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Review article

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Insights into chondrocyte populations in cartilaginous tissues at the single-cell level

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Abstract

Chondrocyte biology is being revolutionized by single-cell multi-omics technologies, revealing cellular heterogeneity within cartilaginous tissues. Although past research has implicated cellular heterogeneity in chondrocyte populations, advances over the past decade in single-cell transcriptomics now enable a more granular, functionally annotated classification of chondrocyte subtypes. These analyses provide crucial insights into the role of these subtypes in cartilage formation, maintenance and disease progression. Chondrocyte populations are implicated in tissue homeostasis, pathogenesis and responses to external stimuli, including pro-inflammatory mediators and novel therapeutic agents. This knowledge opens pathways for developing targeted treatments for diseases such as osteoarthritis and intervertebral disc disease. Insights into the molecular signatures of disease-critical chondrocyte populations provide a foundation for biomarker discovery and therapeutic targeting, and there are exciting opportunities for leveraging these findings to progress regenerative therapies. Spatial and temporal profiling of cellular markers, behaviour and metabolic activity will enhance understanding of disease pathogenesis and chondrosenescence and could possibly enable early intervention for osteoarthritis, thereby preventing irreversible joint damage. Future research must integrate advanced single-cell techniques with computational modelling to unravel the dynamic interplay of chondrocyte populations. These efforts could transform precision medicine in rheumatology, addressing the unmet clinical needs in cartilage-related diseases.

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Sections

Key points

- Musculoskeletal disorders, particularly osteoarthritis and intervertebral disc disease, remain therapeutic challenges owing to a focus on symptom management rather than mechanistic targeting.
- Single-cell RNA sequencing analysis has identified distinct chondrocyte subpopulations in healthy and diseased tissues, overturning the paradigm of chondrocyte homogeneity.
- Cellular diversity mapping through single-cell transcriptomics enables molecular stratification of cartilage degeneration, which forms the basis for disease-modifying therapies.
- Integrating spatial transcriptomics and subcellular proteomics will reveal microenvironment-specific chondrocyte behaviours that are critical for the maintenance of tissue homeostasis, which could better explain how chondrocyte subtypes contribute to tissue homeostasis.
- Machine learning-driven analysis of multi-omics data accelerates the discovery of network-level therapeutic targets for personalized treatment strategies.

Introduction

The increasing prevalence of musculoskeletal disorders, particularly osteoarthritis (OA) and intervertebral disc (IVD) degeneration (IVDD), represents a considerable public health challenge globally[1]. These conditions not only lead to chronic pain and disability but also impose a substantial economic burden on healthcare systems[2]. Current treatment options focus on symptomatic relief rather than addressing the underlying pathophysiological mechanisms. As the population ages, the incidence of these musculoskeletal disorders is expected to rise, necessitating a deeper understanding of their underlying mechanisms to develop effective therapeutic strategies[3].

Chondrocytes, the most abundant and functionally important cell type in cartilage, are essential for skeletal development and musculoskeletal function[4–6]. Chondrocyte phenotypes in developmental cartilage disorders (such as chondrodysplasia) and cartilage following traumatic joint injuries have been reviewed elsewhere[7,8]; however, their specific roles in developmental cartilage disorders remain poorly understood. This Review focuses on how single-cell technologies are unravelling chondrocyte heterogeneity in prevalent degenerative disorders such as OA and IVDD, with implications for biomarker discovery and targeted therapies. Chondrocyte phenotype is determined and maintained by the local physio-chemical microenvironment provided by the cartilage-specific extracellular matrix (ECM)[9]. Previous conceptions of cartilage structure portrayed chondrocytes as nearly uniformly distributed within the ECM, with limited appreciation for their spatial organization or functional diversity. Over the past two decades, imaging-based studies have revealed heterogeneity in cell morphology and distribution, including fibroblast-like chondrocytes with cytoplasmic processes, particularly in non-degenerate cartilage[10–12]. However, advances over the past 5–10 years in multi-omics approaches, which integrate single-cell RNA sequencing (scRNA-seq) and proteomics, have transformed the understanding of the complexity of cellular heterogeneity in cartilage and IVD[13]. Studies using these approaches have uncovered previously unrecognized chondrocyte populations that are associated with both healthy and diseased tissue, providing insights into their specific roles in cartilage formation and maintenance[14]. Defining the cell populations present in different types of cartilage, which we summarize in this Review article, is indispensable for future cartilage tissue engineering strategies, and provides important insights related to pathogenesis. Such insights are critical for identifying potential biomarkers and therapeutic targets for OA, IVDD and other cartilage-related disorders[15]. Moreover, integrating spatial transcriptomics and subcellular proteomics could provide a more comprehensive view of chondrocyte behaviour within their native microenvironment. Mapping the spatial distribution of chondrocyte subtypes and their associated signalling pathways in developing (Box 1 and Fig. 1) and mature cartilage will improve understanding of how these cells contribute to cartilage homeostasis and the progression of degenerative diseases and could also facilitate development of targeted therapies for these conditions.

In this Review we provide a comprehensive update on chondrocyte populations in cartilaginous tissues at the single-cell level in health, disease and senescence, and highlight the applications for these technologies for deciphering the phenotypic cues that could be developed into sensitive, specific biomarkers and therapeutic targets for cartilage disorders in synovial joints or in the IVD.

Cell phenotype and phenotypic markers at the single-cell level

Understanding the diversity of cell phenotypes in joint and spinal tissues is crucial for interpreting their roles in development, homeostasis and disease. Notably, in addition to chondrocytes, synoviocytes and synovial fibroblasts have emerged as central regulators of synovitis in OA, interacting dynamically with chondrocytes to propagate inflammatory mediators and cartilage-degrading pathways under mechanical or metabolic stress[16]. This crosstalk exacerbates disease progression, positioning synoviocytes and synovial fibroblasts as important therapeutic targets alongside chondrocytes. Over the past decade, the integration of high-resolution techniques such as scRNA-seq, spatial transcriptomics and proteomics has enabled unprecedented insight into cell populations across cartilaginous tissues. This section explores how these tools have revealed tissue-specific heterogeneity and phenotypic markers in hyaline cartilage, the meniscus and the IVD, with emphasis on both healthy and pathological contexts.

Cellular complexity in hyaline cartilage

The morphology of chondrocytes within hyaline cartilage varies depending on their function and location within the tissue. In articular cartilage, the characteristic roundish cell morphology is predominantly observed in the chondrocytes of the middle layer, where cells are sparse. These cells are embedded in an ECM that is rich in proteoglycans and collagen type II, which aids in the absorption and distribution of mechanical compressions applied to the joint. By contrast, chondrocytes in the deep zone exhibit an enlarged pre-hypertrophic or hypertrophic appearance and are often arranged in columns oriented perpendicular to the surface. In the superficial layer, which faces the synovial fluid of the joint space and shields the deeper layers from shear stress, chondrocytes are abundant, have a flattened morphology and are tangentially oriented relative to the cartilage surface, as demonstrated by 3D synchrotron imaging of the intact tissue[11,17] (Fig. 2).

