Cytoskeletal-Nuclear Mechanotransduction following Loss of Tension in Tendons

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Intro: 250 words max.... Current: 250

Tendon is a dense connective tissue that facilitates movement by transmitting forces from muscle to bone through a highly organized extracellular matrix (ECM), comprised of collagen fibrils that align parallel to the tendon axis.[2] Tendon cells maintain a state of "tensional homeostasis" by generating intrinsic forces (direct cellular contractility) and/or responding to externally applied mechanical loads.[2,4] Tensional homeostasis is critical as alterations in this process have been implicated in the progression of tendon disease, such as overuse tendinopathies.[4]

Cells can interpret applied mechanical cues into biochemical responses through a process called mechanotransduction.[3] External forces from the surrounding ECM can activate signaling cascades at focal adhesions, which transduce through the actin cytoskeleton to the nucleus via the linker of nucleoskeletal and cytoskeletal (LINC) complex, establishing a direct connection between the nucleus and the ECM.[1,3] As tensional forces from the ECM increase, so does nuclear tension, thus widening the nuclear pores to allow for transcriptional co-activators/factors, such as Yap/Taz, to translocate to the nucleus and alter gene expression.[2]

While it is well established that mechanical loading is critical for the formation and maintenance of tendon tissue, whether this signaling axis from the ECM to the nucleus is required for properly transducing these signals *in situ* is a key gap in knowledge. This study examines the effect of these mechanobiological signaling proteins on the tendon's response to tension loss. These insights may inform the pathogenesis of overuse tendinopathies, as loss of tension from fatigue damage can promote tissue remodeling and aberrant cell differentiation.

Methods/Materials: 250 words max... ~161 words

Tail Tendons from 8 week old mice were harvested, cut into 1.5cm segments, and cultured in media (DMEM, 10% FBS, 1% pen/strep/fungizone) in 12-well non-tissue culture treated plates and allowed to acclimate for 1 day following harvest before pharmacological perturbations were administered. The following transgenic mouse lines were used: Life-Act GFP mice (n=2), and homozygous *Myh9/Myh10* flox/flox mice (n=3). *Myh9/10* expression,

which encodes for non-muscle myosin IIA/B, was ablated utilizing the TAT-Cre system, a cell-permeable Cre fusion protein, with a 5-hour treatment (2.67mM) one day after tail tendon harvest. A subset of tendons was also treated with recombinant human TGF-β (5ng/ul, contractility agonist), Blebbistatin (10μM, actomyosin contractility inhibitor), and/or FAKi (10μM, focal adhesion kinase inhibitor) daily for 12 days. DMSO (10μM) was used as a vehicle control. Individual tendons were imaged daily and their length measured using Fiji/ImageJ to quantify the rate of tendon contraction. Life-act GFP tendons were imaged with AXIO scanner on day 12pt.

Results/Discussion/Conclusion: 350 words max... 311 words

This experiment investigated the necessity of specific proteins in the mechanotransduction signaling axis that acts through the F-actin cytoskeletal network on the ability of tendon cells to contract following loss of tension. Non-muscle myosin II (NMII) motor proteins engage with F-actin to generate contractile forces. To confirm its necessity, expression of Myh9 and Myh10 (genes encoding for the two main isoforms in tendon cells, NMIIA and NMIIB) was deleted from the tail tendons using TAT-Cre recombinase, resulting in explant contractions of only 33% while No TAT-Cre contracted 70% (Fig. 1A). This indicates the necessity of non-muscle myosin II for tendon contraction.

To further examine the role of NMII, tendons were cultured in Blebbistatin, a molecule that inhibits the myosin head from completing the powerstroke in the actomyosin complex. Blebbistatin-treated explants demonstrated minimal contraction (6%), significantly less than DMSO controls (74%) and pro-contractility cytokine TGF-β (92%) (Fig. 1B). When Blebbistatin treatment was challenged with the contractility agonist, TGF-β, only a modest increase in contraction occurred (12%), thereby reinforcing the necessity of NMII in tendon contraction.

Upstream of actomyosin, integrin proteins bind to the ECM and transduce mechanical cues to the cells through focal adhesion kinase (FAK). We inhibited FAK using a small molecule inhibitor (FAKi) and measured only a 20% reduction in tendon length, which increased to 40% when challenged with TGF-β (Fig. 1C).

