Evolution and advancements in genomics and epigenomics in OA research: How far we have come

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Three Decades of Advancements in Osteoarthritis Research: Insights from Transcriptomic, Proteomic, and Metabolomic Studies

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Running headline: Omics in OA research over last 30 years

ABSTRACT

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 rending 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 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 patters within the affected tissues.

Conclusions: Leveraging a multi-omic 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.

Keywords: spatial omics, single cell, RNA-seq, proteins, metabolism

INTRODUCTION

Osteoarthritis (OA) is a complex disease characterized by pain and reduced function, involving both local joint and systemic tissues and fluids. Sociodemographic and clinical factors are associated with incidence and progression of OA[1, 2]. Studies describe multiple OA phenotypes with contributions of these components[3, 4]. Individual patient characteristics can also modify systemic molecular profiles[5], contributing to OA disease. Understanding local and systemic molecular profiles of OA patient populations (endotypes) has been a major recent focus to uncover new targets and disentangle the heterogeneous clinical presentations of OA. This is particularly achieved through advanced omic technologies, including transcriptomics, proteomics and metabolomics.

Transcriptomics analyzes transcripts expressed from the genome[6]. Microarrays were first employed in 1995 as hybridization-based platforms to quantify a set of targeted RNA sequences[7]. This was followed by advanced RNA-seq that captures all sequences, including alternative splice variants and single nucleotide polymorphisms. In 2009[8], single cell (sc)RNA-seq was first performed, permitting the quantification of transcripts in a single cell. scRNA-seq has played a pivotal role in unfolding the cellular composition of diverse tissues, organs or organisms[9]. Single nucleus (sn)RNA-seq follows analogous principles to scRNA-seq, but employs isolated nuclei. While cytosolic RNA information is not captured, snRNA-seq offers advantages including the use of archived tissues, reduced batch variability, and minimal impact on dissociation-induced transcriptional stress[10].

Proteomics, conceived in 1994, refers to the large-scale analysis of proteins, including identification, quantification, post-translational modifications (PTMs), localization, and degradation. The significance of proteins as the primary effectors of biological function, combined with limited overlap with other omics, underscores the importance of protein analysis in understanding OA pathogenesis and improving management[11]. Metabolites are the reactants and products of the myriad of biochemical reactions mediated by proteins. Metabolomic profiling, defined in 1998[12], involves the characterization of thousands of metabolites[13].

Over the past 30-plus years, advancements in transcriptomics, proteomics, and metabolomic technologies have provided insight into the molecular changes occurring in OA tissues and fluids. In this review, we highlight how omic technologies have increased our biological understanding of OA. We also discuss how future spatial transcriptomics could aid in better OA heterogeneity at the tissue level and how multi-omic 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 tools (**Figure 1**). Advancements in microarray technologies, followed by progressive improvements to high-throughput sequencing methods, including RNA-seq, scRNA-seq and snRNA-seq, have led to significant advancements in understanding OA pathology at the level of various tissues. Here, we emphasize the application of transcriptomics in different joint tissues affected by the disease.

Cartilage Transcriptomics

In adult cartilage, chondrocytes, the sole cell type, are organized into various zones where the cells are at different progressive stages of differentiation. 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 comparing healthy/unaffected and OA-damaged cartilage[14]. More comprehensive microarray studies in the early 2000s reported significant differences in differentially expressed genes related to cell proliferation, collagen synthesis or matrix degradation comparing intact and damaged cartilage[15], and differentiated between different grades of OA cartilage specimens demonstrating the potential implication of increased oxidative stress and cell damage in OA chondrocytes[16]. Following studies tried to tackle particular experimental challenges using genome-wide microarrays or different specimen comparison[17-19]. As a result, the list of pathologically-relevant candidate genes was extended, including genes involved in bone formation or skeletal development.

Animal models provide a great opportunity to uncover additional genes and signaling pathways that are regulated at the transcriptional level during early OA when human tissue is more scarcely available. Appleton et al. performed one of the first studies investigating transcriptional changes in chondrocytes in a surgically induced rat OA model[20] and identified several cytokines, chemokines, and growth factors with implications in early degrading cartilage. 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 the early disease phase,

