ECM-driven metabolic plasticity of human tendon fibroblasts in health and disease

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INTRODUCTION: Tendinopathy, a disorder that results in pain, swelling, and impaired tendon function, is a clinical problem that affects ~3.5 million people in the US.[1] Tendinopathy is caused by failure of tendon to self-repair and is characterized by degenerative extracellular matrix (ECM), changes in the abundance of key ECM proteins, specifically an increase in type III collagen (COL III) relative to type I collagen (COL I) and increased cell death.[2] Several studies have implicated dysregulation of ECM and mitochondrial dysfunction in the progression of tendon pathology.[3] Additionally, oxidative stress, as defined by enhanced production of reactive oxygen species (ROS), has been identified as a factor in tendon disease progression.[1,3] Yet, if and how ECM composition regulates matrix production and the metabolic response by tenocytes remains unknown. In this study, we tested the hypothesis that the cellular metabolism of human tendon fibroblasts from healthy and tendinopathic donors is disease and substrate dependent.

METHODS: All samples were obtained with approval from the Institutional Review Board (HUM00196928). Diseased Achilles tendon biopsies were collected from patients undergoing tissue debridement for chronic insertional tendinopathy. Biopsies were minced and cultured in media (DMEM:F12, 10% FBS, 1% PenStrep) for expansion and frozen at passage 3 for cell culture experiments.[4] All experiments were performed at passages 4-6. Healthy human TFs were obtained from a commercial vendor (ZenBio, Durham, NC). Healthy and diseased human tendon fibroblasts (HTFs) were cultured on COL I (Bovine, 100µg/mL, Advanced BioMatrix) or COL III (Human, 100µg/mL, Advanced BioMatrix) coated onto plasma-activated glass or tissue culture plastic and collected for multiple assays. To assess changes in cell morphology, healthy and diseased HTFs were cultured for 72 hours (h) or 1 week (w), fixed with formaldehyde, stained with Cell Mask Orange, Hoechst, and phalloidin, and imaged using fluorescence microscopy. Cell aspect ratio was measured using Cell Profiler. To visualize and quantify newly synthesized and deposited matrix by cells, HTFs were cultured for 1w, stained with dibenzocyclooctyne (DBCO)-488, fixed, stained with Cell Mask Orange and Hoechst, then imaged using fluorescence microscopy. Cell area, nascent matrix area per cell, and nascent matrix area per cell area were measured using Cell Profiler. Intracellular protein stress response after 96h of substrate contact was measured using protein blot arrays (R&D Systems, Minneapolis, MN). Changes in mitochondrial density was evaluated by culturing healthy and diseased HTFs for 96 hours (h), cells were fixed, stained with MitoTracker Deep Red, Cell Mask Orange, Hoechst and phalloidin, imaged using fluorescence microscopy. Images were computationally cleared using Leica THUNDER software to remove cytosolic autofluorescence. Cell area, number of mitochondria per cell, and mitochondria per cell area was determined in Cell Profiler. After culturing healthy and diseased HTFs for 72h, oxygen consumption was measured for the final 24hours of culture (Resipher), and cells were imaged and counted (BioTek Cytation C10 Confocal Imaging Reader), and oxygen consumption rate was normalized to cell counts. After culturing healthy and diseased HTFs for 72h, L-Lactate production was measured for the final 24h of culture (Glycolysis Cell-Based Assay Kit, Cayman Chemical). Statistical comparisons between groups were performed using a two-way ANOVA with post-hoc Sidak's multiple comparisons to identify specific differences (Disease vs. Healthy; Substrate; GraphPad Prism v10).

RESULTS: Healthy HTFs were more elongated and spindle-like on COL III than COL I over 1 week of culture, however, diseased HTFs did not change their aspect ratio based on ECM condition (Fig. 1A). Nascent ECM labeling revealed healthy HTFs produce significantly more matrix when cultured on COL III as compared to on COL I as well as compared to diseased HTFs on COL III (Fig 1B). Diseased HTFs, however, did not change their ECM production based on culture substrate (Fig 1B). HTFs from diseased donors had higher expression of oxidative stress-associated proteins (Fig. 2A). Based on these results, we next examined changes in mitochondrial number, oxygen consumption, and lactate production (glycolytic metabolism) to identify a potential mechanism responsible for these findings. However, we did not find any differences in the number of mitochondria per cell area (Fig. 2B), oxygen consumption (Fig. 2C), or lactate production (Fig. 2D) due to disease or ECM coating.