In contrast to other cell types, such as mesenchymal stem cells (MSCs), which are recognized by the presence or absence of a defined set of surface markers[18], there is no widely acknowledged set of surface

markers for identifying chondrocytes. Some of the proposed markers (such as CD44, CD73, CD90 and CD105) are non-specific and overlap with MSCs and fibroblasts, and donor variability and methodological challenges hinder consensus[19–21]. This lack of distinct markers might, in part, reflect the unique niche in which chondrocytes reside (embedded within a dense ECM and largely isolated from direct cell–cell contact), which might result in limited biological pressure to maintain a robust repertoire of cell-surface proteins for intercellular communication. Instead, depending on their location, chondrocytes from various zones exhibit differences in the expression of specific markers associated with their unique ECM. Mature chondrocytes in the middle layer express characteristic cartilage components such as collagen type II, IX and XI, aggrecan and link protein[22]. By contrast, pre-hypertrophic and hypertrophic chondrocytes in the deep zone and calcified zone are marked by the presence of collagen type X[23]. Cells in the superficial zone are exclusive producers of lubricin (also known as proteoglycan-4 (PRG4)), a surface protein crucial for joint lubrication. A small proportion of superficial zone chondrocytes in non-degenerate cartilage express collagen type I and largely lack expression of the collagen types typical of deeper layers of articular cartilage, such as collagen type II[24], which challenges earlier assumptions about the absence of collagen type I in healthy hyaline cartilage[10]. In addition to zonal variation, transcriptomic evidence from OA cartilage reveals the presence of *METRNL*$^+$ and *PRG4*$^+$ chondrocyte subtypes, which seem to reflect early and intermediate states of dedifferentiation and are regulated by Hippo signalling via Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ), a key pathway in cartilage remodelling[25].

Although a small number of studies previously described cells in cartilage with progenitor-like features[26,27], the dominant paradigm for many years viewed cartilage as being composed of a nearly uniform cell population[28]. Therefore, the recognition that chondrocytes are in fact not a uniform population predates single-cell sequencing. Previous morphological and immunohistochemical studies using confocal microscopy, cytoskeletal staining and protein-level markers have highlighted heterogeneity in chondrocyte morphology and phenotype in situ[10–12,29,30]. These investigations revealed differences in cell volume, cytoplasmic projections and differential expression of collagen types I and VI, IL-1β and ECM-degrading enzymes, such as ADAMTS4, within macroscopically healthy cartilage.

Although these foundational studies underscored the functional diversity of chondrocytes, the advent of single-cell and spatial multi-omics has dramatically enhanced the resolution of this heterogeneity, revealing transcriptionally distinct subtypes and their roles in homeostasis, inflammation and degeneration. Only advancements in scRNA-seq technologies over the past decade have revealed the full extent of heterogeneity within cartilaginous tissues. Further supporting this heterogeneity, seven transcriptionally distinct chondrocyte subpopulations were identified in OA cartilage, including stress-metabolizing and ECM-synthesis-related subtypes that dominate at early and late stages of damage, respectively[31]; these shifts highlight functional transitions during OA progression. Cellular subpopulations with distinct phenotypes have been identified in the different layers of articular cartilage[32,33] but also from weight and non-weight-bearing areas of articular cartilage tissues[34]. In rheumatoid arthritis (RA), studies using single-cell transcriptomics have also revealed immune-associated chondrocyte populations with distinct spatial distributions depending on mechanical load, which emphasizes the relevance of tissue location even under inflammatory conditions[35].

Box 1 | Signalling pathways involved in chondrogenesis

Cartilage development starts with cartilage progenitor cells differentiating from mesenchymal stem cells. Limb cartilage originates from the sclerotome, whereas head cartilage derives from the cranial neural crest. Articular cartilage progenitors arise from the interzone at future joint sites. Limb development begins with the condensation of cartilage progenitor cells into chondrogenic nodules (a process mediated by cell junctions), which enhances local gradients of chondrogenic growth factors (Fig. 1). This condensation commits mesenchymal cells to the chondrogenic lineage, a process that requires the activation of numerous signalling pathways.

Meniscus fibrocartilage development starts with interzone cells derived from embryonic mesenchyme. The gene signature associated with meniscus development is unique and differs from that of cartilage and ligament development, with the *IGF1*, *GDF5*, *LGR5*, *SCX* and *GLI1* pathways having prominent roles.

Intervertebral disc formation shares regulatory factors with chondrogenesis, but shows key differences in cell types and tissue composition. The annulus fibrosus and cartilaginous endplates are mesenchymal in origin, whereas the nucleus pulposus develops from the notochord, initially containing notochordal cells replaced by chondrocyte-like cells (Fig. 1). SOX9 is essential for nucleus pulposus and annulus fibrosus development, with annulus fibrosus and cartilaginous endplate cells derived from SCX and SOX9 double-positive progenitors.

A separate scRNA-seq study that focused on healthy and OA human articular cartilage also identified seven distinct chondrocyte subpopulations, providing a high-resolution transcriptional map of cell types within macroscopically healthy tissue[36]. In addition, single-cell transcriptomic advances enable a more granular and functionally annotated classification of chondrocyte subtypes than previous in situ immunolabelling-based studies that suggested phenotypic heterogeneity among chondrocytes[12]. These seven clusters were classified as fibrocartilage chondrocytes-1 and fibrocartilage chondrocytes-2 (expressing *SH3BGRL3*, *S100A6*, *MYL9* and *IGFBP5*, *LMCD1*, respectively), cartilage progenitor cells-1 and cartilage progenitor cells-2 (which express *KIAA0101*, *BIRC5* and *CDC20*, *UBE2C*, *CENPF*, *KIAA0101*, *BIRC5*, respectively), regulatory chondrocytes (expressing *EIF5A*, *PGK1*, *ANXA1*, *TUBA1A*), pre-hypertrophic chondrocytes (expressing *SOX9*, *COL9A3*, *COL11A1*) and homeostatic chondrocytes (expressing *TXNIP*, *IFITM3*, *GDF15* and *TIMP1*). The most apparent differences between healthy and OA cartilage were an enrichment of regulatory and pre-hypertrophic chondrocytes in OA and an abundance of cartilage progenitor cells in healthy cartilage. A subpopulation of hypertrophic chondrocytes (expressing *SLC39A14* and *COL10A1*) and distinct from hypertrophic chondrocytes in healthy cartilage was further identified in the superficial region of damaged cartilage in human OA tissue[36] (Supplementary Table 1).

