Spatial Transcriptomics and Canonical Correlation Analysis Reveal Cellular Niches in Human Ankle Synovium

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INTRODUCTION: Osteoarthritis (**OA**) of the ankle is a debilitating disease, influenced by patient characteristics and tissue-specific biology, particularly the synovium of the ankle. Recent work has identified a significant shift in the genetic profile of synovium from patients with minimal signs of ankle OA versus those with end-stage ankle OA using bulk RNA-sequencing [1]. However, bulk RNA-sequencing loses the spatial and cellular context describing the native cellular environment/niches that could improve understanding of synovial biology. Spatial transcriptomics (**ST**) has emerged as a transformative technology to improve spatial resolution to near single-cell resolution [2], yet it has not been used for ankle synovium. Most ST analyses, however, do not incorporate the histological detail of the nucleus and cellular insights in routine hematoxylin and eosin (**H&E**) images in ST pipelines. Computationally integrating these genetic profiles with the cellular and nuclear morphology can be exploited by using canonical correlation analysis (**CCA**). *This study aimed to identify spatially informed cellular niches and to link genetic and histological nuclear/cellular morphology using ST and CCA, respectively, in the synovium of ankle OA.*

METHODS: *IRB-Approved Tissue Collection:* Synovium was harvested from patients (n = 3 female; n = 5 male) undergoing routine ankle surgeries and assigned to OA severity using Outerbridge (OB) scores: healthy (n = 2; OB 0), mild OA (n = 2; OB 1), and end-stage OA (n = 4; OB 4). *ST Analytical Pipeline:* After H&E staining and imaging at 20x magnification on a slide scanner (Zeis Axioscan), synovium was processed for the standard ST pipeline using the 10x Genomics Visium V2 platform (spot size ~50 µm). Sequenced data were then imported, cleaned, and analyzed with Seurat [3] in R to identify cellular clusters or "niches" and their shared genetic profile across all samples. The color of H&E images was standardized using Python [4]. *Extracting Nucleus, Cytoplasmic, and Cellular Morphology and Staining Intensities:* To link the H&E morphology and staining information of the nucleus, cytoplasm, and cell (nucleus plus cytoplasms) to the genes from each spot in ST, spatial-specific boundaries (i.e., spot size) from ST were imported and overlaid on standardized H&E images using QuPath software [5]. Next, standard nucleus detection with shape and intensity measurements (i.e., nucleus, cytoplasmic, and cellular size/shape and hematoxylin and eosin staining intensity) was performed in QuPath. *CCA Data Integration:* Exported per-spot nucleus, cytoplasmic, and cellular from H&E images and ST-derived gene counts were standardized and integrated using CCA in Python programming to correlate features between both datasets [2, 6].

RESULTS: ST analysis identified 1480 shared spatial variant genes leading to 7 distinct cellular "clusters/niches" in ankle synovium across healthy, mild OA, and end-stage OA. Qualitatively (Figure 1A), these clusters appear spatially around the lining layer versus the subintima and between dense connective tissues versus loosely/capillary-rich tissue with fatty-like tissue. Quantitatively (Figure 1B), the shift of clusters from healthy to end-stage indicates a change in phenotypical expression from healthy synovium (cluster 5), predominantly end-stage (cluster 0), and a combination of stages. While numerous genes are defining the cellular clusters there were a few notable up and down genes (up/down) that were inversed for some clusters: cluster 0 (TRPV4/ADAMTS15); cluster 1 (SELE/RGS2); cluster 2 (MMP1/GPR68); cluster 3 (ANGPTL7/MMP9); cluster 4 (GPR68/NEURL2); cluster 5 (ADAMTS15/GPR68); and cluster 6 (ACAN/NEURL2). CCA showed positive and negative associations between genes and histological measures of nucleus, cell, and cytoplasm (Figure 2).

DISCUSSION: Overall, ST analysis and CCA highlighted a set of gene signatures that characterize spatial niches in ankle OA synovium. ST and CCA results appear to converge on similar biological processes. These include, and are not limited to, immune signaling and information, extracellular matrix and tissue remodeling, chromatin remodeling, mitochondrial metabolism, neural signaling, cell cycle progression, and cell apoptosis. CCA uncovered histology-linked genetic information: 1) large, fibroblast-shaped, heterogeneous cells (eosin heterogeneity, eccentricity, large nuclei) associated with KIF17, MORC1, and MUC16, and 2) those that are smaller, denser, and more eosinophilic, associated with low MT-ND1, LENG8, and DDAH2. Ongoing work is exploring other computational approaches like ST multi-vIEW representation learning [2] to uncover spatially informed insights driving these spatial niches, as well as merging additional multi-omics and histological datasets. Uncovering the ankle synovium's complex biology will require a holistic approach with ST and CCA.

SIGNIFICANCE/CLINICAL RELEVANCE: ST and CCA uncovered spatially informed cellular niches characterized in the ankle synovium that span healthy and OA severity. Spatially informed cellular niches will help to reveal the next generation of therapeutic targets in the synovium for ankle OA.

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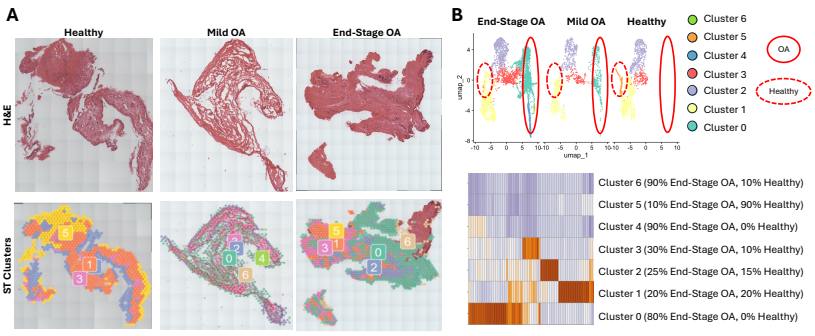

Figure 1. ST analysis demonstrates qualitatively (A) and quantitatively (B &C) a significant shift in the presence of specific clusters from healthy to end-stage OA. The Uniform Manifold Approximation and Projection (UMAP) plot and gene heatmap show genes driving the separation that describe the phenotypes.

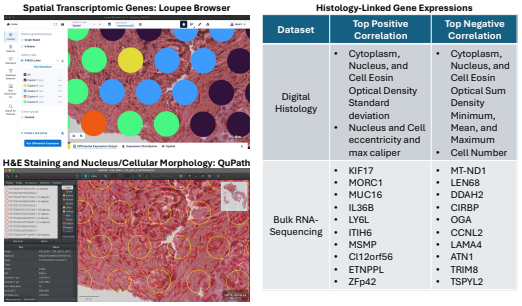

Figure 2. Example of synovium spot-sizes with ST in Loupe Browser (from 10x Genomics) and nucleus/cellular insights in QuPath software; including the H&E-based morphology and staining intensity with per-spot ST gene counts, yielded distinct genes that were positively and negatively associated with histology features and genes.