with skin-lesioned NC/Nga mice increased long-duration scratching, and indicated that this phenomenon might be similar to alloknesis. We examined the effect of topically applied PGD2 on the alloknesis in these mice. Significant increase of long-duration scratching was observed following intradermal injection of a pruritogen (histamine, serotonin, compound 48/80), algogen (acetylcholine, bradykinin, capsaicin), or saline in BALB/c mice cohabited with skin-lesioned NC/Nga mice for 1 week, but not in normal BALB/c mice (non-cohabited mice). Moreover, topically applied PGD2 significantly suppressed the long-duration scratching induced by the above substances in the cohabited BALB/c mice, but not in the normal BALB/c mice. In conclusion, the results suggest that the anti-pruritic efficacy of PGD2 is normalized may be related to its reversal of the hypersensitivity of primary afferents; thus, PGD2 exhibited a suppressive effect on alloknesis.

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Autoimmune bullous diseases in a northwestern region of Romania

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Autoimmune bullous diseases are organ specific diseases characterized by autoreactive T and B cells specific for structural proteins of the skin. Based on clinical, histo- and immunopathological findings, the diferent autoimmune blistering disorders may classified as pemphigus and pemphigoid diseases or the epidermolysis bullosa acquisita. While in the incidence and prevalence of autoimmune blistering diseases is known in different countries, their epidemiology has not yet been addressed in Romania. Therefore, in the present study we investigated between 2001 and 2007 a total of 116 patients patients with autoimmune blistering diseases from the Northwestern region of Romania. The diagnosis was based on the clinical, histo- and immunohistological as well as serological findings. Interestingly, pemphigus vulgaris (PV) was the most common disease representing 58,6% (68 cases). 40 cases (34,4%) were diagnosed with bullous pemphigoid (BP) and 8 cases (6,9%) with other autoimmune subepidermal diseases. The mean incidence was 0.4/100,000 for PV and 0.25/100,000 inhabitants for BP. Both for PV and BP disease severity strongly correlated with ELISA levels of autoantibodies against desmoglein 3 and BP180, respectively, rather than correlating with titers of circulating autoantibodies as revealed by indirect immunoflorescence microscopy. In conclusion, PV has a higher incidence than BP in a North-Western region of Romania. The fact that the life expectancy in Romania (71.3 yrs) is lower compared with Western countries (> 80 yrs) may provide an explanation for why PV (mean onset age of 53.4) is more frequently diagnose compared with BP (mean onset age of 73.6) in Romania compared with other countries.

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Analysis of regulatory T cells in melanoma

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CD4+CD25+ regulatory T cell (Treg) has been reported in cancer microenvironment. Involvement of CD4+CD25+ Treg for immune suppression is supposed as one of the obstacles in cancer immunotherapy. However little is known how Tregs were induced in cancer. Furthermore a key question is raised whether Tregs might be induced during antigen-based immunotherapy. In this study, we analyzed phenotype and cytokine profile in tumor infiltrating T lymphocytes (TILs) and PBMCs from melanoma patients. TILs were isolated and established from fresh metastatic melanoma. PBMCs from HLA-A*2 or HLA-A*24 positive melanoma patients were stimulated with peptides of melanoma antigens, including MART-1, gp100, tyrosinase, MAGE-1 and MAGE-3. Phenotype of TILs and T cells after *in vitro* sensitization was analyzed by flow cytometry, including expression of CD25 and foxP3. Cytokine production from T cells after *in vitro* sensitization was measured, as well as TILs and tumors. CD25+foxP3+ Tregs were detected at 0.7 – 2.4% in TILs. CD25+foxP3+ Tregs were detected 0.5 - 3.3% in PBMCs after *in vitro* sensitization with antigenic peptides, compared with 0 – 1% in un-stimulated PBMCs. Immune suppressive cytokines were released from tumors, but neither from TILs nor T cells after in vitro sensitization. We demonstrated that CD25+foxP3+Tregs were preferentially detected in T cells after *in vitro* sensitization. These results suggested that Tregs may be induced during antigen-based immunotherapy.

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Significance of S100A4 in the pathogenesis of psoriasis

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The metastasis-associated protein S100A4 belongs to the large family of calcium-binding proteins that appears to play regulatory roles in several biological activities. A prognostic role of S100A4 has been documented for patients with some types of cancer. Recently it has been shown that S100A4 is upregulated in the chronic inflammatory disease rheumatoid arthritis. S100A4 has not previous been studied in psoriasis. We wanted to study the expression of S100A4 in lesional (PP), non-lesional (PN) skin samples taken from patients with psoriasis in comparison with healthy controls (NN). Biopsies were stained for S100A4 ($n_{PP,PN}=10$, $n_{NN}=4$), and found was a significant increase of S100A4 expression in the dermis of PP compared to PN (1.8 fold) and NN (4.2 fold). Furthermore, the S100A4 protein was found released from PP biopsies assessed by sandwich ELISA of the supernatant ($n_{pp}=9$). Next we studied the effect of blocking S100A4 protein in a human SCID mouse xenograft model for psoriasis. Polyclonal S100A4 antibodies was given 100µg x 3 or 200µg x 3 ($n_{100}=2$, $n_{200}=2$) and in controls 200µg IgG x 3 ($n_{ctrl}=3$) in week one followed by half dose administration in week two. One week after the last treatment the mice were sacrificed and biopsies were obtained. We found that blocking S100A4 in psoriasis lead down to an 82% decrease in epidermal thickness, decrease in the number of proliferating cells and decrease in S100A4 expression. The strong S100A4 expression in the dermis indicates a potential pathogenic role of S100A4 in psoriasis. This was supported by the positive effect of treatment with antibodies against S100A4 in a SCID mouse model of psoriasis, thus further studies are needed. The increased S100A4 protein in psoriasis lesions may, as seen in cancer, be involved in the increased microvascularity and hyperproliferation seen in psoriasis.

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Mercury compounds induce phenotypic activation of human DCs via an oxidative stress

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Thimerosal (sodium ethylmercurithiosalicylate) is a water-soluble preservative composed of ethyl mercury and thiosalicylic acid. Thimerosal is known as a contact sensitizer responsible for inducing contact dermatitis and it possesses effective bacteriostatic and fungistatic properties. Some cases of generalized cutaneous reactions to thimerosal containing vaccines and a high frequency of sensitization in atopic children have been described. However the mechanisms by which thimerosal and mercury compounds modulate the immune system are still poorly understood. In this context, the aim of this study is to evaluate the role of the oxidative stress on dendritic cell (DCs) phenotypic activation. To determine whether chemicals such as thimerosal, mercuric derivatives or dinitrochlorobenzene induce DCs activation, DCs derived from human monocytes and the monocytic cell line U937 were used. Phenotypic activation of DCs was assessed by cytometry for the expression of selected surface maturation markers (CD86, CD54 and HLA-DR). A cross-talk between reactive oxygen species (ROS) and DCs activation was demonstrated using antioxidant such as N-acetyl-cysteine. In order to study the oxidative stress induced by sensitizers, ROS generation was detected by flow cytometry using several specific fluoroprobes (H2DCF-DA, DHE and DHR123). Glutathione measurement was assessed by cytofluorometry (DSSA and monobromobimane probes) in addition to HPLC-MS quantification. These studies will allow to better manipulate DCs for their use in cancer vaccinology and in inflammatory diseases.

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Pimecrolimus but not betamethasone sustains elevated antimicrobial protein expression in atopic dermatitis

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Reduced antimicrobial peptide (AMP) expression in atopic dermatitis (AD) has been described in one study, where it was hypothesized that this may be a reason for the increased rate of bacterial infection often associated with AD. Very recent studies, however, showed an increased expression of several AMPs in AD. We investigated whether topically applied drugs affect AMP expression in AD. In a randomized, double-blinded left-right comparison study, 15 patients with AD were treated on one upper limb with pimecrolimus and on the other with betamethasone twice daily for three weeks. Immunohistology was performed with specific antibodies for the AMPs psoriasin, RNase7, human beta-defensin 2 (HBD2), human beta-defensin 3 (HBD3), cathelicidin (LL-37), and the alpha-defensins HNP1-3. Before treatment, we found pronounced expression of psoriasin in AD in the upper spinous, granular, and horny layers. Bethamethasone treatment led to a significant reduction in psoriasin expression in most patients, whereas pimecrolimus led to only a slight reduction. HBD2, HBD3, and RNase7 showed a weak to moderate staining in the epidermis in AD before treatment. After treatment with betamethasone, staining intensity was reduced or disappeared, whereas pimecrolimus treatment led to only slightly reduced staining. We did not find a significant immunoreactivity for LL37 and HNP1-3 in AD before or after treatment. We conclude that treatment of AD with betamethasone, in contrast to pimecrolimus, results in a reduction of the AD-associated increased protein expression of several AMPs in most of the patients. The treatment-associated reduction of AMPs is probably related to a broad reduction in protein synthesis affected by the corticosteroid, which further increases the risk of skin infections. In contrast, pimecrolimus does not seem to significantly affect the skin's ability to express AMP and sustain antibacterial defense.

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S. aureus and S. aureus-derived lipoteichoic acid attentuate immediate-hypersensitivity reactions independent of TLR2

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Because IgE/mast cell (MC) interactions significantly contribute to allergic inflammation, we asked whether S. aureus and staphylococcal-derived products, common trigger factors of several allergic disorders, can selectively inhibit MC activation. First, we passively sensitized C57BL/6 mice intraperitoneally with DNP-specific IgE with or without heat-inactivated S. aureus followed by rectal temperature measurements upon DNP challenge. Interestingly, S. aureus-treated mice began to recover significantly faster from anaphylaxis. To identify the signals involved in this response, we next tested the S. aureus cell-wall products lipoteichoic acid (LTA) and the lipopeptide homologue MALP-2 showing both a similar downregulation of anaphylactic responses as S. aureus treated-mice, with LTA-treatment resulting in stronger inhibition. Interestingly, LTA and MALP-2 were only found to be beneficial when applied before as opposed to after the allergen challenge. To further elucidate the protective effects of LTA we quantified the levels of MC mediators by ELISA. The amount of histamine was severely reduced in the peritoneal lavage fluid of IgE+LTA-treated mice 30 minutes after challenge (18.75 ng/ml vs. 30.5 ng/ml in IgE+vehicle/DNP group; p=0.01). Also, de-novo synthesized leukotrienes were measured in the supernatants of IgE+LTA/DNP-stimulated peritoneal MCs (PMCs). Notably, LTA dramatically decreased leukotriene levels as compared to IgE+vehicle/DNP-stimulated PMCs (66 pg/ml vs. 6251 pg/ml; p=0.05) indicating that this pathway was affected as well. To clarify the underlying mechanisms, IgE+LTA-treated PMCs were subjected to FACS analysis of FcepsilonRI expression, which was downregulated by almost 60% (p=0.003). Intriguingly, this process occurred independently of TLR2, as FcepsilonRI expression was also downregulated by LTA in TLR2-deficient PMCs (70% reduction; p=0.002). Collectively, these data suggest that S. aureus-derived LTA may be a feasible therapeutic target to control MC-mediated type-I allergic reactions.

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A conserved C-terminal fragment of Ifapsoriasin is a selectively *Pseudomonas ssp.*-killing antimicrobial peptide

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In spite of permanent contact with soil bacteria mammalian skin is usually not infected. We hypothesized that healthy skin produces certain factor(s) that particularly protect the skin from infection by those bacteria, e.g. *Pseudomonas aeruginosa* (*P. a.*). Upon screening of heel stratum corneum extracts for antimicrobial peptides (AMPs) a peptide fragment of ifapsoriasin (IFPS), a novel skin- and mucosa-specific 248kDa S100 fusion type protein (SFTP) could be identified in *P.a.*-killing HPLC-fractions. Like the prototypical family member profilaggrin, IFPS also contains apart from an S100 domain an EF-hand domain, a spacer sequence, two glycine/serine-rich tandem repeat domains, and a unique C-terminus. To investigate the possible function of IFPS, three 7.5 – 15.5 kDa peptides representing different parts of the whole molecule were recombinantly expressed and tested for antimicrobial activity. Solely a C-terminal fragment, which included the last repeat domain and the C-terminus, was found to be highly active only against *P. a.* and some other soil bacteria. Analyses of further generated additional recombinant C-terminal IFPS peptides identified an antimicrobial 30 amino acid fragment, that was found to be highly conserved in putative IFPS-orthologs of more than 25 mammalian species. To analyze IFPS expression in skin in more detail, antibodies against different IFPS domains were generated and localized immunoreactive IFPS in the uppermost cell layers of stratum granulosum and in the stratum corneum, especially at hands and feet, where it was also strongly expressed in eccrine sweat glands. Western blot analyses suggested processing of IFPS with release of C-terminal fragments, which were further found at the surface of the tongue and in sweat. Identification of IFPS at the surface of skin and mucosa, particularly where moisture-requiring soil bacteria may come into contact and potentially may grow, suggests IFPS as a novel innate defense factor that may protect body surfaces from infection by soil bacteria.

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Platelet-activating factor down-regulates aggregation and LMP-1 expression of Epstein-Barr virus-transformed B lymphoblastoid cells

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B lymphocytes can be immortalized by Epstein-Barr virus (EBV) infection. EBV-transformed B lymphoblastoid cells (BLCs) preferentially aggregates in vitro. The detail mechanism of the aggregation has not been elucidated to date although the role of EBV nuclear antigen (EBNA) and latent membrane protein (LMP) has been ascertained. We here report the influence of platelet-activating factor (PAF) on BLC. PAF is a biologically active ether phospholipid produced by many kinds of cells including eosinophil and mast cell and predominantly involved in inflammatory and immune responses. PAF significantly reduced aggregation of BLCs and down-regulated LMP-1 expression. The suppression of BLC aggregation facilitated more clonal proliferation of BLCs and the down-regulated LMP-1 expression reduced a susceptibility of PAF-pulsed BLC to autologous LMP-1-specific CTL line. The findings suggest a possible participation of PAF in development and clinical course of EBV-associated disorders.

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Rosai-Dorfman disease-derived B lymphocytes have a chemotactic effect on monocytes

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Rosai-Dorfman disease is a rare non-neoplastic histiocytosis of unknown etiology. This disease is generally considered a benign self-limited proliferation of histiocytes, and often shows spontaneous remission although chronic persistent course has also been recognized even after extensive therapeutic intervention. Typical histological findings exhibit a dermal or subcutaneous dense infiltrate of histiocytes with varing population of lymphocytes and emperipolesis. The histogenetic detail of the preferential infiltration of histiocytes has been unknown. We previously showed that B lymphocytes isolated from cutaneous mass of Rosai-Dorfman disease expressed macrophage chemotactic protein (MCP) 1-3 and macrophage infiltrating factor (MIP) 1, 2 at mRNA level, and that those B lymphocytes served as a chemoattractant to allogeneic monocytes. The present study further evaluated the expression of MCPs and MIPs at protein level and the B lymphocytes effect on autologous monocytes derived from cutaneous lesion by migration assay using modified Boyden chamber. The results suggested a partial contribution of lymphocytes to migration of histiocytes although the initial step in histogenetic process of Rosai-Dorfman disease has still been unclear.

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Impaired migration of dendritic cells and enhanced contact sensitivity responses in transgenic mice with lymphatic dysfunction

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K-cyclin transgenic (Tg) mice, in which the Kaposi's sarcoma-associated herpesvirus K-cyclin gene is expressed specifically in lymphatic endothelial cells, show severe lymphatic dysfunction (Blood. 2005 105: 2356-63). Here, we investigated the role of the lymphatic system in contact sensitivity responses by comparing K-cyclin Tg mice and wild-type littermates. The mice were sensitized by applying 2,4-dinitrofluorobenzene (DNFB) or FITC onto shaved abdominal skin. Thickness of ears was measured 24 hr following challenge. Surprisingly, K-cyclin Tg mice showed stronger contact sensitivity responses compared to wild-type littermates (1.13 ± 0.29 mm vs. 0.45 ± 0.27 mm, P<0.01). Wild-type mice transferred with DNFB-sensitized lymphocytes obtained from inguinal lymph nodes either from wild-type or K-cyclin Tg mice showed very low contact sensitivity responses (0.18 ± 0.08 mm and 0.23 ± 0.21 mm, respectively). By contrast, K-cyclin Tg mice transferred with DNFB-sensitized lymphocytes showed strong contact sensitivity responses (0.83 ± 0.56 mm and 0.73 ± 0.57 mm, respectively). In addition, K-cyclin Tg mice showed stronger irritant responses compared to wild-type littermates following application of croton oil (0.88 ± 0.35 mm vs. 0.41 ± 0.12 mm, P<0.01). Next, we documented rare FITC+I-A+ dendritic cells in draining lymph nodes of K-cyclin Tg mice 24 to 72 hr following skin application of FITC, suggesting that skin-derived dendritic cells do not migrate efficiently into draining lymph nodes of K-cyclin Tg mice. Interestingly, impaired dendritic cell migration associated with peripheral lymphatic dysfunction leads to enhanced, and not diminished, allergic and irritant contact sensitivity responses in skin.

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Tumor necrosis factor-alpha induces the production of stem cell factor in human neurofibroma cells derived from neurofibromatosis 1

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Neurofibromatosis 1 (NF1) is characterized by multiple development of neurofibroma (NF) with abundant mast cells infiltration. Although the size is various, some NFs considerably grow despite of the benign tumor. This implies the independent contributory factor, except for NF1 gene, to mitogenic activity of NF cells. We previously showed that tumor necrosis factor-alpha (TNF-alpha) significantly accelerated the proliferation of NF1-derived NF cells *in vitro*. Intratumorous expression of TNF-alpha was immunohistologically found in only mast cells but not other component cells. The findings revealed a concrete role of mast cells in NF growth. In this study, we examined whether TNF-alpha-stimulated NF cells could produce cytokines relevant for mast cells in return. TNF-alpha induced the synthesis of stem cell factor (SCF) in all four kinds of NF cells while IL-3 production was partially found and IL-6 production was not detected. SCF is a multifunctional cytokine associated with survival, differentiation, migration and proliferation of mast cells. Our results suggest a possible mutual action via TNF-alpha and SCF between NF cells and mast cells.

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Inducible nitric oxide synthase regulates dendritic cell migration and contributes to contact hypersensitivity

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Contact hypersensitivity is a T lymphocyte-mediated acquired skin immune reaction induced by hapten exposure. While it is known that nitric oxide (NO) plays important roles in various physiological conditions, and that one of NO synthases, inducible NO synthase (iNOS), is induced by external stimuli, the roles of iNOS in the skin remain mostly unknown. Here we sought to evaluate the role of iNOS in the mouse skin immune responses using an iNOS specific inhibitor and iNOS knockout mice. Initially we confirmed that iNOS was expressed intracytoplasmically in murine Langerhans cells (LCs) among epidermal cell (EC) suspensions using iNOS antibody. We then investigated the involvement of iNOS in a contact hypersensitivity (CHS) reaction, and found that a CHS response induced by FITC as a hapten was enhanced by iNOS inhibitor applied during the sensitization period. Accordingly, iNOS inhibitor significantly augumented the FITC-induced migration of cutaneous dendritic cells (DCs) including Langerin+ LCs and Langerin- dermal DCs in the draining lymph nodes. We then evaluated the roles of iNOS on LCs *in vitro*. On the contrary to *in vivo* results, iNOS inhibitor did not enhance, but rather suppressed the maturation and T cell stimulatory activities of LCs assessed by flowcytometry and cell harvester, respectively. Such inhibitory effects by iNOS inhibitor was not observed in iNOS knockout-derived LCs, confirming the specificity of iNOS inhibitor. These results suggest that iNOS has biphasic activities, suppressing cutaneous DC migration, enhancing the costimulatory molecule expression and T cell stimulatory capacity, and that the resultant overall role of iNOS produced by LCs under external stimuli suppresses a CHS response during sensitization phase.

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Proinflammatory slanDC (6-sulfoLacNAc+ dendritic cells) are present in the inflammatory infiltrate of atopic dermatitis, display an increased expression of CD86 in blood and retain their strong capacity to produce IL-12 and TNFα

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Dendritic cells are suggested to play a central role in the pathogenesis of atopic dermatitis (AD). We previously described the population of 6-sulfo LacNAC expressing dendritic cells, called slanDC (Immunity 2002). SlanDC are the largest population of human blood DC and were shown to prime naïve antigen specific T cells, to produce the highest levels of IL-12 among peripheral blood leukocytes and to program strong Th1-responses (Immunity 2006). In this study we asked whether slanDC may contribute to the immunopathology of atopic dermatitis. With the help a slanDC-specific mAb biopsies obtained from patients with chronic AD lesions (n=15) were studied. SlanDC expressing high levels of CD11c and HLA-DR were frequently found in the perivascular inflammatory infiltrate in all tissues studied. The epidermis was always devoid of slanDC. When studying the phenotype of slanDC in blood of patients with AD (n=16) we observed a significantly higher expression of CD86 compared to healthy controls. SlanDC cultured in the absence of any stimuli displayed a strong upregulation of HLA-DR, CD83 and CD86. In accordance with their high proinflammatory capacity described previously by our group, LPS-stimulated slanDC of patients with AD displayed a much higher production of TNFα as well as IL-12p40/70 compared to monocytes (p= .007 and .0001). Furthermore, slanDC of patients with AD showed an unaltered capacity to produce high levels of IL-12, as studied on the single cells level. Taken together we demonstrate the presence of proinflammatory slanDC in lesional skin of atopic dermatitis and describe slanDC in blood expressing high levels of the costimulatory molecule CD86. Moreover, slanDC in blood of patients with AD can serve as high level producers of IL-12 as well as TNFα. We therefore conclude that slanDC may significantly contribute to the immunopathology seen in chronic AD.

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Sweat antigen induces histamine release from basophils of patients with cholinergic urticaria, associated with high serum IgE concentration and large eosinophil number

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We have previously demonstrated that semi-purified human sweat antigen causes skin reactions and histamine release from basophils of patients with atopic dermatitis (AD) via specific IgE. Patients with cholinergic urticaria (ChU) also develop skin reactions and histamine release of basophils in response to autologous sweat. Our purpose was to study whether patients with ChU may share the sensitivity against sweat antigen with patients with AD and to study clinical characteristics among ChU patients with histamine release activity of basophils. The standard sweat antigen (SA) that induces histamine release from basophils of patients with AD was prepared by concanavalin A, anion-exchange, and reverse-phase chromatographies. Relations between basophile reactivity against SA and clinical features of patients with ChU were analyzed. Twenty-four out of 33 patients with ChU showed positive responses (> 5%) in histamine release test (HRT) with SA, whereas none of healthy controls did so. Patients with positive responses in HRT showed higher incidence of atopic dermatitis, bronchial asthma, allergic conjunctivitis or allergic rhinitis than patients with negative response. The patients with positive responses also showed higher levels of serum IgE and peripheral blood eosinophils, which were correlated with degrees of histamine release. Approximately 70% of patients with ChU are sensitive to SA as many of the patients with AD are. Moreover, ChU patients with sweat hypersensitivity were associated with atopic backgroud. The sweat allergy in patients with ChU may be developed on atopic diathesis .

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Increased cell surface Ku70/Ku80 expression on T cells following apoptosis or activation inducing signals

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Ku, a heterodimeric protein consisting of two subunits, Ku70 and Ku80, is involved in non-homologous end-joining (NHEJ) of the DNA double-strand breaks, telomere maintenance, cell survival, DNA-dependent helicase activity and VDJ recombination. Nuclear Ku70/80 plays a central role in repair of double-strand breaks. Cytoplasmic Ku70 has an anti-apoptotic role via inhibition of Bax translocation to mitochondria. Membrane-bound Ku, has been proposed to play a role in cell-cell, cell-extracellular matrix adhesion and tumor metastasis. We have previously demonstrated increased Ku70 immunostaining in cutaneous T cell lymphoma (CTCL) patients' Pautrier's microabscesses. In this study we show that 100% of normal and CTCL peripheral blood T cells express intracellular Ku70/80; by contrast 3-15% of both normal and malignant T cells express surface Ku70/80. Apoptosis-inducing stimuli, such as UV, but also TCR-dependent and independent T cell activation caused significant upregulation of cell surface Ku70/80 from 10% to 60% following T cell activation or 54% and 71% after 25 and 72mJ/cm2 UVB, respectively. Upregulated surface expression was also observed in CTCL cells, and augmented by pretreatment with trichostatin A. Increased membrane-bound Ku70/80 was not inhibited by cycloheximide, suggesting that cell surface Ku70/Ku80 detection was not due to de novo protein synthesis. Cell surface Ku70/80 upregulation was independent of caspase activation, suggesting that translocation occurs prior to apoptosis. Our results suggest that activation as well as apoptotic stimuli lead to caspase-independent increased surface Ku expression on T cells which may augment phagocytosis by macrophages or a signal required for apoptosis. Increased Ku70 surface expression and enhanced apoptosis by HDAC inhibitors in combination with UV suggests a potential therapeutic treatment of CTCL.

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Visualization of the human beta-defensin-2 in cultured keratinocytes

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The antimicrobial peptide human beta-defensin-2 (hBD-2) belongs to the innate defense system of the human skin. *In vivo* it is inducibly expressed in the upper (differentiated) layers of the epidermis. On the ultra-structural level hBD-2 is located in lamellar bodies (LB), specific organelles implicated in the export of substances to the stratum corneum. In spite of such specific localization *in situ*, expression of the hBD-2 can be stimulated in cultured primary keratinocytes. Primary keratinocytes are lacking the lamellar bodies and it is unclear how hBD-2 is stored and secreted in these cells. Herein we have visualized hBD-2 localization in primary, undifferentiated keratinocytes by fluorescent microscopy. As a model we have used primary foreskin keratinocytes cultured at low calcium concentration. hBD-2 expression was induced by the addition of IL-17 (20ng/ml) for 24 hours. The level of the hBD-2 expression was determined by ELISA. Expression of hBD-2 on the high level was revealed just in one part of the cell population, namely in the cells growing above the basic layer of cells. In these cells hBD-2 is localized in discrete granules in the cytoplasm. Very often these granules associate with the outer part of the nuclear envelope and build up conglomerates in the middle part of the cell. No colocalisation of the granules with the endoplasmatic reticulum or Golgi apparatus was observed. We suppose that the expression of the hBD-2 in cell culture reminds the situation *in vivo*: cells exhibiting hBD-2 expression level are higher differentiated cells

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NLR activity in contact hypersensitivity: a role of langerhans cells and/or keratinocytes

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We have previously shown that contact hypersensitivity (CHS) due to contact sensitizers (CS), such as dinitrochlorobenzene, is dependent on the inflammasome. This protein complex, composed of a Nod-like receptor (NLR) and the adaptor protein ASC, regulates the activation of caspase-1 and, subsequently, of the pro-inflammatory cytokines IL-1β and IL-18. We found that the importance of the inflammasome is mainly restricted to the sensitization phase of CHS. At this stage, Langerhans cells and bone marrow dendritic cells are thought to play a more important role than T, B, NK and Mast cells, although this is debated. We set to determine which cells require an inflammasome to prime T cells in CHS, using both *in vitro* studies on primary cells, and *in vivo* studies in mice. Surprisingly, we found that neither Langerhans cells lines nor primary dendritic cells can activate IL-1β in response to contact sensitizers. However, both cells responded well to classic LS containing the NLR activator muramyldipeptide. Primary T, B, NK, Mast cells and similar cell lines although failed to respond to CS, whereas keratinocytes secreted significant amounts of IL-1β. Similarly, UV irradiation, another inflammasome-trigger, induced significant response in keratinocytes only. Taken together, these data may suggest that keratinocytes are the main target of CS. To further refine this question, we have generated chimeric mice that either contain an inflammasome-deficient bone-marrow or skin. CHS responses in these mice suggest that radio-resistant skin resident cells are the major source of inflammasome-driven IL-1β. We are currently generating LC specific inflammasome deficient mice, which will allow us to definitely identify the cells targeted by CS.

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Ablation of mature mast cells in adult mice based on Cre/loxP-mediated recombination

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Mast cells play a crucial role in allergies and host defence against pathogens. In addition, mast cells were implicated in autoimmune processes, cancer, tissue repair, and transplant tolerance. Investigation of this important cell type, however, was severely hampered by the scarcity of mast cells in tissues. Herein, we use the Cre/loxP recombination system for conditional mast cell-specific mutagenesis in vivo. A transgenic mouse line was generated that expresses the Cre recombinase under the control of the mast cell protease 5 (Mcpt5) promotor. In a bacterial artificial chromosome (BAC) containing the Mcpt5 locus along with abundant flanking DNA, we replaced the coding region of exon 1 by a Cre cassette. The BAC insert was pronucleus injected and Mcpt5-Cre transgenic mice were crossed to a Rosa26-EYFP reporter line to demonstrate mast cell-specific Cre-mediated recombination. Double transgenic animals showed highly efficient recombination in mast cells, but not in other cell types. Next, Mcpt5-Cre mice were mated to the iDTR transgenic line, which expresses a human diphtheria toxin receptor upon Cre-mediated deletion of a loxP-flanked stop element. After intraperitoneal injections of diphtheria toxin, mast cells were no longer detectable in peritoneal cavity or skin demonstrating that this cross allows inducible ablation of the mast cell lineage in vivo. Investigating mast cell numbers in skin biopsies at different time points after ablation of mast cells, we observed exponential mast cell repopulation over several weeks compatible with proliferation of a limited population of local precursors of cutaneous mast cells. Our new mouse models will allow to elucidate mast cell-specific functions and to get new insights into mast cell biology.

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Activation of innate and adaptive immune responses in early Ixodes ricinus tick bite lesions in humans. Histopathologic and immunohistochemical study of 15 patients.

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Ixodes ricinus (IR) ticks are the vector for many important pathogens. The immune response directed towards infested uninfected ticks has not yet been examined in human skin. 15 patients (m:f=8:7, median age 35yrs, 18-70yrs) with a recent IR bite (< 48hrs ago, tick still attached) were included. Exclusion criteria were expanded local inflammation, previous topical treatment, and extracutaneous symptoms acutely or during a 6wks follow-up. A 4mm punch biopsy was taken in all patients from the bite site. Serial sections of formalin-fixed, paraffin-embedded biopsy specimens were investigated by histopathology and immunohistochemistry (IHC). Mouse anti-human monoclonal 1st antibodies to leukocyte differentiation antigens CD11c, CD68, CD4, CD8, and CD20 were from Dako (Glostrup, DK) or Novocastra (Newcastle, UK). Binding was detected by an indirect streptavidin-biotin method with a goat anti-mouse/rabbit 2nd antibody (Dako REAL, Dako). Semi quantitative grading of IHC staining from 0-3 was performed by 2 blinded observers. Histopathology showed spongiosis, epidermal vesicles or necrosis around tick fragments, and papillary edema in all cases. A moderate to severe primarily perivascular and superficial dermal infiltrate, sometimes extending into the panniculus was equally composed of eosinophils, neutrophils, histiocytes, and lymphocytes. IHC grading revealed a predominance of dendritic cells (mean score (ms) 1.9) over histiocytes (ms 1.1). The number of CD4+ (ms 1.5) and CD8+ (ms 1.2) lymphocytes was comparably moderate, whereas B cells were infrequent (ms 0.5). B cells more than T cells were clustered in primarily perivascular location throughout the dermis, whereas dendritic cells and histiocytes were found more scattered with predominance for the deep dermis. Early IR tick bite lesions are histopathologically characterized by an intense mixed inflammatory infiltrate. IHC reflects both, activation of an innate and adaptive, particularly cell-mediated immune response.

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A novel inducible transgenic mouse model for aggressive systemic mastocytosis

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Mastocytosis is characterized by abnormal numbers of mast cells in skin and internal organs. Clinical manifestations range from mild increases in cutaneous mast cells to severe forms of mast cell leukemia. The disease is associated with point mutations of the KIT gene, which result in constitutive signalling of the Kit receptor. While these mutations are most likely key events in pathogenesis, their relevance for the different forms of the disease is unclear. We have now generated a transgenic mouse model based on a bacterial artificial chromosome (BAC) containing the entire KIT gene along with abundant flanking DNA. In addition to the activating point mutation, we introduced genetic switch elements allowing for Cre/loxP-mediated control of transgene expression. Early general expression results in perinatal lethality due to dysregulation of hematopoesis with excessive leukocytosis. This is in accordance with the absence of germline inheritance of the corresponding mutation in human mastocytosis. Furthermore, our model also allows a specific induction of the transgene in adult mice. The analysis demonstrated an aggressive form of mastocytosis following induction of Cre expression by type I interferon. The disease of the adult animals is characterized by diffuse infiltration of the skin with high numbers of mast cells and we also observed multiple focal accumulations. About half of the animals develop additional hematopoetic neoplasms. Our new model provides clues for the understanding of the molecular pathogenesis of mastocytosis and will be a valuable tool in the establishment of new therapeutic approaches.

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Expression profiling of differentially regulated genes in activated and resting human memory versus naïve T cells

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Immune system dysfunction can contribute to inflammatory cutaneous diseases. Memory T cells, which are important effectors of the immune response, are localized to sites of cutaneous pathology. We hypothesize that investigating the transformation of naïve T cells into memory T cells by their genetic profiles may provide insight into the role of abnormal memory T cells in disease. To identify gene expression differences between naïve and memory T cells, we compared global gene expression using Affymetrix oligo-microarrays in activated and resting memory and naïve CD4+ T cells (n=3 sets). Gene sets were screened for difference in expression by 5-fold or more. We verified these differences at the transcriptional level using quantitative reverse transcription PCR and the translational level by Western blots. Microarray analysis revealed 13 genes up-regulated at least five-fold in activated memory versus naïve T cells. These included cytokines, signal transduction ligands and receptors, synaptic proteins, and cell communication and nucleic acid binding molecules. All but one gene had at least five-fold mRNA elevation, and half of them had increased protein levels. 10 genes, which encoded receptors including chemokine receptors, signal transduction molecules, and transcription factors, were discovered to be increased at least five-fold in resting memory versus naïve T cells. Seven had up-regulated mRNA levels of at least five-fold, and three had elevated protein levels by previous studies. Microarray analysis yielded 4 genes up-regulated by at least five-fold in resting naïve versus memory T cells. These genes represented a nucleotide exchange factor, a receptor, and two hypothetical proteins. All were confirmed to have elevated mRNA levels of at least five-fold, and two demonstrated increased protein levels. Identifying specific genes differentially regulated in memory and naïve T cells not only sheds light on memory T cell development but also adds to a growing list of candidate genes that may drive the evolution of cutaneous T cell mediated diseases.

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Pathway characterization of infliximab treatment from *in situ* analysis of psoriatic plaques

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Our purpose was to establish a molecular profile of psoriasis (PsO) to define the early and late panel of biological processes and pathways involved in the resolution of PsO upon IFX treatment. Gene expression profile was evaluated in skin biopsies collected prior to treatment(baseline,BL)and at day 3 and wk 10 from 70 pts treated with PBO,3-or5mg/kg IFX at wks 0,2,and 6 from a 50wk phase 3 trial of 835 PsO patients evaluating the safety and efficacy of IFX induction monotherapy(3- and 5mg/kg)followed by 4 regimens of IFX maintenance monotherapy(scheduled either q8 wks or PRN for each of the 2 doses). Normal skin samples(N) collected from normal healthy volunteers were analyzed. A molecular profile of PsO was established comparing N to BL samples showing the activation of numerous biological processes and pathways. Genes included in either the Th1 pathway such as STAT1,IL-8 and Mx1,or genes from the Th17 pathway such as IL17,DEFB4,CCL20 and IL22 were upregulated when compared to normal skin.Upon IFX treatment,pro-inflammatory cytokines,chemokines,immune response-related genes,and genes involved in cell cycle,proliferation and division,and defense responses were part of the IFX treatment effects.These effects were measurable as early as day 3 and were more pronounced at wk 10.Genes included in the Th1 and Th17 pathways were down-regulated.None of the genes identified comparing PBO post-treatment samples in both treatment groups to BL samples overlapped with those identified after IFX treatment.A response signature,defined by comparing responders to non-responders,showed a predominance of metabolic-related pathways.A cross comparison between the post-treatment gene expression changes and the response signature at wk10 highlighted genes involved in cell cycle-related pathways while excluding any immune response or metabolism-related pathways.In conclusion, IFX significantly impacted the Th17 pathway which has a role in reducing epidermal hyperplasia upon TNF inhibition.These findings confirm that antiTNF therapy impacts various biological processes and pathways in PsO.

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TRAIL-expressing plasmacytoid dendritic cells induce T Cell apoptosis in viremic HIV-1 patients

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It has been suggested that HIV-1 interacts with toll-like receptor-7 (TLR-7) on plasmacytoid dendritic cells (pDCs). We recently found that pDCs stimulated with TLR-7 ligands are induced to express tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and mediate death in responsive cancer cells. We therefore investigated the phenotype and function of pDCs in HIV-1 viremia and their possible role in triggering death in permissive T cells. The surface expression of TRAIL and its pro-apoptotic receptors TRAIL-R1/2 as well as anti-apoptotic TRAIL-R3/4 was determined on subsets of circulating leukocyte subsets gained from viremic HIV-1-infected patients, HIV-1 patients on HAART with a stable viral load below the limit of quantification and healthy controls. We also analyzed the lytic properties of patient-derived pDCs against purified $CD4^+$ T cells. While pDCs from healthy donors or treated recipients had no surface expression of TRAIL, this molecule was significantly upregulated on pDCs of untreated viremic patients. The apoptosis-inducing TRAIL-R1 was found to be expressed on $CD4^+$ T cells of viremic and non-viremic HIV-1 patients but not on those of healthy subjects. Importantly though, HAART recipients additionally displayed the anti-apoptotic TRAIL-R4 on these cells. Cytotoxicity assays with purified cell subsets from viremic HIV-1 patients revealed lytic properties of pDCs against $CD4^+$ T cells, in contrast to pDCs from healthy controls or treated patients. In *ex vivo* experiments with PBMCs from viremic HIV-1 donors, depletion of pDCs and inhibition of TRAIL or IFNα resulted in reduced $CD4^+$ T cell apoptosis. Depletion of $CD8^+$ T cells or monocytes from viremic HIV-1 patients had no effect on the apoptosis of $CD4^+$ T cells. Our data provide evidence that pDCs of viremic HIV-1 patients have the potential to kill permissive $CD4^+$ T cells in a TRAIL-dependent manner and, thus, could contribute to T cell depletion in HIV-infected individuals.

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The chemokine receptor CCR3 participates in tissue remodeling during atopic skin inflammation

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Atopic dermatitis is a chronic inflammatory skin disease with tissue remodeling. Although recent studies provided insight into the recruitment and activation pathways of leukocytes the underlying mechanisms of tissue remodeling in atopic skin inflammation remain elusive. Our objective was to identify chemokine-mediated communication pathways regulating tissue remodeling during atopic skin inflammation. Analysis of CCR3 expression and that of relevant chemokines in atopic vs. non-atopic skin inflammation and in healthy skin, and on cultured skin structural cells were characterized by qPCR. Immunofluorescent microscopy, flow cytometry analysis were performed on cultured fibroblasts. Cellular origin of chemokine receptor and ligands were determined by immunohistochemistry. Functional relevance were demonstrated by intracellular calcium mobilization, *in vitro*-wound repair, cell migration, and BrdU assays. Here we demonstrate the abundant expression of the chemokine receptor CCR3 *in vitro* and *in vivo*. Among its ligands CCL26 is significantly up-regulated in atopic when compared to healthy skin. *In vivo*, epidermal keratinocytes show abundant CCL26 expression in lesional atopic skin. In structural cells of the skin, TH2-cytokines are dominant inducers of CCL26 expression. CCL26 enhances fibroblast migration and *in vitro* wound repair through CCR3 signaling, but has no effect on fibroblast proliferation. Taken together, human dermal fibroblasts express functionally active CCR3 on their cell surface. In atopic skin inflammation CCL26 induces fibroblast migration and repair resulting in skin-related tissue remodeling.

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T helper 17 cells maintain their continual presence in psoriasis through a CCL20-CCR6-mediated positive chemotactic feedback loop

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Interleukin (IL)-23 is over-expressed by both keratinocytes (KC) and dendritic cells in psoriasis and serves as a key survival and proliferation factor for T helper (Th) 17 cells, types of T cells that have recently been implicated in psoriasis pathogenesis. Human Th17 cells secrete IL-17A, IL-22, and tumor necrosis factor (TNF)-α, and have also been shown to express CC chemokine receptor (CCR) 6, a chemokine receptor involved in leukocyte trafficking into skin and other tissues. The purpose of this study was to explore mechanisms of how Th17 cells traffic into inflamed skin. By immunostaining for IL-17A, IL-22, and CCR6, we found numerous Th17 cells present in psoriatic plaques, but not in normal human skin. Using both normal human KC in monolayer cultures and stratified human KC in reconstructed human epidermis, we found that Th17 cytokines (IL-17A, IL-22, TNF-α) markedly increased mRNA and protein expression of CC chemokine ligand (CCL) 20, a CCR6 ligand. In psoriasis lesions, CCL20 was abundantly expressed by KC, and numerous CCR6+ leukocytes were present in dermal papillae adjacent to CCL20+ KC. Lastly, we show that subcutaneous injection with IL-17A, IL-22, and TNF-α up-regulated mRNA and protein expression of CCL20 in murine epidermis, and also led to influx of CCR6+ leukocytes into treated skin. In summary, Th17 cells produce cytokines that stimulate KC to produce CCL20, which in turn is capable of recruiting additional CCR6+ Th17 cells into skin. These data provide a potential explanation of how Th17 cells maintain their continual presence in psoriasis through a CCL20-CCR6-mediated positive chemotactic feedback loop.

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Differential effects of irritants and contact allergens on keratinocytes and intact skin *in vitro*

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The irritant effect of potent contact sensitisers may be important in potentiating their immunogenicity. The nature of irritancy is not understood, but may reflect activation of innate immune mechanisms in resident skin cells which potentiates antigen presentation. We have compared the effects of a contact sensitiser (DNCB) and an irritant (croton oil) on primary keratinocytes and intact skin *in vitro*. Keratinocytes were isolated from healthy skin and stimulated with 3% croton oil or 3μM DNCB for 6 hrs. Human skin samples (8mm punch biopsies) were mounted in Franz diffusion chambers and the epidermis treated with 3% croton oil or 0.25% DNCB in acetone for 6 hours. Expression of IL-1β, IL-6, IL-8, IL-12, IL-18 and TNFα mRNA was determined by RT-PCR. Cytokine mRNA levels were expressed as relative units(RU) above untreated samples. The addition of croton oil to intact skin(n=5) increased expression of mRNA for IL-1β and IL-18(median RU 2.0 and 4.6, respectively). DNCB caused an increase in the expression of IL-1β, IL-8 and IL-18(RU medians 4.9, 6.8 and 2.3, respectively in skin). By comparison, keratinocytes (n=5) exposed to croton oil demonstrated an increase in the expression of IL-6(median RU 7.3), IL-8(median RU 10.6), IL-12p40(median RU 4.2) and TNFα(median RU 3.4), whereas those exposed to DNCB only IL-6 showed >2 fold increase(median RU 2.2). Thus, croton oil induced greater expression of inflammatory cytokines by keratinocytes whereas DNCB induced a greater expression of cytokines in skin. This difference could reflect differences in penetration into skin and/or it may reflect the activation of other cell types such as Langerhans' cells, by the more immunogenic DNCB. It is possible that the combination of both keratinocyte and *ex vivo* skin systems could be a useful screening tool to investigate new chemicals for irritant and immunogenic properties. However, the results highlight the importance of using whole human skin for investigating the mechanisms which are likely to involve interactions of different cell types.

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Macrophage mediated UVB-induced immunosuppression occurs via IL-6

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Cutaneous UVB exposure leads to suppression of immune responses and has been shown to be mediated by the immunosuppressive cytokine IL-10. We have previously shown that monocytes/macrophages are important cellular mediators of immunosuppression and have identified the cytokine IL-6 as a critical factor, as IL-6 deficient mice do not demonstrate UV-induced immunosuppression. Using FITC skin-painting to guide isolation of skin-originating cells that migrated into draining lymph nodes (DLNs), we demonstrate here that topical application of the contact sensitizer DNFB on UVB-irradiated skin leads to a robust increase in F4/80+ macrophages and little increase in CD11c+ dendritic cells (DCs) in the DLNs. The proportion of FITC+F4/80+CD11c- macrophages in normal vs. UVB-irradiated skin following FITC/DNFB application was 4.9 ± 2.6 % and 24.0 ± 0.7 % ($p<0.05$), respectively (n=2). In contrast, the proportion of FITC+CD11c+F4/80- DCs remained relatively unchanged, 9.9 ± 1.3 % and 9.4 ± 1.6 %. Furthermore, the majority (81.6 ± 9.3%, n=2) of F4/80+ cells in the DLN were FITC+. Isolation and ex vivo re-stimulation of the FITC+/F4/80+/CD11c- macrophages in contrast to FITC+/F4/80-/CD11c+ DCs were found to produce high levels of IL-6 as analyzed by ELISPOT assay; 353.9±58.1 vs. 5.10±2.61 SFC/104 cells, respectively ($p=0.02$, n=3). Interestingly, IL-6 expression appeared to be confined to monocyte/macrophage and DCs populations as the remaining F4/80-/CD45+ leukocyte/lymphocyte fraction of the DLN did not produce IL-6 assessed by ELISPOT. We also analyzed DLN populations for IL-10 production and observe that FITC+/F4/80+/CD11c- macrophages and not the FITC+/F4/80-/CD11c+ DCs were a source of IL-10 (110.7±24.0 vs. 0.67±0.26 SFC/104 cells, respectively, $p<0.05$, n=3). Thus, skin infiltrating macrophages following UVB exposure migrate to the DLN where they may participate in suppression of immune responses by coordinated release of IL-6 and induction of IL-10.

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Human cutaneous squamous cell carcinoma is associated with poorly functioning myeloid dendritic cells and regulatory T cells

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We studied phenotype and stimulatory potential of DCs from human SCC and adjacent patient matched, site matched, non-tumor bearing skin in order to determine whether tumor associated DCs differed in phenotype and stimulatory potential from those associated with non tumor bearing skin. Tissue blocks were evaluated by immuno-histochemistry and positive cells were counted manually with area measures computed using NIH IMAGE 6.1. We found high numbers of CD1a+ dendritic cells infiltrating epithelial tumor nests and high numbers of CD11c+ cells in the peritumoral infiltrate. High numbers of SCC was associated with high numbers of CD25+ cells and Foxp3+ cells. Triple label immunofluorescence confirmed the presence of CD4+CD25+Foxp3+ regulatory T cells. DC crawl outs from SCC and non tumor bearing skin obtained by sorting for CD11c+ HLA-DRHi (>99% pure) were by analyzed by FACS for surface marker expression and co-cultured with allogeneic T cells to assess stimulatory potential. T cell proliferation was measured by CFSE dilution. DCs from tumor bearing skin and non-tumor bearing skin expressed CD83 and CD86. However, SCC associated DCs were less potent stimulators of allogeneic T cell proliferation than DCs from adjacent non-tumor bearing skin. In addition, culture with cytokine cocktail to promote DC maturation enhanced stimulatory potential of DCs from non tumor bearing skin but did not affect DCs from SCC. These findings indicate the following: (1) The peritumoral infiltrate surrounding squamous cell carcinoma contains high numbers of myeloid DCs and T regs; (2) DC émigrés from human SCC express CD83 and CD86, but are not potent stimulators of T cell proliferation. This may provide insight into a mechanism by which SCC might evade immunosurveillance.

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TLR7-, 8- and 9-activated peripheral blood dendritic cells acquire subtype-specific cytotoxic activity

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We have previously shown that peripheral blood dendritic cells (DCs) acquire cytotoxic activity in response to Toll-like receptor (TLR) 7/8 stimulation. Whereas plasmacytoid DCs (pDCs) mediate death by tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), myeloid DCs (mDCs) acquire the lytic molecules granzyme B and perforin. To better understand the biological significance of this finding we now asked whether cytotoxic activity can be induced in DCs by ligands to TLRs other than TLR7/8. mDCs and pDCs were isolated from peripheral blood and cultured with ligands for TLR1-9 for 12h. The expression of TRAIL, intracellular lytic molecules and costimulatory molecules (CD40, CD80, CD83, CD86) before and after culture was assessed by FACS analysis. Virtually all pDCs expressed TRAIL after TLR7, TLR7/8 and TLR9 stimulation, but not in response to other TLR ligands or when unstimulated. In the case of TLR7/8, but not TLR9 stimulation, this event was accompanied by an up-regulation of the costimulatory molecules CD40, CD80, CD83 and CD86. In contrast to pDCs, the vast majority of TLR7/8-stimulated mDCs expressed perforin and granzyme B, but not TRAIL. This phenomenon was not observed when using other TLR ligands, including CpG, for stimulation. Similar to what has been observed with pDCs, TLR7/8 stimulation of mDCs induced maturation of these cells. Our data indicate that DC cytotoxicity is strongly and exclusively linked to the occupancy of cytoplasmic rather than membrane-bound TLRs engaged in host defense during infection with intracellular microorganisms.

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Th17 T cells resident in normal human skin

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We reported previously that non-inflamed human skin contains a large population of skin homing memory effector T cells with a broad range of antigen specificity. Such T cells in other settings are typically committed with regard to cytokine polarization (i.e., Th1, Th2, Th17). Using T cells isolated from short term skin explant cultures, we analyzed production of IL-4, IL-17, IFNγ and TNFα via intracellular flow cytometry after stimulation with PMA/ionomycin. Production of IL-4 was limited to <5% of cells, while IFNγ was produced by 15-20% of cells. A mean of 39% (n=10 donors) and up to 63% of total skin T cells produced IL-17; the vast majority of IL-17 producing T cells did not produce IFNγ. IL-17 producing cells were exclusively $CD4^+$, and strongly expressed CLA, CCR4, and CCR6. These cells could be expanded by IL-15, either alone or in conjunction with IL-2 or IL-23. Therefore, significant numbers of skin homing Th17 memory effector cells are present in normal skin. Surprisingly, another unique population of T cells was also observed. Up to 80% of T cells extracted from normal skin produced TNFα. While many of these produced either IL-17 and IFNγ in addition, roughly 50% produced only TNFα without any evidence of IFNγ, IL-17, or IL-4 production. These cells also had the phenotype of skin homing effector memory cells, with abundant CLA, CCR4, and CCR6 expression. In summary, these data demonstrate that the population of T cells in normal skin is significantly different from the population of T cells in peripheral blood. In addition to their skin homing receptors, the profile of cytokine expression is distinct and unique. Classic polarized populations of Th17, Th1, and Th2 exist in normal skin, but Th17 cells are more abundant than Th1 and Th2 cells. A new population of T cells, not previously described, that expresses TNFα but not other subset defining T cell cytokines, also exists in normal skin. These T cells constitute a pool of diverse antigen experienced memory T cells generated by the interaction of skin with its environment.

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An investigation of prolactin expression in psoriasis

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It is well recognised that psoriasis may be precipitated and/or exacerbated by psychological stress. Prolactin (PRL) might mediate this effect. As a key stress hormone PRL is immediately up-regulated by physical and psychological stress. At the cutaneous level, it may mediate vascular expansion in the dermis, keratinocyte proliferation, and a T cell predominant infiltrate; the key pathogenic features of psoriasis. Using immunohistochemistry we investigated the expression of both PRL and PRL-receptor (PRL-R) in skin from healthy controls (NN;n=10; males=9, female=1), compared to involved (PP) and uninvolved (PN) skin from patients with early onset chronic plaque psoriasis (n=10; males=5, females=5), aged between 18 and 55 years. Primary PRL and PRL-R antibodies were applied, incubated overnight, and the Vectastain® Elite ABC detection system was used. Quantification was percentage of positively stained epidermal cells. Repeated measures analysis of variance was used to detect any significant difference between the groups. Both PRL and PRL-R were present in the epidermis, dermal fibroblasts and sweat glands in NN skin. PRL and PRL-R expression was significantly increased in PP samples throughout the epidermis, particularly in the basal layer, when compared to NN skin (p=0.0001). There was no significant difference in expression of PRL and PRL-R between PN and NN skin (p=0.77 and p=0.25 respectively). This study is the first to identify increased PRL and PRL-R expression in plaques of psoriasis, which likely represents local cutaneous production. PRL may contribute to a pro-inflammatory local milieu; facilitating T cell trafficking into the skin by enhancing interferon-γ (gamma)induced chemokine production in keratinocytes. Future studies will investigate the control and regulation of cutaneous PRL and PRL-R production

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Murine and human langerhans cells divide in the epidermis

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Despite recent advances, the ontogeny of Langerhans cells (LC) and the mechanisms of population homeostasis remain incompletely resolved. Recruitment of $GR\text{-}1^{Hi}$ precursors from the circulation and proliferation of LC or their precursors in the cutaneous microenvironment have both been proposed as mechanisms for maintenance of epidermal LC numbers. However, the nature of such in situ proliferation has not been defined. In this study we have shown, both in mouse and human epidermis, that LC undergo mitosis in situ as visualized by nuclear expression of the cell cycle marker Ki67. Epidermal sheets were obtained from non-inflamed normal skin taken from 4 week old mice and human plastic surgery specimens. Ki67 expression was analysed by confocal microscopy in at least $1x10^3$ LC per sample, detected according to IA-IE expression in mouse epidermis and of Langerin in human epidermis. In both species, a notable fraction of epidermal LC expressed Ki67 (7.36 ± 0.99% in mice and 3.84% in human skin). In both species $Ki67^+$ LC typically displayed an elongated morphology with an extended nucleus or were found in pairs. $GR\text{-}1^{Hi}$ precursors were previously shown to cluster and proliferate in the dermis at which stage no Langerin expression was observed. Our cells are thus clearly different from this population. The data presented here clearly indicate that LC, despite being terminally differentiated DC, maintain the ability to undergo mitosis in the periphery and we therefore hypothesise that this mechanism is, at least in part, responsible for the maintenance of the epidermal LC population *in vivo*.

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Expression and regulation of interleukin-32 in human keratinocytes

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Interleukin 32 (IL-32) is a pro-inflammatory cytokine, originally described as NK4, a product of IL-2 activated NK and T cells. More recently identified as a major IL-18-stimulated protein in epithelial cells, IL-32 is a potent inducer of TNF-α, via activation of the NF-κB signalling pathway, in addition to IL-6 and a number of chemokines. *In vivo*, strong IL-32 expression has been demonstrated in rheumatoid arthritis and inflammatory bowel disease tissue and has been shown to correlate with levels of systemic inflammation. Following the identification of IL-32 in a variety of other epithelial cell types, we were interested in examining keratinocytes and *ex vivo* cutaneous tissue for this novel cytokine. Second passage normal human keratinocytes, cultured in the presence or absence of either IL-1α (100 ng/ml) or IFN-γ (20 ng/ml) for 24 hours, were subjected to transcriptional profiling using Affymetrix HG-U133A microarrays. Negligible quantities of IL-32 mRNA were transcribed constitutively, however, both IL-1α and IFN-γ significantly up-regulated IL-32 (means: 2.5-fold and 16.8-fold, respectively; n=6 donors); the only cytokine examined to be induced by both stimuli. Real-time RT-PCR analysis confirmed IL-32 mRNA induction in *ex vivo*, organ-cultured normal skin following IFN-γ stimulation at 30 ng/ml and above. Analysis of primary keratinocyte supernatants from 2 further donors using a cytokine proteome array verified IL-32 induction at the protein level by IL-1α and demonstrated extracellular release of this molecule. Intriguingly, preliminary analysis of psoriatic biopsies suggests an approximate 3-fold down-regulation of IL-32 mRNA in chronic lesional skin, compared with paired non-lesional samples (n=3). These data represent the first demonstration of IL-32 in cutaneous tissue. The inducibility of this molecule by a range of pro-inflammatory cytokines known to be involved in chronic, cutaneous inflammatory dermatoses and its apparent dysregulation in psoriasis suggest that further studies on the effects of IL-32 in these diseases are warranted.

