Shared and Unique Cellular and Molecular Features of Torn Anterior Cruciate Ligament and Matched Patella Tendon Autograft

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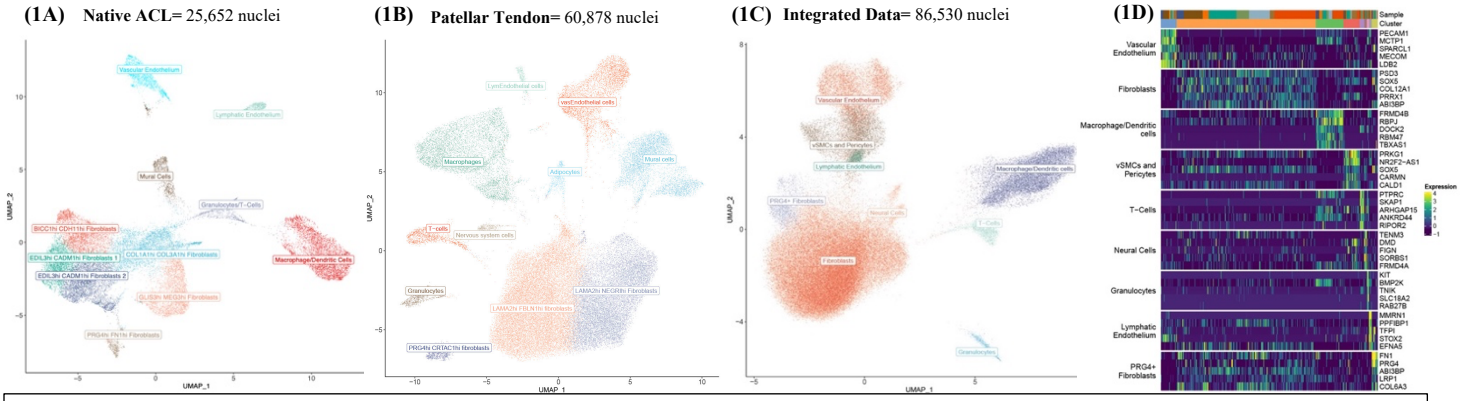
INTRODUCTION: Anterior cruciate ligament (ACL) tears represent a significant challenge in orthopaedic medicine, affecting over 2 million people annually and accounting for more than 50% of all knee injuries. Due to the ligament's poor intrinsic healing capacity, these tears lead to significant functional impairment and often require surgical reconstruction. Bone-patellar tendon-bone (BPTB) autografts are considered the gold standard for ACL reconstruction (ACLR), particularly for athletes and other active populations. Following ACLR, tendon grafts undergo a process of remodeling or "ligamentization" in which the transplanted tendon transforms structurally into tissue that more closely resembles that of a ligament (Amiel et al.,1986). However, while the histological differences between the patellar tendon and the native ACL are well-documented, the underlying cellular and molecular differences between the two soft tissues remain poorly understood. This study uses single-nuclei RNA sequencing (snRNA-Seq) to characterize the cellular and molecular features of matched ACL and patellar tendon tissues, aiming to elucidate the shared and unique signaling mechanisms that underpin graft remodeling.

