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Recent advances in omics and the integration of multi-omics in osteoarthritis research

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Abstract

Osteoarthritis (OA) is a complex disorder driven by the combination of environmental and genetic factors. Given its high global prevalence and heterogeneity, developing effective and personalized treatment methods is crucial. This requires identifying new disease mechanisms, drug targets, and biomarkers. Various omics approaches have been applied to identify OA-related genes, pathways, and biomarkers, including genomics, epigenomics, transcriptomics, proteomics, and metabolomics. These omics studies have generated vast datasets that are shaping the field of OA research. The emergence of high-resolution methodologies, such as single-cell and spatial omics techniques, further enhances our ability to dissect molecular complexities within the OA microenvironment. By integrating these multi-layered datasets, researchers can uncover central signaling hubs and disease mechanisms, ultimately facilitating the development of targeted therapies and precision medicine approaches for OA treatment.

Keywords Osteoarthritis, Multi-omics, Spatial omics, Single cell omics, GWAS, Artificial intelligence

Introduction

Osteoarthritis (OA) is one of the most prevalent chronic diseases, affecting over 500 million people, accounting for approximately 7% of the world's population [1], substantially burdening public health systems. OA is a disease of the joint as a whole organ, involving profound cell-cell interaction during the disease's progress and symptom manifestation. Articular chondrocytes are the resident cells within articular cartilage that biomechanically maintain a smooth and elastic surface at the end of bones, allowing low-friction movement. These long-lived and mostly postmitotic cells have both anabolic and catabolic functions in remodeling the cartilage extracellular matrix (ECM) in a healthy state. However, upon damage or stress, articular chondrocytes lose proper homeostasis and undergo degeneration, leading to fibrosis, scarring, ECM loss, and mineralization, etc. The cartilage changes further impact the activities of osteoblast and osteoclast lineages in the subchondral bone, resulting in the alterations of bone architecture and the growth of osteophytes, further aggravating OA symptoms. Synoviocytes, the lining cells of the synovium, become activated in response to inflammatory mediators, releasing pro-inflammatory cytokines and proteases that further exacerbate joint damage and pain in OA. In a feedforward mechanism, immune cells, such as macrophages and T lymphocytes, infiltrate the joint and produce inflammatory cytokines and proteases, thereby potentiating joint inflammation and damage. These complex OA-associated changes in cell properties and activities, cell-cell interaction, and signaling crosstalk, together with the heterogeneous genetic backgrounds of OA patients, baffled the development of OA treatments. Currently, there is a lack of FDA-approved therapies that can modify the trajectory of OA progression [2]. It calls for a more comprehensive

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understanding of OA as an organ disease associated with systemic molecular changes in the organism and intricate changes in the tissue microenvironment. Moreover, OA diagnosis is usually late because articular cartilage lacks innervation, so the initial cartilage damage is usually non-symptomatic; thus, the diagnosed cases are often at a more advanced disease stage (joint pain and stiffness), which is more difficult to manage clinically [3]. Therefore, early OA diagnosis is important for managing OA progression, but it urgently requires the discovery of new and effective biomarkers. The recently developed multi-omics approaches and downstream data integration provide an unprecedented opportunity for us to move the needle forward. This review highlights some selected recent advances in OA research that have included omics analyses or integrated multiple omics datasets to further our understanding of OA.

The advantages and limitations of omics studies

High-throughput technologies have changed science by enabling the investigation of highly complex molecular events within cells and tissues. Now, high-throughput omics approaches encompass a wide array of molecular measurement techniques that will allow an integrated view of the biology underlying the molecular landscape of OA.

Transcriptomics

Transcriptomics studies the complete set of RNA molecules produced by the genome. It provides insights into gene expression patterns, alternative splicing events, and regulatory mechanisms that control gene expression. Most research in OA focuses on characterizing transcriptomic changes in cartilage, subchondral bone, and synovium, as they are directly associated with the disease and the most accessible samples for study. For example, a large transcriptome analysis involving 124 patients examined paired samples of low- and high-OA knee articular cartilage, revealing alterations in the expression of 38 coding RNA and 25 long non-coding RNA genes, alongside isoform and splicing changes [4]. Furthermore, transcriptomic profiling in a mouse model revealed that *microRNA-17 (miR-17)* is highly expressed in the superficial and middle zones of the articular cartilage, where it plays a protective role against cartilage degradation induced by DMM (destabilization of the medial meniscus) surgery [5]. The study also demonstrated that *growth differentiation factor 5 (GDF- 5)* upregulated endogenous *miR-17* expression, enhancing its protective function against OA. In addition to cartilage degradation, changes in the microarchitecture of subchondral bone are a distinct and prominent hallmark of OA. Increasing evidence suggests that cells within the subchondral bone contribute to both the onset and progression of OA [6, 7]. An RNA-seq study found that *CNTNAP2* and *STMN2* were significantly upregulated in the subchondral bone of OA lesions from human hips and knees [6]. Additionally, their analysis identified upregulation of *IL11* and *CHADL* in both OA subchondral bone and cartilage. A recent study published in 2024 by Jiang et al. identified transcriptional alterations in genes such as *MMP13*, *MMP1*, *MMP2*, *APOD*, *IL6*, *TNFAIP6*, *FCER1G*, and *IGF1* in the synovium of OA patients, which are implicated in regulating extracellular matrix metabolism and inflammatory processes [8].

While transcriptomics enables understanding RNA-level changes in OA, several limitations should be considered. The presence of RNA does not always correlate with protein abundance due to post-transcriptional regulation, including variations in translation efficiency and protein degradation. Notably, standard RNA-seq and commonly used analytical tools primarily measure gene expression and are not well-suited for detecting alternative splicing, RNA editing, or miRNA-mediated regulation of gene expression. Additionally, they provide limited insight into different RNA processing stages, such as unspliced precursor RNAs or intermediate transcripts, which can significantly impact gene function. The detection and quantification of non-coding RNAs, including long non-coding RNAs and transposable element-derived transcripts, remain challenging, particularly in repetitive regions where multi-mapping reads are often discarded or randomly assigned. Importantly, while transcriptomics provides insights into gene expression, integrating other omics approaches, such as epigenomics, is essential to uncovering how the genome is regulated to permit or suppress these changes in gene expression.

Epigenomics

Epigenomics enables the global, unbiased identification of molecular modifications, such as DNA methylation, histone methylation or acetylation, and regulatory RNAs that govern chromatin accessibility and that influence gene expression without altering the underlying DNA sequence.

