Aging disrupts the pericellular microenvironment of articular cartilage in the knee joint

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INTRODUCTION: Growing evidence indicates that the pericellular matrix (PCM) surrounding articular chondrocytes regulates cartilage homeostasis by mediating biochemical and biomechanical signals.[1,2] Disruption of this microenvironment through alterations in PCM composition, structure, or mechanical properties has been implicated in the onset and progression of knee osteoarthritis (KOA). Although aging is the strongest risk factor for KOA, the contribution of age-related PCM remodeling to disease development remains poorly defined. To this end, this study aimed to mechanistically investigate the relationship between the PCM profile and age-related KOA. For this purpose, we applied a comprehensive and unbiased approach that combined literature mining and multi-omics analyses with histological validation, leveraging both our established murine model of age-related KOA[3] and a genetically modified model to directly interrogate the function of type VI collagen, a major PCM component.[1]

METHODS: To systematically define the PCM protein network, we first performed a systematic literature review integrated with PubPular-based literature-mining to generate an unbiased list of 34 PCM-associated proteins. This protein list served as the basis for all subsequent analyses. First, we mapped these proteins onto a publicly available single-cell RNA sequencing dataset of healthy and osteoarthritic human cartilage from older adult donors (GSE255460).[4] Next, to dissect the influence of aging on the PCM, we performed mass spectrometry on articular cartilage from the knee joints of male C57BL/6 mice at three different ages (n=5/group): young (4-6 months), middle-aged (10-14 months), and aged (21-24 months) following IACUC approval. Principal component analysis (PCA) of the murine proteome was then performed after filtering the 34 PCM proteins. Findings from single-cell RNA sequencing and proteomics were validated by immunofluorescence on decalcified paraffin joint sections collected from age-matched C57BL/6 male mice used for mass spectrometry. To define the functional role of type VI collagen, we used Col6a1KO mice that were generated by crossing NSG (NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ) mice with Col6a1$^{GT/GT}$ mice. Statistical analyses included linear regression and Wilcoxon signed-rank tests, with $p < 0.05$ considered significant. We used only male mice to isolate aging effects while minimizing sex hormone–related influences, as age-induced hormonal shifts affect osteoarthritis progression.[5] All *in vivo* studies followed the ARRIVE guidelines to ensure rigor, reproducibility, and transparent reporting.

RESULTS: Single-cell RNA sequencing of human cartilage revealed that PCM genes were predominantly enriched in a fibrocartilage chondrocyte (FC) cluster, which has uniquely emerged in osteoarthritic cartilage (**Fig. 1A**). The FC cluster was characterized by significant enrichment of extracellular matrix organization, collagen metabolic processes, and the pericellular matrix (**Fig. 1B**), which are due, at least in part, to enrichment of type VI collagen and type III collagen (**Fig. 1C**). Further, PCM genes were upregulated in the weight-bearing regions compared to non-weight-bearing regions within human osteoarthritic cartilage, suggesting a region-dependent enrichment. Further examining shifts in the PCM in the context of aging, PCA of murine cartilage revealed clear segregation of young, middle-aged, and aged samples, indicating progressive remodeling of the PCM over time (**Fig. 1D**). Consistent with human dataset, among the PCM proteins, type VI collagen progressively accumulated with age, whereas type III collagen decreased (**Fig. 1E**). Immunofluorescence analyses of murine cartilage samples validated these findings, as evidenced by increased type VI collagen and decreased type III collagen levels in aged cartilage compared to young counterparts (**Fig. 1F**). Interestingly, increased type VI collagen expression was most pronounced in weight-bearing, fibrotic, and degenerated regions, consistent with the spatial patterns observed in human osteoarthritic cartilage (**Fig. 1A-C**). Finally, Col6a1KO mice displayed compromised cartilage integrity and an inflamed, fibrotic synovium, suggesting that type VI collagen protects against fibrotic conversion in the knee joint.

DISCUSSION: Multi-omics analyses of human and murine model systems, integrated with a curated PCM protein network, revealed PCM remodeling in a region- and age-dependent manner, with PCM accumulation localized to degenerated fibrocartilage. Considering results from genetically modified Col6a1-deficient mice, type VI collagen appears to suppress fibrotic conversion, potentially acting as a negative feedback regulator in the progression of age-related KOA. Together, these findings provide novel mechanistic insights into age-related KOA and suggest that the PCM is a dynamic modulator of cartilage homeostasis and a potential target for intervention for elderly population.

SIGNIFICANCE: The results from this work indicate a novel understanding of the PCM's role in driving age-related KOA through an aberrant remodeling process. Understanding the collagen remodeling process that occurs in the PCM offers a new diagnostic tool for KOA progression and helps to identify a possible therapeutic target for KOA.

REFERENCES: [1]Zhao et al 2022. [2]Guilak et al. 2018. [3]Iijima et al. 2023. [4]Fan et al. 2024. [5]Gilmer et al. 2025. [6]Pullig et al. 1999.

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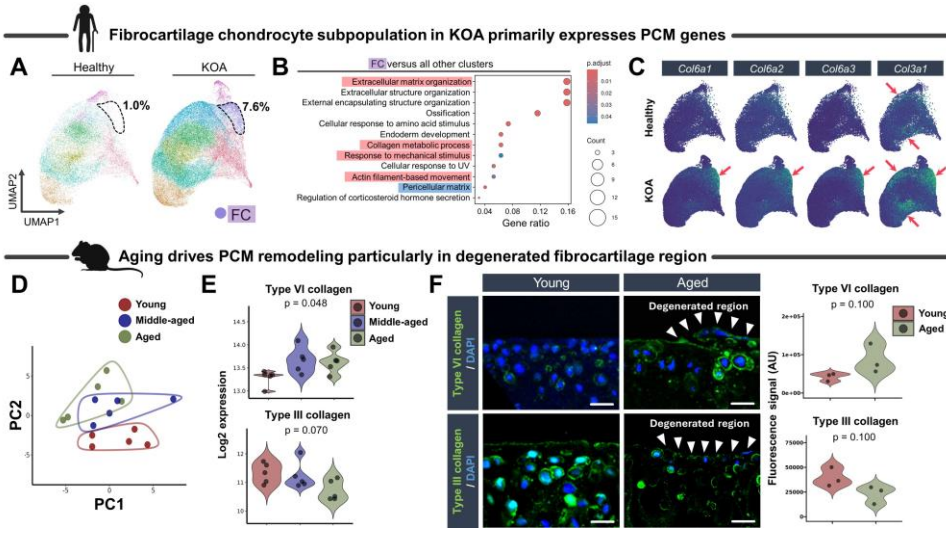

Figure 1: Changes in PCM composition with aging in human and murine samples **A** Single cell RNA sequencing comparing human cartilage between healthy and KOA human samples. **B** Characterization of fibrocartilage chondrocyte (FC) cluster, showing pericellular matrix upregulation. **C** Comparison of collagen gene expression between healthy and KOA joints. **D** PCA plot of mass spectrometry data from young, middle aged, and aged mice (n=5/group). **E** Comparison of collagen abundance from mass spectrometry data across different aged groups. **F** Immunofluorescence images stained with either type III or VI collagen (green) and nuclei (blue) with fluorescence intensity comparison between young and aged samples (n=3/group).