MRTF/SRF Signaling Is Required for Postnatal Tendon Maturation

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INTRODUCTION: Postnatal tendon maturation is marked by increasing tendon length and cross-sectional area, structural changes, and increasing mechanical properties[1]. Physiological loading is critical as postnatal paralysis impairs fibril maturation and functional properties[2,3]. Despite the importance of tendon as a load bearing tissue, little is known about the mechanotransduction pathways that regulate tendon biology. A few recent studies implicated the YAP/TAZ mechanotransduction signaling pathway in postnatal tendon matrix gene expression and catabolism[4,5]. However, the structural and functional properties of tendons were not assessed. Although MRTF/SRF signaling is a known mechanotransduction pathway, its role in tendon development remains largely unexplored[6]. Previously, we showed that MRTF signaling regulates *in vitro* embryonic tendon cell differentiation, but the relevance of MRTF signaling in tendon development *in vivo* remains unknown. In this study, we used deletion of *Mrtfa* (global) and *Mrtfb* (targeted) in *Scx*-lineage cells to investigate the role of MRTF signaling in postnatal tendon development *in vivo*. We hypothesized that MRTF signaling is required for maintenance of tendon cell identity and maturation.

METHODS: *Mrtfa*$^{-/-}$;*Mrtfb*$^{f/f}$ mice were crossed to *ScxCre* mice to target deletion in tendons (dKO) and analyzed at P56[7,8]. *ScxmCherry* was incorporated to facilitate visualization of tendons. Whole mount brightfield and fluorescent images of the Achilles tendon were used to measure tendon length. Tibia length was assessed via fluoroscopy (n=3). Transmission electron microscopy (TEM) was used to assess collagen fibrils (n=3) and analyzed using a custom MATLAB script[9]. Tensile testing was performed on tendons (0.5N preload for 1min, ramp to failure at 1%/s) (n=7-8). RNA-seq was performed from dissected Achilles tendons (n=3-4). Significantly differentially expressed genes were defined by $\log_2$FC > 1.25 and p-adj. < 0.05. Statistics were performed with student's t-tests with significance set at p<0.05. Male and female mice were used equally in all analyses; all mouse work was carried out under IACUC approval.

RESULTS: Achilles tendon length was significantly shorter in dKO mice compared to heterozygous littermate controls, even when normalized by body weight and tibia length (which were both reduced in dKO) (**Fig 1A**). TEM showed a dramatic shift towards a smaller fibril diameters, suggesting a defect in fibril maturation (**Fig 1C**). Consistent with this finding, elastic modulus was also reduced in dKO, although there was no difference in failure stress or in tendon cross-sectional area (**Fig 1B**). RNA-seq revealed that dKO tendons were generally transcriptionally similar to WT at P56, with only a handful of dysregulated genes (12 upregulated and 14 downregulated) compared to *Cre*-negative controls (**Fig 2A,B**). Interestingly, upregulated genes in the dKO tendons included genes related to chondrogenesis and ossification (*Col2a1, Alpl, Runx1*) suggesting that loss of MRTF signaling may result in phenotypic instability. This was further supported by calcification detected in the dKO tendons (TEM images, not shown), but not in the *Cre*- littermates, indicating heterotopic ossification (HO) in dKO tendons. Pathway analysis of the RNA-seq data revealed a strong suppression of TGFβ signaling in dKO tendons compared to controls (**Fig 2C**), suggesting that impaired mechanosensing may disrupt key pathways involved in tendon growth and homeostasis.

