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Title

Deep cellular profiling reveals a pathological fibroblast population in human Achilles tendinopathy

Introduction

The forefront of personalized care lies in the understanding of the disease pathophysiology. In rheumatology, biologicals have revolutionized the treatment of inflammatory joint diseases. By selectively modifying the course of synovial inflammation, joints are saved. To date, no such efficient treatment exists for tendinopathy. Advanced analytically techniques, such as single cell RNAseq, will greatly enhanced our insight in tendon pathobiology. Commendable studies have examined human tendinopathic tissue using such techniques. However, heterogeneity in the tendinopathic samples and the lack of anatomically matched control tissue has confounded interpretation of the data.

Material & Methods

Tendinopathic (n=15) and healthy control (n=10) Achilles tendon midportion samples were compared. Diseased samples are collected during surgical procedures for longstanding Achilles tendinopathy in patients that did not respond to conservative treatment. Healthy tissue came from age and sex matched organ donors. First, the healthy and pathological status of the tendon was assessed by the conventional histological Bonar score. Additionally, the existing scoring system was automatated by the use of digital microscopy and artificial intelligence (QuPath software).In addition to histological analysis, three complementary techniques were used to interrogate the cellular biology of the tendon. Cells were enzymatically isolated from the tissues and analyzed by flow cytometry. RNA was extracted from the whole tendon tissue and the individual cells to perform bulk and single cell RNAseq respectively.

Results

Overall Bonar scores were higher in tendinopathic samples compared to control, but no differences were found in cellularity. Digital microscopy of entire sections (5 x 5mm) revealed a 2-fold increase in cell number in tendinopathic tendons compared to control tendons. Automated whole slide grading showed excellent correlation with the conventional Bonar scores for these subtopics for collagen arrangement (Spearman $r = 0.73$, $p<0.0001$) and ground substance (Spearman $r = 0.64$, $p=0.002$).

Analysis of tendon cells by flow cytometry, did not demonstrate changes in relative number of immune or endothelial cells, but did show an increase in the frequency of CD90+ fibroblasts in tendinopathic tendons. Bulk RNAseq revealed >1800 differentially expressed genes. Sub-analysis of the fibroblast gene expression by single cell RNAseq revealed tendinopathic fibroblasts to form a distinct cluster from healthy fibroblasts, defined by a high level of extracellular matrix genes (COL1A1, TNC) and fibroblast activation genes (THY1, SCX).

Discussion

Histological validation of tendon samples has been improved by the use of digital microscopy and artificial intelligence. A robust dataset to deeply profile the human Achilles tendon tissue has been generated and a pathological subtype of fibroblast has been identified in tendinopathy. Further analysis of this dataset will lead to identification of biological pathways in tendinopathy. This work could help in the identification of therapeutic target for the treatment of tendon disease.