Osteoarthritic Synovium and Systemic Sclerosis Skin Share Conserved Fibroblast Transcriptome Patterns

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INTRODUCTION: Fibrosis of the synovium is a hallmark of osteoarthritis (OA), where it is a primary clinical correlate to joint pain[1], while fibrosis of skin, lungs and other organs is the hallmark of systemic sclerosis, SSc. Both OA synovium and SSc skin exhibit extracellular matrix changes, ectopic chondrogenesis and mineralization, nociceptive sensitization, and focal inflammatory infiltrates. Studies directly comparing the pathophysiology of these two conditions are lacking entirely, but involvement of multiple conserved pathomechanisms suggest that these diseases may share common cellular and molecular patterns. SSc fibroblasts from affected tissues and organs have been shown to display well-conserved patterns of fibroblast signaling and differentiation[2,3], but these studies did not include OA synovium in their comparisons. Thus, the nature and extent of conserved fibroblast subsets and their associated signaling patterns in OA synovium and SSc skin remains uncharacterized. Here, we utilized human single cell RNA sequencing (scRNA-seq) data to compare fibroblast gene expression patterns in OA and SSc tissues.

METHODS: A systematic literature review was conducted to identify and aggregate scRNA-seq datasets from human OA synovium and SSc skin. In total, 4 published datasets each for OA and SSc were identified and incorporated into an integrated analysis. Within each disease, scRNAseq data from individual studies were imported into R and integrated using the Seurat package. Each dataset was filtered to exclude multiplets and low-quality cells. The remaining cells were clustered and identified. Fibroblast populations from OA and SSc datasets were isolated using a panel of pan-fibroblast marker genes (e.g. PDGFRA$^+$, PDGFRB$^+$, VIM$^+$) and re-integrated into a joint data object comprising all fibroblasts from both OA and SSc datasets. The resultant fibroblast object was clustered into subpopulations with distinct gene signatures. Differential abundance analysis between OA and SSc was performed using the Milo R package. Conserved and differentially-expressed gene markers (DEGs) were calculated within each subcluster, with genes expressed at $P_{adj} < 0.05$ retained for further analysis. DEG lists were submitted to Metascape[4] for gene ontology (GO) analysis. Trajectory analysis was performed independently within OA and SSc datasets using the Monocle R package to predict differentiation trajectories. To determine the root node for this analysis, a gene module was calculated for the gene panel (PI16$^+$, DPP4$^+$, CD34$^+$, CD248$^+$, FBN1$^+$), defining a population of fibroblasts previously characterized as pan-tissue progenitors[2,5], and the root node was placed within this population of cells.

RESULTS: In total, 13,761 and 12,203 fibroblasts were analyzed from human OA and SSc datasets, respectively. Clustering analysis of these datasets revealed 9 subclusters with distinct gene signatures (Fig. 1A,B), labeled according to their top 2 gene markers. Differential abundance analysis of these subclusters revealed 1 distinct cluster unique to OA that express markers of synovial lining fibroblasts (PRG4$^+$, THY1$^-$)[5], and three distinct clusters unique to SSc (Fig. 1C). Both SSc-specific and OA-specific clusters expressed genes involved in GO terms related to Wnt signaling, while SSc-specific clusters also expressed genes involved in RHO/ROCK signaling, both of which are known to be contributory to fibrosis[6,7] (Fig. 1D right). Five subclusters were shared between diseases; GO analysis of conserved genes (Fig 1D left) within two of these clusters (APOE$^+$, CXCL12$^+$ and CXCL2$^+$, CXCL3$^+$ clusters) revealed GO terms related to inflammatory and immune signaling, primarily via cytokine and chemokine pathways including TNF and IL18, suggesting these inflammatory pathways are well-conserved between OA and SSc in these clusters. Two conserved clusters (SFRP1$^+$, COL1A1$^+$ and IGFBP6$^+$, MFAP5$^+$) expressed genes within GO terms related to matrisome secretion, while also participating in disease-related signaling pathways (angiogenesis and RHO/ROCK signaling, respectively), suggesting that matrix deposition in fibrosis may also be well-conserved between OA and SSc fibroblasts, and may be influenced by disease-related signaling. Trajectory analyses of both OA and SSc fibroblasts predicted nodes within the subpopulation of cells expressing progenitor gene markers (Fig. 1E), consistent with pan-tissue fibroblast progenitors[2,5], which were used as the root nodes for the analyses. Clusters unique to OA and SSc had the highest predicted pseudotime values, with most also containing a terminal node, suggesting that these clusters represent distinct fibroblast differentiation endpoints. SSc-specific clusters were predicted to arise from progenitors via differentiation through the APOE$^+$ and CXCL2$^+$ clusters (associated with cytokine/chemokine secretion and inflammatory signaling). In OA, multiple differentiation trajectories were predicted, suggesting that no single obvious trajectory predominated, though multiple potential routes through the APOE$^+$ and CXCL2$^+$ clusters were consistent with predicted SSc fibroblast trajectories.

