Type III Collagen Expression Decreases During Neonatal Tendon Development and is Unchanged in Early Neonatal Tendon Healing

Margaret K. Tamburro[1], Jaclyn A. Carlson,[1,2] Miranda K. Doro,[1] Stephanie N. Weiss,[1] Susan W. Volk,[2] Louis J. Soslowsky[1]
[1]McKay Orthopaedic Research Laboratory, University of Pennsylvania, Philadelphia, PA
[2]School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA
Margaret.Tamburro@pennmedicine.upenn.edu

Disclosures: Margaret K. Tamburro (N), Jaclyn A. Carlson (N), Miranda K. Doro (N), Stephanie N. Weiss (N), Susan W. Volk (N), Louis J. Soslowsky (N)

INTRODUCTION: After tendon injury, fibrovascular scarring leads to inferior tendon function and high re-injury risk. Specifically, poor and insufficient remodeling of the provisional, type III collagen (Col3)-rich matrix to a highly aligned, type I collagen (Col1)-rich matrix results in a disorganized and weak matrix throughout healing. Much like the early healing matrix in adult tendon, developing embryonic tendon contains high levels of Col3 [1]. However, the magnitude and timing of *Col3a1* gene expression in the developing and healing neonatal tendon have not been elucidated; this information may provide crucial foundation for investigations of neonatal development and healing as potential mechanisms of superior tendon remodeling from a Col3- to Col1-rich matrix. Therefore, the objective of this study was to define the expression profile of the *Col3a1* gene throughout early neonatal development and healing. We hypothesized that *Col3a1* expression would be highest immediately post-partum and decrease throughout neonatal development. Additionally, we expected healing neonatal tendons to mount a quick and robust Col3 response with increased *Col3a1* expression during early healing timepoints.

METHODS: For investigations of neonatal development, thirty-five right knees from C57/B6 wild-type (WT) mice were harvested at postnatal days 0, 3, 7, 10, and 14 (p0, p3, p7, p10, p14; n ≥ 6/group mixed sex). For investigations of neonatal healing, twelve WT mice received right patellar tendon biopsy punch injury (0.3 mm diameter, performed under 10X magnification; Fig 1A, B) at 7 days of age. Right knees were harvested at 3- and 7-days post-injury, corresponding to p10 and p14 of the mice, respectively (n = 6/group mixed sex). All studies were IACUC approved. For all groups, patella-patellar tendon-tibia complexes were fixed for 4 hours in 4% paraformaldehyde, dissected, and cryo-embedded. Tendons were sectioned coronally (40 µm) and micro-dissected with a 25G needle to ensure proper isolation of the neonatal tendon for developmental ages (p0, p3, p7, p10, p14) or injured matrix for healing timepoints (3 days post-injury/p10, 7 days post-injury/p14). Dissected tendon tissue was digested, and RNA was isolated as described [2]. qPCR for *Col3a1* and *Abl1* (housekeeper) was performed. ΔCt values were calculated with reference to *Abl1* expression ($\Delta Ct = Ct_{Abl1} - Ct_{Col3a1}$). A one-way ANOVA was used to assess differences in *Col3a1* expression between developmental age and healing timepoints. Significance was set at $p < 0.05$.

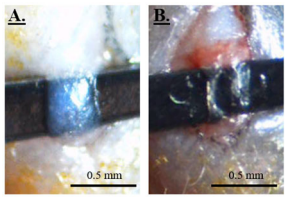

Figure 1: (A) Uninjured p7 patellar tendon. (B) p7 patellar tendon after biopsy punch (0.3 mm diameter) injury.

RESULTS: Supporting our hypothesis in the developing neonatal tendon, *Col3a1* expression was highest at p0 and decreased through p14, representing a 76% decrease in average *Col3a1* expression throughout this period (Fig 2A). Interestingly, *Col3a1* expression was not increased with neonatal injury throughout early healing timepoints. *Col3a1* expression 3 and 7 days after injury was not different from the uninjured baseline at p7 (Fig 2B) or from *Col3a1* expression at corresponding, uninjured developmental timepoints (p10 and p14; Fig 2B).

