Network perturbation predicts shifts in chondrocyte bioenergetic pathways in response to extracellular aging cues

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Introduction: Identifying the causal mediators of aging and tracing their downstream effects in complex, low-turnover tissues—like articular cartilage—remains a significant challenge. Recent advances in omics-based technologies have enabled comprehensive profiling of transcriptomic and proteomic alterations in aging tissues[1,2], including those within the knee joint.[3] Although differential expression analyses are becoming increasingly common in both aging and orthopaedic research, they fail to dynamically simulate the propagation of extracellular perturbations through tissue-specific molecular pathways. Here, we present a computational framework for *in silico* perturbation modeling designed to predict distinct transcriptional responses to age-specific extracellular environmental cues. We exemplify applications of this framework using articular chondrocytes exposed to secretomes derived from infrapatellar fat pads (IFPs)—integral components of the cartilage microenvironment—excised from the knee joints of young and aged animals.

Methods: To establish a foundational model for aging-associated signaling in cartilage, publicly available transcriptomic profiles from knee joints of healthy and osteoarthritic adults[4] were used to construct a weighted gene co-expression network.[5] To identify physiologically relevant perturbation inputs, we performed mass spectrometry-based proteomics on media conditioned with IFPs excised from the knee joints of young and aged male mice (n=4/group), following IACUC approval. We previously found that male IFPs had a greater influence on markers of chondrogenicity in aged chondrocytes.[6] Thus, only IFPs from male mice were used to generate conditioned media. All *in vivo* and *in vitro* studies were performed in accordance with the ARRIVE guidelines to ensure rigor, reproducibility, and transparent reporting. Using the SEPDB secreted protein database[7] and the NicheNet ligand-target model[8], we identified receptors corresponding to secreted proteins identified via mass spectrometry. We then modeled the intracellular propagation of these extracellular signals using the Random Walk with Restart (RWR) algorithm[9] and evaluated the biological relevance of the network inference using Gene Ontology enrichment analysis on ranked gene list outputs. To validate the results from this network model, we benchmarked the *in silico* outputs against RNA-sequencing data from aged chondrocytes treated with IFP-conditioned media that we have previously reported.[6]

Results: A total of 1,294 genes were differentially expressed between healthy and osteoarthritic patients, of which 800 were upregulated and 494 were downregulated in osteoarthritic knee cartilage. This includes the downregulation of chondrogenic genes, such as *Sox9*, and upregulation of catabolic and fibrocartilaginous genes, such as *Mmp13*, *Col1a1*, and *Fap* (Fig. 1A). We then constructed a cartilage-specific transcriptional network using topological overlap matrices and retained the top 50,000 weighted interactions for subsequent analyses. Genes encoding for core components of mitochondrial complex I involved in oxidative phosphorylation, *Ndufb9* and *Ndufab1*, emerged as top hub genes within the network (Fig. 1B). 30 secreted proteins were identified in IFP-conditioned media. After mapping these ligands to their corresponding receptors, we employed the RWR algorithm to simulate the impact of these extracellular ligands on intracellular transcriptional responses and generated a ranked list of genes based on their proximity to receptor seeds in the network. Through GO enrichment analysis of top-ranked genes from the RWR model and differentially expressed genes identified by RNA-sequencing of murine chondrocytes treated with IFP-conditioned media *in vitro*, we identified 50 pathways enriched *in silico* and 46 pathways enriched *in vitro.* Of these, 18 pathways overlapped between the two datasets. Subsequent semantic similarity analysis implicated an additional 9 pathways from the *in silico* dataset as having high functional similarity (semantic similarity score ≥0.70) with the overlapping pathways, leading to reclassification of these pathways as overlapping (Fig. 2). Notably, a substantial portion of the overlapping pathways were associated with mitochondrial dysfunction, including *mitochondrial respiratory chain complex I assembly* and *cellular respiration*, further supporting the biological validity of the computational model in capturing disease-relevant mitochondrial processes.