Single-cell and spatial transcriptomic analysis of healthy and OA human knee articular cartilage identified 33 cell population-specific marker genes that define 11 chondrocyte populations, including 9 known populations and 2 newly defined populations: pre-inflammatory and inflammatory chondrocytes[37]. This study established that the

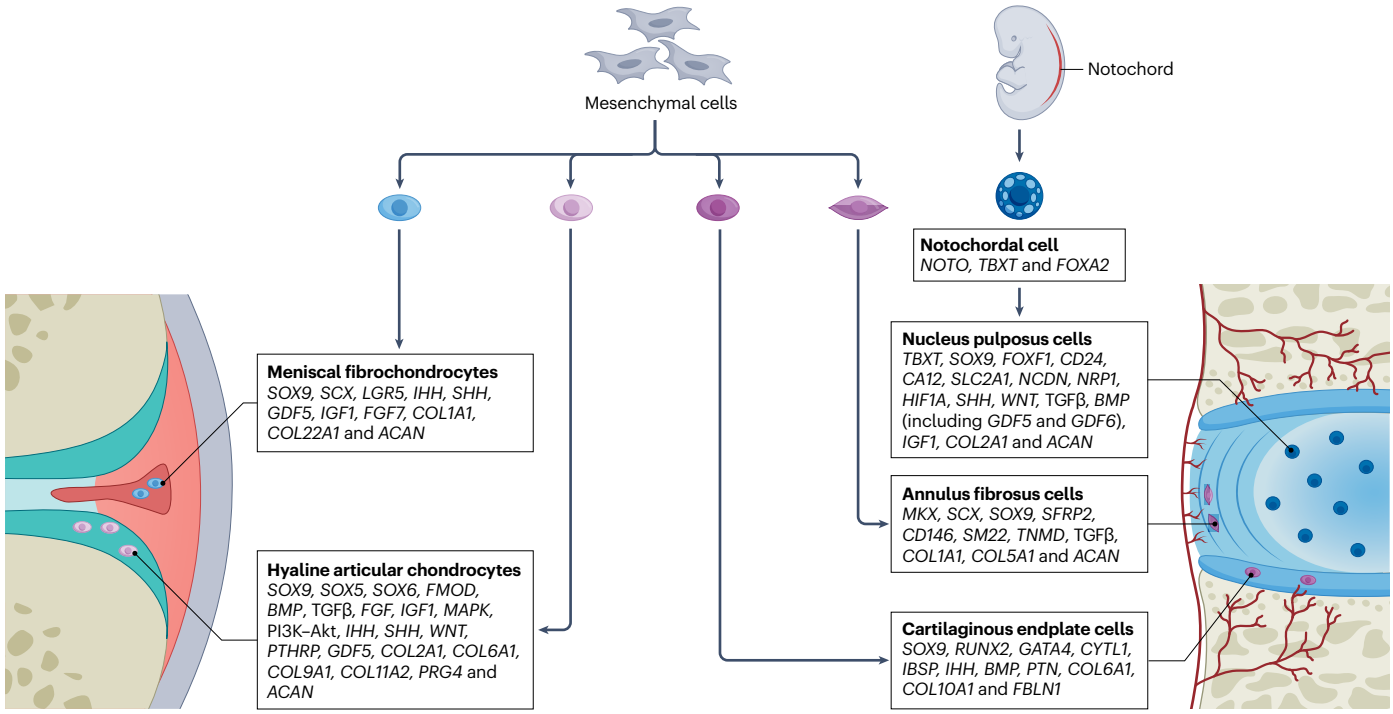

Fig. 1 | Main signalling pathways and markers that regulate the development of cartilaginous tissues. Mesenchymal cells differentiate into progenitor cells giving rise to various chondrogenic lineages, characterized by partially overlapping signalling pathways, and the expression of transcription factors and extracellular matrix (ECM) components. Nucleus pulposus cells are derived from the notochord. Hyaline articular chondrocytes: *SOX9*, *SOX5* and *SOX6* (chondrogenesis); *FMOD* (collagen organization); *BMP*, TGFβ, *FGF*, *IGF1*, *MAPK*, PI3K–Akt and *WNT* (proliferation and differentiation); *IHH*, *SHH* and *PTHRP* (also known as *PTHLH*) (homeostasis and differentiation); *GDF5* (joint development); *COL2A1*, *COL6A1*, *COL9A1*, *COL11A2*, *PRG4* and *ACAN* (ECM components). Meniscal fibrochondrocytes: *SOX9* and *SCX* (differentiation); *LGR5* (progenitor marker); *IHH* and *SHH* (homeostasis and differentiation); *GDF5* (joint development); *IGF1* (proliferation and differentiation); *FGF7* (differentiation); *COL1A1*, *COL22A1* and *ACAN* (ECM components). Annulus fibrosus cells: *MKX*, *SCX* and *SOX9* (differentiation); *SFRP2* (ECM remodelling); *CD146* (also known as *MCAM*; progenitor marker); *SM22* (also known as *TAGLN*; contractile phenotype); *TNMD* (tenomodulin, tendon-like identity); TGFβ (developmental signalling); *COL1A1* and *COL5A1* (tensile strength); *ACAN* (ECM components). Cartilaginous endplate cells: *SOX9* and *RUNX2* (differentiation); *GATA4* and *CYTL1* (boundary formation); *IBSP* (mineralization inhibition); *IHH*, *BMP* and *PTN* (ossification signals, hypertrophy and ECM remodelling); *COL6A1* and *COL10A1* (ECM components); *FBLN1* (ECM organization and mechanical stability). Nucleus pulposus cells: *TBXT*, *SOX9* and *FOXF1* (differentiation); *CD24*, *CA12* and *SLC2A1* (involved in hypoxic and microenvironmental adaptation); *NCDN* (lineage maintenance and ECM stability); *NRP1* (developmental patterning); *HIF1A* (hypoxia response); *SHH*, *WNT*, TGFβ and *BMP* (including *GDF5* and *GDF6*), *IGF1* (developmental signalling); *COL2A1* and *ACAN* (ECM components).

pre-hypertrophic chondrocyte and hypertrophic chondrocyte populations are potentially essential for disease progression in OA, and that the pre-fibrocartilage chondrocyte population, a distinct entity from the previously described fibrocartilage chondrocytes, is a major contributor to the stratification of patients with OA[37]. Another study investigated OA human knee articular chondrocyte populations under different mechanical loading conditions via scRNA-seq[38]. In line with previous studies, 12 chondrocyte subtypes were identified, and their functions, development and interactions with other cells were described. The study also identified a new chondrocyte subset, termed hypertrophic chondrocytes-C. These findings underscore the importance of delineating major cell populations within healthy cartilage and comparing them with pathological cells; such comparisons are key for comprehending the distinct roles of various chondrocyte populations and their respective pathogenic mechanisms, which contribute to the development of diseases such as OA.

In a 2023 study, a subset of chondrocytes with high expression of *SPP1* (also known as osteopontin) was identified in human OA cartilage using scRNA-seq[39]. These *SPP1*$^{+}$ chondrocytes exhibited the highest SenMayo score, a transcriptomic index used to quantify cellular senescence, among all chondrocyte subgroups and demonstrated strong angiogenic potential. Furthermore, the SPP1 signalling network was more abundant in OA cartilage than in healthy cartilage, and the receptor–ligand binding pattern of SPP1–CD44 appeared to have an important role in this network.