When DNA methyltransferases methylate DNA at the 5' position of cytosine within a CpG dinucleotide, this results in 5-methylcytosine, which is strongly associated with gene silencing. Numerous studies have examined DNA methylomes in OA using methods such as DNA methylation arrays, bisulfite sequencing (BS-Seq), whole genome bisulfite sequencing (WGBS), and reduced representation bisulfite sequencing (RRBS). As an example, a study analyzing the methylation profiles of hip and knee OA cartilage using the Illumina Human Methylation 450 Array identified 12 differentially methylated regions

(DMRs) near genes such as *MEIS1*, *GABRG3,* and *RXRA* between hip and knee OA [9]. *MEIS1* is a transcription factor that regulates gene expression, including stem cell pluripotency; *GABRG3* is potentially involved in pain perception, and *RXRA* is linked to bone metabolism, though none have been directly associated with OA. Additionally, a comprehensive epigenomics study utilizing multilayer genomic and epigenomics data, including DNA methylation, miRNA profiles, genetic variations, and mRNA profiles from synoviocytes of rheumatoid arthritis (RA) and OA patients, revealed 523 high-methylated regions specific to OA, with associations to transcription factors like *GLI1*, *RUNX2*, and *TFAP2 A/C* [10]. These high-methylated regions were further linked to networks involved in tissue development and TGF-β signaling. Two similar studies have examined genome-wide DNA methylation profiling to investigate the role of epigenetic modifications in subchondral bone during OA progression. One study examined subchondral bone from patients with hip OA, identifying 7,316 differentially methylated CpG sites in subchondral bone underlying eroded cartilage, most hypomethylated. They also found a strong TGF-β signaling and tumor necrosis factor family signature in differentially methylated genes [11]. Similarly, another study analyzed the DNA methylation profiles of subchondral bone from different regions of the tibial plateau from patients with knee OA, revealing progression-associated methylation alterations [12]. This study highlighted that the epigenetic changes occurred earlier in subchondral bone than in cartilage and identified shared and unique differentially methylated genes involved in tissue repair and skeletal system development.

Histone marks are chemical modifications (such as methylation, acetylation, phosphorylation, and ubiquitination) on the histone proteins around which DNA is wrapped. These epigenetic marks not only act as molecular switches that are key regulators of chromatin structure and gene expression but also serve as docking sites for reader proteins. For example, histone acetylation (e.g., H3K9ac, H3K27ac) is associated with active transcription. Acetylation reduces the positive charge on histones, weakening their interaction with the negatively charged DNA and making the chromatin more accessible to transcription factors and RNA Pol II. Bromodomain-containing proteins, such as BRD4 bind acetylated lysine and help recruit transcription elongation factors, including the P-TEFb complex [13]. Histone methylation can have different effects depending on the specific lysine residue. For instance, H3K4me3 is typically associated with active gene promoter and can be recognized by reader proteins like CHD1, which promotes elongation by facilitating chromatin remodeling, whereas H3 K36 me3 marks the body of actively transcribed genes is recognized by the MORF complex and other factors, indicating elongation [14]. In contrast, H3K27me3 and H3K9me3 are recognized by reader proteins like Polycomb group proteins and HP1, which recruit a silencing complex that can repress the transcription by stabilizing a more compact chromatin state [15]. In OA, aberrant epigenetic modifications play a pivotal role in the dysregulation of gene expression, leading to disease progression.

Several studies used immunohistochemistry or western blot to identify the total changes of specific histone marks in the OA primary tissue instead of genome-wide profiling. For example, in a study examining histone methylation in synovial tissues, increased methylation of H3K79 and upregulation of DOT1L, a histone methyltransferase, were observed in both OA and RA patients, suggesting that histone methylation may play a role in transcriptional activation involved in these diseases [16]. Importantly, another study highlighted the critical role of *Dot1l* in maintaining cartilage homeostasis and protecting against OA in a genetic mouse model, attributing its function to the inhibition of Wnt signaling [17]. In conditions mimicking OA, such as *Dot1 l* deletion or treatment with the *Dot1l* inhibitor EPZ- 5657, the total levels of H3K79me2 were reduced, as confirmed by western blot analysis. ChIP-qPCR (chromatin immunoprecipitation followed by qPCR) was used to examine the enrichment of DOT1L and H3K79me2, revealing that SIRT1 drives hyperactivation of Wnt signaling when *Dot1l* is blocked in chondrocytes. However, while these approaches could pinpoint specific genomic loci, they did not offer a comprehensive and genome-wide profile of epigenetic modifications. As a result, significant shifts in histone mark distribution across the genome can be overlooked, particularly if the total levels of these marks are minimally or not changed. ChIP-seq (chromatin immunoprecipitation followed by sequencing) is used to identify genomic DNA regions that bind to specific DNA-associated proteins, such as transcription factors and post-translationally modified histones. However, ChIP-seq analyses on hard tissue pose challenges due to the requirement for a substantial number of cells and the difficulty of obtaining them. One study used transcription factor ChIP-seq on primary chondrocytes, and the results suggest a crucial role of FOXO1 in regulating cartilage-specific genes in OA chondrocytes. Epigenome profiling methods CUT&RUN and CUT&Tag were developed as alternative approaches to ChIP-seq, offering the significant advantage of requiring fewer cells while providing a higher signal-to-noise ratio compared to ChIP-seq. For example, H3K9me3 CUT&RUN has been used to study skeletal tissue in mice, revealing how TRIM28, a crucial adaptor protein assembling epigenetic silencing complexes,

regulates skeletal stem cell fate and prevents their transition to neural crest-like cells [18]. A study that generated H3K27ac CUT&RUN data in primary human chondrocytes identified OA risk genes, including *SOCS2*, which regulate inflammation in response to cartilage matrix damage [19].

ATAC-seq (assay for transposase-accessible chromatin with sequencing) is another profiling approach to study chromatin accessibility. It is highly efficient and requires fewer cells than ChIP-seq, rendering it more applicable in studying hard tissues [20]. Unlike ChIP-seq, which targets specific histone marks or transcription factors, ATAC-seq offers a global survey of open chromatin regions, providing a broad map of the genome regulatory landscape. Although ATAC-seq does not directly measure transcription factor binding, one can predict the binding of transcription factors based on reference databases by analyzing the sequence motifs within accessible regions. Moreover, ATAC-seq identifies active regulatory elements, such as enhancers and promoters, often characterized by higher chromatin accessibility [21]. ATAC-seq has been conducted to profile the overall accessible chromatin landscape change in comparing injured and intact cartilage tissue and identified strikingly distinct chromatin signatures, notwithstanding patient-to-patient differences [22]. Further analysis revealed that enhancers account for most differentially accessible regions, including enhancers from *BMPR1b*, *WNT5a*, and *FGFR2,* which are all known to play a role in regulating OA pathogenesis. Our recent ATAC-seq study revealed the aberrant activation of endogenous retroviruses (ERV) in OA or aging joints. The findings suggest that the loss of H3K9me3, possibly due to aging or cellular stress, leads to ERV reactivation that contributes to tissue inflammation and OA progression [23].

