# Cellular Senescence Markers in the Infrapatellar Fat Pad During Knee Osteoarthritis

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INTRODUCTION: Osteoarthritis of the knee (KOA) is a degenerative disease that affects the entire knee joint. KOA is characterized by cartilage loss, chronic pain, and impaired mobility. The infrapatellar fat pad (IFP) cushions the anterior knee as a shock absorber, fills the space between joint structures, and provides patellar stability during movement. It is also a highly vascularized and innervated tissue that produces cytokines, adipokines, and growth factors to regulate knee inflammation and bone remodeling. However, in KOA, it becomes fibrotic, and inflamed, contributing to pain and joint degeneration. Cellular senescence is characterized by growth arrest and the secretion of pro-inflammatory molecules. Senescence has been implicated as a key mechanism driving these changes. Senescent cells in the IFP may play a role in KOA pathology. Understanding how senescence differs between healthy donor samples (HDS) and KOA patients, and how it impacts fibroblast, macrophage, adipocyte, and endothelial cells and cell subtypes can help identify novel therapeutic targets that reduce key KOA pathological processes. We hypothesize that KOA drives distinct senescence-associated transcriptional signatures across IFP cell populations and subclusters, with alterations based on obesity status.

METHODS: We analyzed single-nucleus sequencing and bulk gene expression data from HDS and KOA patients to identify senescence-associated genes. Our analysis interrogated marker expression across both pooled IFP tissue and cell type specific and subcluster specific samples from n=21 individuals, of which there are 9 females in total (n=7 with KOA and n=2 HDS) and 12 males in total (n=8 with KOA and n=4 HDS). Differential gene expression analysis was performed comparing KOA vs HDS. We performed Welch's t-tests across groups. Genes were classified as candidate/suggestive if nominal $p < 0.05$ with $|\log_2 FC| \geq 0.1$; highly confident genes were defined by FDR < 0.1 (Benjamini–Hochberg). Senescence-associated genes were then mapped across fibroblast, macrophage, adipocyte, and endothelial subclusters of the IFP. Heatmaps highlighted senescence-associated genes and their regulation within each cell type. Pathway analysis was done via KEGG, GO, and Reactome in significant and suggestive proteins for KOA vs HDS, and in cell type analysis.

RESULTS: Senescence-related proteins TGFB1, FPR3, CCNE2, and FOXP3 have been identified to be statistically significant between KOA and HDS, as determined by the Benjamini–Hochberg method. When pooling all cell types and comparing KOA-IFP vs HDS-IFP, 30 suggestive senescence marker genes were identified and four senescence marker genes (TGFB1, FPR3, CCNE2, and FOXP3) passed FDR correction (Fig i.). In cell type and subcluster specific analysis, we found fibroblasts (Fig. iia), macrophages (Fig. iib), adipocytes (Fig. iic), and endothelial cells (Fig. iid), to have 31, 27, 21, and 44 significantly differentially expressed senescence associated genes respectively.

DISCUSSION: Senescence is confirmed using multiple biomarkers across different cells and tissues which is stratified as nine different hallmarks of senescence. When analyzing all cell types and comparing KOA-IFP vs HDS-IFP, TGFB1, FPR3, and FOXP3 were associated with the senescence associated secretory phenotype (SASP), while CCNE2 was associated with cell cycle arrest, indicating that KOA-IFP may involve multiple hallmarks of senescence when compared to healthy donor IFP. Also, without comparing by KOA status, genes differentially expressed by fibroblast, macrophage, and adipocyte subclusters within the IFP were associated with SASP. Fibroblast subclusters differentially expressed genes such as IGFBP7, MMP3, VEGFC, and VCAM1 which can be associated with extracellular matrix remodeling, fibrosis, angiogenesis, and inflammatory recruitment, therefore fibroblast subclusters within the IFP may impact chronic inflammation associated with KOA. Macrophage subclusters differentially express SASP components like CD55 and IL18 which are associated with immune dysregulation and persistent inflammation. Additionally, adipocyte subclusters differentially express genes such as IGFBP7, MMP2, and AXL which are associated with disrupted metabolic balance, insulin resistance, and obesity-related inflammation and aging. Within endothelial cell subclusters, IGFBP7, VEGFC, CDKN1A, and NAMPT were highly expressed, and are associated with impaired vascular integrity as well as abnormal vessel growth. Additionally, in endothelial cells, there are significant differentially expressed genes within subclusters related to SASP, cycle arrest, and nuclear changes. Overall, these results suggest that KOA-IFP involves multiple hallmarks of senescence, including SASP, nuclear changes, and cell cycle arrest, across diverse cell types, driving chronic inflammation, tissue remodeling, and metabolic-vascular dysfunction that promote KOA progression.

