Disabling mechanosensing via the LINC complex impacts developing tendon mechanics and collagen morphology

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INTRODUCTION: Tendon injuries are common due to aging or overuse during daily activities. As tendons have limited healing capacity, there is ongoing interest in tissue engineering to repair or replace these collagenous and mechanosensitive tissues. Cell-and-scaffold approaches for restoring tendon tissues are promising, but limited by the poor understanding of how mechanical signals regulate tendon development. Immature tendons depend on mechanosensation and mechanotransduction for differentiation and development, but many specific mechanobiological pathways are unknown. The nucleus has emerged as a major regulator of cellular mechanosensation, predominantly via transcriptional changes in response to external mechanical signals that are transduced through the linker of nucleoskeleton and cytoskeleton (LINC) protein complex. Specific roles of LINC in tenogenesis have not been explored, leaving a large knowledge gap regarding the role of nuclear mechanosensing in tendon development and function. Our objective in this study was to investigate how LINC may be regulating tendon development and tenogenesis by disabling LINC-mediated mechanosensing via dominant negative (dn) expression of the Klarsicht, ANC-1, and Syne Homology (KASH) domain, which is necessary for LINC to function. We hypothesized that LINC regulates mechanotransduction in developing tendon, and that disabling LINC would impact tendon mechanical properties and morphology in both a mouse and cellular model of dnKASH. We used Achilles (AT) and tail (TT) tendons as representative load-bearing and limb-positioning tendons, respectively.

METHODS: All animal work was approved by the Boise State University IACUC. Scleraxis-Cre/KASH-LacZ mice and dnKASH mesenchymal stem cells (MSCs) were generated as previously described[1]. C3H10T1/2 cells were used as controls, as previously described[2]. All dnKASH (++) and control (+-) animals were sacrificed at postnatal day 10 (P10) and tendons were dissected and mechanically evaluated via uniaxial tensile testing, or fixed and imaged using scanning electron microscopy, as previously described[3]. C3H10T1/2 and transfected dnKASH mouse MSCs were tenogenically differentiated for up to 21 days using recombinant human TGFβ2[2]. The presence of dnKASH was confirmed by genotyping in the mice, and puromycin selection in the cells. Western Blots (WB) evaluated impacts of dnKASH on proteins associated with tenogenesis in stem cells[3]. Crimp distance was measured as previously described[3]. Differences between dnKASH (++) and control (+-) tendons were assessed by unpaired T-tests with Welch's correction (significance: $p<0.05$).

RESULTS: Mechanical testing showed that disabling the LINC complex via dnKASH significantly impacted AT mechanical properties and cross-sectional area (CSA), and TT CSA (Fig. 1A-D). AT elastic modulus and maximum stress decreased significantly, while CSA increased. TT CSA decreased significantly. Collagen crimp distance was significantly lowered in ++ ATs, and significantly increased in ++ TTs (Fig. 1F-G), and the corresponding force-displacement curves for ATs also showed shortening of the toe region indicative of smaller crimp distance (Fig. 1E). SEM showed no obvious morphological changes to the ++ dnKASH tendons (Fig. 1H-I). WB showed increased N-cadherin, β-catenin, and Connexin-43 production in dnKASH cells, indicating a disruption of TGFβ2-induced tenogenesis (Fig. 1J).

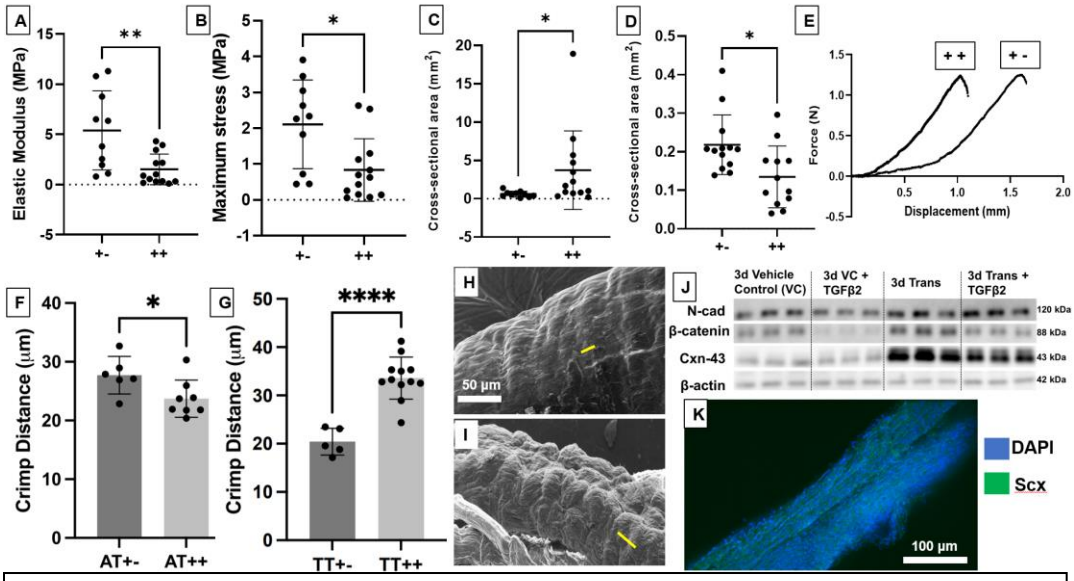

Fig. 1. Tendons from ++ dnKASH mice snow significant changes in mechanical properties. AT elastic modulus (A) and maximum stress (B) decrease, while cross sectional area increases (C). TT cross-sectional area decreases (D). (E) Representative AT force-displacement curves show the ++ dnKASH tendons reach peak force with smaller displacement, consistent with a shorter crimp distance. Crimp distance decreased significantly in dnKASH ATs (F), but increased in ++ TTs (G). AT (H) and TT (I) SEM images show the crimp pattern; yellow lines illustrate crimp distance. WB assays (J) showed changes in tenogenic and mechanosensitive protein levels in transfected cells, compared to control cells, suggesting LINC is necessary for TGFβ2-induced tenogenesis. (K) P10 dnKASH tendons express Scx.

DISCUSSION: dnKASH expression "disconnects" LINC from the cytoskeleton, preventing developing tendons or differentiating tendon cells from sensing their mechanical environment. While it is known that tendons require mechanical signals to develop correctly, it has not previously been shown which specific mechanotransducive pathways are involved. We show for the first time that disruption to the LINC complex specifically impacts tendon mechanics and crimp distance, as well as cellular tenogenic potential. As crimp distance is a function of collagen morphology and is also impacted by tendon mechanosensing, we show that nuclear mechanosensing is likely essential to healthy collagen function in mature tendon. Our prior work shows that removing mechanical signals via spinal cord transection in neonatal rats similarly impacts AT mechanical properties, but does *not* impact crimp distance in ATs or TTs[3]. Our current findings implicate a pathway previously unexplored in tendon development. LINC and KASH deserve further investigation.

SIGNIFICANCE/CLINICAL RELEVANCE: This study provides novel insight into a defined cellular mechanism via which disruptions to mechanical loading may influence tendon development. To our knowledge, no prior study has connected changes to tendon mechanical properties and collagen crimp morphology to a specific mechanotransducive pathway. Our findings may be used to inform tendon tissue engineering that incorporates mechanobiology.

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