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Three decades of advancements in osteoarthritis research: insights from transcriptomic, proteomic, and metabolomic studies

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SUMMARY

Objective: Osteoarthritis (OA) is a complex disease involving contributions from both local joint tissues and systemic sources. Patient characteristics, encompassing sociodemographic and clinical variables, are intricately linked with OA rendering its understanding challenging. Technological advancements have allowed for a comprehensive analysis of transcripts, proteomes and metabolomes in OA tissues/fluids through omic analyses. The objective of this review is to highlight the advancements achieved by omic studies in enhancing our understanding of OA pathogenesis over the last three decades.

Design: We conducted an extensive literature search focusing on transcriptomics, proteomics and metabolomics within the context of OA. Specifically, we explore how these technologies have identified individual transcripts, proteins, and metabolites, as well as distinctive endotype signatures from various body tissues or fluids of OA patients, including insights at the single-cell level, to advance our understanding of this highly complex disease.

Results: Omic studies reveal the description of numerous individual molecules and molecular patterns within OA-associated tissues and fluids. This includes the identification of specific cell (sub)types and associated pathways that contribute to disease mechanisms. However, there remains a necessity to further advance these technologies to delineate the spatial organization of cellular subtypes and molecular patterns within OA-afflicted tissues.

Conclusions: Leveraging a multi-omics approach that integrates datasets from diverse molecular detection technologies, combined with patients' clinical and sociodemographic features, and molecular and regulatory networks, holds promise for identifying unique patient endophenotypes. This holistic approach can illuminate the heterogeneity among OA patients and, in turn, facilitate the development of tailored therapeutic interventions.

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Introduction

Osteoarthritis (OA) is characterized by pain and reduced function, involving both local joint and systemic tissues and fluids. Various sociodemographic and clinical factors are associated with incidence and progression of OA.[1,2] These sociodemographic (e.g., age, sex, BMI etc.) and clinical factors (e.g., chronic pain, comorbidities such as metabolic disorders, inflammation, malalignment etc.) refer to some phenotypes of OA. Studies have described multiple OA phenotypes, with contributions of these components.[3,4] Individual patient characteristics can also modify systemic molecular profiles,[5] contributing to OA. OA endotypes are defined by molecular signatures and associated mechanisms that underly disease pathologies. For instance, low tissue turnover, structural damage, and systemic inflammation endotypes were identified from biochemical markers in urine and serum using two prospective cohorts of patients with OA, with some differences in the proportions of subjects with pain and structural progression between endotype groups.[6] However, all groups had progressors and non-progressors regardless of group, suggesting the biochemical marker endotypes identified are only part of the puzzle. A deeper understanding OA endotypes has recently become a major focus to uncover new targets and disease-associated mechanisms. This is achieved through advanced omic technologies, including transcriptomics, proteomics, and metabolomics. Combining endotypes uncovered from these advanced approaches with OA patient phenotypes may help to uncover distinct endotype-phenotype combinations (endophenotypes) to disentangle OA disease heterogeneity.

Transcriptomics analyzes transcripts expressed from the genome.[7] Proteomics, conceived in 1994,[8] is the large-scale analysis of proteins, including their identification, quantification, post-translational modifications (PTMs), localization, and degradation. Metabolites are the reactants and products of biochemical reactions mediated by proteins. Metabolomics, defined in 1998,[9] involves the characterization of thousands of metabolites.[10]

Over the past 30-plus years, advancements in transcriptomics, proteomics, and metabolomics technologies have provided insight into molecular changes occurring in OA tissues and fluids. In this review, we highlight how these omic technologies have contributed to our biological understanding of OA. We also discuss how spatial-omics could aid in better understanding OA at the tissue level, and how multi-omics approaches can help define OA populations by both endotype and phenotype, unraveling the complex heterogeneity observed in individuals with OA.

Transcriptomics

The field of OA has greatly benefited from high-throughput transcriptomic tools (Fig. 1). Advancements in microarray technologies, followed by progressive improvements to high-throughput sequencing methods, including “bulk” RNA-sequencing (RNA-seq) and single cell (sc)RNA-seq, have led to deeper understanding OA pathophysiology at the tissue, cell, and molecular level. Here, we emphasize the application of transcriptomics in different joint tissues affected by this disease.

Cartilage

In adult articular cartilage tissue, the sole cell type found are chondrocytes that are organized into zones where they have distinct functions to maintain tissue homeostasis and extracellular matrix (ECM) turnover. During OA, there is a shift from homeostasis to catabolism, resulting in cartilage degeneration and loss of chondrocytes.

Early OA studies using DNA array technology focused on disease mechanisms including involvement of matrix metalloproteinases or the imbalance between anabolic and catabolic processes by comparing unaffected and OA-damaged cartilage.[11] More comprehensive microarray studies in the early 2000s reported significant differences in differentially expressed genes related to cell proliferation, collagen synthesis, and ECM degradation when comparing intact and damaged cartilage,[12] and differentiated between different grades of OA cartilage specimens demonstrating the potential implication of increased oxidative stress and cell damage in OA chondrocytes.[13] Following studies tried to tackle specific experimental challenges using genome-wide microarrays and different specimen comparisons.[14–16] As a result, the list of pathologically relevant candidate genes was extended, including genes involved in bone formation and skeletal development.

Since human repair phenotypes are inaccessible and clinical samples are limited for analysis, animal models provide a great opportunity to uncover additional genes and signaling pathways that are regulated at the transcriptional level during early OA. Appleton et al. performed one of the first studies investigating transcriptional changes in chondrocytes using a surgically induced rat OA model[17] and identified chemokines, such as *Cxcr4* and *Ccl2*, with implications in progressing and early degrading cartilage, respectively. Studies in mice undergoing destabilization of the medial meniscus (DMM) surgery to induce OA, aimed to delineate the evolution of changes in chondrocyte gene expression during early disease, focusing on different time-points,[18,19] and dissect the role of previously identified central genes (such as *ADAMTS5*) and aging.[20] These preclinical studies had a crucial role in characterizing the complex pathogenesis of OA cartilage degradation, which is activated upon first disease induction.