A 2024 single-cell study further refined the understanding of chondrocyte populations that are critical for the progression of OA. In a post-traumatic model of OA, pre-inflammatory and inflammatory chondrocyte subtypes emerge early and contribute to disease progression through cytokine-mediated crosstalk[37]. In parallel, angiogenic (*Smoc2*$^{+}$*Angptl7*$^{+}$) and osteogenic (*Col1a1*$^{+}$) chondrocytes have been identified as drivers of pathological vascularization and subchondral bone remodelling in later stages of disease[40]. These findings align with trajectory analyses that reveal time-dependent shifts in chondrocyte states following joint injury, with inflammatory and ECM-degrading signatures progressively dominating the transcriptomic landscape[41].

Similar to single-cell transcriptomics data, cytometry by time of flight (CyTOF) single-cell proteomics using a panel of 33 markers (which included cell-surface receptors, adhesion molecules, signalling mediators and transcription factors) revealed three cartilage progenitor cell (CPC) variants (CPC I–III) in healthy and OA human cartilage[42], which also included the previously identified migratory CPCs. CPC I was characterized by low CD105 and high CD54 (also known as ICAM-1) expression, and very active ERK1–2 signalling; CPC II had high levels of CD73 expression and the CPC III population was enriched for pro-inflammatory pathways, including nuclear factor-κB (NF-κB), signal transducer and activator of transcription 3 (STAT3), β-catenin and hypoxia-inducible factor 2α (HIF2α). Furthermore, a rare chondrocyte population, termed inflammation-amplifying (Inf-A) chondrocytes, was identified in patients with OA using CyTOF-based single-cell proteomics. Despite their atypical signalling profile, these cells were confirmed to express classical chondrogenic markers such as CD44 and SOX9, affirming their chondrocyte identity. They exhibited high levels of IL1R1 (also known as CD121a) and TNFRII (also known as CD120b), as well as exclusive activation of JNK and SMAD1–5 signalling pathways, and accounted for ~2% of the chondrocyte population based on single-cell proteomic and transcriptomic analyses[33]. Owing to the established role of CD24 in mitigating inflammation, CD24-enriched chondrocytes were termed inflammation-dampening chondrocytes and displayed enrichment of inflammation and immune cell trafficking-related pathways. Thus, a combination strategy of enhancing these rare inflammation-dampening chondrocytes and inhibiting the inflammation-amplifying chondrocyte populations could be effective in mitigating inflammation in OA cartilage[42]. In a follow-up study, four senescent CPC populations were identified in human OA cartilage based on $p16^{INK4a}$ expression[43]. These senescent subsets, which included and expanded upon the previously defined CPC I–III populations, exhibited distinct inflammatory and catabolic signalling profiles.

Cellular complexity in the meniscus

The meniscus comprises three zones, the avascular (white) inner zone, the outer vascular (red) zone, and a transitional red–white zone. The avascular inner zone is subject to compressive loading, whereas the outer vascular zone is under tensile and torsional loading[44]. Cells within the meniscus have historically been described as fibrochondrocytes, a mixed phenotype reflecting both fibroblastic and chondrogenic features, although microarray and scRNA-seq analyses have since uncovered specific cell types and gene signatures, both within healthy and OA meniscus and across its distinct inner and outer zones.

In a study in which microarray analysis was used to investigate the differences in transcriptomes between OA and non-OA human meniscal tissues, bone-related genes such as *SPARCL1*, *COL10A1* and *WIF1* were upregulated, whereas *VEGFA* and *POSTN* were downregulated within OA meniscal tissues. Cluster analysis of the array data showed that pro-inflammatory genes were highly expressed in the

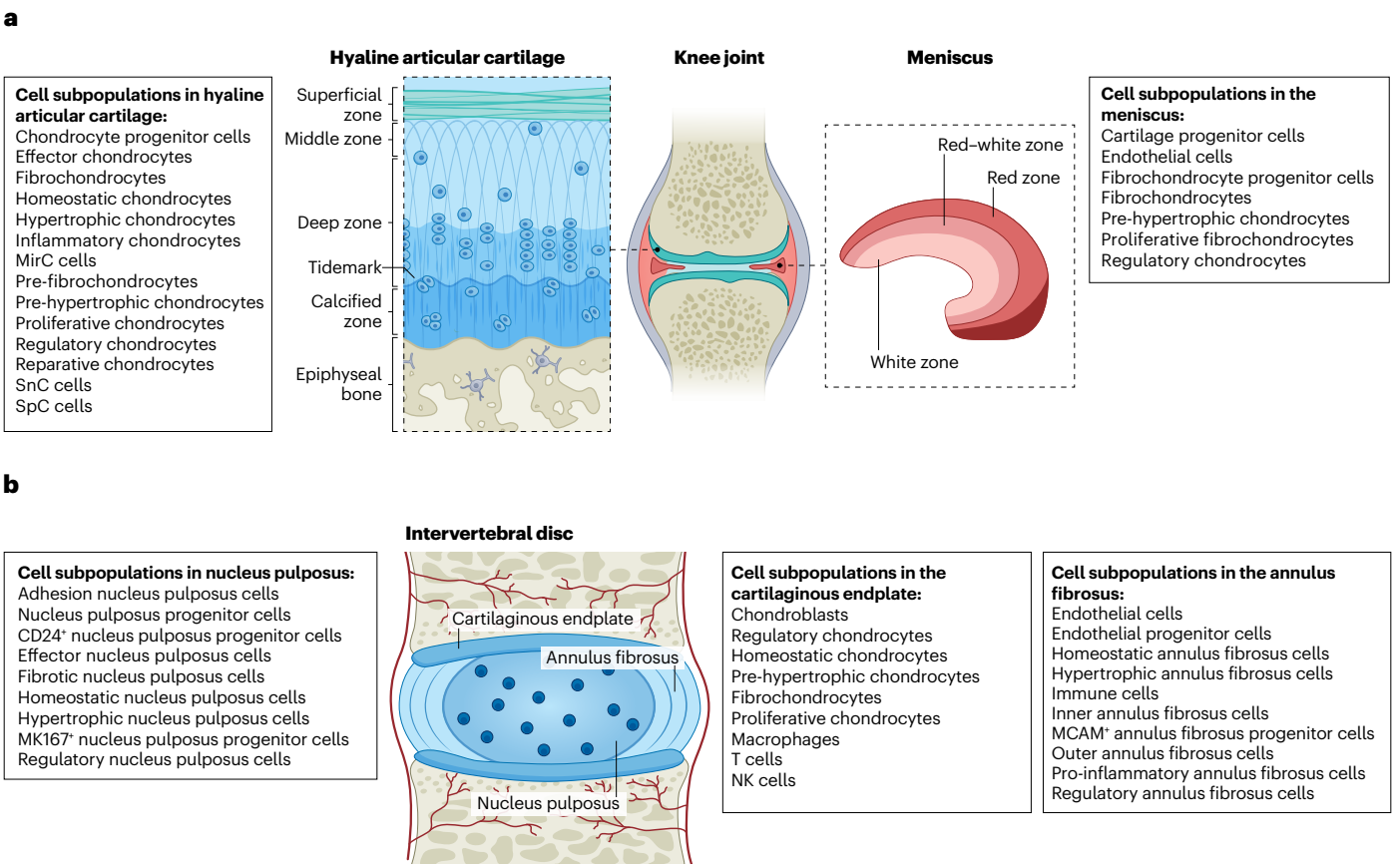

Fig. 2 | Cell populations in cartilaginous tissues. Single-cell RNA sequencing has identified numerous cell populations (only the main populations are shown in this figure) in hyaline articular cartilage and the meniscus (**a**) and the intervertebral disc (**b**). MirC, metal ion-related chondrocyte; NK cell, natural killer cell; SnC, senescent cluster; SpC, splicing chondrocyte.

