Exploring the Cellular and Molecular Landscape of the Healthy Human Patellar Tendon at a Single-cell Resolution

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INTRODUCTION: Patellar tendinopathy is a common but debilitating disorder, particularly in jumping athletes, with limited effective treatment options (Marigi et al., 2022). Current literature suggests that the healthy human patellar tendon is composed of tendon stem cells, tenocytes, and mature fibroblasts that secrete extracellular matrix proteins to maintain its structure. With the advent of single-cell/single-nucleus RNA sequencing (scRNA-seq/snRNA-seq) technologies, high-resolution mapping of the cellular composition and molecular mechanisms of cell types present in the tissues and organs of the human body has become possible. However, analysis of the cellular and molecular features of the healthy patellar tendon at single-cell resolution has not been reported. Using snRNA-seq, this study establishes a reference single-cell atlas of healthy human patellar tendon to further the understanding of cellular homeostasis and the cellular basis for disease.

METHODS: After institutional board review, informed consent was obtained from all study participants. Patellar tendon samples (n=4, [3 males, 1 female], mean age= 25) were collected from patients undergoing anterior cruciate ligament reconstruction with a bone-patellar tendon-bone autograft. Samples were snap-frozen and stored at -80°C. Snap-frozen samples were cut on dry ice and underwent a single-nuclei isolation method using a previously published protocol (J. Mimpen et al., 2021). Isolated nuclei were counted and loaded onto the Chromium Next GEMX Chip (10X Genomics) with a targeted nuclei recovery of 10,000-20,000 nuclei per sample. Samples were then loaded onto the Chromium iX Controller (10X Genomics) and libraries were prepared using the Chromium GEM-X Single Cell 3' Reagent Kits v4 (10X Genomics) according to the manufacturer's instructions. Quality control of cDNA and final libraries was analysed using High Sensitivity ScreenTape assays on a 4150 TapeStation System (Agilent). Final libraries were pooled and sequenced on a NovaSeq X (Illumina) sequencer by the Weill Cornell Genomics Core at a minimum depth of ~20,000 read pairs per expected nucleus.

RESULTS: Following data processing and quality control, the Harmony-integrated snRNA-seq data from 60,878 nuclei were clustered and annotated, identifying 11 clusters of distinct cell types (**Fig. 1A**). The most abundant population was fibroblasts, which subclustered into three transcriptionally distinct subgroups: *PRG4*hi*CRTAC1*hi, *LAMA2*hi*FBLN1*hi, and *LAMA2*hi*NEGR1*hi fibroblasts, each characterized by unique and shared differentially expressed canonical markers. Other identified clusters include endothelial, mural, immune, nervous system cells and adipocytes. Endothelial populations comprised both vascular (*PECAM1*, *VWF*) and lymphatic (*PROX1*, *MMRN1*) cells, while mural cells (vascular smooth muscle cells and pericytes) expressed *PRKG2* and *RCAN2*. The immune cell populations were broadly diverse, including tissue-resident macrophages, T-cells (*SKAP1*, *CD3E*), and granulocytes (*KIT*, *HPGDS*). Other stromal cells identified, such as nervous system cells, expressed *NRXN1* and *NRXN3*, whereas adipocytes expressed *PLIN1* and *ADIPOQ*. Heatmap analysis (**Fig. 1B**) confirmed cell-type-specific marker gene expression and highlighted both shared and unique transcriptional signatures across clusters.

DISCUSSION: This study identifies fibroblasts as the predominant cell type in the healthy patellar tendon and resolves this population into three distinct subtypes. Interestingly, a variation of the *LAMA2*hi*FBLN1*hi subpopulation annotated as *FBLN1*hi fibroblasts in a recent study by J.Y. Mimpen et al. (2025) has also been identified as a major subcluster in the healthy quadriceps tendon. The identification of *LAMA2*hi*NEGR1*hi fibroblasts aligns with previous work showing the presence of *NEGR1*hi fibroblasts in adult and developmental tendons, suggesting a role in mechanosensitivity (Kurjan et al., 2025). The presence of *PRG4*hi*CRTAC1*hi suggests a functional role in synthesizing and secreting lubricin for lubrication (Fu et al., 2025). Beyond the fibroblast populations, vascular and lymphatic endothelial cells, as well as mural cells, were identified, consistent with previous descriptions of other healthy tendons such as the hamstring, Achilles, and quadriceps tendons (J. Y. Mimpen et al., 2024). While immune cells broadly expressed *PTPRC*, *RUNX1*, and *DOCK2*, they were further characterized by a predominance of tissue-resident macrophages (expressing *F13A1*, *MRC1*, *MERTK*), alongside T-cells and granulocytes, consistent with their known roles in immune surveillance and regulation. Cells expressing neural markers such as *NRXN1* and *NRXN3* suggest a role in tendon innervation and display transcriptomic features consistent with glial cells. Finally, adipocytes expressing *PLIN1* and *ADIPOQ* confirm their known function in tendon lipid metabolism. Our ongoing work, which includes spatial RNA sequencing (Xenium), will further elucidate the functional landscape of the cell types and cell-cell communication networks, while also validating the cell types governing cellular homeostasis in the healthy patellar tendon.