It has been proposed to enrich the current, more clinical approaches towards phenotyping to include molecular endotypes derived from omic technology data.[21] Fernández-Tajes et al. found in cartilage microarray data from 23 patients two endotypes with gene expression differences related to inflammatory response,[22] while Soul et al. identified two endotypes in RNA-seq data from 44 patients, but with differences related to oxidative stress, innate immune responses and Wnt signaling.[23] Steinberg et al. reported comparable observations with two endotypes (including inflammation).[24] Yuan et al. identified four patient endotypes when combining different tissues, underlining the importance of OA as a “whole joint disease.”[25] Future studies will leverage advanced computational methods and machine learning to characterize transcriptomic endotypes and their correlation with clinical phenotypes.

The first scRNA-seq study on human OA cartilage was published in 2018, providing evidence for seven molecularly-specific subpopulations and transient states of chondrocytes, as well as novel functional phenotypes with regulatory functions including immunomodulation.[26] The dataset further allowed identification of cartilage progenitor cells (CPCs) that express stem cell-related surface markers. Following studies partly confirmed these initial findings and integrated synovial tissue/cells in the analysis to dissect synovial cell-chondrocyte crosstalk mechanisms.[27] These cell population-based studies uncovered subordinate disease mechanisms that have been submerged in RNA-seq gene expression studies, such as ferroptosis.[28] One of the first studies published applying scRNA-seq to animal models was conducted, demonstrating that most cell clusters described in humans are also present in mouse knee cartilage.[29] A recent methodological approach combined RNA-seq with scRNA-seq, which generated a high confidence (pure) chondrocyte gene signature by avoiding cross-contamination from other tissues, a process necessary in smaller animal models with limited tissue availability.[30] These advancements have led to a deeper understanding of molecularly defined chondrocyte subtypes, including the

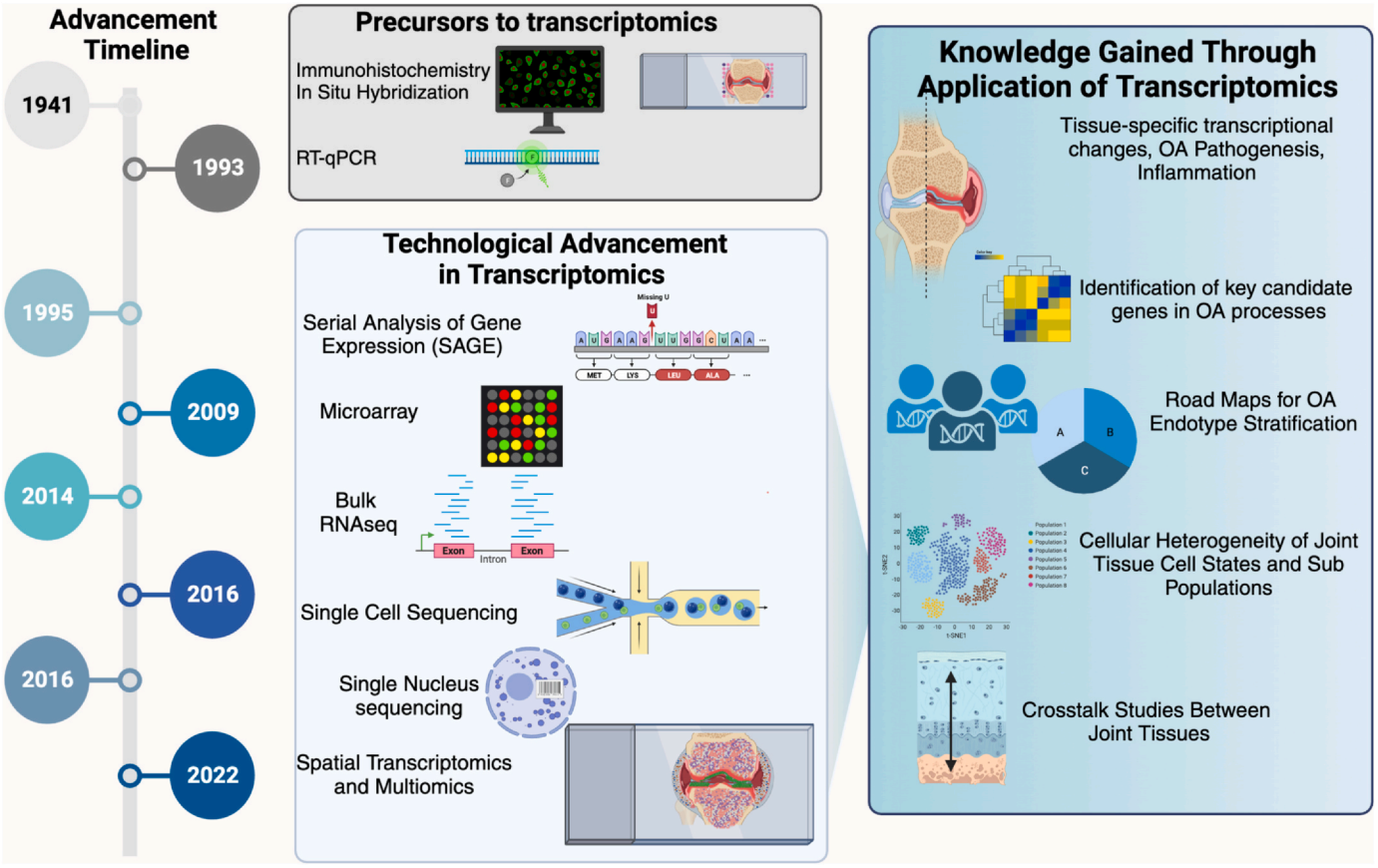

Fig. 1 Osteoarthritis and Cartilage

Transcriptomics – technological advancements and application to OA. Transcriptomics has evolved over time from the advent of SAGE sequencing and microarrays to include high-throughput sequencing techniques. Combining sequencing technology with microarray and imaging has also allowed for the development of spatial transcriptomics. Transcriptomics has advanced the field of OA by enhancing our understanding of tissue-specific and inter-tissue cross-talk, determination of key genes associated with OA, uncovering transcriptomic endotypes, and identifying joint cell heterogeneity.

differentiation between pre-hypertrophic and hypertrophic chondrocytes, and the definition of CPCs, with more recent studies shedding light into synovial-chondrocyte cell interactions and immune cell involvement in OA cartilage degradation.

