Conditional Deletion of Decorin and Biglycan in Mature Mouse Tendons Results in Inferior Mechanical Properties and Delayed Collagen Fiber Realignment

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Introduction

Tendon and ligament injuries occur more commonly with increasing age[1] and there is no clear understanding of the mechanisms underlying the pathophysiology of tendon aging. Two small-leucine rich proteoglycans (SLRPs) known as decorin and biglycan, play an important role regulating the assembly and organization of collagen fibrils during development[2-4]. The absence of decorin has been shown to prevent the normal decline in mechanical properties that occurs with aging in conventional knockout mouse models[5]. However, the impact that these SLRPs have on tendon aging and homeostasis independent of their influence on development has yet to be established. Therefore, the objective of this study was to investigate the acute effects of conditional deletion of decorin alone and both decorin and biglycan in mature uninjured tendons. We hypothesize that the loss of decorin or decorin and biglycan will not acutely alter the mechanical properties of mature, uninjured tendons because they will have undergone normal development and minimal aging will have occurred from the time of gene inactivation to sacrifice.

Materials and Methods

Conditional female $Dcn^{flox/flox}/Bgn^{+/+}$($Dcn^{-/-}$) and $Dcn^{flox/flox}/Bgn^{flox/flox}$ (compound decorin/biglycan null) as well as $Dcn^{+/+}/Bgn^{+/+}$control (WT) mice (n = 16/group) with a TM inducible Cre in the Rosa26 locus were utilized (IACUC approved). To induce Cre excision of the conditional alleles, mature 120 day old mice received three consecutive daily IP injections of tamoxifen (9mg/40g body weight). WT mice received tamoxifen injections to control for potential side effects. Mice were euthanized at 150 days old and whole knees were collected. The patellar tendon-bone complex from one limb of each animal was dissected from the knee and prepared for biomechanical testing as described[5]. Tendons were subjected to a viscoelastic testing protocol as well as ramp to failure. Dynamic collagen fiber realignment was quantified using our established cross-polarization imaging technique as described[5]. A bilinear fit was used to describe the toe and linear regions of the load-displacement curve and peak force, equilibrium force, and percent relaxation during stress-relaxation were quantified. Dynamic modulus and phase angle delta were computed during frequency sweeps at multiple strain levels and fiber realignment, failure stress, and failure load were computed during ramp to failure. From the contralateral limb, histological sections of the patellar tendon-bone complex were prepared and stained using standard techniques. Cell shape and cellularity were calculated using commercial software (Bioquant). One way ANOVAs with post-hoc Bonferroni corrections ($p < 0.05/3$) were used to establish significance between genotypes. Kruskal-Wallis tests with post-hoc Dunn's multiple comparisons were used for non-normal data sets.

Results

Compound decorin/biglycan knockouts exhibited increased percent relaxation during the linear region of the test (5% strain) when compared to tendons from WT controls (Figure 1A) and failed at lower loads than both WT and $Dcn^{-/-}$ tendons (Figure 1B). No significant differences in cross-sectional area, modulus, toe or linear stiffness, or transition strain were found between genotypes. The compound knockouts exhibited increased tan(δ) when compared to WT tendons at nearly all frequencies at both toe (not shown) and linear strain levels (Figure 2). However, there were no significant differences in dynamic modulus across groups. In addition, compound knockouts underwent more realignment at the insertion during the linear region of the test than WT tendons (Figure

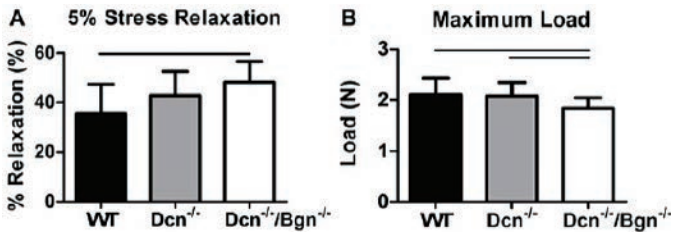

Figure 1. Quasi-static properties of WT, $Dcn^{-/-}$, and $Dcn^{-/-}/Bgn^{-/-}$ patellar tendons. Thirty days after gene inactivation, compound knockouts had increased percent relaxation at 5% strain **(A)** and lower failure loads **(B)**.

