Differential Roles for Decorin and Biglycan in Tendon Aging

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Introduction

Tendon injuries occur more commonly with increasing age[1], yet the mechanisms underlying the process of tendon aging are unclear. Decorin (Dcn) and biglycan (Bgn) are two small leucine-rich proteoglycans (SLRPs) that are regulators of collagen fibrillogenesis and are highly expressed during tendon development[2]. The absence of Dcn has been shown to prevent the normal decline in mechanics with decreasing age[3], while the inducible deletion of Bgn[4] and compound Dcn/Bgn[5] resulted in reduced mechanical and structural properties in mature tendon. However, the roles of Dcn and Bgn on tendon aging, independent of their influence on development, are unknown. Therefore, the objective of this study was to determine the differential roles of Dcn and Bgn during tendon aging. Due to the detrimental effects of Dcn on tendon aging, we hypothesized that the Dcn- and Dcn/Bgn-null mice will show a reduced impact of aging on mechanical and structural properties compared to WT and Bgn-null mice.

Methods

Female $Dcn^{+/+}/Bgn^{+/+}$ control (WT, n = 32), $Dcn^{flox/flox}$ (I-$Dcn^{-/-}$, n = 32), $Bgn^{flox/flox}$ (I-$Bgn^{-/-}$, n=32), and compound $Dcn^{flow/flox}/Bgn^{flox/flox}$ (I-$Dcn^{-/-}/Bgn^{-/-}$, n = 32), mice with a tamoxifen (TM) inducible Cre, (B6.129-Gt(ROSA)26Sortm1(cre/ERT2)Tyj/J, Jackson Labs) were utilized[5] (IACUC approved). Cre excision of the conditional alleles was induced in mature (120 day)[5] mice via three consecutive daily IP injections of tamoxifen (4.5mg/40g body weight). WT mice received TM injections to control for potential side effects. Mice were euthanized at 300 and 570 days of age (n = 16/group/age). The patellar tendon-bone complex from one limb of each animal was dissected and prepared for mechanical testing[6]. Tendons (n = 16) were subjected to a viscoelastic testing protocol of three stress relaxations, each followed by frequency sweeps, with the test culminating in a ramp-to-failure. Percent relaxation was quantified for each stress-relaxation. Samples for transmission electron microscopy (TEM) analysis of fibril structure (n = 4) were fixed *in situ*[5]. Cross sections through the midsubstance of the patellar tendon were examined at 80 kV. Fibril diameter was measured using images from the center of the tendon.

Histological sections of the patellar tendon-bone complex (n = 4) were prepared using standard techniques. Cell shape and cellularity were calculated using commercial software (Bioquant). A two-way ANOVA was performed followed by Bonferroni post-hoc analysis to evaluate the effect of genotype and age on tendon mechanics. Kolmogorov-Smirnov tests were used for the analysis of TEM and histology data. Significance was set at $p < 0.05$.

Results

Genotype significantly affected midsubstance modulus, while age and the interaction between age and genotype did not. Induced deletion of *Dcn* resulted in increased midsubstance elastic modulus at 300d (Figure 1A) versus WT and I-$Dcn^{-/-}/Bgn^{-/-}$. These differences were not present at 570d or in the insertion region (Figure 1B). Stress relaxation at 3% strain (Figure 2A) revealed genotype, age, and the interaction between genotype and age as significant. I-$Dcn^{-/-}/Bgn^{-/-}$ showed increased percent relaxation versus WT, while I-$Dcn^{-/-}$ exhibited decreased percent relaxation. Genotype and age significantly affected stress relaxation at 4% strain (Figure 2B), while the interaction did not. I-$Dcn^{-/-}/Bgn^{-/-}$ displayed increased relaxation versus I-$Dcn^{-/-}$ at 300d, and increased relaxation versus WT and I-$Dcn^{-/-}$ at 570d. Stress relaxation at 5% strain (Figure 2C) showed no significant differences between groups. Dynamic modulus (E*) and phase angle delta (δ) revealed no changes between genotypes, but age and the interaction was significant for I-$Dcn^{-/-}$ for E* and tan(δ) and for I-$Dcn^{-/-}/Bgn^{-/-}$ tan(δ) (Figure 3). I-$Dcn^{-/-}$ E* was decreased at 570d vs 300d, while tan(δ) was increased (Figure 3A-B). I-$Dcn^{-/-}/Bgn^{-/-}$ showed increased tan(δ) at 3% strain for 0.1-5 Hz, and 4% strain at 5 Hz (Figure 3C).

