Unbiased Secretome Profiling Reveals the Beneficial Effects of FGF18 on Mouse Knee Articular Cartilage

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INTRODUCTION: Articular cartilage (AC) degeneration is a central driver of osteoarthritis (OA), a condition that imposes a significant global health burden. This degeneration involves the progressive breakdown of the extracellular matrix (ECM), decreased tissue resilience, and loss of homeostatic signaling. While injury-based models in young animals have illuminated many OA mechanisms, they do not fully capture the molecular complexity of age-related cartilage decline. In healthy cartilage, chondrocytes maintain ECM integrity through a balanced network of anabolic and catabolic processes, supported by a secretome rich in structural and ECM proteins, growth factors, and developmental signaling regulators. In youth, this includes fibroblast growth factors (FGF1, FGF2, FGF18), insulin-like growth factor 1 (IGF1), and BMP antagonists such as Gremlin 1 (GREM1), alongside collagens and proteoglycans. These proteins are essential for ECM synthesis, chondrocyte proliferation, and control of hypertrophy. With aging, this regenerative microenvironment declines. FGF18, an FGFR3 agonist with proven chondrogenic activity in injury models, is currently in clinical trials for OA. However, its capacity to restore molecular programs lost with natural age-related pathologies of cartilage remains unclear. We hypothesized that the AC secretome undergoes marked compositional changes with age, leading to the loss of regenerative factors, such as FGFs and GREM1, and that key developmental signals, like FGF18, can reactivate matrix-regulating pathways in aged cartilage, partially restoring a "youth-like" secretome.

METHODS: Male and female mice were used in this study. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) and performed in compliance with NIH guidelines. AC was micro-dissected from femoral condyles and tibial plateaus of young (three-month-old, n=3) and aged (24-month-old, n=3) wild-type mice using a stereomicroscope, with careful removal of menisci and synovium to ensure purity. Explants from each animal were cultured individually in serum-free DMEM/F12 medium for 24 hours at 37°C and 5% CO_2. Supernatants were collected, centrifuged, digested in solution with trypsin, and subjected to label-free quantitative proteomics via liquid chromatography-tandem mass spectrometry (LC-MS/MS) in data-independent acquisition (DIA) mode. Spectra were searched against the UniProt Mus musculus database using DIA-NN. Intensities were $\log_2$-transformed, filtered to retain proteins with ≥2 valid values in at least one group, and compared between cohorts using Student's t-test with Benjamini–Hochberg correction. For rescue experiments, aged cartilage explants were treated with recombinant human FGF18 (500 ng/mL) for 48 hours under identical culture conditions. Untreated AC explants served as control. Next, the explants were washed, transferred to a new culture plate, and cultured in serum-free media without any stimulation for an additional 24 hours. Supernatants were collated from both cohorts and analyzed by LC-MS/MS. Gene Ontology (GO) and Ingenuity Pathway Analysis (IPA) were performed to identify functional pathways altered by aging and modulated by FGF18.

RESULTS: A total of 841 secreted proteins were identified and validated as extracellular via PANTHER classification. Principal component analysis revealed clear separation between young and aged AC secretomes, with aged profiles showing reduced variance and convergence toward a depleted state. Volcano plot analysis revealed 49 proteins were significantly enriched in young AC, including GREM1, IGF1, FGF1, FGF2, and several ECM components. The proteins enriched in the young mouse AC secretome were strongly associated with GO terms for "extracellular matrix organization," "cartilage development," and "regulation of cell proliferation." In contrast, only six proteins were enriched in aged AC secretome, with functional annotation pointing toward inflammatory and oxidative stress processes. IPA indicated substantial downregulation of FGFR3-mediated FGF18 signaling, IGF1 pathways, and BMP antagonism in aged cartilage. Structural ECM proteins and enzymes involved in ECM remodeling were also reduced, suggesting impaired capacity for matrix repair. FGF18 treatment of aged mouse knee joint AC explants induced notable molecular changes. Secretome analysis revealed increased levels of developmental ligands and enhanced expression of anabolic factors, including ECM components, indicating a youthful profile. Matrix turnover markers suggested partial recovery of ECM synthesis. Pathway analysis confirmed reactivation of FGFR3 signaling and downstream chondrogenic programs, indicating that FGF18 can re-engage regenerative networks even in aged tissue.

DISCUSSION: Our study reveals that the secretome of AC from aged mouse knee joints undergoes profound shifts, characterized by loss of key regenerative growth factors and structural ECM proteins. Reduced FGFs, IGF1, and GREM1 are likely to diminish anabolic responses, compromise BMP regulation, and increase susceptibility to mechanical and inflammatory stress. These molecular deficits align with the functional decline observed in aged cartilage and may underlie its poor capacity for repair. FGF18 rescue experiments demonstrate that aged cartilage retains a degree of plasticity in its molecular profile. While proteoglycan synthesis was minimally impacted, developmental transcription factors and collagen composition were favorably modulated, suggesting partial restoration of matrix turnover capacity. These data point to FGF18-FGFR3 signaling as a viable therapeutic target for counteracting aspects of cartilage aging. Further studies with larger cohorts and extended treatment durations will clarify the durability and scope of these restorative effects, as well as whether combining FGF18 with other interventions could yield broader ECM recovery.

SIGNIFICANCE/CLINICAL RELEVANCE: Given OA's prevalence and the lack of disease-modifying treatments, interventions that preserve or restore cartilage homeostasis are urgently needed. This study identifies the loss of FGFR3-mediated FGF18 signaling as a hallmark of aged AC and shows that exogenous FGF18 can partially restore a youthful secretome and chondrogenic signaling profile. These findings highlight a potential pathway for therapeutic intervention in age-related cartilage degeneration, with implications for delaying OA onset and progression.

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