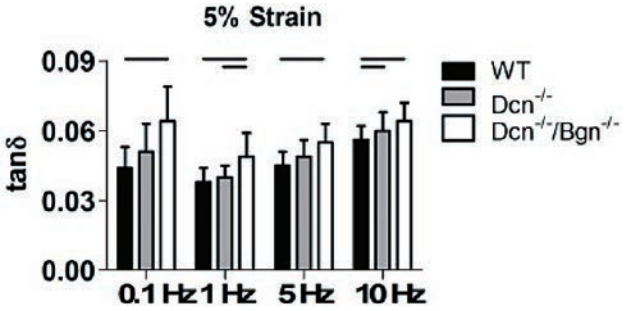

Figure 2. Viscoelastic properties of WT, $Dcn^{-/-}$, and $Dcn^{-/-}/Bgn^{-/-}$ patellar tendons. Compound knockout tendons had significantly increased tan(δ) at 5% strain compared to WT.

3A). The insertion region of WT and $Dcn^{-/-}$ tendons realigned earlier than compound knockout tendons (at 3-5% strain compared to 5-7% strain) (Figure 3B-3D). The midsubstance of WT tendons realigned earlier than both $Dcn^{-/-}$ and $Dcn^{-/-}/Bgn^{-/-}$ tendons (at 1-3% strain compared to 3-5% strain) (not shown). No significant differences in cell density or cell shape were observed across groups (not shown).

Discussion

SLRPs are important for maintaining normal tendon structure and function[5,6]. Contrary to our hypothesis, compound knockout tendons responded inferiorly during dynamic loading compared to WT and $Dcn^{-/-}$ tendons 30 days after gene inactivation. These results suggest that even after a short absence of SLRP expression, tendons deficient in both decorin and biglycan fail at lower loads and are more dissipative of energy during dynamic loading. Furthermore, compound knockout tendons exhibited increased realignment at 5-7% strain, which corresponds to the linear region of the stress-strain curve, consistent with increased fibril sliding during realignment as described previously[7]. Tendons deficient in both SLRPs also realigned at later strains

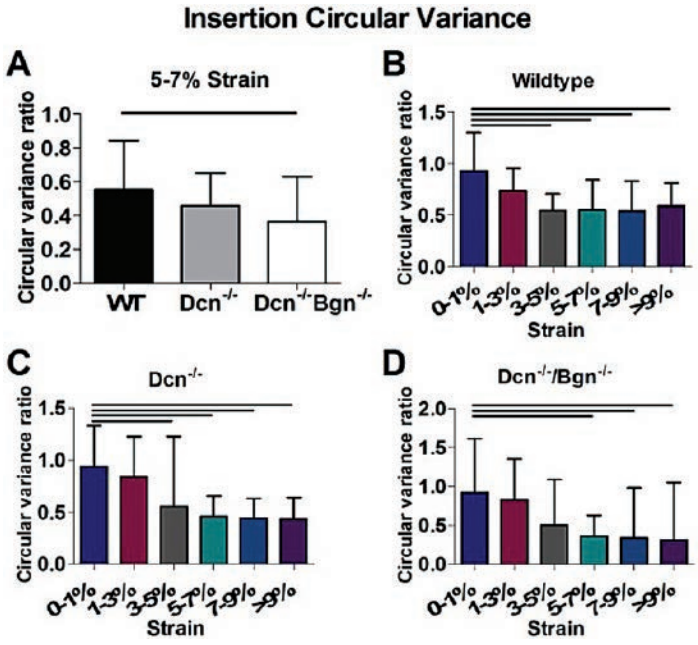

Figure 3. Realignment of WT, $Dcn^{-/-}$, and $Dcn^{-/-}/Bgn^{-/-}$ patellar tendons. $Dcn^{-/-}$/Bgn-/- tendons exhibit increased realignment at 5-7% strain **(A)**. WT **(B)** and $Dcn^{-/-}$ **(C)** tendon insertions realign earlier than $Dcn^{-/-}/Bgn^{-/-}$ **(D)**.