While instrumental in providing critical insights into gene regulation, epigenomic studies come with several limitations. These approaches capture only a snapshot of the dynamic chromatin interactions, making distinguishing the functional vs. non-functional regulatory elements challenging, particularly in non-coding regions. For example, the enhancers are highly cell-type-specific, and identifying functional enhancer regions requires additional evidence, such as transcriptional activity or 3D chromatin interaction data. ChIP-seq, a widely used method for mapping protein-DNA interactions, is highly dependent on antibody specificity and affinity, which can lead to off-target binding or failure to capture the intended protein. DNA methylation data are largely correlative and do not establish causal relationships with gene expression. Most studies focus on CpG methylation, while non-CpG methylation remains less understood and more challenging to detect. Similarly, ATAC-seq, which maps chromatin accessibility, identifies open chromatin regions but does not directly indicate active regulatory elements. To infer functional significance, downstream validation or integration with reference datasets is often required.

Proteomics

Proteins, the final products of genes, are responsible for the phenotypes of cells. Although proteins are translated from mRNA, protein levels are not always correlated with mRNA levels due to the delay of translation, isoforms, or protein half-life. Also, posttranslational modification (PTM) and cleavage profoundly affect the function of proteins, but these changes cannot be detected via transcriptomic analysis [24]. Moreover, secreted proteins might be found at sites different from their synthesis so that solely studying the genome could overlook them.

Proteomics, first defined in 1995 as the large-scale characterization of a cell, tissue, biofluid, or organism's entire protein complement, can resolve these issues [25]. Proteomics aims to study the proteome and obtain a more comprehensive and integrated view of biological specimens rather than each protein individually. This approach enables the comparison of protein expression across samples, helping to identify and quantify novel or disease-specific proteins, also known as protein expression proteomics, and studies including the isolation of protein complexes and characterizing protein signaling or protein-drug interactions are known as functional proteomics [26].

The early use of proteomics was to annotate the genome, as the exon-intron structure of many genes cannot be accurately predicted from genomic data alone. Proteomics studies helped confirm the existence of genes. Conversely, the expansion of proteomics has been driven by large-scale nucleotide sequencing of genomic DNA or RNA. Therefore, integrating both genomic and proteomic information will make it possible to elucidate the physiological mechanisms, such as disease, aging, and the effects of the environment [27].

Currently, proteomic analyses commonly utilize mass spectrometry (MS) techniques, including Surface Enhanced Laser Desorption Ionization (SELDI), Matrix-Assisted Laser Desorption Ionization (MALDI) coupled with time-of-flight (TOF), and gas chromatography MS (GC–MS) or liquid chromatography MS (LC-MS), to identify proteins via mass-to-charge ratios of peptides [28]. The high-throughput capabilities and accuracy of results by MS technology have largely replaced Edman sequencing, which used microsequencing techniques in the 1990s [29]. A study utilized MS-based imaging to compare the distribution of peptides and proteins in human control and OA cartilage [30]. It identified that

specific protein markers, such as cartilage oligomeric matrix protein and fibronectin, are exclusively present in OA cartilage samples. Another study also compared the proteome profiles of healthy menisci, those with mild degeneration, and end-stage OA [31]. A panel of 42 proteins was gradually changed in abundance from healthy to end-stage OA, with lower levels of QSOX1 and higher levels of G6PD in the mild degeneration group, suggesting potential drug targets. Later, a study profiled menisci proteomes from OA patients (n =10) and healthy controls (n =10), allowing us to see a large difference between the medial menisci from OA and healthy. As a result, the metalloproteinase proteins, such as MMP3 and TIMP1, showed higher abundance in the end-stage OA menisci, aligning with the cartilage degradation and inflammatory conditions.

Synovial fluid is easily accessible and may be obtained from OA patients through minimally invasive procedures, making it well-suited for identifying protein biomarkers and facilitating early diagnosis and treatment targets [32–35]. A study utilized the SWATH-MS (Sequential Window Acquisition of All Theoretical MS) approach to analyze the alteration of the synovial fluid proteome in OA. As a result, complement C1r (C1R) and the dickkopf-related protein 2 (DKK2) were identified as potential disease biomarkers or drug targets for OA in synovial fluid [36]. Similarly, another study analyzed the synovial fluid proteome to investigate the protein expression changes during different stages of knee OA [35]. This study compared late-stage OA (arthroplasty), early knee OA (arthroscopy caused due to degenerative meniscal tear), and individuals without knee OA as a control group. Approximately 200 differentially expressed proteins were identified. COL1A1 and COL3A1 were increased in early-stage OA but decreased or even absent in late-stage OA. Additionally, CRTAC1 was exclusively upregulated in late-stage OA, suggesting its potential as a marker for severe cartilage damage.

Glycoproteomics is a branch of proteomics that identifies protein glycosylation, which accounts for the most common post-translational protein modifications [37]. A recent study conducted N-glycoproteomics by comparing cartilage samples from OA patients without and with type 2 diabetes mellitus (DM-OA) [38]. This report identified 847 N-glycosylation sites on 374 proteins, among which, the upregulated glycosylated complement C8α alpha chain in the DM-OA group might augment membrane attack complex activity, exacerbating cartilage destruction. This research presents a comprehensive map of the glycosylation patterns in OA and suggests type 2 diabetes mellitus as an independent risk factor for OA.

Moreover, studies aimed at aligning the structure of protein complexes and location in specific cellular organelles or characterizing all protein-protein interactions are known as structural proteomics [26]. Techniques such as atomic-resolution cryo-electron microscopy (cryoEM) and X-ray crystallography are widely used to determine the structures of undiscovered endogenous large-sized protein complexes and extracellular vesicles (EVs) [39, 40]. Nuclear Magnetic Resonance (NMR) is another powerful technique used for specifically determining medium-sized proteins in solution. These approaches can be applied to unravel the architecture of protein complexes implicated in cartilage breakdown, which is crucial to OA pathology.

However, proteomics studies face several challenges. First, the complex and dynamic nature of the proteome, including protein turnover, variants, and diverse PTMs, complicates quantification. Also, the low-abundance but crucial proteins (such as transcription factors, signaling ligands, or rate-limiting metabolic enzymes) often fall below detection limits or are overshadowed by highly expressed proteins (such as cytoskeleton proteins and extracellular matrix proteins). In addition, the variability in sample preparation, instrument sensitivity, and data acquisition can increase noise and limit the reproducibility across experiments.