Discussion: This study presents and validates a computational framework that integrates tissue-specific transcriptomic data, extracellular proteomic inputs, and network-based propagation modeling. This network-based approach enabled us to predict transcriptional consequences of age-associated extracellular cues *in silico* and revealed that mitochondrial dysfunction represents a convergent pathway disrupted in aged cartilage. Using cartilage-IFP crosstalk as an example, our application of the RWR method to simulate signal propagation from extracellular ligands through a gene co-expression network represents a novel approach for modeling paracrine signaling. Unlike conventional pathway enrichment methods, which are limited by predefined annotations, our diffusion-based approach captures context-specific interactions and permits the quantification of ligand-specific influence across diverse biological modules. Compared to existing methods, such as Cytotalk, which rely on cell-cell communication modeling from single-cell transcriptomic data[10], our approach does not require cell-type resolution and, instead, leverages tissue-specific co-expression topology to infer downstream signaling. Collectively, our integrative framework bridges proteomic and transcriptomic layers to uncover how topological positioning of receptors governs tissue responses to paracrine cues.

Significance: The insights from this model advance our mechanistic understanding of joint aging and provide a scalable approach to identify interventional targets in degenerative musculoskeletal disorders. Additionally, this computational pipeline can be extended to other age-related or disease-associated tissues to support hypothesis generation and therapeutic target discovery.
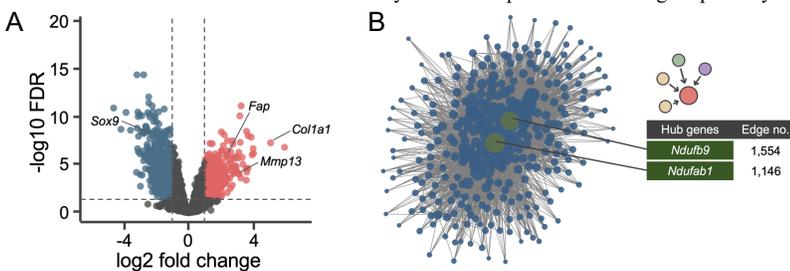

Figure 1: **A.** Volcano plot showing differentially expressed genes between healthy and osteoarthritic knee cartilage. **B.** Weighted gene co-expression network, highlighting top hub genes, *Ndufb9* and *Ndufab1*.

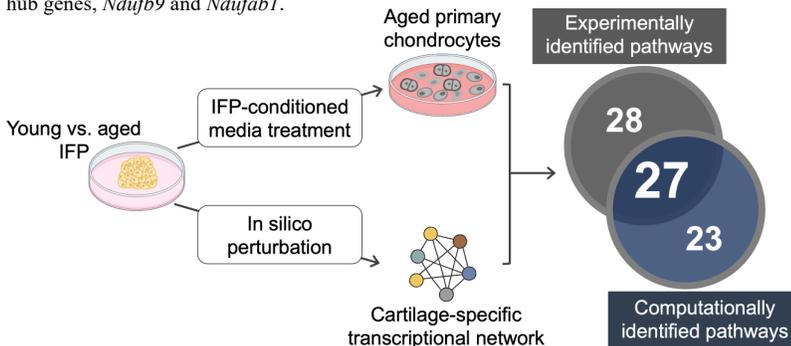| Hub genes	Edge no.
Ndufb9	1,554
Ndufab1	1,146

Figure 2: Schematic overview of comparative analysis between RNA-seq data collected *in vitro* and RWR-based prioritization model. Most overlapping pathways between the two approaches were associated with mitochondrial processes and dysfunction.

References: [1]Huang et al 2025 [2]Takasugi et al 2024 [3]Liu et al 2025 [4]Fisch et al 2018 [5]Langdelf & Horvath 2008 [6]D'Amico, McNeill, & Khay et al 2025 [7]Wang et al 2024 [8]Browaeys et al 2020 [9]Valdeolivas et al 2019 [10]Hu et al 2021