127 focusing on different time-points[21, 22], and dissect the role of previously identified central genes
128 (such as *ADAMTS5*) or ageing[23]. These preclinical studies had a crucial role in characterizing the
129 complex pathogenesis of OA cartilage degradation which is activated upon first disease induction.
130
131 It has been proposed to enrich the current, more clinical approaches towards phenotyping to
132 include molecular endotypes and genotypes derived from omic technology data[24]. Fernández-Tajes
133 et al. found in cartilage microarray data from 23 patients two endotypes with gene expression
134 differences related to inflammatory response [25], while Soul et al. identified two endotypes in RNA-
135 seq data from 44 patients, but with differences related to oxidative stress, innate immune responses
136 and Wnt signaling[26]. Steinberg et al. reported comparable observations with two endotypes
137 (including inflammation) [27]. Yuan et al. identified four patient endotypes when combining different
138 tissues, underlining the importance of OA as a "whole joint disease" [28]. Future studies will leverage
139 advanced computational methods and machine learning algorithms to characterize patient
140 transcriptomic endotypes and their correlation with clinical phenotypes.
141
142 The first scRNA-seq study on human OA cartilage was published at the beginning of 2019,
143 providing evidence for specific subpopulations and transient states of chondrocytes, as well as
144 novel functional phenotypes such as regulatory functions (immunomodulation)[29]. The dataset
145 further allowed identification of cartilage progenitor cells (CPCs) that express stem-cell-related
146 surface markers. Following studies partly confirmed these initial findings and integrated synovial
147 tissue/cells in the analysis to dissect synovial cell-chondrocyte crosstalk mechanisms [30]. These cell
148 population-based studies have uncovered subordinate disease mechanisms which have been
149 submerged in bulk-RNA gene expression studies, such as ferroptosis[31]. 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 mice knee cartilage[32]. A recent methodological approach combined bulk-RNA-seq with scRNA-seq, which showed promising results to generate a high confidence (pure) chondrocyte gene signature, avoiding cross-contamination from other tissue, a process necessary in smaller animal models with limited tissue availability[33]. These advancements have led to a deeper understanding of chondrocyte subtypes and more recent studies are shedding light into synovial-chondrocyte cell interactions and immune cell involvement in OA cartilage degradation.

Synovium Transcriptomics

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[34].

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 [35]. Relevant to fibrosis, Remst et al. found that *PLOD2*, *LOX*, *COL1A1*, *COL5A1*, and *TIMP1* were increased in both end-stage human OA synovial tissue and TGF-β-stimulated human OA synovial fibroblasts [35]. Lambert et al. analyzed human end-stage OA synovium by microarray of normal vs inflamed regions within the same subjects [36]. 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, end-stage RA, and normal synovium showed that

compared to both healthy and RA fibroblasts, 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[37]. 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 bulk RNA-seq facilitated the deeper identification of specific signaling pathways active in the OA synovium. Steinberg et al. performed bulk-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 [38]. 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 bulk-RNA-seq[39]. Low-frequency loading enriched interferon-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 synovial inflammation. Further studies are needed to describe the mechanoreceptors and intracellular signaling responsible for synovium mechanosensitivity.

196 To study macrophage phenotypes in arthritis, flow-sorted synovial tissue-derived macrophages
197 from OA and inflammatory arthritis (RA or psoriatic arthritis) were analyzed by RNA-seq [40]. Two
198 distinct subgroups of OA macrophages were identified, underpinned by 155 differentially-
199 expressed genes: a "classical OA" subset and an "inflammatory-like OA" more similar to
200 macrophages from inflammatory arthritis. The inflammatory-like subset was enriched in pro-
201 inflammatory signaling and cell-cycle genes, which was functionally confirmed by flow cytometry
202 demonstrating that synovial tissue from these patients had a ~2.5-fold greater number of
203 macrophages. Using a model of ACL rupture in mice, Bergman et al. described a divergence of the
204 synovial transcriptome between male and female mice associated with greater progression of
205 PTOA severity, pain behavior, MMP activity, and osteophyte formation in male mice [41]. Male mice
206 had increased gene expression of pro-fibrotic, neuroangiogenic, and ERK signaling genes,
207 indicating that synovitis may be a key driver of the well-documented greater OA severity in male
208 mice across OA models.
209
210 One of the first scRNA-seq datasets of OA synovium was published in 2018, which included
211 characterization of synovial fibroblasts from OA and RA patients [42]. From 337 fibroblasts from
212 two OA and two RA patients, this study identified three primary synovial fibroblast groups: 1) A
213 *CD34*(-), *Thy1*(-) population, now recognized as *PRG4*^{High}, *CLIC5*+ lining/intimal fibroblasts; 2)
214 A *CD34*(-) *Thy1*(+) population localized to the sublining/subintima near blood vessels, and 3) a
215 *CD34*(+) *Thy1*(+), now recognized to represent progenitor-like *DPP4*+/*PI16*+ "universal
216 fibroblasts" [43]. The first whole-synovium OA atlas was published by Chou et al. in 2020 and
217 comprised of ~10,600 synovial cells from three OA patients [44]. The major synovial cell types were
218 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 the 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 [45]. In end-stage OA patients, these fibroblasts expressed genes related to eicosanoid signaling, prostanoid biosynthesis, and 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, in-part, driven 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 [46]. 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"[43]. Differentiation trajectory analysis suggested this cell population gave rise *Acta2*/αSMA+ myofibroblasts that further transition 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 are 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 [47].

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 Transcriptomics

Subchondral bone refers to the cortical, trabecular and marrow adipose tissue compartments beneath articular cartilage. Advanced OA stages display substantial thickening of the subchondral cortical plate and trabeculae. Changes in the composition of subchondral marrow adipose tissue, referred to as bone marrow lesions (BML) are associated with both pain and cartilage volume loss. First insights from histopathological studies showed that during OA, blood vessels and nerves invade the subchondral cortical plate and there is an elevated presence of macrophages, vascular structures, and osteoclasts in subchondral marrow adipose tissue[48-52].

