Determining the Roles of Decorin and Biglycan in Tendon Healing Using Inducible Knockdown at Time of Injury

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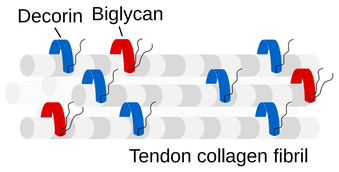
Introduction

- The small leucine-rich proteoglycans (SLRPs), decorin (Dcn) and biglycan (Bgn):
  - Regulate fibrillogenesis and matrix assembly
  - Play important roles throughout tendon healing



- In conventional *Dcn*$^{-/-}$ and *Bgn*$^{-/-}$ mice[1], absence of:
  - Dcn → No improvement in dynamic modulus between 3- and 6-weeks post-injury
  - Bgn → Moderate effect on early tendon healing

} Differential role of SLRPs during healing

- Confounding results in conventional model due to cumulative effects of SLRP deficiency on altered development and growth
  → Isolated roles of Dcn and Bgn on tendon healing are unknown

Objective: To determine the roles of Dcn and Bgn on the mechanical properties of healing tendons in mature mice using inducible knockdown at the time of injury

Hypothesis: Induced knockdown of Dcn, Bgn, and both Dcn/Bgn expression would impair the tendon healing response leading to reduced improvement in mechanical properties post-injury with greater impairment after knockdown of Dcn.

Methods

- Knockdown of Dcn, Bgn, or Dcn/Bgn was induced at time of injury (**Fig 1, 2**).
- At 3 or 6-weeks post-injury, patellar tendon-bone complexes were prepared for mechanical testing and dynamic collagen fiber realignment analysis (**Fig 3**).

Study Design

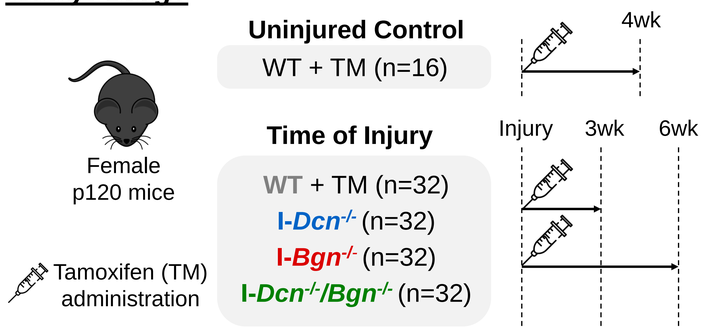
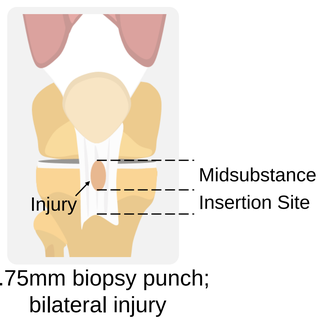


Figure 1: Female *Dcn*$^{+/+}$/*Bgn*$^{+/+}$ (WT), *Dcn*$^{flox/flox}$ (I-*Dcn*$^{-/-}$), *Bgn*$^{flox/flox}$ (I-*Bgn*$^{-/-}$), and compound *Dcn*$^{flox/flox}$/*Bgn*$^{flox/flox}$ (I-*Dcn*$^{-/-}$/*Bgn*$^{-/-}$) mice with a tamoxifen inducible Cre (B6.129-Gt(ROSA)26Sortm1(cre/ERT2)Tyj/J, Jackson Labs) were used[2]. Cre excision was induced via 2 (injured) or 3 (uninjured) consecutive daily IP injections. WT mice received tamoxifen to account for potential side effects.

Patellar Tendon Injury Model



0.75mm biopsy punch; bilateral injury

Figure 2: 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.