Metabolomics

In contrast to proteomics, metabolomics, which also relies on MS or NMR, is an omics approach for globally identifying metabolites in cells, tissues, biofluids, or entire organisms. Metabolites are small molecules that are the end products of the metabolic process, including amino acids, sugars, lipids, nucleotides, and other organic compounds. Metabolomics provides a snapshot of the metabolic state of a system at a specific time. As metabolites are highly dynamic and can change rapidly in response to environmental stimuli, they are more sensitive indicators of physiological states. Due to the rapid shifts in metabolism driven by ongoing cellular reactions, careful attention must be paid to minimize preparation artifacts that could distort the result. Metabolomics studies in OA can yield different results depending on the sample preparation method, such as fresh versus frozen samples, tissue versus primary cultured cells, flow-sorted vs unsorted cells, as well as the timing of processing. These factors can significantly influence the metabolic profile, making it challenging to ensure reliable and reproducible results [41, 42]. Despite these challenges, metabolomics offers unique advantages for understanding disease mechanisms, and a more dynamic view of cellular processes complements other omics approaches.

Altered metabolism within joint tissues has been previously shown to affect OA development [43]. Glucose metabolism has been extensively studied in

chondrocytes, though the role of other energy metabolism pathways with different fuel/substrates, such as fatty acid or amino acid metabolism, has yet to be extensively researched [44]. Notably, glutamine metabolism was found to enhance the inflammatory response in chondrocytes [45]. A significant aspect of metabolic alterations in chondrocytes is mitochondrial dysfunction, which promotes a metabolic shift favoring glycolysis over oxidative phosphorylation, disrupting homeostasis, and altering the balance between catabolic and anabolic activities [43, 46]. Mitochondrial dysfunction disrupts the electron transport chain (ETC), resulting in an imbalanced production and clearance of reactive oxygen species (ROS) and a shift in the availability of metabolite and building blocks. These changes can significantly affect various metabolic pathways [47].

Furthermore, studies utilizing Flight-Secondary Ion Mass Spectrometry (TOF–SIMS) have demonstrated molecular-level differences in lipid distribution between healthy and OA cartilage. Cholesterol and some other fatty acids showed significant localization in OA cartilage, with higher concentrations near the cartilage surface and calcium and phosphate ions accumulated near chondrocytes in OA tissue, suggesting mineral deposition in this area [48]. Metabolic profiling of biological fluids has emerged as a strategy for biomarker diagnostics, capable of early detection, prognosis, and monitoring of diseases [49]. Changes in the composition of synovial fluid may affect its lubricating properties, leading to increased wear and tear on the cartilage and inflammation. Besides harboring a variety of bioactive molecules including growth factors, cytokines, and enzymes, the synovial fluid provides metabolites to nourish chondrocytes, while other metabolites, including chondroitin sulfate degradation products, arginine and proline, nitric oxide, phosphatidylcholine, lysophosphatidylcholine, ceramides, myristate derivatives, and carnitine derivatives, were found to increase in OA [50]. A substantial body of research has also investigated changes in metabolomics of plasma or serum from OA patients, providing valuable insights into the OA-associated metabolic alterations [51–55]. For example, a study investigated plasma metabolites and metabolic pathways in a large cohort. It revealed cystine as a potential metabolic biomarker of radiographic OA severity and early-phase synovitis [52]. Hence, profiling the metabolic changes of joint tissue and synovial fluid can potentially identify novel therapeutic targets and elucidate new mechanisms for OA.

Like other omics, metabolomics has its unique limitations. Metabolite levels are greatly impacted by diet, environment, and physiology, adding variability. Sample collection and storage affect metabolite stability, risking degradation. Different analytical platforms (e.g., LC-MS, GC–MS, NMR) vary in sensitivity and bias, influencing detection. The complexity of metabolic pathways and the difficulty distinguishing de novo synthesis, salvage, and transport pathways further challenges data interpretation, requiring isotopic tracing, robust computational tools, and reliable reference databases.

Single-cell and spatial omics

In contrast to the above bulk omics methods using tissues, high-resolution methodologies, such as single-cell and spatial omics techniques, are revolutionizing our understanding of cell behaviors and microenvironments and enabling precise quantification and visualization of molecular complexities within tissues. They have been applied to OA studies at an accelerating rate.

Single-cell

Single-cell RNA sequencing (scRNA-seq), a powerful technique used to analyze the gene expression of individual cells, has revealed a hierarchy of cell lineages and has enabled the identification of stem cells required for skeletal development [18]. The use of scRNA-seq in OA has revealed previously unknown cellular and molecular characteristics through the progression of the disease, offering valuable insights into the development of precise diagnostic and therapeutic approaches tailored to the distinct molecular profiles observed [56–61]. A recent scRNA-seq study from knee OA patients compared the cellular composition and subpopulation-specific gene expression of normal and OA cartilage in 13 cell subpopulations [62]. Specifically, alterations in gene expression showed an enrichment of the ferroptosis pathway in OA fibrocartilage and inflammatory chondrocyte populations. Pathological angiogenesis is coupled with aberrant osteogenesis, which accelerates OA in subchondral bone tissue. Synovial fibroblasts were associated with end-stage OA pain when they shifted toward a more disease-specific fibroblast pathotype in OA progression, featuring highly expressed HTRA3 and GPX3 [57]. In addition, a recent study profiled articular cartilage from healthy and injured mouse knee joints at single-cell resolution and identified nine chondrocyte subtypes with distinct molecular profiles [59]. The study also highlighted injury-induced early molecular changes in these chondrocytes and compared the molecular similarities between mouse and human chondrocytes, expanding the view of chondrocyte heterogeneity in OA. Another study from the same group using scRNA-seq delineated the temporal dynamics of immune cell recruitment after joint trauma in post-traumatic osteoarthritis (PTOA) [63]. This work identified several immune cell types, such as monocytes and macrophages expanded post-injury, highlighting their role in joint repair.

While scRNA-seq has transformed the understanding of OA at the single-cell level, single-cell epigenetic data has yet to be extensively used for OA study. Single-cell-level epigenetic sequencing methods, such as scATAC-seq, scMNase-seq, scChIP-seq, scBS-seq, and scale scMethyl, have emerged as tools for uncovering the epigenomic characteristics of cellular subtypes [64–67]. Additionally, studies used Cytometry by Time-Of-Flight (CyTOF), a single-cell proteomics technique, to identify and quantify labeled protein epitopes on the surface and interior of individual cells [68]. CyTOF has been used to identify distinct chondrocyte progenitors and inflammation-modulating chondrocyte subpopulations that include a pro-inflammatory population marked by interleukin- 1 receptor one and tumor necrosis factor receptor II and an anti-inflammatory population marked by CD24 [69]. Single-cell omics approaches have enabled profiling a statistically meaningful number of individual cells within a tissue/organism. However, single-cell profiling requires generating a single-cell suspension through mechanical and enzymatic dissociation, which does not preserve the original tissue architecture. Consequently, developing technologies in which spatial information is maintained within the tissue architecture has been a logical next step in the evolution of omics technologies.