Initial omic investigations into human OA subchondral bone and BMLs were conducted during the early to mid-2000s, utilizing microarray technology[53-55]. A landmark study comparing osteoporotic and OA femoral heads yielded initial molecular evidence for increased expression of

pro-angiogenic genes within OA subchondral bone [53]. Functional and pathway analyses unveiled an enrichment of pathways connected to angiogenesis, collagen fibrils, and cell proliferation between medial (sclerotic) and lateral (non-sclerotic) tibial plateaus[56]. A similar approach comparing knee tibia BMLs to controls identified enrichment of pathways related to angiogenesis, cytokine signaling, PDGF and Wnt signaling[55]. Among the potential molecular targets suggested by these studies, *STMN2, IL11* and *CHADL* were confirmed recently using RNA-Seq[57].

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 extracellular matrix remodeling pathway molecular crosstalk [28]. However, the diverse array of cell types present in subchondral bone, including osteocytes, adipocytes, osteoblasts, osteoclasts, vascular cells, immune cells, and progenitor cells in marrow adipose tissue, continues to present a significant challenge in interpreting bulk transcriptomics data. Notably, a preliminary study utilizing scRNA-seq indicated the existence of at least ten distinct molecular cell types within subchondral marrow adipose tissue[58].

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[59], entire bones[60], or larger animal models[61]. 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 [61]. Furthermore, transcriptomic analysis of

aging bone lacking marrow adipose tissue revealed differential expression of several genes associated with human hip or knee OA, such as *Col27a1, Col2a1* and *Col11a1*[60].

Expanding upon the foundation laid by current transcriptomic datasets, further investigations using proteomics [62-64] and lipidomics of subchondral bone will open new avenues for disease endotyping, biomarker discovery and development of novel therapeutic targets.

Meniscus Transcriptomics

Within OA-affected menisci, there are indications of both gross and histologic pathology, characterized by increased water, proteoglycan, and collagen content, and elevated expression of *MMP13*[65]. In a significant advancement, Rai and colleagues[66] performed RNA-seq analysis on meniscus tissue, demonstrating that menisci within OA-afflicted joints exhibit an inflammatory phenotype, contrary to menisci from non-OA joints, which display a repair-oriented phenotype. A subsequent investigation elucidated key biological processes (inflammation, chemotaxis, cytokine-to-cytokine interaction) in the context of OA-related meniscus degradation[67].

Pioneering work in scRNA-seq targeting the 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[68]. In-depth analyses further 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 the advanced stage 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 has augmented our understanding of the spatiotemporal landscape of meniscus gene expression and furnished additional insights into degenerative mechanisms, cellular compositions, and putative treatment strategies for the meniscus. Moving forward, investigations that continue in this trajectory hold the promise of uncovering rare meniscus cell subpopulations and improving our understanding of cell-cell interactions within the microenvironment of this clinically critical tissue.

PROTEOMICS

Proteomic analyses in OA started in ~2004, yet studies were constrained by a plethora of technical limitations, especially regarding sensitivity and throughput (**Figure 2**). The first proteomic study of osteoarthritic chondrocytes [69] was performed by two-dimensional gel electrophoresis. Since then, strategies using nano-LC-MS/MS are most common. By these means, shotgun proteomics studies have elucidated the molecular composition of articular cartilage and other joint tissues. In one of the most exhaustive works in this area, proteomic analyses characterized the different layers of and cartilage from healthy or OA hip and knee tissue[70].

Other studies focused exclusively on the proteins released (secretome) by articular chondrocytes and articular cartilage. One of the first works following this reasoning used an *in vitro* model of bovine calf cartilage explants to analyze proteins released in response to treatment with IL-1β,

TNF-α, or mechanical compression[71]. Subsequently, the secretomes of lesioned and non-lesioned OA human cartilage and healthy tissue were compared leading to the identification of proteins distinctively released by diseased tissue, such as osteoprotegerin and periostin[72]. Synovial fluid and serum from OA patients have also been widely analyzed by LC-MS/MS in proteomic studies, as ideal sources for OA biomarkers. The first in-depth study of the OA synovial fluid proteome was published in 2014[73]. Thereafter, several papers have searched this proteome to find markers of early OA[74]. In serum, a recent study defined a panel of 15 serum proteomic markers to predict OA progression[75].

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[76]. A recent study compared changes in N-glycosylated protein abundance in OA cartilage and controls with traumatic joint injury, identifying several N-glycosylated peptides altered in diseased tissues[77]. In another study, Dong and colleagues carried out a phosphoproteomic analysis comparing lesioned vs preserved (control) OA cartilages identifying >4,000 differential phosphorylated peptides, illustrating the alteration of kinase hubs and transduction pathways in OA[78].