DISCUSSION: This comparative analysis revealed similarities between the OA and the SSc fibroblast transcriptome. Strikingly, most fibroblast subtypes were conserved between the two diseases, including those that secrete matrix molecules and pro-inflammatory cytokines. This indicates that both excessive collagen deposition and pro-inflammatory signaling in OA and SSc may arise through fibroblast subsets shared between the two. Moreover, both OA and SSc exhibited unique, specialized fibroblast clusters, but disease-associated signaling pathways including Wnt and RHO/ROCK signaling were associated with these clusters in both diseases[5,6]. Monocle further predicted that differentiation trajectories towards disease/tissue-specific fibroblast subtypes in OA and SSc may share similarities. Formulating a comprehensive understanding of similarities and differences between these two distinct conditions associated with fibrotic and inflammatory processes can facilitate cross-utilization and development of novel therapeutic paradigms.

SIGNIFICANCE: Understanding conserved patterns of fibrosis in OA and SSc could uncover novel paradigms for fibrosis *per se*. This knowledge could be used to guide the development of fibrosis treatments which target conserved pathways and are therefore more likely to be effective against multiple diseases.

REFERENCES: [1]Collins+ 2016; [2]Buechler+ 2021; [3]Lendahl+ 2022; [4]Zhou+ 2019; [5]Knights+ 2022; [6]Santos and Lagares 2018; [7]Beyer+ 2013

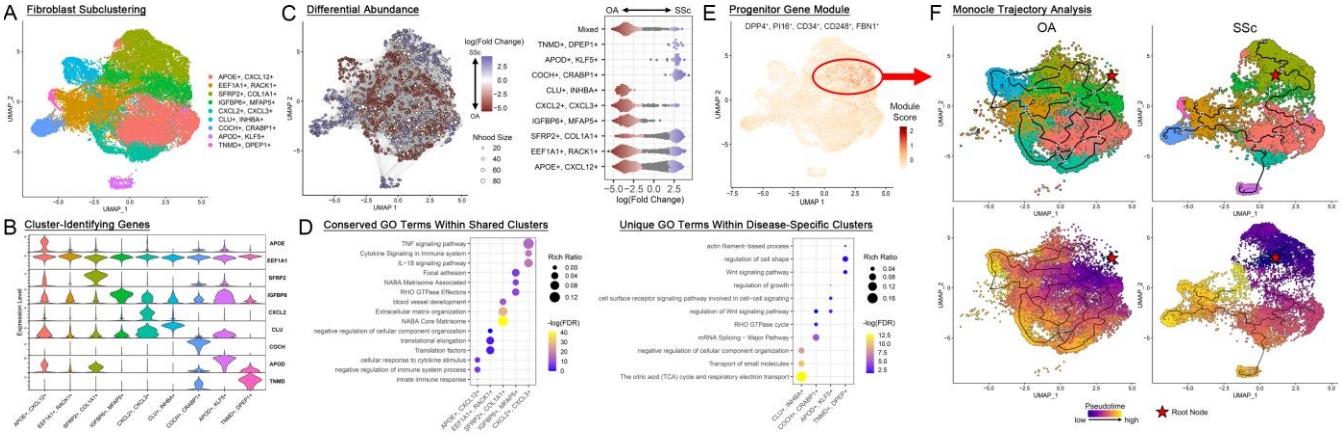

Fig 1. *(A) UMAP of integrated OA and SSc fibroblasts. Clusters were named based on their top 2 cluster-specific genes. (B) Violin plot of top cluster-specific genes. (C) Differential abundance analysis, stratified by cluster. (D) Left - top 3 GO terms from conserved gene markers within each cluster, right - top 3 GO terms from cluster-specific gene markers within each disease-specific cluster. (E) Gene module defining pan-tissue fibroblast progenitors. (F) Monocle trajectory analysis.*