Endogenous Tenocyte Activation Underlies the Regenerative Capacity of the Adult Zebrafish Tendon

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INTRODUCTION: Tendons are essential, frequently injured connective tissues that transmit forces from muscle to bone. Their unique highly ordered, matrix-rich structure is critical for proper function. While adult mammalian tendons heal after acute injuries, endogenous tendon cells, or tenocytes, fail to respond appropriately, resulting in the formation of disorganized fibrovascular scar tissue with impaired function and increased propensity for re-injury.

METHODS: Here, we show that unlike mammals, adult zebrafish tenocytes activate upon injury and fully regenerate the tendon. Using a full tear injury model in the adult zebrafish craniofacial tendon of various transgenic zebrafish lines, we defined the hallmark stages and cellular basis of tendon regeneration through a combination of multiphoton imaging, lineage tracing, transmission electron microscopy, and pharmacological approaches.

RESULTS: Remarkably, we observe that zebrafish tendons regenerate and restore normal collagen matrix ultrastructure by 6 months post-injury (mpi) (Figure 1). Tendon regeneration progresses in three main phases: inflammation within 24 hours post-injury (hpi), cellular proliferation and formation of a cellular bridge between the severed tendon ends at 3-5 days post-injury (dpi), and re-differentiation and matrix remodeling beginning from 5 dpi to 6 mpi. Importantly, we performed tendon cell lineage tracing to demonstrate that pre-existing tenocytes are the main cellular source of regeneration, proliferating and migrating upon injury to ultimately bridge the tendon ends. Finally, we show that pharmacological inhibition of TGF-β signaling severely impairs bridge formation (Figure 2), altogether indicating that TGF-β is required for tenocyte recruitment during regeneration.

DISCUSSION: As tenocytes are not recruited to the defect following an analogous full tear injury in mice[1], our work pinpoints an underlying difference in cellular mechanisms of fibrotic healing versus regeneration following tendon injury. These results thereby aptly position the adult zebrafish tendon as an invaluable comparative system to elucidate mechanisms required for proper matrix restoration and regeneration following injury.

SIGNIFICANCE/CLINICAL RELEVANCE: Collectively, our work debuts one of the only adult tendon regenerative models which may be leveraged to reveal regenerative cues that may enhance pre-existing clinical treatments and potentially inspire innovative strategies to treat tendon injuries.

REFERENCES: 1. Howell, K., Chien, C., Bell, R. et al. Novel Model of Tendon Regeneration Reveals Distinct Cell Mechanisms Underlying Regenerative and Fibrotic Tendon Healing. Sci Rep 7, 45238 (2017). https://doi.org/10.1038/srep45238

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IMAGES AND TABLES:

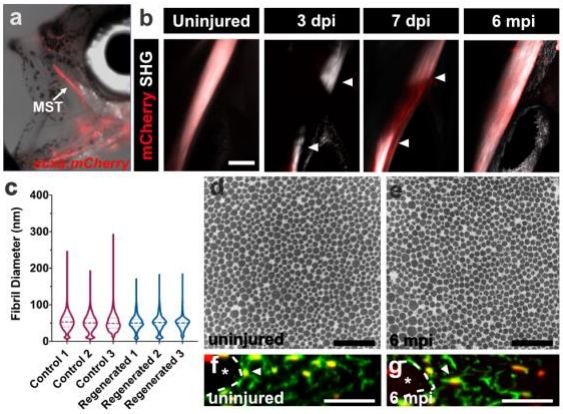

Figure 1. The adult zebrafish tendon can regenerate. (a) DIC image of maxillary superficial tendon (MST) in *scxa:mCherry* fish. (b) 2-photon time course images of *scxa:mCherry* expression in MSTs during homeostasis and after injury. Arrowheads denote tendon ends. (c) Quantification of collagen fibril diameter in uninjured (n=3) and regenerated (n=3) tendons at 6 mpi. (d-e) Representative cross-sectional 50,000x TEM images from uninjured and regenerated tendons at 6 mpi. (f-g) Cross-sectional 2-photon Z-stack projections of uninjured (f) and regenerated (g) tendons in *scxa:mCherry* fish (shown in green). Nuclei shown in red. Dotted lines denote muscle boundary and asterisk denotes muscle. Arrowhead denotes representative tenocyte with long processes. Scale bars denote 100 μm in b, 500 nm in d-e, and 10 μm in f-g. dpi, days post-injury; mpi, months post-injury; SHG, second harmonic generation.

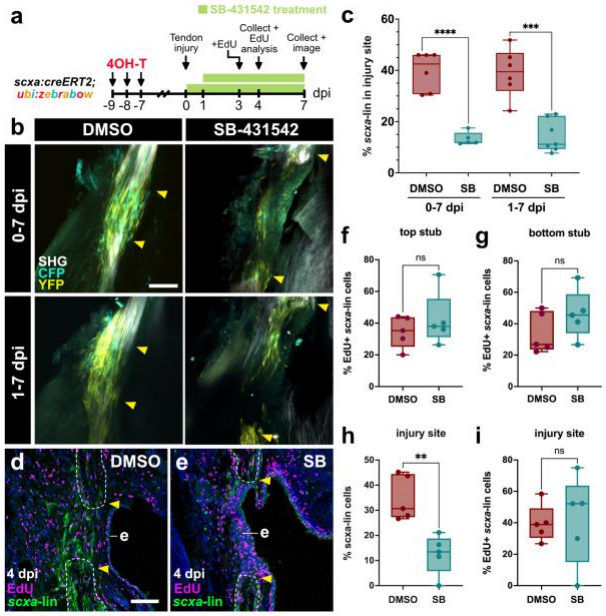

Figure 2. TGF-beta signaling is required for tenocyte recruitment, but not proliferation, during tendon regeneration. (a) Experimental schematic. (b) Representative 2-photon images of *scxa*-lineage cells (CFP+/YFP+) in regenerating tendons at 7 dpi following DMSO or SB-431542 (SB) treatment beginning immediately after injury (0 dpi) or 1 dpi. (c) Quantification of the percentage of *scxa*-lineage cells in the injury site in both treatment regimens. (d-e) EdU staining (magenta) in DMSO or SB-treated *scxa*-lineage traced zebrafish. Dotted lines outline tendon stubs. (f-g) Quantification of EdU+ *scxa*-lineage cells in top (f) and bottom (g) stubs at 7 dpi. (h-i) Quantification of *scxa*-lineage cells and EdU+ *scxa*-lineage cells in the defect at 7 dpi. Yellow arrowheads mark tendon ends. Scale bars denote 100 μm in b, d-e. e, epidermis. ****p<0.0001, ***p<0.001, **p<0.01, ns, not significant.