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# Standardizing single-cell approaches to osteoarthritis: Toward a comprehensive cellular atlas

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ABSTRACT

Osteoarthritis (OA) is a degenerative joint disease marked by progressive cartilage degradation and complex cellular heterogeneity. In recent years, single-cell RNA sequencing (scRNA-seq) has emerged as a powerful tool for dissecting the cellular composition of the osteoarthritic joint. However, constructing a complete and coherent picture of the OA single-cell landscape remains challenging, akin to assembling a puzzle from multiple sets, each with pieces of varying shapes and sizes. This editorial outlines the next steps in advancing single-cell research in OA, emphasizing the need for standardized cell type annotations and comprehensive, integrative access to both historical and newly generated datasets.


Osteoarthritis (OA) is a degenerative joint disease characterized by progressive cartilage degradation and complex cellular heterogeneity. Over the past several years, single-cell RNA sequencing (scRNA-Seq) has emerged as a powerful tool to dissect the cellular composition of the human osteoarthritic joint. However, building a complete picture of the OA single-cell milieu in the articular cartilage from these early datasets is like assembling a puzzle from dozens of different sets, where the pieces differ in size and shape.

Studies spanning from 2019 to 2021 established the main cellular heterogeneity in articular cartilage based on OA gene markers [1–4], whereas later studies (2022–2025) have used the most recent advances in single-cell technologies to analyze larger cell populations and refine cellular annotations [5–14]. But is this enough to understand the cellular mechanisms of OA?

In 2019, the pioneering study by Ji and colleagues [1] provided a new perspective on articular cell populations and how they deviate into pathology during disease progression. They analyzed only OA articular chondrocytes, but later studies included synovial [2], bone and immune lineages [5] with the same goal of identifying new cell populations associated with the pathology. Taken together, these multi-tissue efforts underscore that OA is a genuinely whole-joint disorder in which subchondral bone [5], synovium [2] and other peri-articular tissues cooperate dynamically with cartilage in disease progression. More studies refined cell resolution by increasing the number of analyzed cells from hundreds to tens of thousands. From these, we have been able to reveal unbiased new key players in OA tissues.

While the diversity of single-cell platforms, sequencing depths and ad-hoc labelling schemes has been invaluable for discovery, it has also produced study-specific gene-expression signatures that are hard to align; robust cross-study comparison will require converging on validated cell-type annotations and a shared set of reference markers. Almost every study to date has identified at least one new cell population by differential gene expression, but with scarce functional, morphological or positional information. The lack of standardization in gene annotation and the poor regulation of dataset deposition in public databases makes it difficult to integrate even the relatively small number of studies since 2019 [1].

While some recent studies have attempted to compensate for these different populations by reanalyzing the pioneer papers from 2019 to 2021, others continue to publish new datasets using new marker genes, but sometimes without even making them publicly available. Early studies, despite reanalysis, are rapidly becoming obsolete due to new technical advances in single-cell technology that allow us to profile more cells and more sequencing depth, while the upcoming challenge will be integrating earlier datasets with complementary approaches to transcriptomics such as spatial analysis and chromatin regulatory data (e.g., ATAC-seq).

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| OA Cell Types | Ji et al. [1] | Chou et al. [2] | Fan et al. [11] | Huang et al. [14] |
|---|---|---|---|---|
| Effector Chondrocytes | TF, CHRDL2, TPM4 | - | CHRDL2, FRZB, CYTL1 | - |
| Regulatory Chondrocytes | CKS2, HMOX1 | CHI3L1, CHI3L2 | CHI3L1, CHI3L2 | CFH, LUM, DCN |
| Progenitor Chondrocytes | P3H2, DDX21, UPP1, CDV3, NGF, C3orf52 | - | C11orf96, BMP2, HMGA1 | BHLHE41, CCL20, DUSP6 |
| Pre-hypertrophic Chondrocytes | TGFBI, S100A4, THBS3, ADAMTS5, TPPP3 | COL10A1, IBSP, COL2A1 | PRG4, ABI3BP, CRTAC1 | IL11, MMP3, CXCL3 |
| Fibrocartilage Chondrocytes | COL1A1, TMSB4X, ID3, PRSS23, OLFML3, LAMA4, HES1, SEPW1, ZFP36L1, TPM1, PHPT1, IFI6, ESD, UACA, TCEAL8, ID1, ZFP36L2 | COL1A1, COL1A2, S100A4, PRG4 | MMP2, COL1A1, COL1A2 | MYLK, ACTA2, CTGF |
| Hypertrophic Chondrocytes | WWP2, BHLHE41 | COL10A1, IBSP, JUN | SPP1, IBSP, COL10A1 | FMOD, EBF1, ADAMTS5, ELL2, NEAT1 (HTC-1), FMOD, EBF1, OLFM2, PDGFRB, SCG2 (HTC-2) |
| Homeostatic Chondrocytes | JUN, BRD2, RGS16, CCNL1, SNHG12, DDIT3, TRA2A, RGS2, KMT2E, H2AFX | MMP3, FOSB, JUN | HSPA1B, HSPA1A, HSPA6, DDIT3, JUN | DDIT3, ATF3, GDF15 |
| Reparative Chondrocytes | - | COL2A1, CILP, COL3A1, COMP | CILP2, CILP, OGN | - |
| Pre-Fibrocartilage Chondrocytes | - | IL11, COL2A1, CILP, OGN | COL27A1, PLCG2, WWP2 | PTX3, TAGLN, SPARC |
| Pre-inmflamatory Chondrocytes | - | - | IFI16, IFI27 | - |
| Inmflamatory Chondrocytes | - | - | CXCL8, CD74, GPR183 | - |

