Investigating Tendinopathy Pathogenesis in a Rat Patellar Tendinopathy Model Using RNA-seq

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INTRODUCTION: Calcific tendinopathy (CT) is a debilitating condition characterized by the apatite deposits within the tendon, resulting in chronic pain, functional decline, and reduced exercise tolerance among patients [1]. Despite various clinical treatments, outcomes have been inconsistent, highlighting the need for a better understanding of the underlying pathogenesis [2]. The pathogenesis of CT is multifactorial and complex, with one potential mechanism being the erroneous differentiation of tendon stem/progenitor cells (TSPCs) into chondrocytes or osteoblasts [1, 2]. Additionally, immune-stem cell crosstalk may also play a role in this process, necessitating further investigation [3]. Hence, the objective of this study is to establish a dynamic landscape of tendinopathy development and explore the mechanisms underlying TSPC differentiation and immune-cell crosstalk in CT.

METHODS: To establish a rat model of patellar tendinopathy, 10-12 week-old, Sprague Dawley (SD) rats were used in this study. These rats were randomly divided into three groups: (1) phosphate buffer solution (PBS) injection group; (2) collagenase (250 U)-treated group (COL); and (3) the contralateral tendon served as an intact group. The tendinopathy model was characterized via gross morphology, histopathology, and gene expression analysis. Additionally, transcriptomics investigations were performed on 2-, 4- and 8-week post-surgery to study the tendinopathy pathogenesis (Figure 1A).

RESULTS SECTION: Our findings show that (1) The COL groups exhibited swelling, yellowish tendons, as well as an abundant secretion of mucus compared to the PBS groups (Figure 1B); (2) Histologically, the COL groups showed a complete loss of architecture, irregular fibers, and abnormal tenocytes at week 2. Chondrocyte-like cells, indicated by round cells with lacunar space, were first observed in week 4. At week 8, a large area of calcification and more chondrocyte-like cells were observed (Figure 1B); (3) Based on RNA-seq, the rat patellar tendinopathy group displayed distinct gene expression patterns compared to the control groups. Significant enrichment in gene ontology (GO) patterns and pathways associated with chondrogenesis, osteogenesis, and immune response were observed in the tendinopathy group (such as chondrocyte proliferation, endochondral ossification, response to macrophage colony-stimulating factor and wnt signalling pathway). Cell deconvolution analysis revealed a notable increase in the proportion of specific immune cell types, including T cells and M2 macrophages, while demonstrating a concurrent decrease in the population of immune cells such as dendritic cells throughout the progression of the disease. Moreover, single-sample gene set enrichment analysis (ssGSEA) indicates the presence of a stem cell lineage switch from tenogenic phenotype to chondrogenic phenotype and discernible alterations in immune cell populations within rat patellar tendinopathy. (Figure 1C).

DISCUSSION: The rat tendinopathy model we established demonstrated a phenotype characterized by tendon matrix degradation and heterotopic ossification, which aligns with existing literature. Preliminary transcriptomic data further validated the presence of a cartilaginous transcriptional program and noticeable changes in immune cell populations in the rat patellar tendinopathy. For future investigations, we intend to employ multiparametric flow cytometry analysis to explore the underlying mechanisms associated with immune-stem cell crosstalk as well as cell lineage switch.

SIGNIFICANT: The pathogenesis of CT is characterized by multifactorial complexity. By establishing a robust patellar tendinopathy rat model and conducting comprehensive transcriptomics and flow cytometry analysis, we can effectively address substantial knowledge gaps in the field of intricate tendon biology. This approach allows us to gain insights into the pathological progression of tendinopathy and ultimately provide guidance for optimal tendon regeneration strategies.

REFERENCE: [1] Chianca V *et al.* Acta Biomed. 89(Suppl 1), 186–196, 2018; [2] Millar N L *et al.* Nat Rev Dis Primers. 7, 1, 2021; [3] Russo V *et al.* *Cells*. *11*(3), 434, 2022.

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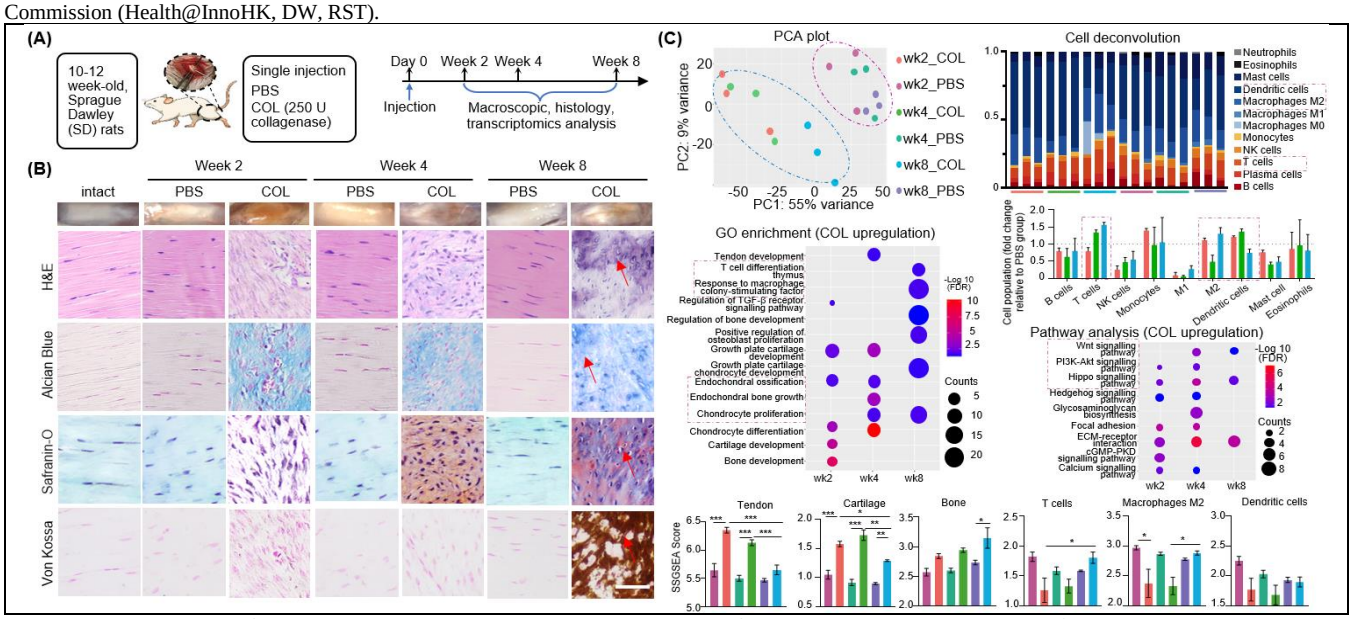

Figure 1: Establishment of a Patellar Tendinopathy Model and Exploration of its Pathogenesis. (A) Schematic of experimental design. **(B)** Gross morphology and histological analysis at 2-, 4- and 8-week post-surgery. All the COL groups showed abnormal tenocyte, hypercellular, increase in sGAG. Week 8 COL group also demonstrates the presence of heterotopic ossification (n=3, scale bar=200μm). **(C)** RNA-Seq analysis of rat patellar tendinopathy model. Cell deconvolution analysis shows the dynamic change of immune cell populations profiles in tendinopathy development. GO, pathway and ssGSEA analysis showed the presence of a cartilaginous transcriptional program and discernible alterations in immune cell populations in rat patellar tendinopathy (n=3; mean ± SEM; ***, $p < 0.001$, **, $p < 0.01$, *, $p < 0.05$).