Novel Classification of Peroneal Tendon Disease Reveals Cellular and Molecular Phenotypes of the Tenosvnovium

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Introduction: Pathologies of the peroneal tendon drive a significant source of lateral ankle pain, often referred to as peroneal tendon disease (**PTD**) [1,2]. PTD encompasses a spectrum of peroneal tendon pathologies (e.g., tendinosis, tenosynovitis, tears, and instability), resulting in inflammation and/or degeneration of the peroneal tendon and surrounding tenosynovium (**TS**). Currently, limited consensus exists on the classification of PTD involving both the peroneal tendon and TS, challenging accurate diagnosis and targeted therapeutic strategies. Previous studies have focused on describing the pathoanatomy (e.g., clinically observed structural tissue changes) of the peroneal tendon in PTD; however, there is limited research at the cellular and transcriptomic level focused on the TS. Holistic knowledge of the genetic composition, biological pathways, and tissue components of TS involved with different hallmarks of PTD may yield potential therapeutic targets and optimize treatment algorithms. We aimed to create a clinically meaningful classification system for PTD incorporating a detailed analysis of genetic, cellular, and tissue changes of the TS surrounding the peroneal tendon.

Methods: Patients undergoing surgery for peroneal tenosynovitis, tendinosis, tendon tears, and/or instability were classified for PTD using our 4-type classification system based on the following criteria: non-inflamed TS with normal appearing tendons (**Type 0**; n = 2); inflammatory TS with normal appearing tendons (**Type 1**; n = 9); inflammatory or degenerative TS with tendinosis or split tear involving less than 50% of tendon substance (**Type 2**; n = 1); inflammatory or degenerative TS with tendinosis or split tear involving greater than 50% of tendon substance (**Type 3**; n = 3); and complete or near complete degenerative rupture (**Type 4**; n = 3). After classifying PTD, TS was processed for bulk RNA sequencing (**RNA-seq**; n = 18; type 0/1 vs type 2-4) and digital histopathology (**DH**; n = 18): RNA-seq defines the gene transcriptomic landscape while DH identifies the cellular architecture of the TS. RNA-seq was performed using the Illumina NovaSeq6000 platform. Differential expression was determined using the R edgeR (3.22.5) package after adjusting via a scaling normalization factor and correcting P-values via the Benjamini Hochberg method. Gene Ontology (**GO**) analysis terms were identified to classify gene properties. For DH, TS was processed for standard paraffin-embedded histology and hematoxylin and eosin staining (n = 3 sections/TS). Stained tissue sections were imaged using a slide scanner and imported into an open-source software, QuPath, for quantification. Briefly, TS was manually traced, and then the number of cells was automatically detected, and cellular density was quantified. A qualitative assessment was performed on the spatial distribution of the cells relative to tissue structures (e.g., fat, fibrous tissue, and vascularity). Kruskal Wallis with Dunn's post-hoc was used to detect differences in cellular density between type 1-4 vs type 0.

Results: We observed increased cellular density (i.e., hypercellularity) with progressive PTD type, qualitatively and quantitatively (Figure 1A, 1B). The most remarkable increase was seen between type 3 and type 4 PTD with complete degeneration or tear of tendon ruptures. There were notable increases in vascular and nerve-like structures of TS for each PTD type (Figure 1A). Qualitatively, increases in cellular density are spatially around vascular/nerve structures and non-fatty-like tissue. Our RNA-seq analysis identified 13,206 differentially expressed genes between normal/inflamed TS with non-degenerative tendon (PTD types 0 and 1) and abnormal TS with torn/degenerative tendon (PTD types 2-4). GO pathway analysis highlighted that genes were associated with biological and molecular pathways of increased inflammatory leukocyte and neutrophil activation/migration, decreased adipocyte differentiation/activity, cellular components of increased collagen, and cytokine signaling (Figure 1C).

Discussion: Our novel classification system created to better define PTD demonstrates unique cellular density in each PTD type and transcriptomic differences between high and low PT types. Our DH findings show that an increasingly progressive disease state is characterized by hypercellularity in the TS. RNA-seq revealed a potential increase in gene pathways related to inflammation and extracellular matrix remodeling of collagen, with a decrease in adipocyte differentiation. Our ongoing work is increasing the sample size to robustly explore differences between each PTD subtype. Improved clarity in biological pathways and cellular composition of each PTD subtype will help optimize surgical strategies and therapeutic targets for PTD.

Significance/Clinical Relevance: The development of a new PTD classification system shows significant cellular and transcriptomic differences in different stages of peroneal tenosynovitis. Better clarifying the PTD subtypes and biological pathways will help identify appropriate surgical treatments and therapeutic targets.

References: [1] Koutsogiannis+, *JBJS Reviews*, 2022; [2] Ferran+, *Sports Medicine*, 2006

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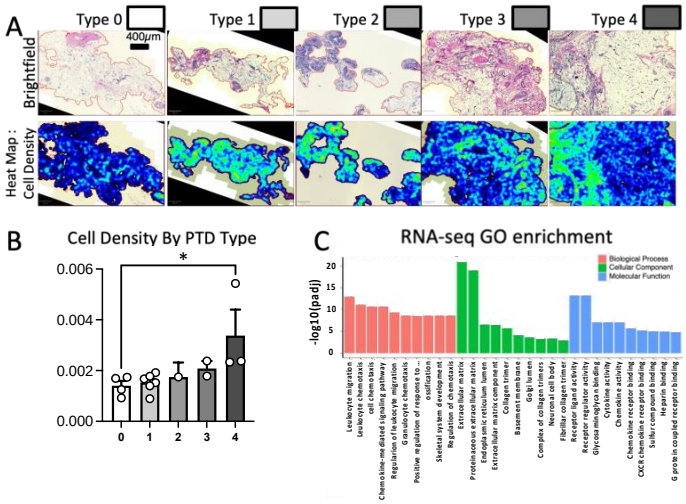

Figure 1: (A) Representative histology images of tenosynovium as a function of PTD classification from type 0 – type 4. Heat maps qualitatively highlight increasing cellular density (blue low density; green is high density) (B) Quantification of cell density (# of cells/total tissue area) as a function of PTD classification type (n = 18; * indicates p<0.05). (C) Significant Gene Ontology (GO) terms. Top 30 significant GO terms reveal increased inflammatory leukocyte migration, neutrophil activation/migration, and extracellular matrix remodeling, with decreased adipocyte differentiation/activity.