Single cell and spatial transcriptomics in human tendon disease indicate dysregulated immune homeostasis

Tendinopathy; encompassing multifactorial tendon disorders characterised by pain and functional limitation remains a significant burden in musculoskeletal medicine.1 Recent findings highlight a key role for immune mediated mechanisms in tendon disease supporting the concept that pivotal immunological and biomechanical factors conventionally associated with inflammatory rheumatic and musculoskeletal diseases (RMDs) are manifest in tendon.2 Single cell technologies3 (scRNAseq) are increasingly applied in rheumatology to identify key cellular phenotypes that drive disease pathogenesis. Despite efforts with small cell numbers and heterogenous tendon biopsies4 there remains no detailed spatial tendon cell atlas to inform translational targeting. Herein, for the first time utilising scRNAseq and spatial transcriptomics (S2), we carry out cell–cell interaction analysis to build an atlas of the dynamic cellular environment that drives the development of chronic human tendon disease.

In healthy (4 biopsies, n=3040 cells) and diseased (5 biopsies, n=19084 cells) tendon we find a mix of endothelial, immune and stromal cells (figure 1A, online supplemental file 1). Each cell type group is present in disease and healthy tissue but with distinct quantitative and qualitative characteristics. Within stromal populations we identified ‘mural type’ stromal cells (figure 1B). Mural cells, which include pericytes, are possible progenitor cells in tendon5 and interestingly, these cells are phenotypically similar to NOTCH3 high mural cells described in rheumatoid arthritis (RA) synovium which can differentiate into fibroblasts following interactions with endothelial cells (ECs) via JAG1.6 Cell–cell interaction and S2 analysis indicate a similar phenomenon could occur within tendinopathy between mural cells and SEMA3G ECs (figure 1F). In all diseased stromal cell populations, there was greater expression of genes for extracellular matrix proteins (eg, COL1A1, COL3A1, FN1, BGN) which is considered the hallmark feature of tendinopathy.

Seven subtypes of PECA1+ ECs were found (figure 1D), including a population of LYVE1+ ECs that produce CCL21 and have been shown to regulate dendritic cell (DC) migration.7 Furthermore, CCL21 is upregulated in these cells in tendinopathy (online supplemental figure S2B). DCs comprise the single largest immune cell population present in normal tendon (figure 1B). Intriguingly, DCs are also present in diseased tendon however, showing therein greater levels of DC activation and lower levels of C1Q genes (regulatory DC markers) (online supplemental figure S2D,E). The activation of DCs and subsequent T cell activation8 in tendinopathy is further evidenced by pathway analysis of differentially upregulated genes in disease (online supplemental figure S3D,E). This activation may in part be due to increased matrix protein expression, such as FN1, which can activate DCs and resulting in alterations in T cell populations within tissue potentially contributing to mechanisms driving disease chronicity. Additionally, we found three populations of macrophages in diseased tendon, one of which, cycling macrophages (figure 1C, online supplemental figure S2A) is unique to diseased tissue. The transcript profile of macrophages in normal tendon most closely resembled tissue repair and debris clearance (figure 1E, online supplemental figure S2D). Within normal tendon we found APP expression in tenocytes which can induce a resolution promoting phenotype in macrophages. However, APP expression was reduced in the stromal compartment in disease coinciding with diminished expression of LYVE1 within diseased tissue macrophages (figure 1E, online supplemental figure S2D). These macrophage subsets have recently been associated with RA remission and we postulate the phenotypic drift away from this phenotype promotes aberrant tissue repair and attendant tendinopathy.9