**Table 1**

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Illustrative list of main marker genes for the most representative cell populations identified in single-cell transcriptomic individual studies, from early to recent research, as reported by the original authors.

To bring consensus to this increasing complexity, several international research consortia such as the Human Cell Atlas [15] and the OARSI initiative have proposed standard annotations to cell types. Their aim is not to erase tissue-specific biology, but to place every single-cell dataset, regardless of platform, depth, or protocol, within a common reference framework so that chondrocytes, synovial fibroblasts, or subchondral osteoblasts are labelled consistently across studies. Such standardization will make true biological differences easier to detect and compare. At present, many of the resulting annotation tools are still being finalized or are not yet fully public, and they have only begun to be applied to disease-driven expression changes in pathologies such as osteoarthritis.

As a growing field, OA single-cell research must now consider the state-of-the-art in cell annotation from recent single-cell studies of both healthy and OA tissue to reach a consensual definition of cell types based on function and tissue localization, and to avoid redundant efforts. We need to establish common ground for future studies, integrating anatomical and positional information with emerging expression profiles of cells involved in disease mechanisms. This is not only crucial for OA but for all musculoskeletal diseases.

One key step will be integrating recent knowledge on OA marker genes, identified through unsupervised clustering, with functional annotations derived from integrative atlas approaches of healthy tissue (i.e., trauma and non-OA related samples). So far, we have only accomplished the first part of this effort:

## Early single-cell discoveries in OA

Since the first single-cell study about OA in 2019 [1], an increasing list of cell populations has emerged as consensus among each new study. This author-defined naming system can be summarized as: **Effector chondrocytes**, which drive tissue degradation by expressing high levels of catabolic enzymes and inflammatory mediators, directly contributing to cartilage breakdown; **Homeostatic chondrocytes**, which maintain cartilage integrity by producing critical extracellular matrix components, serving as a baseline for healthy tissue function; **Fibrochondrocytes**, transitional cells that exhibit characteristics of both chondrocytes and fibroblasts, involved in reparative responses but potentially contributing to aberrant fibrosis when repair fails; **Hypertrophic and pre-hypertrophic chondrocytes**, representing stages in a differentiation pathway that associates with abnormal mineralization in some cases of OA; **Progenitor chondrocytes**, a promising group with stem-like properties and regenerative potential; and **Regulatory chondrocytes**, which produce anti-inflammatory cytokines and growth factors, helping balance destructive and reparative processes in the joint.

Subsequent studies added their own OA cell populations: Chou et al. [2] integrated, for the first time, the analysis of synovial cells in the OA joint. They also described a new cell population, **reparative chondrocytes**, a subset in the articular region with a gene expression profile suggestive of tissue repair and regeneration. These cells express anabolic and repair-associated markers, indicating their potential role in restoring cartilage integrity. Wang et al. [3] provided the first comparative analysis including a high number of non-OA cartilage cells, a valuable control. Fu et al. [4] expanded the scope by comparing OA with other conditions and exploring specific signaling pathways, such as the PGRN/TNFR2/14–3–3ε axis, further refining chondrocyte classification. They better defined fibrocartilage into two distinct populations and described **mitochondrial chondrocytes**, although these seem more relevant to Kashin-Beck disease than OA. These four studies: Ji et al. [1], Chou et al. [2], Wang et al. [3], and Fu et al. [4] can be considered pioneering in single-cell OA research. However, only three of them (the ones led by Ji, Chou, and Fu) have been reanalyzed in depth (up to eighteen times) to refine our understanding of OA joint cell heterogeneity. Unfortunately, Wang et al. [3] dataset is not available in public databases and has therefore not been reanalyzed.