than WT tendons at both the insertion and midsubstance, which may explain their tendency to fail earlier than WT tendons. Previous work found that the normal loss of modulus, increase in tan(δ), and changes in fiber organization that occur in aged mice were prevented in conventional decorin knockout mice while conventional biglycan knockout mice and WT mice aged similarly[5]. The early differences seen in this novel conditional knockout model suggest that the concomitant deletion of both decorin and biglycan after development interferes with the maintenance of the normal tendon response to load at maturity. Therefore, decorin and biglycan may work synergistically to regulate the structure and function of mature tendon. Alternatively, dysfunctional expression of biglycan in $Dcn^{-/-}$ mice may compensate for the absence of decorin and provide protection from some of the changes that occur in the compound knockouts. Future work will focus on the impact that the conditional deletion of decorin and biglycan during maturity has on the mechanical and structural properties of aged tendons.

Conclusions

This study begins to define the interactions between decorin and biglycan in maintaining tendon homeostasis at maturity and will help clarify their role in tendon aging.

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References

1. Buckwalter JA, Heckman JD, Petrie DP; AOA. An AOA critical issue: aging of the North American population: new challenges for orthopaedics. *J Bone Joint Surg Am.* 2003 Apr;85-A(4):748-58. Review. PubMed PMID: 12672854.

2. Schönherr E, Hausser H, Beavan L, Kresse H. Decorin-type I collagen interaction. Presence of separate core protein-binding domains. *J Biol Chem.* 1995 Apr 14;270(15):8877-83. PubMed PMID: 7721795.

3. Schönherr E, Witsch-Prehm P, Harrach B, Robenek H, Rauterberg J, Kresse H. Interaction of biglycan with type I collagen. *J Biol Chem.* 1995 Feb 10;270(6):2776-83. PubMed PMID: 7852349.

4. Zhang G, Ezura Y, Chervoneva I, Robinson PS, Beason DP, Carine ET, Soslowsky LJ, Iozzo RV, Birk DE. Decorin regulates assembly of collagen fibrils and acquisition of biomechanical properties during tendon development. *J Cell Biochem.* 2006 Aug 15;98(6):1436-49. PubMed PMID: 16518859.

5. Dunkman AA, Buckley MR, Mienaltowski MJ, Adams SM, Thomas SJ, Satchell L, Kumar A, Pathmanathan L, Beason DP, Iozzo RV, Birk DE, Soslowsky LJ. Decorin expression is important for age-related changes in tendon structure and mechanical properties. *Matrix Biol.* 2013 Jan;32(1):3-13. doi: 10.1016/j.matbio.2012.11.005. Epub 2012 Nov 23. PubMed PMID: 23178232; PubMed Central PMCID: PMC3615887.

6. Corsi A, Xu T, Chen XD, Boyde A, Liang J, Mankani M, Sommer B, Iozzo RV, Eichstetter I, Robey PG, Bianco P, Young MF. Phenotypic effects of biglycan deficiency are linked to collagen fibril abnormalities, are synergized by decorin deficiency, and mimic Ehlers-Danlos-like changes in bone and other connective tissues. *J Bone Miner Res.* 2002 Jul;17(7):1180-9. PubMed PMID: 12102052.

7. Connizzo BK, Sarver JJ, Han L, Soslowsky LJ. In situ fibril stretch and sliding is location-dependent in mouse supraspinatus tendons. *J Biomech.* 2014 Dec 18;47(16):3794-8. doi: 10.1016/j.jbiomech.2014.10.029. Epub 2014 Oct 31. PubMed PMID: 25468300; PubMed Central PMCID: PMC4261030.