SIGNIFICANCE/CLINICAL RELEVANCE: The IFP is an active site of senescence associated gene expression in KOA. Candidate genes and subcluster-specific differentially expressed senescence genes provide potential targets for interrupting KOA-specific pathogenesis across distinct populations of fibroblast, macrophage, adipocyte, and endothelial cells in joint degeneration.

IMAGES AND TABLES:

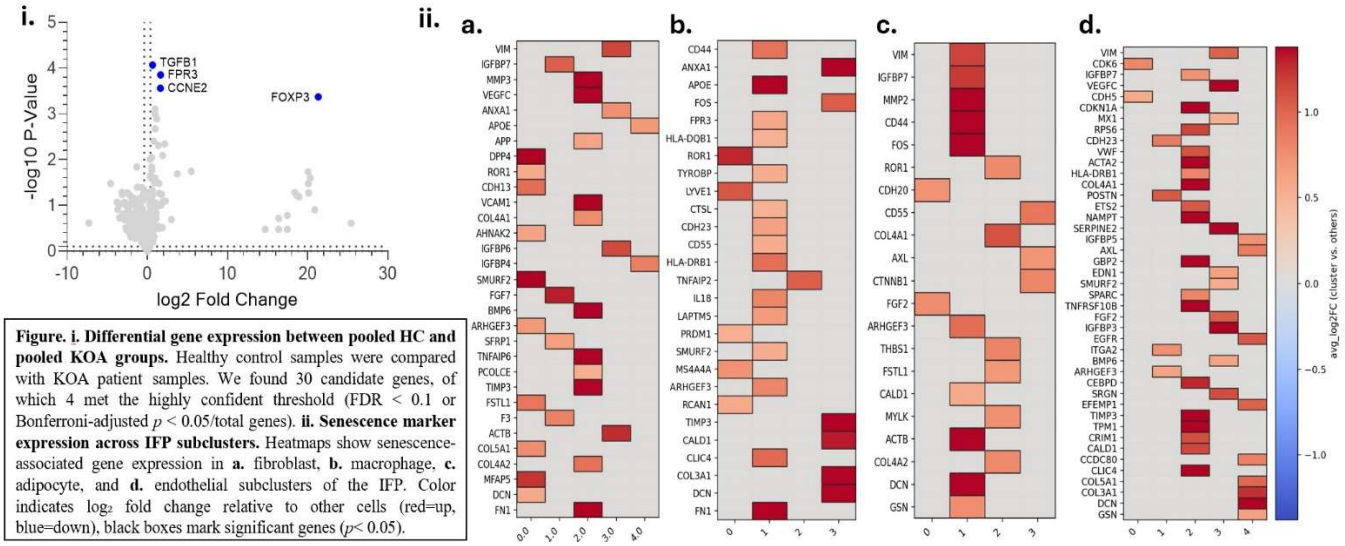


**Figure. i. Differential gene expression between pooled HC and pooled KOA groups.** Healthy control samples were compared with KOA patient samples. We found 30 candidate genes, of which 4 met the highly confident threshold (FDR < 0.1 or Bonferroni-adjusted $p < 0.05$/total genes). **ii. Senescence marker expression across IFP subclusters.** Heatmaps show senescence-associated gene expression in **a.** fibroblast, **b.** macrophage, **c.** adipocyte, and **d.** endothelial subclusters of the IFP. Color indicates $\log_2$ fold change relative to other cells (red=up, blue=down), black boxes mark significant genes ($p < 0.05$).