Spatial omics

Advances in spatial biology techniques now allow intact tissue sections to be examined using various omics approaches. These techniques enable mapping the spatial coordinates of molecular profiles and investigating cells within their tissue microenvironment. Using platforms like NanoString GeoMx, researchers can stain and image RNAs or proteins within whole formalin-fixed paraffin-embedded (FFPE) or fresh frozen tissue sections, followed by sequencing, to analyze spatial gene expression in cartilage. GeoMx allows for the section of regions of interest (ROIs), making it particularly useful for studying specific histological zones in OA cartilage, such as the superficial layer versus the deep zone, to examine differential gene expression related to disease progression. Geo-seq is a laser capture microdissection-based RNA sequencing technique that is used to segment different regions to preserve spatial information before dissociation [70]. Using Geo-seq to study knee cartilage in OA, researchers integrated scRNA-seq data to define the spatial landscape of chondrocytes, revealing that the articular surface was the more transcriptionally active zone than the superficial, middle, and deep zones. Although the laser capture microdissection technique is powerful, its isolation process significantly limits throughput. Recently, microfluidic-based barcoding methods have emerged for spatial transcriptomics, which can be categorized into two main strategies: deterministic barcoding, where cell positions are pre-defined, and random barcoding, where barcodes are assigned without prior knowledge of cell location. Notable examples include Visium/Visium HD/Xenium (10 ×Genomics), HDST, MERFISH, DBiT-seq, Slide-seq, and Spatial Transcriptomics (ST). Among these, Visium is useful for studying broader tissue architecture with a resolution of 55 μm (Visium) or 2 μm (Visium HD), making it suitable for exploring zonal differences in mineralized tissues or synovial tissue. For example, this method has been applied to analyze postnatal growth plate chondrocytes in murine hindlimbs and to map skeletal stem and progenitor cells (SSPCs) in decalcified adult mouse femurs [71, 72]. In contrast, Slide-seq and DBiT-seq provide near single-cell resolution, which could be advantageous for characterizing heterogeneity within chondrocytes or synovial fibroblasts. Moreover, Xenium and MERFISH even offer subcellular resolutions, enabling precise localization of transcripts within individual cells. The team that developed ST demonstrated its efficacy by profiling the three-dimensional spatial transcriptomics of human rheumatoid arthritis synovium tissue [73].

Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) enables the generation of spatially resolved proteomic and metabolomic data in OA [74]. This method offers the advantage of identifying proteins and metabolites from tissues without separation and homogenization while preserving the information on their spatial distribution. MALDI-MSI platform ionization imaging allows the *in-situ* detection of many peptides and proteins in OA cartilage [30]. Applied MALDI-MSI, proteins such as biglycan, prolargin, decorin, aggrecan, fibronectin, and cartilage oligomeric matrix protein were identified and localized. MALDI-MSI is particularly useful for studying metabolic shifts in cartilage and synovium during OA progression, as it can provide spatially resolved insights into lipid metabolism, oxidative stress markers, and glycosylation patterns, which are not captured by the transcriptomic approach. MALDI-MSI has also been used to determine the N-glycome in the cartilage and subchondral bone of knee OA in a spatially resolved manner [75].

Moreover, AtlasXomics technology has demonstrated the potential of spatial epigenome assays, including spatial-ATAC-seq and CUT&Tag tools, which will enable the spatial context of epigenomic modifications within joint tissues. For example, spatial-ATAC-seq could be applied to OA cartilage to determine how chromatin accessibility changes across tissue zones and identify potential regulatory elements involved in OA progression in each of these compartments. Similarly, spatial CUT&Tag could reveal histone modification patterns

attributed to the disease state of joint cells, such as chondrocyte hypertrophy, senescent, inflammation, or other stress responses.

Future research will increasingly combine single-cell and spatially resolved techniques in omic studies to help solve the obstacle of tissue heterogeneity. This will aid in a comprehensive understanding of cellular interactions and the microenvironment in disease progression. The continuous development of new technologies and computational methods in single-cell and spatial technologies will be instrumental in identifying novel and precise therapeutic strategies.

Multi-omics in OA

The joint analysis of the genome, epigenome, transcriptome, proteome, metabolome, or multi-omics transforms our understanding of cell biology in health and disease. Most omics studies in OA have focused on a single technology (Table 1). However, recent advances in multi-omics approaches are providing a more comprehensive and refined view of the complex molecular mechanisms underlying OA.

Multi-omics integration strategies in OA research

Multi-omics allows scientists to gain a more comprehensive understanding of complex biological systems by combining information from various omics approaches based on their resolution (i.e., bulk, single-cell, and spatial omics) and molecules of interest (i.e., genomics, epigenomics, transcriptomics, proteomics, and metabolomics). On the resolution level, each omics technology offers unique benefits by excelling in one type of resolution while having limitations in others (Fig. 1). Bulk omics offers deep molecular profiling by analyzing the entire population of cells within a tissue, making it effective for identifying overall molecular trends. However, it lacks cellular resolution since it averages signals across different cell types, concealing cellular heterogeneity and potentially masking rare cell types or distinct cellular states. Single-cell omics excels in cellular resolution, revealing cellular diversity and allowing detailed study of individual cell types and states within tissues, but lacks spatial context and has limited depth of data. Spatial omics provide spatial resolution by mapping molecular information directly onto tissue sections, preserving spatial relationships between cells and tissue architecture, but have limitations in molecular depth. Thus, multi-omics methodologies that integrate data at bulk, single-cell, and spatial levels are necessary to acquire high-resolution molecular and cellular hierarchy from genome to phenome in OA pathogenesis [76]. A recent study integrated single-cell and spatial transcriptomics to deconvolute the significant cellular heterogeneity in OA tissues and identify novel inflammatory chondrocyte populations as potential therapeutic targets [77]. The study analyzed 19 cartilage samples from 8 OA patients and 3 controls, profiling 135,896 chondrocytes using scRNA-seq (10 × Genomics) to explore cellular heterogeneity. In addition, spatial transcriptomics (Geo-seq) was performed to examine spatial heterogeneity. The scRNA-seq and Geo-seq data were integrated to reveal zone-specific differentially expressed genes in OA and healthy joints. Further integration of these results with bulk RNA-seq data enabled the classification of OA patients into two distinct subtypes. Another study identified distinct cellular populations and transcriptomes in hip synovium mediating the progression of OA by integrating single-cell and spatial transcriptomics using synovial tissues of patients with femoroacetabular impingement and OA [78]. Moreover, the integration of bulk and single-cell RNA-seq has been used to identify 15 pyroptosis-related genes in human OA samples, which could be used as biomarkers for diagnosis and prognosis in OA [79]. Similarly, integrated bulk and single-cell RNA-seq has identified macrophages as a diagnostic marker of early immune cell infiltration during synovial inflammation in OA, as well as several key mediators of macrophage interaction with other cellular populations in the OA microenvironment [80].