OA meniscus, whereas genes associated with tissue regeneration were more prominently expressed in the non-OA meniscus[45]. Healthy (non-OA) meniscus samples were taken from patients with partial meniscus tears that showed no macroscopic evidence for OA or other joint diseases; however, even a partial tear can influence the expression of specific genes, as there could be upregulation or downregulation in post-traumatic inflammation-associated genes upon injury. In one study, scRNA-seq analysis of healthy human meniscus from patients undergoing amputation (mean Kellgren–Lawrence grade 0) compared with OA meniscus (mean Kellgren–Lawrence grade 3) was used to identify specific markers for OA meniscus. In contrast to the microarray analysis discussed earlier in this article, *LCN2*, *RAB27B*, *PRDM1* and *SERPINB2* were upregulated in human OA meniscal tissues compared with healthy tissues, with *LCN2* and *RAB27B* emerging from gene ontology as potential early-stage OA meniscus-specific markers[46]. The expression of both *Lcn2* and *Rab27b* was increased for up to 6 months in spontaneously aged mice with OA[46]. However, the authors did not evaluate human meniscus at different disease stages of OA to observe whether these genes can be used as specific meniscus markers for early OA. Owing to meniscus tears being a potential start point for OA, finding an early-stage marker within the meniscus is vital to prevent the onset of disease[47].

scRNA-seq analysis has also provided a greater understanding of the cell types within the meniscus. Specifically, studies have identified seven cellular populations within the human meniscus: endothelial cells, cartilage progenitor cells, regulatory chondrocytes, fibrochondrocytes, pre-hypertrophic chondrocytes, fibrochondrocyte progenitors (also described as CD146$^+$ pericyte-like cells) and proliferative fibrochondrocytes[48,49] (Supplementary Table 2). The avascular zone of the tissue also contains lymphocytes and myeloid cells, whereas the vascular zone has a greater proportion of endothelial cells and also Schwann cells that correlate with the presence of nerves within this region[48]. In both of the aforementioned studies, the presence of fibrochondrocyte progenitors within the tissue indicates the presence of regenerative populations within both the healthy and degenerative meniscus. A *CD146*$^+$ (a typical pericyte marker, also known as *MCAM*) population that was isolated from healthy human meniscus had a multilineage differentiation capacity and expressed stem cell markers; however, within the degenerative meniscus, a loss of *CD146*$^+$ cells led to an increase in a *CD318*$^+$ (also known as *CDCP1*) cell population that displayed progenitor-like characteristics. The latter population could have a crucial role in meniscal degeneration and has been proven to be a marker for meniscus progenitor populations isolated from degenerative meniscus[50]. *CD318* expression in injured meniscus tissue was reduced upon treatment with TGFβ; thus, *CD138* could be a potential marker for meniscal degeneration[49]. The study supports the presence of progenitor populations within the meniscus described in previous human and bovine in vitro studies[50–53].

At the tissue level, in vivo post-traumatic destabilized medial meniscus (DMM) mouse models of OA have demonstrated pathological mineralization in the lateral joint compartment, a process known as lateral chondrocalcinosis, which can drive medial articular cartilage damage via LEF1 signalling[54]. These findings highlight the relevance of Wnt signalling, as LEF1 acts as a key downstream effector in the canonical Wnt beta-catenin pathway, in regulating meniscal stiffness and pathological mineralization. The data also suggest that alterations originating in the lateral compartment, such as chondrocalcinosis, might contribute to degenerative changes in adjacent joint structures, including the medial articular cartilage.

Cellular complexity in the intervertebral disc

Phenotyping studies using omics technologies at the transcriptome and proteome level have identified a wide range of markers of human notochordal cells[55–58], healthy nucleus pulposus, annulus fibrosus, and cartilaginous endplate (CEP) cells and tissues[59,60], as well as markers of degeneration[59,61–64]. These efforts to understand the nucleus pulposus cell phenotype resulted in the publication of an international consensus statement in 2015 on markers to distinguish nucleus pulposus cells from annulus fibrosus and CEP cells[65]. Nucleus pulposus cells express markers found in human and bovine notochordal cells[66,67], suggesting that at least a proportion of human nucleus pulposus cells are notochord-derived. However, additional progenitor cell populations have been identified within the human and mouse IVD (most notably Tie2$^+$ GD2$^+$ nucleus pulposus progenitor cells[68]), including cells from the nucleus pulposus, annulus fibrosus and CEP, which possess MSC-like properties such as multipotency[69]. Although some of these populations have been proposed to have regenerative potential, they highlight the complexity of IVD formation and the diversity of cells that exist within the disc during development, ageing and degeneration.

scRNA-seq is beginning to provide a more detailed understanding of the cell subpopulations within the human IVD. Comparisons of the cells within the healthy human IVD have revealed differences in transcriptional profiles between nucleus pulposus cells and annulus fibrosus cells[70], and comparisons of non-degenerate and degenerate IVD cells from the same donor have revealed a panel of potential biomarkers of disease[71]. Additionally, multiple distinct cell sub-types within both the human nucleus pulposus and annulus fibrosus have been identified[72–77], with studies showing a shift in IVDD tissues towards populations with a more fibrotic phenotype, populations that might drive angiogenesis and an increased presence of immune cell-like populations, most notably macrophages, when compared with non-degenerate discs. Although the function of these subpopulations requires further investigation and functional validation, the alterations in cell populations might underpin the tissue-level changes observed during degeneration, and these studies highlight the diversity of cell phenotypes present within the human IVD throughout ageing and degeneration (Supplementary Table 3).

Alongside studies investigating cell populations associated with degeneration, scRNA-seq has also enabled identification of a putative *PROCR*$^+$ progenitor cell population within the human nucleus pulposus[78]. Additionally, transcriptomic and protein-level analyses of human and mouse IVD during early embryonic development have identified populations during early (*SOX10*$^+$) and late (cathepsin K$^+$ (encoded by *CTSK*)) IVD formation as well as populations that are responsible for ECM homeostasis (*CTSK*$^+$ and brachyury$^+$ (encoded by *TBXT*))[79]. An integrated analysis of proteome sequencing, bulk RNA sequencing and scRNA-seq data identified *SERPINA1* as a biomarker to regulate or predict the progress of IVDD[80]. Identification and functional characterization of these subpopulations within the adult human IVD could further elucidate their roles in tissue homeostasis and identify progenitor cell populations with potential for therapeutic application.

Ageing, inflammation and chondrosenescence

With age, cartilaginous tissues might gradually become damaged, which can lead to prevalent joint diseases such as OA and IVDD[81]. Notably, these degenerate tissues do not present a widespread apoptotic phenotype[82], leading researchers to investigate causal drivers of structural damage. In the past decade, research has focused on elucidating the role of senescence in OA and IVDD pathophysiology[83].