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Cutaneous graft-versus-host disease: are Langerhans cells implicated?

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Graft-versus-host disease (GVHD) is the major cause of mortality following allogeneic hematopoietic cell transplantation (HCT). The skin is the most frequently affected organ containing a complex population of antigen presenting cells, including the Langerhans cell (LC), which may have a role in the pathogenesis of GVHD. We established in a mouse model that host LCs can survive the transplantation regimen and that the persistence of host LCs is sufficient for cutaneous GVHD but whether this also applies to human transplantation is unknown. We aimed to test the relationship between LC chimerism and GVHD in a large cohort of patients undergoing HCT and to determine the utility of LC chimerism testing as a clinical predictive test of GVHD. We present a multi-center, prospective study of patients undergoing HCT with skin biopsies collected pre-transplant and 2 weeks, 1 month, 3 months, 6 months and 1 year post transplant. LC chimerism was determined in sex-mismatched transplants using combined X-Y fluorescence in-situ hybridization and Langerin immunohistochemistry on paraffinized sections and analyzed using four channel fluorescence microscopy. In six months, 78 skin biopsies have been collected. LC engraftment is highly variable at 1 month post transplant (median 86% donor; range 0-100%, n=15) despite full donor engraftment in the blood, but correlates closely with the intensity of preparative conditioning and prior cutaneous GVHD. Patients given higher dose treatment or experiencing GVHD have more rapid LC engraftment (median 100%; range 82-100%, n=9) compared with patients given reduced conditioning (median 5.5%; range: 0-33%, n=6). Further follow up will determine whether persistence of host LCs confers an increased risk of subsequent GVHD, relapse and transplant-related mortality. Despite complete donor-derived hematopoiesis, there is significant persistence of host LCs in many patients at one month post-transplant. LC chimerism may have the potential to predict transplant outcome with the prospect of tailoring immunosuppression to the individual patient.

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Fcγreceptor activation triggers monocytes to differentiate into immature dendritic cells that promote autologous T cell response.

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Antibodies are potent inducers of inflammation thought Fcγ receptors (FcγRs). The purpose of this study is to elucidate the role of FcγRs in dendritic cell (DC) differentiation and to evaluate the function of these DC. Human peripheral monocytes derived from healthy donors were stimulated by plate-bound IgG and differentiation was measured by flow cytometry. Monocytes differentiated into $CD1b^+$ DCs in a dose dependent manner in response to IgG. Differentiation was blocked by the addition of an anti-CD64 (FcγR I) monoclonal antibody suggesting CD64 was the receptor responsible for $CD1b^+$ DC differentiation. Furthermore, FcγR ligation triggered transcription of GM-CSF mRNA and $CD1b^+$ cell differentiation was suppressed by the addition of an anti-GM-CSF monoclonal antibody. The FcγR-induced $CD1b^+$ cells exhibited an immature dendritic cell (DC)-like phenotype. Finally, the $CD1b^+$ DC induced T cell activation in both autologous and allogeneic mixed lymphocyte reactions. These findings predict that the stimulation of FcγRs induces GM-CSF production that in turn triggers monocyte differentiation into an immature DC population. Furthermore, we speculate that by activating auto-reactive T cells, FcγR-differentiated $CD1b^+$ DCs play an important role in the pathogenesis of immune complex related autoimmune diseases.

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Propionibacterium acnes induces proinflammatory cytokines and antimicrobial peptides in skin-derived dermal cells

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One of the major factors that contributes to the pathogenesis of acne is Propionibacterium acnes. Previously, our lab has shown that P. acnes can induce the production of proinflammatory cytokines, via the activation of Toll-like receptor (TLR) 2, in human monocytes. However, these cells were acquired from the peripheral blood. Given that P. acnes is part of the normal skin flora and proliferates within the pilosebaceous follicles of the skin, determining the interaction between P. acnes and resident cells would be important in understanding the local inflammatory response characteristic of acne. Currently, limited studies exist that directly address this question and therefore, we investigated the role of the resident immune cells in mediating the immune response against P. acnes. We isolated dermal cells directly from normal skin using dispase to separate the epidermis from the dermis and collagenase to yield a single-cell suspension of dermal cells. These cells were found to express CD14 and CD11c, markers for both macrophages and dendritic cells by FACS. In response to stimulation with P. acnes, which contains a TLR2 ligand, the dermal cells release proinflammatory cytokines IL-1β and TNF-α, and the chemokine IL-8. Activation of TLR has been shown to not only lead to production of cytokines in monocytes but also the induction of antimicrobial peptides such as human beta defensins and cathelicidins and we found that in response to P. acnes stimulation, these cells upregulated the mRNA levels for the antimicrobial peptides human beta defensin 2 (hBD2) and cathelicidin. Furthermore, this induction appeared to be regulated through the vitamin D pathway. Taken together, our data suggest that the dermal cells are active participants in the skin's immune response against P. acnes and may contribute to the inflammatory response seen in acne pathogenesis.

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Functional activities of primary skin-derived immune cells

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The skin immune system is comprised of resident immune cells that mediate innate immune responses at the interface of skin and the outside environment. In the epidermis, Langerhans cells are specialized dendritic cells with the capacity to present antigen and activate cells of the adaptive immune response. In the dermis, a key immune cell type is the dermal dendritic cell. Our data indicates that in normal skin there are at least two subpopulations of dermal dendritic-appearing cells: CD1+ dendritic cells (DC) and CD209+ macrophages (MF). These two distinct populations of "dermal dendritic cells" may differentially contribute to innate and adaptive immune responses in skin. It has been difficult to obtain large numbers of dendritic cells (DCs) and macrophages (MF) from normal human skin. As a consequence, most immunologic analyses have relied upon *in vitro* cell models. At least three distinct CD209+ populations have been derived by cytokine treatment of peripheral blood monoytes using IL-15, IL-4 and IL-10. The *in vivo* relevance of these populations remains unclear. We recently have adapted technology to derive resident cells from normal skin, making it possible to directly study CD209+ dermal MF and CD1+ DC to determine whether they can mediate functions of innate immunity. We measured the ability of these populations to be activated via TLRs to mediate the vitamin D-dependent antimicrobial pathway. We demonstrate that while peripheral blood-derived dendritic cells require vitamin D supplementation, skin-derived cells function well in the absence of exogenous vitamin D. In addition, we demonstrate the ability of these populations to be differentially activated via TLRs to induce cytokine and costimulatory molecule production. Finally, we assessed the ability of these populations to present antigen to T cells via MHC class II and CD1. These studies provide important new information about the human innate immune response in the skin and its ability to stimulate adaptive immune responses.

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Lysosomal thiol reductase GILT is essential for MHC class II-restricted processing of melanoma antigen TRP-1

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Presentation of tumor antigens in the context of MHC class II is essential for the activation of CD4+ T lymphocytes, durable CD8+ cytotoxic T lymphocyte responses and effective humoral responses. Melanocyte differentiation antigens including tyrosinase, tyrosinase-related protein (TRP)-1, and TRP-2 are melanosomal integral membrane proteins involved in melanin biosynthesis. Melanosomal membrane proteins contain a dileucine-based sorting signal which serves to target these antigens to the endocytic pathway leading to presentation by MHC class II, and they contain internal disulfide bonds. Melanoma patients generate CD4+ T lymphocytes responsive to class II-restricted epitopes from tyrosinase, TRP-1 and TRP-2. Gamma-interferon inducible lysosomal thiol reductase (GILT) is hypothesized to facilitate the generation of class II binding peptides by reducing proteins in the endocytic compartment and exposing epitopes for class II binding. Here, we demonstrate the importance of GILT in MHC class II-restricted processing of TRP-1 in vitro and in the development of an anti-TRP-1 immune response in vivo. Primary GILT-deficient B cells were unable to process TRP-1 from tumor cell lysates to present a class II-restricted TRP-1 epitope. The localization of TRP-1 to the class II loading compartment and the presence of internal disulfide bonds in TRP-1 likely explain this result. Furthermore, the absence of GILT delayed the onset of TRP-1-specific auto-immune vitiligo in a mouse model. The percentage of TRP-1-specific effector memory T cells was increased following the onset of vitiligo suggesting that T cell activation correlates with disease onset. Therefore, reduction of melanocyte/melanoma antigens by GILT is likely to be important in the development of an anti-melanocyte response in vitiligo and in the immunotherapy of melanoma.

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Increased frequency of T cells with a regulatory phenotype in psoriasis and differential contraction of this population with biologic treatments

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Our expanding knowledge of immunology has led to the development of new biologic therapies. However, very little is known about how these biologic treatments affect the frequency of Tregs in psoriasis, a disease that is thought to be autoimmune in nature. In principle dysfunction and decreased frequencies of Tregs have been described in autoimmune diseases. We sought to characterize the frequency of phenotypic Tregs in psoriasis before and after treatment with efalizumab, etanercept and alefacept. Our results indicate that Tregs are increased in untreated psoriatic lesional skin and peripheral blood. The lesional skin of patients that responded to efalizumab or etanercept exhibits Treg frequencies that gradually decline proportionately with decreasing total T cell numbers. In peripheral blood the frequency of Tregs remained unchanged in patients that received alefacept or etanercept. However, in patients that were treated with efalizumab blood Tregs were "diluted" by generalized lymphocytosis, resulting in lower Treg frequencies. The total blood T cell counts of patients that experienced a psoriasis relapse after discontinuation of efalizumab therapy declined to pretreatment values but Treg counts remained stable. We hypothesize that after cessation of treatment, effector T cells exit the circulation more rapidly than Tregs and enter inflammatory skin sites. Hence, the T-cell population that initiates new psoriasis lesions, including psoriasis flares, may initially have too low a Treg frequency to normally constrain T-cell activation. Overall, these results produce the surprise finding that resolution of inflammation induced by biologic therapies is associated with elimination of most Tregs from previously inflamed skin lesions, rather than accumulation of a Treg population that suppresses pathogenic inflammation.

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Human lymphocyte home to human skin xenografts *in vivo*

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Studies of lymphocyte homing performed *in vitro* or in mice are inherently limited in application to human disease. To address this issue, we have developed a homing model based on mice bearing both human skin xenografts as well as circulating human peripheral blood leukocytes. Of primary importance, a time course study showed that mouse vessel penetration of human skin xenografts begins and human vessels within the grafts gained continuity with the mouse circulation between day 5 and day 10 after engraftment, as identified by i.v. injection of a human specific FITC conjugated lectin (Psophocarpus tetragonolobus) and *in vitro* staining for mouse or human CD31. From day 10 onward, the number of human vessels and the degree of continuity with murine circulation was continuously reduced. Analysis of adhesion molecule expression in human skin xenotransplants revealed that the number of ICAM-1 and E-selectin positive vessels remained low after transplantation and that expression at 10-20 days post transfer was similar to that found in pretransplant human skin. Leukocyte trafficking to human skin under various conditions was studied by adoptive transfer of human peripheral blood mononuclear cells labeled with Cell Tracker Orange into skin grafted mice. After 24h, grafts were harvested and digested with collagenase D and the resultant cell preparation characterized by flow cytometry. In preliminary studies, activation of human skin grafts with TNF-alpha in combination with the chemokine SDF-1 (CXCL12) or TARC (CCL17) resulted in a substantial (up to 300-fold) increase in cell accumulation within grafts compared to TNF-alpha treatment alone. We conclude, therefore, that this dual xenograft model can be used for studies of human leukocyte homing to human skin *in vivo*, but analysis should be restricted to a time frame of 10-20 days after graft implantation and focused by confirmation of species specific vascular staining. Previously published studies using human skin xenografts to study lymphocyte homing should be interpreted with regard to these findings.

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Comprehensive characterization of skin microbiota in patients with atopic dermatitis and normal subjects using T-RFLP method

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Our previous study using bacterial 16S rRNA gene-based clone libraries revealed that the microbiota in healthy human skin include uncultured microorganisms, although the microorganisms in the skin exposed to the disease conditions remain to be examined. To compare the profiles of skin microbiota in 13 patients with atopic dermatitis (AD) and 10 healthy controls, terminal restriction fragment length polymorphism (T-RFLP) analysis of bacterial 16S rRNA gene was applied to 23 swab-scrubbed samples from facial skin. T-RFLP method successfully revealed the complex bacterial members of the skin microbiota as peak patterns of capillary electrophoresis of terminal restriction fragments (T-RFs). Each T-RF peak reflected a microorganism, and the microorganism to which each peak was assigned could be predicted by computer simulation of T-RF length using the nucleotide sequence data of bacterial species residing in the skin. Among 18 species detected in the present study, Stenotrophomonas maltophilia was detected significantly more common in AD patients (5/13 for AD patients vs 0/10 for controls), whilst Dietzia maris was detected significantly more common in normal controls (8/10 for controls vs 2/13 for AD patients). Moreover, Streptococcus species, which are considered uncommon in uninfected skin, were detected in 7 patients and 8 normal controls. Although further study should be undertaken to investigate the roles of these microorganisms in AD, the microbiota were presumed to include hitherto uninvestigated bacterial species in the major population of patients with AD and healthy controls.

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The lysate of allogenic apoptotic non-infected macrophages diminishes *Leishmania major* burden by involvement of Foxp3-positive regulatory T-cells

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Parasites of the genus *Leishmania* cause substantial mortality and morbidity in the developing world. While numerous treatment modalities exist, there is no ideal therapy for leishmaniasis. The purpose of or study was elucidate the mechanisms of regulation of the intracellular parasite clearance by macrophages (Mϕ) during cutaneous leishmaniasis (CL). BALB/c mice received 3 injections subcutaneously with 2-week intervals in between with cell lysate obtained from non-infected bone marrow-derived Mϕ after photodynamic therapy with EtNBSe (PDT). The PDT regimen was chosen in this way that death of Mϕ occurred through apoptotic pathway. The control group of mice received PBS injections. Two weeks after the last injection mice were infected intradermally in the ear with 10^6 metacyclic promastigotes of *L. major*. Mice of the Mϕ+PDT group showed a delayed onset of disease and contained ~ 3.1-logs less parasites than did the controls ($p < 0.05$). Lower levels of cytokines TNF-α and IL-6 (cytometric bead array), and lower levels of chemokines CCL2/MCP-1 and CCL22/MDC (ELISA) were observed in the ear tissues of the mice that received a lysate than did the controls. A percentage of IFN-γ-producing T cells in the draining lymph nodes of the Mϕ+PDT mice was higher than in the control group. We found that a Mϕ death in CL lesions was a result of the attack of the immune system (caspase-3 activation on IHC) than cytopathic effect of the intracellular parasitism. Flow cytometric analysis revealed higher number of F4/80+ (Mϕ) and CD4+CD25+Foxp3+ T regulatory (T reg) cells in the lesions of the Mϕ+PDT mice than did the controls. Correlation analysis showed that the percentage of Mϕ in the skin directly correlates (r = 0.93) with number of T reg. These results demonstrate that T reg may play a role in a reduction of the parasite burden through before unknown mechanisms of the regulation of the Mϕ survival in the inflammatory infiltrate.

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Assessing microbial burden in chronic wounds: comparison of routine cultures, quantitative microbiology, and molecular techniques

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Wounds account for >50% of direct medical costs for skin diseases in the U.S. Expensive systemic antibiotics are commonly prescribed based on qualitative culture (few/moderate/heavy growth). We studied microbial burden in chronic wounds determined by qualitative, quantitative microbiology, real-time PCR (RT-PCR), and metagenomic analysis. We evaluated microbial populations across wounds. 41 chronic wounds in 28 out-patients were assessed. We curetted leading wound edge using a 3mm curette. 12 wounds were evaluated at multiple sites (rim/rim; rim/center). 12 samples were analyzed using 16S clone libraries. Quantitative culture (CFU/gm) was used as gold standard for analyses. Minimum tissue for reproducible measurements was 20mg. 11/14 (79%) of negative qualitative cultures were positive on quantitative microbiology. Qualitative results (Not present/Few/Moderate/Heavy) did not correlate with quantity of organisms on quantitative culture. Our RT-PCR analysis was 92% sensitive. In 8/11 (73%) specimens negative by quantitative culture, RT-PCR identified organisms which could not be grown on culture. 16S clone library analysis demonstrated substantially more complex bacterial communities than culture or PCR. Probability for rim-center pair bacterial counts within 1 log was 95.9% (95% CI: 78.6%-99.3%, p=.0008). MRSA (44.8%) was the most common pathogen, followed by Pseudomonas aeruginosa (27.6%) and Group B Streptococcus (27.6%). There was remarkable consistency of bacterial populations across a wound. Qualitative culture results did not correlate with quantitative results. RT-PCR was a sensitive tool for rapid identification and characterization of bacteria in chronic wounds. 16S clone library analyses make it clear that bacterial populations in wounds are much more complex than demonstrated by culture. The role of microbial flora in wound healing needs to be reassessed using these new analytic methods.

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Pathomorphological characteristics of Malassezia folliculitis

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Malassezia folliculitis is a common pathology seen in young patients. The condition is frequently misdiagnosed or mixed with acne. Administration of antibiotics in such cases does not help to improve the clinical status. Usually small multiple whitish pustules are seen, comedons are absent. Malassezia folliculitis is often associated with seborrhoeic dermatitis of the scalp and skin. Frequently the condition causes psychological problems and sometimes severe itch. Biopsy is performed in severe cases with resistance to therapy and unusual location of folliculitis. Although some investigators founded Malassezia cells in the hair follicules, the pathogenesis of folliculitis development is not fully known. We observed 157 patients (133 men and 24 women) with the clinical picture of folliculitis. Middle age was 21,2±8,5 years. Content of the follicles was extracted and studied by direct microscopic examination and cultured in the Saboraud agar with addition of sterile olive oil. Malassezia was found to be a reason of folliculitis in 47 patients (29,9%). Skin biopsy with STIEFEL 5 mm diameter punch was performed in the skin of the back. Specimen where stained with Gimza staining method. In the surface of the stratum corneum basophilic cells of fungi of different shape are founded (round, oval, S-ahaped). Several cells are founded in stratum spinosum but their size is usually smaller than of those in the upper layers. There is no cell reaction around Malassezia in the epidermis. In derma hyphae of Malassezia are founded, surrounded by infiltrates composed of lymphocytes, macrophags, neutrophyls, and plasmocytes. Malassezia cells are founded also in the ducts of seborrhoeic and sweet glands, and around fat cells situated under the arteriols. This pathomorphological picture suggests that Malassezia yeasts affect not only superficial skin layers but also dermal structures. In Malassezia folliculitis not only yeasts but also hyphae that is a pathogenic form are seen in the specimens. Inflammation infiltrate is seen in deeper layers of the skin that explains the effect of systemic antifugal drugs.

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TLR2 and Nod2 activation increases HIV susceptibility and augments viral replication in Langerhans cells, but not in dermal type Dendritic cells: implications for sexual transmission of HIV

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Numerous studies have shown a higher risk of acquiring HIV infection in the presence of other sexually transmitted diseases (STD), however, biologic mechanisms responsible for enhanced HIV acquisition are unclear. Recent studies have documented Langerhans cells (LC) as initial cellular targets in sexual transmission of HIV. Here, we studied whether microbial components augment HIV infection in LC by activating TLR and Nod pattern recognition receptors. Monocyte-derived LC (mLC) expressed TLR1, 2, 3, 6, and Nod1 and 2, and induced maturation and cytokine production in response to ligand activation through these receptors. Next, mLC were incubated with TLR1-9 and Nod1 and 2 ligands for 24 h before or after HIV infection to assess effects on HIV susceptibility and replication in these cells. Surprisingly, TLR2 (heat-killed *Listeria monocytogenes*) and Nod2 (MDP) ligands dramatically enhanced both HIV susceptibility and replication in mLC, whereas other TLR and Nod ligands did not affect HIV infection. The same infection-enhancing effects were observed when mLC were incubated with other related bacterial components (lipoprotein, LTA, PGN, and whole Gram+ bacteria). Importantly, TLR2 and Nod2 ligands also significantly increased HIV susceptibility in freshly isolated human epidermal LC. In contrast, these ligands decreased both HIV susceptibility and replication in monocyte-derived dendritic cells (mDC). Regarding mechanisms underlying increased HIV infection in LC, we found that TLR2 and Nod2 activation resulted in significant up-regulation of cell surface CD4 on mLC, but not on mDC. Furthermore, in mLC, the activation of these ligands significantly decreased mRNA expression of APOBEC3G/3F, an HIV suppressor factor. Given these data, we hypothesize that ligation of TLR2 and Nod2 by Gram+ bacterial products may underlie enhanced sexual transmission of HIV in the setting of concomitant bacterial STD infection.

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Detection and quantification of gene expression in archival formalin-fixed, paraffin-embedded skin tissue

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Archival formalin-fixed, paraffin-embedded (FFPE) tissue specimens are an excellent resource for retrospective studies. However, the reliable quantification of gene expression in FFPE tissue has previously been subject to limitation due to the quality of RNA that can be extracted from such samples. We aimed to develop a sensitive and reproducible method to analyse the expression of Human Endogenous Retrovirus K (HERV-K) mRNA in archival tissue. This method consisted of real time quantitative TaqMan PCR using primers spanning an amplicon range of 94 to 117 base pairs. Because of the small target size, this approach was optimal for quantitative determination of gene transcript levels in FFPE tissue in which RNA is fragmented. RNA was extracted from FFPE samples using the RNeasy FFPE kit, including on-column DNase digestion (Qiagen). RNA quality and normalisation of gene expression were controlled for by quantification of the spliced mRNA of the housekeeping gene *Hypoxanthine-guanine phosphoribosyltransferase* (*HPRT*) that has been shown previously to be ubiquitously and consistently expressed in all human cells. Primers and probes for *HPRT* were designed to span an intron to prevent annealing to genomic DNA. Using this method, expression of *HPRT* was detected in 100% of archival tissues analysed comprising 8 benign naevi, 8 primary melanoma and 8 normal skin FFPE samples (n=24). Relative target gene expression was calculated using the formula $2^{-\Delta Ct}$ where ΔCt (Cycle threshold) = Ct *target gene* -Ct *HPRT*. HERV-K full length mRNA was found to be expressed at significantly higher levels than *HPRT* in all samples. Good intra- and inter-assay reproducibility was observed. These results suggest that quantitative real time PCR utilising short amplicons is an ideal tool for retrospective analysis of gene expression in archival skin tissue.

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Activation of TLR3 or TLR7 in keratinocytes induces potent soluble antiviral activity and protects against vaccinia virus infection

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Patients with atopic dermatitis have deficient antimicrobial activity in the skin and are predisposed to eczema vaccinatum, a potentially lethal disseminating infection due to vaccinia virus. In this study, we investigated if stimulation of keratinocytes could rescue antimicrobial production and protect against vaccinia. Vaccinia replication was measured in HaCat keratinocytes by plaque assay and realtime PCR for vaccinia DNA-dependent RNA polymerase. A dose-dependent reduction in plaque formation was seen with Poly(I:C), a TLR3 ligand. Treatment for 24 hrs with 25µg/ml Poly(I:C) induced complete inhibition (100%, $p<0.001$). Lesser inhibition was seen at lower doses; 250ng/ml (30%, $p<0.01$), 2.5µg/ml (65%, $p<0.001$). Time course studies with 10µg/ml Poly(I:C) treatment also showed a large reduction in viral RNA synthesis with maximal inhibition at 8 hrs (89% inhibition, $p<0.001$). Stimulation of HaCat cells for 24 hours with 5µg/ml Imiquimod, a TLR7 ligand, also inhibited vaccinia plaque formation (24% inhibition, $p<0.05$) and viral RNA synthesis (68% inhibition). Other stimuli of keratinocyte innate immune responses and antimicrobial peptide synthesis, such as 1,25OH Vitamin D, inactivated vaccinia, or ligands for TLR2/1, 2/6, or 9, did not induce an antiviral response. Anti-vaccinia activity stimulated by Poly(I:C) was transferable from HaCat conditioned media to NIH/3T3 cells (deficient in TLR3), indicating that the inducible antiviral activity is soluble. Analyses of antimicrobial peptides induced by these stimuli suggest that this antiviral activity is more likely associated with hBD2 or 3, but not cathelicidin production. In summary, TLR3 and TLR7 ligands stimulate an antiviral response in HaCat keratinocytes that can completely block virus infection in this system. Understanding the mechanisms of protection induced by TLR3 and TLR7 signaling in keratinocytes offers opportunity to apply a novel therapeutic approach to eczema vaccinatum.

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Reduced Langerhans cell number in human papillomavirus (HPV)-related skin lesions is associated with low expression levels of macrophage inflammatory protein (MIP)-3alpha and E-cadherin

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Background: HPV causes recalcitrant warts for a long period, evading host immune surveillance. On the other hand, viral warts sometimes show spontaneous regression by the induction of immune responses. It has not been elucidated why immune cells are less responsive against HPV and what kind of stimuli induces the immune response against HPV. Objectives: To elucidate the immune escape mechanisms of HPV, we examined the association between the density of dendritic cells (DCs) and the epidermal expression of MIP-3alpha and E-cadherin in HPV associated lesions with or without inflammation. Materials and Methods: Tissue samples obtained from patients with HPV-related skin diseases (common wart, myrmecia, flat wart, condyloma acuminatum, bowenoid papulosis and HPV-related Bowen's disease: HPV-Bowen) with or without inflammation were immunostained with various DC markers, MIP-3alpha or E-cadherin. RT-PCR and in situ hybridization (ISH) was performed to compare MIP-3alpha gene expression by inflammatory common warts with that of non-inflammatory common warts. Results: HPV-related lesions without inflammation showed reduced LC number, decreased MIP-3alpha expression, and altered E-cadherin expression. These changes were recovered in the inflammatory lesions, in which a considerable number of dermal myeloid and plasmacytoid DCs were observed, accompanied by a dominant infiltration of CD8+cells. RT-PCR and ISH also revealed the upregulation of MIP-3alpha gene expression in the inflammatory common warts. Conclusions: Decreased expression of MIP-3alpha and E-cadherin by the lesional keratinocytes may inhibit LC migration into epidermis and its adhesion to keratinocytes. The recovery of MIP-3alpha and E-cadherin expression on keratinocytes may recruit DCs in the skin, resulting in the eradication of HPV-infected cells from the skin.

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The investigation on the distribution of Malassezia yeasts on healthy human skin in Korea by application of 26S rDNA PCR-RFLP

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To isolate, identify, and classify Malassezia yeasts from the normal human skin of Koreans, we sought to implement novel molecular biology technique, namely 26S rDNA PCR-RFLP method. And we carried out to recognize the existence of a difference in the frequency of discovery and qualitative distribution of Malassezia yeasts by different age, sex and body site. Malassezia yeasts were cultured from clinically healthy human skin using scrub-wash technique at five sites (forehead, cheek, chest, upper arm, and thigh) and swabbing technique at scalp in 160 participants comprised of 80 males and 80 females aged from 0 to 80. 26S rDNA PCR-RFLP method was used for molecular analysis. An overall positive culture rate was 62.4% (599/960). As shown in the experiment groups by their age, the positive culture rate was the highest (74.2%) in the age group (AG) 21-30 and 31-40 (89/120). In the experiment groups by different body areas, the scalp showed the highest positive culture rate of 90% (144/160). On analysis of 26S rDNA PCR-RFLP, M. globosa was the most predominant species in the AG 0-10 (32.8%), 11-20 (28.7%), 21-30 (32.3%). M. restricta was identified as predominant species in the AG 41-50 (28.6%), 61-70 (31.7%) and 71-80 (23.3%). According to body site, M. restricta was more frequently recovered in the scalp (57.1%), while M. globosa was more frequently recovered in the chest (38.1%). Especially in this study, M. dermatis, newly isolated Malassezia species from atopic dermatitis patient in Japan, was isolated and identified in 19 cases (1.9%) in healthy subjects. The key is to recognize the existence of a difference in the type of Malassezia species in different AGs as well as body areas, which reflects differing skin lipid levels in various AGs and different body areas. Moreover, 26S rDNA PCR-RFLP analysis which was opted in this study could provide a sensitive and rapid identification system for Malassezia species, which may be applied to epidemiological surveys and clinical practice.

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Cure rate, duration of cure and recurrence rate in onychomycosis according to clinical factors

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The purpose of this study was to evaluate the cure rate, duration required for complete cure and recurrence rate in onychomycosis according to the extent of nail involvement and other clinical factors. Medical records between December 2000 and December 2005 of 207 patients with onychomycosis on the great toe nail were reviewed retrospectively. One year after complete cure, patients underwent a follow-up examination of recurrence. The complete cure rate (CCR) as a whole was 78.3%, duration of cure (DC) was 31.7 weeks, recurrence rate (RR) was 36.0%. CCRs were 92.4% in the nail involvement-under-25% group (A), 81.8% in the group involving 25~50% of the nail (B), 72.2% in the group involving 50~75% of the nail (C), 63.9% in the group involving 75~100% of the nail (D) ($p<0.05$). DCs were 15.8 weeks in group A, 32.9 weeks in group B, 33.5 weeks in group C, and 53.9 weeks in group D ($p<0.05$). RRs were 12.5% in group A, 44.8% in group B, 52% in group C, and 45.2% in group D ($p<0.05$). We did not find any statistically significant difference in CCR, DC, RR according to clinical type and gender. CCRs were 81.1% in the non-old-age group (Y) and 65.8% in the old-age group (O) ($p <0.05$). DCs were 30.3 weeks in group Y and 39.2 weeks in group O ($p<0.05$). RRs were 33.6% in group Y and 50.0% in group O ($p>0.05$). CCRs were 76.1% in itraconazole group (ITR) and 86.1% in terbinafine group (TER), and 53.3% in only-topically-treated group (TO) (between ITR and TER, $p>0.05$). DCs were 28.7 weeks in ITR group, 33.8 weeks in TER group, and 47.6 weeks in TO group (between ITR group and TER, $p>0.05$). RRs were 34.3% in ITR group, 38.5% in TER group, and 33.3% in TO group ($p>0.05$). CCRs were 77.7% in non-diabetes mellitus group (N) and 80.5% in diabetes mellitus group (DM) ($p>0.05$). DCs were 30.2 weeks in group N and 37.7 weeks in group DM ($p<0.05$). RRs were 30.9% in group N and 53.6% in group DM ($p<0.05$). There were statistically significant differences in CCR, DC, and RR depending on the extent of the nail involvement; therefore, it seems important to obtain early treatment.

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Protection against acute and recurrent herpes simplex virus (HSV) type 2 genital disease in guinea pigs after immunization with a dominant-negative recombinant HSV-1

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An ideal vaccine against HSV would be safe and effective against both HSV-1 and HSV-2. CJ9-gD is a novel dominant-negative recombinant HSV-1 that is completely replication-defective, does not establish detectable latent infection in vivo, and expresses high levels of the major HSV-1 antigen glycoprotein D immediately following infection. Previously, we demonstrated that immunization with CJ9-gD protects guinea pigs from primary HSV-1 skin disease and reduces latent infection. In mice CJ9-gD induces strong humoral and cellular immune responses, leading to protection against both HSV-1 and HSV-2 genital disease. As guinea pigs remain the ideal model to study genital herpes and its prevention, we tested CJ9-gD as a vaccine against HSV-2 genital disease in guinea pigs. Animals immunized with CJ9-gD developed at least 700-fold higher titers of HSV-2-specific neutralization antibodies than mock-immunized controls. After challenge with wild-type HSV-2, all 10 control guinea pigs developed multiple genital lesions with an average of 21 lesions per animal. In contrast, only 2 minor lesions were found in 2 of 8 CJ9-gD-immunized animals, representing a 40-fold reduction on the incidence of primary genital lesions in immunized animals ($p < 0.0001$). Immunization significantly reduced the amount and duration of viral shedding, prevented systemic involvement and provided 100% survival, whereas 90% of mock-immunized animals succumbed due to the severity of disease. After recovery from primary infection, immunized animals showed no signs of recurrent disease or viral shedding and carried significantly less latent viral DNA in their dorsal root ganglia than surviving mock-vaccinated controls ($p < 0.0001$). Collectively, we demonstrate that vaccination with the HSV-1 recombinant CJ9-gD elicits strong and protective immune responses against primary and recurrent HSV-2 genital disease and reduces the extent of latent infection.

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Keratinocytes senses poxvirus infection through the mitochondrial antiviral signaling protein (MAVS) and IRF3-dependent pathway

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Skin provides first-line defense against invading microorganisms. Keratinocytes not only form an essential physical barrier to prevent the entry of invaders, but also initiate vigorous innate immunity through the production of inflammatory cytokines, chemokines and antimicrobial peptides upon sensing the "danger signals". How keratinocytes sense viral infections and generate antiviral immune responses is not well understood. Understanding the interplay of poxvirus with the skin immune system might shed light on the pathogenesis of eczema vaccinatum, which is a severe complication of smallpox vaccine characterized by extensive vaccinia virus infection in the skin after vaccination. The vaccinia virus E3L is a virulence gene that encodes dsRNA-binding protein, critical for interfering host antiviral responses. Infection of murine primary keratinocytes with vaccinia virus with deletion of E3L gene but not wild type vaccinia triggers the production of IL-6, CCL4, CCL5 and IFN-β. This effect is dependent on viral gene expression and viral DNA replication. Host antiviral immune responses triggered by ΔE3L virus were completely abolished in keratinocytes derived from IRF3-/- or MAVS-/- mice, but not in those from TLR3-/- or MyD88-/- mice. In addition, ΔE3L virus infection induced activation of IRF3 in keratinocytes from wild type mice, but not from MAVS-/-. Taken all together, these findings provide strong evidence that intracellular dsRNA produced during ΔE3L virus infection in keratinocytes is sensed through the mitochondrial antiviral signaling protein (MAVS) and IRF3-dependent pathway. Wild type vaccinia silences antiviral innate immunity in keratinocytes through sequestration of dsRNA by E3L protein.

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HPV associated mucocutaneous disease post HAART/IRD - patient immunophenotype

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HPV (human papillomavirus) related mucocutaneous disease can be persistent and/or emergent and clinically problematic after HAART (highly active antiretroviral therapy) and may represent part of the spectrum of IRD (immune restoration disease). IRD is increasingly recognised as a common complication of HAART in HIV/AIDS. Between 15 and 25% of patients develop some form of IRD and at least half of these are dermatologic.[1] Most IRD appears related to infective agents. However little is known about host factors in the development of IRD. We identified a cohort (n=24) of HIV positive patients presenting with persistent cutaneous or genital warts and anogenital carcinoma in-situ, and HIV+ controls (n=26), from the same clinic population. Utilising routine blood samples we quantitated haemoglobin (Hb) concentration, percentages of lymphocytes expressing CD4, CD8, CD56, and CD19 markers, CD4/CD8 T-cell ratios, lipid profiles, and HIV viral loads. Analysis was by parametric statistics (Student's *t* test). No significant differences were found for all the variables examined, although with larger numbers, the trends observed might approach statistical significance. The mean CD4/CD8 T-cell ratio for patients was 0.53 (SD 0.38) and for controls 0.73 (SD 0.35), p=0.07; and the mean CD4% for patients was 24.2% (SD 11.1) and for controls 29.5% (SD 8.7), p=0.06. More patients and other immunophenotypic factors are to be studied but preliminary results suggest that post HAART HPV/IRD may be associated with non-immunophenotypic variables such as viral or host immunogenetic factors.

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Actin filaments and microtubules are required for efficient HIV transfer from dendritic cells to T cells via infectious synapses

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Dendritic cells (DC) are the most potent antigen-presenting cells and are widely spread throughout skin and mucosal tissues. It is now well established that HIV can subvert basic functions of DC in order to efficiently target CD4+ T cells via infectious synapses. The goal of this study is to analyze the relative contribution of cytoskeleton actin filaments and microtubules in HIV transfer from DC to CD4+ T cells via infectious synapses, using confocal microscopy, RNA interference (RNAi) and infection transfer assays. We first observed that efficient HIV recruitment to the DC-T cell infectious synapse is dependent on actin and microtubules. Treatment with low doses of actin inhibitors (cytochalasin D, latrunculin A) and microtubule inhibitors (nocodazole, sanguinarine chloride) abrogated HIV relocation from tetraspanin-rich vesicles to the infectious synapse, without inhibiting DC-T cell contacts, as assessed by confocal microscopy. Treatment of DC with these drugs induced clear morphological changes of the tetraspanin-rich compartment without other cellular morphological alterations. We also demonstrated that transfer of infectious particles to target CD4+ T cells is inhibited following treatment of DC with actin and microtubule inhibitors. Next, we investigated the role of modulators of actin filaments and microtubules such as Larg, Cdc42 and RhoA in HIV transfer via DC-T cell infectious synapses. Knock-down of these modulators in DC by RNAi, or inhibition with specific drugs, strongly repressed HIV transfer to CD4+ T cells, thereby further emphasizing the role of actin and microtubules in DC for efficient transmission of HIV infection. Our results also support the putative role of filopodia in HIV transfer through infectious synapses between DC and CD4+ T cells. In summary, this work demonstrates that actin filaments and microtubules are essential for HIV trans-infection of CD4+ T cells via DC. This study opens the way for potential new therapeutic approaches in order to prevent sexual transmission of HIV.

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IL-1R activation is required for IL-17-dependent neutrophil recruitment against *Staphylococcus aureus* skin infections

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We previously reported that *S. aureus*-infected IL-1β- (but not IL-1α) and IL-1R-deficient mice had a severe defect in neutrophil recruitment and control of the infection as compared with wild type mice. Recent studies have demonstrated that IL-17 has been shown to regulate neutrophil recruitment to various peripheral tissues by promoting granulopoiesis and by production of neutrophil chemokines. Since neutrophil recruitment and abscess formation is a key host defense mechanism against *S. aureus* skin infections, we hypothesized that IL-1R activation and IL-17 production were both required for optimal neutrophil recruitment against *S. aureus* skin infections. To test this hypothesis, we used a mouse model of cutaneous infection with *S. aureus* in conjunction with bioluminescent bacteria to track bacterial growth. We found that skin lesions of *S. aureus*-infected IL-17R-deficient mice were substantially larger (2-3 fold) with higher bacterial counts (up to 9-fold) and had a 30% defect in neutrophil recruitment (as measured by myeloperoxidase activity) compared with wildtype control mice ($p<0.05$). This severe phenotype of skin lesions of *S. aureus*-infected IL-17R-deficient mice resembled skin lesions of *S. aureus*-infected IL-1β- or IL-1R-deficient mice. In addition, we found that skin specimens from IL-1R-deficient mice had an absence of induction of IL-17 mRNA at early time points after *S. aureus* infection, suggesting that IL-1R activation was required to produce IL-17. Thus, these data provide evidence that IL-1R activation is required to mediate IL-17-dependent neutrophil recruitment in immunity against *S. aureus* skin infections.

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Staphylococcus epidermidis protects the skin through mutual symbiosis

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Staphylococcus epidermidis (SE), a common skin isolate, lives innocuously on the epithelium. We hypothesized that the antimicrobial barrier of normal skin includes non-pathogenic resident mutualistic microbes. To investigate if a mutualistic relationship exists between SE and mammalian skin, SE was inoculated at 10^4 CFU on freshly excised mouse skin or skin that received a partial-thickness burn. SE grew on healthy skin ($2.2x10^6$CFU) but not on injured skin ($4.7x10^3$CFU; $p<0.05$). To evaluate if SE protects against pathogens, growth of Group A Streptococcus (GAS) was evaluated on human skin containing SE or washed free of bacteria, with survival observed only in the latter. Also, GAS did not proliferate on mouse skin explants that were colonized with 10^6CFU/ml SE. The capacity of SE to kill GAS was due to a soluble factor since supernatants from SE culture, but not S. aureus or GAS, inhibited GAS growth at 8h ($p<0.01$). HPLC purification and MALDI-TOF MS sequencing of purified active fractions from Se identified two peptides: δ-haemolysin (δh) and Phenol Soluble Modulin (PSM). Synthetic peptides based on these sequences confirmed activity against GAS (8μM), E. coli (4-8μM), and S. aureus (4-8μM). The killing mechanism was membrane disruption as vesicles leaked when incubated with δh or PSM, and EM of GAS incubated with peptide showed disruption. SE also was anti-viral as δh reduced vaccinia PFU by 46% ($p<0.05$) and PSM reduced plaque size by ~70%. Targeted deletion of PSM in SE (SEΔPSM) confirmed its antimicrobial action in vivo. Partially purified supernatants from SEΔPSM showed greatly reduced inhibition of GAS by radial diffusion assay ($11.8mm^2$ vs $27.5mm^2$ for WT). These data show that SE acts as a mutual symbiote by growing on the skin while protecting against pathogens. We conclude that the innate immune system of the skin should be redefined to include the cutaneous microbiota.

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Identification of endemic non-tuberculous mycobacterial infection on the Island of Satowan associated with swimming in World War II era bomb craters

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Since World War II, residents of Satowan (n=650), an outer island in Chuuk, FSM, noted a high prevalence of a chronic, progressive and disfiguring skin disease known locally as "spam disease." We evaluated one such patient in Portland, Oregon who developed his plaques while living on Satowan. A culture from lesional skin demonstrated M. marinum in this index case, prompting us to travel to Satowan to examine similar patients. A case-control study was performed on the island of Satowan with cases being defined as patients with chronic, progressive verrucous or keloidal plaques on the extremities with a duration >3 months. Inquiries into various lifestyle and occupational exposures were performed with the aid of local health officials. Biopsies were performed for mycobacterial culture, histopathology and PCR analysis. We examined 45 persons, identified 39 case-patients (point prevalence of 6.0%), and enrolled 98 controls. Most case-patients (75%) had a single plaque, nearly all (92.3%) reported their lesions occurred at previous skin trauma sites. All cases were taro farmers (OR 6.3 with $P<0.01$) and among taro farmers, swimming in water-filled World War II era bomb craters was associated with infection (OR 8.2 with $P<0.01$). Histopathologic sections (n=9) showed a pattern typical of mycobacterial and deep fungal infections. All tissue cultures and special stains were negative for mycobacteria and fungi (n=19), whereas PCR analysis revealed non-tuberculous mycobacterial DNA in 8 of 9 samples analyzed. "Spam disease" is a chronic, progressive skin disease of high prevalence on Satowan. Taro farming and swimming in water-filled World War II era bomb craters have been identified as major risk factors for developing this infection. These risk factors along with tissue culture, histopathology and PCR data reveal M. marinum as likely the causative agent.

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Adrenergic stimuli mediate suppression of keratinocyte antimicrobial activity, which is reversible by the neuroendocrine derived antimicrobial peptide, Catestatin

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Catestatin (Cst) is a neuroendocrine peptide derived from Chromogranin A (encoded by Chga) and was previously known as a nicotinic cholinergic receptor (nAChR) antagonist in the adrenal medulla. Recently, we found that Cst acts as an antimicrobial peptide (AMP) and is expressed in keratinocytes. Here, we sought to further define the function of Cst by examining the ability of mice deficient in Chga to resist skin infection. Chga-/- mice were more susceptible to Group A Streptococcus, as lesions were 40% larger (3.5±0.4mm vs. 2.5±0.2 mm,$P<0.05$) and contained 3X more surviving bacteria ($4x10^7±1x10^7$ CFU/ml vs. $1x10^7±5x10^6$,$P=0.05$)compared to wild-type mice. Chga-/- mice also had a reduction in the AMP cathelicidin, as measured by qPCR, and a decrease in total AMP activity extracted from skin, as purified by HPLC and measured by radial diffusion. Since Cst is an antagonist of nAChR, we hypothesized that a decrease in AMP in Chga-/- mice was due to unopposed suppression by acetylcholine(ACh). This hypothesis was supported by the observation that keratinocytes stimulated by 1,25 vitamin D3 and the TLR2/6 agonist Malp2 increased cathelicidin mRNA by 212±10 fold, but only 46±6 fold when ACh was present; a ca.4.5-fold suppression. This effect was dose dependent, also occurred with nicotine, and was inhibited by pretreatment with Cst. Direct analysis of total extracted AMPs from keratinocytes was also suppressed by ACh and reversed by Cst. Thus, we have identified two previously unknown mechanisms by which keratinocytes protect against infection. Cst can act as an AMP, but also blocks inhibition of other AMPs by ACh. These observations further define our understanding of the link between the neural and immune systems and expand the opportunity for targeted therapy of infection by controlling the AMP defense system.

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Chronic exposure to high-glucose solution increases mast cell degranulation and LTC4 release upon FcεRI engagement

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Diabetics often complain an itch of skin. Mast cell-derived chemical mediators might be involved in the pathogenesis of itch. Chronic exposure to high glucose solution has a variety of effects on cell survival and gene expression. Previous studies suggested that glucose deficiency results in reduction of ATP production, and causes inhibition of histamine release and LTC4 secretion from mast cells upon FcεRI cross-linking. However, effects of high glucose solution on mast cell activation remain unclear. We investigated the effects of high glucose solution on mast cell activation following FcεRI engagement. BMMCs were cultured for 7 days in the high glucose (600mg/dl) or normal glucose (100mg/dl) solution. Mannitol (500mg/dl) was added to normal glucose solution as osmotic control. After glucose exposure, BMMCs were sensitized with IgE, and stimulated with antigen. Chronic exposure to high glucose solution resulted in significant increase in degranulation and LTC4 release. On the other hand, IL-6 production was not affected. There are no significant differences in cell proliferation, division, survival, and FcεRI expression on the cell surface between cells cultured in high glucose solution and cells cultured in normal glucose solution. Interestingly, intracellular production of ATP was not affected, suggesting that degranulation and LTC4 release is increased independently of ATP-synthesis pathway in glycolysis by exposure to high glucose solution. It was reported that methylglyoxal, a product of side pathway in glycolysis, is increased in hyperglycaemia. Aminoguanidine is known as a potent scavenger of methylglyoxal. Enhancement of degranulation and LTC4 secretion was clearly reversed when 1mM aminoguanidine was added in high glucose solution. These results suggest that methylglyoxal or some methylglyoxal-derived molecules may facilitate mast cell degranulation and LTC4 release.

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Relationship among human herpesvirus 6 reactivation, serum interleukin-10 levels, and skin rash / GVHD after allogeneic stem cell transplantation

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Human beta-herpesviruses such as HHV-6, HHV-7, and CMV have a tendency to remain latent in the body after the primary infection, and then reactivate in immunocompromised hosts, including patients after allogeneic stem cell transplantation (allo-SCT). It is known that skin rash usually occurs after allo-SCT. The skin rash is often regarded as a cutaneous sign of acute GVHD. However, the exact cause of the skin rash is usually difficult to identify. Here, we prospectively examined HHV-6, HHV-7, CMV, and EBV DNA in the peripheral blood of patients after allo-SCT at regular intervals, and assessed the relationship among human herpesvirus reactivation, serum cytokine levels, and the appearance of skin rash or GVHD. Fifteen patients who received allo-SCT were enrolled (7 cord blood SCT, 8 peripheral blood SCT). Peripheral blood samples were obtained weekly after allo-SCT. HHV-6, HHV-7, CMV, and EBV DNA were detected in the peripheral blood by PCR, and DNA levels were assessed by real time PCR. The serum levels of IFN-γ, IL-4, IL-10, TNF-α, and soluble IL-2 receptor (sIL-2R) were serially measured. In result, 10 of 15 patients showed maculopapular eruption after allo-SCT and were diagnosed as having GVHD. HHV-6 DNA was detected in all 10 patients with skin rash, while only one of the 5 patients without skin rash was positive for HHV-6 DNA. In 8 of 10 patients with skin rash, HHV-6 DNA levels correlated with the course of cutaneous manifestation. However, there is no relationship between skin rash and the appearance of other herpesviruses such as HHV-7, CMV, or EBV. Furthermore, serum IL-10 and sIL-2R levels increased in association with skin rash. In conclusion, reactivated HHV-6 and IL-10 producing activated T cells may be involved in the pathogenesis of skin rash/GVHD after allo-SCT.

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Expression of LL-37 and human neutrophil peptides in neutrophils of psoriatic skin

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Psoriasis is a common chronic inflammatory skin disease characterized by pink to red scaly papules and plaque. One of characteristic histological findings is the neutrophilic accumulation in the parakeratotic cornified layer. To investigate the role of antimicrobial peptides (AMPs) expressed by infiltrating cells, we performed immunohistochemistry in psoriatic lesions and compared with that of atopic dermatitis (AD). Skin biopsies were obtained from four patients with psoriasis and nie patients with AD after informed consent. 5-μm formalin fixed sections were stained with antibody against human neutrophil peptides (HNPs), LL-37 and granulysin. Immunohistochemical analysis demonstrated that strong staining for LL-37 and HNPs was observed in the infiltrating neutrophils in epidermis of psoriasis.Immunohistochemical analysis of AD lesions demonstrated that a few neutrophils within vessels in dermis were positively stained for these AMPs. Lymphoid cells expressing granulysin were sparse in both psoriasis and AD. In this study, we showed both LL-37 and HNPs were abundantly expressed infiltrating neutrophils in psoriatic epidermis. Recent studies indicate that LL-37 has the regulatory function of inflammation and immune responses such as release of HNPs from neutrophils. LL-37 derived from neutrophils in psoriatic epidermis may contribute to autocrine and/or paracrine release of HNPs from neutrophils. It has also reported that self-DNA coupled with LL-37 activate plasmacytoid dendritic cells (pDCs) and activated pDCs drive autoimmunity in psoriasis. Our observations indicate that the accumulation of neutrophils appears to be important not only in histological diagnosis but also in pathogenesis of psoriasis.

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An effective acne vaccine targeting a cell wall-anchored sialidase of *Propionibacterium acnes*

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Acne vulgaris afflicts more than fifty million people in the United State and the severity of this disorder is associated with the immune response to *Propionibacterium acnes* (*P. acnes*). Systemic therapies for acne target *P. acnes* using antibiotics, or target the follicle with retinoids such as isotretinoin. The former systemic therapy may non-specifically kill skin bacteria, which impacts the homeostasis of skin-resident microflora. The latter systemic treatment is highly effective but also carries a risk of side effects including immune imbalance, hyperlipidemia, and teratogenicity. Thus, despite substantial research into potential new therapies for this common disease, acne is still one of skin diseases that require alternative approach of treatments. Here we create an acne vaccine targeting a cell wall-anchored sialidase of *P. acnes*. The importance of sialidase to disease pathogenesis is shown by treatment of a human sebocyte cell line with recombinant sialidase that increased susceptibility to *P. acnes* cytotoxicity and adhesion. Mice immunized with sialidase elicit a detectable antibody; the anti-sialidase serum effectively neutralized the cytotoxicity of *P. acnes in vitro* and *P. acnes*-induced interleukin-8 (IL-8) production in human sebocytes. Furthermore, the sialidase-immunized mice provided protective immunity against *P. acnes in vivo* as this treatment blocked an increase in ear thickness and release of pro-inflammatory macrophage inflammatory protein (MIP-2) cytokine. The results indicated that acne vaccines open novel therapeutic avenues for acne vulgaris and other *P. acnes*-associated diseases.

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Cellular and molecular events leading to the immune escape phenomenon in mollusucum contagiosum

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Molluscum contagiosum(MC),harboring a lot of MC viruses(MCV), persists in the living layers of the epidermis,evading the host immune responses. MC lesions,however,spontaneously disappear associated with so-called"molluscum reaction". PURPOSE:We investigated the cellular and molecular mechanisms that allow MC to evade the host immune responses,focusing on the followings: MCV-encoded molecules that regulate immune system,cellular gene expression by MCV-infected keratinocytes,and the recruitment of dendritic cells (DC) in MC lesions. Total RNA was extracted from MC lesions,and the expression of MCV-encoded molecules(MC148;viral chemokine,MC159L;viral FLICE-inhibitory protein(v-FLIP))was assessed by RT-PCR.Immunohistochemical examination was performed to detect chemoattractant factors derived from MCV-infected keratinocytes (MIP3α:macrophage inflammatory protein 3α, OPN:osteopontin),DC subtypes and their number determined by CD1a,Langerin,CD83,and CD123. We also performed *in situ* hybridization to detect MIP3α mRNA in the MC lesions. Our results indicated that the MC148 mRNA was detected in all MC lesions, and MC159L was detected in 11 of 12 MC lesions. The expression of chemoattractant factors such as MIP3α and OPN was markedly decreased in the MCV-infected keratinocytes,and Langerhans cells and plasmacytoid DC were also decreased. In the inflammatory MC lesions,however,the expression of keratinocyte-derived chemoattractant factors was up-regulated,and the DC numbers were increased. These results suggest that MC can evade the host immune responses by expression of MCV-encoded immunoregulatory cytokines, and down-regulation of the chemoattractant factors,which interfere DC recruitment in MC lesions.

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Varicella zoster virus inhibition of Toll-like receptor 3 signalling in keratinocytes – a novel immune evasion strategy.

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Varicella zoster virus (VZV) is able to infect and replicate within human keratinocytes. We have previously shown that this results in loss of the ability of infected keratinocytes to respond to IFN-γ. We wished to determine the extent to which VZV is able to disrupt toll-like receptor (TLR) signalling in keratinocytes as this is a crucial mechanism through which keratinocytes respond to infection. Human keratinocytes were infected with either wild-type or vaccine strain VZV and subsequently stimulated with TLR ligands for known receptors on keratinocytes. The expression of ICAM-1 and HLA-class I was up-regulated by TLR3 ligand (poly I:C) but not by TLR4, 7/8 or 9 ligands, suggesting that TLR3 carries an important immune function for keratinocytes. This up-regulation was inhibited in VZV infected keratinocytes. Thus, following TLR3 stimulation, mean HLA class I expression increased significantly from 1391 to 2248 ($p<0.05$) in uninfected keratinocytes but was not significantly increased in infected keratinocytes (an increase from 1108 to 1416). Similarly, following TLR3 stimulation, mean ICAM-1 expression increased significantly from 70 to 192 ($p<0.05$) in uninfected keratinocytes but was not significantly increased in infected keratinocytes (an increase from 49 to 92). By staining with annexin V and cell viability analysis, we showed that the inhibition of TLR3 signalling was not due to increased cell death of infected keratinocytes. Furthermore, by use of IFN-γ blocking antibodies we showed that inhibition of TLR3 signalling is independent of IFN-γ. These data show that VZV is able to inhibit TLR3 signalling in keratinocytes, representing a novel immune evasion strategy by the virus.

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cDNA Microarray analysis of HPV33 induced bowenoid lesions

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Human papillomavirus (HPV) is an important etiologic agent in cervical carcinoma and in some malignant skin tumors including Bowen's diseases and squamous cell carcinomas. To characterize the signaling pathway of HPV-induced malignant skin tumors, we examined HPV33 induced bowenoid lesions, verrucous lesions, and normal skin from the same patient with myelodysplastic syndrome. Previously, we reported that the expression of β-catenin, E-cadherin, and presenilin-1 was down-regulated in HPV33 induced bowenoid lesions when compared to those of verrucous lesions, suggesting that reduced expression of presenilin-1 may result in down-regulation of Notch signaling pathway due to insufficient cleaved Notch in bowenoid lesions (68th annual SID meeting in Los Angeles). In this study, we examined RNA samples from bowenoid and verrucous lesions by using cDNA microarray analysis to characterize other signaling pathways. More than hundreds of genes were differently upregulated in HPV33 induced bowenoied lesions, but there was no significant difference in gene expression related with Notch and Wnt signaling pathways. Langerin and MIP3-α were down-regulated in verrucous lesions when compared with bowenoid lesions and normal skin. This indicates that the decrease in number of Langerhans cells may be necessary to maintain the immunological homeostasis of verrucous lesions, but may be no longer necessary for bowenoid lesions. In bowenoid lesions, the expression of filaggrin and involucrin was down-regulated, but the expression of keratin-17(K-17) was up-regulated. Immunohistochemical analysis showed that K-17 was strongly expressed in the basal layer of the boweonid lesions, suggesting that the abnormal expression of K-17 may result in development of bowenoid lesions. In conclusion, in addition to cell cycle associated genes, immunomodulatory factors and cytoskeleton molecules may be also associated with HPV-induced malignant transformation in skin.