Mechanical Testing and Dynamic Collagen Fiber Realignment Analysis

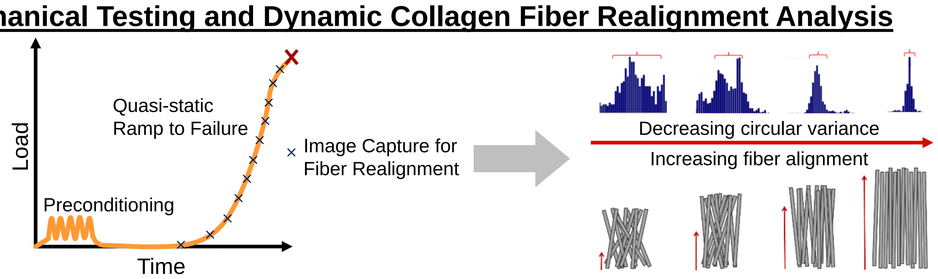


Figure 3: Tendons were subjected to a testing protocol consisting of preconditioning and a quasi-static ramp to failure at 0.3%/s. Dynamic collagen fiber realignment in the insertion site and midsubstance of the tendon was measured throughout the ramp-to-failure using a crossed polarizer setup[4].

Statistics. Significance was set at p≤0.05 and trends at p≤0.1.

- Tendon mechanics: One-way ANOVAs between genotypes with Tukey post-hoc tests were conducted for 3- and 6-week post-injury groups.
- Fiber realignment: Two-way repeated measure ANOVAs with Tukey post-hoc tests (between genotypes) and Bonferroni corrections (between strain levels).

References & Acknowledgments

[1] Dunkman AA et al., Ann Biomed Eng, 2014. [2] Robinson et al., Matrix Biology, 2017. [3] Lin et al., J Biomech, 2006. [4] Dunkman AA et al., Matrix Biology, 2013.

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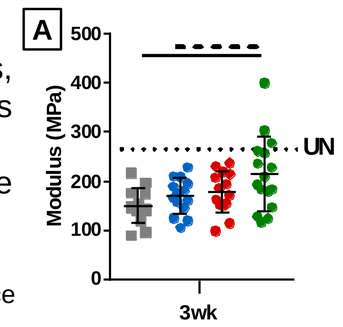
Results

At 3 weeks post-injury (Fig 4),

- Knockdown of Dcn or Bgn alone had no effect on tendon modulus, while knockdown of both Dcn and Bgn led to increased modulus compared to WT.
- Knockdown of Bgn resulted in reduced fiber realignment in the midsubstance, but there were no differences in the insertion site.



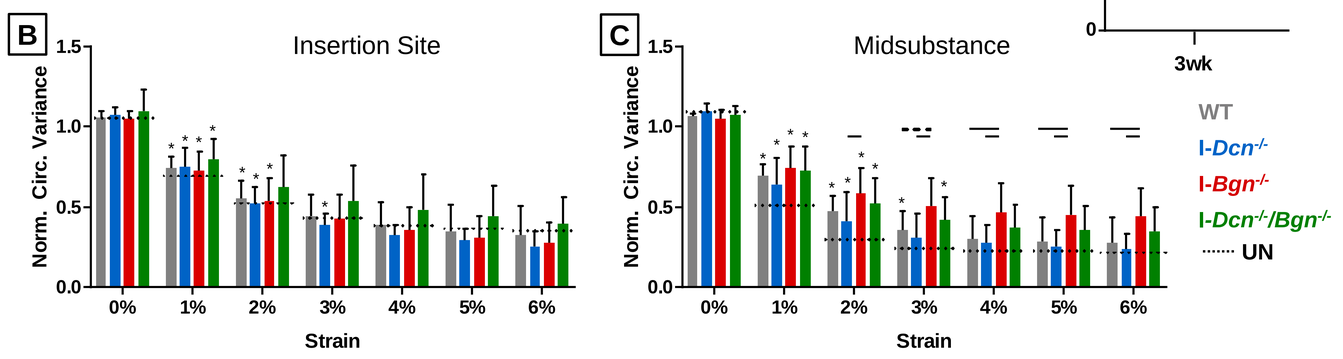
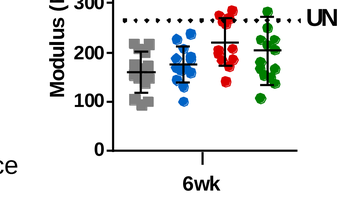