Cell senescence (also termed chondrosenescence in articular cartilage) is characterized by an irreversible halt in cell division[84,85]. Cell senescence increases with age and correlates with progressive tissue degeneration and functional loss[86–89].

Senescence in hyaline cartilage

Senescent cells often display dramatic changes in structure, metabolism and secretory profile, indicating that senescent cells have a pleotropic phenotype. These cells often display an increase in cell volume, senescence-associated β-galactosidase activity, senescence-associated heterochromatic foci and the expression of cell-cycle-related proteins, such as $p16^{INK4a}$, $p19^{ARF}$, $p14^{ARF}$ and $p21^{CIP1}$ (refs. 85,89,90). Moreover, senescent cells contribute to a systemic increase in pro-inflammatory mediators[88], as they secrete exosomes (known as senescence-associated secretory phenotype (SASP)) that contain pro-inflammatory mediators, chemokines (IL-1β, IL6 and CXCL8) and ECM-degrading enzymes, such as matrix metalloproteinases (MMPs) and cathepsins[88,91]. In addition to the systemic effect of senescent cells, it has been suggested that SASP-secreting senescent cells confer a 'bystander effect' that affects neighbouring cells, resulting in further induction or reinforcement of tissue senescence[92,93] (Fig. 3). This process seems to be mediated by SASP-related factors and cytokines, which also contribute to age-related chronic inflammation[90,94]. Notably, mechanical insults to human hyaline articular cartilage contribute to senescence in the superficial zones, displaying telomere erosion and reduced cell doubling[95]. In line with these observations, data from animal models of ageing and post-traumatic OA (such as those that use the anterior cruciate ligament transection procedure) show that the number of senescent cells is highest in the superficial zone (which is directly exposed to mechanical loading)[96]. Given that the superficial zone is enriched in stem-cell populations, the accumulation of senescent chondrocytes might interfere with the regenerative potential of the tissue after loading[97]. Cumulatively, these data suggest that mechanical loading entices the initial emergence of superficial senescent chondrocytes, which could be further increased with time via the SASP-mediated 'bystander effect'. To this end, ageing and the inflammaging process can only contribute to the proportion of chondrosenescence in articular cartilage.

Humans and rodents exhibit a chronological increase in the senescence biomarkers $p14^{ARF}$ and $p16^{INK4a}$, respectively; but these changes are not associated with increased levels of SASP, nor does loss of murine $p16^{INK4a}$ result in a mitigated OA phenotype[98]. Notably, the gradual acquisition of the chondrosenescent phenotype is suggested to be accompanied by chondrocyte hypertrophy and mineralization[99], posing a specialized profile of senescent chondrocytes. Mechanistically, this hypertrophy-related chondrosenescent feature is not fully understood, but evidence shows that cartilage-specific ablation of SIRT1 (which is known to repress senescence[100]) resulted in severe post-traumatically induced ectopic osteophyte formation, meniscal mineralization and cartilage damage[54], accompanied by increased chondrosenescent $p16^{INK4a}$ staining[101]. In a recent study, age-associated transcriptional changes, such as *GATA4* upregulation, impaired chondrocyte ECM synthesis and amplified pro-inflammatory responses, providing a mechanistic link between cellular ageing and OA susceptibility[102].

The relationship between inflammation and cellular senescence in the context of musculoskeletal disorders remains unclear, with both chronic and acute inflammation potentially contributing to the accumulation of senescent cells in ageing tissues or after injury[96,101]. Although chronic inflammation can induce senescence, and anti-inflammatory treatments might clear senescent cells (Fig. 3), the effects of acute injury-related inflammation on senescence are not fully understood, suggesting a complex interplay between these processes.

Most in vivo studies, including studies describing cartilage senescence, are traditionally performed in male mice given that they are reported to harbour a more severe OA phenotype than female mice[103], and therefore the male models better support this chronological accumulation in SASP and senescent cell phenotype[96,104]. In a 2024 study that examined a targeted treatment to enhance SIRT1 activity, aged female mice did not display a senescent phenotype, whereas aged male mice did have the senescent hallmark of H2Aγ[105]. These data insinuate that, at least in preclinical models, different phenotypes of senescence can occur owing to sex-related differences, which should be addressed in future research.

Senescence in the meniscus

The specific association of senescence in meniscal tissues has predominantly focussed on its association with articular cartilage. Thus, studies specifically examining senescence in meniscus are rare and more commonly related to studies investigating the aged meniscus[106]. A study that used gene databases from previous microarray analyses identified four genes (*RRM2*, *AURKB*, *CDK1* and *TIMP1*) and microRNAs associated with these genes in senescent human meniscal tissues[107], whereas another study showed that downregulation of *FOXO1* and *FOXO3* transcription factors in aged meniscal tissues increased susceptibility to OA[108].

A study that aimed to identify specific OA markers using scRNA-seq analysis of healthy and OA human meniscal tissues, found a subset of cells with upregulated expression of fibroblast activating protein and the transcription factor *ZEB1*, and promoted ECM degradation and senescence[109]. Senotherapeutic drugs (therapies that target senescent cells) have yet to be directly applied to meniscal tissues, although the specific mechanisms that induce senescence remain to be elucidated.

Senescence in the intervertebral disc

Senescence often correlates with skeletal ageing, a major risk factor for IVDD and OA[90]. Other pathogenic factors, including oxidative, genotoxic and inflammatory stress, along with nutritional constraints that contribute to IVDD, all correlate with cell senescence. Consequently, senescence has an important role in the pathophysiology of IVDD[110].

Early work showed a positive correlation between $p16^{INK4a}$ expression levels and disc degeneration in patients[87]. Later studies, using $p16^{tdTOM}$ reporter mice, showed increased levels of $p16^{INK4A}$, p21 and senescence burden in aged mouse IVD[111]. These authors, using a model of conditional deletion of $p16^{INK4A}$ ($Acan^{creERT2}$;$p16^{INK4a}$), showed a compensatory role of $p19^{ARF}$ in the senescence process. Although $p16^{INK4A}$ was dispensable for the induction and maintenance of senescence, this study established a causal relationship between $p16^{INK4A}$ with SASP and altered ECM homeostasis. These findings aligned with studies of a mouse model of cyclin dependent kinase inhibitor 2A (*Cdkn2a*; encoding $p16^{INK4A}$) germline deletion, which showed a reduction in oxidative stress and disc degeneration following tail suspension injury[112]. Furthermore, a study using a genetically engineered $p16^{INK4A}$-3MR transgenic mouse model showed that systemic clearance of $p16^{INK4a}$-positive cells ameliorated age-related disc degeneration[113]; mice that lacked $p16^{INK4A}$-positive senescent cells had decreased ECM catabolism and reduced inflammation. These findings support the causal relationship between senescent cells and IVD degeneration[114,115].