Synovium

The synovium is the inner lining of joints. Composed of a heterogeneous population of stromal cells, immune cells, adipocytes, and nerve-, blood vessel-, and lymphatic vessel-associated cells, the synovium undergoes dynamic cellular and molecular changes during OA progression.[31]

Early microarray studies of rodent and human OA synovium revealed a signature of fibroblastic and myeloid enrichment, with regulated genes involved in TGF-β signaling, collagen synthesis and cross-linking, and innate immunity.[32] Relevant to fibrosis, Remst et al. found that *PLOD2*, *LOX*, *COL1A1*, *COL5A1*, and *TIMP1* were increased in end-stage human OA synovial tissue and TGF-β-stimulated human OA synovial fibroblasts.[32] Lambert et al. analyzed human end-stage OA synovium by microarray of normal vs inflamed regions within the same subjects.[33] Inflamed synovial regions overexpressed select cytokines, chemokines, various enzymes, catabolic proteases, and markers of angiogenesis. This was among the first studies to demonstrate anatomic region-dependent variability in the transcriptome of OA synovium. Microarray analysis of cultured synovial fibroblasts from end-stage OA, compared to healthy and end-stage RA synovial tissue, showed that OA fibroblasts exhibited enriched genes related to cell adhesion and actin cytoskeleton, small GTPases and GTPase signal transduction, and neurotrophic mediators, further solidifying the more fibrotic nature of OA synovium.[34] Microarray studies were instrumental in revealing the synovial gene expression signatures of OA, marked by abundant adaptive immune-related and pro-fibrotic processes.

The advent of RNA-seq facilitated deeper identification of specific signaling pathways active in the OA synovium. Steinberg et al. performed RNA-seq analysis of synovium from 113 OA patients and identified two synovial endotypes: one characterized by inflammatory genes and one by ECM- and cell adhesion-related genes.[35] It is unclear whether these endotypes are simply different disease states (e.g., patients with active inflammatory flares) or truly reflect different pathomechanisms. To study the contribution of beneficial (i.e., exercise) and detrimental (i.e., injury and fibrosis) mechanical loading experienced by synovium, Philpott et al. subjected human late-stage OA synovial tissue to low-frequency or high-frequency tensile strain, followed by RNA-seq.[36] Low-frequency loading enriched for pathways reglated to deinterferon-gamma and -alpha responses, Fc receptor signaling, and lysosomal routing, proposed as protective immunomodulatory and inflammation-resolving functions. Conversely, high-frequency loading activated

pathways related to NOD-like receptor signaling and redox stress, increased lactate release, and promoted 3-nitrotyrosine formation, which are detrimental to synovium as they induce synovial inflammation. Further studies are needed to describe mechanoreceptors and intracellular signaling responsible for synovium mechanosensitivity.

To study macrophage phenotypes in arthritis, flow-sorted synovial tissue-derived macrophages from OA and inflammatory arthritis (RA or psoriatic arthritis) were analyzed by RNA-seq.[37] Two distinct subgroups of OA macrophages were identified, underpinned by 155 differentially-expressed genes: a "classical OA" subset and an "inflammatory-like OA" subset more similar to macrophages from inflammatory arthritis. The inflammatory-like subset was enriched in pro-inflammatory signaling and cell-cycle genes, which was functionally confirmed by flow cytometry, demonstrating that synovial tissue from these patients had a ~2.5-fold greater number of macrophages. Using a model of anterior cruciate ligament (ACL) rupture in mice, Bergman et al. described divergence of synovial transcriptomes between male and female mice associated with greater progression of post-traumatic OA severity, pain behavior, matrixmetalloproteinase activity, and osteophyte formation in male mice.[38] Male mice had increased expression of pro-fibrotic, neuroangiogenic, and extracellular signal-regulated kinase signaling genes, indicating that synovitis may be a key driver of the well-documented greater OA severity in male mice across OA models.

One of the first scRNA-seq datasets of OA synovium was published in 2018, which included characterization of synovial fibroblasts from OA and RA patients.[39] From 337 fibroblasts from two OA and two RA patients, this study identified three primary synovial fibroblast groups: 1) A *CD34*(-), *Thy1*(-) population, now recognized as *PRG4*^{High}, *CLIC5*+ lining/intimal fibroblasts; 2) A *CD34*(-) *Thy1*(+) population localized to the sublining/subintima near blood vessels, and 3) a *CD34*(+) *Thy1*(+), now recognized to represent progenitor-like *DPP4*+/*PI16*+ "universal fibroblasts."[40] The first whole-synovium OA atlas was published by Chou et al. in 2020 and comprised ~10,600 synovial cells from three OA patients.[41] Major synovial cell types were identified and characterized by top differentially expressed genes: lining fibroblasts, sublining fibroblasts, smooth muscle cells, endothelial cells, mast cells, T cells, macrophages, dendritic cells, and B cells. This study also constructed a model of synovial-cartilage crosstalk using ligand-receptor expression patterns, demonstrating that synovium is the primary contributor of cytokines and chemokines whereas cartilage is responsible for growth factor and morphogen production.

To understand cellular sources of neurotrophic mediators responsible for OA pain, Nanus et al. demonstrated that OA synovial tissue from sites of joint pain are enriched in synovial fibroblasts expressing neurotrophic mediators, compared to sites with lesser pain.[42] In end-stage OA patients, these fibroblasts expressed genes related to eicosanoid signaling, prostanoid biosynthesis, and insulin growth factor-1 (IGF-1) signaling, all recognized to mediate nociceptive sprouting and pain perception. In early OA painful synovial sites, fibroblasts also expressed genes related to IGF-1 and eicosanoid signaling. Thus, local enrichment of nociceptive nerve fibers giving rise to region-dependent pain signatures is likely driven, in-part, by distinct synovial fibroblast subsets. Defining the molecular regulation of these fibroblasts could produce novel OA pain therapeutics targeting these cells.