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Variation in cell entry activities between *Mycobacterium leprae mce1A* region and *Mycobacterium tuberculosis mce1A* region into different cultured human airway epithelial cells

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Both *M. leprae* and *M. tuberculosis* are pathogenic, acid fast bacillus. The transmission mode of these mycobacterium, however, are different. *M. leprae* enters through the upper airway mucous membranes, and *M. tuberculosis* infects through the lower airway mucous membranes. Our previous studies revealed that the proteins coded in *mce1A* region of *M. tuberculosis* and *M. leprae* are the key pathogenesis factor regarding the mycobacterium's epithelial cell entry. This study aims at further elucidating this role of Mce1A protein on entry into airway mucous membrane epithelial cells by using recombinant *E. coli* expressing the mycobacterium invasion active region (Inv-X) in the outer membrane through the AIDA (adhesion involved in diffuse adherence) system. A comparative study of invasion activities of these recombinant *E. coli* into different cultured human airway mucous membrane epithelial cells was performed. The study revealed that the invasion activity into HEp-2 cells, a laryngeal mucosa epithelial cell strain, by *E. coli* expressing Inv-X from either *M. leprae* or *M. tuberculosis* was about the same. Invasion activity into A549 cells, an alveolus epithelial cell strain, was stronger for *E. coli* expressing Inv-X from *M. tuberculosis* than from *M. leprae*. In contrast, invasion activity into RPMI2650, a nasal mucous membrane cell strain, was stronger for *E. coli* expressing Inv-X from *M. leprae* than from *M. tuberculosis*. It has been believed that *M. leprae* easily proliferates in nasal mucous membranes because of the low temperature of the membranes. This study suggests that *M. leprae* finds its way preferably into nasal mucous membrane via Mce1A protein.

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Overcoming bacterial resistance with true novel antibiotics: The ORFeome of Staphylococcus aureus will connect to downstream 'omics' applications

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The pathogen Staphylococcus aureus is responsible for significant morbidity and mortality in humans through superficial and deep skin infections. Increasing resistance to antibiotic therapy has been noticed and for the methicillin-resistant S. aureus strain only a few antibiotics including vancomycin and linezolid are appropriate drugs. Both substances are members of long known classes of antibiotics and therefore also resistant strains have developed. To find novel classes of antibiotics, which do not elicit resistance within short time, it is necessary to understand the molecular mechanisms of virulence and antibiotics-resistance. Therefore we cloned the entire set of S. aureus protein-encoding open reading frames (ORFs), its ORFeome. Starting from the complete genome sequence of S. aureus strain Mu50, which has 2967 predicted ORFs, we generated a database of protein-coding ORFs and constructed an ORFeome library of 2562 Gateway Entry clones, each containing a defined ORF. The site-specific recombination cloning technology offered fast and efficient cloning and resulted in cloning of 95% of the ORFs in an entry vector. The quality of the ORFeome was examined by DNA sequencing and PCR. Functionality of the ORFeome was shown by recombinant protein production which resulted in 37 positive clones of the 40 tested clones. Furthermore, in a yeast 2-hybrid homodimerisation assay we detected 22 novel homodimers, which are part of the S. aureus interactome. Based on this ORFeome we are now able to analyse the S. aureus interactome providing us with novel targets for antibiotics.

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Immune response to vaccinia virus scarification is modulated in mice that overexpress IL-1α in the epidermis

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Transepidermal inoculation of live vaccinia virus (VV) by scarification induces specific and long-lasting immunity to smallpox. Response to vaccinia scarification reflects the balance of cytokines and immune response elements present in, or recruited to, skin. Dysregulation of this balance, as in patients with atopic skin disease, can lead to persistent viral replication and cause significant morbidity and mortality. To explore the mechanisms regulating the cutaneous immune response to vaccinia, we utilized transgenic mice engineered to over-express the inflammatory cytokine interleukin-1α in the epidermis. Following scarification with vaccinia virus, both transgenic mice and wild-type mice are able to generate local pox reactions similar to those seen in humans. At high doses of vaccinia virus inoculated, IL-1α transgenic mice demonstrated earlier and enhanced T-cell immune responses as well as stronger humoral immunity. Furthermore, IL-1α transgenic mice recruited immune cells more rapidly to the site of inoculation. At limiting doses, however, some IL-1α transgenic mice eliminated the virus without formation of pox lesions or activation of the adaptive immune response. This study suggests IL-1 may have use as an adjuvant to enhance antiviral immunity, however the balance of IL-1 effects on innate and adaptive immune functions will be critical. Development and extension of this model will enhance our understanding of the immune response to vaccinia virus and promote the creation of safer and more effective vaccination strategies.

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Genetic involvement of bacterial sensor molecules *TLR2, DC-SIGN* and *NOD2* in Hansen's disease

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Occurrence of new patients of Hansen's disease (leprosy), caused by *Mycobacterium (M.) leprae* infection, is now almost absent in Japan but is still uncontrolled in developing countries. *M. leprae* invade intracellularly but are weak on their toxicity, therefore some host condition is believed to have a critical role on establishment of the disease. Even after infection, phenotypic heterogeneity is observed including two major extremities; the lepromatous (L-) type, which is multibacillary and associated with hypo-cellular immunity, and the tuberculoid (T-) type, which is paucibacillary and accompanied with hyper-cellular immunity. As one cause of the host response, genetic predisposition has been considered at two stages of the disease progression, establishment of the disease and determination of the phenotype. On the basis of the recent progress of the research on innate immunity, here we analyzed single nucleotide polymorphisms (SNPs) of the genes of major bacterial sensor molecules, *TLR2, DC-SIGN* and *NOD2*, in the Japanese Hansen's disease patients and compared with the healthy controls, or between the L- and the T- types. Although no nonsynonymous polymorphism of *TLR2* and *NOD2* was detected in the patients, among SNPs in the promoter regions, appearance of *TLR2/-16934TT, DC-SIGN/-939CC* and *-336TC* were significantly ($P<0.05$) lower in the female patients compared with the controls. Interestingly, all patients with *TLR2/-16934TT* showed *DC-SIGN/-336TT* with significant difference with the controls, and all controls with *TLR2/-16934AA* showed *DC-SIGN/-871TT*, suggesting some cooperation of these genes on establishment of the disease.

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Skin scarification with Modified Vaccinia Ankara virus provides superior and safer protective immunization strategy against poxvirus challenge

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Although smallpox was successfully eradicated through the global vaccinia virus (VV) immunization campaign in 1979, smallpox vaccination has gained renewed interest due to the considerations of bioterrosim. Available vaccine preparations (Dryvax) are associated with life threatening eczema vaccinatum and necrosum in patients with atopic dermatitis or immunosuppression. The replication deficient VV strain, Modified Vaccinia Ankara (MVA), has been proposed as a safe alternative smallpox vaccine, though its true efficacy has never been tested. Because it fails to replicate in human skin cells, MVA is inoculated exclusively by intradermal, subcutaneous or intramuscular injection, while classic VV vaccines are delivered by scarification. In our studies, we examined the safety and efficacy of MVA immunization via skin scarification in mouse models. Surprising, we demonstrated that MVA skin scarification led to "pox" lesion development at the inoculation site, and strongly induced both humoral and cellular immune responses in a dose-dependent manner. At same dose, MVA skin-scarified mice elicited significantly higher serum VV-specific IgG levels and stronger cellular immune responses compared to mice immunized with MVA by all other transcutaneous injection routes. Most importantly, MVA skin-scarified mice showed 100% protection from a lethal challenge of wild type VV, while the MVA delivered by injection was only partially protective. In addition, even at high doses, MVA was safely inoculated via skin scarification to Rag-/- mice, indicating its safety in immunosuppressed recipients. Our data strongly suggest that MVA skin scarification is both superior and safer in delivering protective immunization, and this mode of delivery of MVA warrants further clinical evaluation in healthy and immunocompromised volunteers.

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Myxoma virus induces type I interferon production in plasmacytoid dendritic cells through MyD88-dependent and IRF3-independent signaling pathway

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Poxviruses are dermatotrophic dsDNA viruses that employ multiple strategies to evade the host immune system. Myxoma virus is a rabbit-specific poxvirus that causes lethal myxomatosis in European rabbits. It has been explored as an oncolytic virus for the treatment of human cancers. Here we report that myxoma virus infection induces both IFN-α, β, and IL12p40 production in murine Flt3L-cultured bone marrow-derived plasmacytoid dendritic cells (pDCs). Co-infection of myxoma virus with wild type vaccinia virus in pDCs significantly attenuated the production of type I interferon. We further demonstrate that this induction effect is independent of the mitochondrial antiviral signaling protein (MAVS) or IRF3, but is dependent of MyD88. In addition, we show that treatment of infected pDCs with chloroquine which interferes with endosomal acidification, suppressed the induction of type I interferon. This result is consistent with our hypothesis that myxoma virus-induced activation of pDCs is mediated by endosomal TLR(s) which utilizes MyD88 as an adaptor. Vaccinia virus attenuates this process possibly through encoded viral proteins that interfere with the recruitment of MyD88 to TLR(s). Taken all together, we have identified a key signaling pathway through which myxoma virus activates pDCs. Because TLR7 and TLR9 have been implicated in sensing viral infection in pDCs, efforts are underway to further determine whether TLR7 and/or TLR9 is involved in this process, and how dsDNA viral replication intermediates are recognized by TLR(s) localized on endosomal membranes.

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Acne vaccine development using a humanized acne microenvironment model: *in vivo* P. acnes-induced host responses

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Acne is a human disease of the sebaceous hair follicle. Unlike human, most animals produce little or no triglycerides in hair follicles to harbor Propionibacterium acnes (P. acnes) a fact that has encumbered the development of novel treatments for acne lesions. Here, we employed a bioengineering approach using a tissue chamber integrated with a dermis-based cell-trapped system (DBCTS) to mimic the *in vivo* microenvironment of acne lesions. Human sebocyte cell lines were grown in DBCTS as a scaffold and inserted into a perforated tissue chamber. After implantation of a tissue chamber bearing human seboytes into ICR mice, P. acnes or PBS was injected into a tissue chamber to induce host immune response. Infiltrated cells such as neutrophil and macrophage were detectable in tissue chamber fluids. In addition, a pro-inflammatory cytokine MIP-2 was elevated after P. acnes injection. In tissue chamber fluids, thirteen proteins including secreted proteins and cell matrix derived from mouse, human cells or P. acnes were identified by proteomics using isotope-coded protein label (ICPL) coupled to NanoLC-MS mass spectrometric analysis. After P. acnes infection, four proteins including fibrinogen, alpha polypeptide, fibrinogen beta chain, S100A9, and serine protease inhibitor A3K altered their abundances in a mimicked acne microenvironment. The acne model has been used for evaluate the efficacy of novel acne vaccines targeting P. acnes virulence proteins. The bioengineered acne model thus provided an *in vivo* microenvironment to study the interaction of host with P. acnes and offered an implement for development of novel anti-acne vaccines.

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Cell-mediated immune responses and zoster-associated pain in herpes zoster patients

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Recurrent herpes zoster (HZ) in immunocompetent patients is uncommon, presumably due to the boosting of varicella-zoster virus (VZV)-specific immunity related to an initial eruption. In this multicenter study, we compared the VZV-specific cell-mediated immune(CMI) responses following an episode of HZ to healthy subjects. A validated interferon-gamma enzyme-linked immunospot (IFN-γ ELISPOT) assay was used to evaluate the variability of the VZV-specific CMI responses in subjects diagnosed with acute HZ (≤ 72 hours of lesion onset) compared to age, race, and gender-matched healthy controls. Blood was drawn for immunogenicity studies at Day 0, 2 weeks, 6 weeks, and 6 months post-enrollment. 88 subjects with acute HZ were enrolled (57 subjects ≥ 60 years of age and 31 subjects between 21 to 59 years), as well as 58 and 31 matched controls, respectively. The HZ subjects had significantly higher ELISPOT responses then their corresponding controls. The geometric mean counts (GMC's) peaked 1-2 weeks after lesion onset. The GMC's in HZ subjects ≥ 60 years of age were 92 [95%CI (49, 174)], 241 [95% CI (150, 387)], and 120 [95% CI (72, 200)] compared to matched control counts which were 22 [95% CI (14, 33)], 20 [95% CI (10, 40)], and 27 [95% CI (16, 45)] at Day 0, 2 weeks, and 6 weeks, respectively. The GMC's in HZ subjects between ages 21 to 59 were 111 [95% CI (54, 227)], 198 [95% CI (122, 322)], and 162 [95% CI (118, 224)] compared to matched controls which were 58 [95% CI (30, 113)], 61 [95% CI (28, 134)], and 40 [95% CI (20, 83)] at Day 0, 2 weeks, and 6 weeks, respectively. The VZV-specific CMI response was substantially boosted by an episode of HZ. Among the healthy controls, older adults had a lower immunity than younger subjects at baseline, but the boosting effect of HZ was comparable for all age groups. This research may help determine if the zoster vaccine can induce VZV-specific CMI responses that correlate with prevention of HZ by comparison of vaccine recipient responses to the responses seen in these HZ subjects.

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Establishment of a skin infection in mice using bioluminescent staphylococcus aureus

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Staphylococcus aureus (S. aureus) skin and soft-tissue infections are becoming increasingly difficult to treat as new patterns of antimicrobial resistance continue to emerge. The need for novel therapeutic options is evident and an optimal murine model of superficial S. aureus infection is needed to examine the potential usefulness and safety profiles of these new therapies. We report a new mouse model of superficial S. aureus infection. Superficial infection is established using a number eleven blade scalpel to make three parallel incisions between the shoulder blades of c57BL/6 mice with subsequent inoculation of bioluminescent S. aureus. Bacterial counts are followed throughout the infection using the Xenogen Optical Imaging system; previous studies have shown correlation between photons/second emmitance and conventionally performed CFU assays. Infected mice develop ulcers that are significantly larger than similarly treated controls. Without treatment bacterial counts return to normal within ten days. Established effective antibiotic treatments have proven to decrease ulcer size and hasten resolution of infection when compared to vehicle. This mouse model of superficial S. aureus infection has proven reproducible and clinically relevant and provides the opportunity to follow bacterial counts in individual mice over the course of an infection without the need to sacrifice at various time points to obtain CFU assays. We propose that this is an ideal model to examine the efficacy of experimental topical anti-staphylococcal agents.

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HSV infection of cutaneous mast cells induces impaired immunological function and apoptosis

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Recent studies have revealed the importance of mast cells in the initiation and regulation of innate and adaptive immune defense. During bacterial infection, mast cell-derived TNFa promotes dendritic cell migration to draining lymph nodes and facilitates bacterial clearance. However, it remains largely unknown whether mast cells are involved in host defense against viruses. Therefore, we investigated whether herpes simplex virus (HSV) infects mast cells and affects their immunological function. We used murine fetal skin-derived cultured mast cells (FSMC), which exhibit important features of connective tissue type mast cells. 24 h after HSV-2 (strain 186) exposure with MOI 100, viral antigen was detected in 26% of FSMC by intracellular staining with anti-HSV-2 mAb. We have previously reported that FSMC express TLR3, which recognize viral dsRNA, and produced proinflammatry cytokines and chemokines in response to the ligands. Nevertheless, HSV infection of FSMC failed to induce the production of TNF-α, IL-6, MIP-1α and MIP-2. Furthermore, upon stimulation by LPS, HSV-infected FSMC produced significantly lower amount of these cytokines and chemokines when compared to uninfected FSMC. As a control, heat-inactivated HSV did not affect the production. In addition, HSV infection did not induce degranulation of FSMC. We next analyzed the apoptosis using an annexin V binding and TUNEL assay. HSV induced apoptosis of FSMC in a virus number-dependent fashion, whereas heat-inactivated HSV did not. Moreover, phosphonoacetic acid (PAA), an inhibitor of viral replication, reduced apoptosis, but not abolished, suggesting that both early and late HSV proteins play roles in apoptotic cell death. These results indicate that HSV infection of FSMCs leads to their apoptosis and inhibition of cytokine and chemokine production. Thus mast cells may participate in the reduction of inflammatory responses and the subsequent acquired immune responses against the virus, and prolong the infection.

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The incidence and risk factors for developing postherpetic neuralgia

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Postherpetic neuralgia (PHN) is a common complication following herpes zoster (HZ). It is characterized by pain that persists for longer than 1 month following resolution of the vesicular rash. This complication is more common in patients older than 50 years. The aims of our study were to determine the incidence of PHN in 132 patients who were hospitalized for treatment of HZ during the period 1994-2007 and to identify risk factors for occurrence of PHN. Sixty per cent were women and 40% were men with a mean age of 65 years (range 13-84 years). The thoracis dermatomes were the most commonly affected (48%), while disseminated herpes zoster was observed in 6 patients. Of these 132 patients, we followed 104 of them and identified that about 80% experienced PHN 1 month at least. The incidence of PHN did not vary between the sexes, but the mean age was about 15 years greater than in patients with acute varicella-zoster virus infection. Patients with herpetic neuralgia who did not develop PHN were significantly younger and had less severe acute pain than PHN patients but were significantly more likely to have severe and widespread rash than patients without persisting pain. Patients with PHN were more likely to have head and neck involvement (34%) than were those with acute herpes zoster (30%). To conclude, increasing age, gangrenous zoster and cervical and trigeminal localization are risk factors for developing PHN in HZ patients. An early treatment is important to identify HZ patients who have an increase risk of developing PHN.

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Varicella zoster virus colocalizes within adnexal stem cell compartments of skin

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Varicella zoster virus (VZV) causes both primary varicella (chickenpox) and herpes zoster (shingles) which results from reactivation of the virus from its latent state in dorsal root ganglia and subsequent movement to the isthmus region of the hair follicle, which overlaps anatomically with the follicular stem cell compartment. We hypothesized that VZV vesicle formation in skin depends on infection of pluripotent stem cells in the hair follicle. Colocalization studies were performed with antibodies directed against putative follicular stem cell markers [MCSP (clone: LHM2), K15 (clones LHK15 and C8/144B)] and the VZV antigen IE63, to see if VZV preferentially infects hair follicle stem cells. Sixteen archival herpes skin biopsies were identified that were positive for the VZV antigen and had follicular involvement on H&E staining. Colocalization studies with the above markers showed prominent VZV infection in the isthmus region and the germinative epithelium, from which the differentiated layers of the hair follicle originate. The sebaceous and eccrine glands, which are also sites of stem cell enrichment showed prominent involvement with VZV. Multinucleated keratinocytes which are characteristic of cutaneous herpes infections showed increased staining with MCSP and K15. To examine if interfollicular stem cells are permissive for VZV infection *in vitro*, a keratinocyte SCC cell line (MET1) was FACS-sorted with anti-CD44 to identify the 'stem cell' rich population and replated to form colonies. IE63 staining localized mainly to the paraclone-like differentiated colonies. Replicate experiments are being performed in primary keratinocytes and other keratinocyte cell lines. This study highlights the pathway of viral spread in herpes zoster and suggests that VZV may initially colocalize within adnexal stem cell compartments of the skin.

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Cyclosporin A induces apoptosis of Epstein-Barr virus-transformed B lymphoblastoid cells

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Cyclosporin A (CsA) has been shown to have antiviral activities against human immunodeficiency virus (HIV), hepatitis C virus (HCV), herpes simplex virus, and vaccinia virus. Most of those CsA effects are thought to be associated with competitive inhibition of cyclophilins A, B, and C, intracellular ligands of cyclosporin A. Some viruses may require the binding to cyclophilins for viral replication although the detail mechanism underlying their responsibility for processing of the viral genome replication. This assumption has been supported by the experimental findings that CsD, a CsA analogue with a similar binding capacity to cyclophilins but much weaker immunosuppressive activity, also had the antiviral effect and FK506 sharing pharmacologic mechanisms that suppress T-cell activation failed to suppress the viral activity. Data exhibited to date suggest the action of CsA without calcineurin/NFAT-mediated pathway but do not elucidate the distinctive mechanism. We here report that CsA but not FK506 induced apoptosis of B lymphoblastoid cells transformed by Epstein-Barr virus whose association with cyclophilin has not been evidenced. The present findings might lead to a possibility to CsA function on B cell lineage and viral replication cascade irrelevant for cyclophilin.

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Implication of human herpes viruses type 6 and 8 in parapsoriasis

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Previous recent studies have suspected the involvement of viral agents and particularly human herpes viruses (HHV) in the development of lymphoproliferative diseases such as cutaneous lymphomas. HHV8 is known to play a role in Kaposi syndrome and in some lymphoproliferative diseases such as Castleman's disease or body-cavity-based lymphoma. Recently, the presence of HHV8 has been reported in parapsoriasis lesions. The etiology of parapsoriasis remains unknown and this pathology may be considered as an early stage of mycosis fungoïdes. HHV6 acts as a lymphotropic virus, responsible for roseola infantum, its implication is suspected in pityriasis rosea. The aim of this study was to investigate the presence of HHV6 and HHV8 in 50 lesional tissues obtained from patients with parapsoriasis, using real-time PCR. The diagnosis of parapsoriasis was confirmed by histology. Total DNA was extracted from cutaneous lesions using the phenol-chloroforme method, and the presence of viral genomes was investigated using real-time PCR. The presence of HHV6 and HHV8 DNA was not detected in any studied sample. This study does not confirm the suspected role of HHV8 in parapsoriasis as suggested by Trento et al, whose results demonstrated the presence of HHV8 using nested-PCR in 10 cutaneous samples of patients with parapsoriasis. The differential prevalence of HHV8 infection (2% in France versus 14% in Italy) does not seem to be sufficient to explain these different results. However, our results are in accordance with those published by Nagore et al that did not detect HHV8 using PCR in 29 cutaneous samples of mycosis fungoïde patients or those from Dupin et al that did not detect HHV8 in 23 mycosis fungoïdes lesions and 5 Sézary syndrome lesions and found 1/6 parapsoriasis positive for HHV8. Regarding HHV6, our results do not suggest the implication of this virus in the pathogenesis of parapsoriasis. In conclusion, the role of HHV6 and HHV8 in the pathogenesis of parapsoriasis cannot be demonstrated.

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Serological relationship between cutaneous human papillomavirus types 5, 8 and 92

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Evidence on a possible association of cutaneous human papillomavirus (HPV) types, especially genus beta-papillomaviruses (PV), and the development of non-melanoma skin cancer (NMSC) is accumulating. Vaccination with virus-like particles (VLP) consisting of self-assembled L1 major capsid protein has been introduced to control anogenital HPV infection. We have examined the serological relationship between beta-PV types 5, 8, and the new type HPV 92, which has been recently isolated from a basal cell carcinoma containing a high number of viral genomes. Following expression by recombinant baculoviruses the L1 major capsid protein of HPV 92 self-assembled into VLP that elicited high-titer antibodies after immunization, similar to that of L1 from HPV 5 and 8. For serotype analyses, hemagglutination inhibition (HAI) assays were used as a surrogate for detection of virion neutralizing antibodies, that correlate with protection from viral infection. Antisera raised against HPV 5 and 8 VLP displayed HAI activity not only against the homologous type, but also against heterologous HPV types 5, 8 and 92, whereas HAI activity of antisera against HPV 92 VLP was restricted to the homologous type. Results of neutralization assays using HPV 5 pseudovirions were consistent with those from HAI assays. Cross-neutralizing immune responses by VLP vaccination against heterologous HPV types may provide broader protection against the multiplicity of HPV types detected in NMSC. If a close link to HPV infection can be conclusively established, our results may provide a basis to further evaluate VLP of beta-PV as candidates for a prophylactic skin-type HPV vaccine, aiming at reducing the incidence of NMSC.

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Extremely high viral loads of HPV26 and HPV88 in squamous cell carcinomas (SCC) of the nail unit in an HIV-immunosuppressed patient

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Infection with high-risk mucosal human papillomavirus (HPV) types has been associated with development of digital SCC. HPV26 is a genital-mucosal type of uncertain dignity that has been very rarely detected in cervical cancer. In an HIV-infected patient suffering from verrucous SCC of all fingertips, HPV26 and a new type, HPV88, were identified. HPV26-specific real-time PCR determined high viral load (56-44.000 copies/cell) in all right hand tumours, whereas left hand SCC had only low copy numbers (0.4-1.2/cell). Conversely, left hand SCC showed very high HPV88 copy numbers with up to $1.3*10^6$/cell, SCC of the right hand low viral copy numbers (0.09-1.6/cell). The extremely high viral loads in these digital SCC suggested a major causative role for the respective dominant HPV type in tumor development, whereas contamination was the likely cause for low levels of HPV DNA detected on the opposite sites. By *in situ* hybridization HPV26E6 DNA was detectable in suprabasal tumor cells indicating productive viral infection. Tumor cells stained positive for p16INK4a, a marker of high-risk HPV-associated neoplasia. No missense or synonymous nucleotidic changes in the E6 ORF were detected, that could account for abnormal biologic properties of this HPV26 isolate as compared to HPV26 prototype. This indicated that productive infection with high-level amplification of the viral genome played a causal role in tumor progression. HPV88 was isolated from one of the SCC and represents a new species of gamma PV. A survey of skin tumors and normal skin biopsies from immunocompetent (n=362) and immunosuppressed (n=26) patients detected HPV88 DNA in 7 specimens. These showed 10-logarithms lower viral loads, implying that HPV88 infection is not generally associated with immunosuppression or SCC. Here we identify HPV26 and 88 as the likely primary causes of digital SCC in an HIV-infected immunosuppressed patient.

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Mycoplasma infection as etiological cofactor in severe cutaneus drug reactions

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Adverse reactions to medicines manifest as skin rashes which are mild and self limited in most of the cases. Many etiological factors are known to act as coinducing agents of the skin symptoms, such as bacterial infections. Infection caused by human Mycoplasma pneumoniae can also result in similar skin symptoms and is known to complicate drug metabolism to result in adverse skin reactions to medicines. The role of other Mycoplasmae in contributing to severe cutaneous adverse reactions (SCAR) is not known. Our aim was to study Mycoplasma DNA in sera of 26 patients with SCARs. Cases were sorted based on etiology into 3 groups: 1. drug induced SCARs proved by lymphocyte transformation test (LTT) 2. SCARs with infections in the preceding 2 weeks; 3. SCARs of both possible origins. A control group of healthy individuals was also tested (n=40). A wide-spectrum and sensitive PCR-based Mycoplasma detection-screening method was used to determine Mycoplasma DNA positivity. 20/26 (77%) SCAR patients' sera were Mycoplasma DNA positive whereas only 12/40 (30%) control individuals showed positivity ($p<0,05$). As expected Mycoplasma presence was significant in the 2. SCAR group vs. control group (13/15 vs. 12/40, $p<0,05$). Even the LTT+ SCAR patients were found to carry Mycoplasma DNA significantly more often then the controls (14/15 vs. 12/40 $p<0,05$). We also found that the maculo-papular SCARs were highly significantly associated with Mycoplasma positivity (10/12 vs. 12/40, $p<0,05$), while SCAR patients with infectious and noninfectious background were not significantly different from each other in that. Studying SCAR patients for the presence of Mycoplasma DNA indicating a current or past infection, we found that the infection is significantly more widely present in SCAR patients, ($p<0,05$) than in controls. Even in patients who were proven to have drug-induced SCARS Mycoplasma DNA positivity was of high prevalence. Our data suggest that Mycoplasma infection is a significant etiological cofactor in many patients with SCAR.

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Proinflammatory Staphylococcal cell wall component lipoteichoic acid potently suppresses skin inflammation by induction of regulatory T cells

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Staphylococcus aureus is a known trigger for inflammatory skin diseases, especially atopic dermatitis. S. aureus sets free enormous amounts of lipoteichoic acid (LTA), a bacterial cell wall component, and it was the aim of this study to reveal the role of LTA for cutaneous immune responses. Therefore, the fluorescein isothiocyanate (FITC) contact hypersensitivity (CHS) model, which mimics atopic dermatitis with high IgE levels and Th2 cells, was established and the effects of LTA on FITC-CHS were studied. The presence of LTA during repeated contacts with FITC enhanced FITC-specific cutaneous inflammation and induced Th1-immunity demonstrating a role of LTA as a type I PAMP. In contrast, limited application of LTA during initial contacts to FITC significantly suppressed lesional T cell inflammatory cytokine expression such as IL-4 and IFN-γ. This LTA application also had functional consequences as T cells from draining lymph nodes demonstrated significantly suppressed proliferation *ex vivo*. In order to understand the mechanisms of this *in vivo* effect, CD4+ T cell activation was analyzed *in vitro* in the absence of other cell types by polyclonal activation. Surprisingly, whereas other TLR2 ligands acted as T cell costimulators, LTA treatment resulted in doubling of CD4+CD25+Foxp3+ cells and IL-10-producing CD4+ T cells compared to control leading to significant suppression of T cell proliferation. Taken together, we identified two opposing biological functions of LTA on cutaneous inflammation: 1) Enhancement of severe or ongoing inflammation through induction of type I immunity as clinically observed in atopic dermatitis and S. aureus colonization. 2) Suppression of mild inflammation by Treg-mediated inhibition. This second effect may be of great relevance as it indicates how on the one hand resident microflora such as S. epidermidis mediates tolerance and even stabilizes the cutaneous immunological barrier and how on the other hand this mechanism could be exploited by cutaneous microorganisms to evade effective immune responses.

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Higher mRNA levels of chemokines and cytokines associated with macrophage activation in erythema migrans skin lesions in patients from the United States than in patients from Austria with Lyme borreliosis

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Erythema migrans (EM) is caused primarily by Borrelia afzelii (Ba) in Europe and solely by Borrelia burgdorferi (Bb) in the United States. Bb infection in the US has previously been associated with faster expansion of EM and with more associated symptoms, compared with Ba infection in Europe. However, reasons for these differences are not yet known. We determined the Borrelia species infecting 67 US or Austrian patients with EM. The clinical pictures and chemokine and cytokine mRNA levels in lesional skin were then compared in the 19 Bb-infected US patients and the 37 Ba-infected Austrian patients, the 2 largest groups. The 19 Bb-infected US patients had faster-expanding EM and a median of 4 associated symptoms, whereas the 37 Ba-infected Austrian patients had slower-expanding EM and usually did not experience associated symptoms. Compared with the EM of Ba-infected Austrian patients, those of Bb-infected US patients had significantly higher mRNA levels of chemokines associated with activation of macrophages, including chemoattractants for neutrophils (CXCL1), macrophages (CCL3, CCL4), and T helper 1 cells (CXCL9, CXCL10, CXCL11). In addition, compared with EM of Austrian patients, EM of US patients tended to have higher mRNA levels of the macrophage-associated proinflammatory cytokines interleukin 1beta and tumor necrosis factor alpha, and they had significantly higher mRNA expression of the anti-inflammatory cytokines interleukin 10 and transforming growth factor beta. EM of Bb-infected US patients expanded faster, were associated with more symptoms, and had higher mRNA levels of macrophage-associated chemokines and cytokines than did EM of Ba-infected Austrian patients.

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Assessment of burn wound infection by swab and biopsy culture: A comparative study

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Wound infection is a frequent and severe complication in burn patients accounting for a high number of mortalities. Although, there are some reports comparing swab and biopsy cultures of wounds of various etiologies or chronic wounds, the findings are different from geographical region to another. Thus, the aim of the study was to evaluate the burn wounds by different bacteriologic methods of surface swab and tissue biopsy culture in our country, Iran, to determine the type and prevalence of the bacterial infection in burn patients. In this study, a prospective evaluation was conducted in Shahid Motahari Burn Center, Tehran, on patients suffering from burn with more than 20% of TBSA admitted in 2001-2002. Wound swab and biopsy specimens were collected within the second week. Patients were followed to the point of their discharge or death. The results revealed that a positive contaminated bacteria growth in 100% of swabs and 89.3% of the biopsies in differential culture media. From these cultures, 18.7% of the biopsies were positive in selective culture media. These different cultures had similar pathogen reports. Of the 75 patients studied, 78.7% died. The frequency rates of positive tissue cultures and bacterial load were not different between the dead and the survived patients. In conclusion, bacteriologic methods do not have enough predictive value to assess the clinical outcome.

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Development of a chronic wound in a diabetic (db/db) mouse by infection with biofilm

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Chronic wounds such as diabetic ulcers, pressure sores, and venous stasis ulcers are a major source of morbidity and mortality. Since chronic ulcers are not homogeneous, systematic study of ulcer therapies is difficult. Our goal is to create a standard chronic wound model. It has been shown that specialized microbial colonies known as biofilm are present in chronic human wounds. The microorganisms present in biofilm are protected from the host defenses, topical antiseptics, and systemic antibiotics. For this reason, biofilm infections persist and may contribute to poor wound healing. We hypothesize that application of biofilm to an already characterized diabetic mouse wound model may create a reproducible chronic wound. Bacterial biofilm was developed by incubating planktonic Pseudomonas aeruginosa (PAO-1) and transferring to polycarbonate membrane filters placed on LB agar plates. The biofilm (~10^8 CFU) was transferred to 6mm wounds created on the dorsal skin of diabetic mice and the wounds were covered with Tegaderm® dressing. The biofilm was transferred to the wounds at several different time points following wounding. The mice died if the biofilm was transferred soon after wounding; however, mice that were allowed to recover from the surgery before biofilm inoculation developed a purulent wound that persisted for weeks. In addition, the timing of Tegaderm® application proved to be a critical variable for purulence and quality of the wound. Ultimately, the development of a reproducible chronic wound in a diabetic mouse will allow *in vivo* testing of potential wound healing therapies.

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Reactivation of Epstein-Barr virus is associated with severe cutaneous and systemic symptoms

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Epstein-Barr virus (EBV) is characterized by "latent" infection, and its reactivation induces various clinical symptoms, including typical and severe hydroa vacciniforme (HV), hypersensitivity to mosquito bites, and infectious mononucleosis (IM). We studied cellular and molecular events upon EBV reactivation from the latent cycle, and correlation with the systemic conditions. Either the latent or lytic cycle was determined by expression of LMP1 or BZLF1 mRNA respectively in skin lesions, the peripheral blood mononuclear cells (PBMC), and cell lines. EBV load was compared in PBMC and serum samples. Cytotoxic T cell (CTL) response in the lesions was detected by immunostaining using anti-TIA-1, granzyme B, and granulysin monoclonal antibodies. EBV-infected cells were detected by *in situ* hybridization using EBER1 probe in the lesional specimen. A reactivation marker, BZLF1 mRNA was present in most cases of severe HV lesions associated with systemic symptoms, whereas no reactivation was found in their PBMC. Although no reactivation occurred in typical HV lesions, the expression levels of LMP1 mRNA increased as compared with those in PBMC. A follow-up study showed that the expression levels of BZLF1 mRNA in the skin lesion and EBV load in not PBMC but serum increased upon exacerbation of the illness. We could induce EBV reactivation with TPA treatment in chronic active EBV infection related NK/T cell lines. These results indicate that EBV reactivation was induced in severe HV lesions, and that up-regulation of LMP1 mRNA occurred in typical HV lesions. Many CTLs carrying cytotoxic molecules were infiltrating in both typical and severe HV lesions, in which a small number of EBV-infected T-cells were present. Taken together, CTL response against the reactivation-related molecules may induce severe HV lesions associated with systemic symptoms. In typical HV lesions, CTL response might be evoked against the latency II-related molecules including LMP1.

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Cellular immune responses following cutaneous viral infection are negatively regulated by Langerhans cells

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Langerhans cells (LC) are a class of highly specialized dendritic cells (DC) uniquely present in epidermis, and constitute the first immunological barrier against pathogen insults. However, the classical paradigm that Ag-carrying LC activate naïve T cells following cutaneous immunization is hypothetical and lacks convincing experimental support. This is largely due to the difficulty of distinguishing the functions of multiple phenotypically overlapping DC subsets in skin and skin-draining lymph nodes (SDLN). Recently a novel LC conditional knock-out mouse model was developed. In this model, a diphtheria toxin receptor tagged with EGFP was expressed under the control of the langerin promoter (Lang-DTREGFP mice). Diphtheria toxin injection specifically depletes LC in epidermis and skin-draining LN. Taking advantage of this model, we investigated the role of LC in the adaptive immune response following epicutaneous infection with vaccinia virus (VV), a highly immunogenic viral infection model mimicking smallpox vaccination in human. We showed that the serum VV-specific IgG was comparable in the LC-depleted and control mice. However, LC depletion led to significantly enhanced proliferation and IFN-gamma production of Ag-specific T cells in SDLN as well as in spleen. There was no effect on homing receptor expression, since activated CD8 T cells expressed comparable level of skin-homing molecules in SDLN with or without LC. Consistent with the stronger T cell response, the cutaneous viral load was significantly lower in the LC-depleted mice than that of the controls. However, unexpectedly, the LC-depleted mice showed greater clinical cutaneous inflammation than the control mice. Taken together, these results suggest that epidermal LC negatively regulate, rather than enhance, Ag-specific T cell responses following cutaneous viral infection. This regulatory function of LC may be important to regulate the strength of the T cell mediated cutaneous immune response and maintain peripheral tolerance to cutaneous Ags.

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Investigation of the resident skin biome through isolation and identification of propionibacterium acnes bacteriophages

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Propionibacterium acnes is an important commensal of human skin, recognized as one key factor in the pathogenesis of acne vulgaris. Given that the skin biome is greatly influenced by the relationship between resident bacteria and their phage, we investigated microcomedones. Adhesive strips were applied to the nose of acne patients to obtain microcomedones, which were subsequently plated directly onto A-media plates. In one of eight samples, plaques were directly visualized and bacteriophage isolated. Twenty-five plaque isolates were observed, ten of which were selected, analyzed by PCR and partial sequence analysis. Several genes were chosen to identify similarities between isolates such as the major and minor tail regions, as well as other unique encoding regions including the portal, amidase, scaffold, and helicase regions. All ten phage isolates were found to be identical. This P. acnes bacteriophage was found to have an 85% similarity to the only other two characterized P. acnes bacteriophages, ATCC 29399-B1 and the PA6 genome. High similarity was seen in the major tail region providing evidence for a bacteriophage specific for P. acnes, while significant diversity was seen among other encoding regions including the tail protein and amidase regions. Through further identification and sequencing of P. acnes bacteriophages comes the potential for genetic manipulation of the host bacterium as well as the use of lytic portions as possible therapy for acne.

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Comparison of antibacterial activities of five medicinal herbs against the *in vitro* growth of bacterial skin pathogens

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Antibiotic resistance continues to be of considerable concern in the treatment of bacterial skin infections. This study investigated the antibacterial activities of five medicinal plants against the in vitro growth of four skin bacteria, namely Staphylococcus aureus, Staphylococcus epidermidis, Propionibacterium acnes and Pseudomonas aeruginosa. The organisms were selected because of their association with many primary cutaneous infections. The essential oils of the following herbs were examined: Ocimum basilicum (basil), Syzgium aromaticum (clove), Mentha x piperita (peppermint), Rosmarinus officinalis (rosemary) and Thymus vulgaris (thyme). The antibacterial activities of the essential oils were determined with the disk diffusion method using bacterial cell cultures of approximately 1.5 x 10^8 CFU/ml. The minimum inhibitory concentrations (MIC) of the essential oils were also determined. In order of decreasing potency, the zone of inhibition of each essential oil were as follows: thyme (30 mm), clove (12 mm), peppermint (10 mm), basil (9 mm) and rosemary (9 mm). Thyme oil was the only essential oil that inhibited the growth of all four bacteria. The results also showed that P. aeruginosa was most insensitive to the essential oils, while P. acnes was most sensitive. The results obtained from this study warrant further investigation into the identification of the active constituents, mechanisms of action and side effects of each essential oil in relation to the prevention and treatment of bacterial skin infections.

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Protective effect against UV-light-induced skin inflammation with topically applied platinum nanoparticles in mice

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Skin is a major target for toxic insult by a broad spectrum of ultraviolet (UV) radiation that is capable of altering its structure and function. It is thought that intracellular reactive oxygen species (ROS) play an important role in the UV-induced inflammatory responses in the skin. Metal nanoparticles have been developed to increase the catalytic activity of metals due to the large surface area of smaller particles. Since some noble metal nanoparticles are reducing catalysts, they may thus be applicable as antioxidants which reduce ROS in a living body. However, there are only a few studies reporting the antioxidant effects of platinum nanoparticles. Recently, we manufactured platinum nanoparticles protected with poly (acrylic acid) were manufactured by reduction with ethanol for 2 hr (PAA-Pt). The protective effect of topically applied PAA-Pt against fluoroquinolone photoallergy induced mice was investigated. Fluoroquinolone photoallergy is inducible in Balb/c mice, when they are sensitized by systemic administration of LFLX (2 mg/0.2ml, i.p) followed by 12 J/cm2 UVA irradiation of the shaved abdomen and challenged with systemic quinolone and UVA exposure of earlobes (20 J/cm2). After UVB exposure (0-25 mJ/cm2) of cultured keratinocytes, DCF-DA was used to detect ROS production in cells. We found that significant ear swelling was observed in the control mice, but this response was inhibited in the earlobes treated with PAA-Pt ointment prior to UVA irradiation. In addition, a marked increase in the production of ROS was observed in UVB-treated keratinocytes, while a decreased generation of oxidant was found in PAA-Pt treated cells. Taken together, these results indicate that topically applied PAA-Pt effectively protects against UV-radiation-induced photoallergy.

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Attenuation of sunburn reaction and UVB-induced oxidative DNA damage with no alterations in UVB-induced skin carcinogenesis in Nrf2 gene-deficient mice

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Ultraviolet (UV) radiation is an important environmental factor in the pathogenesis of skin aging and cancer. Many harmful effects of UV radiation are associated with generation of reactive oxygen species. Cellular antioxidants prevent the occurrence and reduce the severity of UV-induced photoaging and diseases of the skin. The transcription factor, Nrf2, and its negative regulator protein, Keap1, are central regulators of cellular antioxidant responses. We used nrf2-null mice to investigate the roles of the Nrf2–Keap1 system in protection of skin from harmful effects of UVB irradiation. A single irradiation with UVB induced stronger and longer-lasting sunburn reaction in nrf2-null mice. Histological changes, including epidermal necrosis, dermal edema, inflammatory cell infiltration, sunburn cell formation, TUNEL-positive apoptotic cell formation, and accumulation of oxidative DNA products such as 8-hydroxy-2′-deoxyguanosine after UVB irradiation were more prominent in nrf2-null mice. These findings indicate that the Nrf2–Keap1 pathway plays an important role in protection of the skin against acute UVB reactions, including cutaneous cell apoptosis and oxidative damage. However, there were no significant differences in skin carcinogenesis between nrf2-null and wild-type mice exposed to chronic UVB irradiation, suggesting that there is a complex and subtle balance between factors promoting and preventing photocarcinogenesis.

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Protective effect of the Baicalin against DNA damage induced by ultraviolet B irradiation to mouse epidermis

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Purpose: To investigate whether topical application of Baicalin affords protecting Balb/C mice epidermis from UVB-induced cyclobutane pyrimidine dimers (CPDs) formation and its underlying mechanisms. Methods: A DNA damage model of UVB irradiation-induced mice epidermis was established. The immunohistochemical staining, southwestern dot blotting were used for CPDs detection; western blotting was used for P53 detection; reverse transcription-polymerase chain reaction (RT-PCR) was used to detect the mRNA level of Bcl-2 and Bax; terminal deoxynucleotidyl transferase-mediated Dutp nick end labeling (TUNEL) was used to detect apoptotic cells. Results: Topical application of Baicalin on Balb/C mice skin significantly decreased the amount of epidermal CPDs 1,24 and 48 h after 180mJ/cm 2 of UVB irradiation as compared to untreated mice. UVB-induced apoptosis was less pronounced in Baicalin treated mice epidermis which was accompanied by less p53 accumulation and higher Bcl-2/Bax ratio compared to that of untreated mice. Conclusion: Taken together, these results suggest that topical Baicalin prevents DNA photo-damage. Baicalin is therefore a promising protective substance against UVB radiation.

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Study on the UVB Irradiation Induced Photo-damage and (–)-epigallocatechin-3-gallate Protection in BALB/C mouse skin

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Objective To investigate the pathological changes and the apoptosis of epidermal cells in the skin of BALB/C mouse irradiated by chronic ultraviolet B(UVB) and the photoprotective effects of topical application of (–)-epigallocatechin-3-gallate (EGCG) on the skin damaged by UVB. Methods BLAB/C mice were pretreated with EGCG daily then irradiated with different dosages of UVB.The skin samples were obtained after one month and the pathological changes were observed by paraffin section dyeing and the apoptotic cells were detected by TUNEL. Results There were obvious pathological changes in the skin of BALB/C mouse after chronic UVB irradiation, such as hyperkeratosis,acanthosis,sponge like edematization,sunburn cells occurring in the epidermis, oedema in the papillary layer of dermis,telangiectasis,inflammatory cell infiltrating and so on. The intervention of EGCG could reduce such changes induced by UVB irradiation. In addition, EGCG showed to promote apoptosis in chronic UVB irradiation group.Conclusion Chronic UVB irradiation causes noticeable pathological changes in BALB/C mouse skin and topical application of EGCG can protect BALB/C mouse skin from photodamage.EGCG could promote apoptosis after chronic low dosage of UVB irradiation.

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Crosstalk between keratinocytes and fibroblast on activation of UVB-induced MMP-1

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Previous studies have been authorized that alteration of dermal matrix is defined as a major reason of the skin aging initiated by ultraviolet (UV)-lights. On the process of dermal alteration, the effects generally have been focused on dermis and dermal fibroblasts. There is a little study demonstrating contribution of epidermis and keratinocytes to dermal alteration. On metabolism of collagen which is a main component in dermis, matrix metalloproteinases-1 (MMP-1) plays a central role. Because MMP-1 is the major collagenolytic enzyme against native and fibrillate type-Icollagen and also is upregulated in the UV irradiated skin. In this study, our purpose is to identify contribution of keratinocytes and fibroblasts to dermal alteration. Then, we focused on MMP-1 expression after UVB-irradiation in keratinocytes, fibroblasts, and also fibroblasts affected by UVB-irradiated keratinocytes. -Methods- To address the effects of keratinocytes on MMP-1 expression of fibroblasts, fibroblasts were cultured with conditioned medium prepared by UVB-irradiated keratinocytes. In addition, MMP-1 expressions of each cell type, karatinocyte and fibroblast, by UVB-irradiation were examined. MMP-1 expression was measured with the mRNA expression by RT-PCR and the protein level by Western blotting. -Results- It was found that UVB-irradiation to keratinocytes or fibroblasts showed increases of MMP-1 level, respectively. Then, their MMP-1s were predominantly pro-MMP-1 and were secreted as inactive form. On the other hand, the conditioned medium by UVB-irradiated keratinocytes was also strongly upregulated both of mRNA expression and MMP-1 protein of fibroblasts. Interestingly, its MMP-1 was shown as active form. The fact led to a following speculation; UVB-irradiated keratinocytes secreted some substance(s) which exert on fibroblasts to produce/secrete some proteinase(s) realizing activation of MMP-1. The study is a first report on crosstalk between keratinocytes and fibroblasts on activation of UVB-induced MMP-1.

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Photocontact allergic and phototoxic studies of chlorproethazine

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Neuriplege® cream was available as a non-prescription medication in France as a muscle relaxant, until its withdrawal from the market in January 2007. The active ingredient is chlorproethazine (CPE), a derivative of the phenothiazine chlorpromazine. It has been suggested that CPE be included in an upcoming European multi-centre photopatch test study due to prior reports of contact and photocontact allergy. Therefore, prior to its withdrawal from the market, we investigated the photocontact allergic and phototoxic potential of Neuriplege® cream and CPE by means of an *in-vitro* phototoxic study and volunteer photopatch testing. The *in-vitro* phototoxicity study was performed in HaCaT keratinocytes using a neutral red dye phototoxic assay (in triplicate). The concentration of CPE required to reduce dye uptake by 50% was 0.9µg/ml in the presence of 5 Jcm-2 UVA light and 11.9µg/ml without UVA light exposure, showing clear evidence of phototoxicity. A one hour incubation of keratinocytes with CPE was approximately 13 times as toxic to the cells in the presence of UVA light compared to incubation with the drug alone. Of 2 healthy volunteers photopatch tested to Neuriplege® cream, one developed a phototoxic reaction. These 2 volunteers were then photopatch tested to Neuriplege® and CPE, together with 7 additional healthy volunteers. Both of the initial study volunteers experienced a photocontact allergic reaction to Neuriplege® (as is) on re-exposure and one also reacted to 10% CPE in white soft paraffin. Of the 7 volunteers not previously exposed to Neuriplege® (as is), 5 developed phototoxic reactions, with similar but less pronounced phototoxic reactions seen when 10% CPE in white soft paraffin was used. This study demonstrates the strong phototoxic and photocontact allergic sensitisation potential of CPE in Neuriplege® cream. Its previous availability to consumers within the European Union means it is likely that many have been sensitised. Clinicians should be aware of this problem, as Neuriplege® cream is likely to remain in the possession of many patients in the European Union.

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The role of Thrombomodulin in UVB irradiated epidermal keratinocytes

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Thrombomodulin (TM), an endothelial cell surface anticoagulant glycoprotein, acts as a cofactor for protein C. TM has been also detected in epidermal keratinocytes, especially in Malpighian-layer keratinocyte, and has been recognized as a keratinocyte differentiation marker. It is recently reported that TM has cell protective effect. However, the role of TM expressed in the epidermal keratinocytes is not fully characterized. Epidermal keratinocytes are exposed to ultraviolet (UV) rays. High-dose UVB irradiation is known to induce apoptosis of keratinocytes. In the present study, we examined the relation between TM and UVB irradiation in immortalized human epidermal keratinocyte cell line, HaCaT. The mRNA of TM increased with 10 mJ/cm2 UVB and decreased with the irradiation of high (>25 mJ/cm2) UVB dose. TM expression decreased in 2 hours and consequently recovered in 16 hours after 10 mJ/cm2 UVB irradiation in HaCaT cells. The mRNA of TM was up-regulated within 1 hour after 10 mJ/cm2 UVB irradiation. 10 mJ/cm2 UVB induced p38MAPK activation, but not ERK, JNK. SB203580, a specific inhibitor of p38MAPK, inhibited UVB induced cAMP Response Element Binding Protein (CREB) activation and down-regulated TM mRNA level after 10 mJ/cm2 UVB exposure. TM siRNA treated HaCaT cells resulted in reducing cell viability after 10 mJ/cm2 UVB irradiation. In contrast, TM overexpression protects HaCaT cells against UVB. Thus, Our results suggest that p38MAPK-CREB signaling pathway by low-dose UVB induced TM expression and that TM plays important role to protect keratinocytes after low-dose UVB.

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Differential repair of UVB-induced cyclobutane pyrimidine dimers in cultured human skin cells and whole human skin

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Solar ultraviolet (UV) radiation is the most important environmental factor involved in the pathogenesis of skin cancers. UVB radiation, the most energetic part of the solar spectrum, has been identified as the most mutagenic and carcinogenic component. Absorption of UVB photons by DNA induces dimerization of adjacent pyrimidine bases that gives rise to two types of DNA damage, namely cyclobutane pyrimidine dimers (CPD) and pyrimidine (6-4) pyrimidone photoproducts (6-4PP). Although the effect of the chemical structure of CPD on their mutagenic potential has been clearly established, little is known on how the repair machinery is efficient towards the four CPD produced at TT, TC, CT and CC dinucleotides. By using a highly accurate and quantitative HPLC-mass spectrometry assay, we investigated the formation and repair of CPD and 6-4PP in primary cultures of human keratinocytes and fibroblasts as well as in whole human skin. Firstly, we observed that the yield of dimeric lesions was slightly higher in fibroblasts than in keratinocytes and in contrast, that the rate of global repair was higher in this last cell type. In the three types of samples, the repair of 6-4PP was found to be more efficient than that of CPD with no difference in the removal of the TT and TC derivatives. In contrast, a significant influence of the nature of the two modified pyrimidines was observed on the repair rate of CPD. The decreasing order of removal efficiency was the following: C<>T > C<>C > T<>C > T<>T. These data, together with the known intrinsic mutational properties of the lesions, would support the reported UV mutation spectra. A noticeable exception concerns CC dinucleotides that are mutational hotspots with an UV-specific CC to TT tandem mutation, although related bipyrimidine photoproducts are produced in low yields and efficiently repaired.

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UV and H2O2 activate AMPK cell signaling pathway in cultured human skin keratinocytes

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AMP-activated protein kinase or AMPK is an evolutionarily conserved sensor of cellular energy status, activated by a variety of cellular stresses that deplete ATP, and other stresses such as oxidative stresses. However the possible involvement of AMPK in UV and H2O2-induced skin aging has not been fully studied. In this study, we observed that UV and H2O2 induce AMPK activation in cultured human skin keratinocytes (HaCaT cells). UV and H2O2 also activate LKB1, one upstream signal of AMPK in an EGFR dependent manner. Using Compound C, a specific inhibitor of AMPK and AMPK specific siRNA knockdown as well as AMPK activator AICAR, we found that AMPK serves as a positive regulator for p38 and p53 (ser15) activation in HaCat cells in response to UV radiation or H2O2. We also found that AMPK serves as a negative feedback signal against UV-induced mTOR activation in a TSC2 dependent manner. Inhibiting mTOR and activating p53 and p38 may mediate AMPK's pro-apoptotic effect in UV or H2O2 treated cells. Furthermore, activation of AMPK also leads to phosphorylation of acetyl-CoA carboxylase or ACC, the pivotal enzyme of fatty acid synthesis, and PFK2, the key protein of glycolysis in UV-radiated cells, which may explain reduced adipogenesis in UV- or H2O2-treated 3T3-L1 adipocytes and HaCaT cells. Collectively, our results demonstrate that UV and H2O2 induce EGFR/LKB1/AMPK signal pathway in cultured skin keratinocytes, which plays important roles in UV- or H2O2-induced signal transduction and skin aging.

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mTOR activation protects against UVB-induced cell death in cultured skin keratinocytes and dendritic cells

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mTOR (mammalian target of rapamycin) serves as a signal integrator of several upstream signals, including growth factors, nutrients, energy levels, and stresses. We demonstrate in this study that UVB radiation induces mTOR activation in human skin keratinocytes (HaCaT cells) and both cultured mouse dendritic cells (XS 106 cell line) and mouse monocyte derived dendritic cells (MoDC). Using specific inhibitors and gene manipulation methods, we observed that UVB induces mTOR activation is dependent on EGFR-mediated AKT activation. We also found that TSC2/mTOR, downstream of AKT, is involved in UVB-induced S6K activation. UVB radiation also induces activation of AMPK (AMP-activated protein kinase) and p38 as well as its upstream signal LKB1. Our data suggests that LKB1/AMPK/p38 serves as a negative feedback pathway against UVB-induced mTOR activation. We conclude that mTOR activation, together with its upstream signals, serves as a novel survival signal against UVB-induced cell death.

