Identification of novel transcription factors for developing tendons

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INTRODUCTION: Tendons are dense connective tissues that transmit muscle forces to bone. Despite their importance in musculoskeletal function, the critical factors regulating tendon biology during their development have not been fully identified. Since the first identification of *Scx* as a tendon transcription factor[1] only a handful of other transcription factors have been identified for tendon, and none are critical for tendon development (including *Scx*)[2-6]. Although a recent landmark study identified *Ebf* transcription factors as a regulator of tendon induction, this appears restricted to cranial tendons[7]. To identify new transcription factors for the body and limbs, we previously established an *in vitro* model of tendon development using mouse embryonic stem cells (mESCs) and showed high transcriptional fidelity of mESC-derived tenocytes compared to embryonic E14.5 mouse tail tenocytes by single cell RNA sequencing[8]. We then carried out bulk RNA-sequencing at timepoints throughout the fibrous differentiation phase and identified gene modules that had similar expression patterns to *Scx*, the earliest known marker for tendon progenitors. Using this unbiased approach, we now identify several candidate transcription factors, including *Bcl11a*, *Hlx, Mdfi*, *Pknox2, Foxp2,* as well as the long non-coding RNA *Dnm3os;* importantly, none were previously associated with tendon. To test the role of these factors in tendon development, we now use *in situ* hybridization to validate their expression in embryonic mouse limb tendons.

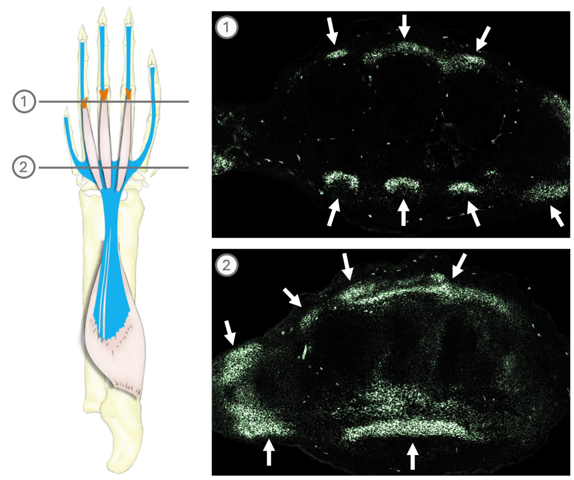

Fig. 1: RNAscope of transverse forelimb sections from E14.5 mouse embryos show *Scx* expression in developing tendons (white arrows).

METHODS: Mouse embryos were collected at E14.5 through timed matings. All procedures were consistent with IACUC guidelines. After harvest, limbs were fixed and embedded for cryosectioning. Multiplex fluorescent *in situ* hybridization (RNAscope) was performed on transverse forelimb cryosections using probes for candidate transcription factors, as well as the known tendon marker *Scx* to visualize tendons.

RESULTS: We first screened candidate factor expression at E14.5 since tendon differentiation and patterning is largely complete at this stage. At two different limb levels, we observed clear *Scx* expression specific to tendons (**Fig 1**). Out of the six candidate factors, *Dnm3os*, *Hlx*, and *Mdfi* appeared specific to tendons (**Fig 2**). *Hlx* and *Dnm3os* appeared more closely correlated with *Scx*+ regions while *Mdfi* was also detected in the developing cartilage, as well as surrounding mesenchyme. The remaining factors, *Pknox2*, *Foxp2*, and *Bcl11a* showed distinct expression in other tissues (**Fig 3**). While *Pknox2* appears to be co-localized to muscle regions as well as in the surrounding mesenchyme, *Foxp2* was detected in a subset of flexor tendon cells (but not extensor tendons) as well as the periosteum. *Bcl11a* was largely restricted to periosteum regions with no expression in limb tendons.