DISCUSSION: In this study, we showed that loss of MRTF signaling during postnatal tendon development gives rise to shorter, weaker, and less mature tendons by P56. While HO formation has been reported for tendon, it is typically observed in the context of age (1 year old mice) or injury; in this study we observed HO formation in young dKO tendons, suggesting that MRTF signaling may be important for maintenance of tendon fate in the postnatal period. Previous studies also reported HO in *Mkx* mutants postnatally that may be due to ectopic hedgehog signaling[10]. RNA-seq analyses of dKO mutants did not show regulation of *Mkx*, however, pathway analysis revealed that TGFβ signaling was suppressed in dKO tendons. Since TGFβ is required for maintenance of tendon fate[11], the regulation of phenotypic conversion may depend on both TGFβ and hedgehog. We also found that tendon marker expression (*Scx, Tnmd, Mkx*) was similar between dKO and WT tendons. Since we only assessed at a single timepoint, ongoing studies will define tendon phenotype at earlier stages. It is also possible that the absence of MRTF signaling results in tendon cells that cannot sense their normal physiological loading environment. This impaired mechanosensing may place the cells in an injury-like state, ultimately leading to the formation of HO. Current analyses will assess whether this loss triggers an inflammatory or injury-like response. Finally, since embryonic phenotypes were not observed with *ScxCre* deletion (not shown), we will also target limb mesenchyme using *Prx1Cre* to test the role of MRTF signaling in embryonic tendon development.

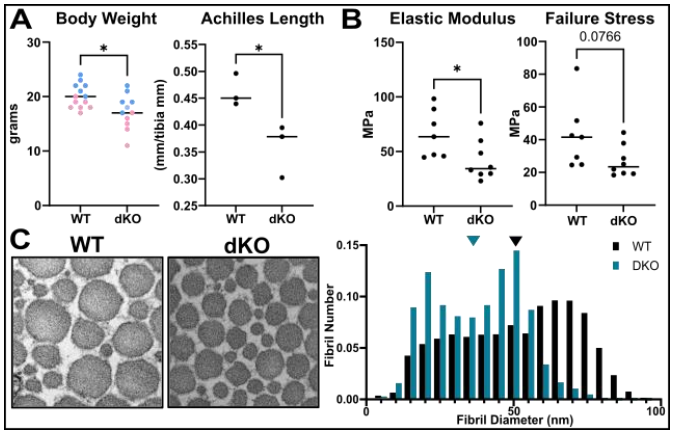

Fig. 1: *Mrtfa/b*^{ScxCre} tendons are structurally and functionally inferior. **A**: *Mrtfa/b*^{ScxCre} are smaller and their Achilles tendons are shorter, even when normalized to their smaller skeleton. **B.** dKO tendons have a lower elastic modulus **C.** dKO tendons have significantly smaller collagen fibrils on average with very few large fibrils. (n=3-8) t-test *p<0.05.

SIGNIFICANCE: Identifying mechanotransduction pathways involved in tendon development will inform future regenerative efforts.

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ACKNOWLEDGEMENTS: We acknowledge NIH/NIAMS R21 AR078966, R01 AR081673 to AHH and T32 AR080744 to ERK.

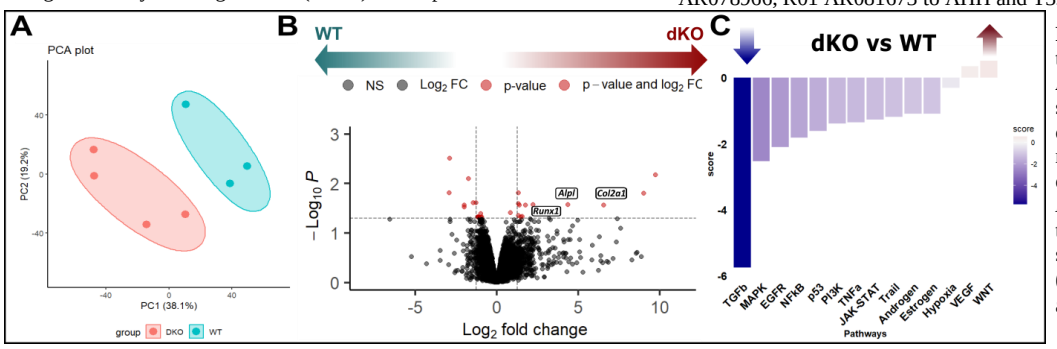

Fig. 2: *Mrtfa/b*^{ScxCre} tendons are transcriptionally distinct by P56. **A**: WT and dKO tendons cluster separately within the principal component space. **B.** Genes related to chondrogenesis and osteogenesis (*Alpl, Col2a1, Runx1*) are upregulated in dKO tendons. **C.** TGFβ signaling is suppressed in dKO tendons. (n=3-4) DEGs $\log_2$FC>1.25 p. adjusted < 0.05.