To describe the emergence of post-traumatic OA-associated synovial fibroblasts, Knights et al. performed scRNA-seq of murine synovium following noninvasive ACL rupture.[43] Seven synovial fibroblast subsets were described, including a *Prg4*^{High} lining fibroblast, which overexpressed the Wnt agonist R-spondin 2 following injury, and four sublining fibroblast populations with unique molecular programs. Among the sublining populations was a *Dpp4+/Pi16+* stromal progenitor, consistent with the "universal fibroblast."[40] Differentiation trajectory analysis suggested this population gave rise to *Acta2*/αSMA+ myofibroblasts that further transitioned into *Prg4*^{High} lining fibroblasts, underpinning synovial lining hyperplasia. *Sox5* was identified as a molecular regulator of *Dpp4+* progenitor-derived lining fibroblasts and R-spondin 2 expression. Subsequent work using a cartilage injury model further demonstrated that *Dpp4+/Pi16+* synovial progenitors were derived from the *Gdf5*-lineage joint interzone, giving rise to *Prg4*-expressing lining fibroblasts, with *Sox5, Foxo1, and Creb5* predicted as transcription factors underpinning lining fibroblast fate.[44]

Robust descriptions of OA synovial immune phenotypes and their origins are still lacking, which remain challenging given the incomplete understanding of genes unique to synovial immune cells, absence of lineage-tracing models that do not overlap between resident and systemically-derived cells, and the short half-life of many immune cells.

Subchondral bone

Subchondral bone refers to cortical, trabecular and marrow adipose tissue (BMAT) compartments beneath articular cartilage. Advanced OA stages display substantial thickening of the subchondral cortical plate and trabeculae. Changes in the composition of BMAT, referred to as bone marrow lesions (BMLs) are associated with pain and cartilage volume loss. First insights from histopathological studies showed blood vessels and nerves invade the subchondral cortical plate during OA, and there is an elevated presence of macrophages, vascular structures, and osteoclasts in BMAT.[45–49]

Initial transcriptomic investigations into human OA subchondral bone and BMLs were conducted during the early to mid-2000s, utilizing microarray technology.[50–52] A landmark study comparing osteoporotic and OA femoral heads yielded initial molecular evidence for increased expression of pro-angiogenic genes within OA subchondral bone.[50] Functional analyses unveiled enrichment of pathways related to angiogenesis, collagen fibrils, and cell proliferation between sclerotic and non-sclerotic tibial plateaus.[53] A similar approach comparing knee tibia BMLs to controls identified angiogenesis, cytokine signaling, PDGF and Wnt signaling pathway enrichment.[52] Among the potential molecular targets suggested by these studies, *STMN2, IL11* and *CHADL* were confirmed recently using RNA-Seq.[54]

Advancements in bioinformatic tools have helped to start unravel cellular and molecular mechanisms driving tissue remodeling in OA. Ligand-receptor mapping of cartilage and subchondral bone RNA-seq profiles inferred increased angiogenesis and ECM remodeling pathway molecular crosstalk.[25] However, the diverse array of cell types present in subchondral bone, including osteocytes, adipocytes, osteoblasts, osteoclasts, vascular cells, immune cells, and progenitor cells in BMAT, continues to present significant challenges in interpreting bulk transcriptomics data. Notably, a preliminary study utilizing scRNA-seq indicated the existence of at least 10 distinct molecular cell types within BMAT.[55]

The size of murine joints is a major constraint to study subchondral bone in models of OA, thus transcriptomic analyses were usually conducted using intact cartilage and bone,[56] entire bones,[57] or larger animal models.[58] Analysis of temporal gene expression profiles following surgical induction of OA revealed upregulation of osteoclast-related genes at early stages, followed by induction of osteoblast-related genes at later stages.[58] Furthermore, transcriptomic analysis of aging bone lacking BMAT revealed differential expression of several genes associated with human hip or knee OA, such as *COL27A1, COL2A1* and *COL11A1.*[57]

Expanding upon the foundation laid by current transcriptomic datasets, further investigations using proteomics[59–61] and lipidomics of subchondral bone will open new avenues for disease endotyping, biomarker discovery and development of novel therapeutic targets.

Meniscus

Within OA-affected menisci, there are indications of gross and histologic pathology, characterized by increased water, proteoglycan, and collagen content, and elevated expression of *MMP13*.[62] In a significant advancement, Brophy et al. performed RNA-seq analysis on meniscus tissue, demonstrating that menisci of OA-afflicted joints exhibit an inflammatory phenotype, contrary to menisci from non-OA joints, which display a repair-oriented phenotype.[63] In addition, this analysis revealed the involvement of epigenetically regulated histone deacetylation in meniscus tears as well as the expression of lncRNAs. A subsequent investigation elucidated key biological processes (inflammation, chemotaxis, cytokine-to-cytokine interaction) in the context of OA-related meniscus degradation.[64]

Subsequently, a pioneering scRNA-seq study of human meniscus identified cellular heterogeneity and changes in the proportions of cell clusters based on disease status. In the context of healthy menisci, five empirically defined cell populations and two novel cell clusters were identified. In stark contrast, OA degenerated menisci revealed four cell clusters, with one being distinctive due to its progenitor cell characteristics.[65] In-depth analyses clarified the cellular composition of the meniscus and the precise manners in which specific cell clusters partake in both development and degenerative processes. For instance, gene transcripts representative of meniscus degeneration (*GAS1*, *RAB3B* and *CD318*) were highly expressed in a unique cell cluster. The peak expression of these genes coincided with advanced stages of meniscus cell differentiation, highlighting an aberrant cellular degenerative response, shedding light on potential mechanisms driving meniscus degeneration and its association with OA progression.

Thus, scRNA-seq data augmented our understanding of the spatiotemporal landscape of meniscus gene expression and furnished additional insights into degenerative mechanisms, cellular compositions, and biological links between meniscus tear and OA. Moving forward, investigations that continue this trajectory hold promise to uncover rare meniscus cell subpopulations and improve our understanding of cell-cell interactions within the microenvironment of this clinically-critical tissue and its participation in OA genesis.