SIGNIFICANCE/CLINICAL RELEVANCE: This study establishes a single-nuclei atlas of the healthy human patellar tendon, providing a reference framework for understanding tissue homeostasis and identifying potential novel therapeutic targets for the treatment of patellar tendinopathy. This work also overcomes the challenges of obtaining rare healthy tendon tissue and generating high-quality transcriptional data from its dense extracellular matrix, enabling a clinically relevant tissue-specific atlas.

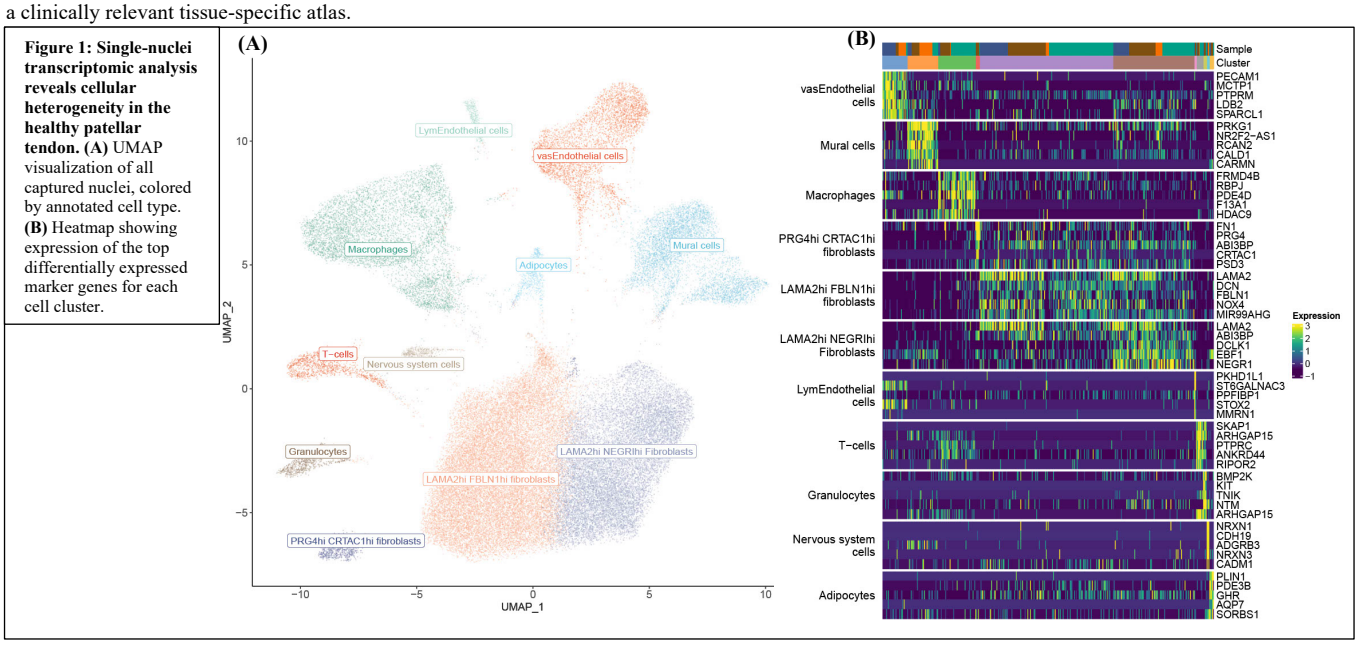

Figure 1: Single-nuclei transcriptomic analysis reveals cellular heterogeneity in the healthy patellar tendon. (A) UMAP visualization of all captured nuclei, colored by annotated cell type. **(B)** Heatmap showing expression of the top differentially expressed marker genes for each cell cluster.