DISCUSSION: Tendinopathy pathogenesis typically includes increased deposition of COL III; yet how this environmental change affects HTF behavior has previously been unclear.[1,5] We show that healthy HTFs exhibit phenotype plasticity when exposed to two different ECMs. Specifically, we showed that COL III, which represented degenerative ECM, induced increased nascent ECM production and cell stress. However, diseased HTFs do not respond to different ECM substrates by generating more nascent ECM or changing their cell shape but rather by increasing oxidative cell stress. Similarly, Heo et al. recently found that healthy HTFs can reorganize their chromatin in response to degenerative chemophysical environments while diseased HTFs do not.[6] This observation was attributed to pre-existing chromatin remodeling that may fix the transcriptional behavior of diseased HTFs compared to healthy, which may explain our observations. Future work will examine the transcriptional and epigenetic response of HTFs on COL I and COL IIII as well as ROS production and mitochondria function to identify the mechanism for our observed findings.

SIGNIFICANCE/CLINICAL RELEVANCE: Tendinopathy has few non-surgical treatment options.[1] Elucidating environmental determinants of HTFs behavior will aid in the design of novel therapies for tendinopathy.

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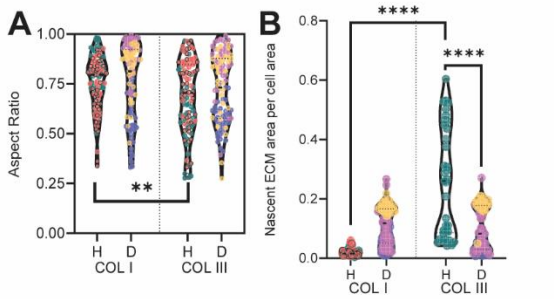

Fig 1. Cell shape and ECM production depends on ECM and disease status A) Healthy HTFs are more spindle-like on COL III after 1 week B) Healthy HTFs increase nascent ECM production on COL III. (H - Healthy, D - Diseased, n > 75 cells from 2 healthy & 3 diseased donors and at least 3 individually repeated experiments, individual symbol colors correspond to specific donors across all experiments, ** p<0.01, ****p<0.001)

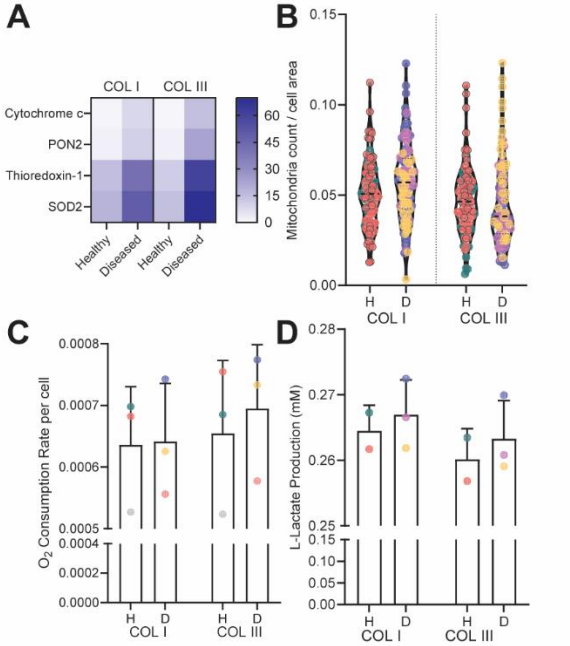

Fig 2. Metabolic response of healthy (H) and diseased (D) HTFs to COL I or COL III. A) Diseased HTFs had increased oxidative stress-associated protein expression on COL I and COL III compared to healthy HTFs B) There were no differences in mitochondria per cell area (n > 75 cells from 2 healthy & 3 diseased donors and at least 3 individually repeated experiments) C) Similarly there were no differences in O2 consumption (n=3 donors per group) or D) L-lactate production (n=2 healthy, 3 diseased donors, individual symbol colors correspond to specific donors across all experiments)