Proteomics

Proteomic analyses in OA started in ~2004, yet studies were constrained by technical limitations, especially regarding sensitivity and throughput (Fig. 2). The first proteomic study of osteoarthritic chondrocytes was performed by two-dimensional gel electrophoresis.[66] Since then, strategies using nano-liquid chromatography-mass spectrometry (LC-MS)/MS are most common. By these means, shotgun proteomics studies have elucidated the molecular composition of articular cartilage and other joint tissues. One of the most exhaustive works in this area characterized the different layers of cartilage from healthy or OA hip and knee tissues,[67] identifying more unique proteins in the superficial layer than in the deep one, such as gelsolin, tenascins or lubricin. This study also showed differences in protein abundance related with the disease state (i.e., decrease of COMP or clusterin) or joint site (i.e., aggrecan core protein or matrilin-3 enriched in hip cartilage, while Chitinase-3-like protein 1 or MMP1 increased in the knee tissue). Most recently, cutting-edge technology combining cytometry with mass spectrometry (mass cytometry) allowed single-cell proteomic analyses in cartilage. A panel of 33 markers was developed for profiling chondrocytes by Cytometry by Time of Flight (CyTOF), which was employed to establish a single-cell atlas for cartilage and revealed "rare" cell populations in the OA tissue.[68] This technology has been subsequently used to map the effects of two DMOAD candidates on chondrocytes isolated from patients with end-stage OA.[69] Altogether, single-cell proteomic analysis has shown a great

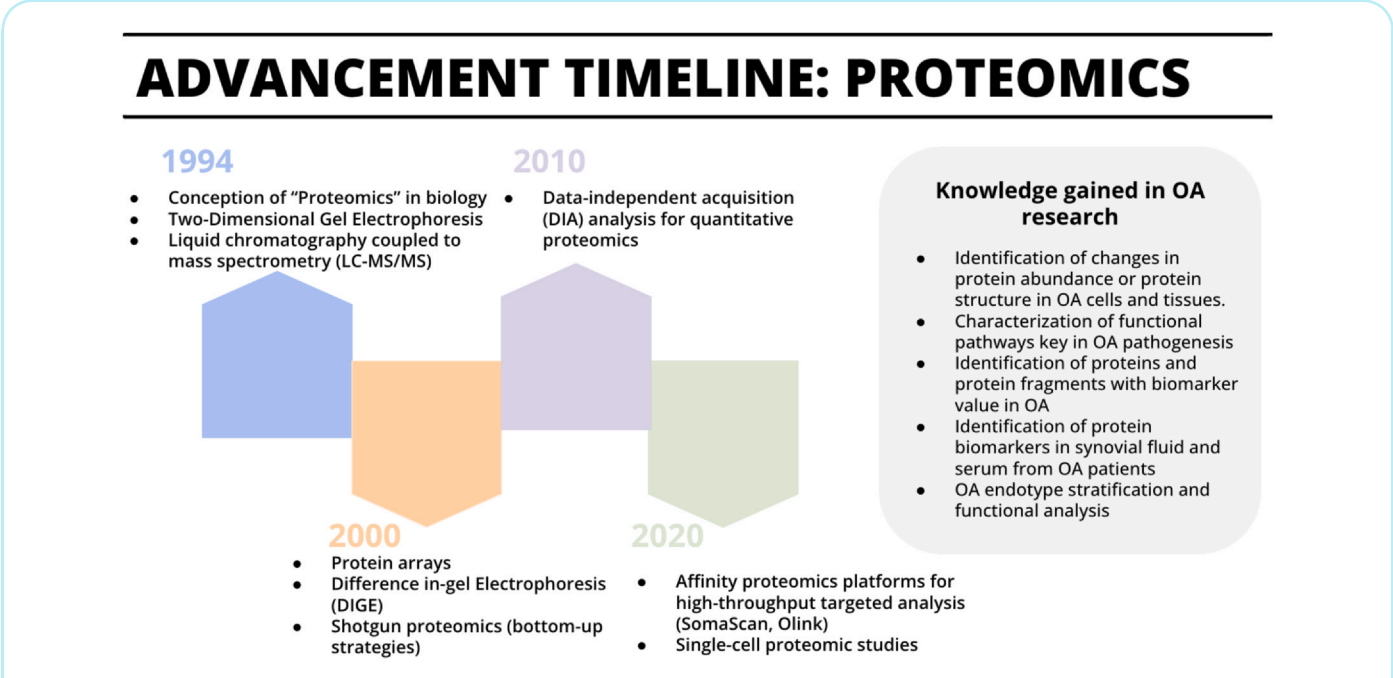

Fig. 2 Osteoarthritis and Cartilage

Proteomics – technological advancements and applications to OA. Proteomics was originally defined in 1994. Technologies have evolved and include mass spectrometry- and affinity-based techniques for high-throughput, targeted, and single cell proteomics. Beginning in 2004, proteomics research in OA has been used to study protein levels, key enriched pathways, and patient endotypes from multiple joint tissues and synovial fluid.

potential for patient stratification in OA, which may be critical in determining precision medicine approaches.

Other proteomic studies focused exclusively on proteins released (secretome) by articular chondrocytes and cartilage. One of the first works following this reasoning used an in vitro model of bovine cartilage explants to analyze proteins released in response to treatment with IL-1β, TNF-α, or mechanical compression.[70] The cytokine treatment caused a decrease in the synthesis of collagen subunits, and increased release of aggrecan and proteins related to innate immunity, while mechanical compression particularly enhanced the release of intracellular proteins. In another study, secretomes of lesioned and non-lesioned OA human cartilage, and healthy tissue, were compared leading to the identification of proteins distinctively released, such as osteoprotegerin and periostin.[71] Synovial fluid and serum from OA patients have also been analyzed by LC-MS/MS in proteomics as ideal sources for OA biomarkers. The first in-depth study of the OA synovial fluid proteome was published in 2014.[72] Thereafter, several papers searched this proteome to find markers of early OA.[73] A recent study defined a panel of 15 serum proteomic markers to predict OA progression,[74] including peptides from cartilage acidic protein 1 (CRTAC1), vitamin D binding protein and the complement C1r subcomponent.