METHODS: This study received approval from an institutional review board, and informed consent was obtained from all participants, under all applicable institutional laws and regulations. ACL remnants (n=4) and matched patella tendon autograft samples (n=4) were collected from patients with subacute ACL tears (n=4, [3 males, 1 female], mean age=25, time from injury to surgery: 52–92 days) undergoing ACLR with a BPTB autograft. Samples were snap-frozen and stored at -80°C. The snap-frozen samples were cut on dry ice and underwent a single-nuclei isolation protocol using a previously published method (Mimpen et al., 2021). Isolated nuclei were counted and loaded onto the Chromium Next GEMX Chip (10X Genomics), targeting a nuclei recovery of 10,000-20,000 nuclei per sample. The 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. 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: Single-nuclei transcriptomic analysis revealed the detailed cellular composition of both the native ACL after subacute ACL rupture and the patellar tendon. Tissue-specific analysis revealed fibroblasts as the abundant population in both the native ACL and patella tendon. The patella tendon was subclustered into three transcriptionally distinct subgroups: *PRG4*hi*CRTAC1*hi, *LAMA2*hi*FBLN1*hi, and *LAMA2*hi*NEGR1*hi fibroblasts (**Fig. 1A**). The native ACL was characterized by four diverse fibroblast subtypes, including distinct *BICC1*hi*CDH11*hi, *EDIL3*hi*CADM1*hi type 1, *EDIL3*hi*CADM1*hi type 2, *GLIS3*hi*MEG3*hi, and *PRG4*hi*FN1*hi populations (**Fig. 1B**). The integrated dataset showed that both tissues share broad cell populations, including fibroblasts (*PRG4*, *COL12A1, SOX5)*, vascular (*PECAM1*, *VWF*) and lymphatic (*PROX1*, *MMRN1*) endothelial cells, mural cells (vSMCs and pericytes, [*PRKG2*, *RCAN2]*), neural cells (*NRXN1*, *NRXN3)*, and diverse immune cells such as macrophages/dendritic cells (*DOCK2, FRMD4B)*, T-cells (*SKAP1*, *CD3E*), and granulocytes (*KIT*, *HPGDS*). Other stromal cells identified were adipocytes that expressed *PLIN1* and *ADIPOQ* (**Fig. 1C**). Heatmap analysis (**Fig. 1D**) confirmed cell-type-specific marker gene expression and highlighted both shared and unique transcriptional signatures across clusters.

DISCUSSION: This study establishes fibroblasts as the predominant cell type in the native ACL and the patellar tendon graft, resolving into five and three subpopulations, respectively. These three patellar fibroblast populations likely represent the homeostatic state of the tendon, similar to the resident and homeostasis-associated fibroblasts that have been identified as being characteristic of healthy tendon tissue. The reactive and heterogeneous profile of the ACL is consistent with prior transcriptomic studies (Brophy et al., 2016; Yang et al., 2023), which demonstrate that injury induces the expansion of specific fibroblast subtypes responsible for driving inflammation and extracellular matrix remodeling. When integrated, it is clear that while both tissues share a foundational architecture of fibroblast, endothelial, mural, adipocyte, neural and immune cell lineages, they diverge significantly at the molecular level. The distinctive sets of fibroblast subtypes identified in the native ACL versus the patellar tendon strongly suggest highly specialized cellular functions adapted to the unique biomechanical demands of each tissue. These intrinsic differences may underlie the challenges of graft maturation and the incomplete "ligamentization" of patellar tendon autografts after ACLR. A limitation of this study is the utilization of ruptured ACL tissue as opposed to healthy ligament, which may introduce heterogeneity into the cell types studied, but obtaining healthy ACL tissue in human patients is rarely feasible. Ongoing work, which includes spatial RNA sequencing (Xenium), will further explore the comparative compositional analysis of the cell types, functional landscape and cell-cell communication networks, while also validating the identified cell types and examining transcriptomic differences in ACL ruptures at various timepoints post-injury.

SIGNIFICANCE/CLINICAL RELEVANCE: The discovery of distinct cell types, especially fibroblast subtypes, between the subacutely ruptured ACL and the patellar tendon graft which is utilized to reconstruct the ACL surgically highlights their unique biomechanical adaptations. Recognizing this divergence provides a foundation for better understanding the process of tendon graft "ligamentization" and for developing targeted strategies to enhance graft remodeling, improve integration, and ultimately optimize surgical outcomes.



Figures: 1) Single-nuclei transcriptomic analysis reveals cellular heterogeneity in the healthy patellar tendon and subacute ACL Tears. (A) UMAP visualization of all captured nuclei for the healthy patella tendon. **B)** UMAP visualization of all captured nuclei for subacute ACL tears. **(C)** UMAP visualization of integrated data. **(D)** Heatmap showing expression of the top differentially expressed marker genes for each cell cluster for integrated Patella and subacute ACL tear samples.