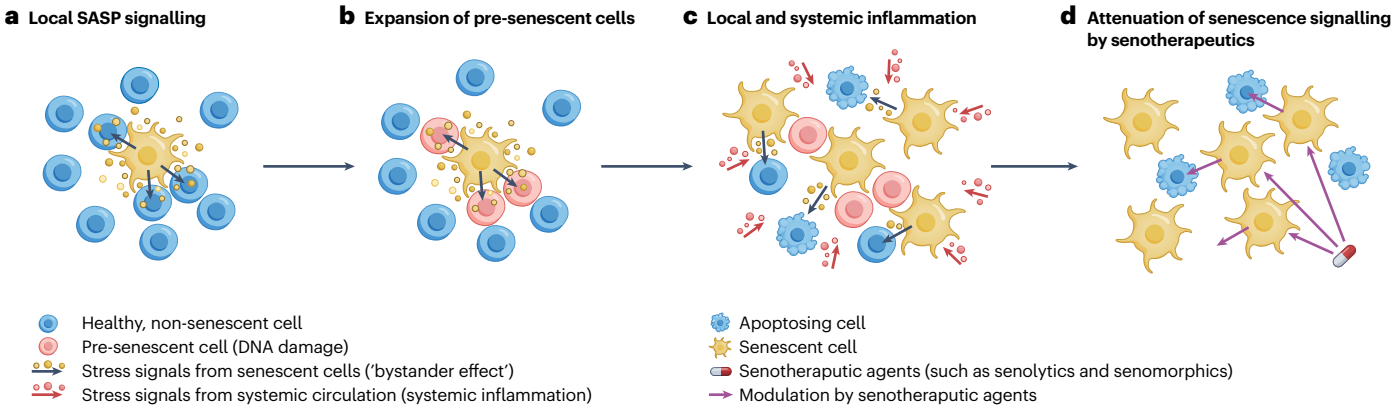

Fig. 3 | Senescence in cartilaginous tissues. A proposed trajectory wherein the frequency of cellular senescence in cartilage increases in proportion to both chronic and acute inflammation. Although the frequency of senescent cells in the tissue is initially low (**a**), healthy cells accumulate DNA damage over time with increasing levels of inflammation and oxidative stress (**b**). Moreover, senescent cells negatively affect neighbouring healthy cells through the production and secretion of senescence-associated secretory phenotype (SASP) factors (known as the 'bystander effect'), which predisposes them to senescence. Various external stimuli, such as trauma, injury and infection, promote further inflammation and amplify the bystander effect, causing healthy, non-senescent cells to undergo apoptosis, whereas senescent cells are more resistant to inflammatory conditions and instead undergo cellular dysfunction. Local (that is, intra-articular) and systemic inflammatory mediators arising from chronic co-morbidities (such as obesity, diabetes and cardiovascular disease) further exacerbate the process (**c**). Senotherapeutic agents selectively induce apoptosis in senescent cells or modulate their secretory phenotype, thereby reducing their accumulation and mitigating the harmful effects of their pro-inflammatory secretome, especially the senescence-inducing effects of SASP factors (**d**).

Another study used scRNA-seq to identify compartment-specific changes in human IVDD and observed gene signatures of cell senescence, as well as a notable reduction in cells, particular stem cells and fibroblast progenitors, expressing markers of immature cell types in both the annulus fibrosus and the nucleus pulposus[116]. Other studies that investigated the cellular heterogeneity during human IVDD have observed changes in processes such as ferroptosis, which, similar to senescence, are linked to oxidative stress and inflammation[117]. A 2024 study using scRNA-seq, identified autophagy-related protein 9 A (*ATG9A*) as a key marker associated with IVDD, whereby ATG9A expression is diminished during degeneration, which suggests reduced autophagic flux[118]. Notably, basal autophagy has a protective function during ageing-related pathologies, and dysregulated autophagy contributes to many pathologies that affect the spinal column[119,120]. However, a 2021 study also showed the importance of autophagy in establishing full senescence through regulated protein stability and the importance of this process during human OA[121]. Further investigations are needed to understand this relationship in the context of IVDD. Although similarities have been noted between other skeletal tissues and the IVD, pathways (such as cGAS–STING) that are linked to cell senescence did not contribute to senescence burden in the ageing mouse with IVD, highlighting the cell and tissue type specificity of the mechanisms driving cell senescence[122].

Senotherapeutic agents are therapies that target cellular senescence and include both senolytics (which reduce inflammation and improve tissue function by removing senescent cells) and senomorphics (which help maintain tissue function by reducing the negative impact of senescent cells, such as chronic inflammation, without eliminating these cells). These therapies show great potential for treating OA and IVDD by targeting senescent cells in affected tissues. However, further research is needed to elucidate the broader effects of senescence on joint health, to develop reliable biomarkers for patient selection and to optimize treatment protocols for disease models and cell-based therapies. For developing novel senotherapeutic strategies, single-cell analysis could be instrumental in identifying specific cellular subpopulations and their roles in senescence, thereby allowing for targeted interventions to mitigate age-related tissue dysfunction. This approach could help to elucidate the complex interactions between senescent cells and their native niche, potentially leading to novel senotherapeutic agents that could improve tissue regeneration and function (Box 2).

Future prospects

Although genomic and transcriptomic analyses, including single-cell transcriptomic analyses, have transformed the understanding of chondrocyte heterogeneity, their use in predicting functional ECM outcomes remains limited. For instance, transcript levels of *ACAN* or *COL2A1* alone do not reflect the sulfation patterns of glycosaminoglycans or the cross-linking density of collagen fibrils, both of which are critical for load-bearing capacity[123–125]. This example highlights the need to better integrate single-cell multi-omics approaches with tissue-level multi-omics analyses, as well as to complement transcriptomics data with direct assessments of ECM biomechanics and post-translational modifications, as gene expression alone might not faithfully predict tissue-level functionality[126].

Notably, differences in study outcomes often arise from methodological and biological variables; for instance, time points can critically influence results: early-stage OA tends to involve transient inflammatory or proliferative chondrocyte states, whereas late-stage disease predominantly exhibits catabolic or senescent populations[127]. Species-specific differences (such as rodent versus human cartilage)[128] and OA induction methods (such as surgical destabilization versus chemical injury)[129] yield distinct pathophysiology, which influences the observed transcriptional profiles. For example,

mechanical injury models predominantly activate mechanosensitive pathways (such as YAP and TAZ)[130], whereas inflammatory models (such as collagenase-induced OA) amplify cytokine-driven responses[131]. Sex-specific differences in hormone signalling and immune regulation might underlie divergent cellular subpopulations in men and women[132]. Furthermore, technical variables such as cell isolation protocols (for example, the effects of enzymatic digestion on stress-response genes) and sequencing depth can skew population distributions[133]. Acknowledging these factors is essential for understanding differences across studies and advancing translational insights into chondrocyte heterogeneity.

Despite advances in the field, several critical questions remain unanswered. The precise molecular mechanisms that regulate the transition of chondrocytes from healthy to pathological states are still poorly understood. Additionally, the role of systemic factors, such as age, sex, metabolic health and mechanical loading, in shaping chondrocyte subtype distributions necessitates further investigation[134]. Addressing these gaps could substantially refine the understanding of cartilage degeneration and inform the development of targeted therapeutic strategies. Longitudinal single-cell analyses of post-traumatic OA models are needed to resolve temporal shifts in chondrocyte subtypes (such as fibrocartilage and pre-inflammatory chondrocytes) and their causal roles in fibrosis and inflammation[37,135].