Further evidence suggesting that the stromal environment may induce inflammatory changes comprises increased expression of MIF (figure 1E, online supplemental figure S2B) in diseased tenocytes that can induce proinflammatory effects via its receptor CD74, which is also upregulated in macrophages from diseased tendon (figure 1E, online supplemental figure S2C). S2 generated indicative data from cell–cell interaction analysis suggests stromal induced immune regulation. As such, we postulate the primary role of the immune compartment within the tendon is to regulate and resolve damage; however, following cumulative microtrauma the fundamental process of debris removal and matrix repair initiated by tenocytes could lead to positive amplification of the immune compartment. We further propose that within diseased tendon immune homeostasis may become imbalanced and activated immune cells, primed by both endothelial and stromal cells, promote a cycle of inflammation and aberrant tissue repair. The inflammatory environment, including cytokine pathways that are unequivocally demonstrated in this preliminary tendon atlas, have been targeted to yield potent immunological interventions in a range of inflammatory RMDs—the potential to target and investigate these pathways in human tendon disease is now compelling.

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Figure 1  Cell composition and interactions of healthy and diseased human tendon. (A) Normal (n=4, human hamstring tendon) and diseased (tendinopathy, n=5, human supraspinatus tendon) human tendon were processed for single cell analysis using Chromium 10x 3′DEG chemistry. Infographic shows number of donors and cells sequenced. Uniform manifold approximation and projection (UMAP) embedding of 22,124 single cells delineating endothelial, immune, stromal tenocyte and stromal mural cells with marker genes. (B) Stromal cells of the tendon. UMAP embedding with gene markers and distribution of seven delineated stromal cell populations from human tendons; mural tenocyte (mT), normal tenocyte1 (nT1), normal tenocyte2 (nT2), diseased tenocyte1 (dT1), diseased tenocyte2 (dT2), diseased tenocyte3 (dT3) and diseased cycling tenocytes (dTc). (C) Immune cells of the tendon. UMAP embedding with gene markers and distribution of 6 delineated immune cell populations from human tendons; dendritic cells (DC), macrophage1 (Macro1), macrophage1 (Macro2), cycling macrophage (Macro-C), T-Cells1 (T-Cell1) and T-Cells2 (T-Cell2). (D) Endothelial cells (EC) of the tendon. UMAP embedding with gene markers and distribution of seven delineated EC populations from human tendons; CD36 high EC (CD36EC), E-Selectin EC (CD62EC), collagen 4 vessel EC (COL4aEC), immune-like EC (ImmuneEC), LYVE1 positive EC (LYVE1EC) and SEMA3G positive EC (SEMA3GEC). (E) Tenocyte–immune interactions in tendon (n=3 healthy, vs 4 diseased). Predicted cell–cell interactions using CellphoneDB statistical framework on human tendon immune and stromal cells. Selected ligand–receptor interactions showing APP and MIF ligand–receptor pairs in tendon stromal and immune cells. Mean of combined gene expression of interaction pairs (Log2 mean) and p value of specificity of interactions. Violin plots of APP and MIF expression in tendon stromal cells from healthy (pink) and diseased (green) tendon. Spatial expression (log2FC) of stromal APP and macrophage LYVE1 in normal human tendon and stromal MIF and macrophage CD74 in tendinopathic tendon visualised on 10x Genomics visium data, boxes highlight areas of coexpression. Violin plots of LYVE1 and CD74 expression in immune cells from healthy (pink) and diseased (green) tendon. Biorender infographic summarising tenocyte–immune cell interactions in tendon disease. (F) EC–tenocyte interactions in tendon. Predicted cell–cell interactions using CellphoneDB statistical framework on selected human tendon endothelial and stromal cells. Ligand–receptor interactions showing NOTCH3 ligand–receptor pairs in tendon endothelial and stromal cells. Mean of combined gene expression of interaction pairs (Log2 mean) and p value of specificity of interactions. Violin plots of NOTCH3 and Jag1 expression in tendon stromal and ECs, respectively from healthy (pink) and diseased (green) tendon. Spatial expression (log2FC) of NOTCH3 and MCAM from mural tenocytes and SEMA3G and JAG1 from SEMA3GEC’s in human diseased tendon visualised on 10x Genomics visium data, boxes highlight areas of coexpression. Biorender graphic of predicted SEMA3GEC and mT interaction in human tendon.
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