Global analysis of specific PTMs has also been explored in OA research. A N-glycome analysis of OA chondrocytes and synoviocytes described an increased binding of galectins due to glycoprotein modifications, which induced proinflammatory markers.[75] Another study compared changes in N-glycosylated protein abundance in OA cartilage and controls with traumatic joint injury, identifying 22 N-glycosylated peptides that were increased in the diseased tissues.[76] In another work, Dong and colleagues performed a phosphoproteomic analysis comparing lesioned vs control OA cartilages, identifying > 4000 differential phosphorylated peptides and illustrating alteration of kinase hubs and transduction pathways in OA.[77]

Notably, ECM protein degradation studies have also been in the spotlight of proteomics. Progression of matrix degradation in response to mechanical damage and cytokine treatment in human tissues was explored by targeted proteomics to measure certain protein domains of collagen, aggrecan and COMP.[78] A further study carried out an analysis of endogenous peptides released from human OA cartilage, identifying specific peptides from prolargin and clusterin that were differentially released from OA knee and hip tissues, respectively, compared to healthy.[79] Finally, recent papers in this area described the massive proteolytic events that take place in OA cartilage, delineating the role of specific proteases such as HtrA1.[80]

Affinity proteomics studies have also been carried out for the discovery or OA biomarkers in body fluids. Novel, large-scale affinity proteomics platforms have been developed to facilitate biomarker discovery and risk prediction, including the aptamer-based SomaScan platform (SomaLogic, Boulder, CO) and the proximity extension assay developed by Olink (Uppsala, Sweden). Using SomaScan, 4792 proteins were measured in plasma of > 37,000 individuals to search for potential protein biomarkers of hip, knee, and/or hand OA, and identify biomarkers for joint replacement.[81] CRTAC1 was found to be the most promising candidate biomarker for OA incidence and was predictive of progression to joint replacement. In two recent studies using the Olink platform, CRTAC1 was also strongly associated with OA severity and progression in a screening of Rotterdam study participants,[82] with its predictive nature validated in a study on > 54,000 individuals from the UK Biobank.[83]

Technological advances in affinity proteomics and CyTOF platforms, along with the exceptional capacity of LC-MS/MS to identify protein fragments and modifications with increasing sensitivity and speed, demonstrates that after 20 years of research, proteomics has reached a high level of maturity in the OA field that turns it into the best tool for large-scale functional research in OA.

Metabolomics

The first reports of metabolomics in OA used nuclear magnetic resonance (NMR)-based metabolomics and meniscectomized guinea pigs.[84] NMR also characterized synovial fluid from various joint diseases, finding similarity between metabolomic profiles from OA, RA, crystal-associated arthritis, and spondylarthritis compared to septic arthritis.[85] NMR-based metabolomic profiling of urine distinguished progressors from non-progressors, finding key discriminatory roles for N-N-dimethylglycine, hippurate, histidine, and trigonelline at 18 months.[86] Several studies used MS to study metabolites in OA. The first MS-based metabolomics study targeted 163 metabolites in serum of knee OA subjects and controls, finding ratios of valine to histidine and xleucine (both leucine and iso-leucine) to histidine significantly different between the groups.[87] In infrapatellar fat pad, lipoxin A_4, thromboxane B_2, and arachidonic acid were key metabolites separating OA from normal tissue by dimensionality reduction.[88]

Metabolomic profiling can analyze OA cultured samples and conditioned media. 13-C labeling of carbon sources showed that OA chondrocytes use the tricarboxylic acid cycle, and patient-matched chondrocytes from OA regions produce more lactate than those from macroscopically normal cartilage.[89] Untargeted metabolomic profiling found extracellular stiffness impacts OA chondrocyte mechanotransduction,[90] suggesting that decreased pericellular matrix stiffness may affect OA pathophysiology. *In vivo* studies support metabolic changes as altered joint mechanotransduction. For example, a single night of wheel exercise induced multiple pathways including amino acid metabolism and synthesis of catecholamines and ubiquinol.[91] Six months of exercise resulted in increased connectivity between local joint-level structural and pathological measures and synovial fluid metabolites.[92] Germ-free mice had decreased variability in synovial fluid metabolomic profiles compared to conventional mice, and in response to joint injury, with metabolite differences related to inflammation and innate immunity.[93] Isotopic labeling in a rat injury model identified potential plasma metabolite biomarkers 2-aminoadipic acid, GABA, and saccharopine.[94] Rats subjected to either high-fat diet or surgical cartilage damage exhibited differences in multiple oxylipins in plasma and synovial fluid.[95] These in vitro and in vivo studies show that metabolomic profiling provides deep insight into OA pathologies, identifies novel disease mechanisms, and finds potential drug targets.

Metabolomic profiling of clinical samples provides detailed insight into the molecular nature of OA. Metabolomic profiles of synovial fluid discriminated between early- and late-stage OA KL grades.[96] Similarly, analysis of post-mortem synovial fluid found distinct endotypes within both early- and late-stage OA.[97] Additional endotypes were discovered by clustering analysis of targeted metabolomic profiles from plasma of OA patients and normal controls.[98] Targeted metabolomic profiling found endotypes within a population of late-stage (KL grades 3–4) OA patients, with key metabolites related to tRNA acylation and B-vitamin metabolism.[99] Targeted metabolomic profiling of plasma from total joint replacement patients found ratios of acetycholine to phosphatidylcholine and phosphatidylcholine-diacyl-C36:4 to isoleucine were associated with post-replacement pain.[100] ^{1}H NMR metabolomics found differences in profiles between inflammatory and fibrotic synovial tissues, as well as correlations between some metabolites detected between synovium and synovial fluid.[101] As technology and biobanks of OA tissues and fluids improve, opportunities to advance human OA research using metabolomic profiling will emerge.

A major challenge of metabolomics is variability (owing in part to dietary and environmental factors) that is hard to control in clinical studies, complicating interpretation of how metabolomic profiles relate to fundamental biology of OA. However, when profiled sequentially in time, metabolite data can provide *bona fide* information for enzymatic activity across multiple pathways simultaneously. Therapeutic interventions can be evaluated through changes in metabolites and metabolomic profiling, which has potential to provide insight into disease endotypes to improve our knowledge of OA and move toward improved clinical treatments.

Spatial-omics

While it has been long appreciated that the articular joint is an organ system,[102] it has been challenging to decode the factors that govern inter-tissue and inter-organ crosstalk (factors outside of the joint organ system that may signal to the joint).[103] Current spatial transcriptomics techniques produce unique molecular identifiers (UMIs) in a capture radius of ~55 μm, and approaches have been developed to identify putative cell-cell communication data [e.g., communication analysis by optimal transport][104] (Fig. 3). Given the relatively recent development of spatial-omics approaches, we will briefly highlight how these technologies have been applied to OA and other rheumatic diseases to illustrate potential utility of these techniques.

