Differential Burden of Senescence in Synovial Fibroblasts of Females and Males at Total Joint Replacement

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INTRODUCTION: Osteoarthritis (OA) is a major cause of disability worldwide and drives significant reductions in quality of life and loss of productivity. Chronological age is a strong risk factor for OA, but biological sex is a compounding factor with females experiencing a higher rate of disease than males. Cellular senescence of various joint tissues has been suggested as a potential driver of age-related OA, with synovial fibroblasts potentially playing a critical role through production of inflammatory cytokines and matrix-degrading molecules as part of the senescence associated secretory phenotype (SASP) [1]. However, senescence is a highly heterogenous phenomenon; previous work in other cell types has shown that different subtypes of senescence may arise from the same initial stress applied to a homogenous population of cells over time [2]. Further, the reliance on transcriptomic data to define senescent cells is a particular challenge in this context given the increasing divergence of gene expression and protein levels during aging [3]. The development of therapeutics that target senescence in the joint, such as senolytics or senomorphics, would benefit from a greater understanding of senescence heterogeneity and the effect of sex. We hypothesize that overall senescence burden and the precise makeup of subtypes of senescent cells differ between biological males and females, possibly driving the observed difference in overall disease burden. To test this hypothesis, we use an iterative immunostaining method to quantify 12 proteins in thousands of synovial cells for 10 female and 10 male donors.

METHODS: Human knee OA synovial tissue was obtained from patients undergoing total knee arthroplasty for advanced OA (n = 20 evenly split between male and female). Samples were from donors aged 55-81 and were selected such that each "pair" of male and female donor was +/- 2 years apart for to account for potential differences in chronological age. A portion of tissue from each sample was enzymatically digested for isolation of synovial cells and passage purified to passage 3 to enrich for synovial fibroblasts. Cells were preserved in liquid nitrogen until all 20 donors required had been collected. All donor samples were then thawed simultaneously for recovery and re-plated on fibronectin-coated 96-well glass bottom plates for fixation. Using a novel single-cell proteomics technique, iterative indirect immunofluorescence imaging (4i), we profiled each donor in triplicate for 12 proteins known to be associated with senescence and the proinflammatory component of the SASP. Single-cell protein features were then calculated and combined using a custom-built analysis pipeline designed for this purpose. Statistical analysis was performed using paired or nested t-tests as appropriate. Multi-protein single cell data was integrated using Potential of Heat-diffusion for Affinity-based Trajectory Embedding (PHATE) to visualize these high-dimensional data.

RESULTS: Female donors, regardless of chronological age, express a higher level of the senescence and OA-associated protein p16 when compared to male donors (p=0.0016, 10 females vs. 10 males by nested t-test, 3000 cells sub-sampled per donor) (Fig. A). Female donors also expressed higher levels of cytoplasmic IL-8, used here as a proxy for secretion of this inflammatory cytokine, as compared to male donors both in aggregate and when pair-matched by age (p=0.0046) (Fig. B). PHATE as a dimensional reduction tool preserves the spatial relationship between cells based on the similarity of their features. Cells nearer to one another have a higher similarity of the aggregate protein features. When the single-cell measures of SASP proteins (p-p65, IL-6, IL-8, p65, and JAK2) are integrated and projected as a PHATE structure, male and female donors cluster separately (Fig. C). This is also true when performed for senescence proteins more commonly associated with cell cycle arrest (p16, p21, p53, p38, RB, p-RB) (not shown). Together, these findings indicate that synovial fibroblasts from female and male donors have significant differences in the levels of both SASP and cell cycle arrest proteins.

DISCUSSION: Our preliminary findings indicate that biological females have a higher synovial fibroblast senescence burden at end-stage disease than males, regardless of chronological age. This increased senescence burden is paired with an increased pro-inflammatory SASP, which could drive OA through signaling between the synovial fibroblasts and chondrocytes. This higher senescence burden at end-stage disease is possibly a result of a more rapid accumulation or slower clearance of senescent cells over the lifespan, potentially leading to the observed higher incidence of knee OA and the earlier average onset. Further work is ongoing to evaluate differences in oxidative stress and DNA damage-associated proteins within this same cohort of donors. We expect that subsequent integration of all single-cell measurements will offer insight into the subtypes of senescent cells present, their relative prevalence, and their likely effect on neighboring tissues.

SIGNIFICANCE/CLINICAL RELEVANCE: Attempts to leverage the link between cellular senescence and OA for disease-modifying therapeutics have so far been unsuccessful. We have identified a significant difference in senescence burden between males and females, with the potential for distinct subsets that emerge from integrating multiple protein-based readouts. A better understanding of the biology underlying these differences may lead to more effective targeting of specific populations of disease-driving senescent cells.

REFERENCES: [1] Diekman BO & Loeser RF. Aging and the emerging role of cellular senescence in osteoarthritis. *Osteoarthritis and Cartilage* 32(4):365-371 (2024). [2] Sessions, GA, Loops, MV, Diekman, BO & Purvis, JE. Multiplexed single-cell imaging reveals diverging subpopulations with distinct senescence phenotypes during long-term senescence induction. *GeroScience* (2025). [3] Ding et al. Comprehensive human proteome profiles across a 50-year lifespan reveal aging trajectories and signatures. *Cell* (2025).

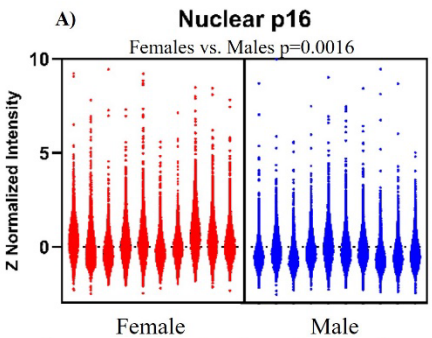

Figure A. Z normalized intensity of nuclear p16 in primary human synovial fibroblasts taken from 10 male and 10 female donors.

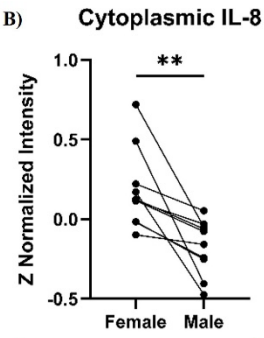

Figure B. Pairwise comparison of cytoplasmic IL-8 in primary human synovial fibroblasts. Plotted as mean of 3000 cells. Lines connect age matched pairs +/- 2 years between male and female.

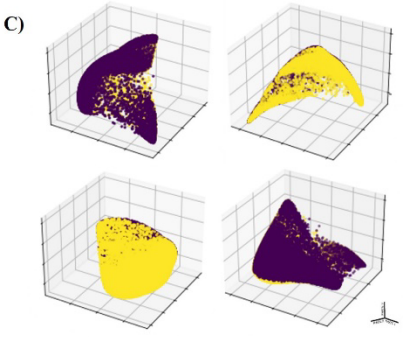

Figure C. Four 90-degree rotations of a 3D PHATE plot of SASP proteins showing the clustering of individual male (yellow) and female (purple) cells in a dimensionally reduced space.