Determining the Roles of Decorin and Biglycan in Tendon Healing Using Conditional Deletion at Time of Injury

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Introduction

Tendon injury leads to a healing cascade of inflammatory, proliferative, and remodeling phases, but the mechanisms underlying these processes remain unclear. Small leucine-rich proteoglycans (SLRPs) such as decorin (Dcn) and biglycan (Bgn) are regulators of fibrillogenesis and matrix assembly and play important roles throughout tendon healing. Previous studies using conventional $Bgn^{-/-}$ and $Dcn^{-/-}$ mice showed that absence of Dcn impaired the healing response with no improvement in dynamic modulus between 3- and 6-weeks post-injury, while absence of Bgn had a moderate effect on early tendon healing, together suggesting differential roles of these SLRPs throughout the injury response.[1] However, these results are confounded by the cumulative effects of SLRP deficiency on altered development and growth, and the isolated roles of Dcn and Bgn on tendon healing are unknown. Therefore, the objective of this study was to determine the regulatory role(s) of Dcn and Bgn on the mechanical properties of healing tendons in mature mice using conditional deletion at the time of tendon injury resulting in an isolation of Dcn, Bgn, and both Dcn/Bgn knockdown. We hypothesized that induced deletion of Dcn, Bgn, and both Dcn and Bgn expression would impair the healing response compared to wild type mice leading to reduced improvement in tendon mechanical properties post-injury. Because Dcn has been shown to mediate all stages of healing while Bgn is primarily important in the inflammatory phase, we hypothesized that deletion of Dcn would result in greater impairment.

Methods

Female $Dcn^{+/+}/Bgn^{+/+}$ control (WT, n = 48), $Dcn^{flox/flox}$ (I-$Dcn^{-/-}$, n = 32), $Bgn^{flox/flox}$ (I-$Bgn^{-/-}$, n = 32), and compound $Dcn^{flox/flox}/Bgn^{flox/flox}$ (I-$Dcn^{-/-}/Bgn^{-/-}$, n = 32) mice with a tamoxifen inducible Cre (B6.129-Gt(ROSA)26Sortm1(cre/ERT2)Tyj/J, Jackson Labs) were utilized[2] (IACUC approved). At 120 days old, Cre excision of conditional alleles was induced in all mice via two (injured mice) or three (uninjured mice) consecutive daily IP injections of tamoxifen. WT mice also received tamoxifen to account for any potential side effects. WT mice (n = 16) were designated as uninjured controls, and remaining mice were divided into 3- or 6-week post-injury groups to represent the early and later remodeling phases of healing (n = 16/genotype/time point). At time of induction, mice in injury groups underwent bilateral patellar tendon injury surgery as described[3] and were sacrificed 3- or 6-weeks later. Uninjured groups were sacrificed at 150 days old. The patellar tendon-bone complex from one limb of each animal was dissected and prepared for mechanical testing to assess potential differential effects in both the midsubstance and insertion regions of the tendon.[4] Tendons were subjected to a testing protocol consisting of preconditioning and a quasi-static ramp to failure. Dynamic collagen fiber realignment was measured throughout the ramp-to-failure using a crossed polarizer setup. Images were used to optically measure moduli in the insertion site and midsubstance regions. To evaluate the effect of genotype on tendon healing, one-way ANOVAs with Bonferroni corrections were conducted for 3- and 6-week post-injury groups. Significance was set at $p \leq 0.05$; trends at $p \leq 0.1$.