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SIRT1 activation confers protection against UVB and H2O2-induced cell death in cultured skin keratinocytes

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SIRT1 is a member of a highly conserved gene family (sirtuins) encoding NAD+-dependent deacetylases, originally found to deacetylate histones leading to increased DNA stability and prolonged survival in yeast and higher organisms, including mammals. SIRT1 has been found to function as a deacetylase for numerous protein targets involved in many cellular pathways, including cellular stress responses, apoptosis, and axonal degeneration. In this study, we found that SIRT1 is expressed in cultured human skin keratinocytes (HaCaT cells). Both UV and H2O2, the major inducers of skin cell damage, down-regulate SIRT1 in a time and dose dependent manner. We observed that reactive oxygen species-mediated JNK activation is involved in this SIRT1 down-regulation. SIRT activator resveratrol protects against UV-and H2O2-induced cell death, while SIRT inhibitors sirtinol and nicotinamide enhance cell death. Activation of SIRT1 negatively regulates UV- and H2O2-induced p53 acetylation, since nicotinamide and sirtinol as well as SIRT1 siRNA enhances UV- and H2O2-induced p53 acetylation while SIRT1 activator resveratrol inhibits it. We also found that SIRT1 activation is involved in UV-induced AMPK and downstream ACC, PFK phosphorylation. Collectively, our data provide new insights into understanding of the molecular mechanisms of UV-induced skin aging, suggesting that SIRT1 activators such as resveratrol could serve as a new anti-skin aging agent.

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Extracellular matrix secreted by senescent fibroblasts induced by UVB promotes cell proliferation in HaCaT cells through PI3K/AKT and ERK signaling pathway

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Chronic exposure to solar ultraviolet radiation (UV) induces photoaging, and ultimately photocarcinogenesis. Senescent human skin fibroblasts (HSFs) in UVB stress-induced premature senescence (UVB-SIPS) share a similar extracellular matrix (ECM) phenotype with other types of senescent fibroblast. ECM from senescent fibroblasts induced by a variety of stresses has been shown to promote preneoplastic and neoplastic epithelial cell growth, a potential mechanism conferring to carcinogenesis. We undertook this study to explore whether the extracellular matrices from UVB-induced senescent fibroblast have any effect on the proliferation of HaCaT cells. The results showed that ECM secreted from HSFs in UVB-SIPS has 13.15% and 29.27% more stimulatory effect on proliferation than ECM secreted from presenescent HSFs and non-ECM, respectively. ECM from fibroblasts in UVB-SIPS activates FAK, ERK, and AKT in HaCaT cells. ERK and PI3K/AKT inhibitors inhibit ECM-induced ERK, AKT activation and cell proliferation. Cytochalasin D, a destructive agent of cytoskeleton, inhibits ECM-induced FAK activation and cell proliferation in HaCaT cells. Collectively, we conclude that ECM secreted from HSFs in UVB-SIPS promotes cell proliferation via ERK and PI3K/AKT pathway and modulation of FAK and cytoskeletal proteins in HaCaT cells. Pharmacological manipulation of those signaling components may lead to the prevention and treatment of skin cancer induced by chronic solar exposure.

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p53-related apoptosis resistance and tumor suppression activity in UVB-induced premature senescent human skin fibroblasts

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Exposure to solar UV irradiation leads to photoaging, immunosuppression, and ultimately carcinogenesis. Cellular senescence is thought to play an important role in tumor suppression and apoptosis resistance. However, the relationships among stress-induced premature senescence or SIPS, tumorigenesis and apoptosis induced by UVB remain unknown. We developed a model of UVB-induced premature senescence in human skin fibroblasts (HSFs). After repetitive five subcytotoxic exposures to UVB at the dose of 10mJ/cm2, biomarkers of senescence are markedly established: senescence-associated β-galactosidase (SA β-gal) activity, growth arrest, and overexpression of senescence-associated genes. First, there is an increase in the proportion of cells positive for SA β-gal activity. Second, there is a loss of replicative potential as assessed by MTT assay. FACS analysis shows that UVB-stressed HSFs are blocked mostly in G1 phase of the cell cycle. And replicative senescence (RS), protein expression of p53, p21WAF-1 and p16INK-4a are increased significantly. Third, the levels of the mRNA of three senescence-associated genes, fibronectin, osteonectin and SM22, are also increased. A real time PCR array to investigate mRNA expression of p53-related genes involved in growth arrest, apoptosis and tumorigenesis indicate that p53, p21, p19, Hdm2, and bax are up-regulated, and bcl, HIF-1α, VEGF are downregulated. Collectively, our data suggest that UVB-induced SIPS play an important role in p53-related apoptosis resistance and tumor suppression activity.

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The use of photoacoustics for distinction among melanin, hemoglobin, and exogenous pigment in skin lesions *in vivo*

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Accurate clinical characterization of pigmented lesions may provide a diagnostic challenge for the dermatologist. Pigment in the skin may arise from melanin, hemoglobin, or exogenous sources. We investigated the clinical use of photoacoustics, or laser-induced ultrasound, to rapidly characterize pigment *in vivo*. Twenty-six patients with clinically apparent melanocytic lesions (n=8), vascular lesions (n=8), or tattoos (n=10) were enrolled from July 2007 to September 2007 for clinical, photoacoustic, and histopathologic evaluation. After clinical evaluation, a photoacoustic signal was generated in each sample using a tunable laser system operating at 422 and 530 nm with a 5 ns pulse duration. The signals were detected by a miniature acoustic sensor embedded in the laser handpiece. We used the ratio of amplitudes of the photoacoustic signals at 422 and 530 nm to distinguish between pigments, with ratios above 2.0 classified as vascular and below 2.0 as melanotic. Incisional biopsies were subsequently performed of all melanocytic and vascular lesions. Clinical, photoacoustic, and histopathologic evaluations were then compared for congruence. Histology confirmed the clinical impression in all specimens. 87.5% (7/8) of both melanotic and vascular lesions were accurately characterized by the photoacoustic apparatus. Waveforms from exogenous pigment (tattoos) were present in both categories and their characterization did not reach statistical significance. The vascular and melanotic ratios were statistically significantly different with a p-value of 0.0011. These findings suggest that photoacoustics may be used to distinguish between melanocytic and vascular skin lesions *in vivo*, and point to potential clinical applications in the future.

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Experimental extracorporeal photopheresis inhibits contact hypersensitivity by 2 mechanisms: generation of interleukin-10 and induction of regulatory T cells

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We have developed a model of extracorporeal photopheresis (ECP) which utilizes contact hypersensitivity (CHS) as a disease model. Leukocytes were obtained from dinitrofluorobencene (DNFB)-sensitized mice and exposed to 8-methoxypsoralen and UVA (PUVA). Cells were injected i.v. into DNFB-sensitized mice. Ear challenge was suppressed in the recipients, indicating that ECP does not only, as shown previously, inhibit the induction but also the elicitation of CHS. When splenocytes obtained from these recipients 3 days after injection of PUVA-treated leukocytes were injected i.v. into naïve mice, sensitization was not induced. This indicated that ECP performed in sensitized mice induces regulatory T cells. Thus, we concluded that the inhibition of CHS was mediated by regulatory T cells (ECP-Treg). We now report that purified ECP-Treg suppress CHS only in naive but not in sensitized mice, indicating the ECP-Treg like UVB-induced Treg suppress only the induction but not the elicitation of CHS. This suggests that ECP may have at least 2 mechanisms of action. One which inhibits the elicitation of CHS and one that drives the generation of Tregs which in turn can inhibit CHS induction. Interleukin (IL)-10 is known to inhibit both the induction and the elicitation of CHS. Thus we analyzed serum obtained from mice after injection of either untreated or PUVA-exposed leukocytes. In mice which had received PUVA-exposed leukocytes significantly elevated IL-10 serum levels were found but not in mice which received untreated leukocytes. Thus we propose that the inhibition of the elicitation phase of CHS upon infusion of PUVA-treated leukocytes is a process which does not require Treg but may be mediated via enhanced IL-10. In parallel, but independently, Treg are induced as demonstrated by the adoptive transfer experiments. Together, this suggests that ECP inhibits both the induction and the elicitation phase of CHS and induces Treg, though most likely through different mechanisms.

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Differential effects of UVA and H2O2 on the expression of cell defence genes in human cutaneous fibroblasts, effects of chronic photo-exposure

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Fibroblasts are the main cell targets of sun exposure due the deep penetration of UVA into the dermis. Recently UVA radiations have been shown to induce not only oxidated bases due to the production of ROS but predominantly the formation of cyclobutane pyrimidine dimers. This suggests that an alternative mechanism is responsible for the adverse effects of UVA. To differentiate cellular response that can be attributed to pure oxidative stress and UVA we used a cDNA macroarray containing 82 genes related to antioxidant defences, DNA repair and extra-cellular matrix (ECM) regulation. Responses to acute in vitro stresses (UVA irradiation and H2O2 treatment) using fibroblasts prepared from skin biopsies taken from Sun-Protected and Sun-Exposed sites of 10 volunteers were studied. In both SE and SP cells: UVA induced the transcription genes involved in DNA repair (APEX, GTF2H1, POLD2, XRCC1), ECM regulation (MMP1 and MMP3) and anti-oxidant defence (SOD3 and SEPW1) whereas only 4 genes were induced by H2O2 (MMP1, MMP3, GPX1 and XRCC1). SE cells displayed specific responses toward UVA and H2O2 with the up-regulation of respectively (AOE372, HMOX1, HMOX2, SOD1, SEPW1, ERCC1, LIG4, TDGg/t, MDM2, SHC1) and (AOE372, SEPW1, ERCC1, LIG4, MPG, XPC, MMP2, FOSB). By contrast 8 genes were up-regulated in SP cells by UVA and only 2 by H2O2. Moreover, a delayed transcription response was observed in SP cells compared to SE cells in response to H2O2. In conclusion, transcripts involved in ECM regulation were induced independently of stress type and sun-exposure status of cells. UVA and H2O2 induced very different pattern of genes expression confirming that effects of UVA are not limited to oxidative stress. SE cells responded more rapidly to oxidative stress suggesting either an adaptation of skin chronically exposed to sunlight or a reaction toward a lack of antioxidant defences.

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DNA repair is inhibited by cyclosporin A but not everolimus in human cells

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Cyclosporin A (CsA) rather than immunosuppressive drugs like everolimus enhances UV-induced skin carcinogenesis. We measured post-UV survival of normal human GM00637 fibroblasts and AG10107 lymphoblasts in the presence of increasing CsA or everolimus concentrations by MTT. Both cell types showed a gradually decreased relative (excludes toxic effects) post-UV cell survival with increasing concentrations of CsA from 0.01 μM to 1.5 μM (e.g. AG10107: 24 % reduction of survival at 0.1 μM CsA vs. 0 μM CsA and 50 J/m2 UVC to cells). In contrast, cells treated with increasing amounts of everolimus (0.25 nM - 100 nM) showed unaltered relative post-UV cell survival. To assess the cellular repair capacity we cotransfected UV-irradiated firefly luciferase and control untreated renilla luciferase reporter plasmids into GM00637 and AG10107 cells. Incubation with increasing concentrations of CsA (0.1 μM - 0.35 μM) resulted in a significantly decreased relative repair capacity in a dose-dependent manner (i.e. AG10107: 15.2 % repair without CsA, 14 % with 0.1 μM CsA, and 7.9 % with 0.35 μM CsA; GM00637: 13 % repair without CsA, 10.6 % with 0.125 μM CsA, and 3 % with 0.2 μM CsA; $p<0.05$). In contrast, incubation of the cells with increasing amounts of everolimus (0.5 nM - 1000 nM) did not impair relative repair (i.e. ~16 % repair in AG10107 with and without everolimus). We also measured removal of UV-induced DNA damage using an ELISA and specific antibodies against CPD or 6-4PP lesions. Incubation of GM00637 and AG10107 cells with CsA led to a reduced DNA photoproduct removal after 24 h (e.g. 3 % vs. 32 % CPD in AG10107 at 0.5 μM CsA vs. 0 μM CsA and 5 % vs. 36 % CPD in GM00637 at 0.1 μM vs. 0 μM CsA; $p<0.05$). As we used therapeutically relevant doses for CsA (0.08 μM - 0.25 μM) and everolimus (3.1 nM - 8.3 nM), our results suggest that CsA but not everolimus impairs cellular repair resulting in skin cancer proneness which is consistent with clinical observations.

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Serotonin $5HT_{2A}$ and PAF receptor signalling has little effect on *cis*-urocanic acid induced production of immunomodulatory mediators in human keratinocytes

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Urocanic acid (UCA) is a major epidermal chromophore that undergoes *trans* to *cis* isomerisation following exposure to solar UV radiation. *Cis*-UCA suppresses cell-mediated immunity in mouse skin, but the molecular events are poorly understood. We previously demonstrated that *cis*-UCA increases the production of immunomodulatory mediators in primary human keratinocytes (HK). Recent studies suggest that *cis*-UCA binds to the serotonin $5HT_{2A}$ receptor and that $5HT_{2A}$ and platelet-activating factor (PAF) receptor antagonists block *cis*-UCA induced immune suppression in mouse skin. However, whether *cis*-UCA exerts effects via these receptors in HK remains unclear. In this study, we examined the involvement of $5HT_{2A}$ and PAF receptors in the ability of *cis*-UCA to stimulate the production of immunomodulatory mediators in HK. Using real-time RT-PCR, $5HT_{2A}$ receptor mRNA was not detected, yet PAF-R mRNA was constitutively expressed in HK (11 donors). Treatment of HK with *cis*-UCA increased prostaglandin E_2 (PGE_2), IL-6, IL-8 and TNFα secretion while 5HT treatment only stimulated IL-6 production (~4.5 times higher than control). Pretreatment with a non-specific 5HT receptor antagonist partially inhibited the IL-6 increase by 5HT, but had no inhibitory effect on mediator production by *cis*-UCA. Intracellular calcium mobilization studies using the human epithelial line KB transduced with the PAF-R demonstrated that treatment with *cis*-UCA but not *trans*-UCA or 5HT resulted in a weak but reproducible Ca^{2+} flux (but not in vector only transduced KB cells). Radioligand binding studies indicated that *cis*-UCA did not bind to PAF-R. Moreover, *cis*-UCA exerted only minimal effects on PGE_2 production in PAF-R-expressing KB cells. Our results suggest that $5HT_{2A}$ and PAF receptor signalling do not appear to be involved in *cis*-UCA-mediated production of immunomodulatory mediators in HK.

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A study of the relationship between the photo-aging dependent T414G mitochondrial DNA mutation with oxidative stress production and senescence in human skin fibroblasts

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Support has grown for the free-radical theory of ageing, and it is now widely accepted that oxidative damage to cellular constituents is an important component of age-dependent decline. Mutations in the human mitochondrial genome (mtDNA) are thought to be one of the causes of age-dependent cellular decline through their detrimental effects on respiration or reactive oxygen species (ROS) production. However for many mutations this link has not been clearly established. We have recently shown that a T414G mutation, within the control region of mtDNA, accumulates in both normal and photo-aged human skin (n=108)(Aging Cell, 2007, Aug;6(4):557-64). Therefore, the aim of the present study was to investigate the phenotypic importance of the T414 mutation in human skin. We observed a differential behaviour of the T414G mutation during repeated long term culture of dermal skin fibroblasts from elderly individuals. This implies the absence of consistent selection against the mutation. To further investigate this, we used flow cytometry cell sorting by autofluorescence to separate the cells based on their degree of senescence (confirmed by β-galactosidase activity, paired t-test $P<0.01$). We showed up to a 7-fold (t-test, $P<0.01$) greater redox probe oxidation (using MitoSOX (mitochondrial specific probe) and DHR123) in the prematurely senescent cells within the proliferating population. In addition using real time PCR assays specifically developed for this study, we observed a higher mtDNA copy number and increased mtDNA damage (strand break assay) in the prematurely senescent cells (t-test, $P<0.01$). A comparison of these parameters with the level of the T414G mutation did not reveal a correlation with any increased or decreased mtDNA copy number, markers of cellular ageing or ROS production. In conclusion, although we cannot exclude the possibility of a very subtle effect on respiration, the current data supports a neutral role of the T414G mutation on ROS production and the onset of cellular senescence in ageing skin.

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Intervention effects of epigallocatechin-3-gallate on photoproducts induced by ultraviolet B in primary human melanocyte

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To investigate the production and clearance of photoproducts induced by ultraviolet B irradiation and the intervention effects of epigallocatechin-3-gallate (EGCG) on Human Primary Melanocyte. Melanocytes were separated from foreskin and cultured with M-254 and HMGS-2 medium. The proliferation rates of melanocytes were detected when cells had been incubated with 10, 20, 40, 80 and 100μg/mL EGCG for 72 h, respectively. The formation of cyclobutane pyrimidine dimers (CPDs) in melanocytes was detected by immunocytochemistry. The production and clearance of CPDs were examined by immunodotblot assay at 0.5 and 24 hours after treatment by UVB and EGCG. It was found that low concentration of EGCG (10-20μg/mL) promoted the proliferation of cultured human melanocyte($P<0.05$). The production of UVB-induced photoproducts was quick, while the clearance was slow. 10,20μg/mL EGCG can facilitate the clearance of photoproducts ($P<0.05$), but had no effect on the formation. We concluded that EGCG can facilitate the clearance of UVB-induced photoproducts in melanocytes.

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Infrared irradiation reduces UVB-induced apoptosis via reduction of DNA damage and regulation of apoptosis-related proteins

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Infrared radiation (IR), a natural component of the solar spectrum, is increasingly used for medical, cosmetic, and wellness purposes. In this setting, artificial IR exposures are quite frequently combined with UV irradiations, e.g. for tanning purposes. The impact of IR on UV-induced carcinogenesis is still unclear. Hence we investigated the interplay between IR-A (760-1400 nm) and UVB with regard to UVB-induced DNA damage and apoptosis. Primary murine keratinocytes were treated with IR-A and 3 h later with UVB. Cells pretreated with IR-A showed a significantly lower apoptotic rate compared to keratinocytes exposed to UVB alone. The same effect was observed *in vivo* when sunburn cells were counted in the epidermis of C57BL/6 mice. Since UV-induced DNA damage is a major molecular trigger for UV-induced apoptosis, we evaluated the amount of thymine dimers *in vitro* and *in vivo*. 16 h after UVB we observed remarkably less thymine dimers in keratinocytes pretreated with IR-A 3 h before UVB than in UVB only treated cells. Shuttle vector assays demonstrated that the reduction of UV-induced DNA damage by IR-A pretreatment may be mediated by enhanced DNA repair. The reducing effect of IR-A on the formation of thymine dimers was not observed in nucleotide excision repair (NER) deficient XPA knock out mice, implying that IR-A pretreatment might induce NER. Intracellular FACS analysis revealed that UVB-induced down-regulation of the anti-apoptotic proteins FLIPL and BCL-XL was prevented by pretreatment with IR-A, whereas the pro-apoptotic protein BAX was downregulated by IR-A alone. Together, these data indicate that IR reduces UV-induced apoptosis both *in vivo* and *in vitro* via the reduction of DNA damage and the induction of anti-apoptotic proteins. The anti-apoptotic effects of IR could support the survival of UV-damaged cells and thus enhance carcinogenesis. Since, however, IR reduces UV-induced DNA damage, the balance between these two effects may be important. Thus, *in vivo* photocarcinogenesis studies are required.

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Photo-protective effects of anti-kertinocyte IgG antibodies in vitiligo patients involve NGF-Trk-Bcl2 pathway

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The pathogenesis of vitiligo remains obscure. Recent evidences indicate that dysfunctional keratinocytes may play a regulatory role in the activation and progression of the disease. We have previously demonstrated the presence of anti-keratinocyte antibodies in the sera of active vitiligo patients. The functional roles of these antibodies remain unclear. Another intriguing phenomenon noted in vitiligo patients is that while keratinocytes in depigmented skin are more vulnerable to trauma, the photo-related damages are not increased in vitiligo skin. The present study seeks to investigate the functional role of anti-keratinocyte IgG antibodies in vitiligo patients in terms of UVB protection. IgG antibodies were obtained from patients with active vitiligo. The titers of anti-keratinocyte antibodies were determined. Cultured keratinocytes were treated with IgG antibodies from vitiligo patients and healthy controls, with or without UVB irradiation. The changes in cytokine profiles, the effects on cellular survival, and the involved molecular pathways were evaluated. The antibodies from vitiligo patients elicit a UVB dose-dependent nerve growth factor release from keratinocytes. In addition, these antibodies also exhibited a stimulatory effect on Bcl-2 expression and an anti-apoptotic effect on UVB-treated cells. Addition of nerve growth factor blocking antibodies abrogated the protective effects vitiligo IgG on UVB-treated keratinocytes. The expression of tyrosine kinase receptor was significantly increased by vitiligo IgG following UVB treatment. In summary, the protective effects of anti-keratinocyte antibodies on UVB-irradiated keratinocytes involve the modulation of NGF-Trk-Bcl2 pathway. These results provide an explanation for lack of increased UVB damages in patients with vitiligo.

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PUVA suppresses experimental autoimmune encephalomyelitis by preventing the pertussis toxin-induced reduction of regulatory T cells

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CD4(+)CD25(+) regulatory T cells (Treg) expressing the lineage marker Foxp3 are key players in immune response regulation. Accumulating evidence strongly suggests that CD4(+)CD25(+) Treg have a pivotal role in suppressing the development of autoimmune diseases. Pertussis toxin (PTx) is a bacterial toxin used to enhance the severity of experimental autoimmune diseases such as experimental autoimmune encephalomyelitis (EAE). *In vivo* administration of PTx promotes Th-17 cell generation and reduces the number and function of CD4(+)CD25(+)FoxP3(+) Treg. We analyzed the effect of psoralen + UVA irradiation (PUVA) on the PTx-induced downregulation of Treg. Mice injected intraperitoneally with 400 ng PTx on days 0 and 1 were treated with psoralen and UVA1 exposure on days 2 and 3. At 24 h after the last irradiation, mice were killed and the spleens removed. A single suspension of splenocytes was stained with anti-mouse CD4, CD25 and FoxP3 antibodies. CD4(+)CD25(+)FoxP3(+) Treg in PTx-treated mice were significantly reduced compared to naïve mice, whereas Treg number in PUVA-treated mice was unchanged. Next, we evaluated the effect of PUVA on EAE induction in an EAE mouse model. Mice were immunized subcutaneously with 100 μg myelin oligodendrocyte glycoprotein (MOG)35-55 on day 0 and injected intraperitoneally with 200 ng PTx on days 0 and 2. At 30 min after intraperitoneal injection with 8-MOP, the shaved backs of the mice were exposed to 10 J/cm2 UVA1 on days 3 and 4. After restimulation with MOG35-55 on day 7, clinical signs were monitored every other day by assessing the EAE score. The mean EAE score in PUVA-treated mice was significantly decreased compared to control MOG-immunized mice. Together these data suggest that PUVA suppresses the induction of EAE, presumably by preventing the PTx-induced reduction of Treg. These data are the first to illustrate the potential therapeutic value of PUVA in various autoimmune diseases.

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Enhancement of the photodynamic effects on human oral squamous cell carcinoma cell lines by treatment with calcipotriol

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5-aminolaevulinic acid (ALA)-based photodynamic therapy (PDT) for patients with skin and oral diseases is a highly sophisticated procedure, but the incidence of disease recurrence after treatment with ALA-based PDT is somewhat alarming. Calcipotriol, an analogue of 1,25-dihydroxyvitamin D3 (1,25(OH)2D3), has been reported to regulate the proliferation and differentiation of keratinocytes. In order to obtain even greater efficacy of ALA-based PDT, we investigated the synergistic effects of calcipotriol as an adjunct to ALA-based PDT for human oral squamous cell carcinoma (SCC) cell lines. Intracellular protoporphyrin IX (PpIX) converted from exogenous ALA in SCC cell lines treated with/without calcipotriol was measured by a fluorescencemeter. Then, the *in vitro* effects of calcipotriol, the cyclooxygenase (COX)-2 selective inhibitor (nimesulide), ALA-based PDT, and their combination on two SCC cell lines, HSC-2 (a COX-2 high expresser) and HSC-4 (a COX-2 non-expresser), were determined by MTT assay and double-staining for annexin V and propidium iodide. The concentration of intracellular PpIX was increased in four of the eight SCC cell lines (50%) treated with calcipotriol. The greatest alteration of intracellular PpIX was found in HSC-4 (1.9-fold). The combination of calcipotriol and ALA-based PDT remarkably inhibited cellular proliferation and induced cellular death of both HSC-2 and HSC-4. Whereas, this morphological damage was more serious in HSC-4 than in HSC-2. Furthermore, these effects were almost equivalent to the synergistic effect of the combination of nimesulide and ALA-based PDT on HSC-2. The present study suggests that treatment with calcipotriol enhances the photodynamic effects on SCC via the accumulation of exogenous ALA-dependent PpIX.

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Dichotomous effect of ultraviolet B radiation on the expression of corneodesmosomal enzymes in epidermal keratinocytes

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The mechanism by which exposure of the skin to ultraviolet B (UVB) radiation induces desquamation of the stratum corneum is unknown. The stratum corneum chymotryptic enzyme (SCCE) and the stratum corneum tryptic enzyme (SCTE) are serine proteases of the kallikrein family that are encoded by the KLK7 and KLK5 genes, respectively. They might play a role in desquamation by degrading corneodesmosomal proteins in the granular layer of the epidermis. The activities of these proteases are regulated by lympho-ephithelial Kazal-type related inhibitor (LEKTI), which is a serine protease inhibitor expressed in the epidermis, encoded by the SPINK5 (serine protease inhibitor Kazal-type 5) gene. To clarify the role of these molecules in UVB-induced desquamation of the stratum corneum, we examined the effect of UVB irradition on the expression of LEKTI (SPINK5), SCTE (KLK5) and SCCE (KLK7) in normal human epidermal keratinocytes and HaCaT cells. UVB irradiation at 30mJ/cm2 significantly reduced the expression of SPINK5, and increased the expression of KLK5 and KLK7, as assessed by real time RT-PCR. UVB irradiation also significantly reduced the expression of LEKTI and increased the expression of SCTE by Western blotting. In addition, UVB irradiation (50 mJ/cm2) of a reconstructed three-dimensional epidermal model reduced the expression of LEKTI and increased the expression of SCTE and SCCE by immunofluorescence analysis. Protease activity, as assessed by in situ zymography, was enhanced in the epidermis following exposure to the same dose of UVB irradiation compared with sham-irradiated epidermis. These results suggest that reduced expression of LEKTI and increased expression of SCCE and SCTE after UVB irradiation in human epidermal keratinocytes may attribute to desquamation of the stratum corneum.

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Intracellular degradation of collagen and elastin by cathepsin K: A novel mechanism of photoaging

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Cathepsin K (catK) is an intracellular lysosomal protease with strong collagenolytic and elastolytic activities, well known for its bone-degrading properties in osteoclasts. It was recently described to be expressed in dermal fibroblasts in scars, which suggests that it also contributes to the homeostasis of the extracellular matrix (ECM) in the skin. UV-induced degradation of collagen and elastin is a hallmark of photoaging and is usually attributed to proteases that are secreted into the extracellular space. To study a possible role of intracellular ECM degradation in photoaging, we examined expression of catK after UV-exposures and internalization and degradation of collagen and elastin in dermal fibroblasts. UVA, but not UVB or ionizing radiation strongly induced catK in a dose- (100 – 500 kJ/m2) and time- (1 – 4 days) dependent manner. This was observed by immunoblotting in cultured fibroblasts and *in-vivo*, by immunostaining of explant skin. No catK expression was seen in sham-irradiated skin. Prominent internalization of FITC-labeled collagen I and IV and elastin was observed in 99 % of cultured fibroblasts. Co-staining of internalized collagen and elastin with the lysosomal protein Lamp1 demonstrated that internalized collagen and elastin localize to lysosomes within 16 hours. At earlier time points (3 – 10 hours), internalized collagen was found mainly in early, Lamp1-negative endosomes. Incubation of fibroblasts with heavily-labeled elastin that fluoresces only upon degradation revealed intracellular, lysosomal elastin degradation in 77 % of cells. Pre-incubation of cells with a highly specific catK inhibitor reduced this number to 24 % (p=0.006). These data show that dermal fibroblasts do internalize and degrade ECM proteins in lysosomes and that this degradation is mediated at least in part by catK. CatK upregulation by UVA exposure strongly suggests a role of these intracellular processes in photoaging and that extracellular ECM degradation is not the only mechanism by which ECM proteins are degraded after UV-exposure.

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Drinking green tea inhibits photocarcinogenesis via rapid repair of damaged DNA (thymine dimers) through enhancement of nucleotide excision repair in mice

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Administration of green tea polyphenols (GTPs) in drinking water inhibits photocarcinogenesis in mice. UV-induced cyclobutane pyrimidine dimers (CPDs) have been implicated in skin cancer initiation. Oral administration of GTPs increases the levels of interleukin (IL)-12 in UV-exposed mouse skin and draining lymph nodes. As IL-12 has been shown to have DNA repair ability, we determined whether chemopreventive effect of drinking GTPs is mediated, at least in part, through IL-12-dependent DNA repair. To investigate this possibility, we used IL-12 knockout [KO] and nucleotide excision repair [NER]-deficient mice. Employing photocarcinogenesis protocol, we found that oral administration of GTPs (0.2%, w/v) inhibits photocarcinogenesis in wild-type mice in terms of tumor incidence and tumor multiplicity, but did not prevent it in IL-12 KO mice. UVB-induced CPD+ cells were significantly reduced or repaired more rapidly in the skin of wild-type mice treated with GTPs than untreated control mice. In contrast, this effect of GTPs was not seen in IL-12 KO mice. However, the numbers of UVB-induced CPD+ cells were significantly reduced in IL-12 KO mice when GTPs-treated mice were s.c. injected with rIL-12 on UV-exposed site. Real-time PCR analysis revealed that the levels of mRNA expression of NER genes [XPC, XPF, XPG, DDB1 and RPA (p14)] in GTPs-treated UV-exposed wild-types were higher than non-GTP-treated UV-exposed wild-types, suggest the role of NER gene expression in DNA repair in GTP-treated mice. This effect of GTPs was not observed in IL-12 KO mice. The treatment of NER-proficient mice with GTPs promoted repair of UVB-induced CPDs but not in NER-deficient mice, suggesting that the nucleotide excision repair mechanism is involved in GTPs-mediated DNA repair. These results indicate that drinking green tea inhibits photocarcinogenesis in mice through IL-12-dependent and NER-mediated DNA repair.

1200

Aberrant DNA methylation patterns lead to the transcriptional silencing of the tumor suppressor genes in UV-exposed skin and tumors

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Cancer epigenetics deal with important processes like DNA methylation and histone modification patterns that can either activate oncogenes or inactivate tumor suppressor genes, the crucial steps in tumor development. Since methylation at critical CG sequences in the genome serves as a signal for silencing tumor suppressor genes, we sought to determine the DNA methylation pattern in the UV-exposed skin and tumors. For this purpose SKH-1 hairless mice were subjected to photocarcinogenesis protocol. When tumor yield was stabilized, skin and tumor samples collected for the analysis. The levels of various epigenetic biomarkers were determined by immunostaining, western blot analysis and real-time PCR. Hypermethylation pattern of DNA was found in UV-exposed skin compared to non-UV-exposed skin. The expression levels of the various DNA methyltransferases, e.g., Dnmt1, Dnmt3a and Dnmt3b, were 2 to 4- folds higher in UV-exposed skin than normal skin. Similar but greater DNA hypermethylation pattern was observed in UV-induced skin tumors. The hypermethylation of DNA in UV-exposed skin may lead to silencing of tumor suppressor genes, thereby contributing to the risk of skin cancer. We also have analyzed the methylation status of the various tumor suppressor genes, like P16INK4a, RASSF1A and CDH1, on UVB exposure through methylation specific PCR and the relative repression levels of their mRNA using real-time PCR, which indicated that the levels of these tumor suppressor genes were downregulated in UV-exposed skin and tumors on hypermethylation. The increased expression of the MeCP2, a methyl binding protein, and histone deacetylase 1 (HDAC 1), in UV-exposed skin shows that the aberrant hypermethylation of DNA recruited the HDAC 1 to the methylated CpGs through MeCP2 which had caused the repressive effect of the genes either by their direct association with DNA or by complexing with HDAC 1. These data suggest that UV-induced hypermethylation in DNA may have a role in skin cancer development.

1201

The effects of intense pulsed light and 5-aminolevulinic acid on the B16 melanoma cells

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Intense pulsed light (IPL) are a well-established, nonablative therapy for skin pigmentation disorders. The adjunctive use of 5-aminolevulinic acid (5-ALA) with IPL enhanced the effects, but the effects and mechanisms of IPL on the pigment cells are not clear. Our study was to investigate the effects of IPL plus 5-ALA on the proliferation, melanogenesis and tyrosinase activity of cultured B16 murine melanoma cells. B16 cells were pretreated with or without ALA and irradiated with IPL (20, 30, 40 J/cm2), also the negative control group and the positive control UVA irradiation group (3, 4, 5 J/cm2) were designed. The MTT spectrophotometry and NaOH assay were used to determine the proliferation and melanogenesis of the B16 cells at 24h, 48h, 3d, 4d, and 5d after irradiation, also the tyrosinase activity was detected. The cell yielding in UVA group increased significantly($P<0.05$) compared to negative control group at 24h, 48h, 3d, 4d, and 5d after UVA irradiation(3, 4J/cm2). IPL (20, 30, 40 J/cm2) or IPL plus ALA did not have any significant effects on cell proliferation. The melanogenesis and tyrosinase activity in UVA group were significantly ($p<0.01$) higher than the negative control group at 24h, 48h, 3d, 4d and 5d after UVA irradiation (3, 4, 5 J/cm2), and they were dose-dependent. The melanogenesis and tyrosinase activity in IPL or IPL plus ALA group were significantly ($p<0.01$) lower than the negative control group at 48h after IPL irradiation, and the melanogenesis and tyrosinase activity in IPL plus ALA group were lower than IPL alone. We demonstrated that UVA could stimulate the proliferation of B16 cells, increase the melanogenesis and tyrosinase activity of the cells. IPL or IPL plus ALA have inhibitory effects on melanogenesis and tyrosinase activity without any influence on cell proliferation in B16 cells. IPL plus ALA had stronger effect than IPL alone. Our data provide the evidence that IPL could be considered as a safe and effective nonablative therapy for skin pigmentation disorders, besides 5-ALA could enhance the effects.

1202

Psoralen+UVA (PUVA) lowers IL-23 levels and clears psoriasis-like skin lesions in TGF-beta1 transgenic mice

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Although psoralen+UVA (PUVA) has been used for a long time as a highly effective treatment for psoriasis, little is known how it exactly acts. We employed transgenic mice expressing human wild-type TGF-beta1 to elucidate the mode of action of PUVA. These mice develop skin lesions with epidermal hyperplasia and inflammatory infiltration, resembling features of psoriasis. K5.TGF-beta1 transgenic Hsd:ICR(CD-1R) mice (age >14wks) were treated on their shaved backs with topical PUVA (10mcg 8-MOP in 100mcl ethanol 15min before 2.5 or 5 J/cm^2 UVA) twice a week for a total of 4 weeks. During the 4-week study period, the specific mouse psoriasis severity score (MPSC) (composed of a rating from 0 to 3 for erythema, infiltration, and scaling) increased in untreated control mice from a mean±SEM of 3.14±0.55 to 4.83±0.43. Both doses of PUVA exhibited a dramatic therapeutic effect by reducing the MPSC to 0.95±0.23 and 1.64±0.24, respectively (ANOVA, $p < 0.05$), whereas UVA alone had no significant effect. This effect of PUVA was paralleled by microscopic alterations, including significant reduction of epidermal hyperplasia and inflammatory infiltrate within the dermis. Importantly, PUVA also down-regulated elevated serum levels of IL-23p19. In addition, there was a trend that PUVA prolonged survival of the K5.TGF-beta1 transgenic mice. The effect of PUVA on IL-23 linked to an improved skin status in the mice is intriguing because anti-IL-12/IL-23 antibody treatment is in the pipeline for approval for psoriasis in humans. Thus, the K5.TGF-beta1 transgenic mouse model may offer the opportunity to learn more about the therapeutic mechanisms of PUVA (including effects on Tregs and Th-17 cells), which may ultimately help to improve the safety and/or to develop other treatments acting like PUVA but possibly having less side effects.

1203

Efficacy of Extracorporeal Photopheresis (ECP) monotherapy in the treatment of Cutaneous T Cell Lymphoma (CTCL)

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A multi-center, open label, single arm clinical trial previously demonstrated the safety and efficacy of ECP as a monotherapy in the treatment of patients with advanced/refractory CTCL (Edelson, et al. 1987). We present a long-term, secondary analysis of these patients in order to further evaluate clinical outcomes and predictors of response for ECP in Mycosis Fungoides (MF) and Sezary Syndrome (SS). 39 patients (pts) who met eligibility criteria were included in the secondary efficacy analysis as the intent-to-treat (ITT) population. 31 pts with generalized erythroderma (GE) and 8 pts with extensive patch plaque (EPP) were treated with standard ECP on 2 consecutive days every 4-5 weeks for 3 months. Skin improvement was calculated by comparing baseline skin score to skin scores on all subsequent treatment dates. The median follow-up of 39 ITT pts was 4 years (range 9 days-7.8 yrs). 29 pts (74%) achieved at least a >50% improvement in skin score, and 16 pts (41%) achieved >90% improvement on ECP. Patients received a median of 12 (range 4-65) or 30.0 (range 12-109) individual ECP treatments to achieve a >50% or >90% response, respectively. The mean times to reach a >50% or >90% response were 8.4 +/- 6 months (median=6.5) or 25.3 +/- 14.9 mos (median=19.6), respectively. The mean duration of a >50% response was 32.5 > 28.6 mos, which included a median of 20 (range 0-153) ECP treatments. Median survival from date of diagnosis was 10.6 yrs and from date of first ECP treatment was 5.4 yrs. In this long-term, follow-up analysis, ECP monotherapy was associated with a significant and durable improvement in skin score in the majority of patients with MF and SS.

1204

Thioredoxin upregulation by 5-aminolaevulinic acid-based photodynamic therapy in human skin squamous cell carcinoma cell line

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5-aminolaevulinic acid-based photodynamic therapy (ALA-PDT) is widely performed in the clinical setting for superficial skin cancers, giving favourable results, but residual tumor and recurrence occasionally occur. Thioredoxin is a common antioxidant that suppresses apoptosis and facilitates cell growth. We investigated the expression of thioredoxin following ALA-PDT in human skin squamous cell carcinoma cell line, HSC-5. ALA-PDT was performed in HSC-5 cells using low-dose (5 J/cm2, 100 mW/cm2) or high-dose (30 J/cm2, 100 mW/cm2) irradiation, and the expression of thioredoxin was measured by Western blotting. An MTT assay was used to assess cell growth following low-dose of multiple irradiations. Cell death was examined by Western blotting for caspase-3 and PARP. Immunofluorescence double-staining using annexin V and propidium iodine was also performed. Expression of Thioredoxin was only observed following low-dose exposure ALA-PDT. Multiple low-dose exposure ALA-PDT significantly proliferated cell growth. With high-dose exposure ALA-PDT, caspase-3 and PARP expression were seen, and cell death due to apoptosis and/or necrosis was observed, but thioredoxin was barely detected. In conclusion, low-dose exposure ALA-PDT increased the expression of thioredoxin and facilitated the growth of HSC-5 cells.

1205

Anti-apoptotic role of S100A8 in X-ray irradiated keratinocytes

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Ionizing radiation is used to treat a lot of cancers, however, it also produced unwanted side effect on normal tissues, such as radiodermatitis. We previously established an animal model for radiodermatitis, and identified many of radiation-induced genes by cDNA microarray. Of the candidates, we chose S100A8 gene for a further study. The aim of this study is to investigate the functional role of S100A8 in X-ray irradiated keratinocytes. RT-PCR and immunohistochemistry were performed to demonstrate the S100A8 induction by X-ray irradiation. HaCaT keratinocytes were transduced with the recombinant adenovirus expressing GFP-S100A8, and then effects on cell cycle and apoptosis were analyzed using flow cytometry and Western blot. X-ray irradiation markedly induced S100A8 expression in the hyperplastic epidermis of mouse. Overexpression of S100A8 by adenoviral transduction led to the enhancement of cell proliferation in the absence and/or presence of X-ray irradiation, as compared with Ad/GFP control group. Furthermore, overexpression of S100A8 significantly protected the X-ray-induced apoptosis. These results suggest that S100A8 have an anti-apoptotic role in X-ray irradiated keratinocytes.

1206

Interleukin-23 reduces UV-induced DNA damage and thereby photoimmunosuppression via induction of DNA repair.

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DNA damage induced by ultraviolet radiation (UV) can result in mutations and subsequently in development of skin cancer. However, the vast majority of UV-induced DNA lesions is removed by the nucleotide excision repair (NER). Induction of apoptosis by UV represents an additional protective mechanism since it eliminates cells which still harbor considerable amounts of DNA damage. The immunomodulatory cytokine interleukin (IL)-12 was shown to reduce UV-induced DNA damage, presumably via induction of NER, and thereby to prevent photoimmunosuppression. IL-23 is a related cytokine since it shares the same p40 chain. We studied whether IL-23 exerts not only structural, but also functional similarity to IL-12. In earlier experiments we showed that IL-23 reduces UV-induced apoptosis of keratinocytes and supports long term survival. Subcutaneous injection of IL-23 into wild type (WT) mice resulted in a remarkable reduction of the number of apoptotic keratinocytes (sunburn cells). Accordingly, significantly reduced UV-induced DNA damage was found in the epidermis upon injection of IL-23. This was not observed in XPA-/- mice which are deficient in NER, suggesting that IL-23, similarly to IL-12, reduces DNA damage via affecting DNA repair. UV-induced DNA damage has been recognized as the major molecular mediator for photoimmunosuppression. Suppression of induction of contact hypersensitivity by UV was prevented upon injection of IL-23 before UV exposure in WT but not in XPA-/- mice, indicating that IL-23 requires functional NER to prevent UV-induced immunosupppression. Taken together, IL-23 can be added to the list of cytokines which exhibit the capacity to remove UV-induced DNA damage presumably via modulation of DNA repair.

1207

Use of skin organ culture to analyze relationships between antioxidant capacity and UVB-induced inflammation

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UVB irradiation increases skin's reactive oxygen species (ROS) and causes DNA/protein/lipids damage, leading to inflammation and photoaging. Limited information is available, however, on the mechanisms that connect oxidative stress with cytokine secretion and inflammatory response in skin. In this work we used skin organ culture to investigate the effects of UV irradiation on oxidative status and on biomarkers of stress and inflammation. 0.5x0.5 cm skin pieces were incubated in DMEM, epidermal side air exposed, and irradiated with UVB (200mJ/cm^2). At various times epidermis and cell medium were analyzed. Total antioxidant capacity was quantified by ferric reducing antioxidant power determination. Uric acid and ascorbic acid levels were determined by HPLC. Protein biomarkers, cytokines and enzymes were analyzed by immuno-assays and by ELISA. Our results show that skin antioxidants decreased 0.5 hr after UVB exposure in parallel to increase in ROS quantities. At 24 hr an increase in apoptotic response (caspase 3 activity) and no change in cell viability (mitochondrial activity) was noted. The MMP1 activity, collagen degradation products and the secretion of TNFα, IL-1β, IL-6 and IL-10 were also markedly increased. Thus demonstrating the development of photoaging and inflammation. Suprisingly, the levels of inducible nitric oxide synthetase (iNOS) were lower 24-72 hr after irradiation, compared to controls. In conclusions, the skin model system responded to UVB by: a. Induction of ROS species production in correlation to reduction of the antioxidants. b. Initiation of stress response and protein damage. c. Apoptosis, cell damage and secretion of cytokines was increased at 24 hr. d. The reduction of iNOS expression raises the possibility that alternative mediators may promote the inflammatory response in UVB-irradiated skin.

1208

Characterization of DNA repair phenotypes from different repair-deficient cell lines with an *in vitro* miniaturized test

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DNA repair mechanisms constitute major defences against agents that cause cancer, degenerative diseases and aging. Xeroderma pigmentosum and Cockayne syndrome are two important DNA repair diseases. The skin of these patients is highly sensitive to UV irradiation. In order to investigate Nucleotide Excision Repair (NER) and Base Excision Repair (BER) functionalities, we developed a highly sensitive microarray plasmid chip to quantify excision/synthesis (ES) activities in cell lysates. Quantitative measurements of cell repair capacities toward several different base lesions (photoproducts, cisplatin adducts, oxidized bases and abasic sites) are obtained simultaneously. Here we studied ES phenotypes of SV40 immortalized fibroblasts from patients with DNA repair deficiencies, either untreated or following UVB irradiation. In unirradiated cells, ES activities were markedly impaired for all lesions tested in XPC, XPD and XPG nuclear extracts compared to the control, whereas XPA, XPB, XPF and CSB nuclear extracts presented almost normal ES activities. The multifactorial role of repair proteins is here one more time demonstrated: some XP proteins are clearly involved in repair of small lesions handled by BER. ES activities in nuclear extracts were also measured 24hrs after UVB irradiation. ES activities of the control toward all lesions were stimulated whereas this stimulation was absent or very low for XPA and XPC extracts, depending on the lesion considered. For example, a stimulation of 70% was observed for repair of photoproducts in extracts of normal cells, and this value dropped to 20 and 0% for XPA and XPC extracts, respectively. For 8-oxoG, this stimulation was 130, 45 and 5% for the same extracts. Modulation of ES activities in response to UVB in the different cell types could reflect differences in repair induction. It remains to be determined if these differences are linked to differences in cell cycle checkpoints activation.

1209

Galectin-3 promotes activation of AKT and protects keratinocytes from apoptosis

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Keratinocytes undergo apoptosis in a variety of physiological and pathological conditions. Galectin-3 is a member of a family of β-galactoside-binding animal lectins expressed abundantly by epithelial cells including keratinocytes. Here we have studied the regulatory role of galectin-3 in keratinocyte apoptosis using cells from gene-targeted galectin-3 null mice. We show that galectin-3 mRNA was significantly upregulated in ultraviolet-B (UVB)-irradiated keratinocytes. Galectin-3-deficient keratinocytes were significantly more sensitive to apoptosis induced by UVB as well as various other stimuli, both *in vitro* and *in vivo*. In addition, galectin-3 deficiency is associated with alterations in the activation of signaling molecules known to be involved in pathways regulating apoptosis. These include the increased activation of c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK), and decreased activation of AKT. Our findings suggest that endogenous galectin-3 is an anti-apoptotic molecule in keratinocytes and may play a role in the development of apoptosis-related skin diseases.

1210

Rapid diagnosis of xeroderma pigmentosum group A by using recombinant adenoviruses

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Xeroderma pigmentosum (XP) is an autosomal recessive disease characterized by sun sensitivity, early onset of freckling and subsequent neoplastic changes on sun-exposed skin. XP is composed of eight different complementation groups, resulting from nonfunctional mutations of genes encoding nucleotide excision repair or post-replication repair enzyme. Complementation group A (XP-A) is the most frequent group in Japan and corresponds to the most severe clinical form. Diagnosis of XP has been carried out by two consecutive procedures of 1) UV sensitivity test of fibroblast cells derived from the patient and 2) complementation tests of the patient cells with each of the known XP fibroblasts. We have constructed recombinant adenoviruses that express each of the XP genes and established rapid and easy grouping of XP. By infecting XP-A cells with XP-A-expressing adenovirus followed by cell counting, we were able to differentiate the UVC sensitivity of XP-A cells infected with XP-A-expressing adenovirus from those with XP-B- or XP-D-expressing adenovirus. Our grouping method of XP by adenovirus infection, UVC irradiation and cell viability assay is a convenient method for the general laboratory without any specific instruments or techniques.

1211

Mutations induced by the UVA component of simulated sunlight predominate in the basal layers of the epidermis in engineered human skin

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Skin cancers are one of the most frequent human tumours. The ultraviolet B (UVB) waveband within sunlight is an important carcinogen, however UVA is also likely be involved. By ascribing mutations to being UVB or UVA induced, we have previously shown that human skin cancers contain similar numbers of UVB and UVA induced mutations, and importantly the UVA mutations were at the base of the tumours. To determine whether this occurred early or late during photocarcinogenesis, and prove that the mutations were UV induced and the dose of sunlight that can cause mutations, we exposed engineered human skin (EHS) to UVA, UVB, or a mixture that resembled sunlight. EHS resemble human skin, modelling differential waveband penetration to the basal dividing keratinocytes, and the complex interplay between epidermal cells. Furthermore we only gave 4 exposures with 0.25 of the dose of sunlight capable of causing sunburn in light skinned Caucasians. Both UVA and UVB induced mutations in over 90% of irradiated EHS, suggesting that sunlight doses achievable during normal daily activities may contribute to skin cancer. UVA- but not UVB-induced mutations predominated in the basal dividing keratinocytes that are thought to give rise to skin tumours. Our studies also show that oxidative base damage may contribute to both UVA and UVB mutagenesis but by different mechanisms. These studies indicate that UVA-induces a larger number of mutations in dividing basal keratinocytes than UVB in the early stages of photocarcinogenesis in human skin, UVA may be an important carcinogen during the early stages of human skin cancer induction.

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The effect of light emitting diode on melanoblasts—- Implication on treatment of vitiligo

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Light emitting diode (LED) in the red and infrared spectrum has efficacy in stimulating wound healing and accelerating optic nerve recovery. Very recently, the LED (630nm) has shown effects on repigmentation of vitiligo. Vitiligo is an acquired pigmentary disorder resulting from melanocyte destruction. Compared to low energy He-Ne laser, which is now used in treating vitiligo, LED with similar wavelength, is cheaper, longer-lasting, and requires less treatment time. However, the molecular mechanism of LED light on the activation of melanocytic cells has not been investigated. In this study, we used NCCmelan5 melanoblast (MB) cell line as models to investigate the effects of LED (630nm) on the proliferation, mitochondrial metabolism, melanin production, migration distances, and photo-protective function of MBs, to formulate the theoretical basis LED's treatment of vitiligo. After LED treatment, MBs showed decrement on total metabolism, cell number, melanin synthesis, and migration after irradiation, however, the mitochondria metabolism per cell was up-regulated. Under serum deprivation, pretreatment with LED (1J/cm2) has shown protective effect against cell death and down-regulated bax/bcl-2 mRNA expression ratio. In this study, we showed that LED (630nm) prevents MBs death and instead of exerting direct stimulatory effect. The mechanism for LED-induced vitiligo repigmentation should be via cyto-protection.

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The role of 12/15 lipoxygenase-isoforms in human keratinocyte, HaCaT

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12/15-Lipoxygenase (12/15-LOX) pathways of arachidonate metabolism have been implicated in the pathology of psoriasis. The inhibition of the 12-LOX expression and 15-LOX overexpression abolished cell proliferation and induced apoptosis in several cancer cell lines. UVB-irradiation is commonly used to treat a wide range of several skin diseases. In this study, we show that 12-LOX expression is down-regulated by UVB-irradiation, which increases of 15-LOX expression in HaCaT cells. Furthermore, 15-LOX metabolites blocked cell cycle progression by down-regulation of 12-LOX expression. Thymidine-incorporation assay and cell cycle analysis revealed that pretreatment of 15-LOX metabolites resulted in an accumulation of cells in the S phase of the cell cycle. The mechanisms underlying 15-LOX-mediated cell cycle arrest were associated with the inhibition of several cell cycle regulators (cyclin-D1, cdk4) and 12-LOX expression. Based on our findings, it indicates that the 15-LOX metabolites may inhibit epidermal hyperplasia by regulating 12-LOX expression

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High-dose UVA1 radiation induces FoxP3$^+$regulatory T cells, but does not affect delayed type hypersensitivity

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UV phototherapy is effective for managing severe chronic dermatitis, such as psoriasis and atopic dermatitis. Previously, we reported that painting contact allergens onto narrow-band UVB (NB-UVB)-exposed skin results in tolerance that is mediated via antigen-specific regulatory T cells (Treg). Furthermore, NB-UVB-induced Treg release interleukin (IL)-10, indicating that they may represent a distinct Treg subtype. UVA1 phototherapy is also a well-tolerated and efficacious treatment for atopic dermatitis. In contrast to NB-UVB phototherapy, the underlying mechanism of UVA1 phototherapy is not well known. To clarify the effect of UVA1, we used an animal model of delayed type hypersensitivity to ovalbumin (OVA). Naïve animals were sensitized with OVA through 50 to 100 mJ/cm^{2}UVA1 (Sellamed 4000; Sellas Sunlight, emission peak at 365 nm)-exposed skin. Seven days later, the left footpads were challenged and 24 hours thereafter footpad swelling was measured. OVA sensitization was not inhibited by exposure to high-dose UVA1. Next, to investigate the effect of UVA1 on dermal dendritic cells (dDC) and epidermal Langerhans cells (LC), frozen tissue sections were prepared from UVA1-exposed skin and stained with anti-mouse major histocompatability complex II. Twenty-four hours after UVA1 irradiation induced the disappearance of dDC from the dermis 24, but did not induce the depletion of LC in the epidermis. To confirm whether Treg were induced in UVA1-irradiated mice, regional lymph nodes were obtained from animals that were sensitized to OVA through UVA1-exposed skin and subsequently stained with anti-CD4, anti-CD25, and anti-FoxP3 antibodies. CD4$^+$CD25$^+$FoxP3$^+$Treg were significantly increased in UVA1-irradiated mice compared to sensitized control mice, but they did not release IL-10. Taken together, these data indicate that Treg induced by UVA1 does not affect delayed type hypersensitivity to OVA. Antigen presentation by dDC may play an essential role in inducing tolerance.

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TLR2 and TLR4 mediate UV-induced Cox-2 and IL-6 expression, MAPKs phosphorylation and AP-1 activation

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Toll-like receptor (TLR) in the skin is a first line of defense against invasion of the microbes and induces inflammatory responses. Myeloid differentiation factor 88 (MyD88) is an adaptor protein for the TLRs family. In this study, we investigated the role of TLR2, TLR4 and MyD88 in UV-induced skin responses. First, we demonstrated that UV irradiation induced Cox-2 and IL-6 expression less in the keratinocytes transfected with TLR2, TLR4 and MyD88 siRNA than in those transfected with control siRNA. Secondly, UV-induced Cox-2 and IL-6 expression was prevented by DN MyD88 overexpression. This inhibition of UV-induced Cox-2 and IL-6 expression was due to diminished AP-1 and NF-kB activation by DN MyD88. Moreover, we demonstrated that UV irradiation induced JNK and ERK phosphorylation, and AP-1 activation higher in the HEK293T cells transfected with TLR2 or TLR4 overexpressing vector than in those transfected with empty vector. In addition, UV irradiation induced immediate interaction of TLR2 and MyD88 in keratinocytes. These results suggest that TLR2, TLR4 and MyD88 may play an important role in UV-induced skin inflammation.