Integration of individual omics approaches based on their molecules of interest also provides valuable insights into the pathogenesis of OA. This process identifies correlations and associations between different molecular layers and reveals interactions that may be undetectable when using a single approach. For example, genes that contribute to OA progression, such as *AQP1*, *COL1A1*, and *CLEC3B*, were identified in a study that took an integrated multi-omics approach that included analysis of DNA CpG methylation, RNA expression, and protein expression data in human knee OA tissue [81]. In another ChIP-seq and RNA-seq-based study of primary chondrocytes isolated from human talar cartilage, *SOCS2* expression was identified as an OA risk factor because of its role in the resolution of inflammation following cartilage matrix damage [19]. Moreover, the integration of metabolomics with transcriptomics in another study identified specific and common metabolites and genes involved in the progression of OA and RA [82].

However, multi-omics integration presents several challenges. The heterogeneity of datasets, arising from differences in sample preparation, experimental platforms, and data processing pipelines, complicates direct comparisons across omics layers. Data dimensionality and scale differences—such as the vast number of detected transcripts compared to the relatively limited number of metabolites—make integration difficult.

Table 1 Omics approach

Omics Approach	Technology	Methodology	Advantages	Limitations
Genomics	Genome-wide association studies (GWAS)	Identifies genetic variants associated with traits/diseases by comparing SNP frequencies in large populations	Identifies genetic risk factors; applicable to large cohorts	Cannot directly identify causal genes; most risk variants are in non-coding regions
Transcriptomics	Bulk RNA-seq	Measures gene expression levels across all cell types in a sample	High sensitivity for detecting differential gene expression	Lacks cellular resolution; signal is averaged across cell populations
	Single-cell RNA-seq (scRNA-seq)	Measures gene expression at the single-cell level	Reveals cellular heterogeneity; identifies rare cell types	High dropout rates; limited detection of low-abundance transcripts
	Spatial transcriptomics	Maps gene expression onto tissue sections while preserving spatial context	Links gene expression to tissue architecture	Lower sequencing depth than scRNA-seq; requires specialized imaging platforms
Epigenomics	Chromatin Immunoprecipitation Sequencing (ChIP-seq); Cut&Run; Cut&Tag	Captures DNA–protein interactions by immunoprecipitating specific histone modifications or transcription factors, followed by sequencing	Identifies histone modification and transcription factor binding sites	Requires high input DNA; limited resolution; requires optimized protocols; cannot distinguish allele-specific binding
	Chromatin accessibility (ATAC-seq)	Maps open chromatin regions to identify active regulatory elements	Identifies active enhancers and promoters; links non-coding variants to regulatory activity	Prefers fresh tissue; cannot directly measure transcription; requires prediction of regulatory elements
	DNA Methylation (Bisulfite Sequencing, WGBS, RRBS)	Measures DNA methylation at cytosine residues within CpG dinucleotides, linking DNA methylation with gene silencing or activation	Provides insights into gene silencing and activation via methylation patterns	Data is correlative; does not establish causality; limited to CpG methylation
	Single-cell chromatin accessibility (scATAC-seq)	Measures open chromatin at single-cell resolution	Identifies cell-type-specific regulatory elements	Sparse data; higher sequencing cost
	Spatial epigenomics (Spatial-CUT&RUN)	Profiles histone modifications or transcription factors in intact tissue sections while preserving spatial context	Resolves epigenetic landscapes in specific tissue structures	Lower throughput than bulk ChIP-seq; requires optimized protocols
Proteomics	Mass spectrometry-based proteomics	Identifies and quantifies proteins based on their mass-to-charge ratio	Directly measures functional molecules (proteins)	Limited by dynamic range; post-translational modifications require specialized analysis
Metabolomics	Liquid chromatography-mass spectrometry (LC-MS)	Detects and quantifies metabolites in biological samples	Identifies metabolic pathways altered in disease	High variability; metabolite identification is challenging