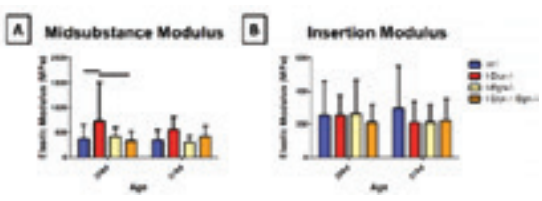

Figure 1. Quasi-static mechanical properties. Deletion of decorin resulted in increased midsubstance elastic modulus at 300d compared to WT and I-$Dcn^{-/-}/Bgn^{-/-}$ **(A)**. No differences were found at 570d or in the insertion **(B)**.

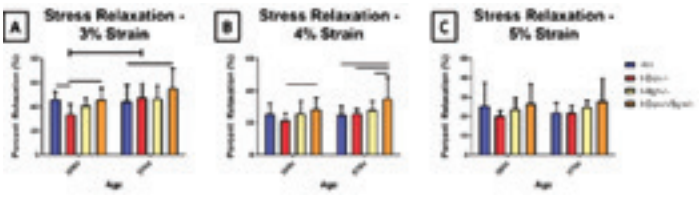

Figure 2. Stress Relaxation. I-*Dcn*$^{-/-}$/*Bgn*$^{-/-}$ showe d increased stress relaxation at 3% and 4% strain at 300d & 570d **(A, B)**. At 3% strain, 570d I-*Dcn*$^{-/-}$ stress relaxation was increased vs 300dI-*Dcn*$^{-/-}$ **(A)**. No changes were seen at 5% strain **(C)**.

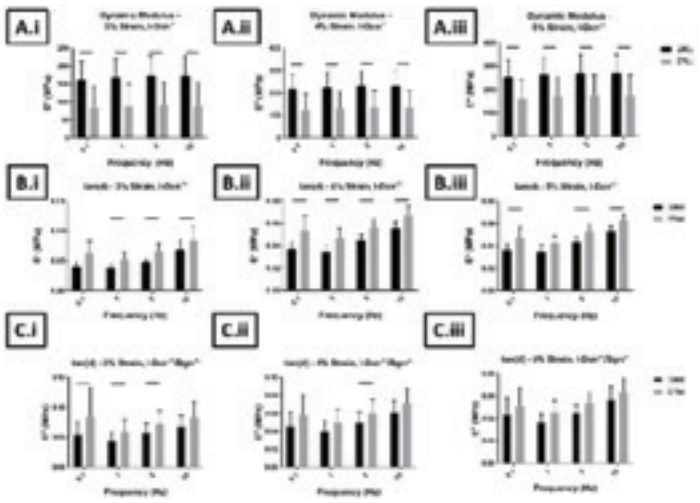

Figure 3. Age-related changes in viscoelast c mechanics. 570d I-*Dcn*$^{-/-}$ resulted in increased dynamic modulus and increased tan(δ) vs 300d I-*Dcn*$^{-/-}$ **(A, B)**. 570d I-*Dcn*$^{-/-}$/*Bgn*$^{-/-}$ resulted in increased tan(δ) at 3%strain and lower frequencies vs 300d **(C.i)**. These differences were remained present at 4% strain and 5 Hz **(C.ii)**. No changes were seen between 300d and 570d I-*Dcn*$^{-/-}$/*Bgn*$^{-/-}$ in dynamic modulus at any strain or frequency, or tan(δ) at 5% strain.

