Collagen V Haploinsufficiency Results in Delayed Healing and Altered Wound Matrix Post-Injury in Murine Tendons

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Disclosures

None

Introduction

Patients with Classic Ehlers-Danlos syndrome (*c*EDS), a disorder characterized most commonly by *COL5A1* haploinsufficiency, suffer from tissue hyperelasticity, skin hyperextensibility, tendon/ligament fragility and abnormal wound healing[1,2]. Collagen V (ColV) haploinsufficiency leads to abnormal tissue development and altered collagen assembly, and mechanical loading of the mouse patellar tendon shows a delay in healing[3] and alterations in stiffness and dynamic modulus post-injury[4]. Furthermore, human studies have shown that females have decreased collagen synthesis[5] and altered gene expression during repair[6], likely influencing the healing potential of *c*EDS tendons.

The objective of this study was to determine the effect of ColV deficiency in female mice on wound matrix formation and the resultant structure-function relationships when mechanical load is applied post-injury. We hypothesized that ColV deficiency will have effects post-injury, resulting in increased fibril diameter and cellularity and decreased mechanical properties, leading to a delayed healing response when compared to wild-type tendons.

Methods

Adult female wild-type (WT) C57/BL6 and heterozygous *Col5a1*$^{+/-}$ mice, a model for *c*EDS, at 120 days of age (n = 84) were used (IACUC approved). Mice were randomly divided into uninjured and injured groups, with injured mice undergoing bilateral patellar tendon injury surgery as previously described[7]. Injured mice were sacrificed early in the proliferative phase at 1-week (1w), early in the remodeling phase at 3-weeks (3w) or later in the remodeling phase at 6 weeks (6w). Uninjured age-matched mice were also sacrificed. Uninjured and injured patellar tendons of both genotypes were assessed.

Gene Expression and Transmission Electron Microscopy (TEM)

Real-time PCR was done as previously described[8]. Each sample (n = 4) was run in duplicate and data was analyzed using StepOne software v2.0. Samples for TEM analysis of fibril structure (n = 4) were fixed *in situ* and processed as described[9].

Mechanics and Histology:

The patella-patellar tendon-tibia complexes were dissected and prepared for mechanical testing (n = 12)[10]. Tendons were subjected to a viscoelastic testing protocol containing three stress relaxations cycles followed by frequency sweeps, culminating in a ramp-to-failure. Dynamic collagen fiber realignment was quantified using cross-polarization imaging during the ramp-to-failure[10]. Histological sections of the patellar tendon-bone complex (n = 4) were prepared using standard techniques. Cellularity was calculated using a standard grading scale.

Statistics

Two-way ANOVAs with post-hoc Bonferroni tests were used to assess the effects of genotype and time on gene expression. Two-way repeated measures ANOVAs with post-hoc Bonferroni tests were used to assess the changes in realignment for increasing strain levels. Kruskal-Wallis non-parametric one-way ANOVA followed by post-hoc Dunn's test for multiple comparisons were used for histologic data. Significance was set at $p \leq 0.05$.

Results

Col5a1 expression was significantly increased in WT tendons at 1w and 3w post-injury (PI) compared to uninjured controls. However, no significant changes in *Col5a1* expression were seen following injury in *Col5a1*$^{+/-}$ tendons (Figure 1). Genotypic differences in *Col5a1* expression were seen at 1w and 3w PI (Figure 1). Fibrils from the mid-substance of WT and *Col5a1*$^{+/-}$ tendons are shown in Figure 2A, with injured tendons having a dominant population of smaller diameter fibrils. Uninjured WT and *Col5a1*$^{+/-}$ distributions were comparable (data not shown), however, distinctly different distributions for WT and *Col5a1*$^{+/-}$ fibrils PI were seen, with *Col5a1*$^{+/-}$ fibrils being larger and more broadly distributed (Figure 2B). Further, WT and *Col5a1*$^{+/-}$ tendons realigned through 5% strain, with *Col5a1*$^{+/-}$ tendons continuing