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Thrombospondin-1 prevented UV-induced reduction of Type I procollagen expression in cultured human dermal fibroblasts

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TSP-1 activates TGF-β1 by binding to the LSKL sequence in the latency-associated protein (LAP). It has been reported that the expression of TSP-1 was decreased by UV irradiation in keratinocytes and *in vivo*. However, it was not well known about the effect of UV on TSP-1 expression in cultured dermal fibroblasts and the role of TSP-1 on type I procollagen after UV exposure. In this study, we investigated that the effects of TSP-1 on UV-induced decrease of type I procollagen expression in cultured human dermal fibroblasts. UV irradiation induced the expression of TSP-1 mRNA and protein, and activated Akt, a downstream target of the PI3K pathways. UV-induced TSP-1 expression was greatly suppressed by the inhibition of PI3K, Akt and mTOR. Furthermore, we demonstrated that the TSP-1 blocking peptide, LSKL, and knockdown of TSP-1 by siRNA augmented UV-induced decrease of type I procollagen. Moreover, recombinant human TSP-1 protein treatment with latent TGF-β1 increased the phosphorylation of Smad3 and the type I procollagen expression in both UV irradiated- and non- irradiated control cells. In conclusion, our results suggest that UV irradiation induces TSP-1 via PI3K-Akt-mTOR pathway and TSP-1 alleviates UV-induced decrease of type I procollagen expression in cultured human dermal fibroblasts.

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Examination of ultraviolet B response in normal human keratinocytes utilizing iTRAQ combined to 2D LC-MS/MS

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Keratinocytes, the main component of the epidermis, are the primary cells naturally exposed to and protecting the underlying tissue from ultraviolet-B irradiation (UVB). Comprehensive analysis of UVB-induced changes in levels of proteins expressed and released by normal human keratinocytes (NHK) may assist in the discovery of potential biomarkers for and improve understanding of UVB effects on the skin. This study determined changes in the proteome of cultured NHKs at 12 and 24 h after UVB (10 mJ/cm^2) using iTRAQ combined with 2D LC-MS/MS. Several distinct proteins identified with >95% confidence were differentially expressed, 47 at 12 h and 29 at 24 h after UVB. At 12 h after UVB, the expression of 22 proteins was increased, including several involved in the apoptosis signaling pathway (caspases and proteins associated with mitochondrial dysfunction). On the other hand, the expression of 25 proteins was significantly decreased, including several involved in metabolic pathways (glycolysis/gluconeogenesis, metabolism of propanoate, pyruvate, ascorbate/aldarate and fatty acids, and degradation of valine, leucine and isoleucine). Surprisingly, 24 h after UVB the changes in expression observed at 12 h after UVB reversed. For example, the expression of 6 proteins increased at 12 h after UVB (α-actinin 4, annexin A2 isoform 1, ATP synthase, filamin (A & B), and ribosomal S13) was significantly decreased at 24 h after UVB. Proteins with previously reported apoptotic and metabolic functions under different UVB treatments and from signaling pathways in keratinocytes were identified. This study represents a comprehensive inventory of proteins expressed by NHK under UVB and demonstrates the utility of combining iTRAQ with 2D LC-MS/MS and bioinformatics analysis for comprehensive identification of potential biomarkers of NHKs following physicochemical challenge.

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UV irradiation reduces lipid synthesis in subcutaneous fat tissues by interleukin (IL)-6 released from keratinocytes or fibroblasts

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Although subcutaneous fat is a basic component of the skin, the changes of fat metabolism in photoaged human skin is poorly understood. In this study, we investigated the alteration of lipid metabolism in the subcutaneous fat tissues by photoaging process and acute UV irradiation in human skin. Our results showed that free fatty acid (FFA) and triglyceride (TG) amounts in subcutaneous fat tissues of photoaged and acutely UV-irradiated human skin in vivo were significantly decreased. The expressions of most lipogenic enzymes and the transcription factor, sterol regulatory element binding protein (SREBP1), were also markedly decreased, although UV is not likely to reach down to fat tissue directly. To elucidate the mechanism by which lipid synthesis was reduced by UV in subcutaneous fat tissue, we checked the alteration of lipid metabolism in the human primary adipocytes by using co-culture system and conditioned media-culture system and performed cytokine array. We found that the expression of genes related to lipid synthesis in cultured human adipocytes in vitro was decreased by the treatment of culture media from UV-irradiated keratinocytes or fibroblasts. When we measured the levels of 175 different proteins in culture media from keratinocytes and fibroblasts 24h post-irradiation (100mJ/cm^2), significant number of proteins were increased or decreased by UV irradiation in both keratinocytes and fibroblasts culture media. Especially, interleukin (IL)-6, which is known to decrease the expression of lipogenic genes in adipocytes, was greatly increased by UV. Furthermore, the blocking IL-6 activity in culture media from keratinocytes and fibroblasts by using neutralizing anti-IL-6 antibodies prevented UV-induced decrease of the genes related to lipid synthesis in adipocytes. Taken together, our results suggest that reduction of the lipid synthesis in cultured human adipocytes may be mediated by cytokines including IL-6 released from UV-irradiated keratinocytes or fibroblasts.

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Non-linear optics for monitoring laser-skin reaction *in vivo*

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Various light therapies have been employed in dermatology. The results depend on the immediate light-skin reaction and the subsequent tissue regeneration and remodeling. Multiphoton microscopy has become popular in biomedical imaging recently. By use of femtosecond laser, it is able to generate tissue autofluorescence (AF) and second harmonic generation (SHG) without staining. The aim of this study is to validate the non-linear optics of multiphoton microscopy as a non-invasive imaging modality for monitoring laser-skin reaction *in vivo*. Mouse skin is irradiated with Erbium:YAG laser at different fluence and immediately imaged under a multiphoton microscope. The alterations of cutaneous non-linear optical properties including AF and SHG are evaluated by both morphological and quantitative analysis. At low fluence, Erbium:YAG laser selectively loosens the compact stratum corneum without detectable damage to the viable skin. At higher fluence, ablation of the epidermal and dermal structures can be visualized. In addition, the residual thermal zone can be clearly demonstrated by the changes in the AF and SHG signals. Since SHG is generated from intact collagen fibers, the collagen damage in the residual thermal zone can be quantified by a ratiometric analysis of AF and SHG signals. In conclusion, the disruption of stratum corneum, keratinocytes and dermal extracellular matrix by laser can be visualized by multiphoton imaging with subcellular resolution. Multiphoton microscope can be a non-invasive tool for monitoring the laser-skin reaction *in vivo*.

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Photodynamic therapy of actinic keratoses with two different concentrations of methyl aminolevulinate and home-based daylight exposure – a randomized, controlled, double-blinded study

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Photodynamic therapy (PDT) is a highly effective treatment of actinic keratoses (AK) but is however time consuming and often painful for the patient. Home-based daylight-PDT would make the treatment independent of the clinic and less painful due to the continuous activation of small amounts of porphyrins during its formation. The objective of this randomized controlled study was to compare response rates and adverse effects after PDT using two different concentrations of methyl aminolevulinate (MAL) and home-based daylight exposure. Twenty-nine patients with AK of the face and scalp were treated with MAL-PDT in two symmetric areas. One area was treated with 16% MAL cream and the other area 8% MAL cream. Patients were sent home and instructed to spend as much as possible of the remaining day outside in daylight. Patients scored pain in the treatment area using a pain diary and light exposure were monitored with an electronic dosimeter wristwatch. We found no significant difference in the treatment effect between the two cream concentrations ($p=0.37$), with a complete response rate at 3 months follow-up of 77% in the 16% area compared with 80% in the 8% area. Patients spend in average 210 minutes out door (range 62-372 minutes). Patients had mild to moderate pain during daylight exposure with a mean maximal pain score of 3.7 (SD±2.4), which is significantly lower than pain scores found during PDT of AK in face and scalp using conventional PDT. Erythema and crusting occurred in both treatments and were similar to inflammation seen after conventional PDT. In conclusion, PDT of AK by continuous activation of porphyrins by daylight proved to be an effective treatment of AK. PDT using daylight activation will make the treatment of these extremely common premalignant tumors more time and cost effective, and more convenient for the patient.

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Short and long term effects of NADPH depletion by a G6PDH inhibitor in human dermal fibroblasts

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It is authorized that cellar redox potential plays a critical role in aging process. Intracellular ROS increase by down of redox potential initiates dysfunction of cells. In a case of fibroblasts, ROS is lowered collagen synthesis and enhanced collagen degradation by MMPs expression. Persistent increasing of ROS level in cells will be one of in vitro models in skin aging study. We focused on NADPH depletion to achieve the persistent-ROS increasing in cells. In metabolism of cells, NADPH is mainly produced by glucose 6-phosphate dehydrogenase (G6PDH) and isocitrate dehydrogenase (ICDH). The study was conducted to elucidate cellular responses under depletion of NADPH using inhibitors of both enzymes. Results: First of all, we assessed contribution of G6PDH and ICDH on NADPH production by using each inhibitor, 6-aminonicotiamide (6AN) and oxalimalic acid (OxA). In fibroblasts, 6AN showed a dose-dependent decline of NADPH-dependent resorufin production, in contrast with OxA. In general, the depletion of NADPH expresses an elevation of oxidative stress. We found increasing of reactive oxygen species production when applying with 6AN for 24 h. 6AN-treatment for 24 h surprisingly showed resistance elevation against oxidative stress such as UVB and H2O2. At the time, the in-situ staining of SH-group gave significant increasing of SH-group in cells. On the other hand, 6-days treatment with 6AN exhibited lower scavenging ability against H2O2 than control cells, and coincidently lower staining on SH-group. Furthermore, 6AN on 6-days treatment accelerated protein carbonylation. Discussion: Intracellular NADPH depletion by 6AN treatment apparently showed prospective elevation of ROS generation, and also unexpected and temporal elevation of resistance against ROS and H2O2-scavenging. The results were specurated that the SH increasing contributed to enhancement of resistance against ROS. The temporal elevation may be demonstrated as one of rescue reactions. Addressing the rescue mechanisms will realize a new approach against aging process.

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Photoprotective effects of lycopene for type I collagen in UVA-irradiated human skin fibroblasts

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Chronic exposure of human skin to UV radiation is a major environmental factor that has serious adverse effects on the structure and function of skin. These changes are referred to as photoaging, i.e., wrinkles and pigmentation. UV radiation induces oxidative stress to the skin. Therefore, antioxidant treatment is considered to be useful for reducing the UV-induced injury. Lycopene is the main carotenoid in tomatoes and has been identified in human skin. Lycopene is an acyclic carotenoid with 11 linearly-arranged conjugated double-bonds and is a powerful antioxidant. We investigated the effect of lycopene on type I collagen, which is the major structural component of the skin and is suggested to be a cause of photoaging in UVA irradiation of cultured human skin fibroblasts. Human skin fibroblasts were cultured and subjected to lycopene treatment for 24 hours. The cells were then irradiated with UVA (30 J/cm2). The UVA-irradiated cells were cultured further for 24 hours. The amount of type I collagen released into the cell culture medium was measured by Western blot analysis. Lycopene treatment of non-irradiated cells increased the amount of type I collagen in the culture medium. UVA irradiation significantly decreased the release of type I collagen in the medium. Lycopene treatment of UVA-irradiated cells induced a 1.3-fold rise in type I collagen release into the medium. These results suggest that lycopene increases collagen synthesis and inhibits UVA-induced collagen reduction in human fibroblasts. Lycopene might therefore be effective against photoaging.

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Calcitriol activated by Ultraviolet B preferentially affected fibroblast growth factor-23

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Fibroblast growth factor 23 (FGF-23) is known as a phosphaturic factor regulating phosphate homeostasis. Several studies suggest that dietary phosphate, serum phosphate and 1,25-dihydroxyvitamin D3 (1,25(OH)2D3, calcitriol) are candidate regulators of FGF-23. While the human studies, which modulated the dietary phosphate showed in rather controversial results, manipulation of the active vitamin D definitely affected FGF-23 in animals. This study was conducted to elucidate the relationship between active vitamin D directly stimulated by ultraviolet B (UVB) exposure and FGF-23 level in human. Ten healthy young adults were recruited to get the UVB exposure thrice a week at sub-minimal erythemal dose (MED) with gradual increment by 10% for 4 weeks. We analyzed serum calcium, serum phosphate, mineral-related hormones such as 25-hydroxyvitamin D3 (25(OH)D, calcidiol), 1,25(OH)2D3, parathyroid hormone (PTH), FGF-23, and bone turnover markers. Vitamin D insufficient group comprised 90% among the enrolled volunteers. 25(OH)D increased by 215% (19.8 ng/mL to 40.5 ng/mL, $p < 0.001$) after 4 weeks of UVB exposure. While 1,25(OH)2D3 increased by 175% (49.9 pg/mL to 64.4 pg/mL, $p < 0.001$) then both level decreased to 141%, 130% respectively after 4 weeks of withdrawal. No significant changes in serum phosphate or parathyroid hormone were noticed. C-telopeptide peaked at 2nd week then decreased, while osteocalcin increased gradually. The significant increase of FGF-23 was detected at 4th weeks after withdrawal of UVB (27.8 pg/mL to 41.4 pg/mL, $p < 0.05$). UVB exposure effectively increased calcitriol and calcidiol with delayed stimulatory effect on FGF-23. This result showed that FGF-23 in human was also directly affected by calcitriol even without any significant changes of serum phosphate or PTH.

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Detection of COP1 in normal human keratinocytes and its changes after UVB irradiation

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UVB irradiation has been indicated to trigger a broad range of gene expression responses in human cells. However, factors governing these events are still not well understood. Human COP1 has been shown as a tumor-suppressor p53 interacting protein; it increases p53 turnover in an ubiquitin-dependent fashion. The aim of our work was to detect COP1 in keratinocytes and to elucidate whether COP1 protein has any function in UVB response of human keratinocytes. For that we have used a vector-based RNA silencing approach, real-time RT-PCR, Western blot, immunocytochemistry and immunohistochemistry. Immunocytochemical stainings revealed that COP1 protein was expressed both in the nucleus and the cytoplasm of cultured keratinocytes. In the epidermis, the basal layer of keratinocytes expressed the highest level of COP1 protein. Upon UVB irradiation, a significant decrease in COP1 mRNA expression has been detected. In order to access the function of COP1 in UVB response, a vector-based silencing method has been applied. Real-time RT-PCR experiments revealed successful gene specific COP1 silencing and Western blot experiments showed the up-regulation of p53 protein in the COP1-silenced cells. The COP1-silenced cells were irradiated with UVB light. UVB irradiation resulted on an additional decrease in COP1 mRNA expression, while the expression of p53 protein was further increased in these cells. Our results suggest a negative regulatory role for COP1 in the UVB response of keratinocytes.

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Survivin siRNA causes cell cycle arrest in G2/M and increases UVB-induced apoptosis in human keratinocytes

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Survivin is a bifunctional member of IAP (Inhibitor of Apoptosis Proteins) family, as it is able to both inhibit apoptosis and to regulate cell cycle. Survivin is strongly expressed in keratinocyte stem cell. At the subcellular level, highly proliferating keratinocytes express survivin in the nucleus, while survivin is detected in the cytoplasm of more differentiated cells. Keratinocytes also express pro- and anti-apoptotic survivin splice variants. The aim of the present study is to evaluate the functions of survivin and its variants in human keratinocytes. Survivin siRNA induces an accumulation of keratinocytes in the G2/M phase of the cell cycle. Moreover, survivin siRNA leads to a cell cycle arrest at 24-48 after transfection. At this time-point, siRNA-treated keratinocytes appear flatter than mock and form smaller colonies. At 120hrs, there are no big colonies, keratinocytes appear seriously damaged, and a lot of vacuoles are present in the cells. Finally, a rapid increase of the sub-G1 rate in siRNA treated keratinocytes is observed starting at 48hrs. Survivin expression decreases after UVB irradiation in a time- and dose-dependent manner. Survivin-2a and survivin-3B seem to increase after UVB treatment, while survivin-2B and survivin-DEx3 seem to decrease 24hrs after UVB irradiation. 5 mJ/cm2 UVB blocks the cells in G2/M phase of the cell cycle, while 50 mJ/cm2 UVB induces apoptosis. UVB-induced apoptosis is significantly increased in siRNA treated K, as compared to mock. siRNA treatment also induces a reduction of cell viability and of 3H-thymidine incorporation. Taken together, these results indicate that not only survivin and its isoforms interfere with UVB-induced apoptosis, but wilde-type survivin seems also to modulate cell cycle in human keratinocytes.

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Janus-faced nature of transcription-coupled DNA repair in the skin

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Eukaryotic cells have evolved various mechanisms to cope with environmentally-induced DNA damage. In this regard, removal of UV-radiation-induced DNA photoproducts occurs via nucleotide excision repair, which comprises two distinct mechanisms: global genome repair, which acts on the whole genome, and transcription-coupled repair (TCR), which serves to repair actively transcribed genes. It is generally assumed that maintenance of genomic integrity by DNA repair mechanisms provides protection against cancer as well as aging. In order to study the relative importance of TCR for the prevention of photoaging versus photoarcinogenesis of the skin, in the present study, TCR-deficient CSB-knockout mice were crossed into the hairless background and subsequently subjected to a chronic UVA irradiation protocol. To our surprise, CSB-deficient hairless mice were resistant against photoaging. After receiving a cumulative dose of 750 J/cm^2 UVA, wildtype littermates showed the formation of skin wrinkles, whereas CSB-deficient hairless mice did not. Wrinkle formation in TCR-proficient mice was accompanied by a 3-fold upregulation of MMP-13 expression in irradiated skin areas, whereas the tissue specific inhibitor TIMP-1 was only slighty (=1.3-fold) elevated. In marked contrast, CSB-hairless mice showed a 2-fold upregulation of TIMP-1, but only a 1.3-fold induction of MMP-13. A similar difference was observed when other genes of known pathogenetic relevance for photoaging were assessed. Accordingly, UVA induced expression of IL-6 and VEGF in wildtype mice, but did not increase it above baseline levels in TCR-deficient hairless mice. The exact opposite was observed when UVA radiation exposures were continued and mice analyzed for tumor formation. After a cumulative dose of 1300 J/cm^2 UVA, 60 % of the CSB-deficient hairless mice had developed one or multiple skin papillomas, whereas the skin of all wildtype mice was tumor-free. These results indicate that TCR protects the skin against UVA-radiation-induced photocarcinogenesis, but allows or even promotes photoaging.

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Over-expression of Mcl-1 in mouse epidermis induces epidermal hyperplasia and resistance to UV apoptosis

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Mcl-1 is an anti-apoptotic Bcl-2 family member whose rapid down-regulation is required for UV apoptosis in cultured cells. To explore the function of Mcl-1 *in vivo*, we generated transgenic mice expressing human Mcl-1 under control of the K5 promoter. Mcl-1 transgenic pups were identified by genomic DNA PCR and appeared normal by gross examination. Expression of human Mcl-1 protein in transgenic mice was confirmed by RT-PCR, immunofluorescence and Western blot. Immunofluorescence revealed over-expression and perinuclear localization of Mcl-1 in basal keratinocytes. Mcl-1 transgenic mice had >2-fold increase in epidermal thickness ($p<0.001$), and this epidermal hyperplasia was associated with elevated expression of proliferation markers Ki67, PCNA and keratin 6. Epidermal differentiation markers keratin 1, filaggrin, and loricrin were also expressed at higher levels in Mcl-1 transgenic mice, indicating that Mcl-1 over-expression alters normal epidermal homeostasis. To determine if ectopic Mcl-1 expression could protect keratinocytes against UV apoptosis *in vivo*, control and Mcl-1 mice were exposed to a single dose of UV (70 mJ/cm^2 UVB) and apoptosis assessed by Bak up-regulation and caspase-3 activation after 24 hours. Mcl-1 transgenic mice maintained higher Mcl-1 expression compared to control mice after UV exposure, and had reduced apoptosis. PKCδ, a known mediator of UV apoptosis in keratinocytes, was phosphorylated at tyrosine 311 and cleaved to generate a catalytic fragment in the control mice exposed to UV, but these activating modifications of PKCδ were reduced in UV-irradiated Mcl-1 mice. Taken together, these data indicate that not only can Mcl-1 protect from UV apoptosis in the epidermis, but that it can alter epidermal homeostasis by inducing epidermal hyperplasia and enhancing differentiation marker expression, suggesting a role in keratinocyte biology beyond its canonical anti-apoptotic function.

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Role of Sirt1 in ultraviolet radiation mediated transformation of skin keratinocytes

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Sirtuins are nicotinamide adenine dinucleotide (NAD(+))-dependent deacetylases which have been shown to play a critical role in the process of aging. Sirt1 is the most well studied member of the sirtuin family that has been shown to be linked to cell longevity through a number of biological functions including gene silencing, apoptosis, fatty acid metabolism, oxidative stress and regulation of cellular life span. Here, we tested the hypothesis that Sirt1 plays a critical role in the UV response including UV-transformation of skin keratinocytes. Employing normal human epidermal keratinocytes (NHEK), hTERT immortalized keratinocytes (N/TERT-1) and HaCaT immortalized keratinocytes, we determined the effect of repeated exposure of UVA and UVB (alone or in combination) on Sirt1. Studies have shown that repeated UVA and/or UVB exposure lead to a transformed phenotype in HaCaT keratinocytes. We found that single UVA or UVB exposure of NHEK cells resulted in an initial increase followed by a decrease in Sirt1 levels. Interestingly, single UVA (0.1 to 0.4 J/cm2) or UVB (10 to 200 mJ/cm2) treatment to N/TERT-1 or HaCaT keratinocytes resulted in a dose-dependent decrease in Sirt1 protein levels and activity. However, both Sirt1 protein and activity levels were found to be appreciably increased as keratinocytes became transformed following repeated UVA and UVB. Interestingly, this increased Sirt1 activity was associated with a decrease in Cu/Zn SOD levels in both N/TERT-1 and HaCaT keratinocytes. Further, treatment of UV-transformed N/TERT-1 and HaCaT keratinocytes with Sirt1 inhibitor nicotinamide resulted in an induction of cell cycle arrest and apoptosis. Taken together, our data suggests that Sirt1 plays an important role in UV response including keratinocyte cell transformation. Based on our study, we suggest that Sirt1 regulation should be evaluated as a strategy for the management of UV-damages including skin cancer and skin aging.

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A phenolic antioxidant protects primary human keratinocytes against ultraviolet B-induced DNA damage

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Chronic exposure to UVB radiation is one of the leading causes of skin cancer. Therefore, it is important to identify therapeutic agents that can offer protection against the deleterious effects of UVB-related skin damage. Photocarcinogenesis is largely caused by direct UV-induced DNA damage that leads to mutagenic photoproducts, of which cyclobutane pyrimidine dimers (CPDs) are the most common. Polyphenols are a class of potent antioxidants and recent data suggest that they may play a role in mitigating certain molecular mechanisms involved in photocarcinogenesis. In this study, we demonstrated that pharmacologic doses of a common polyphenol protects primary human keratinocytes against the formation of CPDs by UVB. Incubation of cells with one micromole of the compound for 2 hours before irradiation was sufficient to produce protective effects against UVB in human keratinocytes. The polyphenol does not need to be present during irradiation to produce the effect. Furthermore, we provide data indicating that the functional consequence of decreased CPD formation is inhibition of the apoptotic response of keratinocytes including down-modulation of certain pro-apoptotic proteins, including FoxO3a. Finally, we also show by TUNEL assay that this polyphenol has similar protective effects in UVB-irradiated human skin explants, an *in vivo* model for acute UVB-damage. Polyphenols are therefore promising candidates for the chemoprevention of UVB-induced skin cancer.

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***In vivo* three-dimensional birefringence analysis of photoaged skin wrinkles using polarization-sensitive spectral domain optical coherence tomography (PS-SD-OCT)**

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Dermal degeneration of extracellular matrix components such as collagen and elastin is considered an important factor for wrinkle formation in photoaged skin. However, exactly how dermal degeneration forms wrinkles is unknown. PS-SD-OCT permits non-invasive three dimensional quantitative analysis of dermal birefringence, mainly due to collagen fiber. In this study, we examined dermal birefringence around a wrinkle three-dimensionaly. Areas (3mm x 6mm) including a horizontal wrinkle of crow's feet of 19 Japanese elderly male volunteers (average age: 72) were measured by PS-SD-OCT and observed by CCD camera. The three-dimensional morphology of silicon replicas of the same areas was analyzed by Voxelan. Skin visco-elasticity was also measured by Cutometer. Our algorism for three-dimensional analysis of upper dermal birefringence provided the horizontal map of the phase-retardation slope corresponding to the observed area automatically, and a parameter with the averaged phase-retardation slope. The change of upper dermal birefringence specific to wrinkle part was not observed clearly from the horizontal map of the phase-retardation slope. However, the averaged phase-retardation slope of the observed areas showed a significant negative correlation with the total volume of wrinkles of the same areas measured by Voxelan. The parameters measured by Cutometer did not correlate with the total volume of wrinkles. These results suggest upper dermal degeneration may be involved in wrinkle formation. Analysis of dermal birefringence using PS-SD-OCT will be useful for understanding wrinkle formation and diagnosis of photoaging.

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Increases in neutrophil infiltration and neutrophil elastase by chronic low-dose UV irradiation in murine skin

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The accumulation of imperfectly repaired skin extracellular matrix (ECM) after exposure to sunlight leads to photoaging. It was reported that neutrophils infiltrate following acute UV radiation (above minimal erythema dose) and release proteolytic enzymes such as neutrophil elastase and matrix metalloproteinases (MMPs). To determine the roles of neutrophils and neutrophil elastase in photoaging, we investigated neutrophil infiltration and neutrophil elastase activity in a chronically UV-irradiated hairless mice photoaging model. Furthermore, to estimate the influence of neutrophil elastase on ECM damage, we examined the effects of neutrophil elastase on MMPs activation in vitro. Mice were irradiated with sub-erythema doses of UVB 3 times/week for 10 weeks and skin specimens were observed under light microscopy (LM) and transmission electron microscopy (TEM). Elastolytic activity of neutrophil elastase in tissue specimens was assessed by in situ zymography. To investigate the activation of MMP-2 by neutrophil elastase, normal human dermal fibroblasts were treated with neutrophil elastase and activation of MMP-2 in culture media was measured by gelatin zymography. After 5 and 10 weeks of irradiation, neutrophil infiltration was observed by LM and TEM. Numbers of dermal neutrophils were increased at both 5 and 10 weeks in UVB-irradiated mouse dermis. Neutrophil elastase activity was also increased in UVB-irradiated dermis. Of course, histological features of photodamaged skin matrix such as collagen fiber damage and dermal-epidermal junction damage were observed in neutrophil-infiltrated skin. Furthermore, in vitro examination showed that activated MMP-2 in fibroblast culture media increases following neutrophil elastase treatment. These results support the concept that neutrophil elastase is responsible, at least partially, for ECM damage in sun-exposed skin.

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Human primary keratinocytes exhibit alterations in thioredoxin and p53 pathway gene expression after combined exposure to UV and di-nitrobenzene sulfonic acid(DNBS)

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Cutaneous inflammation in response to a hapten is attenuated by prior exposure to UV irradiation. We have previously shown by proteomic profiling, that exposure of HaCaT keratinocytes to the chemical sensitizer, DNBS, following UV irradiation results in unique alterations in NADPH oxidase pathways, including Thioredoxin (Trx) and the calcium-binding NADPH oxidase, NOX5. MetaCore® analysis identified an association of p53 with several of the altered proteins. Since HaCaT cells bear key mutations in p53, we decided to employ human foreskin keratinocytes (HFKs) to further probe the p53 pathway. Gene expression profiling and pathway analysis of HFKs after exposure to either UV alone, DNBS alone, or UV and DNBS combined, revealed increased expression of p53-regulated genes following UV (e.g. MDM2, RRM2B, thrombospodin1) and several genes upstream of p53 showed decreased expression after UV and DNBS (e.g. Akt3, PI3KC, PTEN). For the first time, we also identified an Nrf2 -mediated oxidative stress response employed by HFKs following DNBS exposure. A key enzyme in the NRf2-pathway, Thioredoxin reductase (TrxR) was highly expressed following DNBS treatment alone and in the combined UV and DNBS condition. TrxR expression is induced by p53 and in turn, increased levels of TrxR provide negative feedback for p53 transcription. Therefore, increased levels of TrxR in the combined UV and DNBS exposure condition may account for the decreased expression of several p53 pathway genes. In addition, electrophilic compounds, such as DNBS, can bind to TrxR and modify it, resulting in p53 disruption. Hence, p53-induced apoptosis and cell cycle alterations due to UV irradiation are altered by subsequent exposure to DNBS, most likely through modified activity and increased expression of TrxR. p53 induces NFkB activation and thus immunosuppression following UV and DNBS may be the result of altered p53-related gene expression via NFkB signaling.

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Correlation between the dermal water content and physical properties of aged skin

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Many reports demonstrate that the physical properties of aged skin differ from those of young skin. In the case of photo-aged skin, the differences are mainly caused by qualitative and quantitative changes of extracellular matrix components such as collagen and elastin. Ex vivo experiments have demonstrated increases in the dermal water content of aged skin of the cheek and the mobile water content of aged skin of the thigh, compared with young skin. Our in vivo experiments have also shown a higher dermal water content in aged skin compared with that in young skin. There have been no earlier reports, however, on any correlation between the dermal water content and physical properties of aged skin. We thus decided to examine whether the dermal water content is correlated with the physical properties of aged skin.The physical properties of the forearm skin of 30 healthy older subjects (average: 64 years old) and 30 healthy younger subjects (average: 21 years old) were evaluated with cutometer, reviscometer, and ballistometer. The water content of the forearm skin was evaluated with a confocal Raman spectrometer.Our results confirmed that the elasticity of aged skin was significantly lower than that of young skin. The final distention (Uf) and visco part (Ua) were also lower in aged skin than in young skin, and the parameters of the other instruments showed differences as well. The dermal water content of aged skin was significantly higher than that of young skin. The dermal water content was positively correlated with the Uf and Ua in the older subjects, but not in the young subjects. It was not correlated with other parameters of cutometer, such as Ur/Uf and Uv/Ue. There was also no correlation between the dermal water content and parameters of the reviscometer or ballistometer. The water content of the stratum corneum and the epidermis were not correlated with any of the physical properties. In conclusion, these results suggest that the dermal water content reflects the physical properties of aged skin.

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Basal and UV-induced MMP-1 expression are inhibited by p53 in human dermal fibroblasts

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Ultraviolet (UV) triggers induction of matrix metalloproteinase-1 (MMP-1) and also induces the rapid appearance of p53 protein in the epidermis and dermal fibroblasts. However, little is known about the role of p53 on MMP-1 expression in human skin. Here, we investigated the effects of p53 on basal and UV-induced MMP-1 in human dermal fibroblasts. To verify the role of p53 on MMP-1 expression, adenoviral p53 (Ad-p53) gene was infected to human dermal fibroblasts. Our results showed that basal and UV-induced MMP-1 expression was prevented by Ad-p53. In contrast, p53 inhibitor, pifithrin-a augmented the UV-induced MMP-1 expression. On the other hand, p53 activator, etoposide, decreased the MMP-1 expression in both normal and p53-overexpressed cells. In addition, MMP-1 expression was not changed by etoposide and/or pifithrin-a in HaCaT cells, which have mutation of p53. Taken together, we suggest that p53 inhibits basal and UV-induced MMP-1 expression in human dermal fibroblasts.

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Decreased repair of singlet oxygen-induced DNA damage in xeroderma pigmentosum cells

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Xeroderma pigmentosum (XP) is an autosomal recessive disorder including groups (A through G) with defective nucleotide excision repair (NER) and a variant form with defective translesional DNA repair of UV-induced DNA damage. Patients with XP have severe sun sensitivity and a very high frequency of skin cancers on sun-exposed areas. In addition, 30 % (especially 60 % in Japan) of XP patients show progressive neurological degeneration. About 55% of XP patients in Japan belong to the severest form of group A and all of XPA patients, some of XPD cases, and rarely subjects in other XP groups have serious neurological symptoms, however, the molecular mechanism of neurological abnormalities remains unproven. Recently the relation of neurodegeneration to oxygen free radical (not UV)-induced DNA damages has been implicated. Here we studied the repair of singlet oxygen-induced DNA damage in XP cells. We treated an expression vector of luciferase gene (pLuc) with rose bengal (RB) followed by visible light irradiation (Xenon lamp), which procedure can generate various oxidative DNA damages such as 8-oxoguanine in the plasmid by a photodynamic reaction in a dose of RB dependent manner. Thereafter, we transfected damaged plasmid into various groups of 17 XP, 2 CS and 5 normal primary fibroblasts lines and measured DNA repair capacity (DRC) of the cells estimated by luciferase activity after plasmid host cell reactivation for 48 hours. We found that XPA and XPD cells showed extremely lower level of DRC than that of normal cells, however XPC, XPD/CS, XPF, XPG and CS cells represented slightly decreased or similar DRC compared to normal cells. These data suggest that insufficient repair of oxidative DNA damage may partly play a role in skin carcinogenesis and neurological degeneration in XP.

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Phospholipase Cε plays a crucial role in ultraviolet B-induced skin inflammation

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Phospholipase C (PLC) ε is a novel class of phosphoinositide-specific PLC, identified as a downstream effector of Ras family small GTPases (Ras, Rap1, and Rap2). PLCε is also activated by a small GTPase RhoA and heterotrimeric G proteins. These multiple regulatory mechanisms enable PLCε to mediate signals from a wide variety of cell surface receptors, including receptor tyrosine kinases and G protein-coupled receptors. The purpose of this study is to investigate the role of PLCε in the skin response to acute and sub-acute ultraviolet B (UVB) irradiation using PLCε knockout mice. We irradiated wild-type and PLCε knockout mice with a single shot of UVB at a dose of 2.5 kJ/m^2 and examined the skin pathologically at 1, 2, and 7 days after irradiation. We found that UVB-induced keratinocyte apoptosis at days 1 and 2, which was assessed by TUNEL assay, was considerably suppressed in PLCε knockout mice compared to that in wild-type mice. Also, the extent of neutrophil infiltration into the epidermis in PLCε knockout mice at days 1 and 2 was smaller than that in wild-type mice. We next examined the effect of sub-acute irradiation of UVB on the mice skin. We irradiated wild-type and PLCε knockout mice with UVB at a dose of 2.5 kJ/m^2 three times a week for 4 weeks. At the end of the irradiation the texture of the skin surface was smoother and skin stiffness was softer in PLCε knockout mice than in wild-type mice. These results indicate that PLCε plays a crucial role in UVB-induced skin inflammation and demonstrate the importance of PLCε in cutaneous photobiology.

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High-throughput screening for gene function related to UV response using reverse transfection of siRNAs from solid surface

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UV irradiation induces various cellular responses such as apoptosis, cell proliferation, and DNA repair, concomitantly with numerous alterations of gene expressions in the skin. Though the genes responsible for these cellular processes are thought to influence the cellular UV sensitivity, the individual gene functions related to UV response remain unclear. Our group developed an siRNA reverse transfection system from a solid surface as a tool to find the genes affecting the cellular UV sensitivity. This system allows for the transfection of hundreds of different siRNAs, and the effects of siRNA on cellular physiology can be examined in a single experiment. We optimized this platform for cultured human skin fibroblasts and applied it to a screening for candidate genes related to cellular UV sensitivity. In the screening of 331 genes known to be involved in DNA repair, cell cycle, check point, and apoptosis, the down-regulation of 13 genes remarkably reduced the cell viability of the UV-irradiated cells with little or no effects on the viability of non-UV-irradiated cells. Seven of these 13 genes are reported to be involved in nucleotide excision repair (NER), and the other 6 are reported to be involved in apoptosis. In a colony-forming assay for one of the 13 genes, fibroblasts transfected with the siRNA directed against the gene became more sensitive to UV. These findings indicate that our high-throughput assay is a useful tool for the analysis of gene functions responsible for cellular UV sensitivity. This system is likely to help us identify the unsolved molecular mechanisms behind the UV sensitivity of the skin in individuals.

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Protection against photoaging *in vitro* and *in vivo* animal models by isoflavone extract from soybean cake

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Ultraviolet (UV) irradiation causes pathological changes in the skin such as photoaging and skin cancer. Acute UV exposure of the skin causes erythema, inflammation, proliferation and differentiation. Twelve isoflavones are found in soybean and are present in four chemical forms including malonylglucosides, acetylglucosides, glucosides and aglycones. The aim of this study was to evaluate the antiphotoaging activities of the isoflavone extract from soybean cake *in vitro* and *in vivo* animal models. *In vivo* study, hairless mice was irradiated by UVB (150 mJ/cm^2/day for 7 days). The thickness of epidermis, cyclooxygenase-2 (COX-2) and catalase were used to evaluate the antioxidant and anti-inflammatory activities of isoflavone extract. *In vitro* study, human keratinocyte (HaCaT) cell line was used to investigate the inhibition of p38, c-Jun N-terminal kinase (JNK) and extracellular signal regulated kinase (ERK)1/2 phosphorylations. Our results found that the thickness of epidermis and expression of COX-2 after the UVB exposure were increased than control group. However, the catalase concentration was decreased in control group after UVB exposure. Topical treatment with 1 and 3 mg/ml isoflavone extract before UVB irritation, the thickness of epidermis and expression of COX-2 were decreased. The catalase concentration was increased in treatment of isoflavone extract. *In vitro* study, the treatment of isoflavone extract also decreases the UVB-induced cell death. In addition, we observed that the phosphorylation of ERK1/2 was apparently inhibited by the treatment of isoflavone extract. Moreover, we found that acetylglucoside fraction of isoflavone extract was more antiphotoaging activity than the other fractions. Thus, the isoflavone extract from soybean cake is suitable for developing as an antiphotoaging agents for skin care.

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Photoprotection effect of curcumin by down-regulation of UVB-induced cytokines in HaCaT keratinocytes and in vivo

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Excessive UVB irradiation induces expressions of cytokines such as IL-6, IL-8, and TNF-α, in keratinocytes, and resulting in acute inflammation in skin. Curcumin, extracted from the rhizomes of Curcuma longa, has been described as having antioxidant, anti-inflammatory, and anti-carcinogenic properties. It still remains to be elucidated whether curcumin, a natural product exerting immunomodulatory properties, inhibits the UVB-induced expressions of IL-6, IL-8, and TNF-α in HaCaT keratinocytes cell lines. In this study we investigated the effects of curcumin in expressions of IL-6, IL-8, and TNF-α in HaCaT cells and molecular mechanisms underlying inhibitory properties of curcumin. UVB-treated HaCaT cells clearly showed that increased expression of IL-6, IL-8, and TNF-alpha by a dose-dependent manner of UVB (0, 100, 200, 300 mJ/cm2). According to RT-PCR analysis, expressions of IL-6 and TNF-α, but not IL-8, were increased 12 h after UVB-irradiation. Interestingly, pretreatment of curcumin (100 uM) dramatically reduced the expressions of UVB-induced IL-6 and TNF-α. Furthermore activations of MAPKs (p38, JNK, ERK), playing key roles in expressions of cytokines, were partially attenuated by pretreatment of curcumin in UVB-treated HaCaT cells. These results collectively suggest that curcumin may inhibit IL-6 and TNF-α expressions by suppressing MAPKs in UVB-treated HaCaT cells. Furthermore, the apply of ointment containing curcumin markedly protected the erythema and skin atropy in UVB-irradiated hairless nude mouse, due to down-regulation of IL-6 and TNF-α. Thus, our data showed that curcumin may be applied as a promising sun protective agent through modulation of UVB-induced inflammatory cytokines such as IL-6 and TNF-α.

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Multiphoton characterization of infection pathogens

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We performed multiphoton imaging on four fungi and four Candida of medical significance. Intense autofluoresence can be detected from spores and hyphae, especially the cell wall and septum. We analyze the emission spectrum and it shows that fungi and candida of various species have different characteristic fingerprint emission spectrum: Microsporum canis has intense emission in the blue light, Trichophyton mentagrophytes shows more intense emission in the yellow light, Aspergillus flavus has a preferential emission in the red light, and Fusarium solani has a characteristic autofluorescence in the blue light. Our images also show that the Candida tropicalis has an elongated shape, the Candida albicans and Candida krusei has a circular shape, and the Candida glabrata has an elliptical shape. Our result shows that multiphoton microscopy can be used as a noninvasive tool for differentiating fungal and Candida species.

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Effect of UVB irradiation on calcium handling and purinoreceptor expression of keratinocytes

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Nowadays different tumours are responsible for a major part of mortality. Epidermal tumours are neoplastic processes, fifth of this being the invasive melanoma malignum form. UVB irradiation is mostly responsible for the epidermal tumours causing mutations in the DNA and hyperproliferation of the mutant epidermal keratinocytes. It has been shown that intracellular calcium concentration and certain purinergic receptors have important role in cell proliferation or apoptosis, but little information is available about the changes on calcium homeostasis and purinoreceptor expression caused by the irradiation. First we investigated the time dependent effects of one minute long UVB irradiation (60mJ/cm2) on the proliferation of cultured HaCaT keratinocytes using MTT colorimetric assay. The percentage of viable cells significantly decreased compared to control at 24 hours after the irradiation (42.9%), and this change was more pronounced at 36 (65.5%) or at 48 (75%) hours. Increasing the duration of irradiation caused more intensive cell death. We also investigated the purinoreceptor expression pattern and its changes following the UVB irradiation using RT-PCR, immunocytochemistry and Western blot technique. The ionotropic P2X7 receptor, which is involved in apoptosis, showed total degradation after the treatment probably caused by monomerisation. The irradiation generated downregulation of P2X1 receptor which was detectable with all techniques. The expression pattern of P2X3 receptor also altered following irradiation, PCR data revealed partial degradation of this protein. Fluorescent intracellular calcium measurements revealed that both the resting calcium concentration and the amplitude of ATP-evoked calcium transients decreased following the UVB irradiation (from 109±9 nM to 84±10 nM and from 261±35 to 120±31 nM, respectively). These results suggest that UVB irradiation could affect the expression of purinergic receptors and the calcium responses which could be in part responsible for the oncogen properties of the irradiation.

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Reduction of photodamage from daily sub-erythemal UVR exposure by sunscreens may depend on spectral balance rather than sun protection factor(SPF)

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Sunscreens were developed to protect from erythema after a single erythemal exposure. Their efficacy is assessed via their sun protection factor (SPF), i.e. primarily a measure of UVB protection, by simulating intense solar UVB exposure at low latitudes. SPF gives no indication of UVA protection. However, protection from erythema per se cannot assure protection against molecular and cellular damage by either UVB or UVA. Thus, chronic non-erythemal solar exposure is likely to be a major cause of premature skin ageing and photocarcinogenesis. Such damage could, in theory, be prevented by daily care products containing sunscreens. In an exploratory study, we investigated the effects of repeated sub-erythemal exposure with a solar spectrum more typical of Northern latitudes; i.e. having a greater UVA: UVB ratio than the COLIPA spectrum used for SPF testing. Concomitantly, we explored the effect of two UV-absorbing day-care preparations and the influence of their spectral absorbance; one absorbed primarily in the UVB range (COLIPA SPF=8.6±1.4) and the other was broad spectrum (COLIPA SPF=7±1.6). The buttocks of skin type I/II volunteers (n=8) were irradiated daily with 0.3MED for either 6 or 13 days after which 4mm punch biopsies were taken, and processed for routine immunohistochemistry. Erythema was documented by photography and reflectance spectroscopy. Sections were evaluated for DNA damage (cyclobutane pyrimidine dimers, p53 expression, apoptosis), Langerhans cell numbers, and for signs of oxidative stress (hydroxy-2′-deoxyguanosine (8-OHdG)). As will be shown, and in accordance with recent clinical data, a balanced protection over the whole UV range is required for effective protection, including cumulative erythema. Moreover, to rely on standard SPF values when formulating UV-protecting day creams may markedly underestimate UVA damage and provide false security, especially for persons of skin type I/II where adaptive protection from chronic UV-exposure may be limited.

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The effect of UV (A/B) and narrow band blue light (NBBL) on IL-1α and TNFα: *In vitro* co-culture system

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UV radiation on keratinocytes have long been studied on the signal transduction pathways and various cytokine releases to evaluate the inflammatory potential. Stimulating of keratinocytes with UV releases IL-1α and causes inflammation responses via chemotaxis of inflammatory cells and other inflammatory mediators. The monocyte/macrophage is reported to be a major contributor to the development and progression of local and systemic inflammatory responses of skin. An attempt was made to establish an *in vitro* co-culture system using keratinocytes and macrophages. Normal human epidermal keratinocytes (NHEK) and murine macrophages (RAW 264.7) were used. RAW 264.7 cells were irradiated with UVA(0-5J/m^2),UVB(0-40mJ/cm^2), and NBBL(0-20mJ/cm^2). IL-1α and TNFα were assessed in the supernatant of keratinocytes using ELISA assay after 48 hour co-culture treatment. 5-aminolevulinic acid (ALA) was used to evaluate the possible modulation of inflammatory responses of the co-culture system. Preliminary data showed that UV (A/B) radiation induced a 2-4 fold increase in IL-1α levels. TNFα production also increased but to a lesser extend. ALA had minimal effect on cytokine production at the tested concentrations ($1X10^{-5}$ to $1X10^{-6}$ mM). Macrophages irradiated with NBBL had less effect on cytokine production of keratinocytes. These results expand the properties of cytokine production in modulating the inflammatory process and will facilitate testing of its photo-therapeutic applications in different inflammatory skin conditions.

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Antioxidative and photoprotective effects of topical formulations containing coffeeberry

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Excessive exposure to solar ultraviolet (UV) radiation is implicated as the main cause of skin cancer and aging. UVB radiation causes the generation of reactive oxygen species and other free radicals, which in turn can cause serious injury to epidermal cell membranes, DNA, and proteins. Oxidative stress can be prevented by antioxidants, which act by scavenging free radicals, binding metal ions, or removing damaged molecules. While the skin contains endogenous antioxidants such as vitamins C & E, ubiquinones, and glutathione, these are often overwhelmed. Botanical antioxidants in combination with sunscreens have shown promise in preventing the adverse effects of UV radiation on the skin. In particular, extracts from the fruit of Coffea arabica (coffeeberry), contain potent polyphenols including chlorogenic acid, condensed proanthrocyanidins, quinic acid, and ferrulic acid, and have been shown to modulate the expression of collagens, connective tissue growth factor, and metalloproteinases. The aim of this study was to investigate the antioxidant and photoprotective activity of topical formulations containing coffeeberry using various in vitro models. Antioxidant activity was evaluated using the oxygen radical absorbance capacity (ORAC) assay. UVB protection was assessed using reconstructed human epidermis and ex vivo human skin cultures, where cultures were irradiated within 2 hrs after application of test formulations. UVB-induced DNA damage was assessed based on the presence of thymine dimers and upregulation of p53. The activity of coffeeberry formulations was studied in comparison to commercially available products containing such antioxidants as licorice and green tea extracts. The results show that coffeeberry formulations are comparable or superior to the other antioxidant formulations tested depending on the biomarkers evaluated. For example, ORAC scores for coffeeberry were approximately 10-fold higher than that of green tea. Topical formulations containing coffeeberry may be a novel alternative for those seeking to prevent damage caused by sun exposure.

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Nanoparticle skin penetration - Effect of UVR

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Engineered nanoparticles (<100 nm) have become commonplace in numerous technological and consumer applications. For example, TiO2 and ZnO nanoparticles (NP) are formulated into cosmetic products and sunscreens for their UVR protective effect. Concerns have escalated that chronic NP skin exposure could pose a health risk. In vitro studies show NPs are cytotoxic to skin cells, producing reactive oxygen species and inflammatory responses. Existing *ex vivo* data on NP skin penetration is ambiguous and *in vivo* studies are lacking. The purpose of this study is to investigate the effect of NP skin penetration and translocation using an *in vivo* SKH-1 hairless mouse model. A key hypothesis of this research is that NP surface chemistry and hydrodynamic size govern penetration. Highly fluorescent semiconductor quantum dots (QDs), which are easy to monitor using fluorescence microscopy are used as surrogates for predicting penetration of metal oxide NPs. We investigated effect of QD surface chemistry, size and skin status including normal vs. sunburned (270 mJ/cm2 UVB). Skin was harvested for analysis 8 or 24 hr after application of QD applied in glycerol. Results from TEM, fluorescent and Confocal microscopies indicate that QD tend to localize around hair follicles and skin defects. The UV induced epidermal repair response increases penetration but only a small % of QDs applied penetrate into viable epidermis. Results suggest QD permeate through lipid lamella in the SC. This research provides primary knowledge on the penetration of QD through skin in vivo and serves as a model for understanding permeability of the barrier when altered by UV exposure.

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Skin ageing in Chinese women correlated to photoexposure

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Few data exist correlating the morphological and immunohistochemical changes of ageing skin between photoexposed and non-exposed skin of the same patient, even less knowledge in the Asian population. In this study, we designed a clinical study to compare sun-exposed skin and unexposed skin in Chinese women from different age groups. The volunteers were recruited in the southern part of China. A total of 30 healthy Chinese women, aged between 22 and 63 years were divided into young and old group. Photos were taken from the skin and skin punch biopsies (3 mm) were taken from the anterior surface of the upper arm (protected area) and the posterior surface of the forearm (exposed area). The samples were fixed in formaldehyde for further analysis. The epidermal and stratum corneum thickness at exposed area is thinner than unexposed area. The thickness increases with age and peak the highest between 40-60 and decrease afterwards. This enhanced hypertrophy with aging is probably a sign for physical protection of the skin from UV light. As expected, the sun-exposed skin expressed significantly more melanine than the non-exposed skin areas, however this overexpression of Melanin was only significantly increased in the age group 60-70. In addition, further investigation was conducted on caveolin-1. In epidermis, the caveolin-1 expression is increased after photoexposure in keratinocytes (especially for younger group), but decreased with age only in the photoexposed skin. In dermis, no significant changes in photoprotected zone were observed. However, in photoexposed zone, the caveolin-1 expression decreased with age. Caveolin-1 expression decreases with age in epidermis and dermis under UV influence. Therefore Caveolin-1 expression could be an indicator for UV-induced senescence in skin. The expression of different opioid receptors in skin was measured and showed interesting correlation to the above-mentioned results. The opioid receptor expression is changed with age, but not with photoexposure.

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Ski-infiltrating macrophages migrate to draining lymph nodes and produce IL-10 in UV-induced tolerance

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Low dose UVB exposure induces antigen-specific unresponsiveness to antigen(s) introduced through UV-irradiated skin (tolerance). Recently, regulatory T (Treg) cells that mediate the tolerance have been identified in the draining lymph nodes (DLN) of UV-irradiated skin. However, the mechanism how suppressive signals are transmitted from the skin to the DLN remains unclear. To investigate the mechanism we performed detailed time course analyses of cytokines in mouse DLN, using real-time PCR, and protein analysis. IL-12p40 mRNA and protein expression, as well as IL-12p70 protein was upregulated after application of the contact sensitizer 2,4 dinitro-1-fluorobenzene (DNFB) to normal skin. The cellular source of IL-12p40 mRNA was CD11c+ cells. By contrast, following DNFB application to UV-irradiated skin (UV+DNFB), IL-12p40 mRNA was not upregulated, and DLN IL-12p40 and p70 proteins were reduced. UVB irradiation alone did not upregulate IL-10 mRNA, but UV+DNFB upregulated IL-10 mRNA as early as 3-6h after DNFB application. This IL-10 spike immediately preceded a decrease of IL-12p40 mRNA from the level observed following UV, and IL-10 was produced by F4/80+, MCP-1 producing cells in the DLN. The infiltration of F4/80+ cells into UV-irradiated skin was followed by a rapid and remarkable increase of F4/80+CD11c- cells in the DLN 3h following DNFB application. That these F4/80+ cells originated from the skin was confirmed by FITC tagging. ELISPOT assay demonstrated that FITC+F4/80+CD11c- flow-sorted cells in the DLN produce IL-10. We conclude that macrophages which infiltrated into the skin following UVB exposure are capable of rapid migration to the DLN. This required triggering by contact sensitizers. Production of IL-10 by macrophage, in conjunction with IL-12 inhibition in the DLN likely reflects a role as mobile suppressive mediators for locally induced UV-tolerance.

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HIF-1α induction after UVB shows a biphasic pattern in HaCaT cells

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Hypoxia in the skin is an initiator of various events that have long-term effects, such as chronic degenerative dermo-epidermal changes, inflammation, photoaging and cancer. In these processes vascular endothelial growth factor (VEGF) plays a crucial role; also, it is known to be affected by ultraviolet radiation. Hypoxia inducible factor-1 (HIF-1) closely regulates the expression of VEGF in several experimental settings. In this work we studied the impact of a single UVB irradiation on the level of HIF-1 in HaCaT cells by Western blots at several time points after a single irradiation. The physiological importance was confirmed by measuring downstream target gene expressions by quantitative real-time PCR. UVB treatment resulted in an initial depression in HIF-1α that was followed by a subsequent prolonged increase. If cells were exposed to an additional UVB irradiation, another decrease in HIF-1α was provoked, similar to the original effect. These changes followed a strict timeline and were dose-dependent. The potential role of the PI3K/AKT pathway was examined to explore the mechanism underlying the upregulation of HIF-1α upon UVB irradiation. No change was observed in the total level of AKT after UVB treatment, however, its phosphorylation level was found to be markedly higher. In accordance with these observations, wortmannin, an inhibitor of PI3-kinase effectively blocked the increase in HIF-1α. In agreement with previous findings, UVB irradiation increased VEGF and HO-1 mRNA levels determined by quantitative real time PCR. It is concluded that changes in HIF-1α expression underlie the alterations in expression of VEGF upon UVB irradiation. It is supposed that UVB effects in the skin are partially mediated by the PI3K/AKT pathway.

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At temperate geographic latitudes labelled SPF may overestimate photoprotection by topical sunscreens

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Historically, topical sunscreens were designed to protect from erythema 24 hours after sun exposure. Their efficacy is assessed via their sun protection factor (SPF) which is primarily a measure of UVB protection, and test conditions are similar all over the world: Erythema prevention is quantified in volunteers via exposure to an intense source of simulated solar UVR with the high UVB to UVA ratio typical for low latitudes, i.e. a "worst case" situation for UVB damage. Under given test conditions, the SPF of a given sunscreen is independent of its ratio of UVB to UVA protection. However, this is not the case at latitudes outside those emulated in SPF test protocols; e.g. two products of SPF = 10, yet with different UVA protection, will have different "SPFs". Hence, the degree of protection against damage caused by sub-erythemal UVR exposure can also be expected to vary. Thus, its labelled SPF may overestimate a sunscreen's level of protection. We mimicked the situation of every day sun exposure at temperate latitudes and exposed skin type I/II volunteers (n = 8) daily for up to two weeks to sub-erythemal doses of UVR from a source with a UVB to UVA ratio more typical of Northern Europe. Evaluation of the cumulative erythema by photography and reflectance spectroscopy showed that two sunscreens protected less than expected by their SPFs and that a primarily UVB sunscreen performed less well than a broad-spectrum product. These data disclose the limitations of the SPF approach under "real life" conditions and support the use of broad-spectrum sunscreens where the extent of protection is less dependent on the emission spectrum of the test source or of real life UVR. Furthermore, they may contribute to a better understanding of the reported lack of protection of sunscreens against chronic UVR effects.