ECM protein degradation studies have also been in the spotlight. 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[79]. A further study carried out a hypothesis-free analysis of endogenous peptides released from human OA cartilage, identifying specific peptides from prolargin and clusterin differentially released from

OA knee and hip tissues, respectively, compared to healthy [80]. One of the most recent papers in this area described in detail the massive proteolytic events that take place in OA cartilage, delineating the role of specific proteases such as HtrA1[81].

Affinity proteomics studies have also been carried out for the discovery or OA biomarkers in body fluids. One of the first studies using protein microarrays in OA research identified C3, ITIH1 and S100A6 as significantly elevated in serum from OA patients compared to controls. In recent years, novel large-scale affinity proteomics platforms have been developed to facilitate biomarker discovery and risk prediction including the oligonucleotide aptamer-based SomaScan platform (SomaLogic, Boulder, CO) and the proximity extension assay (PEA) developed by Olink (Uppsala, Sweden). Using SomaScan, 4,792 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[82]. 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, CRTAC1was also found strongly associated with OA severity and progression in a screening on 3,517 Rotterdam study participants[83]. Its predictive nature for OA risk and progression to total knee arthroscopy was validated in a study on >54,000 individuals from the UK Biobank[84]. Thus, multiplexed affinity proteomics strategies have demonstrated value to analyze thousands of proteins in large cohorts for OA biomarker discovery.

The technological advances in affinity proteomics 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 NMR-based metabolomics and meniscectomized guinea pigs[85]. 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[86]. 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 [87]. Several studies used mass spectrometry (MS) to study metabolites in OA. The first MS-based metabolomics study targeted 163 metabolites in serum of knee OA subjects and controls, finding that the ratios of valine to histidine and xleucine (both leucine and iso-leucine) to histidine as key metabolite ratios significantly different between the groups[88]. In infrapatellar fat pad, lipoxin A_4, thromboxane B_2, and arachidonic acid were key metabolites separating OA from normal tissue by dimensionality reduction[89].

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 that patient-matched chondrocytes from OA regions produced more lactate than those from macroscopically normal cartilage[90]. Untargeted metabolomic profiling found that extracellular stiffness impacts OA chondrocyte mechanotransduction[91], 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[92]. Six months of exercise resulted in increased connectivity between local joint-level structural and pathological measures and synovial fluid metabolites[93]. Germ-free mice had substantially 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[94]. Isotopic labeling in a rat injury model identified potential plasma metabolite biomarkers 2-aminoadipic acid, GABA, and saccharopine[95]. Rats subjected to either high-fat diet or surgical cartilage damage exhibited differences in multiple oxylipins in plasma and synovial fluid[96]. 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[97]. Similarly, analysis of post-mortem synovial fluid found distinct endotypes within both early- and late-stage OA[98]. Additional endotypes were discovered by clustering analysis targeted metabolomic profiles from plasma of OA patients and normal controls[99]. Targeted metabolomic profiling found endotypes within a population of late-stage (KL grades 3-4) OA patients with key metabolites suggesting tRNA acylation and B-vitamin metabolism[100]. Targeted metabolomic profiling of plasma from total joint replacement patients found that the ratios of acetycholine to phosphatidylcholine and phosphatidylcholine-diacyl-C36:4 to isoleucine were associated with post-replacement pain[101]. ^{1}H-NMR metabolomics found differences in profiles between inflammatory and fibrotic synovial tissues, as well as correlations between some metabolites that were jointly detected between synovium and synovial fluid[102]. As technology and biobanks of OA

tissues and fluids improve, many opportunities to advance human OA research using metabolomic profiling will emerge.

A major challenge of metabolomics is variability (e.g. dietary and environmental factors) that is hard to control in clinical studies which complicates 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. Metabolomic profiling also has potential to provide insight into disease endotypes and future studies can 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[103], 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[104]). Current spatial transcriptomics techniques produce unique molecular identifiers (UMIs) in a capture radius of ~50 μm, and approaches have been developed to identify putative cell-cell communication data [e.g., communication analysis by optimal transport (COMMOT)][105]. Since spatial-omics approaches have been recently developed, we will instead mention a few brief examples of how these technologies have been used in OA and other rheumatic diseases to illustrate the 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[106]. In psoriatic arthritis, spatial transcriptomics was used to identify molecular signatures that separate early and severe disease states[107]. Synovial biopsies from RA patients were evaluated using spatial transcriptomics to identify changes in B cells concordant with alterations in tissue architecture, helping disentangle whether plasma cells are present before or after tissue remodeling[108].

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[109]. 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[110]. Opportunities to assess spatial proteomics in OA have been reviewed elsewhere [111]. As new approaches are developed, reach single cell resolutions (UMIs of 5-10 μm) and are capable of multi-omic measures, we are uniquely positioned to rapidly integrate these technologies to overcome the lack of spatial information in musculoskeletal crosstalk research. For example, there are methods to determine single cell-resolution transcriptomic information using image-seq [112].

