

# Integrative scRNA-seq and spatial transcriptomics uncovers distinct macrophage-fibroblast cross-talk in human hip synovium between patients with femoroacetabular impingement and osteoarthritis

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**INTRODUCTION:** Hip osteoarthritis (OA) affects one in four people by the age of 85, and it is linked to abnormal hip morphology including Cam-type femoroacetabular impingement (FAI)<sup>1</sup>. During flexion and internal rotation of the hip, the osseous protrusion from femoral head-neck junction impinges on the acetabulum, inducing cartilage delamination and leading to the development of hip OA<sup>2</sup>. Thus, FAI has been considered as a unique early-phase hip OA model for studying regulators implicated in disease progression. It is well-documented that inflammatory cytokines derived from the synovial cells such as fibroblasts and macrophages can promote OA in the knee. However, the unique synovial cytokine profiles between knee and hip indicate that hip OA is immunologically distinct from knee OA<sup>3</sup>. Thereby, synovial cellular composition and cell-cell crosstalk in human hip OA synovium is yet to be determined. By exploring disease markers and synovial cell-cell interactions across different disease status (i.e., FAI vs. hip OA), we will better understand underlying signaling pathways and predict potential targets for hip OA treatments. In this study, we hypothesize that synovial tissues from FAI and hip OA patients exhibit distinct transcriptomics and cell-cell interactions. We aim to identify the underlying molecular mechanisms underlying hip OA pathogenesis from FAI by integrating innovative single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics (Spatial-seq) approaches.

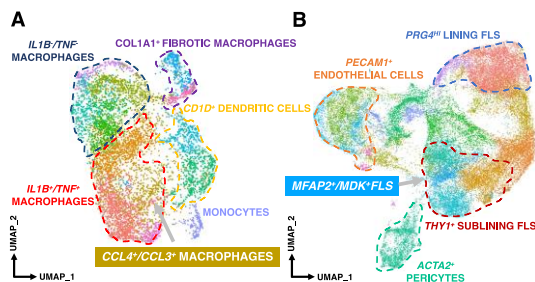
**METHODS:** De-identified human hip synovial tissues were harvested from patients diagnosed with Cam FAI or OA (secondary to FAI) according to approved IRB protocols at the University of Rochester (UR). Tissue samples (five FAI and three OA patients) were submitted to UR Genomics Research Center for scRNA-seq and Spatial-seq (10X Genomics). Distinct cell populations were annotated based on unbiased clustering and expression of differentially expressed genes (DEGs), and later mapped to Spatial-seq datasets using Seurat R package<sup>4</sup>. Cell-cell crosstalk (i.e., ligand-receptor interaction) and downstream activated genes were identified by MultiNicheNet R package<sup>4</sup>. Functional analysis for identified cell subsets was annotated by Gene Ontology (GO) term<sup>5,6</sup>.

**RESULTS:** Upon unsupervised clustering myeloid cells, NK cells, non-hematopoietic cells, and endothelial cells were identified as major conserved cell groups between FAI and hip OA synovial tissues (data not shown due to limited space). Subsequent clustering of  $CD45^+/CD14^+$  cells further yielded 5 distinct cell types including pro-inflammatory macrophages (MΦ), anti-inflammatory MΦ, and fibrotic MΦ, and dendritic cells (Fig. 1A). Re-clustering  $CD45^-$  non-hematopoietic cells resulted in 4 different cell groups: lining and sublining fibroblast-like synoviocytes (FLS) as well as endothelial cells and pericytes (Fig. 1B). Compared to FAI synovium, OA synovium exhibited 1.4-, 1.75- and 2.65-fold increase in  $CCL4^+/CCL3^+$  macrophages (Fig. 1A),  $MFAP2^+/MDK^+$  sublining FLS, and endothelial cells (Fig. 1B), respectively. Cell populations identified by scRNA-seq were then mapped to Spatial-seq datasets to visualize their spatial locations in the FAI and hip OA synovium. For example, we observed that  $CCL4^+/CCL3^+$  macrophages and  $MFAP2^+/MDK^+$  FLS are spatially adjacent to each other, suggesting potential cell-cell interactions between these two populations (Fig. 2). Using MultiNicheNet R package, we identified that CSF1-SIRPA (Signal Regulatory Protein  $\alpha$ ) signaling pathway between  $CCL4^+/CCL3^+$  MΦ and  $MFAP2^+/MDK^+$  FLS activates distinct downstream gene sets across different disease status (i.e., FAI vs. hip OA synovium) (Fig. 3).

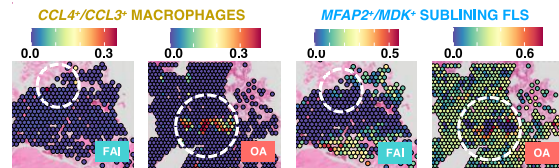
**DISCUSSION:** Our scRNA-seq analysis revealed unique transcriptomic patterns in the synovial cells between FAI (i.e., early-phase OA) and hip OA patients. The finding of significantly increased endothelial cells in OA vs. FAI synovium is supported by the evidence of increased vasculature in OA<sup>7</sup>. Increased vasculature in OA synovium may promote infiltration of inflammatory myeloid cells into the synovium, promoting joint inflammation and disease progression. Furthermore, investigating cellular interactions along with their activated downstream genes allows us to elucidate not only the dynamic changes in the cell-cell crosstalk but also their potential implications during OA progression at the molecular level. As a proof of concept, we identified that  $CCL4^+/CCL3^+$  MΦ may modulate inflammation and cell survival of synovial  $MFAP2^+/MDK^+$  FLS in hip OA by activating CXCL8, IL17R, and BCL2A1 via CSF1-SIRPA signaling pathway. This result aligns with the previous studies reporting that CSF1 secretion may promote the survival, proliferation, and differentiation of macrophages, monocytes and osteoclasts, and its inhibition leads to a decreased inflammation in human RA synovium<sup>8</sup>. However, the role of CSF1 ligand in hip OA pathogenesis is yet to be determined. Interestingly, ANXA4 (Annexin-4), CMKLR1 (Resolvin E1 Receptor), and *TNFSF10* were predicted as activated genes downstream of CSF1-SIRPA pathway between  $CCL4^+/CCL3^+$  MΦ and  $MFAP2^+/MDK^+$  FLS in the FAI synovium. Our GO analysis suggests these three genes are involved in negative regulation of NF- $\kappa$ B signaling, providing a possible explanation why FAI synovium is less inflamed versus OA synovium<sup>9</sup>. Our results further suggest that targeting distinct signaling molecules at different disease stages may be required to prevent hip OA progression from FAI.

**SIGNIFICANCE/CLINICAL RELEVANCE:** Approximately a quarter billion patients worldwide has been diagnosed with hip OA. We believe that our findings will reveal key genetic markers related to hip OA progression and facilitate the development of targeted drug deliveries for therapeutic applications.

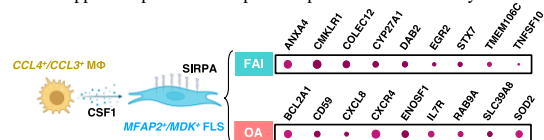
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**Fig. 1:** (A)  $CD45^+/CD14^+$  cells:  $IL1B^+/TNF^+$ ,  $IL1B^+/TNF^-$  and  $COL1A1^+$  macrophages, monocytes, and dendritic cells. (B)  $CD45^-$  cells:  $PRG4^{high}$  lining FLS,  $THY1^+$  sublining FLS,  $ACTA2^+$  pericytes, and  $PECAM1^+$  endothelial cells. (A) and (B) are conserved cell populations between FAI and OA synovium. Note  $CCL4^+/CCL3^+$  macrophages and  $MFAP2^+/MDK^+$  sublining FLS were significantly increased in the OA synovium.



**Fig. 2:**  $CCL4^+/CCL3^+$  macrophages and  $MFAP2^+/MDK^+$  sublining FLS are located close to each other when mapped to spatial transcriptomic spots in FAI and OA synovium.



**Fig. 3:** Activated genes downstream of CSF1-SIRPA signaling pathway between  $CCL4^+/CCL3^+$  macrophages and  $MFAP2^+/MDK^+$  FLS that are upregulated in FAI (top) and OA (bottom). More intense color of the dots represents stronger support of downstream activation by prior literature. Larger size indicates stronger correlation between L-R interaction and target genes.