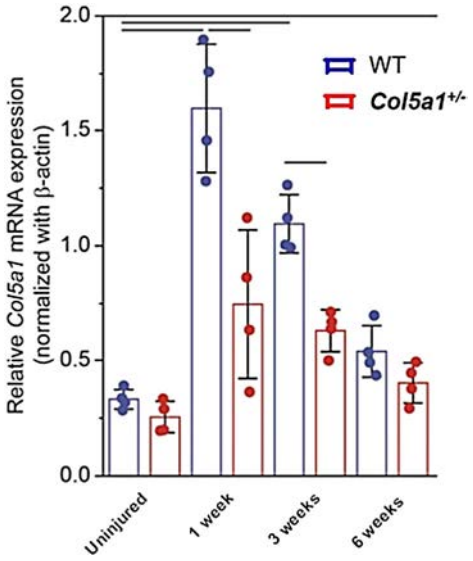

Figure 1. *Col5a1* expression increases 1w and 3w PI in WT but not in *Col5a1*$^{+/-}$ tendons. Solid lines denote significance.

to realign through 6% strain (Figure 3A). Lastly, significant differences in cellularity (Figure 3B) were seen between uninjured and both 1w and 3w samples in both genotypes, and between 1w and both 3 and 6w samples, with no difference between genotypes at any time-point. *Col5a1*$^{+/-}$ tendons had a significant increase in cellularity persisting to 6w PI when compared to uninjured tendons (Figure 3B).

Discussion

ColV plays a key role in fibrillogenesis, matrix remodeling and response to injury, affecting the structure and function of healing tendon. The lack of an increase in *Col5a1* expression 1w PI in *Col5a1*$^{+/-}$ tendons would affect all stages of later healing and indicates a reduction in regulation of fibrillogenesis throughout healing. WT *Col5a1* expression returns to uninjured and *Col5a1*$^{+/-}$ levels by 6w PI injury, indicating that the early increase affects fibril diameter, mechanical properties and cellularity throughout healing. Without the initial increase in ColV following injury, fibrillogenesis is less regulated, resulting in a broader distribution of fibril diameter and a shift to larger fibrils of *Col5a1*$^{+/-}$ tendons PI, which explains the delayed realignment and is supported by previous work[11]. Following injury, a shift towards smaller fibrils was expected as these are new fibrils from collagen secreting myofibroblasts to fill the injury void. Additionally, increased cellularity in *Col5a1*$^{+/-}$ tendons would alter the matrix alignment and architecture, weakening the tissue and affecting mechanical properties. The persistence of increased cellularity in *Col5a1*$^{+/-}$ tendons at 6w PI is consistent with viscoelastic[3] and fatigue data[4], which indicates a delayed healing response