Figure 4: At 3-weeks post-injury, (A) I-*Dcn*$^{-/-}$/ *Bgn*$^{-/-}$ mice had significantly greater modulus compared to WT and a trending increase compared to I-*Dcn*$^{-/-}$. (B) At the insertion site, there were no differences in fiber realignment between genotypes; however, I-*Dcn*$^{-/-}$ tendons continued to realign at higher strains. (C) At the midsubstance, I-*Bgn*$^{-/-}$ tendons exhibited reduced realignment compared to WT and I-*Dcn*$^{-/-}$ tendons. However, both I-*Dcn*$^{-/-}$ and I-*Bgn*$^{-/-}$ tendons realigned quicker than WT and I-*Dcn*$^{-/-}$/ *Bgn*$^{-/-}$ tendons. (—p≤0.05, ---p≤0.1 between genotypes; *p≤0.05 between previous strain level)

At 6 weeks post-injury (Fig 5),

- Knockdown of Dcn had no effect on tendon modulus, while knockdown of Bgn led to greater tendon modulus compared to WT and I-*Dcn*$^{-/-}$ tendons.
- No differences in fiber realignment were observed between genotypes at the insertion site or midsubstance.



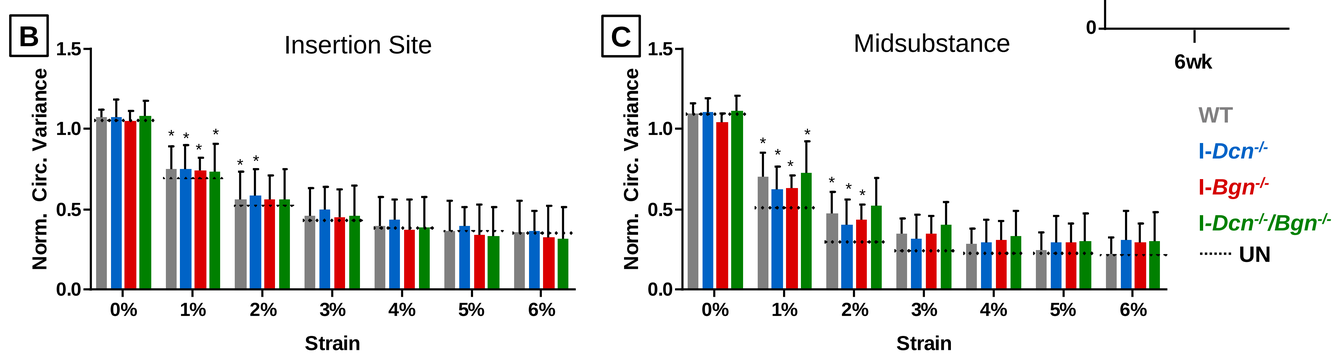


Figure 5: At 6-weeks post-injury, (A) modulus in I-*Bgn*$^{-/-}$ tendons was significantly higher than WT and I-*Dcn*$^{-/-}$, while I-*Dcn*$^{-/-}$/ *Bgn*$^{-/-}$ mice had a trending increase compared to WT. There were no differences in fiber realignment between genotypes at the (B) insertion site or (C) midsubstance. However, I-*Dcn*$^{-/-}$/*Bgn*$^{-/-}$ tendons realigned quicker in both regions. (—p≤0.05, ---p≤0.1 between genotypes; *p≤0.05 from previous strain level)

Discussion

- Contrary to our hypothesis, induced knockdown of Dcn or Bgn did not impair the healing response compared to WT control animals.
  - Findings contrast those observed in the conventional *Dcn*$^{-/-}$ and *Bgn*$^{-/-}$ model[1] suggesting that SLRP deficiency during growth may impair the tendon healing response.
- Modulus in I-*Dcn*$^{-/-}$/*Bgn*$^{-/-}$ tendons at 3-weeks as well as modulus in I-*Bgn*$^{-/-}$ and I-*Dcn*$^{-/-}$/*Bgn*$^{-/-}$ tendons at 6-weeks was greater compared to WT tendons.
  - Induced knockdown of Bgn may have a beneficial effect on tendon mechanics post-injury, possibly through modulation of the inflammatory and fibrotic response.
- Increased circular variance in the I-*Bgn*$^{-/-}$ groups in the midsubstance at 3-weeks reveal that knockdown of Bgn may alter how fibers in healing tendons respond to changes in load.
- Ongoing work: Assess changes in gene expression, matrix composition, and fibril structure

Conclusion

Induced knockdown of biglycan at time of injury had a positive effect on tendon mechanics, while induced knockdown of decorin had no effect. Given that induced knockdown of biglycan also affected fiber realignment, it is necessary to further elucidate the isolated roles of decorin and biglycan in tendon injury.