Decoding post-menopausal osteoarthritis via multi-omics networks at single-cell resolution

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INTRODUCTION: Hormonal shifts that accompany menopause, particularly estrogen decline, have profound effects on joint health. Prior studies suggest that hormonal loss contributes compromised cartilage integrity in the knee joint owing to low-grade inflammation, altered extracellular matrix homeostasis and subsequent collagen deposition in the articular cartilage.[1] This potentially shifts chondrocytes toward a more catabolic or fibroblast-like phenotype. However, the precise molecular mechanisms linking estrogen deficiency to cartilage degeneration remain poorly defined, partly due to methodological limitations of bulk RNA profiling and histological approaches, which mask subpopulation-specific responses. Recently, transcriptomic analyses based on single-cell RNA sequencing datasets have revealed that articular cartilage is composed of multiple transcriptionally distinct chondrocyte subpopulations, including fibrocartilage-like, inflammatory, hypertrophic, and homeostatic lineages.[2] We aim to systematically examine how hormone-sensitive signaling pathways reshape the structure of chondrocyte communication networks or drive fibrotic reprogramming in a subpopulation-specific manner. A multi-layered systems biology approach has been employed to dissect how menopausal hormonal shifts impact cartilage at the cellular and molecular levels.

METHODS: The single-cell RNA sequencing dataset (GSE267616), derived from femoral condyle of C57BL/6 young female mice subjected to bilateral ovariectomy (OVX) and corresponding controls (n = 1/group), was accessed and processed using the Seurat R package (version 5.2.0), followed by SCTransform normalization using the glmGamPoi method for variance stabilization.[3] Chondrocyte subpopulations were annotated using the ScType framework for automated cell type annotation and the proportion of each subpopulation was calculated for each sample. Representative marker genes were reviewed for key populations. A similar pipeline was employed on the single-cell RNA human articular cartilage dataset (GSE255460), which included samples from healthy older individuals (n = 3) and patients with knee osteoarthritis (n = 8).[4] Additionally, a co-expression network was mapped for control and OVX groups to understand the changes occurring in the transcriptional coordination across subpopulations due to menopause related changes. To further confirm the results with a physiologically relevant murine model, proteomic datasets from the 4-vinylcyclohexene diepoxide (VCD) treated menopausal mice and vehicle-treated controls were obtained by mass spectrometry (n = 5/group). Compared to the OVX model, treatment with VCD causes follicle depletion, thus closely mimicking the natural menopausal transition that occurs in humans.[5] The datasets from VCD-treated and control mice were analyzed by log-transformed protein abundance. Gene signatures were derived from the OVX dataset, and the GVSA R package was employed to estimate enrichment scores for each subpopulation gene set.[6] Using integrative non-negative matrix factorization (iNMF), we inferred hormonally regulated intercellular signaling networks in human osteoarthritic cartilage.

RESULTS: Using the single-cell RNA sequencing dataset of the OVX mouse model, a marked expansion of *Col1a1*$^{+}$ fibrocartilage chondrocytes (FCs) was observed (**Fig. 1A**). Furthermore, co-expression network construction revealed a reduction of several modules present in the control models along with increased module fragmentation within FCs in OVX model, suggesting disrupted transcriptional coordination (**Fig. 1B**). Specifically, certain immune response pathways were significantly enriched highlighting functional reprogramming in the FC subpopulation under hormone-deficient conditions. Notably, *Icam1* and *Thy1* were uniquely expressed in FCs, suggesting potential surface markers to distinguish this population. This fibrotic signature was recapitulated in the chemically induced, physiologically relevant VCD menopause model, where longitudinal cartilage proteomics demonstrated progressive enrichment of FC-associated proteins, by menopause onset (day 115) (**Fig. 1C**). Moreover, single-cell RNA sequencing of human osteoarthritic cartilage similarly showed FC expansion and functional shifts at transcriptome level when compared to healthy counterparts (**Fig. 1D)**. Finally, the construction of chondrocyte interaction networks through iNMF revealed FCs as emergent hubs in osteoarthritic cartilage within a hormone sensitive network.

DISCUSSION: Through this work, changes in chondrocyte signaling networks and fibrotic reprogramming associated with hormonal loss accompanying menopause were studied. This was achieved by integrating single-cell transcriptomics and proteomics across murine and human datasets. Integrative network analyses positioned FCs as central hubs of intercellular signaling, highlighting their potential role in orchestrating tissue-level responses to hormonal loss. Our findings position the fibrotic chondrocyte subpopulation, FCs, as key mediators of postmenopausal osteoarthritis and highlight hormone-sensitive intercellular communication as a key driver of disease progression in postmenopausal individuals. Further work will focus on validating these findings with histological and cytometric analyses.

SIGNIFICANCE: Elucidating the gene programs associated with key chondrocyte subpopulations (i.e FCs) will aid the understanding of the mechanisms of cartilage degeneration occurring due to menopause induced hormonal shift. Furthermore, the FC-specific surface markers identified in this study can provide new avenues for the development of targeted drug delivery platforms for treating post-menopausal osteoarthritis.

REFERENCES: [1]Gilmer et al. 2025. [2]Matta et al. 2025. [3]Liu et al. 2025. [4]Fan Y et al. 2024. [5]Brooks et al. 2016. [6]Hänzelmann et al. 2013.

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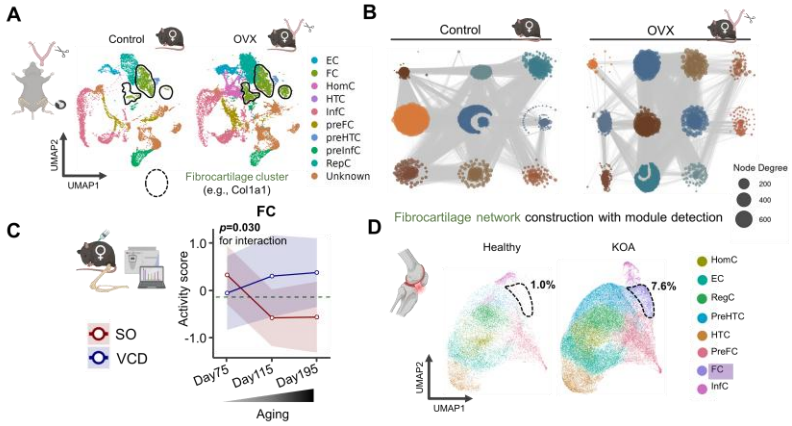

Figure 1: A. UMAP visualization showing nine transcriptionally distinct chondrocyte subpopulations identified in cartilage of OVX model compared to control mice. A relative increase is seen in the fibrocartilage chondrocyte (dotted area) in the OVX group. **B.** Distinct gene modules across control (nine modules) and OVX (eleven modules) identified through co-expression network construction. **C.** UMAP visualization of chondrocyte subpopulations in human arthritic cartilage tissue. **D.** Mass spectrometry-based proteomics from articular cartilage in the knee joint (day 75, 115, 195; n=5/group/time) followed by sample-specific GSVA using chondrocyte subpopulation gene signatures, showing increased activity of FCs in VCD-treated menopausal mice.