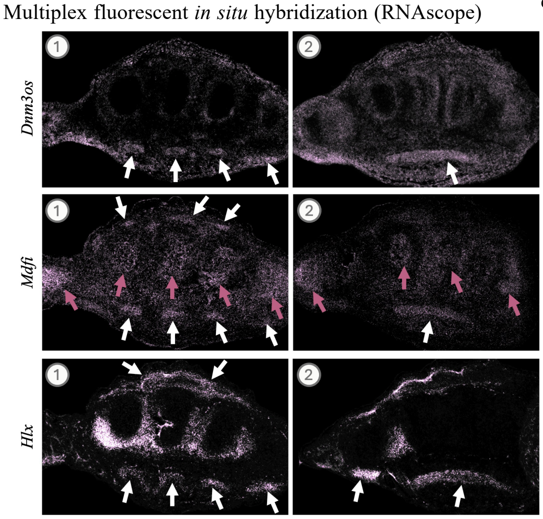

Fig. 2: RNAscope of transverse forelimb sections from E14.5 mouse embryos show *Dnm3os*, *Mdfi*, and *Hlx* expression in *Scx*+ developing tendons (white) and other tissues (cartilage: pink).

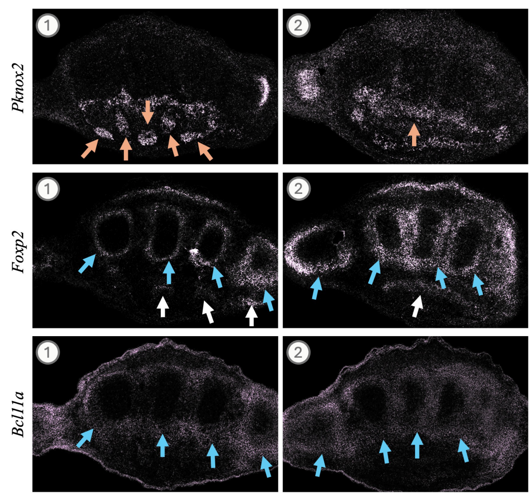

Fig. 3: RNAscope of transverse forelimb sections from E14.5 mouse embryos show *Pknox2*, *Foxp2*, and *Bcl11a* expression primarily in non-tendon musculoskeletal tissues (periosteum: blue, muscle: orange).

DISCUSSION: In this study, we identified *Dnm3os*, *Mdfi*, and *Hlx* as potential regulators of tendon development and validated our unbiased screening approach using mESC tenogenesis to model development. Although several candidate factors showed expression in periosteum, it is important to note that *Scx* is also detected in these regions. The relatively broad expression of *Scx* has now been confirmed in multiple studies demonstrating *Scx* expression in multiple types of connective tissues including muscle connective tissue, lung fibroblasts, and others[9-11]. *Dnm3os* is known to be expressed in the somite and limb bud, with null mutants showing skeletal deformity and lethality at one month of age[12]. Previous studies for *Hlx* function showed critical roles in hematopoiesis, as well as liver and gut development[13]. While the role of *Hlx* in tendon has not been investigated, a very early study suggested expression in the sub-compartment of the sclerotome that is now known to be the syndetome[14]. In contrast, *Mdfi* is known to be highly expressed in the sclerotome during mouse embryogenesis and inhibits the function of myogenic proteins[15]. Taken together, we hypothesize that these factors are regulators of tendon differentiation during embryonic development. Ongoing studies will determine the role of these genes *in vitro* with knockdown during mESC tenogenesis and *in vivo* with targeted deletion in tendon and limb mesenchymal cells.

SIGNIFICANCE: Identifying the regulators of tendon cell fate will improve understanding of tendon biology and provide target genes to focus on when developing cell-based therapies for tendon replacement.

REFERENCES: [1]Schweitzer+, Dev, 2001. [2]Guerquin+, JCI, 2013. [3]Ito+, PNAS, 2010. [4]Murchison+, Dev, 2007. [5]Liu+, JOR, 2015. [6]Liu+, Front. Cell Dev. Biol, 2021. [7]Niu+ Dev Cell, 2024. [8]Kaji+, Nat Comm, 2021. [9]Giordani+ Mol Cell, 2019. [10]Wang+ Cell Rep, 2025. [11]Ramírez-Aragón+ Int J Mol Sci, 2020. [12]Watanabe+ Dev Dyn, 2008. [13]Hentsch+, Genes Dev, 1996. [14]Lints+, Dev Dyn, 1996. [15]Kraut+, EMBO J, 1998.

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