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L-ergothioneine and its transporter OCTN1 participate in regulating the redox status of the skin

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To limit the effects of reactive oxygen species, cells employ a defense system consisting of antioxidant enzymatic activities and small non-enzymatic molecules. L-ergothioneine (EGT) is a dietary histidine derivative with an Oxygen Radical Absorption Capacity 1500-fold that of an equimolar amount of the vitamin E analog Trolox. EGT accumulates in cells normally subjected to high levels of oxidative stress, suggesting a function in their antioxidative defenses. However, only cells that express the specific transporter/receptor OCTN1, can internalize EGT and benefit from its potent antioxidant properties. The efficacy of EGT treatment in neutralizing the oxidative damage in human skin depends critically on whether the skin cells produce OCTN1. We have determined that both in culture and as resident cells the dermal fibroblasts and the epidermal keratinocytes synthesize OCTN1 mRNA and protein. Accordingly, we have observed that dermal fibroblasts treated with EGT show lower peroxide levels following solar simulating UV irradiation than those treated with the CoQ10 analog idebenone. Likewise, keratinocytes treated with EGT for 7 days have a two-fold higher antioxidant capacity than the matching untreated controls. In the epidermis OCTN1 protein is localized predominantly in the basal and granular layers, which are most relevant for the antioxidant function of EGT. Remarkably, OCTN1 mRNA is translated into a number of protein isoforms localizing from the cell periphery to the nucleus. Their relative amounts and subcellular expression profile vary with the physiologic status of the cells. At high levels ROS are toxic. However, lower concentrations of ROS are required for physiologic processes, including activities of signal transduction pathways and transcription factors. Thus, maintaining proper ROS concentrations should be highly regulated. The complex and dynamic expression profile of OCTN1 suggests that together with its ligand, EGT, it may be an integral part of a ROS regulatory mechanism.

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Upregulation of Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and its receptor, FP by ultraviolet light exposure

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Since selective cyclooxygenase-2 (COX-2) inhibitors have been shown to increase the risk of heart attacks, new drugs are being developed that target prostaglandin E (PGE_2) synthases that convert PGH_2 to PGE_2. However such drugs are likely to increase levels of another epidermal prostaglandin, $PGF_{2\alpha}$. This PG has been shown to be a potential tumor promoter in mouse skin, and elevated levels of $PGF_{2\alpha}$ and its receptor FP have been shown to stimulate colorectal cancer invasiveness. In order to investigate the potential risk for enhanced tumorigenesis PGE synthase inhibitors may pose, regulation of $PGF_{2\alpha}$ and FP in response to UV light were examined. Ten human subjects were exposed to graded doses of solar simulated UV light, and their minimal erythema dose (MED) determined 24 hours later. Sun exposed areas of the neck were then exposed to 3 times the subject's MED. 24 hours later, biopsies from irradiated and from a chronically sun exposed site 2 cm away were obtained. Sun protected skin was also biopsied. Immunohistochemical staining revealed very little FP or COX-2 expression in sun protected skin. Chronically exposed skin expressed about 20% more COX-2 and FP on average, with some individuals expressing 3 fold more. Skin exposed to 3 MED exhibited a 3 fold induction of COX-2 and 2 fold of FP 24 hours post irradiation. Patchy, intense and dermal COX-2 upregulation correlated with intense clinical erythema. FP expression was diffuse in the epidermis. Cultured human keratinocytes were also examined to determine the effect of UV exposure on FP expression and $PGF_{2\alpha}$ synthesis. Confluent keratinocytes were exposed to 40 mj/cm^2 UVB. 24 hours later, supernatants and protein were harvested for $PGF_{2\alpha}$ determination by ELISA and FP receptor expression by Western blot. $PGF_{2\alpha}$ levels were increased 5 fold over basal. FP protein expression was also increased 2 fold by UVB light *in vitro*. In summary, we have shown that both $PGF_{2\alpha}$ and its receptor FP are upregulated by UV light in humans. These data suggest that further work examining their role in photocarcinogenesis is warranted.

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Targeted broadband UVB phototherapy improves disorders characterized by increased dermal matrix

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A variety of skin disorders are characterized by localized induration (firmness) of skin due to increased dermal matrix deposition. Ultraviolet (UV) irradiation can break down dermal matrix through induction of matrix metalloproteinases. Targeted broadband UVB (tUVB) through a handheld fiber-optic cable allows concentrated irradiation to specific areas of interest. Through an open study, we investigated whether tUVB may improve clinical appearance and firmness of selected disorders with increased dermal matrix (n=15), including granuloma annulare (GA, n=5), keloids and hypertrophic scars (HS, n=6), and dermatofibroma (DF, n=4). Affected sites were treated with tUVB (peaks 302 & 312nm; T500x, Daavlin) up to 3 times per week for 16 weeks. After determining minimal erythema dose (MED) for each patient, initial treatment began with 2xMED, with subsequent escalation in dose as tolerated (mean cumulative dose = 41J/cm2). Firmness of lesional skin was assessed objectively by the durometer at week 0, 4, 8, 12 and 16. The global response was graded on a 5-point scale [-1= worsening, 0= no change, 1= somewhat improved, 2= much improved, 3= clear]. At the end of therapy, there was significant softening of lesions with an average reduction in durometer readings of 11% (range 0.5%–34%; p=0.003). Four patients (3-GA, 1-DF) demonstrated grade 2 [much improved], 9 patients (2-GA, 2-keloids, 3-HS, 2-DF) grade 1 [somewhat improved] responses, and 2 patients (1-HS, 1-DF) were non-responders. Among the responders (n=13), those who received tUVB 3x per week demonstrated better clinical outcome than those treated less frequently (mean durometer reduction: 14% vs. 7%; mean global response: 1.7 vs. 1.0 [p<0.001]). Our pilot study suggests that tUVB has the potential to improve clinical appearance of disorders characterized by increased dermal matrix. With additional clinical experience and treatment optimization, tUVB may become an effective phototherapy for this group of disorders, in which there are currently limited therapeutic options.

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Mathematical modeling of collagenase-1 induction by ultraviolet A1 in human skin *in vivo*: an approach to optimize antifibrosis therapy

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Ultraviolet A1 (UVA1) phototherapy improves skin thickening due to collagen deposition disorders, such as scleroderma. This antifibrotic effect is mediated in large part by UVA1-induced collagenase. UVA1 (longer λ) penetrates deeper into the dermis than UVB/A2 and may therefore modulate collagen metabolism to a greater degree. Furthermore, UVA1 is less erythemogenic to the skin than UVB/A2, making it a safer light source for photomedicine. Previously, we showed that UVA1 induction of collagenase is dose dependent. This effect is greatly attenuated by darker natural skin pigmentation. In addition, UVA1 dose-dependently induces skin pigmentation (tanning), which in turn dampens subsequent inducibility of collagenase by UVA1. Reports of variable UVA1 effectiveness in fibrotic skin by different therapy regimens are likely due to these interdependent factors. In an attempt to design and customize a UVA1 treatment regimen for optimal collagenase induction in human skin, a mathematical model consisting of a system of two differential equations was developed. These equations were fit to 393 data points generated from 47 normal human subjects irradiated with varying doses and frequencies of UVA1. The equations consider tanning response of subjects according to L* values as determined by a colorimeter, as well as collagenase-1 mRNA expression (fold induction) in biopsy specimens as determined by RT-PCR. The model was tested for predictability, and simulations were found to be consistent with our available data. We therefore believe that this model will allow for evidence-based, customized determination of an optimal antifibrotic phototherapy regimen with UVA1. A software program of this model could conveniently enable physicians and phototherapists to tailor UVA1 dosing and scheduling specific to patients' natural skin color and produce sustained collagenase induction for maximal treatment response.

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PPARγ antagonism alters UVB-induced acute responses in SKH-1 mouse epidermis

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In SKH-1 mice, ultraviolet B (UVB) irradiation induces an acute response characterized by cyclooxygenase-2 (COX-2) induction, as well as an early phase of apoptosis that is followed by a delayed hyperplastic response. Previously, we demonstrated that UVB exposure produces oxidized lipid species that act as potent peroxisome proliferator activated receptor gamma (PPARγ) agonists. In human keratinocytes, PPARγ activation in turn is at least partially responsible for UVB-induced prostaglandin E2 (PGE2) production *in vitro*. Given the known role of cyclooxygenase-2 (COX-2) induction in UVB-induced acute responses, we hypothesized that PPARγ activation may play an important role in regulating UVB-induced COX-2 expression, apoptosis, and delayed hyperproliferation. Using SKH-1 mice, we tested this idea using topical application of a highly specific PPARγ antagonist, GW9662. Mice were pretreated with GW9662 (0.01, 0.1, & 1 mM), then irradiated with 1500 J/square meter of UVB. Mice were sacrificed at 24 & 72 hrs and assessed for COX-2 mRNA expression (24 & 72 hrs), apoptosis (24 hrs), and proliferation (72 hrs). By quantitative RT-PCR, GW9662 pretreatment produced a dose-dependent inhibition of UVB-induced COX-2 expression at both 24 & 72 hrs. Apoptosis (as measured by activated caspase 3) was augmented by GW9662 in a dose-dependent manner. Given that COX-2 induction is thought to suppress apoptosis, this raises the possibility that PPARγ suppresses apoptosis via a COX-2 dependent mechanism. UVB-induced delayed hyperproliferation was assessed by BrdU incorporation, Ki-67, and phosphorylated histone H3 (P-H3) immunohistochemistry. Surprisingly, GW9662 augmented UVB-induced proliferation using all three proliferation markers. Given that PPARγ is necessary for COX-2 induction and UVB-induced COX-2 induction is mitogenic, this suggests that PPARγ suppresses growth independent of COX-2. In summary, our data strongly suggests a prominent role for epidermal PPARγ in regulating UVB-induced acute epidermal responses.

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Sustained trimethylation of histone H3 lysine 4 in the MKP-1 promoter region occurs after a single exposure to UVB

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Mitogen-activated protein kinase (MAPK) phosphatase-1 (MKP-1) plays an important role in regulating inflammatory responses by acting as a negative regulator of the MAPKs p38 and JNK. In addition, by regulating the JNK pathway, MKP-1 has been shown to play an important role in the response to ultraviolet radiation (UVR)-induced DNA damage. While histone modifications play an important role in controlling gene expression, their role in the transcriptional response to UVR has not been completely characterized. To examine the impact of UVR on histone lysine methylation, we analyzed the effect of UVR on histone H3 lysine 4 (H3K4) methylation at the promoter region of the MKP-1 gene. After a single UVB exposure of 40 mJ/cm2, there is an increase in H3K4 methylation within the promoter region of MKP-1. This increased methylation is present 8 hours after irradiation, peaks at 24 hours and is sustained for at least 72 hours. This suggests that a single exposure to UVB can induce stable histone modifications within the MKP-1 promoter region. This time course suggests that these changes may be sustained after cell division. Given the role of MKP-1 in controlling inflammation and DNA damage responses it will be important to fully characterize how its expression is regulated by UVR. Since histone modifications play a central role in gene expression, it will also be important to identify those histone modifications altered by UVR, their stability, and the pathways and enzymes involved in their regulation.

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UVB generated Platelet-activating factor agonist formation involves EGF-R-mediated reactive oxygen species

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Ultraviolet B radiation (UVB) exerts immunosuppressive effects which have been shown to be a major risk factor for skin cancer. Recent studies have implicated the lipid mediator Platelet-activating factor (PAF) in UVB-mediated effects, especially systemic immunosuppression. Previously, our group has demonstrated that UVB irradiation of keratinocytes stimulates PAF and oxidized glycerophosphocholines (ox-GPCs) with PAF-receptor agonistic activity (J. Biol. Chem. 280:35448-35455, 2005). The objective of the present studies is to define the mechanisms by which UVB stimulates PAF agonists. UVB irradiation of human epidermal KB cells resulted in both increased levels of reactive oxygen species (ROS) as well as PAF-R agonistic activity, and both were inhibited by anti-oxidants vitamin C and N-acetyl cysteine. Moreover, pre-treatment of KB cells with the EGF-R antagonist PD168393 also inhibited UVB-mediated ROS and PAF agonists. The next studies examined the ability of a systemic antioxidant to block UVB-mediated immunosuppression *in vivo*. C57BL6 mice were fed standard chow or chow supplemented with 10g/kg vitamin C for 10 days. Mice were then irradiated with UVB or treated with intraperitoneal injection of the PAF agonist CPAF, and dinitrofluorobenzene-induced delayed type contact hypersensitivity (CHS) was assessed. UVB irradiation inhibited CHS in mice fed standard chow, yet had no effect on mice fed vitamin C-enriched chow. However, CPAF treatment inhibited CHS equally in standard versus vitamin C-enriched chow. These studies suggest that UVB-mediated systemic immunosuppression is due to EGFR-stimulated ROS which results in PAF agonist formation.

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A topical antioxidant solution containing vitamins C and E with ferulic acid protects human skin from UV-induced gene induction of inflammatory mediators.

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Sunlight exposure generates oxidative stress in skin that can result in photoaging and skin cancer. The body uses low molecular weight antioxidants to neutralize the reactive oxidants that cause modification of nucleic acids, proteins and lipids; but protection achieved by oral ingestion is limited. To augment antioxidant protection, we have developed a a topical formulation that contains physiologic antioxidants, vitamins C and E, stabilized by a potent plant antioxidant, ferulic acid; optimized for chemical stability, percutaneous absorption, and photoprotection (J Invest Dermatol 125:826-832, 2005). The inflammatory response to sunlight exposure is one mechanism of damage to skin. We examined the mRNA expression of inflammatory cytokines after exposure to solar-simulated radiation in the skin to determine if topical anti-oxidants may inhibit their expression. An aqueous solution containing 15% L-ascorbic acid, 1% α-tocopherol and 0.5% trans ferulic acid (CEferulic) and a vehicle control solution were applied (2mg/cm2) daily for four days to 2 sites on the back of 8 human volunteers. On day four, each patch received 2 MED of solar-simulated irradiation. One day later, each patch and normal untreated skin were biopsied, mRNA isolated, cDNA prepared, and samples analyzed for IL-1α, IL-6, IL-8, IL-10 and TNFα by RTPCR. mRNA expressions in the vehicle treated skin was increased by UV irradiation (Mean Ratio Vehicle Treated/Normal Skin mRNA expression: IL-1 α = 7.19, IL-6 = 244.5, IL-8 = 275; IL-10 = 5.62; TNF-α = 2.46)(Wilcoxon signed ranks $p < 0.01$). Antioxidant treatment significantly reduced mean stimulation of IL-1α=49%,IL-6=65%, IL-8=90%, IL-10=65% (Wilcoxon signed ranks $p < 0.01$) and reduced TNFα expression(16%). These findings suggest that topical CEferulic provides substantial protection against UV-induced stimulation of proinflammatory cytokine mRNA expression which may be important in the prevention of the inflammatory response to sun exposure.

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Weak DNA damage responses after UVA increase mutation formation at UVA-induced DNA photoproducts

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While UVA generates less pyrimidine dimers (PDs) than UVB with equi-mutagenic doses, there is good evidence that PDs cause mutations not only with UVB, but also with UVA. We hypothesize that a weaker anti-mutagenic cellular response is responsible for a higher rate of mutation formation at PDs with UVA, as compared to UVB. To characterize the cellular reactions to UVA and UVB, and to correlate those to the mutagenic outcome, we exposed skin fibroblasts to the same doses of UVA (100, 200, 300 kJ/m2) or UVB (100, 200, 300 J/m2) that we have previously shown to be equi-mutagenic in those cells and performed detailed functional analysis of cell cycle kinetics using bivariate FACS and of DNA repair efficacy using two different DNA repair assays. We found that UVA induced a dose-dependent slowing of DNA replication very rapidly within 45 min, and a brief S-phase arrest after 90 min. Within two hours, UVA-irradiated cells restarted normal DNA replication. In contrast, UVB-irradiated cells started to arrest in S-phase only after 8 hours, but maintained this arrest for at least 48 hours. Activation (phosphorylation) of the known S-phase arrest mediator p95 was observed only with UVB, but not with UVA. A dose-dependent G1/S-arrest was observed after UVB, but was completely absent after UVA. Consistent with this observation, the G1/S-arrest mediator p53 was found to be activated very rapidly within 15 min after UVB, which persisted for at least 24 hours, while UVA activated p53 only briefly and much less prominently after 2 hours. The p53-dependent DNA repair protein XPC was induced more by UVB than by UVA. DNA repair efficiency, however, was not improved after UVB, likely due to damage to the DNA repair machinery itself. This was supported by the observation that DNA repair efficiency was worse after UVB-irradiation when p53 was inhibited by siRNA. Our data demonstrate not only much weaker, but also qualitatively different DNA damage responses after equi-mutagenic doses of UVA and UVB. These differences are very likely to affect rate of mutation formation and support our hypothesis.

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Melanin Quantification of *in vitro* and *in vivo* analysis through infrared fluorescence

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Of all of the chromophores present in the skin, melanin is the primary substance whose main physiologic function is to interact with light. The biological structure, property and function of melanin have become of considerable interest in understanding its role in pathological skin conditions. However, because there are no identifying spectral bands or peaks in the melanin absorption spectra, there are no effective methods to quantify melanin *in vivo*. Our recent study showed that melanin exhibits prominent autofluorescence under near infrared (NIR) excitation; therefore, it is hypothesized that NIR autofluorescence has direct correlation with melanin expression. Our objective is to measure *in vitro* NIR autofluorescence of various melanin concentrations in solution or suspension and *in vivo* measurements of normal and pathologic skin lesions to establish a melanin quantification method. Synthetic DOPA and Sepia melanin were prepared in deionized distilled water and in NH4OH. Both *in vitro* melanin samples and *in vivo* human skin NIR autoreflectances were measured under 785 nm excitation. Results: Both types of melanin in water showed similar fluorescence patterns in water: (i) signal intensity was increased at lower concentrations and reached its maxima at 2.50-5.00 mg/mL, (ii) when melanin concentrations were increased above 5.00 mg/mL fluorescence intensity was decreased. Melanin in NH4OH showed similar patterns. *In vivo* examinations have shown fluorescence to be higher when pigment is increased in normal skin, but in pathological conditions such as heavily pigmented melanoma fluorescence is decreased, which are consistent with the *in vitro* findings. In conclusion, we measured the NIR autofluorescence of melanin *in vitro* and *in vivo*. Preliminary results show that NIR autofluorescence is a promising method in quantification of melanin *in vivo*.

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Spectroscopic characterization of dynamics of pigment reactions of human skin to UVA radiation

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The response of human skin to UVA irradiation has been assessed visually and histologically. The goal of this investigation was to determine the contribution of major skin chromophores responsible for visually perceived changes. Twelve healthy volunteers were exposed to a number of doses (47, 22, 15 J/cm2) broadband UVA radiation (320 – 400 nm) at a fluence rate of 20 mW/cm2. The skin reactions were assessed clinically and with a diffuse reflectance spectroscopy from 30 seconds to 2 weeks. Skin reactions observed following broadband UVA radiation ranged from strong erythema reaction for light-complexion individuals to pigment with no erythema for melano-competent individuals. Skin chromophores including melanin, oxygenated hemoglobin, deoxygenated hemoglobin and scattering were monitored over time (immediately after exposure, 1, 2, 3, 24 hours, 1 and 2 weeks). Melanin pigmentation exhibited a biphasic process with a maximum immediately after exposure, with a rapid decrease, reaching a minimum at 3 hours, followed by a continuous increase for 2 weeks. Oxygenated hemoglobin showed a maximum immediately after exposure, reaching minimum 2 hours post-exposure, followed by a second maximum at 24 hours, suggesting a distinct biphasic process from that of melanin. Scattering parameter progressively increased over the period of 2 weeks. UVA produced pigmentation with a spectroscopic signature distinct from that of native pigment, represented as a decrease in absorbance in the blue wavelength region. This spectral feature disappeared 2 hours post-exposure and yielded a pigment whose spectral shape resembles UVB-induced pigment and native pigment, indicating *de novo* melanogenesis. In conclusion, pigment formed in human skin following exposure to broadband UVA radiation includes contribution from melanin and oxygenated and deoxygenated hemoglobin as well as scattering. Melanin alone cannot account for the observed pigmentary changes due to UVA exposure.

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Occupational photocontact allergy produced by carprofen

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A 42-year old had recurrent episodes of facial dermatitis that she attributed to working with Rimadyl® (a non-steroidal anti-inflammatory drug [NSAID], carprofen, used in veterinary medicine). By the time she attended the dermatology clinic this problem had caused her to resign from her work in a pharmaceutical factory. Patch testing and photopatch testing showed photoallergy to carprofen. The same year, a 47-year old was admitted with erythrodermic dermatitis. Chronic actinic dermatitis was considered, amongst other possible diagnoses, but excluded by normal phototesting and patch testing. Later, it became evident that remissions of dermatitis, which tended to affect exposed sites more than covered sites, coincided with periods off work. She was a secretary in the same factory that the first patient had worked in. Her dermatitis remained undiagnosed; further investigation included patch testing and photopatch testing to carprofen. She showed both contact allergy and photoallergy. Although her exposure was indirect these results did appear relevant and her skin only completely cleared once she got a new job. There are limited reports of patch testing and photopatch testing to this chemical so we tested controls. We stopped this after one of three controls developed active photoallergic sensitisation. After occupational health input to the factory, several members of staff there were referred of whom a further 3 were found to have carprofen photoallergy. Subsequently, a similar single case of occupational photoallergy to carprofen has been investigated and reported from another centre (Reference). This NSAID appears to be a potent photoallergen with sensitisation possible after limited exposure. Photopatch testing to non-standard series test agents should be considered in all patients with undiagnosed exposed site dermatitis. Reference: Walker SL, Ead RD, Beck MH. Occupational photoallergic contact dermatitis in a pharmaceutical worker manufacturing carprofen, a canine nonsteroidal anti-inflammatory drug.

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A new melanoma antigen FABP7 is a potential target for diagnosis, immunotherapy, and molecular target therapy

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The identification of molecules preferentially expressed in cancer cells is important for development of new diagnostic and therapeutic methods. We attempted to identify over-expressed molecules in melanoma by comparing an expression profile of melanoma with those of various normal tissues using GeneChip, and identified FABP7. Actual expression was confirmed by RT-PCR, Northern blot, Western blot, and immunohistochemical analyses. Function of FABP7 was evaluated using cell proliferation and Matrigel invasion assays with FABP7 specific siRNAs and FABP7 cDNA transfection. Immunogenicity of FABP7 was evaluated by detecting IgG Ab specific for FABP7 in sera from patients using Western blot and phage assays. Immunohistochemical study revealed that FABP7 was expressed in 11 of 15 melanoma tissues. By down-regulating the FABP7 expression with the FABP7 specific siRNAs, *in vitro* cell proliferation, and Matrigel invasion of some melanoma cell lines were significantly inhibited. Over-expression of FABP7 in a FABP7 negative embryonic kidney cell line by transfecting the FABP7 cDNA, resulted in enhanced cell proliferation and Matrigel invasion, indicating that FABP7 plays some roles on malignant phenotypes of melanoma cells. IgG Abs specific for the phage or bacterial recombinant FABP7 protein were detected in 14 of 25 (56%) or 8 of 31 (26%) sera from melanoma patients, respectively, but not in any sera from healthy individuals, indicating that FABP7 is an immunogenic antigen in melanoma patients. These results indicated that FABP7 is a potential target for development of new diagnostic and therapeutic methods, including immunotherapy and molecular target therapy.

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Culture characteristics of melanocytes from vitiligo patients

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Vitiligo is a pigmetary disorder of unknown cause characterized by depigmented patches due to destruction of melanocytes. The purpose of this study was to investigate the morphological feaatures of cultured melanocytes from normal and vitiligo patients. Melanonocytes were isolated and cultured from skin of normal and vitiligo patients using melanocte growth media. Cultured melanocytes were examined for morphological changes with the help of inverted microscope. For this study we cultured five normal and 15 vitiligo patient melnocytes. Vitiligo and normal melanocytes showed some significant morphological differences, these were: 1)Dendrites were less in number, less in branching, small in size and thick as compared to melanocytes of normal, which were more in number, branching, large in size and thin. 2)Dendrites stopped growing after branching and were blunt at the edges as compared to normal melanocytes, which grow even after branching. 3)Dendrites showed less or no connections with each other as compared to normal melanocytes dendrites, which were connected to each other. 4) Vitiligo melanocytes had more number of melanosomes as compared to normal melanocytes. In conclusion, melanocytes cultured from vitiligo patients showed some significant morphological differences which have not been reported earlier.

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Expression of heat shock protein 105 and 70 in malignant melanoma and benign malanocytic nevi

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Heat Shock Protein(HSP) restores immature proteins or denatured proteins caused by cell injuries, plays an important role in the immune system, and thus protects cells. Also some heat shock proteins (HSPs) are elevated substantially in malignant tumors developed in diverse organs. But the expressions of HSP in association with melanoma, the most invasive type among skin cancers, have not been studied. Therefore, by various methods, we examined the expression pattern of heat shock protein 70 and 105 in melanoma, benign melanocytic nevi and normal tissue. The specimens of 2 of malignant melanoma, Benign melanocytic nevi and normal skin were analyzed for heat shock protein 70 and 105 expressions using Western blot analysis. And the specimens of 8 of malignant melanoma, 16 of Benign melanocytic nevi and 2 of normal skin were analyzed for heat shock protein 105 expression by immunohistochemical studies. The western blot analysis showed that heat shock protein 70 was all overexpressed in normal tissue, benign melanocytic nevi and malignant melanoma. On the other hand, regarding heat shock protein 105, it was hardly expressed in normal tissues and benign melanocytic nevi. But in HeLa cells and malignant melanoma, heat shock protein 105 was overexpressed. And immunohistochemical examination of HSP 105 showed similar to the result of Western blot analysis. Normal skin and benign melanocytic nevus were expressed weakly. But Malignant melanoma was expressed very strongly. In our study, the HSP 70 was overexpressed in all of 3 tissue types. But HSP 105 was hardly expressed in normal tissues and benign melanocytic nevi, but overexpressed in malignant melanoma. Theresore, HSP 105 is thought to be a tumor-associated antigen of malignant melanoma, and by more developed studies, it may be applied widely to its diagnosis and treatment, and the prediction of prognosis.

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The inhibitory effect of phytoclear-el1 on melanogenesis

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Phytoclear-EL1, an extract from Euphorbia lathyris seeds, has a whitening effect due to inhibition of gene expression and tyrosinase activity. Although many studies have been reported inhibitory effect of arbutin on melanogenesis, there are almost no reports for inhibitory effect of phytoclear-EL1. The purpose of this study was to investigate the inhibitory effect of phytoclear-EL1 on melanogenesis with respect to the *in vitro* and *in vivo* pigmentary system. Our methods included cultured B-16 melanoma cells and thirty human volunteers were used for *in vitro* and *in vivo* studies, respectively. Also, the effect of phytoclear-EL1 on UVB-induced hyperpigmented lesions was examined through a subjective and objective measurement. The number, melanin contents and tyrosinase activity of cultured B-16 melanoma cells decreased in the group applied with 5μg/ml phytoclear-EL1 as compared to that of the control group *in vitro*($p<0.05$). *In vivo* study, the 0.2% phytoclear-EL1-applied group showed higher values than that of the control group with respect to mean L values before and 7 weeks after applying phytoclear-EL1. The inhibitory effect of phytoclear-EL1 among groups in decreasing order was as follows: 0.2% phytoclear-EL1 6 positive control (3% arbutin) > placebo B > placebo D. These results demonstrate that phytoclear-EL1 *in vivo* and *in vitro* have an inhibitory effect on melanogenesis. Phytoclear-EL1 may be suggested as a candidate compound that controls hyperpigmentary disorders. Phytoclear-EL(Euphorbia lathyris)1, Melanogenesis, non-invasive skin measurement of pigmentation.

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Targeting glycogen synthase kinase-3 in human melanoma

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The roles of GSK-3/beta-catenin signaling in melanoma progression have not been clearly defined. In our functional survey of specific melanoma-associated alterations, we found a surprising anti-oncogenic role of GSK-3/beta-catenin signaling. Introduction of an activated or stabilized form of beta-catenin antagonized oncogene-driven transformation of human epidermal melanocytes (HEMs). This antagonistic effect was phenocopied by treatment of HEMs and oncogene-driven HEMs with a small molecular GSK-3 inhibitor, BIO. Whereas the growth of primary HEMs and HEMs engineered with oncogenes are exquisitely sensitive to inhibition by BIO treatment, the growth of melanoma cell lines derived from patients, in contrast, showed variable sensitivities to GSK-3 inhibition. Current efforts to define melanoma expression signatures for GSK-3 inhibitor susceptibility and responsiveness may shed light on potentially novel prognostic markers and therapeutic targets.

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Expression of human endogenous retrovirus K in melanoma

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Human Endogenous Retroviruses (HERVs) have been implicated in the pathogenesis of cutaneous malignant melanoma (MM). HERV sequences comprise about 8% of the human genome and represent a reservoir of potentially pathogenic genes. The aim of this study was to detect and quantify the expression of HERV-K *pol*, *env* and *rec* genes in human MM cell lines and archival primary MM. A new real-time quantitative PCR method to analyse the expression of HERV-K mRNA in archival, formalin-fixed paraffin-embedded (FFPE) tissue was developed, utilising short amplicons to avoid problems of RNA fragmentation. Specificity of the PCR-amplified products was confirmed by sequencing. Studies of HERV-K gene expression in two MM cell lines (WM2664 and A375) showed expression of full-length but not spliced *env* or *rec* mRNA in WM2664, whereas expression of full-length and spliced *rec* mRNA, but not spliced *env* mRNA, was observed in A375. Full-length mRNA and unspliced *env* mRNA was found to be expressed in all archival tissue samples studied, comprising benign naevi (n = 8) and primary MM (n = 8). No correlation was observed between the levels of expression and the clinico-pathological subtype or Breslow thickness of MM. There were no significant differences in the levels of expression of *pol* and unspliced *env* between naevi and MM. None of the naevi but 2/8 MM expressed *rec*. The expression of spliced *rec* mRNA in primary MM and benign naevi has not previously been reported. Rec, a viral accessory protein, physically and functionally interacts with the promyelocytic leukemia zinc finger (PLZF) tumour suppressor and it has been suggested that Rec may be oncogenic by derepressing c-myc through the inhibition of PLZF. This work has successfully explored a new real-time PCR method that can quantify HERV-K gene expression in FFPE samples. It has been used to observe full length HERV-K mRNA expression in MM cell lines and all primary MM and benign naevi studied. The expression of *rec* by some MM requires further investigation.

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CD147/basigin plays an important role in tumor glycolysis in association with monocarboxylate transporter

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Cancer cells require glycolysis for cellular energy resulting in excessive levels of production and the secretion of lactate. Lactate reduces extracelluar pH and then contributes to the invasion, angiogenesis, metastasis, and multi-drug resistance of tumor cells. The human cell-surface molecule CD147/basigin is over-expressed in many tumor cells and has been implicated in a specific- and strong interaction with the monocarboxylate transporter-1 (MCT1) that mediates the transport of lactate through the plasma membrane. Here, we examined whether CD147/basigin, highly expressed in melanoma cells, is involved, via the association with MCT1, in the transport of lactate, the end product of glycolysis. We used RNAi technology to knock down the expression of CD147 in A375 melanoma cells, and RT-PCR and Western blots to study its expression. The co-localization of CD147 and MCT1 on the cell membrane was determined by confocal microscopy analysis. The rate of glycolysis was assessed by measuring the extracelluar lactate level spectrophotometrically. We found that A375 cells manifested remarkably higher CD147 and MCT1 expression than normal human melanocytes (NHMC), and CD147/basigin and MCT1 were co-localized on the A375 cell membrane. The silencing of CD147/basigin in A375 cells clearly down-regulated the expression of MCT1 and abrogated the co-localization of CD147/basigin and MCT1. The glycolysis rate was remarkably higher in A375 cells than NHMC ($p<0.001$); it was significantly decreased ($p<0.001$) by inhibiting the expression of CD147. Our findings strongly suggest that highly-expressed CD147 interacts with MCT1 on the plasma membrane and plays a key role in tumor-cell glycolysis. The novel function of CD147/basigin in tumor-cell glycolysis may be fundamental in tumorigenesis and may provide new insights into the prevention of tumorigenesis and progression of malignant melanoma in association with CD147/basigin.

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The involvement of p53 in senile lentigo

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Benign pigmentations were once a focus in the cosmetic field as aesthetic defects, but now, this phenomenon draws more attention from outside the cosmetics world. Recent studies revealed the mechanism that p53, a tumor-suppressor protein, promotes pigmentation following UV irradiation by direct transcriptional activation of pro-opiomelanocortin (POMC) in the skin. Given that p53 can be activated by various kinds of cellular stress including aging, this finding may explain the occurrence of age spots (senile lentigo). The aim of this study was to confirm the involvement of p53 in the hyperpigmentation in senile lentigo (SL). First, we investigated the presence of p53 by immunohistochemistry in lesional and perilesional skins. As a result, we found stronger expression of p53 in the lesional epidermis than in the perilesional epidermis. To examine the impact of the difference in p53 expression levels, we analyzed gene expressions of p53 transcriptional targets (CDKN1A, GADD45A, MDM2) in both epidermis and determined that expression levels of these genes were significantly higher in the lesional epidermis than in the perilesional epidermis. We then speculated that p53 contributes to melanogenesis via the paracrine pathway involved in the epidermal hyperpigmentation. Therefore, we assessed the expression of stem cell factor (SCF) and endothelin-1 (ET-1) in cultured keratinocytes treated with and without 5-fluorouracil (5-FU), a known inducer of p53. As a result, 5-FU activated p53, which in turn resulted in increased gene expressions of these cytokines. Our findings suggest that accumulation of p53 caused by long-term sun exposure and aging regulates chronic hyperpigmentation via the paracrine melanogenic cytokines.

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Persistent molecular changes after repetitive *in situ* UV exposure of human skin

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The incidence of melanoma has tripled in the past 4 decades, and epidemiological and laboratory data provide strong evidence that UV radiation is a major causative factor for this malignancy. We investigated long-term consequences of repetitive UV exposures of human skin in situ. We examined two sets of biopsies taken 1- 4 yrs after repetitive UV exposures to 95% UVA/5% UVB (Source 1, 6 subjects) or with Solar Simulated Radiation (Source 2, 4 subjects). Changes in protein expression of melanogenic factors (tyrosinase, MART-1, MITF), growth factors and their receptors (SCF, c-kit, bFGF, FGFR-1, ET-1, ETBR, HGF, GM-CSF), adhesion molecules (β-catenin, E-, and N-cadherins), cell cycle proteins (PCNA, cyclins D1 and E2) as well as Bcl-2, DKK-1 and DKK-3 were analyzed by fluorescence immunohistochemistry. Changes in RNA levels (for ETBR) were detected by tissue in situ hybridization. More than 1 yr after UV exposure 1/6 of Source 1 and 3/4 of Source 2-irradiated samples still showed a significantly increased expression of tyrosinase. Most of the molecular markers examined showed no detectable changes in their expression at >1 yr after UV irradiation. While an increase in ETBR protein expression was detected in 3/10 UV-treated (2/6 Source 1 and 1/4 Source 2) subjects, ET-1 protein expression and ETBR mRNA expression showed no change. In summary, among 20 factors examined, only tyrosinase and ETBR protein expression, and only in some subjects, were elevated at >1 yr post UV exposure. This suggests that persistent post-UV exposure changes are very minor and show a considerable subject-to-subject variation. A possibility that changes in the expression of the ETBR protein trigger downstream activation of abnormal melanocyte proliferation (and, hence, might potentially lead to melanoma) deserves further investigation.

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Tris (dibenzylideneacetone) dipalladium (Tris DBA), an N-myristoyltransferase 1 inhibitor, is effective against melanoma growth *in vitro* and *in vivo*

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Melanoma is a solid tumor that is notoriously resistant to chemotherapy, and its incidence is rapidly increasing. Recently, several signaling pathways have been demonstrated to contribute to melanoma tumorigenesis, including constitutive activation of MAP kinase, Akt and Stat-3. The activation of multiple pathways may account in part for the difficulty in treatment of melanoma. In a recent screen of compounds, we found that an organopalladium complex showed significant antiproliferative activity against melanoma cells. This complex, tris (dibenzylideneacetone) dipalladium (Tris DBA), has activity against B16 murine and A375 human melanoma *in vivo*. Tris DBA inhibits several signaling pathways including activation of MAP kinase, Akt, Stat-3 and S6 kinase activation, suggesting an upstream target. Tris DBA was found to be a potent inhibitor of N-myristoyltransferase 1 (NMT-1), which is required for optimal activity of membrane based signaling molecules. Perhaps the most well studied membrane signaling molecule is c-src, which may be upstream of MAP kinase, Akt, Stat-3 and S6 kinase. Tris DBA treatment decreased expression of c-src. Tris DBA is thus a novel compound that is a member of a class of noble metal complexes with potential antitumor activity. Further preclinical evaluation of Tris DBA and related complexes is warranted.

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Effective immunotherapy with α-GalCer-loaded embryonic stem cell-derived dendritic cells expressing multiple antigens against B16 melanoma

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Genetically manipulated dendritic cells (DCs) expressing cancer antigens are considered to be a promising means for cancer immunotherapy. However, many cancer vaccine trials using DCs expressing cancer antigens have been reported without a satisfactory result. It is expected that in vivo transfer of DCs simultaneously presenting cancer antigens and α-galactosylseramide (α-GalCer) stimulate both tumor reactive T cells and NKT cells, thus resulting in a potent anti-cancer immunity. We have established a method to generate dendritic cells from murine embryonic stem cells (ES-DCs). Genetic modification of murine ES-DCs can be accomplished without the use of viral vectors, by the introduction of gene-expression vector plasmids into undifferentiated ES cells by electroporation and subsequent induction of differentiation of the transfectant ES cell clones to ES-DCs. ES-DC technology therefore provides us a novel means for cancer immunotherapy. We examined the effects of multiple antigen-targeted immunotherapies by ES-DCs expressing melanoma associated antigens (GPC3, SPARC, TRP2 and hgp100) expressed in B16 melanoma. CTLs specific to each antigen were sensitized by the *in vivo* transfer of ES-DCs transfected with either murine GPC3, SPARC, TRP2 or hgp100 gene. The simultaneous *in vivo* transfer of these ES-DCs protected the recipient mice from a high dose subcutaneous challenge with B16-F10 melanoma. Then, the effect of α-GalCer-loaded ES-DCs on peritoneal dissemination and spontaneous lung metastasis of B16-BL6/Luc, a melanoma stably expressing luciferase, was evaluated. α-GalCer-loaded ES-DCs expressing multiple antigens showed a significantly greater effect on inhibiting the growth of tumor cells than vehicle loaded ES-DCs or α-GalCer loaded ES-DCs without antigens. In conclusion, α-GalCer-loaded ES-DCs expressing multiple antigens induce a potent therapeutic effect against B16 melanoma.

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Matricellular protein tenascin-C is a key component in the melanoma stem cell niche that maintains side-population

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Tenascin-C is a member of matricellular proteins that are modulators of cell-matrix interactions and cell functions. Although over the last two decades it has been extensively described that the production of tenascin-C was highly upregulated in melanoma and other tumors, little is known about its functions for tumor growth, survival, and metastasis. Recent studies indicate of tenascin-C's role in the stem cell niche. The aim of the study was to clarify the role for tenascin-C in melanoma. Microarray experiments revealed a correlation of melanoma progression with increase in tenascin-C mRNA. Western blot analysis showed that tenascin-C was strongly upregulated when melanoma cells from the same patients were grown as 3D spheres (enriched for stem cells), compared to 2D (adherent) cells. Tenascin-C knock-down, mediated by shRNA-lentiviruses, significantly increased attachment of melanoma cells from spheres when compared to control cells. Tenascin-C was shown by others to alter the response of neural stem cells to BMP-4. While BMP-4 treatment of melanoma cells induced neuronal cell-like trans-differentiation, knockdown of tenascin-C inhibited the effect of BMP-4 on melanoma cell trans-differentiation in 3D spheres. The side population (SP) cells have been shown to exhibit stem cell characteristics and they are candidates for dormant tumor cells because of their high efflux/drug resistance activities. Melanoma cell lines cultured as 3D spheres contained SP cells up to 10% and these were highly tumorigenic in NOD/SCID mice. The tenascin-C knockdown decreased the number of the SP cells from 10% to 0.1% in the melanoma spheres. Expression of multiple ABC transporters including ABCG2 was downregulated by the tenascin-C knockdown. These data suggest that tenascin-C has a critical role in drug resistance of melanoma cells by contributing to the niche for stem cells.

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Inhibition of MEK with AZD6244 is cytostatic as a monotherapy in melanoma, but cytotoxic when combined with docetaxel leading to tumor regression *in vitro* and *in vivo*

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Disseminated melanoma is highly therapy-resistant. The finding that 66% of melanomas harbor the activating BRAFV600E mutation has raised expectations for targeting the Ras/RAF/MEK/ERK pathway in melanoma. This study addresses the anti-melanoma activity of the MEK inhibitor AZD6244 (ARRY-142886). We recently have demonstrated that growing melanoma cells as 3D collagen-implanted spheroids enhances resistance to the MEK inhibitor U0126. Here we investigated the anti-melanoma activity of AZD6244 in 2D cell culture, the 3D spheroid model and an *in vivo* model. In 2D cell culture, AZD6244 was cytostatic and reduced the growth of melanoma cells in a concentration-dependent fashion through induction of G1-phase cell cycle arrest. In our 3D spheroid model the effects of AZD6244 were largely cytostatic and reversible, with drug washout leading to spheroid re-growth. 1205Lu cells were grown as tumor xenografts in SCID mice. After tumor establishment, mice were dosed twice daily with 0, 10 or 30 mg/kg AZD6244 orally. AZD6244 treatment decreased phospho-ERK in the tumors and significantly suppressed tumor growth. The original tumors remained viable, suggesting that AZD6244 monotherapy was largely cytostatic, and not pro-apoptotic in this model. Co-administration of AZD6244 (30 mg/kg) with docetaxel (15mg/kg) led to tumor regression, indicating the potential for MEK inhibitor/chemotherapy drug combinations. For the first time, we show here an inhibitor that directly targets the MAPK pathway in melanoma to correlate *in vitro* and *in vivo* data. AZD6244 fully inhibits growth at well-tolerated doses *in vivo*. Because AZD6244 is largely cytostatic in these melanoma models, it is important to define effective drug combination partners that lead to tumor regression. Here, we have shown that the co-administration of AZD6244 with a commonly used taxane, docetaxel, leads to a reduction in the size of the established melanoma xenografts.

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Assessment of the extent of partner assisted total body skin self-examination in an intervention designed to increase performance

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This study determined the extent of total body skin self-examination (SSE) in people with a history of melanoma, who reported getting partner assistance. Subjects were enrolled in a randomized trial of an educational intervention with either the patient or the patient and their partner. At the baseline visit, they participated in a demonstration of the ABCDE (asymmetry, border irregularity, color variegation, diameter ≥6 mm, and evolution) rule and a 15 minute skills training session. During the 4-month study period, subjects recorded concerning lesion(s) onto a numbered body map. At the follow-up visit, the subjects received a total body skin examination by a dermatologist who recorded areas of concerning lesions. We hypothesize that subjects with partner assistance recorded concerning lesions in areas of the skin that were easily visualized and not considered sexually sensitive (NS) and were less likely to record concerning lesions in hard to see body areas (HNS) or sexually charged areas including the genitalia (HS). Out of the 130 participants in the study, 56 subjects reported partner assistance (SPA). Results show that there were fewer lesions of concern recorded by SPA on the body maps in HS (mean = 0.60 lesions) and HNS (3.57 lesions), as compared to NS (9.00 lesions). There was also a difference in the mean number of lesions recorded in HS by SPA as compared to the dermatologist. While lesions existed in HNS and HS, SPA were either not examining these areas or unable to examine them with to same degree as NH. This may be attributed to issues of body and genital self-image that are affected by cultural upbringing and societal conservative norms. Although further research is needed, our results indicate that specifically addressing HNS and HS during SSE intervention may be warranted to increase patient adherence with complete total body skin examinations.

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AMPK activation mediates the de-pigmentation effects of EGF in cultured skin melanocytes

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AMP-activated protein kinase or AMPK is an evolutionarily conserved sensor of cellular energy status, activated by a variety of cellular stresses that deplete ATP as well as other stresses such as oxidative stresses or UV radiation. However, the possible involvement of AMPK in melanogenesis or skin pigmentation has not been fully studied. In this study, we found that EGF induces down-regulation of melanogenesis in cultured mouse skin melanocytes (B10BR cell line). EGF induces AMPK activation in EGFR/LKB1 dependent manner. AMPK inhibitor compound c and AMPK specific siRNA attenuate EGF-induced down-regulation of melanogenesis or de-pigmentation in B10BR cells. We also found that EGF treatment down-regulates tyrosinase in a MITF independent manner. And this effect was also mediated by AMPK activation. Furthermore, activation of AMPK also negatively regulates mTOR activation which is involved in melanogenesis in B10BR cells. Taken together, our results provide new insights into the molecular mechanisms of EGF-induced de-pigmentation, suggesting that modulation of AMPK pathway may lead to darkening or whitening of the human skin in pursuit of cosmetics purposes.

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Development of a multi-gene classifier to determine the risk of melanoma in suspicious pigmented nevi by non-invasive tape stripping

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Melanoma is diagnosed by histopathologic examination of biopsies in only 3-10% of suspicious pigmented nevi. Previously, we demonstrated that RNA, harvested by non-invasive Epidermal Genetic Information Retrieval (EGIR) tape stripping, can differentiate normal skin from melanoma and atypical nevi. The objective of this study is to develop a multi-gene classifier to determine the risk that a suspicious pigmented nevus contains melanoma by means of a non-invasive, EGIR-based assay. Suspicious lesions were tape stripped and then biopsied as per standard of care. RNA isolated from the tape by EGIR was amplified and assayed on the Affymetrix U133 plus 2.0 GeneChip. Microarray data were normalized using the GCRMA algorithm and the resulting data from melanomas and nevi were randomly divided into training and testing sets. Two different analytic methods, prediction analysis for microarrays (PAM) and random forest (RF), were used for classification and class prediction. A 20 gene classifier was constructed using PAM. The sensitivity, specificity, and accuracy of this classifier, determined from the test dataset, was 100%, 91%, and 92%, respectively. In comparison, the sensitivity, specificity, and accuracy of a 27 gene classifier developed from RF was respectively 94%, 89%, and 91%. These results indicate that PAM is superior to RF for class prediction of melanoma, demonstrating high sensitivity without sacrificing specificity. Furthermore, the PAM-generated 20 gene classifier provides a basis for a clinical assay that can be used to determine the risk that a suspicious pigmented lesion contains melanoma.

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Identification of cells initiating human melanomas

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Tumour-initiating cells capable of self-renewal and differentiation, which are responsible for tumour growth, have been identified in human haematological malignancies and solid cancers. If such minority populations are associated with tumour progression in human patients, specific targeting of tumour-initiating cells could be a strategy to eradicate cancers currently resistant to systemic therapy. Here we identify a subpopulation enriched for human malignant-melanoma-initiating cells (MMIC) defined by expression of the chemoresistance mediator ABCB5 and show that specific targeting of this tumorigenic minority population inhibits tumour growth. ABCB5+ tumour cells detected in human melanoma patients show a primitive molecular phenotype and correlate with clinical melanoma progression. In serial human-to-mouse xenotransplantation experiments, ABCB5+ melanoma cells possess greater tumorigenic capacity than ABCB5− bulk populations and re-establish clinical tumour heterogeneity. *In vivo* genetic lineage tracking demonstrates a specific capacity of ABCB5+ subpopulations for self-renewal and differentiation, because ABCB5+ cancer cells generate both ABCB5+ and ABCB5− progeny, whereas ABCB5− tumour populations give rise, at lower rates, exclusively to ABCB5− cells. In an initial proof-of-principle analysis, designed to test the hypothesis that MMIC are also required for growth of established tumours, systemic administration of a monoclonal antibody directed at ABCB5, shown to be capable of inducing antibody-dependent cell-mediated cytotoxicity in ABCB5+ MMIC, exerted tumour-inhibitory effects. Identification of tumour-initiating cells with enhanced abundance in more advanced disease but susceptibility to specific targeting through a defining chemoresistance determinant has important implications for cancer therapy.

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A small interfering p53R2-targeting RNA inhibited the proliferation and enhanced the chemosensitivity of malignant melanoma

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Malignant melanoma (MM) is an aggressive and chemoresistant skin cancer, characterized by rapid metastasis and poor prognosis. Development of innovative therapies with improved efficacy is critical for treatment of MM. The p53R2 gene, encoding the ribonucleotide reductase (RR) small subunit 2 homologue, is induced by several stress signal including DNA–damaging agents that activate p53. The p53R2 gene product increases the deoxynuclotide triphosphate (dNTP) pool in the nucleus, which facilitates DNA repair and synthesis. In this study, we examined the expression and clinicopathological significance of p53R2 in MM. The positive p53R2 was expressed in 60 of 76 subjects (78.9 %) examined. Expression of p53R2 was correlated with depth of invasion and stage with statistic significance. To investigate the roles p53R2 in MM, KHm4 MM cell line and p53R2-targeting small interfering (si) RNA were employed. P53R2, highly expressed in KHm4 cells, was successfully knocked down by the siRNA. Treatment of KHm4 cells with p53R2-targeting siRNA inhibited cell growth by approximately 35 %, suggesting that p53R2 play a critical role in melanoma proliferation. Moreover, p53R2-targeting siRNA inhibit the growth of KHm4 cells in association with anti-cancer agents such as DITC or nimustin compared with anti-cancer agents alone. These findings suggest that silencing of the p53R2 gene by siRNA enhanced the chemosensitivity to DTIC and nimustion. Taken together, p53R2 could be a therapeutic target enhancing the effectiveness of chemotherapy in p53R2 positive melanoma.

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Inhibition of melanoma cell growth by re-expression of protein kinase C beta

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Protein kinase C (PKC) is a heterogeneous family of serine/threonine protein kinases that have diverse biological effects in normal and neoplastic melanocytes. Normal melanocytes require chronic PKC activation for growth in culture and undergo apoptosis when the phorbol ester TPA is removed from their growth media. In contrast, melanoma cells are growth inhibited or induced to undergo apoptosis when TPA is added to their growth media. To further explore the mechanism(s) behind this differential response to PKC activation, we analyzed the PKC isoform expression profile of normal human melanocytes, HVP16 E6/E7 immortalized melanocytes, and a panel of melanoma cell lines. We found consistent loss of PKCβ and up-regulation of PKCζ in immortalized melanocytes and melanoma lines at both the protein and mRNA levels. Since PKCζ is an atypical PKC isoform which is not activated by TPA, we focused on the functional role of PKCβ down-regulation in melanoma cells. To determine if the loss of PKCβ in melanoma cells played a role in their tumorigenic phenotype or was simply due to reduced differentiation function, we re-expressed PKCβ in melanoma cells and observed a dramatic loss of colony formation in soft agar, indicating that PKCβ loss in melanoma is important for neoplastic growth. PKCβ activation has also been linked to the oxidative stress response, and we found that the PKCβ inhibitor hispidin significantly inhibited UV-induced reactive oxygen species in normal melanocytes. Taken together, these results indicate that the down-regulation of PKCβ in melanoma promotes enhanced growth, possibly by reducing oxidative stress within melanoma cells. Since melanomas frequently have constitutive ERK activation, we hypothesize that PKC activation by TPA would cause over-stimulation of ERK which can inhibit melanoma cell growth. Thus, reduced PKCβ levels in melanoma may be a mechanism to dampen ERK signaling, especially under conditions of oxidative stress when PKCβ becomes activated. Therefore, pharmacologic agents which up-regulate PKCβ may be effective in the treatment of melanoma.

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Activity and expression of matrix metaloproteinases in vitiligo patients

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Vitiligo is a depigmenting disease of uncertain aetiopathogenesis. The purpose of this study was to investigate the matrix metaloproteinases of cultured melanocytes from normal subjects and perilesional and normal skin of vitiligo patients using serum free melanocyte growth media. Melanocytes were isolated and cultured from normal individual and perilesional and normal skin of vitiligo patient using serum free melanocyte growth media. Gelatin zymography was done to determine the activity of gelatinases (MMP-2 and MMP-9) using supernatant of cultured melanocytes of normal individual and perilesional and normal skin of vitiligo patient. RT-PCR: To check the expression of MMP-1, MMP-9 and TIMP-1 using cultured melanocytes of perilesional and normal skin of vitiligo patients. For this cDNA was formed using RNA extracted from the cultured melanocytes. cDNA was then used for checking the expression of MMP-1, MMP-9 and TIMP-1. Vitiligo patients showed more activity of MMP-9 and MMP-2 as compared to the normal skin. Even Perilesional melanocytes of vitiligo patient showed more activity of MMP-9 and MMP-2 and more expression of MMP-1 and MMP-9 as compared to the normal skin melanocytes of vitiligo patient; where as the expression of TIMP-1 is less in vitiligo perilesional melanocytes. In conclusion, vitiligo patient and normal individual showed differences in matrix metaloproteinases. Perilesional and normal skin melanocytes of vitiligo patient showed differences in matrix metaloproteinases. Increase in the expression and activity of matrix metaloproteinases in vitiligo might play a role in detachment of melanocyte and their death.

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Delayed diagnosis of melanoma due to laser treatment in twelve patients

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Laser therapy of melanocytic lesions is a controversial issue. Since targeted treatments by quality-switched laser systems exist, benign epidermal and dermal pigmented lesions can be addressed effectively. The incidence of malignant melanoma is still rising and early diagnosis with small tumour thickness is of enormous prognostic value. Twelve patients from our University Hospital are presented. They underwent laser therapy at a surgeon's, general practitioner's or dermatologist's private practice and revealed melanoma at the location of laser treatment. Concentrating studies of the records for all patients were performed. Clinical, histological and photo-optical data were collected and evaluated. Along with literature review findings, all information was evaluated with special interest in time and reason of diagnostic delay. A delay of diagnosis was revealed, with a time from laser treatment to melanoma-diagnosis from 5.75 to 84 months. The mean delay was 33.29 months in patients from our University Hospital and 36.1 months in twelve patients from literature. Two patients already died of melanoma and three are stage IV showing progressive disease with distant metastases. There were cases of initial clinical misdiagnosis as well as histological misdiagnosis due to insufficient samples. Lentigo maligna was treated in four cases. In three patients initial histological findings of benign nevi could retrospectively be approved. Possible explanations for these findings include inaccurate treatment margins, insufficient histological specimen, recurrence of deeper or non-pigmented melanocytic cells and the unverified impact of laser on transformation from benign into malignant melanocytes. We conclude that laser therapy can delay the diagnosis of melanoma, therefore with a negative impact on the prognosis. Our patients, just as many as worldwide literature presented during the past twenty years, reveal an underestimated medical problem. Exact dermatological diagnosis and histological proof is indispensable.

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Overexpression of mRNA-binding protein CRD-BP in malignant melanomas

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Wnt/β-catenin signaling pathway plays an important role in embryogenesis, stem cell maintenance, tumorigenesis, and aging. Here, we show that RNA-binding protein, CRD-BP (a transcriptional target of Wnt signaling pathway) is highly expressed in primary human malignant melanomas and melanoma cell lines with activated Wnt/β-catenin signaling pathway. Up-regulation of CRD-BP is associated with an elevated level of β-TrCP1 ubiquitin ligase receptor and activation of NF-κB signaling. Our results show that CRD-BP expression is important for melanoma cell proliferation and survival. Knockdown of CRD-BP in melanoma cells results in inhibition of NF-κB-dependent-transcription and induction of apoptosis. Inhibition of CRD-BP expression also resulted in suppression of melanoma cells growth in vitro. The employment of CRD-BP as a novel molecular marker of malignant melanomas and as a potential target for melanoma prevention and treatment is discussed.