Results

WT, I-$Dcn^{-/-}$, and I-$Bgn^{-/-}$ mice had significantly reduced insertion site modulus compared to uninjured controls at both 3- and 6-weeks post-injury, while insertion site modulus was reduced in I-$Dcn^{-/-}/Bgn^{-/-}$ mice only at 6-weeks (Fig. 1A,B). Midsubstance modulus in I-$Dcn^{-/-}$ mice was significantly lower than uninjured and I-$Dcn^{-/-}/Bgn^{-/-}$ groups and trended lower compared to I-$Bgn^{-/-}$ mice 3-weeks post-injury (Fig 1C). Similarly, midsubstance modulus was significantly lower in I-$Dcn^{-/-}$ mice compared to uninjured and I-$Bgn^{-/-}$ groups 6-weeks post-injury (Fig 1D). Midsubstance modulus in I-$Dcn^{-/-}/Bgn^{-/-}$ mice also trended lower compared to I-$Bgn^{-/-}$ mice (Fig 1D). For failure properties, maximum stress trended lower in I-$Dcn^{-/-}$ and I-$Dcn^{-/-}/Bgn^{-/-}$ groups compared to uninjured mice 3-weeks post-injury (Fig 1E), while maximum stress trended lower in I-$Dcn^{-/-}/Bgn^{-/-}$ mice compared to I-$Bgn^{-/-}$ mice at 6-weeks (Fig 1F). Finally, normalized circular variance in the midsubstance at 3-weeks was higher (indicating less collagen fiber alignment) in I-$Bgn^{-/-}$ and I-$Dcn^{-/-}/Bgn^{-/-}$ groups at strains between 1 and

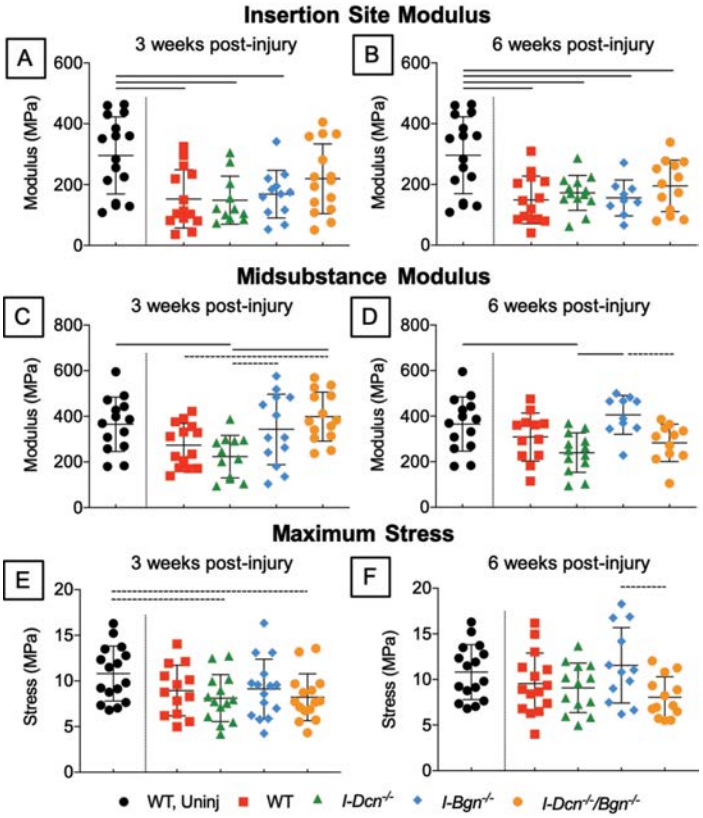

Figure 1. Quasi-static mechanical properties. Insertion site modulus was lower in injured tendons at (A) 3- and (B) 6-weeks post-injury. Only I-Dcn$^{-/-}$ groups exhibited reduced midsubstance modulus compared to uninjured at both (C) 3- and (D) 6-weeks post-injury. Maximum stress trended lower in I-Dcn$^{-/-}$ and I-Dcn$^{-/-}$/Bgn$^{-/-}$ compared to uninjured 3-weeks post-injury but (F) not at 6-weeks post-injury. Solid lines denote significance for $p < 0.05$ while dashed lines denote trends for $p < 0.1$.

4%. (Fig 2C). Few differences were observed at the insertion site or at 6-weeks (Fig 2A,B,D).