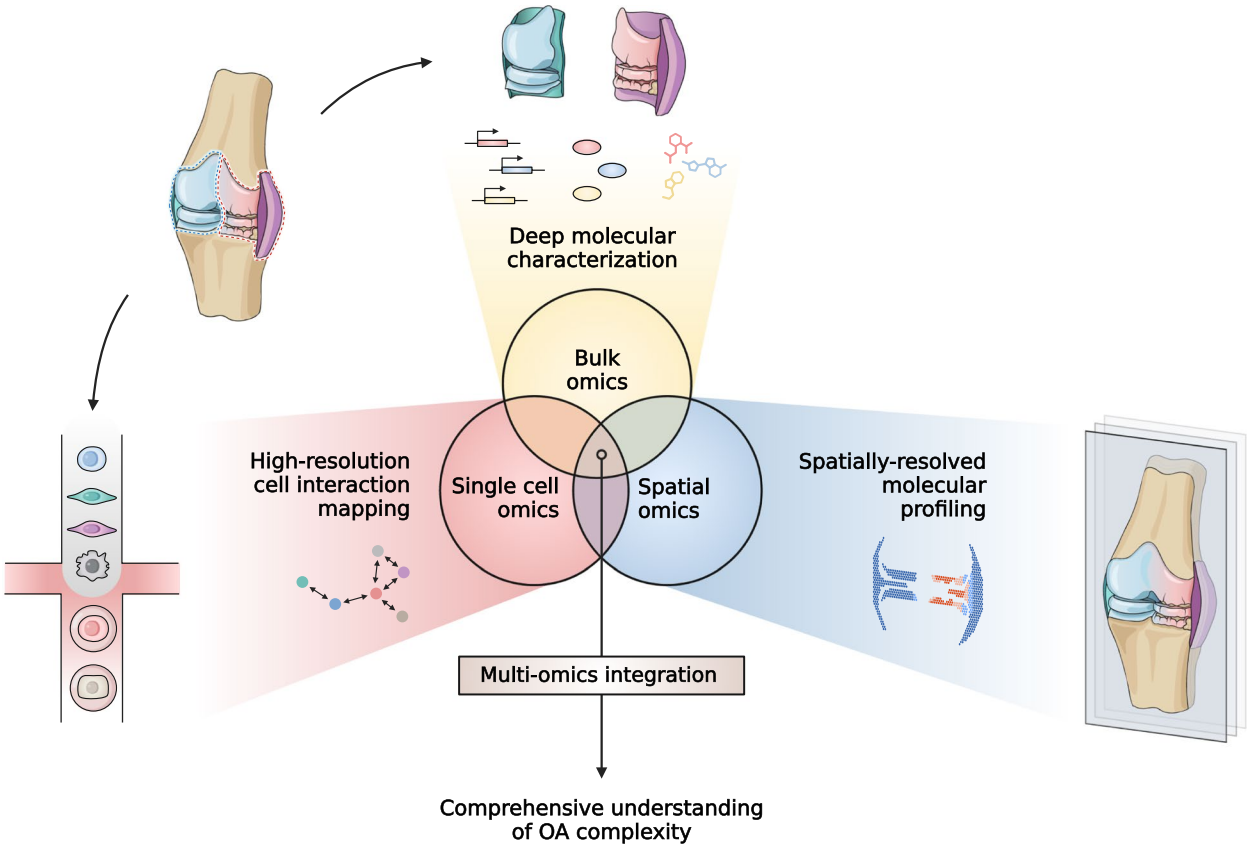

Fig. 1 Multi-omics integration strategies in OA research. Integration of bulk, single-cell, and spatial omics technologies enables a comprehensive understanding of complex molecular and cellular mechanisms underlying OA pathogenesis through maximizing depth of molecular profiling (yellow sector), resolution of cell-cell interactions (red sector), and spatial information (blue sector)

Additionally, batch effects and technical biases from different omics platforms can introduce noise and require careful normalization strategies. Another challenge is the lack of standardized bioinformatics tools capable of harmonizing diverse omics data while preserving biological relevance. Current computational methods, such as principal component analysis (PCA) and partial least squares regression (PLS), help reduce data complexity. However, integrating heterogeneous datasets still requires advanced algorithms and well-annotated reference databases. Artificial intelligence (AI) models offer increased precision in predicting disease outcomes and treatment responses and have transformed radiology for early-stage OA diagnosis over the last decade [83]. AI is also poised to revolutionize multi-omics by enhancing data processing and analytical capabilities. Machine learning and deep learning algorithms can efficiently handle vast amounts of data from diverse omics studies, uncovering patterns and relationships that might remain hidden using traditional analysis. For example, linking enhancers and other non-coding DNA regions to target genes, identifying functional genetic variations, predicting complex protein structures using approaches like AlphaFold, identifying potential drug targets, and distinguishing cell morphology through spatial omics and imaging mass cytometry may all be enhanced by AI-assisted technology [84].

Elucidating OA etiology by integrating GWAS and omics data

Another important use of multi-omics is to facilitate the validation and refinement of genome-wide association studies (GWAS)-produced candidate etiologies for diseases (Fig. 2). GWAS has uncovered thousands of susceptibility loci associated with complex traits and diseases in large cohorts [85]. To date, 633 lead single nucleotide polymorphisms (SNPs; P-value $\leq 5 \times 10^{-8}$) and 367 genes have been associated with increased OA risk, according to the GWAS Catalog (https://www.ebi.ac.uk/gwas/). Meta-analyses of existing data have enhanced the statistical power of GWAS studies. They validate genetic associations of diverse populations and identify genetic effects that may be missed in individual studies, providing more reliable insights into the genetic basis of OA. For

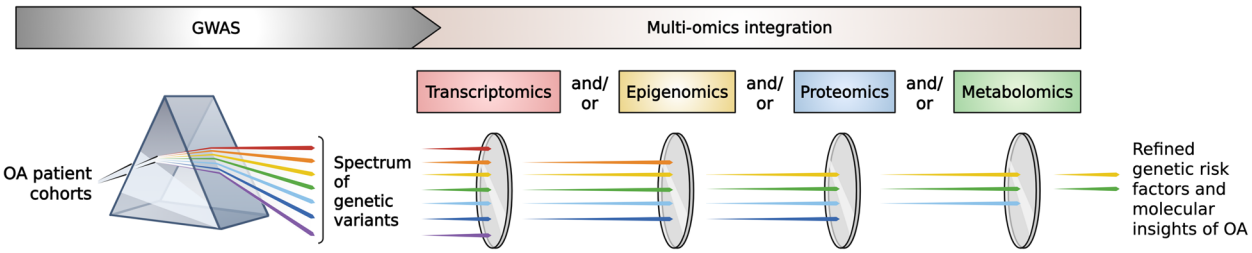

Fig. 2 The integration of GWAS studies with multi-omics enables the identification and refinement of OA etiologies and related molecular mechanisms. GWAS analyses revealed a spectrum of genetic factors associated with OA. Subsequent integration with selective omics approaches, such as transcriptomics, epigenomics, proteomics, and metabolomics, based on their relevance to the properties of candidate genes, allows the reduction/refinement of the candidate gene list and provides deeper molecular insight into how these etiologies influence OA progression

example, a GWAS meta-analysis identified 52 sequence variants associated with knee or hip OA, including ten variants that had not previously been reported [86]. In addition, by meta-analyses using GWAS data from 800,000 individuals among 13 international OA cohorts, the arcOGEN consortium identified 100 risk variants from 11 OA phenotypes [87]. However, identifying causal genes using GWAS remains challenging. It requires other biological validations, with some caveats: **1)** because of its polygenic nature, OA arises from the interplay of both genetic and environmental factors, making it challenging to identify the specific genes that cause OA and the exact mechanisms by which they contribute to disease development; **2)** Considerable heterogeneity in OA study populations arises from differences in disease severity, age, sex, ethnicity, and other factors, which can affect the ability to detect genetic associations; **3)** While GWAS identified risk variants linked to OA, making a mechanistic connection with disease is difficult because most genetic changes occur in non-coding regions; and **4)** Genetic findings alone do not provide a clear understanding of the distinct functions of different cell types within affected joints. Capturing the molecular landscape of OA in primary joint tissue is necessary. Integrating these genetic findings with multiple levels of omics analysis, such as transcriptomics, epigenomics, proteomics, and metabolomics of the primary tissue, represents a promising approach to advancing our understanding of the etiology of OA [4, 88–91].