## Recent advances and limitations

Since 2022, newer studies have allowed a more accurate representation of OA cell heterogeneity. Hu et al. [5] and Lv et al. [6] introduced more stringent quality controls and extended their sampling beyond cartilage into adjacent subchondral bone and synovium offering the first cell-resolved glimpse of cross-tissue crosstalk that cartilage-only studies miss and highlighting processes

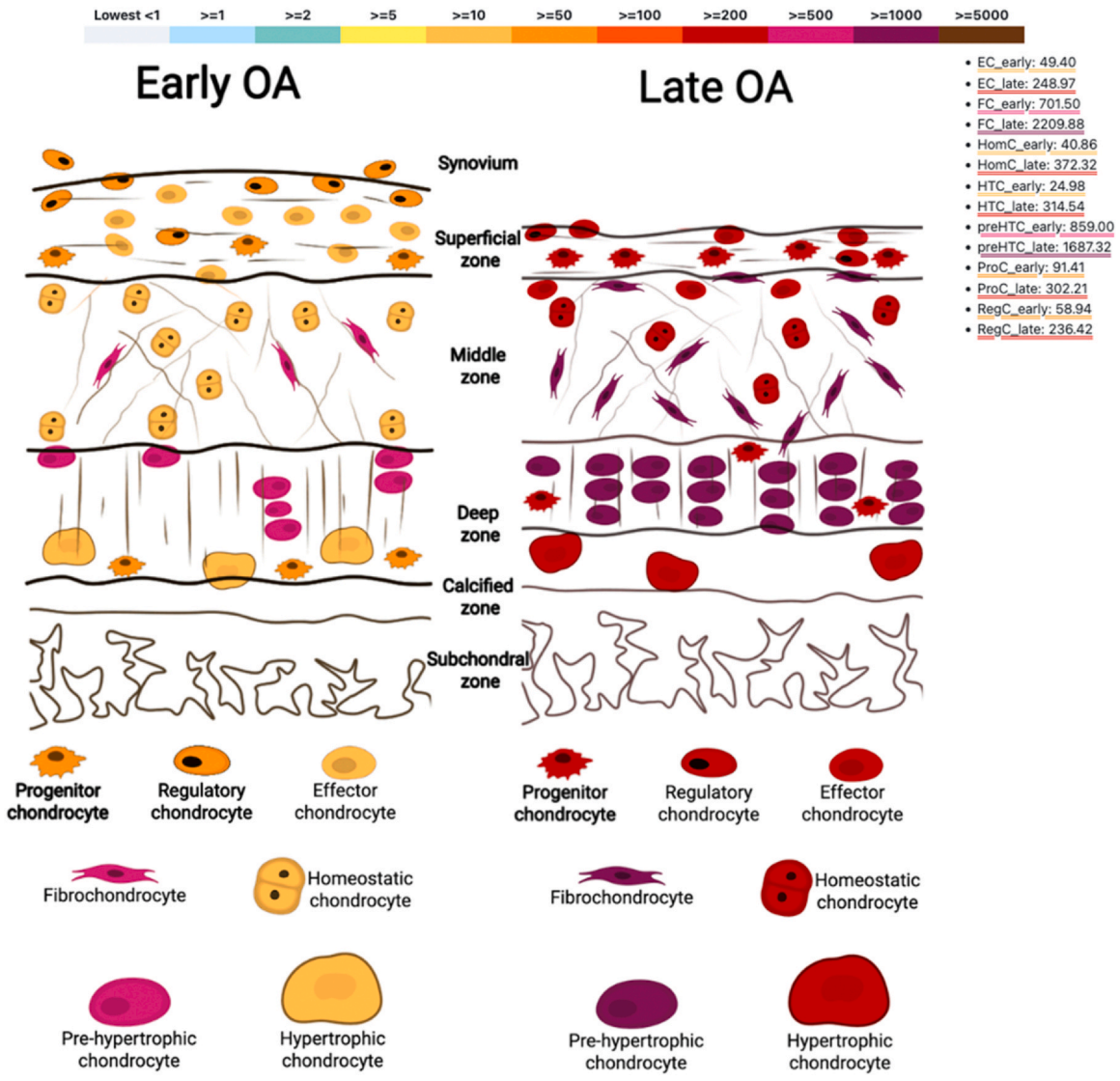


**Fig. 1**

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A comprehensive, interpretative and interactive atlas tool based on the pioneer study by Ji et al. (2019). The seven cell populations identified in this study are represented within an interpretative layout of the articular cartilage, highlighting the expression of the OA marker gene *S100A4*, which is associated with a pathogenic fibroblast-like phenotype and inflammation (https://www.boneatlas.uma.es).