Groups 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 develop[113]. It would be useful to develop similar strategies and goals to integrate these technologies

to bridge the gap of limited spatial information in OA joints and tissues to answer a multitude of open questions about OA as a collective research community.

Multi-omics

Most individual omic studies in OA have focused on a single technology. However, the integration of multiple omic-technologies on biological samples from the same individual may help to uncover links between transcriptomic, proteomic and metabolomic signatures, among other omic technologies, that could aid in defining novel molecular profiles of individual OA patients, generating patient-specific endotypes (**Figure 3**). A current multi-omic approach is integrating scRNA-seq with spatial sequencing, using deconvolution approaches to help define cell populations spatially[114]. In addition, ATAC-seq and RNA-seq can be performed at the single cell level to identify open chromatin regions linked to RNA gene expression[115], with computational approaches to identify putative transcription factors able to bind accessible DNA regions to regulate transcriptomic programs. However, a number of challenges exist for multi-omic analyses Including differences in initial data handing, individual omic data heterogeneity and noise, computational constraints, and data interpretations [116, 117]. However, various approaches have been proposed to investigate multi-omic data including correlation analyses [118], network-based approaches[118-120], supervised or unsupervised machine learning [121], among others [122, 123]. Some tools and visualization portals are available to aid in multi-omic analysis and interpretation [123, 124].

In addition to generating multi-omic OA endotypes, integration with patient sociodemographic and clinical variables (phenotypes) to generate endophenotypes will be critical (**Figure 3**). Not surprisingly, there are associations between signatures of omic datasets and OA risk factors[125-129],

as well as omic signatures and measures of OA disease[45, 130-134], 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-omic and clinical variables together to uncover consensus clusters[135], essentially endophenotypes. Determining how endophenotypes relate to disease prognosis and therapeutic efficacy will be vital to improve outcomes for patients with OA.

CONCLUSIONS

Overall, the utilization of transcriptomics, proteomics and metabolomics technologies since their inception has made remarkable strides in advancing our understanding of molecular, cellular and tissue contributions to 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) integrating multi-omic 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 incorporation of specific OA endophenotypes into study designs, and ultimately improve study outcomes and interpretations for precision medicine in OA.

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Figure Legends

Figure 1. 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.

Figure 2. 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.

Figure 3. Multi-omic integration for identification of OA endophenotypes. Combining sociodemographic, clinical, and omic-technology data can inform of OA patient endophenotypes through integrative, computational and machine learning methods. Uncovering OA endophenotypes can enable precision medicine approaches for treatment and enhanced therapeutic discovery.