Transcriptomics data have been widely integrated with GWAS to help prioritize genes and putative causal variants involved in tissues and cell types [92]. A well-established example is the integration of genomic and gene expression of matching samples to identify genetic variants that influence expression levels of genes, termed expression quantitative trait loci (eQTLs) on a genome-wide scale [93, 94]. This approach has identified genetic variants associated with the expression of proximal genes in chondrocytes and synoviocytes from OA joints [95]. Metabolomics integrated with disease GWAS has been reported to identify the genetic association with metabolite level, known as metabolite quantitative trait loci (metabQTL) [96]. A study integrated transcriptomics results of 49 human tissues and metabolomics results of 1,391 plasma metabolites together with GWAS associations for 2,861 diseases/traits from the GTEx project [97, 98]. Despite the extensive scope of the investigation, the specific molecular landscape of OA-relevant tissues still needs to be better characterized. Methylation quantitative trait loci (mQTL) studies have identified genetic variants linked to particular CpG sites and their corresponding DNA methylation levels [91, 99]. A study combined GWAS data with ChIP-seq and RNA-seq datasets from human primary chondrocytes isolated via enzymatic digestion from human talar cartilage. A study utilized chromatin accessibility profiling (ATAC-seq), DNA methylation profiling, and RNA-seq of articular cartilage from OA patients to identify the dysregulation of regulatory elements in non-coding sequences, such as enhancers [90]. The enrichment of OA SNPs at enhancers suggests that the genetic variations may perturb gene regulation and contribute to OA susceptibility. Another study used chondrocyte ATAC-seq profiling to examine how nucleotide changes within evolutionarily constrained developmental regulatory sequences underlie genetic OA risk [100]. The authors proposed that the genetic variants in regulatory sequences associated with knee development and maintenance might be selected against due to the negative impact on fitness caused by an inefficient bipedal gait. Therefore, these findings suggest that epigenomics data, such as chromatin accessibility mapping, can identify loci in non-coding regions and link an evolutionarily novel aspect of human anatomy to OA pathogenesis. Moreover, a recent study published in 2024 by Bitter et al. generated the first whole genome chromosome conformation analysis (Hi-C) map of primary chondrocytes from OA patients [101]. They integrated with the ATAC-seq mentioned above data and confirmed

the genetic variants associated with OA residing within enhancer-promoter chromatin loop anchors.

Beyond genetic factors, phenomics–the comprehensive study of an organism's observable traits–captures the complex interplay of molecular, environmental, and lifestyle factors contributing to OA heterogeneity (Fig. 2). A recent 2024 phenome-wide causal association study (PheWASs) by Mei et al. identified 133 unique phenotypic traits with potential causal links to OA, including well-established risk factors such as obesity, BMI, and meniscus derangement, as well as novel associations with socioeconomic, cardiovascular, and psychiatric conditions [102]. Unlike GWAS, which examines many genetic variants for their association with a single phenotype, PheWAS focuses on the impact of a single genetic variant across a wide range of phenotypes. Although PheWAS has limitations, particularly in distinguishing true pleiotropy from confounding effects caused by linkage disequilibrium (LD) [103], it could nevertheless expand our ability to identify new genetic variances that might not have been considered in the original GWAS.

These studies highlight the critical role of OA-associated genetic variants in chondrocytes, impacting gene transcription, metabolites, DNA methylation, and regulatory element activity, and the disease-causing genetic variants can be effectively refined by the combination of multi-omics.

Conclusion

The development of OA is influenced by genetics, aging, and environmental factors, necessitating a comprehensive approach to unravel its complexities. In this endeavor, the advent of omics technologies has ushered in noteworthy progress, facilitating the unbiased identification of novel biomarkers and disease mechanisms and the development of promising treatment strategies. To fully realize the potential of omics in OA research, it is essential to integrate various omics methodologies in the context of multi-omics systems biomedicine. This approach combines data from genomics, transcriptomics, proteomics, and metabolomics, which can lead to a better understanding of the molecular mechanisms underlying OA. Moreover, due to the cellular heterogeneity of OA, conventional multi-omics using bulk tissues could not effectively elucidate the intricate signaling crosstalk in the OA microenvironment; the newly developed/developing omics technologies that provide single-cell resolution and spatial information would enable us to achieve a comprehensive understanding of OA. In addition, integrating research findings derived from clinical samples of OA patients and relevant animal models is pivotal [104, 105]. This amalgamation enriches the applicability of scientific insights, bridging the gap between fundamental research and clinical practice. As omics technologies continue to evolve, the incorporation of AI holds great promise for advancing OA research. AI can optimize data integration, predict disease progression, and assist in identifying novel therapeutic targets by analyzing vast and complex datasets. A firm commitment to interdisciplinary research and the consistent use of advanced omics methodologies will undoubtedly aid in improving the understanding, diagnosis, and treatment of OA.

Abbreviations

OA	Osteoarthritis
ECM	Extracellular matrix
microRNA-17	*miR-17*
BS-Seq	Bisulfite sequencing
WGBS	Whole genome bisulfite sequencing
RRBS	Reduced representation bisulfite sequencing
DMRs	Differentially methylated regions
RA	Rheumatoid arthritis
ChIP-qPCR	Chromatin immunoprecipitation followed by qPCR
ChIP-seq	Chromatin immunoprecipitation followed by sequencing
ATAC-seq	Assay for transposase-accessible chromatin with sequencing
ERV	Endogenous retroviruses
PTM	Posttranslational modification
MS	Mass spectrometry
SELDI	Surface Enhanced Laser Desorption Ionization
MALDI	Matrix-Assisted Laser Desorption Ionization
GC-MS	Gas chromatography MS
LC-MS	Liquid chromatography MS
SWATH-MS	Sequential Window Acquisition of All Theoretical MS
DM	Diabetes mellitus
cryoEM	Cryo-electron microscopy
EVs	Extracellular vesicles
scRNA-seq	Single-cell RNA sequencing
PTOA	Post-traumatic osteoarthritis
CyTOF	Cytometry by Time-Of-Flight
FFPE	Formalin-fixed paraffin-embedded
MALDI-MSI	Matrix-assisted laser desorption/ionization mass spectrometry imaging
PCA	Principal component analysis
PLS	Partial least squares regression
AI	Artificial intelligence
GWAS	Genome-wide association studies
eQTLs	Expression quantitative trait loci
metabQTL	Metabolite quantitative trait loci
mQTL	Methylation quantitative trait loci
PheWAS	Phenome-wide association study
LD	Linkage disequilibrium

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Authors' contributions

Y.L. and V.M. contributed equally. Writing–original draft: Y.L.; Figures: V.M.; Writing–review and editing: T.Y., V.M., and D.B.; Funding acquisition: T.Y.; Project administration: T.Y. and Y.L.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not Applicable.

Competing interests
The authors declare no competing interests.

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