like oxidative stress and ferroptosis. Complementary work by Swahn et al. [7], Li et al. [8], and Zhang et al. [9] enriched the field through multi-tissue integration, refined clustering algorithms, and analyses of less-studied joints like the temporomandibular joint. Taken together, these datasets span knee [5], hip [6], synovium/meniscus [7] and temporomandibular joint cartilage [9], providing the beginning of a multi-site test-bed for harmonization pipelines that must preserve true regional biology while correcting technical batch effects. The most recent research by Li [8], Fan [11], Wang [12], Sun [13], and Huang (2025) [14] has leveraged the latest advances to analyze tens of thousands of cells, identifying novel subpopulations (e.g., inflammatory, splicing-related, and metal ion-associated chondrocytes) and applying pseudotime analyses to map differentiation trajectories. Notably, genes such as SERPINE2 and MAFB were ranked among the top differentially expressed features in knee cartilage [5], hip cartilage [6] and synovial-meniscal tissue [7]. However, their precise molecular roles in OA remain undefined, this convergent, dataset-agnostic signal exemplifies the unbiased key players that standardization efforts aim to elevate into future consensus marker panels. Also, to translate these gains into a truly interoperable resource, we must now adopt a consensus two-step collagenase dissociation protocol, anchoring human states to the murine post-traumatic OA atlas, and embedding CellChat-derived ligand-receptor maps within the Skeletal Atlas.

## The need for standardization and a comprehensive OA cell atlas

At this point, we are reaching optimal profiling of individual cell gene expression and a more stable annotation of OA-related cell types (see Table 1) in individual studies. Why then do different studies still yield such different results? We need functional standardization and consensus biological annotation before continuing

to describe new OA-specific cell types. While the description of cellular heterogeneity has been invaluable for understanding OA, these discoveries miss the foundational cell types in healthy cartilage. We still do not know how or from where many of these OA-associated cells emerge, nor can we confidently locate them within the joint anatomy. A more comprehensive interpretation of the data collected so far is needed to advance toward understanding the cellular mechanisms underlying OA.

Our group, along with others, has initiated a consortium to integrate recent and future datasets into a **multi-omic atlas of the skeleton** (www.skeletalatlas.uma.es). This Skeletal Atlas already includes older bulk RNA-seq and early single-cell studies such as Ji et al. [1]. The current v0.5 release is cartilage-centric; the next update will integrate single-cell synovial [2] and subchondral-bone [5] layers and add cross-tissue interaction views, advancing the Skeletal Atlas toward a comprehensive whole-joint resource. This atlas is a work in progress; we are re-annotating every published dataset to a common ontology and generating a merged matrix, and future single-cell studies that follow the standardization and validation criteria outlined in this editorial will help close the remaining knowledge gaps on the way to a definitive OA single-cell atlas. Thus, the challenge of developing a fully interpretative and interactive atlas remains. We need to gather more spatial information about the discovered OA cell populations and establish standardized cell annotations aligned with initiatives like the Human Cell Atlas [15] (see Fig. 1).

In conclusion, although significant progress has been made in elucidating the cellular heterogeneity in OA, the field now faces the critical challenge of standardizing cell annotations to build a coherent and comprehensive cellular atlas of the OA joint. Future efforts must focus on integrating multi-omic and spatial data from both pioneering and recent single-cell studies into unified frameworks, such as those proposed by the Human Cell Atlas and collaborative efforts like the Skeletal Atlas. By harmonizing the diverse methodologies, gene annotations, and positional data currently scattered across studies, researchers will finally be able to unravel the true origins and interactions of OA-associated cell populations, paving the way for more targeted and effective therapeutic interventions.

## Author contributions

IDS: acquisition, analysis, interpretation of data and writing. NFP: Conception, analysis, design of work and writing. ID: Conception, design, interpretation and writing.

## Declaration of Competing Interest

No conflict of interest.

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