Figure 1

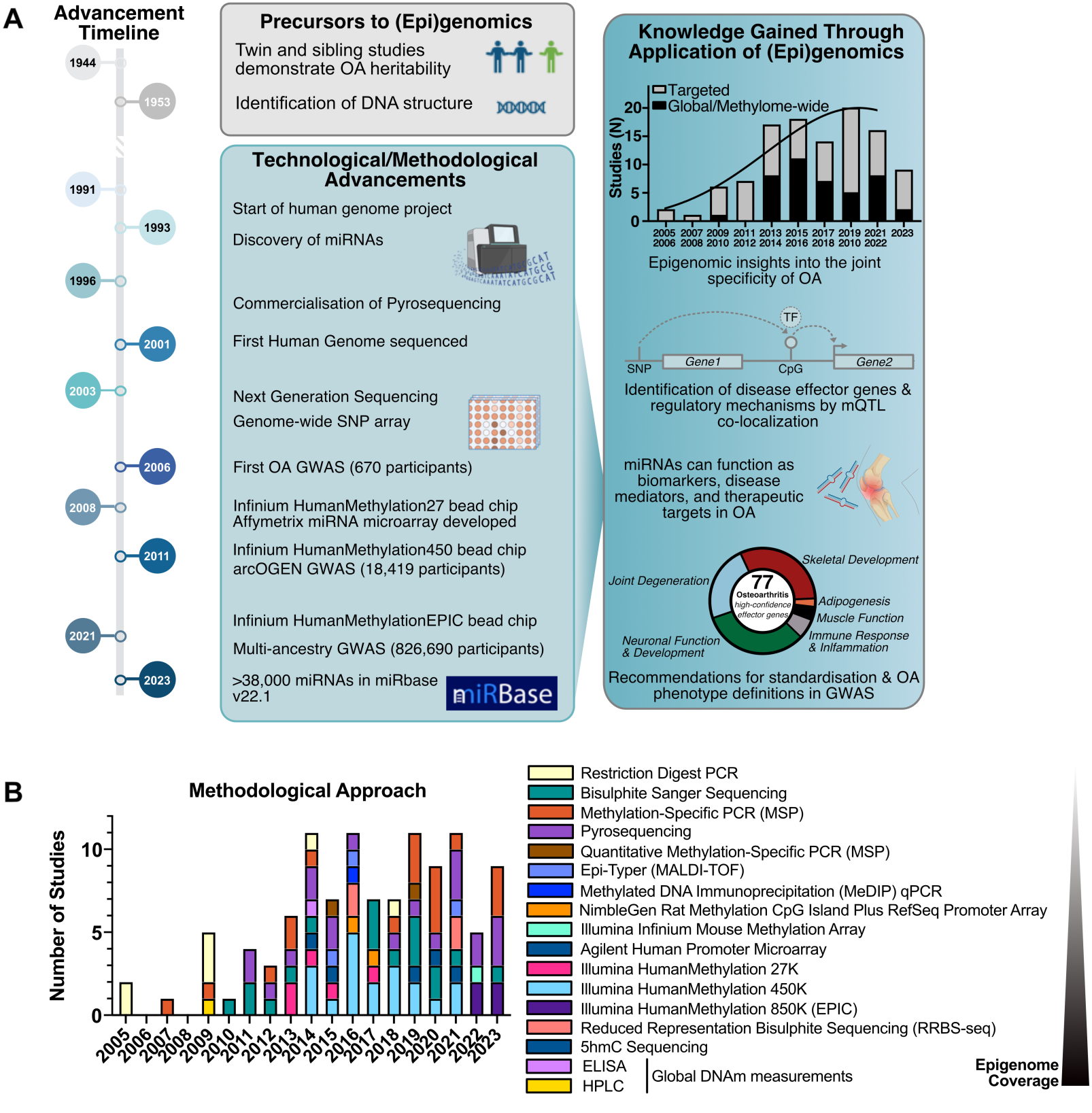

A
Advancement Timeline
1944
1953
1991
1993
1996
2001
2003
2006
2008
2011
2021
2023
Precursors to (Epi)genomics
Twin and sibling studies demonstrate OA heritability
Identification of DNA structure
Technological/Methodological Advancements
Start of human genome project
Discovery of miRNAs
Commercialisation of Pyrosequencing
First Human Genome sequenced
Next Generation Sequencing
Genome-wide SNP array
First OA GWAS (670 participants)
Infinium HumanMethylation27 bead chip
Affymetrix miRNA microarray developed
Infinium HumanMethylation450 bead chip
arcOGEN GWAS (18,419 participants)
Infinium HumanMethylationEPIC bead chip
Multi-ancestry GWAS (826,690 participants)
>38,000 miRNAs in miRbase v22.1
miRBase
Knowledge Gained Through Application of (Epi)genomics
Targeted
Global/Methylome-wide
Studies (N)
0
5
10
15
20
2005 2006
2007 2008
2009 2010
2011 2012
2013 2014
2015 2016
2017 2018
2019 2010
2021 2022
2023
Epigenomic insights into the joint specificity of OA
TF
SNP
Gene1
CpG
Gene2
Identification of disease effector genes & regulatory mechanisms by mQTL co-localization
miRNAs can function as biomarkers, disease mediators, and therapeutic targets in OA
77
Osteoarthritis
high-confidence effector genes
Skeletal Development
Adipogenesis
Muscle Function
Immune Response & Inlfammation
Neuronal Function & Development
Joint Degeneration
Recommendations for standardisation & OA phenotype definitions in GWAS
B
Methodological Approach
Number of Studies
0
5
10
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
2021
2022
2023
Restriction Digest PCR
Bisulphite Sanger Sequencing
Methylation-Specific PCR (MSP)
Pyrosequencing
Quantitative Methylation-Specific PCR (MSP)
Epi-Typer (MALDI-TOF)
Methylated DNA Immunoprecipitation (MeDIP) qPCR
NimbleGen Rat Methylation CpG Island Plus RefSeq Promoter Array
Illumina Infinium Mouse Methylation Array
Agilent Human Promoter Microarray
Illumina HumanMethylation 27K
Illumina HumanMethylation 450K
Illumina HumanMethylation 850K (EPIC)
Reduced Representation Bisulphite Sequencing (RRBS-seq)
5hmC Sequencing
ELISA
HPLC
Global DNAm measurements
Epigenome Coverage

Figure 2

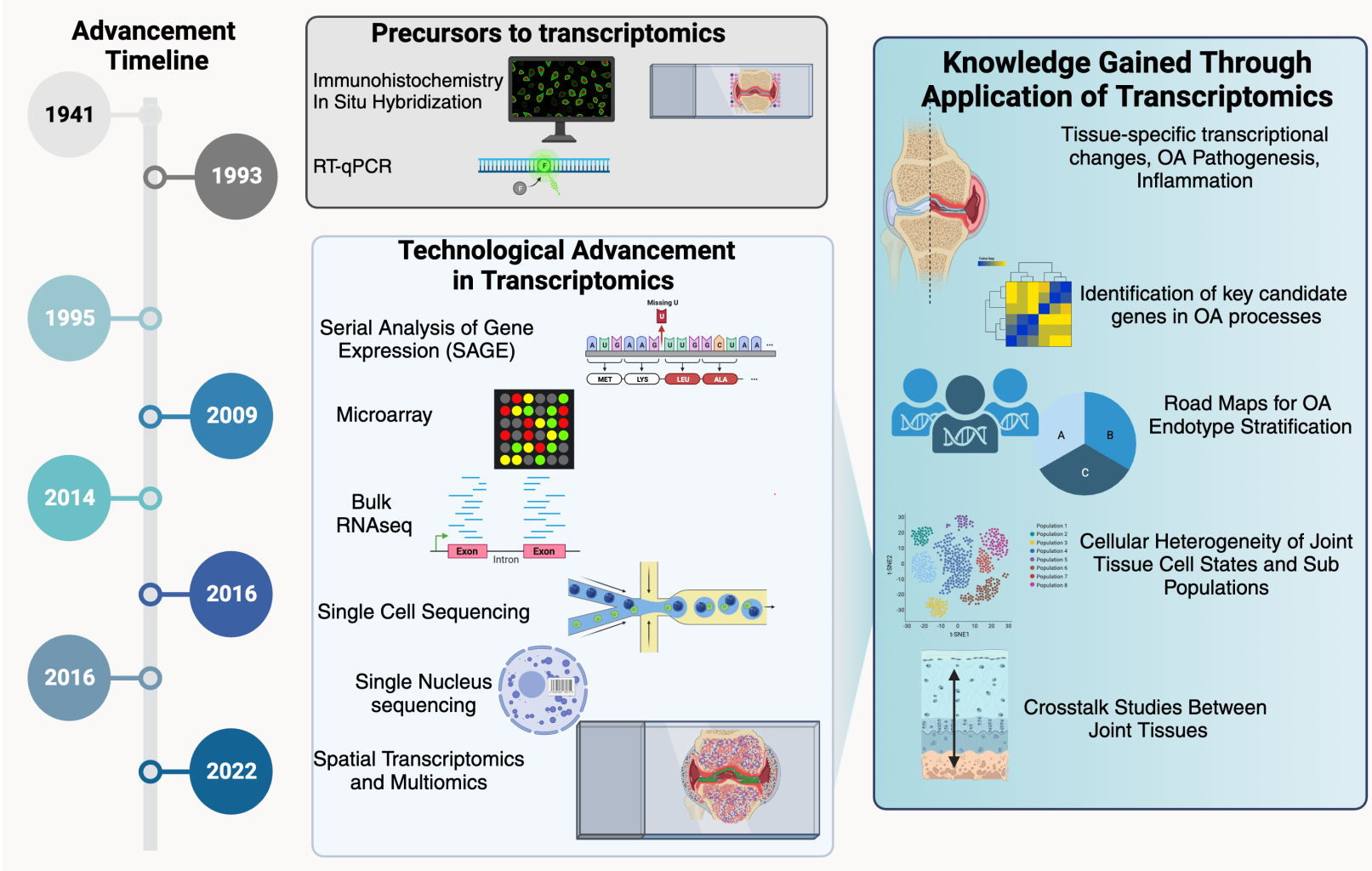
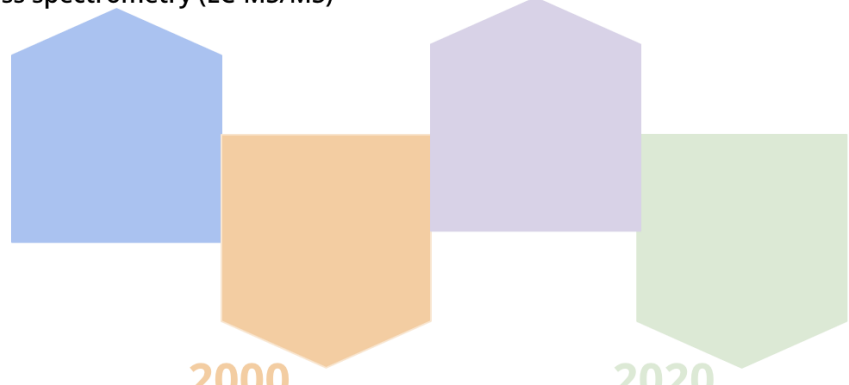

Advancement Timeline
1941
1993
1995
2009
2014
2016
2016
2022
Precursors to transcriptomics
Immunohistochemistry
In Situ Hybridization
RT-qPCR
Technological Advancement in Transcriptomics
Serial Analysis of Gene Expression (SAGE)
Missing U
MET
LYS
LEU
ALA
Microarray
Bulk RNAseq
Exon
Intron
Exon
Single Cell Sequencing
Single Nucleus sequencing
Spatial Transcriptomics and Multiomics
Knowledge Gained Through Application of Transcriptomics
Tissue-specific transcriptional changes, OA Pathogenesis, Inflammation
Identification of key candidate genes in OA processes
Road Maps for OA Endotype Stratification
A
B
C
Cellular Heterogeneity of Joint Tissue Cell States and Sub Populations
Crosstalk Studies Between Joint Tissues

Figure 3

ADVANCEMENT TIMELINE: PROTEOMICS



1994

- Conception of "Proteomics" in biology
- Two-Dimensional Gel Electrophoresis
- Liquid chromatography coupled to mass spectrometry (LC-MS/MS)