Critically evaluating the translatability of animal models to human OA, particularly given the anatomical disparities in cartilage thickness and biomechanical loading patterns between quadrupedal rodents and bipedal humans[136], is crucial. Although animal models such as anterior cruciate ligament (ACL) rupture or DMM provide controlled systems for studying OA progression, they often fail to replicate the chronic, multifactorial nature of human disease, which involves ageing, systemic inflammation and cumulative mechanical stress[136,137]. Notably, cartilage that is classified as 'non-degenerate' in OA joints might still exhibit molecular alterations owing to prolonged exposure to pro-inflammatory mediators and abnormal mechanical stresses, as evidenced by proteomic and transcriptomic profiling[34,138]. Even in macroscopically intact regions, osteoarthritic chondrocytes can display upregulated catabolic pathways (such as MMPs, ADAMTS4 and ADAMTS5) and reduced anabolic activity[138], highlighting the need for cautious interpretation of 'healthy' cartilage.

Looking to the future, integrating spatially resolved multi-omics technologies, such as spatial transcriptomics and proteomics, will enable chondrocyte subsets to be mapped within their native niches. Such approaches could reveal dynamic changes in cellular behaviour during disease progression and provide insights into the molecular drivers of cartilage disorders. Moreover, longitudinal studies using these technologies might help to identify biomarkers for early detection of joint diseases, offering opportunities for timely and more effective interventions.

We propose that future applications of machine learning and artificial intelligence for the analysis of complex multi-omics datasets will uncover previously unrecognized patterns in chondrocyte gene expression and interactions, potentially leading to the discovery of novel therapeutic networks and safer druggable targets. Furthermore, developing predictive models on the basis of patient-specific data could facilitate personalized medicine approaches, tailoring treatments to individual disease trajectories. From a translational perspective, these findings have the potential to substantially improve clinical outcomes for patients affected by conditions such as OA and IVDD. Distinct chondrocyte phenotypes could offer new opportunities to refine current therapeutic strategies. In OA, inflammatory chondrocyte subsets represent potential targets for biologic therapies aimed at suppressing catabolic signalling, whereas progenitor-like populations could be harnessed for regeneration. Therapies that target these inflammatory chondrocyte subtypes could mitigate cartilage degradation in OA and enhancing the regenerative potential of homeostatic or reparative subpopulations could improve cartilage repair. Conversely, failed OA trials targeting broad-spectrum MMPs highlight the need for subtype-specific approaches to avoid disrupting homeostatic ECM maintenance. For meniscus-tissue engineering, hypertrophic chondrocyte subsets, which drive calcification in degenerated menisci, could be selectively inhibited, whereas ECM-producing phenotypes might be expanded to enhance graft integration. Similarly, in IVDD, nucleus pulposus cells with notochord-like signatures show enhanced proteoglycan synthesis[139], suggesting their potential in cell-based IVD regeneration. Therefore, future efforts should explore combined approaches that simultaneously suppress pro-inflammatory pathways and activate regenerative ones, optimizing therapeutic efficacy (Box 3).

Conclusions

Single-cell technologies are transforming the understanding of chondrocyte heterogeneity and functionality across cartilage types and disease states. Distinct chondrocyte subtypes have been identified in

Box 2 | Senotherapeutic agents for joint diseases

Senotherapeutic agents, which aim to modulate or eliminate senescent cells, are emerging as potential treatments for osteoarthritis (OA) and intervertebral disc (IVD) degeneration (IVDD). Intra-articular administration of senotherapeutic modulators has shown promise in reducing OA severity by modulating senescent chondrocytes in preclinical rodent models. Additionally, senolytics can also induce apoptosis in senescent IVD cells, thereby mitigating IVDD.

Most research focuses on the effects of senescence on chondrocytes during OA and IVDD but less is known about its role in other joint tissues or pain transmission. Senotherapeutic drugs have yet to show notable progression in clinical trials, which suggests that improved patient selection using senescence-related biomarkers is needed for more effective and quantifiable clinical outcomes.

Acute post-traumatic OA models might require different senotherapeutic drug dosages or administration methods compared with age-induced OA models. However, it should be noted that intra-articular administration of senotherapeutic agents is likely to target the senescent chondrocyte population that is located in the superficial zone, eliminating their detrimental effects on the tissue and promoting a pro-regeneration milieu. Similarly, the efficacy of cell transplantation approaches could be affected by the chondrosenescent environment, highlighting the need to consider the 'seno-severity' of the host. Future research should focus on understanding the broader effects of senescence on the entire joint and use biomarkers to identify suitable candidates for senotherapeutic therapies. Pretreatment with senotherapeutic agents could potentially enhance the outcomes of cell-based therapies by creating a more favourable environment for transplanted cells.

Box 3 | Exploiting chondrocyte heterogeneity for cartilaginous tissue regeneration

Current cartilage repair strategies, such as microfracture, autologous chondrocyte implantation and matrix-assisted chondrocyte implantation, face issues with tissue quality and durability. Future tissue engineering approaches that leverage the functional diversity of chondrocyte subpopulations that are emerging could be promising targeted therapies. Using mesenchymal stem cells or engineered cells that have been differentiated into specific chondrocyte subtypes could also enhance cartilage regeneration.

Meniscal regeneration focuses on stem cells and meniscal progenitor cells, potentially involving culturing these cells in 3D bioprinted scaffolds. Discovering specific progenitor populations within the meniscus, particularly from healthy and osteoarthritis tissues, could lead to better translational therapies for treating small defects or even whole meniscal tissues.

Approaches to intervertebral disc regeneration include intradiscal injections, gene therapies and cell implantation. Autologous and allogeneic cells, including nucleus pulposus and annulus fibrosus cells, chondrocytes and stem cells, are currently being tested; however, the harsh microenvironment of the degenerate disc poses challenges, necessitating pre-conditioning to improve cell survival. Future strategies might involve regenerative therapies without cell implantation and building on the secretome of notochordal cells.

hyaline articular cartilage, meniscal cartilage and the IVD, which exhibit unique gene expression profiles that correlate with their functional roles in health and disease. Understanding how these cells interact within their native niches and with cells in other joint compartments is crucial for developing more effective regenerative therapies. A deeper understanding of the cellular and molecular diversity of these cell populations, their crosstalk and relative influence can help to develop therapeutic candidates that can tilt the inflammatory and catabolic balance towards restoration of homeostasis and tissue regeneration. This approach will be particularly beneficial in the early stages of disease pathogenesis and progression. An enhanced knowledge of cartilage biology and its molecular regulation is invaluable, not only for understanding joint disorders but also for bone trauma repair. This paradigm shift will open up new avenues for targeted therapeutic strategies in diseases such as OA and IVDD. The identification of key molecular markers associated with specific chondrocyte states could lead to novel biomarkers for early diagnosis and therapeutic targets for these disorders.

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