Elucidating injury-site specific regenerative programs to rebuild the tendon

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INTRODUCTION: Tendons are essential highly ordered, matrix-rich connective tissues that transmit forces from muscle to bone. Acute tendon injuries are common, with tears frequently occurring at two distinct injury sites: the tendon midbody or the tendon-bone attachment site, called the enthesis. Unfortunately, adult mammalian tendons cannot regenerate after severe acute injuries. Tendon cells, or tenocytes, fail to respond and instead, disorganized, functionally impaired scar tissue forms which is prone to re-rupture.[1] Despite clinical demand, current treatments are limited and variable in efficacy, impacting patient quality of life. Better treatments are needed. However, therapeutic advancement has stalled in part due to the lack of adult regenerative models which can be leveraged to identify mechanisms required to properly rebuild the tendon. We have recently shown that unlike their mammalian counterparts, the adult zebrafish tendon can regenerate after full tear injuries to the midbody[2] or enthesis.[3] Yet, the molecular and cellular basis of tendon regeneration remains largely unknown.

METHODS: Here, we demonstrate that unlike their mammalian counterparts, adult zebrafish tenocytes can proliferate, migrate, and regenerate both the tendon midbody[2] and enthesis following full tear injuries. To examine mechanisms governing tenocyte activation at distinct injury sites, we performed single cell (sc)RNA-sequencing, spatial transcriptomics (MERSCOPE), lineage tracing, and chemical/genetic perturbations during homeostasis and regeneration in both injury models to elucidate molecular mechanisms required for regenerating different parts of the tendon. All experiments were performed according to approved MGH IACUC protocol number 2012N000167, using animals of both sexes with an even distribution and a sample size of 4-8 animals per condition. One way ANOVA and unpaired two-tailed t-tests were employed for statistical analyses.

RESULTS: We molecularly characterize and spatially map zebrafish tendon cell populations during homeostasis and regeneration (Figure 1-2). Interestingly, we identify common early injury-responsive cell states which diverge into site-specific regenerative programs driven by differential activation of TGF-beta and/or canonical Wnt in the midbody or enthesis, respectively (Figure 3). While early chemical inhibition of TGF-beta signaling completely impairs re-attachment of tendon ends during midbody regeneration,[2] similar treatment after enthesis injury results in less severe effects, with the majority able to re-form attachments between the tendon and bone, collectively indicating TGF-beta signaling may be less essential for enthesis regeneration. In contrast, we find that Wnt signaling is selectively highly activated in response to enthesis injury. Early global chemical and genetic inhibition of Wnt signaling significantly increases proliferation, while early chemical hyper-activation of canonical Wnt signaling significantly decreases proliferation, tenocyte recruitment, and disrupts tendon-bone re-attachment during enthesis regeneration. These data suggest that the activation levels of Wnt must be tightly controlled to ensure proper enthesis regeneration, as high levels may be detrimental. Finally, we present a cross-species tendon cell atlas and elucidate evolutionarily conserved cell types across zebrafish, mouse, and human (Figure 1).

DISCUSSION: Altogether, our findings elucidate distinct molecular programs governed by TGF-beta/Wnt which are required to regenerate different parts of the tendon and may inform the strategic design of novel therapeutic approaches to treat different types of tendon injuries. Ongoing work is centered on translating these findings into analogous adult mammalian injury contexts to investigate potential effects on healing outcomes.

SIGNIFICANCE/CLINICAL RELEVANCE: Collectively, our work positions the adult zebrafish as an invaluable model for the field and establishes a foundational molecular and spatial map of tendon regeneration, ultimately opening avenues for performing cross-species comparative studies to uncover mechanisms driving regeneration versus fibrosis.

REFERENCES: 1. Howell, K. *et al. Sci Rep*, 2017. 7: p. 45238. **2.** Tsai, S.L. *et al. NPJ Regen Med*, 2023. 8(1): p. 52. **3.** Noedl, M.T. *et al.* In prep.

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IMAGES AND TABLES:

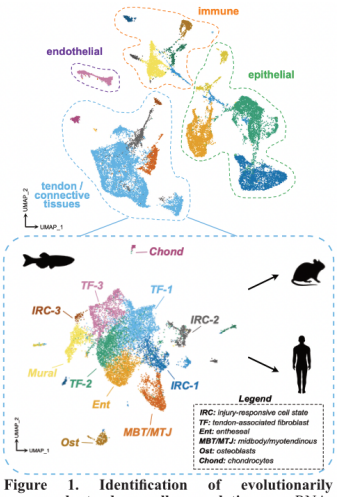

Figure 1. Identification of evolutionarily conserved tendon cell populations. scRNA-sequencing identified adult zebrafish tendon subpopulations. Cross-species mapping onto mouse and human single cell data revealed conserved cell-types and genetic markers across-species.

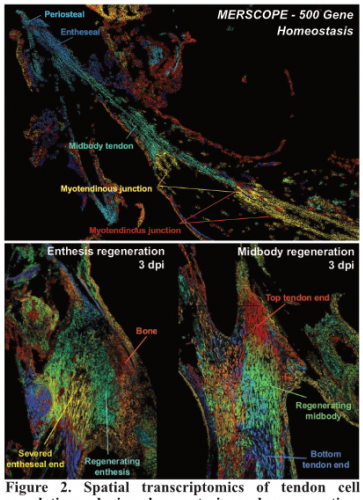

Figure 2. Spatial transcriptomics of tendon cell populations during homeostasis and regeneration. MERSCOPE was performed using a custom 500-gene panel on uninjured (top) and regenerating enthesis/midbody (bottom) adult zebrafish tendons at 3 days post-injury (dpi).

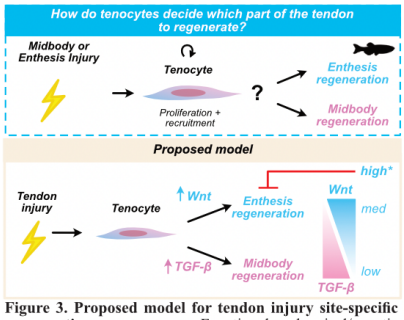

Figure 3. Proposed model for tendon injury site-specific regenerative programs. Functional chemical/genetic perturbation experiments collectively support a model in which tenocytes are specified to regenerate the midbody or enthesis depending on differential activation of TGF-beta/canonical Wnt signaling. In addition, aberrantly high levels of Wnt are detrimental for enthesis regeneration.