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The 3,3,4,4,5,5-hexahydroxystilbene impairs melanoma progression

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Stilbenes comprise a group of polyphenolic compounds and exert beneficial effects on various malignancies. We aimed to increase the anti-tumor effects of stilbenes by generating a novel stilbene derivative, 3,3′,4,4′,5,5′-hexahydroxystilbene termed M8. In vitro, M8 dramatically inhibits the proliferation of human melanoma cells including the metastatic M24 met melanoma cell line. Cell cycle analyses of this metastatic cell line showed that the compound promotes G2/M arrest accompanied with a dose- and time- dependent upregulation of p21. To further evaluate the underlying mechanisms, we performed proteomic analysis of the M24 met cell line. Proteomic analysis revealed that M8 interferes with pathways, which are critically involved in the regulation of tumor progression and metastasis, DNA damage and p53 activation. Consistently, p53 and proteins involved in the mismatch repair machinery and in the double strand break repair were upregulated by M8. In a comet assay M8 was shown to induce a robust tail moment. DNA damage is induced by an M8 dependent release of nitric oxide (NO) and a subsequent nitrotyrosination of the reactive residue Tyr-69 in the actin sequence as verified by mass spectrometry. Moreover, M8 inhibits cell migration as shown in a Matrigel assay via blockade of ROCK-1 in vitro. We confirmed our data in vivo in a metastatic human melanoma SCID mouse model. In this model, we demonstrate that M8 significantly impairs tumor growth and inhibits cell proliferation. Importantly, M8 also interferes with the metastatic process, as M8 treatment could prevent metastatic spread of melanoma cells to distant lymphnodes in vivo. Our data indicate that M8 treatment is a novel and potent approach for the treatment of metastasising melanoma.

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Minocycline protects against H2O2-induced melanocyte apoptosis through inhibiting JNK and p38 activity

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Vitiligo is an acquired and progressive disorder manifested by selective destruction of melanocytes in the skin. The level of hydrogen peroxide or H2O2 has been reported to increase in plasma as well as in lesional skin of vitiligo patients, which is thought to contribute to the disappearance of melanocytes in vitiligo. JNK and p38 play an important role in nerve cell apoptosis induced by oxidative stress while minocycline possesses anti-oxidant activity and blocks oxidative stress-induced neurotoxicity. To investigate whether minocycline protects against H2O2-induced melanocyte apoptosis and to further study the molecular mechanism, we treated cultured mouse melanocytes (B10BR) with H2O2 in the presence or absence of minocycline. We observed that minocycline protects melanocytes against H2O2-induced apoptosis in a dose-dependent manner. H2O2 treatment induces activation of JNK and p38 as well as downstream signal components such as caspase 3 which serves as an apoptosis signal in most cells. Treatment with minocycline significantly inhibits H2O2-induced activation of JNK and p38, and the activation of caspase 3. Collectively, we conclude that minocycline has protective effects on melanocytes *in vitro* against H2O2-induced cell apoptosis and the action of minocycline is related to the inhibition of JNK, p38 and the downstream signal caspase 3. Our findings suggest that minocycline, clinically well-tolerated, safe and efficacious against rheumatoid arthritis, may be useful to prevent the melanocytes loss in the early stage of vitiligo.

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Molecular analysis of melanin in melasma

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Melasma is an acquired abnormal hyperpigmentation of the face of unknown origin. It is associated with pregnancy and estrogen administration. It has to be separated from other hyperpigmented conditions. There is little knowledge regarding its pathogenesis. It is not clear whether the mechanisms that lead to excessive skin colour are the same in each patient. In general it is assumed that melasma is due to excessive melanin production, In general it is assumed that Melasma is due to excessive melanin production, but considerations have not been made regarding excessive retention of melanin or abnormal metabolism of this molecule. We studied one male and 34 female melasma patients between 18 and 42 years of age who were not taking estrogens, had not been in treatment for the past 6 months, and were otherwise healthy. Tape stripping was used to obtain keratinocytes from the skin lesions, from adjacent normal skin, and from normal facial skin of age matched controls. Samples were analyzed for melanin content using visible spectroscopy and Raman spectroscopy. Differences in the amount of melanin among the three groups by the former method were not significant. Only three patients showed different compounds besides melanin which likely are choromophores of unknown nature. However, with Raman spectroscopy, a different molecular structure of altered melanin was found in 11 patients that were not clinically different from the others. It is not apparent if melanin exteriorization is a significant feature in melasma based on these findings. However, due to the Raman spectroscopy we conclude that melasma is a heterogeneous condition and more research is necessary to obtain better therapeutic approaches.

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Intracellular degradation of collagen by cathepsin K in melanoma cells – an alternative mechanism of melanoma invasion

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Degradation of extracellular matrix (ECM) proteins is a prerequisite for tumor invasion and is commonly thought to be mediated by proteases in the extracellular space. Cathepsin K (catK) is a lysosomal protease with strong collagenolytic activities well known for its bone-degrading properties in osteoclasts. It was recently described to be expressed in melanoma cells. A contribution of catK to melanoma invasion was suggested by the observation that a catK inhibitor strongly reduced invasion of melanoma cells through a collagen IV matrix. CatK-mediated degradation of bone collagen takes place within lysosomes after endocytosis of collagen and after secretion of catK into the highly acidic extracellular bone resorption lacunae. The pH in melanomas is probably not low enough to permit extracellular catK activity, as the pH optimum of catK is 5.5. We therefore hypothesized that catK contributes to melanoma invasion by mediating intracellular collagen degradation. We incubated two melanoma lines that strongly express catK with FITC-labeled collagen I or IV, and found that 14.3 % and 17.7 % of the cells exhibited internalized collagen within 24 hours. This collagen was located in lysosomes, as it was shown to co-stain with the lysosomal protein Lamp1. At earlier time points (3–10 hours), internalized collagen was found mainly in early, Lamp1-negative endosomes. Treatment of the melanoma cells with a highly specific catK inhibitor increased the percentage of cells with intracellular collagen 2.1- and 2.6-fold (p=0.03 for both melanoma lines). This indicates that the expression of catK limits detection of internalized collagen through rapid degradation. Our data demonstrate that intracellular degradation of collagen does take place within lysosomes of melanoma cells, and that catK contributes to this process, at least in part. Clinical trials targeting extracellular collagen degradation have failed. We suggest targeting intracellular collagen degradation, e.g. through inhibition of catK, as an alternative therapeutic approach to inhibit melanoma invasion.

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Serum melanin-related metabolites levels in psoriasis patients undergoing narrowband ultraviolet B phototherapy

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Ultraviolet-B (UVB) radiation from sunlight can result in sunburns and/or suntans. Narrowband UV-B (NBUVB), a new light source which is not yet widely available, is effective for treating generalised psoriasis without the use of psoralens. We measured the time course of changes in serum levels of melanin-related metabolites, 5-S-cysteinyldopa (5-S-CD) and 6-hydroxy-5-methoxyindole-2-carboxylic acid (6H5MI2C) in psoriasis patients undergoing NBUVB phototherapy. There were no studies on therapeutic UVB effects on serum these metabolites of human subjects. Eleven Japanese patients with generalised psoriasis vulgaris received NBUVB treatment 5 times per week, with an initial dose of 0.1 J/cm2. The dose was increased by 10% to 20% per treatment for more than 20 treatments. Serum samples were taken before, and 3, 7, 10, 14, 28 days after the phototherapy. After 4 weeks of NBUVB treatment, 9 of 11 patients were in remission, confirming the effectiveness of NBUVB for treating Japanese psoriasis patients. Two patients were dropped from the investigation before day 28 because of other complications. The mean level of 5-S-CD in serum was significantly elevated on days 7, 10 14, and 28 compared with that before the phototherapy. The serum 5-S-CD level peaked on day 10. The mean 5-S-CD level of psoriasis patients before treatment was elevated compared with normal subjects. The mean 6H5MI2C level in serum was significantly elevated on days 10 and 14 compared with that before the phototherapy. The serum 6H5MI2C level peaked on day 10. The mean 6H5MI2C level of psoriasis patients before treatment was not elevated compared with normal subjects. In conclusion, Serum 5-S-CD and 6H5MI2C levels were significantly increased by therapeutic UVB exposure. Sustained levels of 5-S-CD in psoriasis patients suggests they were pre-exposed to less-qualified UV which cause skin injury.

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Cadherin profile of normal human melanocytes *in situ* and in cell culture

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During the development and progression of malignant melanomas, an important role has been attributed to cell-cell adhesion molecules, notably to those of the cadherin superfamily. We have previously shown that in cultured melanoma cell lines the repertoire of cadherins is more heterogeneous than thought and that a subtype of melanoma cells contains, in addition to classical cadherins, the desmosomal transmembrane protein desmoglein 2 (Dsg2) as a solitary plasma membrane protein. Therefore, we have now examined homo- and heterotypic cell adhesions of normal epidermal melanocytes by confocal laser scanning, electron and immunoelectron microscopy, using sections of healthy human epidermis as well as monocultures of normal melanocytes and melanocyte-keratinocyte co-cultures. In normal human epidermis, melanocytes and keratinocytes are connected by small, plaque-bearing *puncta adhaerentia*, containing E- and P-cadherin and the set of plaque proteins typical of adhering junctions, while desmosomes and desmosome-specific proteins are absent. In cultured melanocytes, sucrose gradient centrifugation and co-immunoprecipitation show heterotypic protein complexes of E- and P-cadherin. Moreover, when taken in culture, melanocytes start to synthesize Dsg2 as a solitary desmosomal protein, a finding confirmed by Western blot, PCR and MALDI-TOF analyses. This protein can be co-immunoprecipitated with actin but occurs separate from the classical cadherins. As Dsg2 has been found in cultured melanocytes but not *in situ* in melanocytes residing in the basal epidermis, we hypothesize that its synthesis might involve proliferation. Indeed, immunostainings of tissue microarrays have demonstrated Dsg2 in a subset of nevi and of primary melanomas. The diagnostic and prognostic significance of this finding and the specific subtypes of Dsg2-positive tumors will have to be clarified in the future.

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Evaluation of the whitening effect of Brassicaceae extract on the skin. *In vitro* and *in vivo* studies

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Skin whitening has become a topic of considerable interest for consumers of personal care products across the globe. As a result, much research effort in the industry has focused on the development of skin whitening agents. In this study, we used image analysis in combination with Fontana-Masson staining and digital photography to evaluate the whitening effect of Brassicaceae extract on the skin. Comparative studies were conducted using 1% Brassicaceae extract for 72 hours, or 1% arbutin, known for its whitening effect, or a combination of both, on ex vivo skin. Melanin content was visualized by Fontana-Masson staining. Both Brassicaceae extract and arbutin-treated skin exhibited a significant decrease in melanin content. To confirm visual observations and quantify the results, image analysis was performed using several image processing steps and analysis of luminosity histograms. All data were normalized to the length of the basal layer within a given histological section. Normalized integrated values of histograms revealed a 49% decrease in melanin content in Brassicaceae extract-treated skin samples, compared to only 32% in arbutin-treated skin. Combination of the two products did not improve the Brassicaceae extract's whitening effect (-43%). A simple, blind *in vivo* study with 3% Brassicaceae extract was also carried out on 15 volunteers for 29 days. The volunteers applied the cream on the forearm twice a day. The results showed a very significant decrease in the melanin index. To confirm this result, we performed image analysis of photographs of age spots, using the same method described above. The luminosity of the spots was compared before and after treatment. An increase in luminosity was observed, which corresponds to a decrease in the spots' melanin content. Improvement reached 27% in some volunteers. These results demonstrate that Brassicaceae extract decreases skin pigmentation and can be of great use in whitening skin care products.

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The effects of preservatives on melanogenesis in a three-dimensional human epidermal model

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Melanogenesis in skin is stimulated in response to environmental factors, UV radiation, and chemical compounds. We examined whether the ingredients of dermatological formulations affect melanogenesis in vitro. The alkyl esters of *p*-hydroxybenzoic acid (PHBA), known as parabens, were used as representative preservatives. Because parabens are rapidly absorbed and metabolized into PHBA, which is less toxic than the parabens, we compared the effects of parabens, PHBA, and benzoic acid (BA). Normal human melanocytes were cultured in a medium containing a low concentration of methyl paraben (MP), and the melanin content was subsequently measured. Long-term exposure to MP significantly increased the melanin contents compared to the control, and dose-dependently. We then examined the effects of MP, ethyl paraben (EP), PHBA, and BA on melanogenesis using a three-dimensional reconstituted human epidermal culture model (MelanoDerm™). Thin sections of MelanoDerm™ stained with Fontana-Masson were observed microscopically. Parabens induced an increase in melanin content after 12 days. The melanin content was subsequently measured. Compared with the control, the treatment with 3 mM MP, and 3 mM EP significantly increased the melanin content by approximately 1.47-fold and 1.58-fold, respectively, after 12 days. PHBA and BA, however, had no effect on the melanin content. These results suggest that parabens are potent in inducers of melanogenesis, but that the metabolites of parabens, PHBA and BA, have no effect of melanogenesis. Additionally, the protein profiles of MelanoDerm™ treated with MP and the control were compared by two-dimensional electrophoresis. The expression of 26 proteins was increased and the expression of 36 proteins was down-regulated as a result of the treatment with MP. The identification of these proteins is currently in progress. Furthermore, to clarify the mechanism of the enhancement of melanin synthesis by parabens, a comparison of the protein expressions resulting from PHBA and methyl paraben treatments is required.

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Expression of metabotropic glutamate receptor 1 and phosphorylated extracellular signal-regulated kinase 1/2 in human melanocytic lesions

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Metabotropic glutamate receptor subtype 1 (mGluR1) is a G protein coupled receptor which mediates the activation of extracellular signal-regulated kinase (ERK) 1/2, and is functionally expressed in the central nervous system. Ectopic expression of mGluR1 in melanocytes has been shown to induce melanoma development in transgenic mice. In this study, we investigated the expression of mGluR1 and phosphorylated ERK (pERK) 1/2 in 48 cases of melanoma tissues consisting of 12 superficial spreading melanomas (SSM), 10 nodular melanomas (NM), 8 lentigo maligna melanomas (LMM), and 18 acral lentiginous melanomas (ALM) as well as 24 cases of nevi consisting of 16 common nevi (NCN), five blue nevi (BN), and three Spitz nevi using immunohistochemistry. In addition, mutation in BRAF exon 15 was also examined by direct sequencing. Expression of mGluR1 and pERK was identified in 12 (100%) and 12 (100%) in SSM, 10 (100%) and eight (80%) in NM, seven (88%) and six (75%) in LMM, 17 (94%) and 15 (83%) in ALM, nine (56%) and nine (56%) in NCN, one (20%) and one (20%) in BN, and three (100%) and three (100%) in Spitz nevi, respectively. Exon 15 BRAF mutation was observed in six (50%) in SSM, two (20%) in NM, one (13%) in LMM, and three of 16 (19%) in ALM, six (38%) in NCN, none (0%) in BN, and none (0%) in Spitz nevi. These results indicate that mGluR1 expression might be involved in early process of acquisition of autonomous growth of melanocytes, and further, despite the low frequency of BRAF mutation in some types of melanomas, the ERK signaling pathway is constitutively activated in the majority of melanomas possibly through mGluR1 expression.

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Melanocyte reservoir in Leukotrichia - Preliminary study

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Hair follicle melanocytes consist of two morphologically and functionally different types: pigmented mealnocyte, present in the infundibulum and bulb, and amelanotic melnocyte(AMMC), present in the outer root sheath. AMMC form the melanocyte reservoir in human skin. In the natural hair graying with age, overall and specific depletion of bulb melanocytes and AMMCs of human hair was established. Vitiligo on hairy areas like the scalp, eyebrows and pubic areas is frequentely associated with leukotrichia. Surgical treatment is commonly recommended because repigmentation of the white hair in vitiligo is usually difficult. It has been believed that white hair in vitiligo suggests the depletion of the melanocyte reservoir in the hair follicle. However, no study has revealed that white hair shows depletion of AMMCs. This study aimed to identify AMMC as a melanocyte reservoir in the white hair of vitiligo patients. Immunochemical stain using the antibody NKI/beteb, which recognize premelanosome-related antigens, revealed the presence of AMMC in the outer root sheath of depigmented hair of vitiligo. This result suggests the difficulty of repigmentation of leukotrichia is not due to absence of melanocyte reservoir, but other unknown cause, although there will be difference of time course for response between patients with and without leukotrichia.

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Polyclonality of BRAF mutations in acquired melanocytic nevus and primary melanoma

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Melanocytic nevi are thought to be senescent clones of melanocytes that acquired an oncogenic BRAF mutation, which is considered to be a crucial step in the initiation of melanocytic neoplasia. In this study, however, we observed a mosaic distribution of BRAFV600E and wild-type nevus cells within the lesions of acquired melanocytic nevus (AMN) by using laser-assisted microdissection followed by PCR amplification and sequencing of the BRAF exon 15. Furthermore, subcloning and subsequent sequencing in AMN from patients who were G/T heterozygotes for the single nucleotide polymorphism rs7801086, mapped about 2kb telomeric from the BRAF exon 15, showed that subclones harboring BRAFV600E mutation were accompanied by both the G allele and the T allele, indicating that even nevus cells with the same BRAFV600E mutation originated from different cells. In addition, we also showed polyclonality of BRAF mutations in a subset of primary melanomas by a sensitive shifted termination assay for detecting BRAFV600E mutation as well as subcloning and subsequent sequencing. These results clearly indicate that BRAF mutation is not an initial event in melanocytic neoplasia, and challenges a currently accepted model of BRAFV600E-induced nevus and melanoma formation.

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The roles of oxidase for melanogenesis in PML-1 melanoma cells

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Acral lentiginous melanoma (ALM) is the most common type of melanoma in Asian people. In this study, we obtained metastasized melanoma tissues from a Korean ALM patient, and then established the melanoma cell line named as PML-1 (Primary culture of Melanoma cell derived from Lymph node). The expression of the cell cycle regulators such as Rb (retinoblastoma), p53, p21 and cyclin D1 was significantly down-regulated in PML-1 cells as compared with normal melanocytes. To identify the genes differentially regulated in PML-1 melanoma cells, we performed oligonucleotide microarray, two dimensional electrophoresis and MALDI-TOF analyses. As a result, we obtained 9 candidates that showed highly up- and/or down-regulated expression in melanoma cells. Interestingly, PML-1 showed light brown pigmentation and the down-regulation of melanogenesis marker expression, including Tyrosinase, MITF and TRP1. Based on these results, we chose one interesting gene, oxidase, for a further study. RT-PCR and Western blot demonstrated that oxidase was markedly decreased in PML-1 cells. The melanogenesis in melanocytes was significantly decreased by an addition of oxidase inhibitors, while the overexpression of oxidase by recombinant adenovirus clearly induced the expression of melanogenesis markers in PML-1 cells. These results suggest that oxidase may have impact on melanogenesis positively.

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The AKT pathway and survivin expression are down-regulated by the anti-aging hormone Klotho in human melanoma cell lines

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Malignant melanoma is a highly aggressive cancer that frequently resists various chemotherapies. Inhibition of the EGFR/Ras-PI3K-AKT and Ras-RAF-MAP pathways are considered to overcome this problem. In vitro, suppressing the AKT pathway significantly prohibits melanomas from obtaining invasive/metastatic potential as well as resistance against chemotherapy. Unfortunately, in a phase II clinical trial, AKT pathway inhibitors were not effective as a single agent and so therapeutic synergism with other anti-cancer therapies is currently being considered. However, the wide spectrum of toxicities exhibited by AKT inhibitors overlaps with those of other anti-cancer therapies, thus limiting the choices for determining a safe combination. Therefore, it will be necessary to find a less toxic natural substance to suppress the AKT pathway. *Klotho* is an aging suppressor gene and extends life span when overexpressed in mice. Klotho protein has been demonstrated to function as a hormone that inhibits insulin-like growth factor-1 (IGF-1)-AKT pathway. We produced recombinant mouse/human Klotho proteins. We studied the anti-cancer potency of these proteins against human melanoma cell lines to determine the safety of Klotho as an AKT inhibitor. Klotho protein inhibited the proliferation of several melanoma cell lines including SkMel28 in a dose-dependent manner; it also significantly stimulated the proliferation of human dermal fibroblasts. We investigated the mechanism of Klotho action using SkMel28 as a representative cell line model. Flow cytometric and DNA fragmentation analyses indicated that Klotho induced G2/M cell cycle arrest and apoptosis. Apoptosis appeared to be caused by the decreased expression of survivin, which is up-regulated by the AKT pathway. Survivin overexpression has been reported as one of the main causes for the resistance of melanomas against chemotherapy. In conclusion, because of its synergic effect with other cancer therapies, Klotho protein has the potential to be a therapeutic agent in treatments of melanoma.

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Regulation of melanocyte dendricity by β-catenin

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β-catenin, downstream of canonical Wnt signaling, is multifunction protein and involved in the development and transformation of melanocytes. Moreover, beta-catenin is highly expressed in primary and metastatic melanoma tissues. However, it has not been well elucidated whether β-catenin affects the dendricity of melanocytes. In this study, we investigated the effect of β-catenin using the recombinant adenovirus system. When primary melanocytes were transduced with recombinant adenoviruses expressing β-catenin and TOPflash, the luciferase activity was induced over 10-fold, confirming the intracellular Wnt signaling activation. Interestingly, overexpression of β-catenin led to the decrease of dendrite formation in primary cultured melanocytes. Consistent with this result, the expression of dendrite formation-related molecules, such as Cdc42 and Rac1, was markedly repressed by overexpression of β-catenin. Furthermore, phosphorylation of p38 MAPK was significantly reduced by overexpression of β-catenin, potentiating the inhibitory role for dendrite formation. In cell morphology, β-catenin overexpressed melanocytes resemble somewhat epithelial cell shape, raising the possibility that β-catenin plays a key role in melanoma development. Together, our results implicate the potential importance of β-catenin in the melanocyte dendrite formation and melanoma progression.

1298

Development of optimal guideline for effective dendritic cell immunotherapy in mouse melanoma

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Dendritic cell(DC) immunotherapy is a strong candidate for the treatment of incurable cancers especially malignant melanoma. Nevertheless, the proper guideline of DC immunotherapy does not exist. The absence of the guideline is also an obstacle to clinical trials of DC immunotherapy. So we conducted this study in order to develop an optimal guideline for the effective DC immunotherapy in mouse malignant melanoma. Mouse bone marrow-derived DCs were stimulated with tumor antigen alone or tumor antigen plus a cocktail (anti-CD40 antibody +TNF-α+ IL-1β) for 8, 24 or 48 hours and the characteristics of these DCs, such as surface molecules (CD40, CD80, CD86, MHC class II, CCR7), cytokines(IL-12, IFN-γ, and IL-10), DC-induced T cell proliferation *in vitro*, and the production of IFN-γ by those cells, were evaluated. Mice with melanoma were then treated with DCs stimulated with tumor antigen alone and tumor antigen plus cocktail for 8 or 48 hours. The tumor size and survival rate of these mice were then evaluated. In order to develop optimal guideline toward the determination of proper cell types for DC immunotherapy in mouse melanoma, the functional characteristics of DCs were matched with the clinical efficacy of DC immunotherapy. Beneficial clinical effects such as a reduction of tumor size and an increased survival rate were best observed in the group treated with DCs stimulated for eight hours with tumor antigen plus cocktail. The single prominent characteristic of DCs stimulated for eight hours with tumor antigen plus cocktail was an elevated IL-12 secretion. The cytokine IL-12 was not secreted by other DCs. Consequently, proper production of IL-12 was found to be an important functional guideline for DCs immunotherapy in mouse melanoma.

1299

Neurotrophins and their receptors stimulate melanoma cell proliferation and migration

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Melanoma is a highly aggressive skin tumor that originates in epidermis from melanocytes. As melanocytes share with the nervous system the common neuroectodermal origin and express all neurotrophins (NT). It has been shown that NGF and NT-3 are important in the progression of melanoma to the brain-metastatic phenotype. We wanted to evaluated the expression and function of NT and their receptors in melanoma. We report that primary and metastatic melanoma cell lines (A375, Mewo, Me272, WM115, WM266-4 and SK-MEL-28) synthesize and secrete all NT. Moreover, melanoma cells express the low- (p75NTR) and the high-affinity tyrosin kinase NT receptors (Trk). The inhibition of Trk receptors by either K252a ($p<0.01$ in all cell lines) or by Trk/Fc chimeras ($p<0.01$ in WM115; $p<0.05$-0.01 in WM266-4 and Skmel28 for TrkA and TrkC) prevents proliferation, as shown by MTT assay, indicating that autocrine NT are responsible for this effect. NT-3, NT-4 and nerve growth factor (NGF) induce cell migration, with a stronger effect on metastatic cell lines, as shown by Boyden/Matrigel chambers and by chemokinesis assays ($p<0.01$). Transfection with p75NTR siRNA or treatment with K252a inihibit NT-induced melanoma cell migration, indicating that both the low- and the high-affinity NT receptors mediate this effect. All melanoma cell lines express the p75NTR co-receptor sortilin. ProNGF, that binds p75NTR-sortilin complex, fails to induce apoptosis (TUNEL and MTT assay), but stimulates migration in melanoma cells ($p<0.01$ in all cell lines). Transfection with p75NTR siRNA prevents pro-NGF-induced melanoma cell migration. These results indicate that NT through their receptors play a critical role in the progression and invasion of melanoma.

1300

Genome wide siRNAi-based functional genomics identifies autophagy as a required biological process for melanin production in human cells

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Melanin protects the skin and eyes from the harmful effects of UV irradiation, regulates neurological function in the human brain, and is required for sound conduction in the inner ear. Aberrant regulation of melanogenesis underlies skin disorders (melasma and vitiligo), neurologic disorders (Parkinson's disease), auditory disorders (Waardenburg's syndrome), opthalmologic disorders (age related macular degeneration), and carcinogenesis (melanoma). Genetic and biochemical evidence have identified the core synthetic and regulatory machinery involved in melanin production, but have not fully identified the spectrum of secretory processes required for melanin production. RNA-mediated interference (RNAi)-based functional genomics provides the opportunity to identify novel, previously unappreciated biological processes required for melanin production. We have combined a high-throughput cell-based one-well/one-gene screening platform with a genome-wide synthetic library of chemically synthesized small interfering RNAs to identify autophagy as a biological pathway required for melanin biogenesis. Critical mediators of autophagy in melanoma cells were identified to regulate melanin production and tyrosinase expression in MNT-1 melanoma cells and darkly and lightly pigmented human melanocytes. Several mediators of autophagy were identified to directly regulate tyrosinase production while others regulate melanosome secretion. C57B6 mice haploinsufficient in autophagosome formation (beclin1 +/- mice) had a grey hair phenotype, consistent with the hypothesis that autophagosome formation is required for melanin production. Together, these results identify autophagy as a required process in melanin biogenesis.

1301

DC-HIL's correlation with melanoma growth and its suppression of the anti-tumor CD8 response make it a promising target for anti-melanoma therapy

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Previously we discovered that DC-HIL expressed by antigen presenting cells is a potent negative regulator of T cell activation, exerting its inhibitory function by binding to syndecan-4 (SD-4) expressed on activated (not resting) $CD4^+$ or $CD8^+$ cells. More recently we showed DC-HIL to be expressed constitutively by mouse (B16) and human (SK-MEL) melanoma cell lines at levels even higher than on APC. These findings led us to posit that DC-HIL may account for melanoma's ability to evade immunosurveillance. To address this hypothesis, we generated B16 cells (siRNA-B16) with DC-HIL expression knocked-down by siRNA (90% less) and examined their growth following subcutaneous injection in syngeneic B6 mice. Not only did the siRNA-B16 cells show markedly reduced growth compared to control B16 cells (0.5 vs. 4.0 cm^3 21 days after inoculation), but survival of mice injected with siRNA-B16 (vs. control B16) was dramatically higher (85% vs. 4% at 21 days). It should be noted that both siRNA-B16 and control cells expressed similar MHC class I H-2D/L levels and proliferation rates *in vitro* (MTT assay). We next examined effects of knocked-down DC-HIL expression on anti-tumor CD8 activity. Because B16 cells are weakly antigenic, we used ovalbumin (OVA)-reactive $CD8^+$ T cells from OT-1 mice as effectors and OVA-pulsed siRNA-B16 (vs. B16 control) cells as targets in a 51Cr release assay (10:1 E:T ratio). siRNA-B16 cells were lysed more markedly than control. We also noted inducible expression of SD-4 on OVA-reactive $CD8^+$ cells following coculture with OVA-pulsed (but not with untreated) B16 cells, and enhanced activation of $CD8^+$ cells following coculture with anti-SD-4 (but not control) Ab. Our findings unmask an important role for the DC-HIL/SD-4 pathway in anti-melanoma immunity that can be exploited for pharmacotherapeutic benefit.

1302

Active Nras and Braf provide anchorage-independent survival signals for melanoma cells in part by down-regulating Bim expression

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BRaf and NRas are often activated in melanoma, yet their roles in producing inherent survival signals are not fully understood. In this study, we investigated how $Nras^{Q61K}$ and $Braf^{V600E}$ contribute to melanoma's resistance to apoptosis induced by detachment from the extracellular matrix (anoikis) through regulating Bcl-2 family members. We transiently transfected a human melanocyte cell line PIG1 with $Braf^{V600E}$, $Nras^{Q61K}$, or myristoylated Akt3 expression vectors and subjected the lysates to Western Blot analysis. Results indicated that $Nras^{Q61K}$ and $Braf^{V600E}$ down-regulated the pro-apoptotic Bim protein. Next, we treated human melanoma cells with U0126 (an inhibitor of Mek) to examine the effects of MAPK (Erk) down-regulation. Western blot analysis suggested that blocking MAPK signals in melanoma cells up-regulated Bim. Bim is one of the main pro-apoptotic mediators of detachment-induced cell death (anoikis). Western blot analysis showed that detachment increased Bim expression (~5 fold) in melanocytes but not in melanoma cells. Further, Annexin V staining indicated that detachment induced apoptosis significantly in melanocytes, but it only induced apoptosis in melanoma cells that were simultaneously treated with U0126. Blocking Bim expression using RNAi vectors inhibited anoikis in melanocytes significantly, and it also decreased apoptosis induced by combination of detachment and U0126 treatment in melanoma cells. In summary, this report indicates that $Nras^{Q61K}$ and $Braf^{V600E}$ contribute to melanoma's resistance to apoptosis in part by down-regulating Bim expression, suggesting that Bim is a possible treatment target for overriding melanoma's inherent defenses.

1303

Endothelin-1 and stem cell factor are involved in regulating the proliferation and differentiation of human epidermal melanocytes *in vitro*

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Melanocytes characterized by the activities of tyrosinase, tyrosinase-related protein (TRP)-1 and TRP-2 as well as by melanosomes and dendrites are located mainly in the epidermis, dermis and hair bulb of the human skin. However, the mechanism of the proliferation and differentiation of human epidermal melanocytes is largely unknown. It is investigated in this study what factors are involved in regulating the proliferation and differentiation of human epidermal melanocytes derived from the primary culture of foreskin using serum-free culture. Dibutyryl adenosine 3′:5-cyclic monophosphate (DBcAMP) and basic fibroblast growth factor (bFGF) induced the dedifferentiation of melanocytes in serial cultures. Melanocytes gradually lost their pigments and became melanoblast-like cells in this medium. Of growth factors and cytokines tested, endothelin-1 (ET-1) and stem cell factor (SCF) stimulated the proliferation of these melanoblast-like cells in this medium. ET-1 stimulated the differentiation of human melanocytes in a medium supplemented with DBcAMP and/or L-tyrosine. Moreover, ET-1 and SCF synergistically stimulated the proliferation of human epidermal melanocytes in this medium. These results suggest that ET-1 and SCF are involved in regulating the proliferation and differentiation of human epidermal melanocytes in cooperation with cAMP, bFGF and L-tyrosine.

1304

Role of ING4 in human melanoma cell migration, invasion, and patient survival

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Melanoma is the most lethal form of skin cancer with a high mortality rate due to rapid metastasis. The incidence of melanoma has drastically increased over the past decades. Currently, there is no effective treatment for metastatic melanoma. ING4, a member of Inhibitor of Growth tumor suppressor family, has been shown to diminish colony-forming efficiency, induce p53-dependent apoptosis and arrest cell cycle at G2/M phase. In this study, we investigated the role of ING4 in human melanoma pathogenesis *in vitro* and *in vivo*. We found that overexpression of ING4 suppressed melanoma cell migration by 65% when compared with the empty vector control ($P = 0.0005$, t-test). Overexpression of ING4 also inhibits RhoA activity and Rock-mediated formation of stress fiber ($P = 0.0002$, t-test) in melanoma cells. Moreover, our data showed that overexpression of ING4 inhibits melanoma cell invasion by 50% through Boyden chamber assay when compared with vector control ($P = 0.005$, t-test), and ING4-overexpressing melanoma cell showed significantly reduced MMP-2 and MMP-9 activity by 25% and 61% compared with vector control ($P = 0.01$ and 0.008, respectively, t-test). Furthermore, using tissue microarray and immunohistochemistry staining, we found that ING4 expression is significantly decreased in malignant melanoma when compared with dysplastic nevi ($P < 0.001$, χ^2 test) and reduced ING4 staining is associated with melanoma thickness. Strikingly, reduced ING4 staining is also significantly correlated with poor overall and disease-specific 5-year survival of primary melanoma patients ($P < 0.001$ for both, χ^2 test). In addition, Cox regression analysis revealed that reduced ING4 staining is an independent factor for the poor prognosis of patients with primary melanomas ($P = 0.025$, $RR = 2.60$). Taken together, this study highlighted the importance of ING4 in melanoma pathogenesis and provided a promising prognostic marker as well as a potential therapeutical target for human melanoma.

1305

Electron microscopic alterations in melasma patients treated with collimated low fluence Q-switched Nd:YAG laser

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There is no entirely reliable treatment method for melasma that has been established. Although various kinds of lasers including Nd-YAG have been tried to treat melasma, the results were still controversial. Recently, we experienced clinical and histopathological improvements of melasma patients treated with low fluence Q-switched Nd:YAG laser with pulse width less than 5-7nsec and with Top Hat beam mode through collimation method. 10 out of 17 (58.8%) patients in the split-face study showed "GOOD" (50-75% improvement) and above in its effectiveness. No case of full recurrence was examined and partial recurrence was detected in 12 patients during 2 months' follow-up examination. To our opinion, this treatment method is a new concept that can be described as selective photothermolysis with minimum thermal damage and inflammation reaction to affected tissues by pigmentation. We consider this treatment method should be regarded as 'minimized selective photothermolysis' that can provide a new effective treatment for melasma. In this follow-up study, we investigated *in vivo* ultrastructural changes in the melanosome, using transmission electron microscopy, induced by collimated low fluence Q-switched Nd:YAG laser, and compared before and after treatments. Sequential changes as multi-pass treatments were also compared.

1306

Increased expression of NGFR and NEP in the lesional skin of melasma

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The pathogenesis of melasma has not yet been clearly demonstrated. Manifestations of melasma including the remarkable symmetry of hyperpigmented lesions and the distribution related with trigeminal nerves, suggest that the neural involvement could play a part in the pathogenic mechanisms of pigmentation. Recently, neuropeptides, which are molecules liberated from fine nerve endings in the hypopigmentary diseases including vitiligo, are known to have an important role in the regulation of melanogenesis but they were not evaluated in relation to any hyperpigmentary diseases. The aim of this study was to evaluate whether or not some neuropeptides, neurotrophins and their receptors were associated with the pathogenesis of melasma. To investigate the involvement of neuronal system and neuropeptides in melasma, we examined the expression of NGFR and NEP in the melasma lesional and non-lesional skins. Skin biopsies were obtained from the lesional and non-lesional facial skins of 6 Korean women with melasma. The confocal laser scanning microscopic examination and western blot was performed. In result, The lesional skins of melasma showed markedly increased expression of nerve growth factor receptor (NGFR) and neutral endopoeptidase (NEP) compared with non-lesional skin. No difference could be detected with regard to the intensity of immunoreactivity for calcitonin gene-related peptide (CGRP), calcitonin gene-related peptide receptor (CGRPR), substance P (SP), substance P receptor (SPR). In conclusion, I suggest that neuroactive molecules including NGF is one of the critical factors for the pathogenesis of melasma, which may effect directly to the microenvironment around melanocytes via a NGFr-IR nerve fiber pathway, and increased NEP in melasma has a important role in regulation of melanogenesis. This study was supported by by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (KRF-2007-331-E00002)

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Foxn1 is down regulated in human gray hair

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Foxn1 (Whn, Hfh11) is a transcription factor and its loss results in the nude phenotype of rodents and humans. Recently, Foxn1 was identified as an activator of the pigment recipient phenotype (1). bFGF is a growth factor and Foxn1 target, and this growth factor is a signal released by recipient keratinocytes. In human hair, pigmentation is an important phenotype, and there is considerable interest as to why this pigmentation is lost with age (hair graying). In this study, we compared the expression levels of Foxn1, AFF4 and bFGF in pigmented hair and gray hair that were plucked from the same volunteer. AFF4 is also a transcription factor that can associate with Foxn1. Furthermore, expression sites of Foxn1 and AFF4 in human hair follicles were studied. Gray and pigmented hairs were collected from six volunteers (40 to 49 year old Japanese males). Total RNAs were isolated from hair follicles by Trizol, and these RNAs were reverse transcribed to cDNAs. Expression levels of these molecules were analyzed by qPCR. In gray hair, these three molecules were down regulated compared with pigmented hair. In human skin with pigmented hair, Foxn1 is expressed in epidermal and hair keratinocytes, while AFF4 is expressed in hair keratinocytes but not in epidermis. These results suggest that Foxn1 and AFF4 have important roles in pigmentation of human hair.

1308

A case of malignant melanoma on a medium-sized congenital melanocytic nevus: The approach for molecular characterization

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A 45 year-old Japanese woman was refereed to our clinic with a 2-year history of enlarging black tumor, 17 X 12 mm in size on her lumbar region. The lesion was arising on a black plaque, 3 X 4 cm in size that she had noticed after birth. She had suffered from right breast cancer 8-year before and cervical cancer last year. Histological examination showed malignant melanoma (MM) on a congenital melanocytic nevus (CMN). In order to elucidate the pathogenesis of MM on a medium-sized CMN, we sought to perform molecular characterization in the tumor. First, we examined somatic mutations of NRAS gene in MM and CMN. Genomic DNAs extracted from Paraffin-embedded tissues were amplified by PCR with a pair of primers for exon 3 of NRAS gene, and the PCR products were directly sequenced. Activated somatic mutations of Q61K were identified in both of MM and CMN. Second, since she had suffered from multiple cancers, we investigated microsatellite instability in the pathogenesis of MM. Genomic DNAs were amplified by PCR with 5 pairs of primers for microsatellite markers, and the PCR products were electrophoresed on polyacrylamide gels. No microsatellite instability was observed in MM. Next, we performed array comparative genomic hybridization (aCGH) analysis. Genomic DNAs extracted from frozen tissues of MM and CMN including normal skin were used for test and reference in aCGH analysis, respectively. Loss of chromosome 8p21.1-23.3 and 9p13.3-24.3, and gain of chromosome 15q11.2-13.1, 15q21.3, and 20p12.3-13 were detected in MM. One of familial MM genes, CDKN2A, is mapped in 9p21 among them. These results indicate that MM cells should be originated in CMN cells, and loss of CDKN2A and loss or gain of unknown MM candidate genes might be relevant to the development of MM on a medium-sized CMN.

1309

Identification of alpha-enolase autoantibody in sera derived from a patient with melanoma by autoantibodiomics

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Melanoma is a malignant tumor of melanocytes occurring predominantly in skin but it may also arise in the bowel and the eye. Despite many years of intensive laboratory and clinical research, the sole effective cure for the disease is surgical resection of primary tumor. To identify candidate biomarkers for the chemical diagnosis of melanoma, we applied a novel proteomics-base technique targeting autoantibodies in patient sera. We examined sera from 20 patients with melanoma and 10 healthy controls in this study. Mixed soluble proteins of C32TG and G361 cell lines as melanoma-associated antigens were separated with two-dimensional polyacrylamide gel electrophoresis, transferred on a PVDF membrane and then reacted with diluted serum samples as a primary antibody. After ECL detection of some positive spots on the PVDF membrane, the corresponding positive spots were excited from a silver staining gel, digested with TPCK trypsin and then the tryptic digests were analyzed by MALDI/TOFMS. The MASCOT database search following mass finger printing (MFP) were identified an alpha-enolase in the positive spot. We reconfirmed the positive spot on the PVDF membrane using a monoclonal anti alpha-enolase antibody. From the results of mass finger printing (PMF), we identified total of found 12 tryptic peptides derived from a protein, alpha-enolase (sequence coverage=45.9%, score=63) and the MS/MS analysis at m/z=1908.38, were consistent with the 163rd to 179th amino acids of the protein, we identified the sequence, L-A-M-Q-E-F-M-I-L-P-V-G-A-A-N-F-R of the alpha-enolase. Autoantibody against alpha-enolase in sera was detected with high frequencies during patient follow-up. In conclusion, we first identified alpha-enolase autoantibody in sera derived from a melanoma patient. However, it is still unclear why the autoantibody would be produced and appear in serum.However, the autoantibody might be a candidate biomarker to diagnose melanoma.

1310

The impact of neonatal blue light phototherapy on dysplastic nevus development

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In our present study we aimed to investigate the long-term impact of blue light phototherapy of neonatal jaundice on dysplastic nevus development. Firstly, we compared the prevalence of clinically atypical melanocytic nevi in a group of adolescents and young adults (n=747, age range=14-18 years) with or without a documented history of neonatal blue light phototherapy. As blue light phototherapy has been used for the treatment of neonatal jaundice in Hungary since 1968, then in a 21-to-71-year old healthy adult population (n=618) we investigated whether there is a difference in the occurrence of dysplastic nevi between those born before and those born after 1968. Our results revealed that neonatal blue light phototherapy was associated with a significantly higher prevalence of dysplastic nevi in teenagers (chi-square=4.08, p=0.0433, OR 1.43, 95 % CI 1.010-2.026). We additionally found that the prevalence of dysplastic nevi was significantly higher in those born in or after 1968 than in those born before 1968 (chi-square=17.26, p=0.00003, OR 2.12, 95% CI: 1.48-3.04). The emission spectrum of the blue light lamp was between 370 and 600 nm, with maximum at 450 nm. Approximately 0.3% of the emitted light comprised UVA radiation. The wavelengths of blue light and UV light are adjacent, so they might have partly similar biological effects. Besides inducing melanocyte proliferation, UV irradiation has profound immunosuppressive and immunomodulatory effects, and it is well established that immunosuppression markedly increases the risk of nevus formation. In view of the special characteristics of the newborn skin and immune system, intensive neonatal phototherapy may have a considerable effect on the immature melanocytes of the epidermis. These new epidemiological data suggest that neonatal blue light phototherapy could well be a risk factor for dysplastic nevus development.

1311

Effect of histidine residue modification on tyrosinase activity and conformation

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We found that histidine modification via bromide acetate or diethylpyridinecarbamate (DEPC) conspicuously inactivated tyrosinase in a parabolic noncompetitive inhibition manner. To see whether histidine modification also induced conformational change of tyrosinase, the ANS-binding fluorescence measurements were conducted. The results showed that the bromide acetate binding to tyrosinase directly induced conformational change of tyrosinase. The substrate reactions in the presence of DEPC were shown to the time course of histidine modification, which follows the first order kinetics. We suggested that histidine modification by external chemical ligand-binding affected the substrate's accessibility due to the conformational changes and thus, the complex type of inhibition was occurred. Our results provided information regarding the role of histidine residues at the active site of tyrosinase during catalysis.

1312

Aphidicolin as a possible modulating agent for melanoma antigen expression in melanoma immunotherapy

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Efforts to enhance anti-tumor immunity have led to identification of numerous tumor-associated antigens, but vaccination with such antigens have shown limited efficacy because melanoma is well known to escape immune destruction by selective down-regulation of the very antigens that are being targeted with the tumor vaccine. Even if the vaccine is able to raise highly specific cellular and humoral immunity, loss of the melanoma antigen in melanoma cells impedes its effectiveness. Thus we try to find new agents that augment melanoma antigen expression for improving effectiveness of the melanoma immunotherapy. To easily identify novel agents to up-regulate melanoma antigen gene expression in melanomas, we successfully established high-throughput screening systems. That is, constructions of minimal Melan-A/MART-1 promoter linked to the coding sequence of Green fluorescence protein (GFP) were transfected into the melanoma cell line, MUX expressing a low level of Melan-A/MART. And we obtained a clone of the stably-trransfected cell, which could produce GFP according to a stimulation to the Melan-A/MART-1 promoter. Amount of GFP produced could be easily estimated as the fluorescence intensity by the flow cytometry. In fact, the system can detect the promoter stimulation as fluorescence of GFP. Using the system, we screened 95 chemicals and found 11 candidate agents. We further confirmed that 10 out of 11 agents could really increased the expression of Melan-A/MART-1 protein in MUX cells as well as most other 5 melanoma cell lines of MU89, MM96L+, MM96L-, A375, and SK-MEL-28. The most effective stimulator of 10 agents is aphidicolin, a DNA polymerase inhibitor, which was capable of enhancing expression of both mRNAs and proteins of other melanoma antigens such as gp100, tyrosinase, tyrosinase-related protein 1 (TRP1) and dopachrome tautomerase (DCT). As aphidicolin has a potential for enhancing the expression of several melanocyte-associated antigens, it is expected to be helpful for clinical melanoma immunotherapy.

1313

Gsk3β inhibition promotes melanogenesis in Mouse B16 Melanoma cells and human normal melanocytes

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Glycogen synthase kinase 3β (GSK3β) is implicated in many biological events, including embrionic development, cell differentiation and apoptosis. GSK3β also plays a key role in Wnt/β-catenin pathway. Even if the master regulator of pigmentation MITF-M is a target for Wnt pathway, at the moment the regulatory role of GSK3β in the control of melanogenesis is not completely understood. In this study we evaluated the effect of the inhibition of GSK3β activity in the regulation of melanocytes differentiation. Exposure of murine melanoma cell line B16 and normal human melanocytes to GSK3β specific inhibitors (SB216763, SB415286, BIO and LiCl) resulted in a dose-dependent increase of the differentiation-associated markers level of melanoma cells such as melanin synthesis, tyrosinase activity and protein expression, which were accompanied with morphological changes. The differentiation of B16 cells induced by GSK3β inhibition was closely associated with growth inhibition, without evidence of cell death. Among the four GSK3β inhibitors studied, BIO, which is described as the more selective one, demonstrated the highest capacity to stimulate melanogenesis. In addition, we excluded the possibility that melanogenesis induction was due to an aspecific effects of GSK3β inhibitors using siRNA methodology in place of pharmacological inhibition. Moreover, we demonstrated that even if treatment of B16 cells with siRNA-β-catenin completely abolished the promelanogenic effect of GSK3β inhibition, the over-expression of a constitutively active mutant form of β-catenin (pCS2β-cat-mut) only slightly increased pigmentation, which demonstrated that activation of β-catenin is required but is not sufficient to induce the expression of melanogenic enzymes. All these results demonstrated that GSK3β is implicated in melanogenesis regulation and that pharmacological inhibition of its activity could influence melanin synthesis through mechanisms that are probably not restricted to Wnt/β-catenin pathway activation.

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A new model for melanoma progression, phenotype switching

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Recent expression profiling of melanoma cell lines identified two transcription signatures respectively corresponding with proliferative and invasive cellular phenotypes. A model derived from these findings predicts that *in vivo* melanoma cells may switch between these states. Here, DNA microarray-characterized cell lines were subjected to in vitro characterization before subcutaneous injection into immunocompromised mice. Tumor growth rates were measured and post-excision samples were assessed by immunohistochemistry to identify invasive and proliferative signature cells. *In vitro* tests showed that proliferative signature melanoma cells are faster growing but less motile than invasive signature cells. *In vivo* proliferative signature cells initiated tumor growth in 14 +/- 3 days post injection. By comparison, invasive signature cells required a significantly longer ($p < 0.001$) period of 59 +/- 11 days. Immunohistochemistry showed that regardless of the seed cell signature, tumors showed evidence for both proliferative and invasive cell types. These data indicate that melanoma cells undergo transcriptional signature switching *in vivo* likely regulated by local microenvironmental conditions. Our findings challenge previous models of melanoma progression which evoke one-way changes in gene expression. We present a new model for melanoma progression which accounts for transcription signature plasticity and provides a more rational context for explaining observed melanoma biology.

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Involvement of arylhydrocarbon receptor (AhR-)signaling in skin melanogenesis

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In previous studies we have shown that human epidermal keratinocytes express a functionally active AhR. Accordingly, exposure of human keratinocytes to UVB radiation caused the formation of the AhR ligand 6-formylindolo[3,2-b]carbazole (FICZ) from intracellular tryptophan. Binding of this high-affinity ligand to the cytoplasmic AhR triggered a signaling response which was shown in-vitro as well as in-vivo to be an essential part of the UVB stress response. For decades, dioxin is known to be a strong and functionally active AhR ligand and in humans, dioxin intoxication is associated with mucosal and skin hyperpigmentation. Therefore, in the present study AhR expression was assessed in melanocytes. We now report that primary murine and human melanocytes express the AhR at the mRNA and protein level. AhR expression in this cell type was of functional relevance because stimulation with the AhR ligands FICZ or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induced CYP4501A1/1B1, marker enzymes of the AhR signaling pathway. Induction was abrogated in murine melanocytes by the chemical antagonist MNF and in melanocytes from AhR knockout (AhR-/-) mice. Interestingly, in human primary melanocytes TCDD and FICZ caused induction of the melanogenic enzyme tyrosinase. This induction was AhR dependent, because it could be inhibited with MNF. In cultured murine melanocytes AhR triggering induced mRNA expression of the enzymes tyrosinase and tyrp-1, and eventually increased melanin production. In addition, melanin content and melanocyte density was greatly reduced in the skin of AhR -/- mice, as compared with wildtype litter mates. Thus, the AhR is functionally involved in skin melanogenesis and modulation of AhR signaling may offer a previously unrecognized possibility to manipulate skin pigmentation.

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Intracellular signaling mechanisms involved in endothelin-1-stimulated expression of metallothionein in human melanocytes

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Metallothioneins(MT), one of stress-inducible endogenous proteins has the capacity to scavenge reactive oxygen metabolite and plays a homeostatic role in ultraviolet light (UV)-induced cellular damages. Endothelin-1 is secreted by UVB exposed keratinocytes, which stimulates proliferation and melanization of epidermal melanocytes. Transferred melanin into keratinocytes play an important role in preventing UV-induced skin damages. In this study, we found that ET-1(10nM) stimulates the gene expression of MT in cultured human melanocytes and examined the intracellular signaling mechanisms leading to the increased MT expression. The increased expression of MT was accompanied by phosphorylation of ERK and JNK, but not p38MAPK. As for signaling involved in AP-1, ET-1 elicited a striking increase in the expression of c-Jun and c-Fos at the gene and protein levels. While the increased gene expression of c-jun was significantly inhibited by MEK and/or JNK inhibitors, that of c-fos was significantly abolished by MEK inhibitor but not by JNK inhibitor. The same inhibitory pattern was also seen for the protein expression of c-Jun and c-Fos. Consistently, ET-1-stimulated gene expression of MT was significantly suppressed by MEK inhibitor and/or JNK inhibitor. These findings suggest that the ET-1 stimulated expression of MT is mediated via the enhanced expression of AP-1 including c-Fos and c-Jun which is dependent on the activation of signaling linkages of Raf-MEK-ERK and JNK.

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Non invasive *in-vivo* NIR-FT Raman spectroscopy differentiates malignant melanoma from malignant melanoma suspicious nevi

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There is a need for new methods for non-invasive, preoperative diagnosis of malignant melanoma (MM). Raman spectroscopy is a non-destructive method to analyze molecular structure. We investigated the chemical structure of melanin, proteins and lipids in the skin *in vivo* using Raman spectroscopy. We obtained Raman spectra of 46 pigmented lesions, which clinically and dermoscopically had been classified as suspected for MM. All lesions were excised; 26 lesions were histopathologically diagnosed as MM whereas 20 represented benign nevi (NV). Raman spectra were measured from 0 - 3500 cm-1 (wavenumbers), and analyzed in the region from 1100 to 1800 cm-1 normally dominated by protein and lipid bands, and above 2600 cm-1 for water content. The main feature of MM spectra, when compared with NV and normal skin, was the presence of the novel peaks at 1605 cm-1 and at 1550 cm-1. Moreover, we detected peak shifts from 1255 cm-1 to wavenumbers around 1300 to 1340 cm-1. The peaks at 1550 cm-1 and 1605 cm-1 are likely to arise from increased amount of melanin and hemoglobin in the lesions. We found a high correlation (r2=0.85) between the thickness of the MM lesions and the intensity of the peaks at 1550 cm-1 and 1605 cm-1, together with the intensity ratio 1310 cm-1/1255 cm-1 and skin water content (wide peak around 3250 cm-1). Using binary logistic regression we show that the diagnosis of MM versus clinically suspicious NV can be performed with a sensitivity of 81% and a specificity of 70%, with peak intensity at 1550 cm-1 and intensity ratio 1310 cm-1/1255 cm-1 as significant parameters.

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Ultradeformable liposomes for delivery of small interfering RNA (siRNA) into human primary melanocytes

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It is our aim to optimize a system to deliver siRNA molecules to human epidermis. More specifically, we want to block the expression of a specific Myosin Va exon F containing isoform that is physiologically involved in melanosome transport in human melanocytes. Ultradeformable cationic liposomes (UCL) were prepared and investigated as carriers for *in vitro* transfection of human primary melanocytes. A range of DOTAP/NaChol lipids were formulated from different w/w ratios (6:1, 8:1 and 10:1) of the cationic bilayer-forming lipid 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) and the edge activator sodium cholate. The resulting particles have diameters of about 85 nm and were all positively charged. *In vitro* transfection efficiency of siRNA/UCL lipoplexes was assessed by measuring the expression level of Myosin Va exon F in human primary melanocytes by means of QPCR, against which the siRNA was designed. The optimal ratio of siRNA to liposome was 1:14 (w/w), which corresponds to a charge ratio (+/-) of 4. All transfections were carried out in serum free medium and the best result was obtained with a DOTAP/NaChol ratio of 6:1 (w/w) which revealed a knockdown of 77% at the mRNA level. Complete complexation and protection of siRNA from enzymatic degradation was achieved as judged by gel retardation assays. By using a CellTiter Glo® assay, the 6:1 formulation turned out to be the least cytotoxic of the three. Stability tests showed that after 4 weeks of preservation in HEPES buffer (pH 7.4) at 4°C, the complexes were still able to silence Myosin Va exon F upto 67%. Inside the cell however, at 37°C, the complexes are less stable; after 2 weeks the silencing capacity decreases to 42%. These results were also confirmed on protein level. To conclude, our in vitro data demonstrate that DOTAP/NaChol liposomes can transfect siRNA into human primary melanocytes in an efficient way. Studies to evaluate their penetration capacity through human skin are currently ongoing.