Discussion

This study investigated the roles for Dcn and Bgn in determining tendon mechanics after injury using conditional deletion of Dcn, Bgn, and both Dcn and Bgn at the time of injury. As hypothesized, results revealed that absence of Dcn negatively impacts tendon healing. Modulus within the midsubstance region, the location where the injury is introduced, was only significantly lower in I-*Dcn*$^{-/-}$ mice at both 3- and 6- weeks post-injury compared to uninjured controls.This healing response is consistent with our previous studies using conventional *Dcn*$^{-/-}$ mice,[1] further highlighting the critical role of Dcn in all stages of tendon healing. However, contrary to our hypothesis, induced knockout of Bgn did not impair the healing response compared to WT control animals. These findings contrast those observed in the conventional *Bgn*$^{-/-}$ model suggesting that altered growth, especially considering the important role of Bgn in tendon development and fibrillogenesis, may impair the tendon healing response. Interestingly, midsubstance modulus in I-*Dcn*$^{-/-}$/*Bgn*$^{-/-}$ mice was significantly greater than I-*Dcn*$^{-/-}$ mice 3-weeks post-

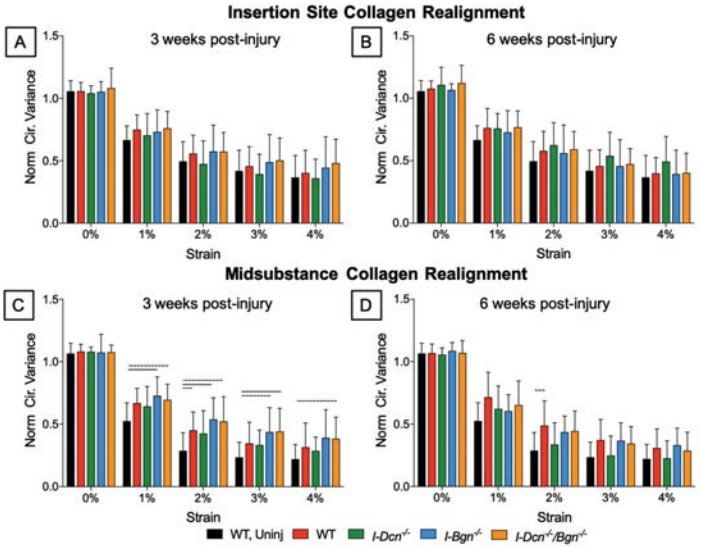

Figure 2. Collagen fiber realignment. There were no differences in collagen fiber realignment at (A) 3- or (B) 6-weeks post-injury within the insertion site region. However, (C) normalized circular variance was higher in I-Bgn$^{-/-}$ and I-Dcn$^{-/-}$/Bgn$^{-/-}$ groups at values of strain between 1 and 4%, and these differences were not sustained to (D) 6-weeks post-injury. Solid lines denote significance for $p < 0.05$ while dashed lines denote trends for $p < 0.1$.

injury, indicating there may be a compensatory or protective effect for Bgn against detrimental changes due to deletion of Dcn. However, these differences were not evident in the insertion site, suggesting the regulatory roles of decorin and biglycan are regionally dependent. Additionally, increased circular variance in both I-*Bgn*$^{-/-}$ and I-*Dcn*$^{-/-}$/*Bgn*$^{-/-}$ groups in the midsubstance at 3-weeks reveal that deletion of Bgn may alter how fibers in healing tendons respond to changes in load. However, the mechanisms driving differences in tendon modulus during healing remain unknown, and ongoing work to assess changes in gene expression, matrix composition, and fibril structure will further elucidate how Dcn and Bgn impact tendon healing.

Significance

In contrast to biglycan, induced deletion of decorin at time of injury has a detrimental effect on mechanics of healing tendons. Elucidating the isolated roles of decorin and biglycan in the response to tendon injury will contribute largely to understanding mechanisms that drive poor tendon healing.

Acknowledgements

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References

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