Col1a1 Expression Decreases while *Col3a1* Expression Increases after Neonatal Tendon Injury

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Introduction

Injured tendons in adult mammals heal with collagen III (Col3)-rich fibrovascular scarring. Col3 fibrils organize as a meshwork to resist low levels of multiaxial tensile strain.[1] In contrast, type I collagen (Col1) fibrils, the primary component of healthy tendon, align in parallel in uninjured tendons to resist high levels of uniaxial tensile strain. Consequently, healed tendons have compromised function and higher re-injury risk. Unlike injured adult tendons, neonatal tendons heal quickly with full recovery of functional properties.[2] Contributions of Col3 to the enhanced neonatal healing response are beginning to be explored; given the recently demonstrated dynamic nature of *Col3a1* expression throughout development,[3] *Col3a1* expression during neonatal healing must be assessed relative to appropriately matched developmental controls. Furthermore, given the significance of coordinated *Col1a1* and *Col3a1* expression in matrix development,[4] *Col3a1* expression dynamics should be contextualized in terms of Col1a1 expression dynamics to better understand regulation of matrix formation during neonatal development and healing. Therefore, the objective of this study was to define the dynamics of *Col1a1* and *Col3a1* expression following neonatal tendon injury. We hypothesized that, compared to physiologic developmental baseline, both *Col1a1* and *Col3a1* expression would increase in early neonatal tendon healing and return to developmental baseline by 21 days-post injury, reflecting a healing response that is more efficient and complete than adult healing.

Methods

C57/B6 wild-type mice (n = 9, mixed sex) received left patellar tendon biopsy punch injury (0.3mm diameter, performed under 10X magnification) at postnatal day 7 (p7). Right patellar tendons served as uninjured developmental controls. Mice were sacrificed at 7-days post injury (p14, n = 5) and 21-days post injury (p28, n = 4). At the time of sacrifice, left and right patellar tendons were isolated and homogenized in TRIzolTM (Invitrogen, Thermo, Waltham, MA). RNA was extracted, converted to cDNA, and pre-amplified (14 cycles) with TaqMan assays for *Col3a1* and *Abl1* as described.[5] qPCR was performed for Col1a1, Col3a1, and *Abl1* (housekeeper). ΔCt values were calculated with reference to *Abl1* expression, and fold change (FC) was calculated relative to uninjured developmental controls ($2^{\Delta\Delta Ct}$). Repeated measures two-way ANOVAs (injury status, timepoint) with multiple comparisons were used to assess differences in gene expression ($\alpha = 0.05$).

Results

Col1a1 expression increased while *Col3a1* expression was unchanged throughout physiologic development from p14 to p28 (Fig. 1A-B). Early after neonatal injury, at 7 days post-injury, *Col1a1* expression was decreased while *Col3a1* expression was increased compared to physiologic baseline (Fig. 1A-C). As healing progressed through 21 days-post injury, *Col1a1* expression increased but remained below physiologic baseline while *Col3a1* expression decreased but remained above physiologic baseline (Fig. 1A-C).

Discussion

We defined *Col1a1* and *Col3a1* expression dynamics during early and late healing to better understand matrix regulation during neonatal healing. In development, from p14 to p28, we observed an increase in *Col1a1* expression while *Col3a1* expression remained consistent. This complements previous work showing *Col3a1* expression decreases from p0 to p143 and suggests that expression may plateau at p14, remaining consistent through p28.3 Dynamic *Col1a1* expression in the context of stable *Col3a1* expression from p14 to p28 may implicate a changing Col3:Col1 ratio as important in physiologic tendon development.

Injury disrupts physiologic *Col1a1* and *Col3a1* expression in the healing neonatal tendon, with no return to baseline in late healing; *Col1a1* expression decreases while *Col3a1* expression increases. This *Col3a1*

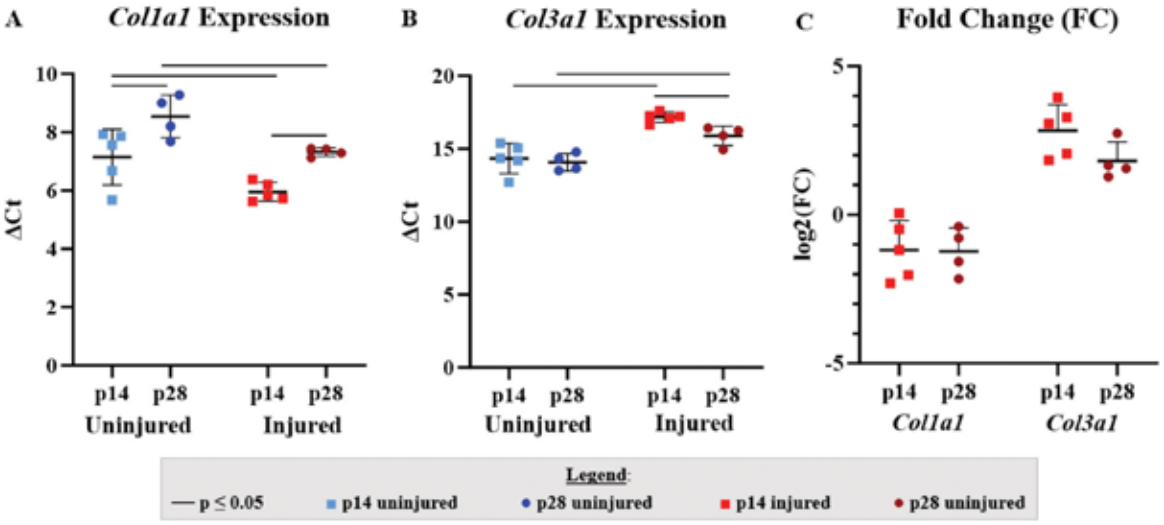

Figure 1. (A) *Col1a1* expression increases from p14 to p28 in uninjured development and is decreased at 7- and 14-days post-injury; **(B)** *Col3a1* expression is unchanged from p14 to p28 in uninjured development and is increased at 7- and 14-days post-injury; **(C)** Compared to uninjured, developmental baseline, injured tendons have a reduced *Col1a1* FC and increased Col3a1 FC at early and late healing timepoints.

expression increase parallels mature tendon healing, implying that neonatal injury may serve as a model of superior healing which is translatable to the adult condition.[6] Moreover, these expression dynamics may indicate that the neonatal healing process is ongoing at 21 days post-injury. Notably, in healing neonatal Achilles tendons, mechanical properties are recovered by 21 days post-injury.[2] As such, neonatal healing may restore mechanical properties despite persistent gene expression changes, or alternatively, this study may highlight differences in patellar and Achilles tendon healing. Mechanical assessment of healing neonatal patellar tendons should be pursued to further understand these possibilities.

Results from this study should be interpreted with consideration of the limitations of comparing injured and uninjured contralateral limbs. Systemic effects of injury on the contralateral limb have the capacity to influence tendon gene expression.[7] Additionally, as this investigation focused gene expression, future work will assess Col1 and Col3 protein amounts, offering a more comprehensive understanding of tissue healing. Nevertheless, this investigation demonstrates similarities in the *Col3a1* expression response in neonatal and adult tendon healing, highlighting the importance of continued study of mechanisms of improved healing in neonates and contributions of Col3 to superior neonatal healing to ultimately identify translatable targets for improving mature healing.

References

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