While approaches have been established to assess the spatial immune cell milieu, they have yet to be used in the OA context, despite established protocols for formalin fixed, paraffin embedded and frozen tissues.[105] In psoriatic arthritis, spatial transcriptomics was used to identify molecular signatures that separate early and severe disease states.[106] Synovial biopsies from RA patients were evaluated using spatial transcriptomics to identify changes in B cells

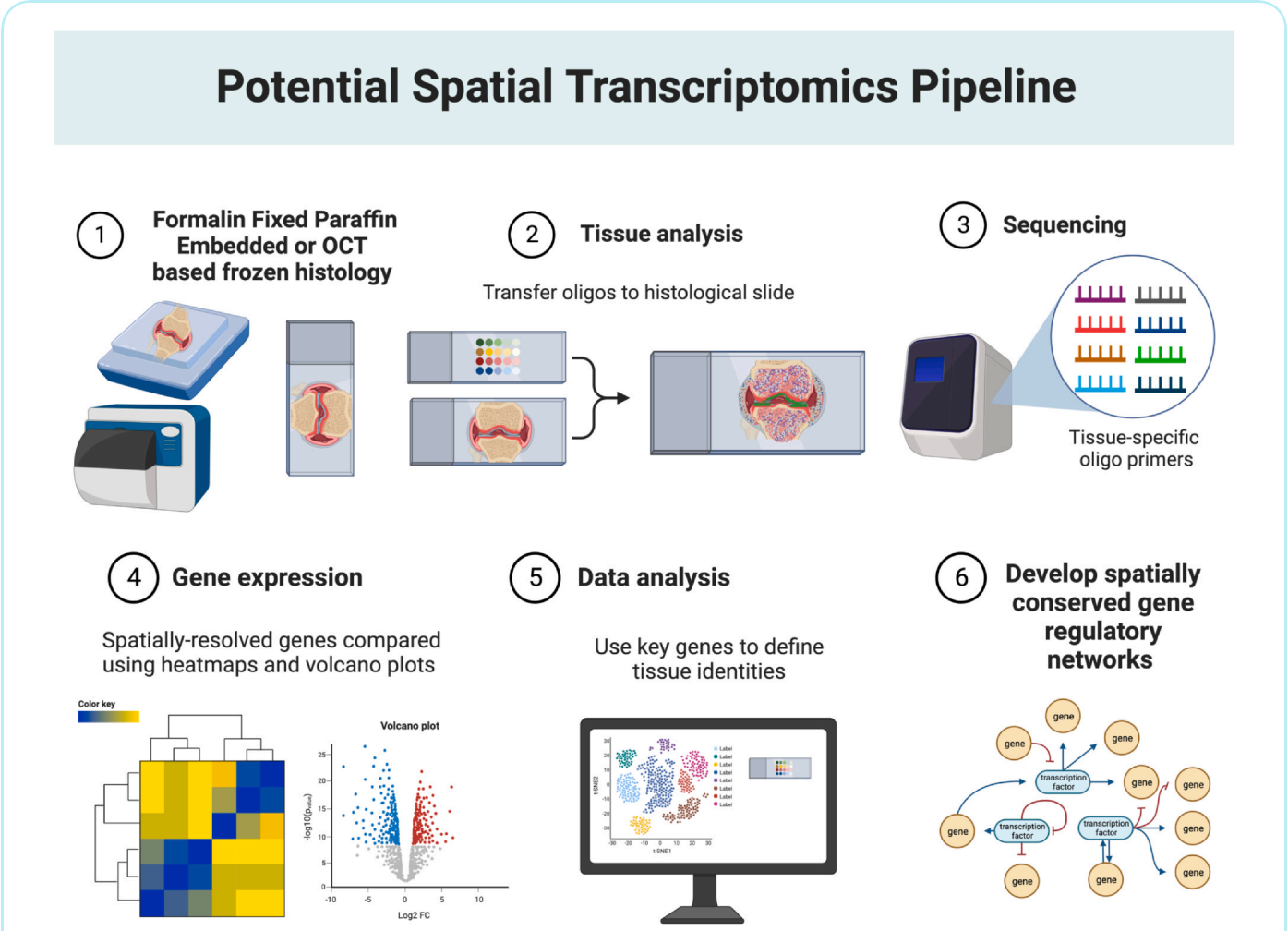

Fig. 3 Osteoarthritis and Cartilage

Spatial transcriptomics pipeline. Fixed or frozen tissue is embedded in paraffin or OCT as required by the specific protocol (1). Next, using a kit from 10×, nanostring, or others, the tissue is prepared, for example, by removing paraffin using xylenes, dried, and typically kept cold until the assay is performed. The same slide or serial slide is used for staining by hematoxylin and eosin which will later be used to resolve spatial information with sequencing data. The tissue is transferred to a barcoded slide using protocols and kits provided by manufacturers (2). The barcoded slide is imaged and either the full genome or a limited dataset is sequenced, pending which spatial technique is selected (3). Then, gene expression data are generated (4), and data is analyzed and processed (5) to determine whole section and tissue specific changes in gene expression (6).

concordant with alterations in tissue architecture, helping disentangle whether plasma cells are present before or after tissue remodeling.[107]

Spatial metabolomic information can also be observed using matrix-assisted laser desorption ionization (MALDI)-MS imaging. Rocha and colleagues used this approach to characterize lipodomic profiles of synovial tissues in OA vs. RA and psoriatic arthritis. This approach yielded characteristic lipidomic profiles from OA patients that may help identify pathophysiological mechanisms in OA.[108] MALDI-MS images have been integrated with label-free proteomics to illustrate that OA cartilage of subjects with and without type 2 diabetes have differential lipid and protein profiles.[109] For example, these spatial analyses revealed that patients with type 2 diabetes who were OA negative had more phosphatidylcholine and sphingomyelin species, compared to patients with type 2 diabetes and OA who had more lysolipids.[109] This observation confirms that phosphatidylcholine and sphingomyelin species are key elements of healthy cartilage.[109] Furthermore, phospholipid content differed in superficial and deep zones of cartilage, which would have not been detectable without these spatial analyses. Opportunities to assess spatial proteomics in OA have been reviewed elsewhere.[110] As emerging approaches reach single-cell resolutions (UMIs of 5–10 μm) and are capable of multi-omic measures, we are uniquely positioned to integrate these technologies to overcome the lack of spatial information in musculoskeletal crosstalk research as exemplified by the methods showcasing single cell-resolution transcriptomic information utilizing image-seq.[111]

Researchers have delineated roadmaps for building cell atlases involving single and spatial multi-omic assessments, which outline a vision and strategic direction for investigation as these technologies advance.[112] It would be useful to develop similar strategies and goals to integrate such technologies to bridge the gap between limited spatial information in OA joint tissues and answering open questions about OA phenotypes as a collective research community.