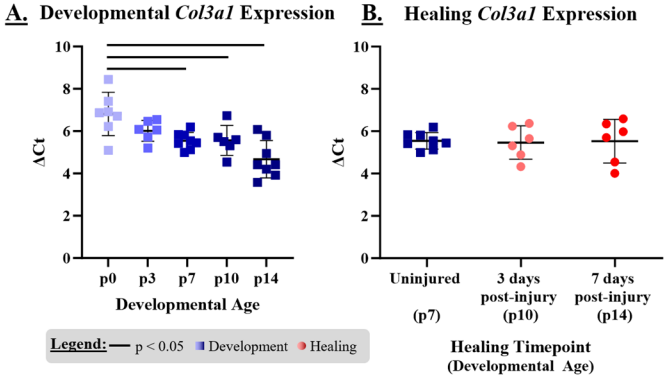

Figure 2: (A) Throughout postnatal development, *Col3a1* expression decreases. (B) After injury induced at p7, *Col3a1* expression is not increased 3 or 7 days after injury.

DISCUSSION: In this study, we defined the expression profile of the *Col3a1* gene throughout early neonatal development and healing to provide crucial foundation for investigations of neonatal development and healing as potential mechanisms of superior tendon remodeling.

Development is regarded as the ideal physiologic process for tendon matrix formation. Many regenerative approaches seek to recapitulate development, making the study of a key component of the developing tendon matrix, Col3, an important foundational step. *Col3a1* expression was previously known to be high *in utero* [1], and the current study is the first to measure the decrease in *Col3a1* expression in early neonatal development. Given the importance of temporally coordinated *Col3a1* expression in other developing, fibroblast-rich tissues [3], this *Col3a1* expression decrease may implicate Col3 in regulation of neonatal tendon development. Moreover, the temporal profile of *Col3a1* expression during neonatal development follows the same temporal profile of *Col3a1* expression during mature tendon healing [4] where expression is high after injury and decreases as healing progresses. Encouragingly, this highlights commonalities between neonatal development and mature healing which may be leveragable in approaches that seek to improve mature healing through biomimicry of neonatal development. Further research is evaluating additional developmental timepoints to identify when homeostatic *Col3a1* expression is achieved.

Neonatal tendon healing is another model of improved tendon matrix formation as neonatal healing is superior in speed and quality [5, 6] to mature healing. Given the similarities between healing in neonatal and mature contexts, neonatal tendon healing has become a favorable model for investigations of improved healing. Interestingly in the current study, neonatal injury did not affect overall *Col3a1* expression during early healing. This indicates a significant deviation from mechanisms of mature tendon healing where dramatically increased *Col3a1* expression is considered a hallmark of the healing response. Our previous investigations of mature mice (same C57/B6 strain) demonstrate increased *Col3a1* expression in early healing (Fig 3) [7, unpublished]. Given the improved healing observed in neonatal tendon, this finding may reveal potential for *Col3a1* modulation as a therapeutic method for improved tendon healing. Additional earlier and later healing timepoints are being explored to understand the complete temporal profile of *Col3a1* expression after neonatal tendon injury. Furthermore, immunostaining for Col3 will be completed for all developmental and healing timepoints to evaluate protein translation to add to the gene expression findings from the current study.

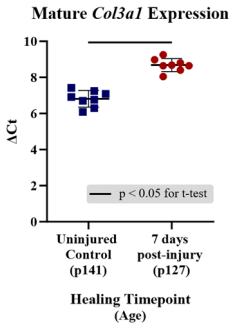

Figure 3: *Col3a1* expression during early tendon healing increases in mature mice [7, unpublished].

SIGNIFICANCE/CLINICAL RELEVANCE: Understanding temporal and mechanistic dynamics of neonatal tendon development and healing may highlight novel targets for improving tendon healing through regenerative approaches.

REFERENCES: [1] Birk et al, Eur J Cell Biol, 1997. [2] Leiphart et al, ORS, 2022. [3] Niederreither et al, Matrix Biol, 1995. [4] Dyment et al, PLoS ONE, 2013. [5] Ansorge et al, JOR, 2012. [6] Howell et al, Sci Rep, 2017. [7] Leahy et al, JOR, 2023.

ACKNOWLEDGEMENTS: This study was funded by NIH R01GM124091, R01AR080029, F31AR082282 and the Penn Center for Musculoskeletal Disorders (P30AR069619). The authors thank Nat Thurlow and Ashley Fung for their assistance with this work.