2000

- Protein arrays
- Difference in-gel Electrophoresis (DIGE)
- Shotgun proteomics (bottom-up strategies)

2010

- Data-independent acquisition (DIA) analysis for quantitative proteomics

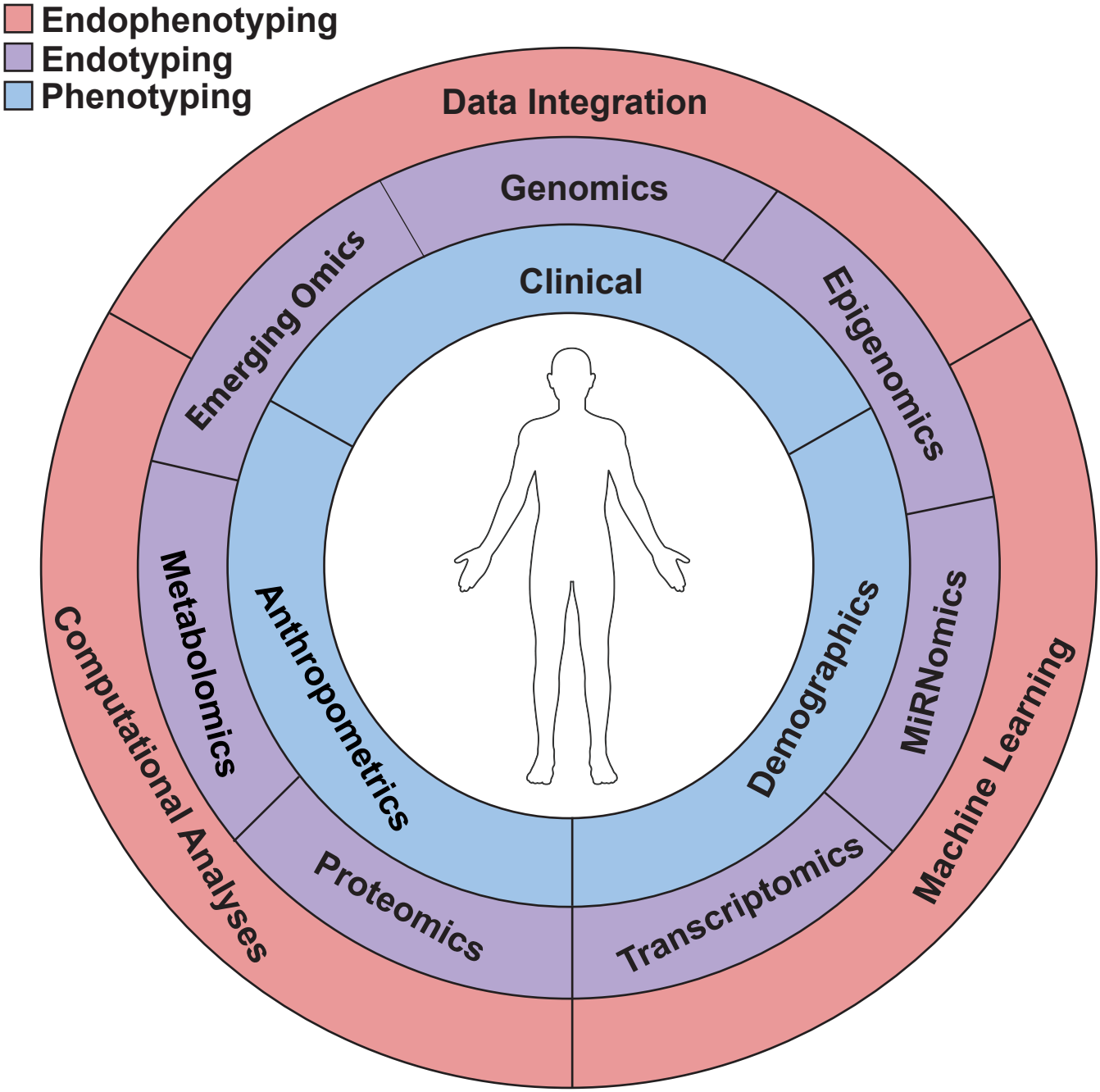
2020

- Affinity proteomics platforms for high-throughput targeted analysis (SomaScan, Olink)
- Single-cell proteomic studies

Knowledge gained in OA research

- Identification of changes in protein abundance or protein structure in OA cells and tissues.
- Characterization of functional pathways key in OA pathogenesis
- Identification of proteins and protein fragments with biomarker value in OA
- Identification of protein biomarkers in synovial fluid and serum from OA patients
- OA endotype stratification and functional analysis

Figure 4


Endophenotyping
Endotyping
Phenotyping
Data Integration
Genomics
Epigenomics
MiRNomics
Transcriptomics
Proteomics
Metabolomics
Emerging Omics
Clinical
Anthropometrics
Demographics
Machine Learning
Computational Analyses