To visualize actin remodeling across different pharmacological treatments, images of Life-act GFP tendons were taken on day 12. Fluorescence remained high in Blebbistatin-treated tendons, potentially because of reduced F-actin turnover. However, DMSO and TGF-β-treated explants exhibited lower fluorescence, perhaps because increased contractility induced higher F-actin remodeling.

Together, these findings suggest that NMII is essential for tendon contraction and mechanotransduction. Genetic deletion or pharmacological inhibition of NMII reduced tendon contractibility even with pro-contractile signals. In addition, upstream activation of FAK in focal adhesions is also critical for tendon cell contraction, indicating the importance of these complexes in cell-ECM interactions. Future studies will investigate the short and long-term cell responses to loss-of-tension to inform potential mechanisms of overuse tendinopathy disease pathogenesis following fatigue load damage to the tendon ECM.

Figure 1

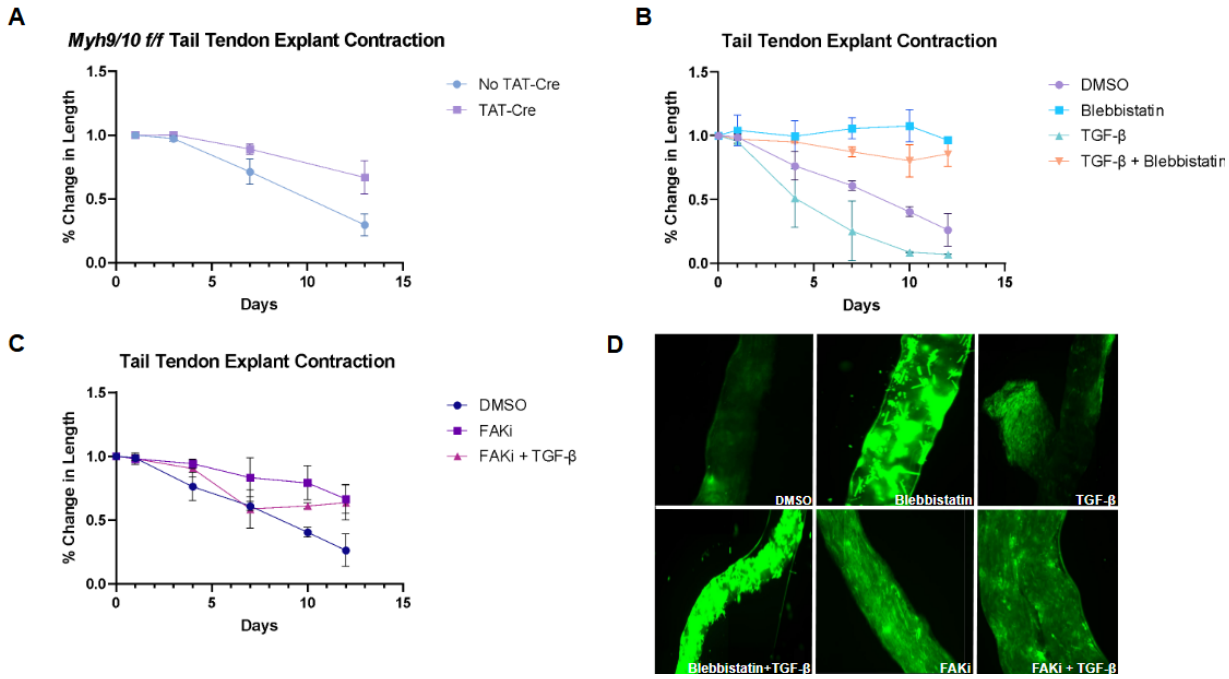

Figure 1: NMII and FAK inhibition abrogates tendon contraction. (A) Tail tendons from floxed Mhy9/10 mice exhibited reduced contraction-mediated shortening when treated with TAT-Cre compared to tendons treated with control media. Quantification of tail tendon lengths recorded at specific timepoints over 12 days (0, 1, 4, 7, 10, 12 days) normalized to the original tail length at day 0. (*B/C)* Inhibition of NMII with Blebbistatin and FAK with FAKi attenuated tail contraction despite the presence of the contractility-promoting agonist, TGF-β. (*D)* Fluorescent imaging of Life-Act GFP tail tendon fascicles at 12dpt. Enhanced fluorescence intensity was observed in the tendons cultured in inhibitory treatments (Blebbistatin and FAKi) instead of the contractility-inducing agonist TGF-β.

Sources:

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