Identification of a new population of mechanosensitive Tnn+ progenitors to form enthesis fibrocartilage

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Disclosures

INTRODUCTION:
The tendon enthesis (bone-tendon interface) constitutes the specialized junction anchoring tendons to skeletal elements, serving dual mechanical roles in securing soft tissue attachments and transmitting musculoskeletal forces to osseous surfaces. Histologically, this transitional zone is characterized by a stratified fibrocartilaginous layer exhibiting spatial gradients in cellular organization and mineralization patterns. Clinically, entheseal complexes (e.g., rotator cuff, Achilles tendon, patellar ligament) demonstrate particular vulnerability to traumatic rupture and post-surgical failure, with current regenerative approaches showing limited capacity to restore native fibrocartilaginous organization. How, then, does the enthesis fibrocartilage is formed and what is the upstream cell sources that drive the formation of a mineral gradient fibrocartilage? Elucidating the identity of enthesis-resident progenitors is critical for advancing regenerative strategies, particularly in the context of the long-standing question of how fibrocartilage forms at tendon-bone junctions under mechanical loading.
METHODS: This study used wild-type C57BL/6 mice and transgenic mouse models (including Tnn-CreART2, Rosa26-tdTomato-DTR, etc.). All animal experiments have been approved by the Animal Ethics Committee of Central South University (No. 2022020058) and comply with relevant Chinese laws and regulations. This study used Tnn-CreERT2 to mate with Rosa26-tdTomato or Rosa26-DTR mice to obtain lineage tracing or conditional knockout models. Tamoxifen induces Cre recombinase activity, while Diphtheria Toxin (DT) induces Tnn $^{+}$ cell ablation. Inject botulinum toxin (BTX) into the suprascapular muscle to simulate a tendon mechanical unloading model. This study systematically analyzed the bone tendon interface using single-cell and spatial transcriptomics, and performed three-dimensional structural analysis using synchrotron radiation micro-CT, H&E、Toluidine Blue、 Immunohistochemical (IHC) and immunofluorescence (IF) staining were used for histological analysis. After the data conforms to a normal distribution, ANOVA and Tukey post hoc tests are used, and $P<0.05$ is considered significant.
RESULTS SECTION: This study identified Tnn^{+} progenitor cells through spatial transcriptomics and scRNA seq. Lineage tracing showed that Tnn $^{+}$ cells were distributed at multiple bone tendon insertion points (rotator cuff, cruciate ligament, Achilles tendon, etc.), but not in articular cartilage. Ablation of Tnn^{+} cells leads to poor development of fibrocartilage, decreased mechanical properties, and reduced mineralization. BTX induced mechanical unloading of bone tendons leads to a decrease in the number and volume of fibrocartilage cells, as well as a decrease in COL2A1 expression. ScRNA seq showed a significant decrease in the proportion of Tnn $^{+}$ cells and the expression of cartilage formation related genes in the mechanical unloading group.
DISCUSSION: In this study, we observed a decrease in the quantity of Tnn+ enthesis progenitors following the removal of muscle loading. We also found that Tnn gene expression was downregulated in unloaded entheses, suggesting that Tnn expression in enthesis progenitors is mechanosensitive. However, the current study did not elucidate the precise biological function of the TNN protein within the tendon enthesis, nor did it investigate the specific cellular mechanisms by which mechanical loading influences Tnn gene expression. Further in vitro experiments will be necessary in future research to address these important questions.
SIGNIFICANCE/CLINICAL RELEVANCE: (1-2 sentences): Identification of mechanosensitive Tnn^{+} progenitor cells as critical drivers of enthesis fibrocartilage formation provides a novel cellular target for regenerative therapies aimed at improving tendon-bone healing, with potential to enhance treatment outcomes for injuries to entheseal complexes such as rotator cuff tears and Achilles tendon ruptures.