Multi-omics

Most individual omic studies in OA have focused on a single technology. However, the integration of multiple omics technologies on biological samples from the same individual may help to uncover links between transcriptomic, proteomic and metabolomic signatures, among other technologies, that could aid in defining novel molecular profiles of individual OA patients, generating patient-specific endotypes (Fig. 4). A current multi-omics approach is integrating scRNA-seq with spatial sequencing, using deconvolution approaches to define cell populations spatially.[113] In addition, scATAC-seq and scRNA-seq can be performed to

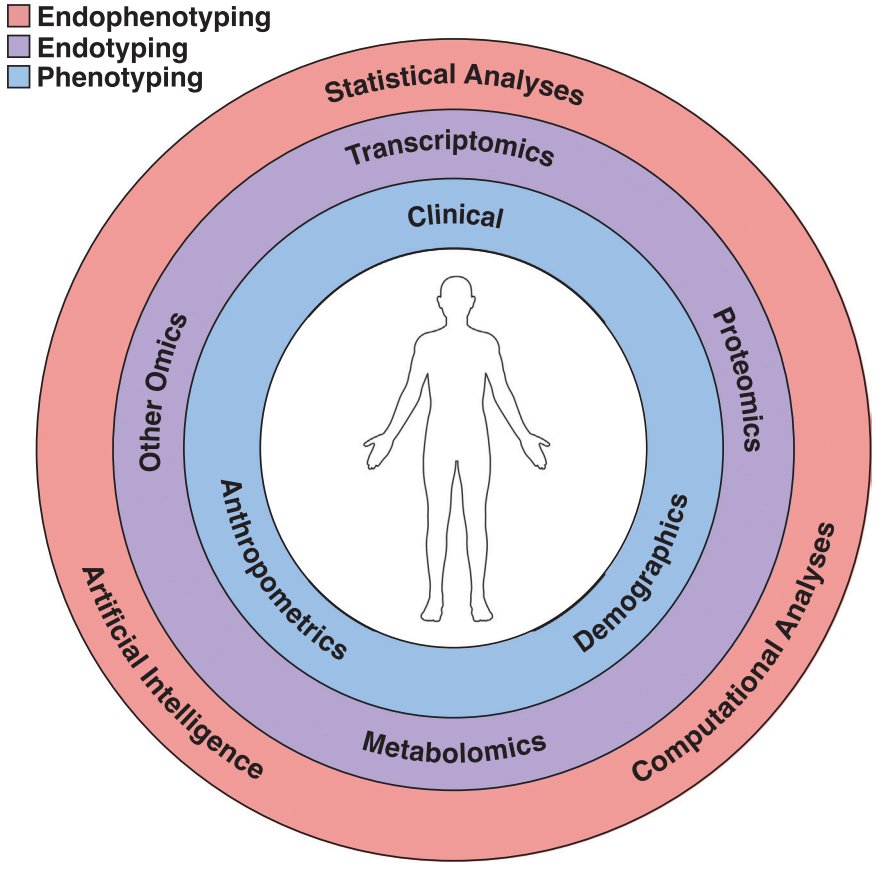

Fig. 4

Osteoarthritis and Cartilage

Multi-omics integration for identification of OA endophenotypes. Combining sociodemographic, clinical, and omic-technology data can inform of OA patient endophenotypes through statistical analyses, integrative computational analyses, and artificial intelligence. Of note, multiple endotype-phenotype combinations are possible using this approach. Uncovering OA endophenotypes can enable precision medicine approaches for treatment and enhanced therapeutic discovery.

identify open chromatin regions linked to RNA gene expression,[114] with computational approaches used to predict transcription factors able to bind accessible DNA regions and regulate transcriptomic programs.

Several challenges exist for multi-omics analyses including differences in initial data handling, individual omic data heterogeneity and noise, computational constraints, and data interpretations.[115,116] However, various approaches have been proposed to investigate multi-omics data including correlation analyses,[117] network-based approaches,[117–119] supervised or unsupervised machine learning,[120] among others.[121,122] Tools and visualization portals can aid in multi-omics analysis and interpretation.[122,123]

In addition to generating multi-omics OA endotypes, integration with patient sociodemographic and clinical variables (phenotypes) to generate endophenotypes will be critical (Fig. 4). Not surprisingly, there are associations between signatures of omic datasets and OA risk factors,[124–128] as well as omic signatures and measures of OA disease,[42,129–133] suggesting linkages across OA disease, omic patterns, and patient phenotypes. Integrating patient-level characteristics with biological omic data may be helpful in unraveling the heterogeneity of OA patients and therapeutic outcomes. Some tools have incorporated the ability to investigate multi-omics and clinical variables together to uncover consensus clusters,[134] essentially endophenotypes. Although currently undefined, multiple endotypes may underlie individual patient phenotypes, aiding in our understanding of OA patient heterogeneity and possibly explaining differences in OA outcomes based on phenotype alone. Determining how endophenotypes relate to disease prognosis and therapeutic efficacy will be vital to improve outcomes for patients with OA.

Conclusions

Since their inception, the utilization of transcriptomics, proteomics and metabolomics technologies has made remarkable strides in advancing our understanding of molecular, cellular, and tissue contributions to OA disease pathology and joint homeostasis. As omic technologies have progressed, so too has the breadth and depth of information collected regarding transcriptomes, proteomes and metabolomes in OA.

The next goals of omic research in OA appear clear: 1) further technological improvements and novel additions to omic methodologies; 2) expansion of omic datasets to include OA patient phenotypes and the incorporation of spatial information; and 3) analysis of multi-omics datasets from various platforms and patient-level data to define OA patient endophenotypes. These goals are poised to enhance our understanding of OA heterogeneity, improve the integration of specific OA endophenotypes into study designs, and ultimately improve study outcomes and interpretations for more effective OA precision medicine approaches.

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Author contributions

All authors made equal contributions to the conception and design of the manuscript, drafting and critical review for important intellectual content. All authors approved the final version to be published. All authors agree to be accountable for all aspects of the manuscript and ensure that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of interest

None.

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