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RNA interference of myosin Va exon F isoforms in primary human melanocytes: an alternative to treat hyperpigmentation

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The movement of melanosomes within human melanocytes is actin-dependent and mediated through the formation of a Rab27a/Slac2-a/Myosin Va (MyoVa) protein complex. We previously reported that only the melanocyte-specific exon F isoforms of MyoVa are involved in melanosome transport to the dendrite extremities. Here we used RNAi to downregulate the expression of the MyoVa exon F isoforms in primary human melanocytes and investigated the effect on intracellular melanosome transport. A significant and specific silencing of MyoVa exon F isoforms was determined by QPCR analysis after transfecting transient siRNAs into human melanocytes. Next, a lentiviral vector system (expressing a shRNA against exon F) was developed to obtain a long-term silencing model in hard-to-transfect melanocyte cells. Infection of primary melanocytes with these lentiviruses resulted in a stable and significant knockdown of the MyoVa exon F isoforms both at the mRNA and protein level as compared to MOCK-transduced cells. Immunofluorescence microscopy showed that knockdown of the exon F isoforms resulted in a blockage of intramelanocytic melanosome transport due to the inability to form the Rab27a/Slac2-a/MyoVa tripartite complex. Perinuclear accumulation of melanosomes was confirmed by bright field microscopy. Further analysis of MyoVa exon F knockdown experiments revealed that the stability of Rab27a, but not Slac2-a, was affected. Both proteins remained however on the melanosomal membrane as was obvious from their relocation to the perinuclear region of the cells. Interestingly, the observed phenotypic effect in this study (ie. clustering of melanosomes around cell nuclei) is the same as that seen in melanocytes from patients with human Griscelli syndrome causing hypopigmentation of the skin. We conclude that our RNAi-based strategy provides a novel tool to block the intracellular melanosome movement in primary human melanocytes and may become an innovative drug to treat hyperpigmentation.

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The use of mitochondrial DNA as a "Biosensor" for UVR induced skin damage: Implications for personalized sun care and effective product formulation

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The use of mitochondrial DNA (mtDNA) damage as a reliable and highly sensitive biomarker of ultraviolet radiation exposure (UVR) in both the dermis and epidermis has been successfully pioneered by ourselves and others (CED,2006;31(4)548-52). In addition, the sensitivity of the mtDNA biomarker enables a clear distinction of DNA damage even between occasional versus usually sun-exposed skin (JID, 2006,126,408-415). This is important as non-melanoma skin cancers occur more commonly on usually sun-exposed sites. Our previous work has identified a specific 3895bp mitochondrial genome deletion which behaves as "biosensor" for both acute and long term UV-induced damage in both human epidermis and dermis (JID, 2004,123,1020-1024). Recently, this marker has been investigated for its presence in the upper layers of the epidermis. The findings have been correlated with the melanocortin 1 receptor gene (MC1R) status of the samples. This gene is key for controlling skin pigmentation and germline variants are associated with increased risk for melanoma and non melanoma skin cancer in Caucasians. A variety of different sun-exposed body sites from 111 volunteers were sampled using a swab technique and the frequency of the mtDNA deletion and MC1R status was assessed with specific real time PCR assays. The mean deletion frequency, from rarely sun exposed sites, such as the heel, was significantly different from the deletion frequency of sun exposed sites, such as the nose ($p < 0.0001$). This data suggests that the upper layers of the skin predominantly containing the stratum corneum is a reliable site for assessing mtDNA damage caused by UVR. Moreover, a scale of DNA damage can be deduced from the data which has provided an opportunity to develop products and formulations with measurable outcomes.

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Melanosomes aggregated to the nucleus yield protection against high doses of UV-B radiation

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The purpose of the study was to investigate the photoprotective effect of melanosomes found to associate with the nucleus in a human melanocyte cell line upon induction of melanogenesis. Induction of melanogenesis in melanocytes was performed by cultivating the human melanocytes (LND-1 cells), in media using ammonium chloride and elevated levels of L-tyrosine. Purification of the nucleus was done by ultracentrifugation and the photoprotective effect of melanin was determined through DNA lesions determined using XL-PCR and quantified using Q-PCR. Purification of the nucleus of melanocytes cultivated with and without induction of melanogenesis showed aggregation of induced melanin to the nucleus. Results indicated that melanin was membrane associated rather than coupled directly with DNA. Non-induced and induced cells as well as nuclei of cells treated similarly were submitted to UV-B radiation of varying doses ranging from 0 to 100 mJ/cm2. The photoprotective effect of melanin was investigated by quantifying DNA lesions of the UV irradiated cells and nuclei. Investigating the levels of DNA damages in induced versus non-induced whole cells showed no significant differences between the levels from 0 to 50 mJ/cm2. Melanin was found to yield a protective effect only at high doses of UV radiation (100 mJ/cm2). Similar findings were obtained upon irradiation purified nuclei of induced and non-induced melanocytes. Conclusively, we showed for the first time that melanin aggregates to the nucleus of melanocytes apparently at the membrane in the human melanocyte cell line, LND-1. Furthermore, we showed that melanin protects nucleus against high but not low doses of UV-B radiation in purified nucleus as well as nucleus within living melanocytes.

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The Silver locus product (gp100) as a new tool for the analysis of melanosome transfer in human melanocyte - keratinocyte co-culture

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Melanosome transfer from melanocytes to keratinocytes in skin and hair remains the unique example of organelle sharing by one mammalian somatic cell type to another. These melanocyte-specific lysosome-related organelles are transferred to keratinocytes of the epidermis and anagen hair bulb where they are thought to protect keratinocytes against UV irradiation, at least in the epidermis. However, the mechanism(s) responsible for this transfer has long remained one of the most enigmatic of heterotypic cell interactions. While there have been many attempts to study this process, significant progress has been hindered by the absence of an adequate *in vitro* model. During our ongoing study of melanocyte: keratinocyte interactions in skin we developed a novel in vitro assay that exploits the specificity of SILV/Pmel17/gp100 expression for melanosome /melanin granules. Using fully-matched cultures keratinocytes and melanocytes isolated from normal healthy epidermis together with double immuno-fluorescence, we have determined that gp100 is a surprisingly useful tracker of transferred melanin. Moreover, transferred melanin granules emit a fluorescence signal facilitating ready quantification of melanin transfer levels between melanocytes and keratinocytes. This quantitative approach was validated using known inducers/inhibitors of melanocyte phenotype. This assay further confirmed that cyto-phagocytosis of melanocytic elements by keratinocytes is one route for melanin incorporation into keratinocytes. Howoever, a role for the recently proposed filopodium as a direct conduit for melanin transfer was substantiated using this novel approach. In conclusion, this assay promises to significantly aid our investigations of the molecular basis of melanosome transfer and offers a new tool for the clinical evaluation of melanocyte modulators.

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Proopiomelanocortin (POMC), the melanocortin precursor, is secreted by human epidermal keratinocytes and melanocytes and stimulates melanogenesis

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Proopiomelanocortin (POMC) is a 241-amino acid precursor polypeptide neurohormone that is expressed in corticotropes (anterior pituitary); melanotropes (intermediate pituitary lobe); neurons in hypothalamus and brainstem; as well as by skin. POMC has long been known to be a source of several biologically-active cleavage products including the melanocortins (ACTH, α-MSH) and β-endorphin. However, POMC processing is incomplete in the pituitary and hypothalamus leading to release of the precursor, POMC. This study examined POMC processing in human skin and the effect of POMC on the melanocortin-1 receptor (MC-1R) and melanocyte regulation. POMC was secreted by both human epidermal keratinocytes and matched epidermal melanocytes. Much lower levels of α-MSH were secreted and only by keratinocytes. Neither cell type released ACTH. Nevertheless the full POMC processing machinery was detected in both melanocytes and keratinocytes. Cell extracts contained significantly more ACTH than POMC, although α-MSH was only detected in keratinocytes. In contrast, hair follicle melanocytes secreted both POMC and α-MSH and this was enhanced in response to corticotrophin releasing factor (CRF) via the CRF1 receptor. In cells stably transfected with MC-1R, POMC stimulated cAMP, albeit with lower potency than ACTH and α-MSH. POMC also increased melanogenesis and dendricity in human pigment cells. The release of POMC from skin cells and its functional activity at MC-1R, highlights the importance of POMC processing as a key regulatory event in the skin.

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A lentivirus-based genetic screen for genes involved in melanoma metastasis

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Melanoma is the deadliest form of skin cancer with 5-year survival rates of less than 50% if it metastasizes. To date, no good treatment options exist for metastatic melanoma. Therefore, we sought to use a genetic screen to identify genes involved in metastasis of melanoma in mice. To begin the genetic screen, the murine melanoma cell line B16 was subcloned by limiting dilution. The resulting subclones were injected into the footpad of common γ chain/ Rag2 double knockout mice (γc-/-, Rag2-/-) and screened for metastases. A single non-metastatic B16 subclone, B16-F0-E, was selected for use in the screen. Next, a lentivirus expressing GFP was used to randomly mutate the B16-F0-E cell line. The F0-E cell line was infected with the GFP-lentivirus at a low titer to minimize the chances of multiple insertions. The lentivirus infected B16-F0-E cells, 25% of which were GFP positive by flow cytometry, were then injected into the foot pad of γc-/-, Rag2-/- mice. After 18 days the mice were analyzed for the presence of metastases. Mice injected with lentivirus infected B16 melanoma cells developed nearly 8 times as many metastases as compared to mock infected B16 cells. The metastases were isolated from these mice and we currently have 27 clones from these metastases that are positive for a lentiviral insertion. The metastatic potential of these clones was verified by re-injecting them into γc-/-, Rag2-/- mice and screening for metastases. The lentiviral insertion sites in the most metastatic clones were identified using a variation of bubble-PCR. The sites of lentiviral insertion identified thus far include RNA-binding proteins, transcription factors and proteins involved in stress-responses. The role of these proteins in melanoma metastasis is currently being investigated.

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Inhibition of polo-like kinase 1 activity causes G2/M phase cell cycle arrest and induction of apoptosis in human melanoma cells

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Melanoma is the most dangerous and lethal skin cancer and metastasizes aggressively if not diagnosed early. Because the current preventive and treatment options have not been able to effectively manage this disease, novel mechanism and target-based approaches are needed to combat this deadly neoplasm. A better understanding of mechanism of melanoma development and progression may provide the basis for the rational design of specific targets and therapeutic strategies for the management of melanoma. Polo-like kinase 1 (Plk1) is a member of a family of mitotic serine/threonine kinases, which are highly conserved among eukaryotes and have been shown to play critical role in cell cycle progression, especially during cell division. Elevated Plk1 expression has been shown in some human cancers and is believed to be an indicator of poor prognosis and disease outcome. In this study, we tested the hypothesis that Plk 1 plays a critical role in melanoma and the inhibition of Plk1 will impart anti-proliferative effect in human melanoma cells. Employing normal human melanocytes and multiple melanoma cancer cell lines viz. WM35, WM-115, Sk-Mel-28, G-361, A-375, and Hs-294T, we determined the levels of Plk1 in melanoma cells versus normal melanocytes. Our data demonstrated that Plk1 is overexpressed, both at mRNA and protein levels in all melanoma cell lines tested when compared to normal melanocytes. Treatment of WM-35, WM-115, A-375 and Hs-294T cells with a small molecule ATP-competitive inhibitor of Plk1 viz. GW843682X resulted in a significant decrease in cell proliferation and viability of melanoma cells as assessed by Trypan blue exclusion assay. Further, as shown by the DNA cell cycle analysis, Plk1 inhibition by GW843682X resulted in a significant G2/M cell cycle arrest of all the melanoma cell lines. GW843682X also caused a significant induction of apoptosis as evident from an increased sub-G0/G1 cell population and cleavage of PARP. This study suggests that Plk1 plays a role in melanoma development and could serve as a viable target for the management of this neoplasm.

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Autocrine and paracrine regulation of human melanoma by interleukin-1β

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Interleukin-1 (IL-1)β is a pleiotropic pro-inflammatory cytokine and a major inducer of inflammation. Although it has been shown to be associated with tumor promotion, immunosuppression, and chemoresistance, the roles of IL-1β in the progression of human melanoma are not fully understood. In this study, we examined IL-1β expression and secretion in human melanoma cell lines and investigated its role *in vitro* and *in vivo*. The analysis of 10 human melanoma cell lines derived from various stages showed that advanced-stage human melanoma cell lines constitutively produced and secreted IL-1β whereas early-stage human melanoma cell lines did not produce or secrete IL-1β. Although the production of IL-1β was induced or enhanced by IL-1 in all human melanoma cell lines, the secretion of IL-1β was only enhanced in advanced-stage melanoma cell lines. When IL-1 receptor was blocked with IL-1Ra treatment, the secretion of IL-1β and other cytokines/chemokines such as IL-6, IL-8, MCP-1, Gro-α and GM-CSF were significantly decreased, suggesting that IL-1β secreted from advanced-stage human melanoma cell lines is biologically active as an autocrine factor. It also suggests that other cytokines/chemokines represent a downstream event of an IL-1β-initiated signal in advanced-stage human melanoma. Furthermore, IL-1β secreted from advanced-stage human melanoma cell lines up-regulated IL-1 in THP-1 or HaCat cells, suggesting its paracrine role. Matrigel plug experiments in nude mice showed that advanced-stage human melanoma cell lines survived and recruited host stromal cells whereas the treatment with IL-1Ra reduced the number of melanoma cells and stromal cells*in vivo*. Our findings indicate that the secretion of IL-1β from human melanoma contributes to tumor progression by acting as an autocrine and paracrine factor to induce other cytokines and chemokines *in vitro* and recruit stromal cells *in vivo*.

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The internal repeat domain of the melanosomal matrix protein PMEL17/GP100 is required for fibrillogenesis

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Over 125 pigmentation-related genes have been identified to date. Of those, PMEL17/GP100 has been widely studied as a melanoma-specific antigen as well as a protein required for the fibrillogenesis in melanosomes. PMEL17 is synthesized, glycosylated, processed, and delivered to melanosomes, allowing them to mature from amorphous round vesicles to elongated fibrillar structures. In contrast to other melanosomal proteins such as TYR and TYRP1, the processing and sorting of PMEL17 is highly complex. Monoclonal antibody HMB45 is commonly used for melanoma detection, but has the added advantage that it specifically reacts with sialylated PMEL17 in the fibrillar matrix in melanosomes. In this study, we generated mutant forms of PMEL17 to clarify the subdomain of PMEL17 required for formation of the fibrillar matrix, a process critical to pigmentation. The internal proline/serine/threonine-rich repeat domain (called the RPT domain) of PMEL17 undergoes variable proteolytic cleavage. Deletion of the RPT domain abolished its recognition by HMB45 and its capacity to form fibrils, but did not affect the vesicular trafficking of PEL17. Truncation of the C-terminal domain did not significantly affect the processing or trafficking of PMEL17, but, in contrast, deletion of the N-terminal domain abrogated both. We conclude that the RPT domain is essential for its function in generating the fibrillar matrix of melanosomes and that the luminal domain is necessary for its correct processing but is not for trafficking to those organelles.

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Rad51 siRNA delivered by HVJ-E increased the sensitivity of dacarbazine to melanoma

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Dacarbazine (DTIC) is one of the most popular alkylating agents to treat melanoma patients. In the process of DTIC treatment, however, melanoma is known to become resistant to DTIC, suggesting induction of DNA repair molecules, such as Rad51, to repair DNA double-strand breaks (DSBs) caused by DTIC. Western blot and real-time PCR analysis indicated that DTIC dose- and time-dependently induced Rad51 expression in human and mouse melanoma cell lines in culture, but not Ku70, which is a main molecule for non-homologous DNA end-joining. These data suggest that knockdown of Rad51 before and after DTIC treatment may overcome resistance of melanoma to DTIC. To test this hypothesis, we developed a novel strategy to knock-down Rad51 in melanoma by introducing Rad51 siRNA with our novel drug delivery system, hemagglutinating virus of Japan envelope (HVJ-E). After UV-irradiation, therapeutic molecules such as genes, proteins and siRNA are efficiently encapsulated into inactive HVJ particle to generate HVJ-E. Using HVJ-E, those molecules can be directly introduced into the cytoplasm of target cells by membrane fusion. We first introduced Rad51 siRNA to the cultured mouse melanoma line B16-F10. Twenty-four hours after Rad51 siRNA introduction, Rad51 expression was almost completely suppressed as compared to the control cells. In colony formation assay of the melanoma cells before and after DTIC treatment, the number of the colonies of the cells transferred with Rad51 siRNA was significantly decreased by DTIC treatment than that of the mock-transferred cells. Annexin V and trypan blue staining indicated higher susceptibility to DTIC treatment in Rad51 siRNA-introduced melanoma cells. Although further study is necessary to confirm this result in vivo, these data presented here suggest that Rad51 siRNA delivery by HVJ-E may increase therapeutic efficacy of DTIC treatment in melanoma patients.

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Development of a novel histopathologic classification for folliculotropic melanomas – a rare and poorly characterized entity

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N Somani, MP Piliang, WF Bergfeld Dermatology/Dermatopathology, Cleveland Clinic, Cleveland, Ohio. Folliculotropic melanoma is a rare and poorly characterized entity with only 6 cases in the current literature. Folliculotropism is not uncommon in lentigo maligna melanoma where superficial sheathing of the follicle occurs. However, deep and extensive follicular melanomatous invasion (FMI) is rare and lacks characterization. Furthermore, practices for reporting FMI vary. Although the biologic behavior of melanomas with deep FMI is unknown, one report suggests that it confers a worse prognosis. Our objective was to characterize the histopathologic spectrum of FMI and the current reporting practices at our institution. We examined folliculotropic melanomas (FM) (n=27) seen over a 15 year period. Careful examination has allowed us to classify FMI into 5 distinct histopathological patterns: sheathing (see below), follicular junctional nests (4 cases), follicular expansile nests or bulbar invasive nests (2), parafollicular invasive nests (5), and follicular nevoid melanoma (3). Sheathing of the follicular epithelium was most common and was the only pattern present in 13 cases. It is likely to be more common than detected by our search due to under-reporting. In all cases, sheathing extended to the isthmus. Tumor bulk and depth of follicular involvement was greater in the other patterns. Next, our evaluation of pathology reports revealed marked variability in measurement and reporting practices. Measurement of Breslow's depth alone may exaggerate risk; while, failure to report the depth of follicular nests may underestimate risk. Providing measures of both thickness (from the follicle) and depth (Breslow measurement from the granular layer) may be more accurate and useful for FMI. Guidelines for standard of care and implications on prognosis require definition. We propose that our classification will provide a valuable tool to logically categorize FMI and we will correlate histologic patterns with biologic behavior in future studies.

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D type cyclin expression in cultured normal human adult melanocytes

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Normal human adult epidermal melanocytes cultured in chemical-free Mel-mix® medium proliferate rapidly, cells become bipolar, melanin content and expression of differentiation antigens tyrosinase related protein-1 (TRP-1) and c-Kit decrease, indicating that melanocytes "dedifferentiate" in the chemical-free *in vitro* culture. There is evidence that D type cyclins have other functions beside cell cycle regulation. It has been shown that D type cyclins are differentially expressed in HaCaT keratinocytes and cyclin D3 has a role in the terminal differentiation of some cell types. We were interested to see whether D type cyclins are differentially expressed in melanocytes. We compared D-type cyclin expressions in undifferentiated (TRP-1$^-$c-Kit$^-$) and differentiated (TRP-1$^+$c-Kit$^+$) melanocytes. Although all three D type cyclin mRNAs were expressed in cultured melanocytes, only cyclin D1 and D3 proteins were detectable in the cultured cells. Using the same antibodies all three cyclin proteins were detectable in melanoma, but benign nevoid lesions expressed only D1 and D3 cyclins, similar to the cultured normal cells. The expression of both D1 and D3 cyclins were weaker in fully differentiated TRP-1$^+$c-Kit$^+$ cells compared to the TRP-1$^-$c-Kit$^-$ dedifferentiated melanocytes. Interestingly, we detected high expression of cyclin D3 protein in the undifferentiated melanocytes. Cholera-toxin and TPA (phorbol 12-myristate 13-acetate) treatment resulted in "redifferentiation" of cultured melanocytes based on TRP-1 expression and cell morphology but there was no change in cyclin D1 and D3 expressions. In conclusion, in normal cultured melanocytes D3 cyclin expression does not seem to characterize the more differentiated cells, in addition we found that cyclin D2 protein expressed only in melanoma.

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The effects of Paeoniae lactiflorae radix decoction and its hydrolysates on melanin

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In Asia, women were deeply influenced by the concept that a white complexion is powerful enough to hide a number of faults. Skin colors are related to the numbers, types and distribution of melanins, which are produced by the melanocyte on the basal layer of epidermal. In the process of melanins formation, tyrosinase dominates the rate limiting steps to transform tyrosine into dopaquinone. So far, the effect of whitening agents such as arbutin and hydroquinone are not satisfied. Scientists have been making efforts to find whitening agents from natural products. Many Chinese herbs are used to modify the skin color from the ancient times. Paeonia lactiflorae radix (PLR) was believed in its whitening effect, but the ethanolic extract didn't show good inhibition on mushroom tyrosinase in former research. In this study, the effects of PLR decoction and its hydrolysates on melanin were assessed. Their effects on melanogenesis were evaluated by mushroom tyrosinase and mouse melanoma cells. The results showed that the decoction of PLR had little effect on melanogenesis, whereas the hydrolysate inhibited the activity of tyrosinase and melanogenesis without influence the expression of tyrosinase. The hydrolysate rather than the decoction might be a better candidate for developing products in whitening cosmeceuticals. Key words: Paeonia lactiflorae radix, hydrolysate, melanoma

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The glutathione peroxidase mimic *Ebselen* is a depigmenting agent that inhibits melanin synthesis, melanosomal transfer, and alters melanosome dispersion in keratinocytes

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Three major factors determine the mammalian skin colour. The amount of melanin synthesized in melanocytes, the rate of transfer of melanosomes into the keratinocytes and the type of distribution of melanosomes within keratinocyte cytoplasm (melanosome aggregation vs. cytoplasmic dispersion). Herein we introduce the glutathione peroxidase mimic *Ebselen* as a novel skin-depigmenting agent with three different mechanisms of action. *Ebselen* in non-cytotoxic concentrations inhibited melanin synthesis in cultured B16 melanocytes. In three-dimensional epidermal reconstructs (Melanoderm™), a 0.5% *Ebselen* formula decreased the amount of melanin to 50% of that of control after two weeks of treatment with no cytotoxicity against melanocytes or keratinocytes. *In vivo*, *Ebselen* produced skin depigmentation after 12 days of topical application on black guinea pig skin and significantly reduced the amount of epidermal melanin without altering the number of DOPA-positive melanocytes. Histochemical, immunohistochemical and quantitative analyses through electron microscopic studies performed on melanocyte-keratinocyte co-cultures as well as black guinea pig skin confirmed that, independently of its melanogenesis inhibiting property, *Ebselen* significantly reduced the rate of melanosomal transfer from melanocytes to neighbouring keratinocytes. Finally, *Ebselen* inhibited melanosomal dispersion in keratinocytes and caused the eventually transferred melanosomes to cluster and to form melanosomal aggregates in the keratinocyte cytoplasm *in vivo*. Melanosomal re-distribution is a well-known mechanism for tegument colour changes in low vertebrates, and the glutathione peroxidase mimic *Ebselen* appears to be the first molecule capable modulating this process in mammalian skin.

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Melanoma cells remember their origins

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Human primary and metastatic melanoma lesions frequently show heterogeneous staining pattern for melanocytic marker genes (e.g. Tyr, Melan-A). Cell cultures derived from metastases also show variable expression for these markers, and microarray analyses reveal two major expression signatures present in melanoma cell line libraries. One signature (proliferative) shows up-regulation of genes involved in melanocytic differentiation, while the other (invasive) shows up-regulation of factors involved in modifying the extracellular environment. These expression profiles correlate with phenotypic characteristics of proliferation, motility and growth factor sensitivity. We believe that expression of the melanocytic master-regulator Mitf is critical to the phenotype. Indeed, interruption of Mitf expression via siRNA reduceds growth factor susceptibility by 60%, a characteristic of the invasive phenotype. We have derived a model in which melanoma cells may switch between proliferation and invasion to drive disease progression. Here we interpret a clinical case (thick primary and metastasis to the gall bladder) in the context of our phenotype switching model. Both primary and metastatic lesions show heterogeneity of staining for melanocytic markers, an aspect explained by our model. However, we also show evidence for microenvironment-dependent behaviour in the metastasis which demonstrates that programs reminiscent of healthy melanocytes may yet be recalled by melanoma cells. Melanoma cells encountering basal membrane structures in distal locations seek isolation from their peers and re-express dendrite structures, in vivo behaviours which they are thought to have been lost during progression.

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Structural and functional basis for the melanocyte damage in vitiligo

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Epidermal melanocytes from vitiligo subjects are reported to be highly susceptible to physical and chemical oxidative stress. Previously, we proposed possible biochemical marker of the oxidative stress and basis for the disappearance of melanocytes. The aim of the present study was to better characterize the possible membrane defect and the degree of susceptibility to peroxidative stimuli in primary epidermal melanocytes of 5 vitiligo patients and healthy subjects. We evaluated the pattern of the total membrane fatty acids (GC-MS method), the transmebrane distribution of the cardiolipin (FACS analysis of the fluorescence intensity of NAO), and the degree of membrane lipoperoxidation (FACS and confocal analysis of the fluorescence pattern of C11-BODIPY581/591). Moreover, we evaluated the modulatory activity of two different pro-oxidant stimuli (7-30 mJ/cm2 UVB and 50-500 uM tBOOH) on the intracellular pathway associated with Ca++ fluxes. In particular, MAPKinases phosphorylation pattern was evaluated. We observed a different transmembrane cardiolipin distribution and lipid peroxidation in vitiligo melanocytes. The exposure to the peroxidative stimuli gave rise to a pronounced alteration of these parameters and affected the intracellular Ca++ fluxes. In conclusion, we described a possible intracellular pathway, involving the structure and the function of the lipid envelope and the kinetic of calcium, leading to the increased susceptibility to mild peroxidative stimuli.

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Stage-specific effects of Hepatocyte Growth Factor on epithelial-mesenchymal transcriptional regulators in melanocytic cells

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Hepatocyte growth factor/Scatter Factor (HGF/SF) is a known mitogen, motogen, and morphogen for many epithelial cells. It is physiologically secreted by cells of mesenchymal origin and is supposed to be crucial for melanomagenesis. Previous work has shown that HGF down-regulates E-cadherin in melanoma cell lines. Mechanisms of HGF induced down-regulation of cadherins in melanoma are unknown. Based on recent reports, which showed that the Snail superfamily of zinc-finger transcription factors represses E-cadherin and up-regulates N-cadherin, we hypothesized that HGF down-regulation of E-cadherin may be mediated through these transcription factors or Twist. Melanocytes and six melanoma cell lines were probed for changes in protein levels by immunoblotting of Snail, Slug and Twist after exposure to recombinant HGF for 8 hrs at 10 or 50ng/ml. After nuclear extraction of exposed and unexposed cells the subcellular location of these proteins was determined. Cell extracts from cell lines derived from human foreskin (FOM101), superficial melanomas (WM35, WM164 and WM793) and metastatic melanomas (WM9, WM278 and 1205Lu) were used. In all cell lines transcription factors Slug and Snail were mainly localized in the nuclear fraction. Melanoma cell lines expressed Snail, Slug and Twist in a cell type and stage-specific manner. Exposure to HGF modulated Snail, Slug and Twist in melanocytic cells at different levels. In melanoma cell lines from early lesions, Slug shifted from the cytoplasm into the nucleus, whereas Snail expression was uneffected. However, in cell lines from metastatic melanoma, Slug and Snail were down-regulated whereas Twist was up-regulated. Our data propose a stage-dependent model of HGF induced changes in epithelial-mesenchymal transcriptional regulators, whereupon Slug expression is affected by HGF in early melanoma lesions, whereas Twist in metastatic melanoma.

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Role of MMP-13 in melanoma invasion and metastasis

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Tumor invasion and metastasis of malignant melanoma have been shown to require proteolytic degradation of the extracellular environment achieved primarily by enzymes of the matrix metalloproteinases (MMP) family. We have previously shown, that increased enzyme activity is localized at the border of tumor cells and the adjacent peritumoral connective tissue emphasizing the crucial role of tumor-stroma interactions in the regulation of MMP activity. In order to prove the role of melanoma-stroma cross-talk and the importance of stroma-derived MMP-1 in the invasion process, we investigated the invasiveness of melanoma cells upon intradermal injection in mice with complete inactivation of MMP-13, the murine orthologue of human MMP-1. Tumor growth was significantly impaired in MMP-13-/- mice and most significant at early time points as compared to wild type littermates. Moreover, metastasis to various organs was reduced to 17,6 vs 30% in lungs, 2,9 vs. 30% in the liver. Strikingly, ablation of MMP-13 completely abrogated formation of metastasis in the heart (0 vs 40%). Decreased tumor growth in MMP-13-/- mice was associated with reduced blood vessel density. In addition, by magnet resonance imaging of tumor-bearing animals decreased blood vessel permeability in the tumors was measured. These data suggest an important role of MMP-13 in tumor growth and an unexpected role in organ specific metastasis of melanoma cells.

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Comparison of the depigmenting properties of retinoic acid and retinaldehyde

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Topical natural retinoids such as retinoic acid (RA) and retinaldehyde (RAL) are used for the prevention and treatment of photoageing and have depigmenting properties We used various *in vitro* and *in vivo* models to compare the depigmenting activities of RA and RAL. In cultured murine B16 melanocytes, the non-cytotoxic concentration of 1 µM of RA and RAL decreased the melanin content by 61% and 77%, respectively. The genomic analysis by qPCR showed no modulation of the genes involved in melanin synthesis and melanosome maturation, such as tyrosinase, TRP1, TRP2 (dopachrome tautomerase), and pmel17 (silver). Topical application of RA and RAL 0.05% on mouse tail skin for 3 weeks decreased the melanin content by 50% and 80%, respectively, and decreased the density of active melanocytes by 50% and 75%, respectively, as shown by the ability of melanocytes to convert DOPA to melanin. When applied to the ears of black guinea pigs, 0.01% RAL decreased epidermal melanin by 50%; electron microscopy analysis showed that the density of melanosomes in keratinocytes was decreased by 62%, whereas the density of melanosomes in melanocytes was unchanged. Thus RAL, the natural precursor of RA, which is less irritating when applied on human skin, showed better depigmenting properties than RA in various models. This indicates that RAL should be considered as a key partner in topical depigmenting preparations.

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Healthcare utilization by the elderly and impact on diagnosis of melanoma

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Although an earlier stage of disease at melanoma diagnosis correlates with improved survival, melanoma outcomes have progressively worsened for those over age 65 and no formal screening programs currently exist. We sought to examine whether increased utilization of healthcare for those over age 65 resulted in an earlier stage at melanoma diagnosis. Data regarding patients (age >67 years) diagnosed with melanoma with staging were analyzed from the Surveillance, Epidemiology, and End Results-Medicare datasets from 1991-1998. As patients often have multiple visits leading to a diagnosis of melanoma, we included visits two years preceding this event. Using outpatient visits as a surrogate for healthcare utilization we evaluated National Claims History (NCH) records. ICD-9 codes were used to evaluate severity of co-morbidities and the Charlson Index was calculated. To determine specialty and number of physicians visited, only records with a Healthcare Financing Administration provider specialty number and unique physician identification number were used. Of the 7,111 Medicare patients the mean age at melanoma diagnosis was 75.9 years, 59% were female, 85% were urban dwelling, and 51% were non-married. Women had more visits than men (22.04, 20.88, p=0.04). Urban dwellers compared to rural dwellers also had more visits (21.94, 18.21, p<0.0001) as did non-married vs. married patients (21.97, 20.72, p=0.02). For those with no co-morbidities, the mean number of visits was higher for stage 0 vs. stage 4 melanomas (16.3, 14.3, p=0.07). This trend was not true for those with co-morbidities. Outpatient visits by those without co-morbidities may be preventive health visits with greater focus on skin exams leading to earlier diagnosis. Visits for those with co-morbidities may focus on non-dermatologic problems. Greater healthcare utilization for those without co-morbidities is associated with earlier diagnosis of melanoma however Medicare beneficiaries with co-morbidities may benefit from scheduled preventive skin exams.

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Oncogenic BRAF(V600E) induces melanoma neuronal differentiation via downregulation of Notch target gene HES1

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Activating mutation in BRAF gene is the earliest genetic change found in majority of melanomas as well as benign melanocytic nevi. Neoplastic melanocytes also often exhibit characteristics of neuronal differentiation, including expression of post-mitotic, terminal neuronal differentiation marker microtubule associated protein 2 (MAP2). Earlier, we showed that MAP2 expression in cutaneous melanoma correlates with better prognosis. Although BRAF is a neuronal cell-enriched RAF kinase and its activity is associated with neuronal development, the role of BRAF in neuronal differentiation of melanoma has not been investigated. We found that cell lines expressing mutant BRAFV600E (mBRAF) have more MAP2 promoter activity compared to those with wild type BRAF or mutant RAS suggesting a role for BRAF in regulation of neuronal gene expression in melanoma. Overexpression of oncogenic mBRAF not only activated MAP2 expression in metastatic melanoma cells but also caused senescence-associated growth inhibition. Transfection studies with MAP2 promoter-reporter constructs showed that activation of MAP2 transcription by mBRAF requires HES1 binding N box in the MAP2 promoter. RT-PCR and western blot analyses showed that mBRAF overexpression downregulated HES1 mRNA with concomitant decrease in the levels of cleaved Notch, a regulator of HES1 expression. Pharmacological inhibition studies showed that Notch-independent and MAP kinase-independent pathways are involved in inhibition of HES1 expression by mBRAF. Our data suggest that HES1 is a critical factor in BRAF-induced neuronal differentiation of melanoma. Together with the observation that clinical trials of melanoma therapy with BRAF kinase inhibitor proved to be ineffective, our data suggest that a combination of BRAF kinase inhibition with Hes1 down regulation may be a more effective therapy for melanoma.

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Terfenadine-induced apoptosis on human melanoma cells involves p73-dependent and –independent cellular signalling pathways

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In the present study we have analyzed the signalling pathways involved in apoptosis induced by terfenadine (TEF), an H1 histamine receptor antagonist, on A375 human melanoma cells. To evaluate protein levels of different signalling molecules, Western Blot analysis and flow citometry were performed. Electron and confocal microscopy analysis were used in order to visualize morphological changes and subcellular localization of specific molecules, respectively. Finally, to evaluate cell viability, an XTT assay was performed. In previous work, we found that 10μM TEF induced DNA damage and caspase-2 dependent apoptosis. In the present work, we have analyzed the signalling pathways activated by DNA damage in cells treated with 7.5 and 10 μM TEF. In both cases, ATM (Ataxia Telangiectasia Mutated) was activated, but p53 activation was not detected at any dose of treatment. By contrast, an increase of p73 expression was found only when cells were treated with lower TEF doses. In this case, we have also observed a very significant increase of ROS production, release of cytochrome c and the expression of Noxa and PUMA proteins, indicating the involvement of mithocondrial pathway in the apoptosis induced by TEF. These results suggest that, although higher doses of TEF induce caspase-2-dependent apoptosis, lower doses induce apoptosis through p73 signalling pathway activation. Moreover, we have observed the formation of autophagosomes in these treated melanoma cells and the expression of Atg7 and LC3 proteins, suggesting that autophagy may also play a role in the cell death induced by TEF.

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Characterization of melanocyte label-retaining cells (LRCs) by microarray analysis

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Characterization and experimental manipulation of melanocyte stem cells (MSCs) will be important to understand mechanisms regulating melanocyte self-renewal in the hair follicle and other cutaneous sites, to determine the consequences of oncogene expression in MSCs, and to develop methods to activate MSCs to produce differentiated progeny. Previous studies describing MSCs have utilized dopachrome tautomerase (Dct) expression and BrdU label retention, as an indication of cellular quiescence in vivo, to identify MSCs in the lower permanent portion (LPP) of the murine hair follicle. Keratinocyte LRCs with stem cell properties have been identified with a bitransgenic system featuring cell type-specific expression of a histone 2B-green fluorescent protein (H2BGFP) fusion protein in a doxycycline-dependent manner. We have used Dct-tTA ('Tet-Off') transgenic mice in conjunction with TRE-H2BGFP transgenic mice to identify and isolate melanocyte LRCs in murine skin and hair follicles. H2BGFP expression is observed in numerous cells of the outer root sheath of anagen follicles at P3, P14, and later ages in the absence of doxycycline. However, after 3-5 weeks of doxycycline administration, the number of H2BGFP-expressing cells is drastically reduced, as determined by flow cytometry and fluorescence microscopy. H2BGFP LRCs in Dct-tTA;TRE-H2BGFP double-transgenic mice were detected in the LPP by colocalization with CD34, a murine bulge marker. Fluorescence-activated cell sorting of Dct-H2BGFP LRCs, and their non-label retaining counterparts, followed by microarray analysis was used to obtain gene expression profiles for these respective cell populations. Multivariate analysis of pooled microarray profiles, with false discovery proportion <0.25 and confidence=80%, identified 234 genes significantly different between these two cell populations. Validation of these findings should be useful for identifying new markers of MSCs.

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Involvement of histamine in malignant progression of human melanoma

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The purpose of our study is to analyze the involvement of histamine in the malignant progression of human melanoma. Cell cultures of melanocytes extracted from biopsies of nevus and primary melanomas were used and a XTT cell proliferation assay was performed to determinate the role of histamine in cell proliferation. Zymography assays were carried out to measure the activity of metalloproteases in the supernatants of cell cultures. Finally, an enzyme-immunoassay (ELISA) was performed to determinate blood serum levels of histamine in 94 patients with melanoma (51 patients with good evolution and 43 with metastasis) and 28 healthy controls. Both normal melanocytes and melanoma cells showed an statistically significant increase in cell growth when cultured in the presence of histamine (CI>95%, p<0,05). Moreover, an increase in the activity of metalloproteases 2 and 9 in the supernatants of cultured histamine-treated melanoma cells was observed. On the other hand, blood serum levels of histamine in the melanoma patients were significantly elevated (6,45±0,64 nM) when compared to those of controls (2,46±0,46 nM), but no differences were observed between patients with good evolution (6,19±0,78 nM) and those who presented metastasis (6,75±1,08nM).These results suggest that histamine can be involved in malignant progression of human melanoma by stimulation of melanocyte proliferation and synthesis of the necessary metalloproteases to disrupt the basal membrane of the epidermis. Nevertheless, our results also demonstrate that blood serum histamine levels are not useful as a prognostic marker in human melanoma.

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Topical pTT pretreatment decreases and delays development of melanomas in UV-irradiated mice

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Sunburns in childhood are a risk factor for melanoma, and melanoma-prone p19 knockout mice expressing H-ras driven by a tyrosinase promoter (Tyr-Hras/p19KO mice) develop more melanomas if UV-irradiated as neonates. Topical application of thymidine dinucleotide (pTT) reduces UV-induced mutations as well as SCC and BCC in genetically predisposed mice. To determine whether pTT reduces melanoma development in UV-irradiated Tyr-Hras/p19KO mice (N=21 to 28/group), we topically applied 100 μM pTT or diluent alone to pups on neonatal days 1 and 2 and sham or UV irradiated (10 mJ/cm^2) them on day 3. All mice were evaluated weekly for tumors ≥1 mm in diameter. At sacrifice, all tumors stained positively for MART-1, confirming their melanocytic origin. In pTT versus vehicle pre-treated mice, onset of melanomas in the sham-irradiated groups was delayed by 4 weeks and in the UV-irradiated groups by 5 weeks. By the end of the study (21 weeks), when most melanomas are expected to have already developed in Tyr-Hras/p19KO mice, in the UV-irradiated groups 4% of pTT mice vs 39% of control mice had died or were euthanized because of tumor burden; and pTT pretreatment significantly increased melanoma-free survival (p<0.04, Kaplan-Meyer survival analysis). At week 18, 92% of pTT/UV mice were free of melanomas vs 48% of vehicle/UVB mice, and this decrease in tumor prevalence persisted through week 21 (72% free of melanomas in pTT/UV vs 43% in vehicle/UV groups). At week 21 there were 77.5% fewer melanomas/mouse on average (0.36±0.2 vs. 1.6±0.3, p<0.01) and a 91.6% decrease in average melanoma burden (27.3±12.7 vs. 325.1±100.7 mm^3 of tumor volume per mouse, p<0.0001) in pTT vs vehicle-treated UV-irradiated mice. There were no significant differences in melanoma burden or tumor-free survival in the sham irradiated groups. Our data demonstrate that two topical pTT applications prior to UV irradiation in the neonatal period reduce and delay UV-induced melanoma development in melanoma-prone mice.

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The role of p73 in human melanocyte DNA damage responses

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Some neural crest-derived cell types and several malignant cell lines have been reported to induce p73, a homolog of p53, in response to DNA damage. Because melanomas, unlike other UV-induced skin cancers, rarely show p53 mutations early in their evolution, and because epidermal melanocytes are neural crest-derived and p73 mutations have been reported in melanomas, we asked whether p73 rather than p53 is the more critical tumor suppressor and DNA damage response protein in melanoctyes. p73 is expressed as both a trans-activating p73 (TAp73) variant with the same transcriptional targets as p53 and an amino terminus truncated dominant negative p73 (ΔNp73) variant that comparably blocks transcriptional activity of both TAp73 and p53. Increased ΔNp73 is reported in many malignancies and the ratio of ΔNp73 to TAp73 is believed to be important in tumorigenesis. By immunoblot analysis, primary human melanocytes had readily detectable TAp73 and very low ΔNp73, while keratinocytes and fibroblasts showed the opposite pattern. In cultures treated with 10 μM cisplatin, a DNA cross-linking agent shown to induce TAp73 in some cell types, vs. vehicle alone, keratinocytes and fibroblasts showed no change in TAp73 or ΔNp73, but melanocytes increased TAp73 by 2-3 fold at 15min – 1hr without affecting ΔNp73. Telomere homolog oligonucleotides (T-oligos) are thought to mimic DNA damage (telomere loop disruption) caused by UV irradiation, while UV damages multiple cell components. Treatment of melanocytes with T-oligos (40 μM GTTAGGGTTAG) increased TAp73 by 2-3 fold and decreased ΔNp73 by 50-70% at 24 and 48 hours, while p53 was unchanged. UVB irradiation of melanocytes (30 mJ/cm2) also induced TAp73 by 2-3 fold 4-48 hours after the irradiation while p53 was transiently induced by up to 5-fold at 4 hours and up to 2-fold at 24 hours after irradiation. These data suggest that p73 may play a larger role in response to DNA damage in melanocytes than in keratinocytes or fibroblasts. This offers a testable hypothesis regarding the relative lack of p53 mutations in primary melanomas.

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Down-regulation of catalase expression and activity in human scalp hair follicle melanocytes is associated with hair graying

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Several theories have been advanced to explain the dramatic age-related loss of pigment from hair (canities). The emerging consensus is that canities results from a combination of reactive oxygen species (ROS)-associated damage in hair follicle melanocytes (HFM) coupled with an impaired anti-oxidant status, and a failure of melanocyte stem cell renewal. In this study we examined catalase protein expression in situ and its activity in cultured HFM derived from young and aged donors. Melanocyte survival, catalase expression and activity, and the expression of p38 stress kinase were examined after HFM exposure to the oxidant hydrogen peroxide (H2O2) from both exogenous and endogenous sources. Furthermore, the effect of anti-oxidants on HFM survival, catalase expression and activity, and p38 kinase expression was examined. Catalase expression was reduced in HFM in situ with advancing donor age, while its expression/activity was found to be higher in young donor HFM in vitro compared to aged donor cells. However, no age-related change in catalase expression/activity was observed in matched dermal fibroblasts. Catalase expression and activity was up-regulated by exogenous and endogenous H2O2 in young, but not, aged donor HFM, which also correlating with higher cell survival after H2O2 exposure of young donor cells. Indeed, HFM from aged donors expressed higher p38 stress kinase baseline levels compared to young donor cells, and this was further increased substantially in aged donor HFM only after exposure to ex-/endogenous H2O2. The addition of two anti-oxidants (Vitamin E and Quercetin) to H2O2-treated HFM from aged donors resulted in increased cell survival and increased catalase expression and activity. Thus, an age-dependant decrease in catalase expression/activity in HFM supports a reduction in anti-oxidant defence capability in hair pigmentation loss with age.

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The most aggressive melanoma tumors have the most cancer stem cells.

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Melanoma remains, by far, the most serious skin cancer, and is among the most chemotherapy-resistant malignancies. While prevention and early detection improve, the incidence of melanoma continues to rise in the U.S. and among most fair-skinned populations worldwide. Although the central role of cancer stem cells has been rigorously established in maintaining myeloid leukemia, breast, brain and other cancers through xeno-transplantation studies, the role of melanoma stem cells (MSCs) in initiating the neoplasm and driving growth remains to be rigorously established. We sought to determine the existence of a cancer stem cell in freshly obtained human melanoma samples and to determine whether MSC frequency was correlated with tumor aggressiveness. Here we show first, that a population of human melanoma cells with high levels of aldehyde dehydrogenase (ALDH) is enriched in cells able to form a tumor when transplanted into NODSCID mice. Second, melanomas with increased MSC frequency are more aggressive as assessed by the histopathology of the primary tumor, the rapidity of growth both *in vitro* and in a murine xeno-transplantation model, and the decreased time from original diagnosis to metastasis. The identification of MSCs distinct from bulk tumor cells, as well as the ability to serially transplant this fraction of cells, provides strong support for a self-renewing MSC and a hierarchical organization of cellular differentiation in melanoma. Furthermore, since increased frequency of MSCs in a lesion was associated with high-risk lesions, these studies highlight the need to target this cell as an avenue to more effective melanoma therapy.

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The leading edge of vitiligo skin lesions contain a higher density of CD11c$^+$ and blood derived dendritic cell antigen-1$^+$ myeloid dendritic cells than non-lesional (pigmented) skin.

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Elucidation of the populations of dendritic cells (DCs) underlying the immune environment of lesional (depigmented skin), leading edge (junction between lesional and non-lesional skin) and non-lesional (pigmented skin) skin biopsies is essential for clarifying the pathogenesis of vitiligo and developing effective clinical therapies. In our study, we characterized and quantified via immunohistochemistry the DC populations present in non-lesional, lesional and leading edge skin biopsies of 20 patients with vitiligo. Here, we found that there is a higher density of CD11c$^+$ and blood DC antigen-1$^+$ (BDCA-1) myeloid DCs in the leading edge of vitiligo lesions than in paired non-lesional and lesional skin biopsies. In the leading edge of vitiligo skin biopsies, both CD11c$^+$ DCs and langerin$^+$ Langerhans cells localized near the dermal-epidermal junction and some CD11c$^+$ DCs were present in the epidermis. Furthermore, few cells stained for CD83$^+$,a DC maturation marker. Thus, we hypothesize that CD11c$^+$, BDCA-1$^+$ and CD83$^-$ myeloid DCs, and langerin$^+$ Langerhans cells subpopulations of lymphocytes appear to play a larger role in the pathogenesis and progression of vitiligo than previously thought.

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Antiproliferative and proapoptotic effects of histone deacetylase inhibitor vorinostat in human melanoma

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Malignant melanoma is notoriously resistant to intrinsic and extrinsic apoptotic pathways and thus its response to standard chemotherapy is poor. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) are the key enzymes that regulate the chromatin structure and function via virtue of acetylation and deacetylation of the acetyl group from the lysine residues of the core nucleosomal histones. Aberrant expression and function of these enzymes has been associated with various malignancies. HDAC inhibitors (HDACIs) have recently evolved as a novel class of antineoplastic agents that promote an open chromatin structure through acetylation of histones, thus regulating the transcription, expression and function of genes involved in proliferation, differentiation, survival and apoptosis. Vorinostat (Zolinza™), an inhibitor of class I and II HDACs, is FDA approved for the treatment of cutaneous manifestations in patients with CTCL who have progressive, persistent or recurrent disease on or following 2 systemic therapies. We tested the efficacy of this compound in amelanotic and metastatic melanoma cell lines A-375 and Hs-294T. After 48 hours, treatment with vorinostat (0, 5, 7.5 and 10μM) resulted in a dose dependent inhibition of growth, viability and colony formation that was greater in A-375 cells than Hs-294T cells (up to 81% reduction in viability). Cell cycle analysis showed a higher percentage of the sub G-1 population in A-375 cells compared to Hs-294T cells, as well as G2-M arrest in both the cell types. To further elucidate the mechanism of action of the drug, we tested its effect on PARP cleavage and Bcl2. Western blot analysis revealed decreased expression of anti-apoptotic Bcl2 and the proteolysis of PARP to its stable 85 kDa fragment (an early marker of programmed cell death). These results suggest that vorinostat might impart its growth inhibitory and pro-apoptotic effects at least in part via cell cycle regulatory and Bcl2 pathways. Further studies are ongoing to confirm and extend these findings.

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Inhibitory effect of *Sophora japonica* on tyrosinase activity and melanin synthesis in murine melanoma cells

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Melanin synthesis is related to tyrosinase presence and indicative of active metabolic state. Tyrosinase is known to accelerate the melanin biosynthesis in melanocyte, so tyrosinase inhibitors hinder the melanin biosynthesis and are useful for the material used in cosmetics as skin-whitening agents. *Sophora japonica* (H17) is a traditional herb medicine used to treat arteriosclerosis, hypertension and other disorders. H17 extracts showed over 50% inhibition of free radical scavenging effects by DPPH assay method and 36% inhibitory effect against tyrosinase activity by the mushroom tyrosinase assay, at a concentration of 100 ug/ml. Melanin biosynthesis was estimated from B16 mouse melanoma cell, which treated with 10 nM of α-MSH and with or without H17 extracts. H17 extracts inhibited (>40%) melanin synthesis at 100 ug/ml, but it did not effect on cell proliferation. According to RT-PCR and western blot methods, tyrosinase mRNA and protein were decreased when H17 extracts was treated in B16 mouse melanoma cells. For this reason, H17 extract inhibits tyrosinase activity, tyrosinase production and melanin biosynthesis. Hence, H17 extract contains a potent agent for skin whitening and skin therapeutic agent.

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Blood-based biomarkers of malignant melanoma by gene expression analysis

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Developing analytical methodologies to detect and identify biomarkers is highly valuable for the early diagnosis and management of cancer patients. Peripheral blood represents systemic processes including immune responses and cellular communications. We hypothesized that blood-based biomarkers using peripheral blood cells from melanoma patients and high-throughput qRT-PCR methodology would provide new biomarkers for melanoma. We first performed cDNA microarray (Human Genome U133 Plus 2.0 array) analysis using blood samples from stage IV melanoma patients, and selected 72 candidate genes. We then performed high-throughput qRT-PCR with the collaboration of SourceMDx using whole blood samples obtained from 45 newly diagnosed melanoma patients (stage I to IV) and 50 normal subjects. Forty-one genes were confirmed to be differentially expressed in melanoma blood (from stage I to IV) compared with normal blood. Many genes are unique sets not previously described in cancer tissues or disease blood, but were differentially expressed in the blood of melanoma patients, even from stage I/II melanoma patients. An automated search procedure in the program GOLDMineR® (Graphical Ordinal Logit Displays based on Monotonic Regression) was then used to implement a stepwise logit analysis for selecting the predictor target genes that distinguish melanoma from normals. We have found that only 2 genes were necessary to classify melanoma patients accurately (sensitivity 93%, specificity 90%, and R-square 0.731). Blood-based biomarkers have several advantages over tissue-based biomarkers. Blood sampling is less invasive and can be used as a screening test to diagnose diseases. Repeated blood sampling is feasible to monitor disease activities and treatment responses. Our report provides the first comprehensive study that analyzes blood-based genetic biomarkers of malignant melanoma.

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Identification of growth-promoting networks differentially activated in CDKN2A wild-type melanoma tumors

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Inactivation of p16 through homozygous deletions, mutations and promoter methylation is a frequent molecular event in melanoma, leading to cell cycle deregulation and contributing to tumorigenesis. We hypothesized that melanoma tumors with wild-type p16 differentially target alternative growth-regulatory mechanisms to achieve the same final state of uncontrolled growth as tumors with p16 aberrations. Identification of such mechanisms could increase our understanding of the melanoma oncogenesis and facilitate targeted therapy development. Mutational status of p16 locus was evaluated in a set of 70 human melanoma tumors using a combination of array-based Comparative Genomic Hybridization (aCGH), targeted sequencing of p16 locus and promoter methylation analysis. In parallel, global gene expression profiling was performed on the entire dataset. Using the p16 gene status as a separator, a supervised analysis of gene expression profiles was carried out. This analysis identified a set of 230 genes with statistically significant difference in expression between the two groups. Knowledge mining approaches were used to map the identified genes to the known gene networks. A cluster of growth regulatory genes was identified using this approach. Significant number of genes in this cluster are overexpressed in p16 wild-type tumors and appear to target growth-promoting networks such as FGFR activation network (e.g. HOX2B and EPHA4), MAP kinase signaling (e.g. NLK) or cell cycle progression (e.g. MYBL2). Similarly, genes with negative growth-regulatory functions mapping to this cluster show decreased expression in p16 wild-type melanoma tumors. Our data demonstrates a useful approach for integration of genomic data and identifies growth regulatory networks that are selectively activated in melanoma tumors with wild-type p16. These types of approaches have potential to elucidate molecular mechanism underlying melanoma and other cancers and facilitate targeted development of tailored therapy.

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Polymorphisms of p53 and its negative regulator MDM2 in human melanoma

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The purpose of our study was to examine 1) the relationship between melanoma risk and a single nucleotide polymorphism in *p53* (a tumor suppressor gene observed in more than 50% of human tumors yet uncommonly mutated in melanoma) that results in the Arg72Pro substitution, and 2) the association between Arg72Pro and *MDM2 SNP309*, a recently described polymorphism in a key negative regulator of p53; the relationship between p53 Arg72Pro and melanoma remains controversial, and its association with *MDM2 SNP309* has not been previously reported in melanoma patients. 298 melanoma patients (126 female, 172 male, 57.5 median age) prospectively enrolled in the NYU Interdisciplinary Melanoma Cooperative Group were studied. DNA was isolated from human blood leukocytes and analyzed for p53 Arg72Pro and *MDM2 SNP309* status by PCR-RFLP. Associations between p53 Arg72Pro, *MDM2 SNP309*, and clinicopathologic features of melanoma were analyzed using chi-square, Fisher's exact, and Wilcoxon tests, as appropriate. The distribution of p53 Arg72Pro genotypes (RR: 55.0%, RP: 37.6%, PP: 7.4%) did not differ from published controls. In addition, no differences were observed between men (PP: 7.3%) and women (PP: 7.6%). There was no association between p53 Arg72Pro and *MDM2 SNP309* in men (p=.68) or in women (p=.74). Median tumor thickness (RR: 0.72 mm; RP: 0.86 mm; PP: 1.56 mm; p=.117) and proportion of cases with ulceration (RR: 12%; RP: 17%; PP: 33%; p=.088) varied with p53 Arg72Pro genotype, however these associations failed to reach statistical significance. These data suggest that p53 Arg72Pro is unlikely to affect melanoma risk. In addition, there was no association between p53 Arg72Pro and *MDM2 SNP309* genotypes in melanoma patients. Expansion of this study to include matched controls is required before a firm conclusion regarding the role of p53 Arg72Pro in melanoma can be drawn.

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Inhibition of PAX3 by TGF-β modulates melanocytes viability

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As a key regulator of microphthalmia-associated transcription factor (MITF), paired box 3 (PAX3) is essential in the melanocyte lineage. Here we provide biochemical evidence demonstrating that PAX3 in skin is directly inhibited by TGF-β/SMAD. Whereas TGF-β in keratinocytes is repressed by UV, PAX3 in melanocytes is up-regulated by loss of repression of TGF-β/SMAD. The repression of TGF-β in keratinocytes by UV is associated with UV-induced melanocytes activation and pigmentation. Furthermore, PAX3 is highly expressed in melanomas and correlates with clinical characters of melanomas. PAX3 is commonly expressed in sun-exposed sites, metastatic melanomas and young patients. Moreover, PAX3 contributes to growth of primary melanocytes and melanoma cells and thus has potential as a therapeutic target in melanoma treatment.