The role of pathogenic CD90+ fibroblast subsets in chronic tendinopathy

Alejandro Gomez Collignon[1], Christopher Buckley[2], Adrian Kendal[1], Stephanie G. Dakin[1]
[1]Botnar Research Center, University of Oxford, UK, [2]Kennedy Institute of Rheumatology, University of Oxford, UK
alejandro.gomez@ndorms.ox.ac.uk

DISCLOSURES: No conflicts of interest.

INTRODUCTION: Tendon disorders, such as tendinopathy and rupture, account for significant healthcare expenditure due to sports injuries and the challenges of ageing societies. To comprehend the cellular foundation of fibrosis and inflammation in tendinopathies, we analyzed *ex vivo* and *in vitro* healthy and diseased human tenocyte clusters at the transcriptomic and proteomic level. We focused our bioinformatic analyses on tenocyte clusters that express the pathogenic CD90+ surface marker. Subsequent mechanistic experiments were designed to explore the functional role of CD90 within these specific tenocyte clusters. The primary objective of this study is to unravel the functional significance of these distinct tendon fibroblast populations and to investigate the mechanistic contributions of CD90 in the molecular pathogenesis of tendinopathy.

METHODS: We conducted CITE-Sequencing of *ex vivo* and *in vitro* human tendon cells, utilizing the 10x Genomics platform coupled with BioLegend surface marker antibodies. We used Seurat for fibroblast population analysis, Monocle for cell trajectory analysis, EnrichR for gene ontology enrichment, and ENSEMBL reference databases for cross-species cell type matching. In situ geographical analysis of fibroblast cell type markers was executed with multiparameter immunohistochemistry. Additionally, we assessed the impact of CD90 expression on fibroblast functions such as proliferation, migration, cytokine production, and differentiation to myofibroblasts. We achieved CD90 knockdown using Dharmacon's on Target THY1 siRNA and contrasted results against control fibroblasts treated with a scrambled siRNA sequence. Pro-inflammatory cytokine production (Mesoscale Discovery), wound healing capacity (Cell Biolabs CytoSelect™ Migration Assay), cell proliferation (DAPI automated counting), and fibroblast-to-myofibroblast transition (α-SMA fluorescent labeling) were then systematically evaluated.

RESULTS: The six identified CD90+ fibroblast subsets demonstrate unique gene expression patterns, with gene ontology enrichment suggesting distinct functional roles. Variations in the cell numbers and relative proportions of each fibroblast cluster were evident across healthy and diseased states. PTX3+ and POSTN+ clusters express genes related to collagen fibril synthesis and extracellular matrix organization. NOTCH3+ cluster is a mural tenocyte, APOD+ cluster is associated with chemoattractants and inflammation, and PRG4+ cluster shows expression patterns associated with chondrogenesis and repair. Seven distinct *in vitro* fibroblast clusters were found in cultured tenocytes, with four fibroblast clusters overlapping between *in vitro* and *ex vivo* data sets. CD90 knockdown in cultured fibroblasts reduced THY1 mRNA levels by >98%. CD90 knockdown in fibroblasts sourced from five tendinopathic donors led to a statistically significant reduction in IL6 and IL8 production levels, 50% decline in fibroblast proliferation, and a 35% reduction in myofibroblast differentiation and fiber formation. In the wound healing assay, CD90+ knockdown cells exhibited a delayed healing trajectory, attributed to both diminished cell division and hampered migration.

DISCUSSION: Constructing a comprehensive human tendon cell atlas that encompasses both healthy and diseased states advances our understanding of the cellular and molecular factors driving inflammation, fibrosis, and impaired healing. Further research is pivotal in elucidating the unique biological roles of different fibroblast subtypes in tendon health and pathology. Moreover, a diseased tendon atlas can shed light on interactions between pathogenic CD90+ tendon cells and adjacent cells, potentially unveiling innovative therapeutic strategies targeting specific CD90+ tendon subsets.

SIGNIFICANCE/CLINICAL RELEVANCE: Our findings enrich our knowledge of cellular landscapes in healthy and diseased tendons and underscore the pro-inflammatory and pro-fibrotic tendencies of CD90 in tendon fibroblasts. Modulating CD90 expression in chronic tendinopathy and other fibrotic diseases presents a promising therapeutic avenue.

REFERENCES: Kendal *et al. Nature, Sci Rep* **10,** 13939, 2020, Dakin et al. *Nature reviews. Rheumatology* vol. 14,12, 2018

IMAGES AND TABLES:

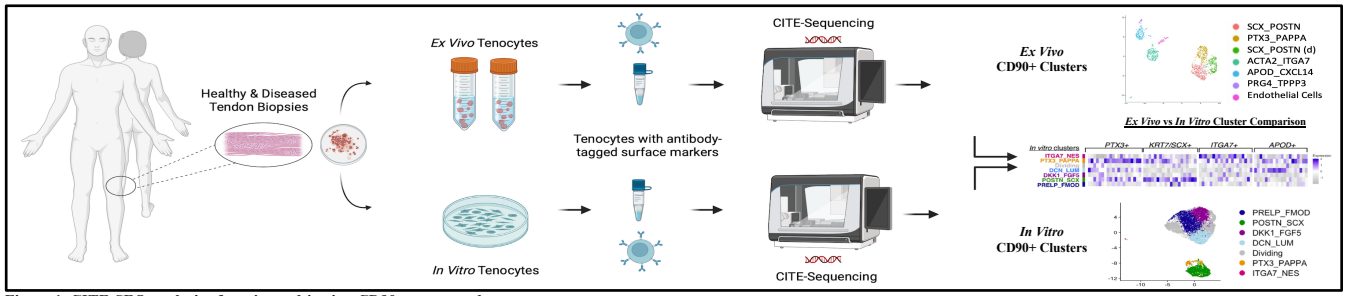

Figure 1. CITE-SEQ analysis of *ex vivo* and *in vitro* CD90+ tenocyte clusters

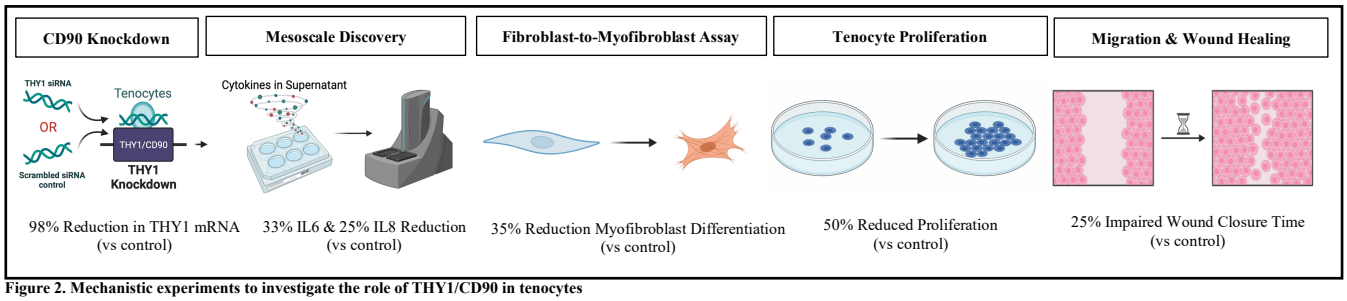

Figure 2. Mechanistic experiments to investigate the role of THY1/CD90 in tenocytes