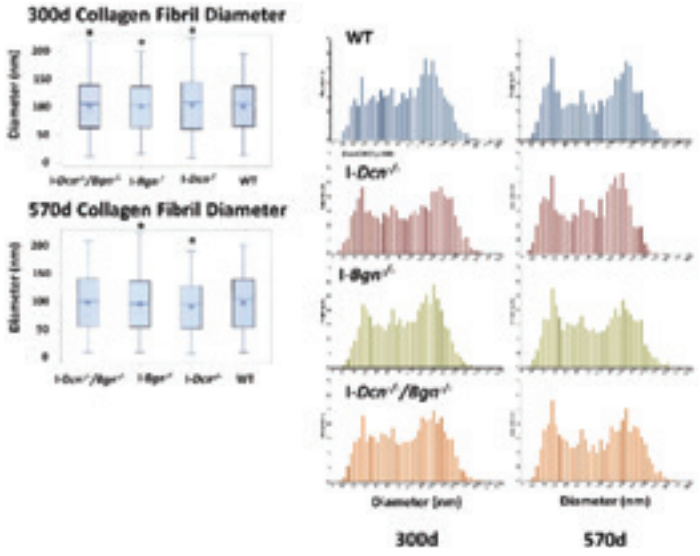

Figure 4. TEM analysis of patellar tendon collagen fibril diameter. I-*Dcn*$^{-/-}$ and I-*Bgn*$^{-/-}$ showed altered collagen fibril diameter at 300d & 570d versus WT. I-*Dcn*$^{-/-}$/*Bgn*$^{-/-}$ resulted in an altered fibril diameter at 300d, but not 570d. * denotes significance vs WT

TEM analysis revealed that I-*Dcn*$^{-/-}$ and I-*Bgn*$^{-/-}$ had altered fibril diameters vs WT at 300d and 570d (Figure 4). I-*Dcn*$^{-/-}$/*Bgn*$^{-/-}$ showed altered fibril diameter versus WT at 300d, but not 570d. Notably, I-*Dcn*$^{-/-}$ revealed increased fibril diameter heterogeneity, an increased maximum fibril diameter, and decreased minimum fibril diameter at 300d. At 570d, I-*Dcn*$^{-/-}$ fibril diameter was reduced versus WT in quartiles 2-4. Histology revealed no significant differences for cell shape or cellularity between any genotypes at 300d or 570d.

Discussion

Supporting our hypothesis, the absence of Dcn resulted in a reduced impact from aging on tendon mechanics, including an increased midsubstance elastic modulus and decreased stress relaxation at 300 day vs WT. Additionally, at 300d, I-*Dcn*$^{-/-}$ had an improved dynamic modulus and phase angle vs I-*Dcn*$^{-/-}$ at 570d, indicating limitations to the extent that the absence of Dcn can improve tendon mechanics during aging. I-*Dcn*$^{-/-}$ also resulted in significant alterations in collagen fibril diameter compared to WT at both 300 and 570 day. At 300 day, the fibril diameter heterogeneity was increased, while at 570 day, there was a reduction in fibril diameter in the upper 75% of the distribution. Contrary to our hypothesis, the absence of both Dcn and Bgn did not result in an improved aging phenotype, with no changes in midsubstance or insertion modulus, and increased stress relaxation versus WT and I-*Dcn*$^{-/}$. The absence of Bgn resulted in an altered fibril diameter distribution with no changes in mechanics. A role for both Dcn and Bgn was revealed in the maintenance of tendon structure at 300 and 570 days. These results support previous work examining the effects of Dcn during tendon aging, which showed no changes in mechanics between mature and aged Dcn knockout mice, while WT and Bgn knockout mice showed declining mechanics with age[3]. Further, reduced viscoelastic and elastic mechanics in I-*Dcn*$^{-/-}$/*Bgn*$^{-/-}$ versus WT at 150 day[5], provides evidence that I-*Dcn*$^{-/-}$/*Bgn*$^{-/-}$ has a distinct phenotype from both I-*Dcn*$^{-/-}$ and I-*Bgn*$^{-/-}$, when it was previously hypothesized that I-*Dcn*$^{-/-}$/*Bgn*$^{-/-}$ would result in a true Dcn knockout phenotype, without compensatory effects of Bgn. Overall, this study provides evidence for the detrimental effects of Dcn on tendon aging and the vital role of both Dcn and Bgn in regulating tendon structure.

This study demonstrates that Dcn and Bgn play important differential roles in regulation of tendon structure during aging, with the absence of Dcn resulting in an improved tendon aging phenotype.

References

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