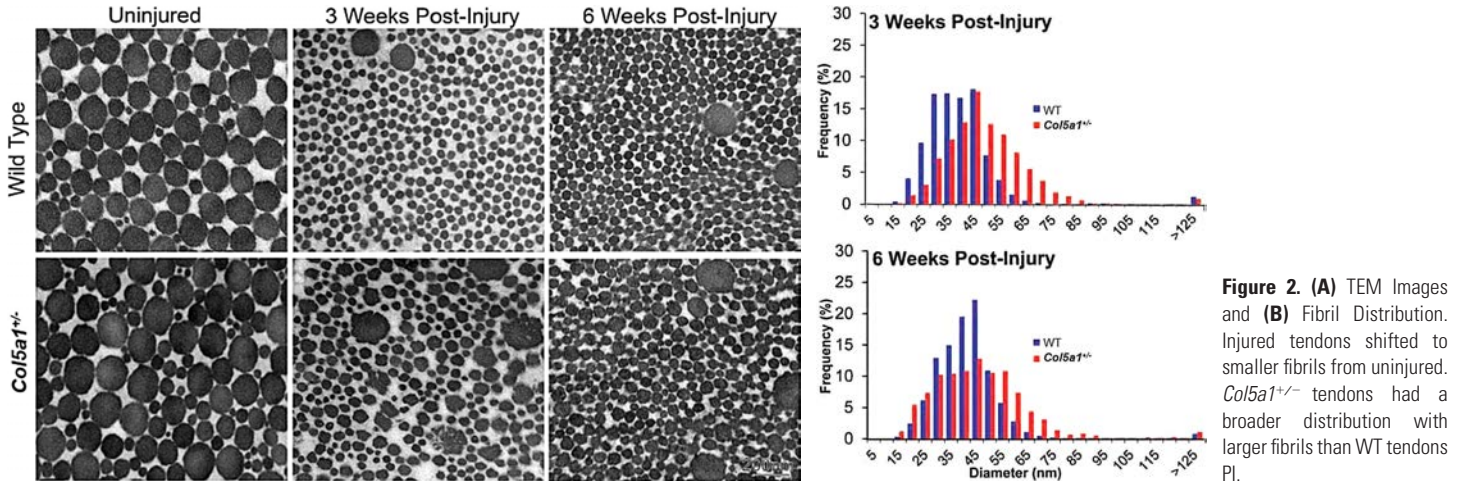

Figure 2. (A) TEM Images and **(B)** Fibril Distribution. Injured tendons shifted to smaller fibrils from uninjured. *Col5a1*$^{+/-}$ tendons had a broader distribution with larger fibrils than WT tendons PI.

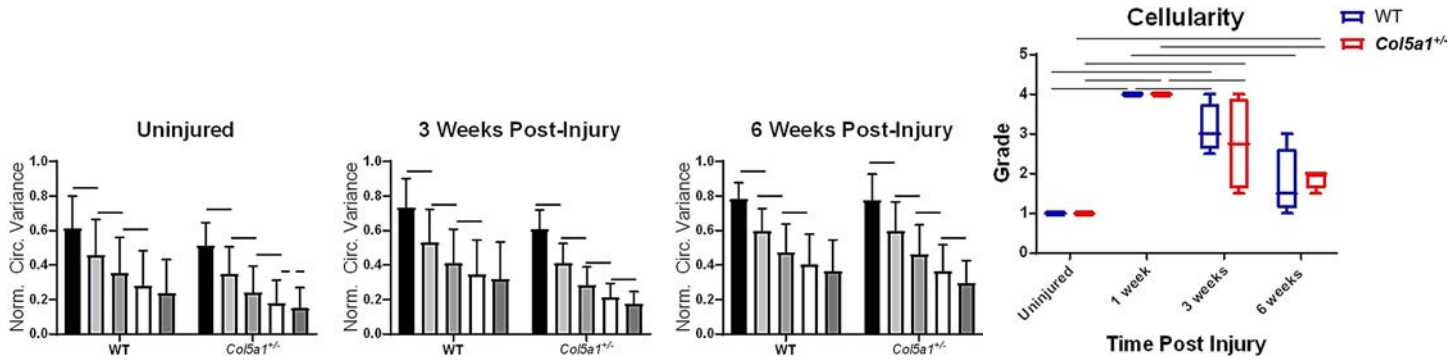

Figure 3. (**A**) Midsubstance Fiber Realignment. *Col5a1*$^{+/-}$ uninjured and injured tendons realign through 6% strain, while WT realign through 5% strain. **(B)** Tendons of both genotypes increased significantly in cellularity 1 and 3w PI when compared to uninjured, this increase persisted to 6w PI in *Col5a1*$^{+/-}$ tendons. Differences in cellularity were also seen between 1w and both 3 and 6w PI in both genotypes. Solid lines denote significance.

in *Col5a1*$^{+/-}$ tendons. Qualitative comparisons to male data show *Col5a1*$^{+/-}$ fibril distribution was similar to WT and cellularity returned to uninjured levels by 6w PI, unlike the data shown, indicating that sex affects outcomes of reduction in ColV[12]. Future directions may include later healing time points to better understand the extent of the delayed healing.

Significance

This study indicates that the lack of an early increase in *Col5a1* expression PI in *Col5a1*$^{+/-}$ tendons influences matrix architecture, alignment, and cellularity throughout tendon healing, demonstrating altered and delayed healing compared to WT tendons.

Acknowledgements

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