Mustn1 expression accompanies mural cell infiltration during Achilles tendon repair

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INTRODUCTION: Musculoskeletal temporally activated novel gene (Mustn1) is a 9.2kDa microprotein that has been extensively studied within musculoskeletal tissues, specifically during muscle regeneration and skeletal repair. Mustn1 has been reported within tendon tissues, but never been explored. We recently mapped Mustn1 onto various open-source, single-cell transcriptomic datasets from various tissues of the hindlimb. Within musculoskeletal tissues, across multiple datasets, Mustn1 and Acta2 (gene coding the protein alpha smooth muscle actin, aSMA) exhibit highly selective coexpression within mural cell states. We hypothesized that this Mustn1 and Acta2 coexpression would be localized to mural cell expansion in response to tendon injury.

METHODS: In this study, we perform partial resection of the right Achilles tendon (when viewed dorsally) 2mm above the calcaneal enthesis. Transgenic mice harboring the Sox9-CreER, Ai9-tdTomato and CXCL12-GFP alleles were used to pulse-chase the sox9-lineage and their relationship to CXCL12-expressing cells. Tamoxifen injections were administered intraperitoneal at a concentration 75mg/kg. One bolus dose was administered at p21, a 7day washout period was observed, followed by injury at p28. Immunohistochemistry of Acta2 and Mustn1 was performed on isolated Achilles tendon tissues at 7day, 14days, 28day and 56days after injury. Males and female mice (n=5) were used for each timepoint and this study was approved by the Institutional Animal Care and Use Committee Protocol 2023RLRMH02. Research reported in this study was supported by grant R15HD092931 (MH), from the Eunice Kennedy Shriver National Institute of Child Health & Human Development of the National Institutes of Health, and an Institutional Support for Research and Creativity (ISRC) Grant from NYIT (MH).

RESULTS SECTION: Preliminary data demonstrates that the sox9-lineage does not infiltrate the repair site at 1 to 2 weeks post-injury. Sox9-lineage red cells can be observed to populate the remodeling tendon, as well as regions of heterotopic ossification , at 4 and 8 weeks post-injury. Cxcl12 expressing cells are observed localized to the sheath in the uninjured Achilles, but expression becomes wide spread in regions of healing tendon at 2-weeks post-injury. aSMA expression is also localized to the tendon sheath in uninjured tissue. Mustn1 and aSMA expression localize to large vessel infiltration of the healing Achilles tendon at 2 weeks and 4 weeks post-injury.

DISCUSSION: When mapping Cxcl12, Mustn1 and Acta2 expression onto the cell states of the uninjured Achilles tendon, endothelial and perivascular localization was observed. Immunostaining confirm that these cell states localize to the tendon sheath of the uninjured Achilles. Post-injury, our initial findings demonstrate a delayed expansion of the sox9-lineage in the healing Achilles tendon, while Cxcl12 expression becomes widespread much earlier within the healing Achilles. Immunostaining of aSMA and Mustn1 confirm expression within mural cell infiltration of the healing Achilles tendon at 2 weeks post-injury. These findings are still in their infancy and further studies will quantify infiltration and proliferation of cell states by timepoint.

SIGNIFICANCE/CLINICAL RELEVANCE: (1-2 sentences): This study confirms the cellular heterogeneity present in the Achilles tendon, specifically within the sheath of the uninjured Achilles. Targeting these high-resolution cell states during Achilles tendon repair pose the promise of increasing the viability of tendon remodeling.

REFERENCES:
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IMAGES AND TABLES: