Knockdown of Decorin and Biglycan During the Early Proliferative and Remodeling Phases of Tendon Healing Alters Gene Expression and Fibril Morphology

Thomas P. Leahy, BS[1]
Ashley K. Fung, M.Eng[1]
Stephanie N. Weiss, MD[1]
Sheila M. Adams, BS[2]
Nathaniel A. Dyment, PhD[1]
David E. Birk, PhD[2]
Louis J. Soslowsky, PhD[1]

[1]McKay Orthopaedic Research Laboratory, University of Pennsylvania, Philadelphia, PA

[2]Department of Molecular Pharmacology and Physiology, University of South Florida, Tampa, FL

Introduction

Tendon matrix consists of highly organized collagen fibrils with small leucine rich proteoglycans (SLRPs) bound to the fibril surface. The SLRPs decorin (gene: *Dcn*) and biglycan (gene: *Bgn*) play a critical role in regulating fibrillogenesis during tendon development and following tendon injury.[1-3] Previous studies have demonstrated that *Bgn* knockdown alone or in tandem with *Dcn* knockdown during healing resulted in improved tendon mechanical properties, regardless of knockdown induction timepoint.[4-5] Surprisingly, *Dcn* knockdown alone had no measurable effect on healing tendon mechanical properties. While these prior studies demonstrated that knockdown of SLRPs could improve tendon mechanical properties, they did not define the mechanism by which SLRP knockdown altered the biological processes and matrix structure within the healing tendon. Therefore, the objective of this study was to define the roles of decorin and biglycan in modulating tendon morphology, gene expression, and collagen ultrastructure throughout the phases of tendon healing. We hypothesized that *Bgn* knockdown alone or in tandem with *Dcn* knockdown would lead to faster recovery of healthy tendon properties, including increased tendon-specific extracellular matrix gene expression, reduced scarred matrix, and a return to an uninjured distribution of collagen fibril sizes.

Methods

Study Design

Female wildtype (WT, n = 36), $Dcn^{flox/flox}$ (I-$Dcn^{-/-}$, n = 36), $Bgn^{flox/flox}$ (I-$Bgn^{-/-}$, n = 36), and compound $Dcn^{flow/flox}$/$Bgn^{flox/flox}$ (I-$Dcn^{-/-}$/$Bgn^{-/-}$, n = 36) mice with a tamoxifen (TM) inducible Cre, (B6.129-Gt(ROSA)26Sortm1(cre/ERT2)Tyj/J, Jackson Labs) were utilized (IACUC approved). At maturity (120 days), mice underwent bilateral patellar tendon injury surgery as described.[1,3] Following surgery, Cre excision of the conditional alleles was induced via two consecutive daily IP injections of TM (2 mg/40g body weight). WT mice received TM injections at 120 days and were divided between the uninjured control group, which was sacrificed at 150 days, and injured groups sacrificed at 3 or ± weeks postinjury. Mice from inducible knockdown genotypes underwent surgery and were evenly divided between Cre-induction during the early proliferative period (TM injections beginning at 5 days post injury, termed TM5) or during the remodeling period (TM injections beginning at 21 days post injury, termed TM21). TM5 animals were sacrificed at 3 or ± weeks postinjury, while TM21 mice were sacrificed at ± weeks postinjury (n = 16/genotype/induction timepoint/sacrifice timepoint).

Gene

Injured patellar tendons were isolated for RNA extraction and cDNA reverse transcription. Pre-amplified cDNA was loaded into a Fluidigm 96.96 Dynamic Array with Taqman assays to probe expression levels of 96 target genes relevant for tendon healing (n = 4/genotype/sacrifice timepoint).

Histology

Whole knees were fixed, decalcified, paraffin embedded, sectioned in the transverse plane, and stained with toluidine blue (n = 4/genotype/sacrifice timepoint). Images were used to quantify scarred area within the injured patellar tendons.

Transmission Electron Microscopy (TEM)

For TEM, injured patellar tendons were fixed, embedded in epon, sectioned at 85 nm, stained, and digitally imaged at 60,000x. Collagen fibril distributions were quantified from images captured within the healing region (n = 4/genotype/sacrifice timepoint).

Statistics

For gene expression and scar area, comparisons were made at each induction-sacrifice timepoint combination using three separate one-way ANOVAs with Tukey post-hoc

tests (significance at $p \leq 0.05$; trends at $p \leq 0.1$). For collagen fibril size distributions, comparisons were made at each induction-sacrifice timepoint combination with Kolmogorov–Smirnov tests (significance at $p \leq 0.05$).

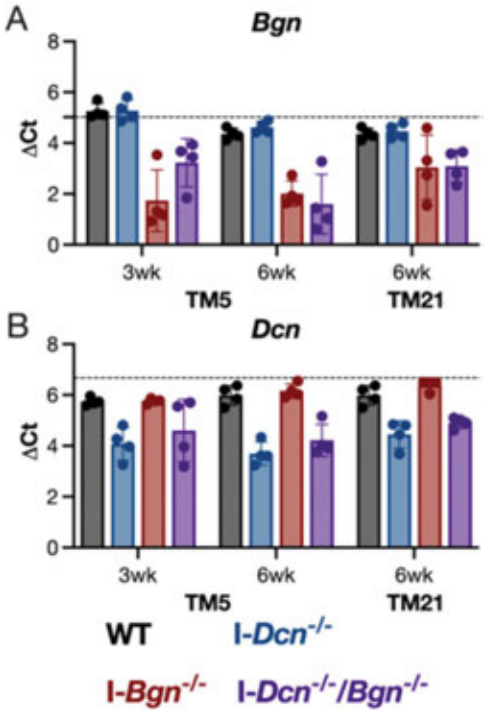

Figure 1. (A) *Bgn* and **(B)** *Dcn* demonstrated expected decreases in expression when targeted for knockdown with at least a trending difference relative to other groups, except for WT vs I-Dcn$^{-/-}$/Bgn$^{-/-}$ at TM5-3wk ($p = 0.15$). ΔCt was calculated by subtracting the gene Ct from average housekeeping Ct (Abl1 and Rps17).

Results

All knockdown groups demonstrated expected decreases in the targeted genes (Figure 1). Further analysis at 3 weeks postinjury revealed increased expression of genes associated with matrix remodeling, inflammation, and activated fibroblasts in the TM5 I-*Dcn*$^{-/-}$/*Bgn*$^{-/-}$ group relative to all other groups (Figure 3). At ± weeks postinjury, the TM5 I-*Bgn*$^{-/-}$ and I-*Dcn*$^{-/-}$/*Bgn*$^{-/-}$ groups displayed increased expression of matrix remodeling genes, including Adamts5, Fbn1, Loxl2, and Mmp2, relative to the TM5 WT and I-*Dcn*$^{-/-}$ groups. In the TM21 groups, the increased expression of similar matrix remodeling genes was maintained in I-*Bgn*$^{-/-}$ tendons but not I-*Dcn*$^{-/-}$/*Bgn*$^{-/-}$ tendons. While there were no differences in relative scar area between groups (data not shown), fibril size distributions were significantly different between all groups compared (Figure 2).

Discussion

Consistent with our hypothesis, the I-*Bgn*$^{-/-}$ and I-*Dcn*$^{-/-}$/*Bgn*$^{-/-}$ tendons demonstrated increased expression of matrix remodeling genes relative to WT and I-*Dcn*$^{-/-}$ tendons at ± weeks postinjury, which is consistent with improved mechanical properties in these groups.[4,5] Interestingly, increased expression of these genes depended on induction timepoint, as this was observed in both I-*Bgn*$^{-/-}$ and I-*Dcn*$^{-/-}$/*Bgn*$^{-/-}$ groups at TM5 but only in the I-*Bgn*$^{-/-}$ group at TM21. This suggests that *Dcn* has a more prominent role between 5 and 21 days postinjury. Contrary to our hypothesis, we did not observe reduced scarred matrix nor a return to an uninjured distribution of collagen fibrils in I-*Bgn*$^{-/-}$ and I-*Dcn*$^{-/-}$/*Bgn*$^{-/-}$ tendons. While the I-*Bgn*$^{-/-}$ group exhibited a narrower

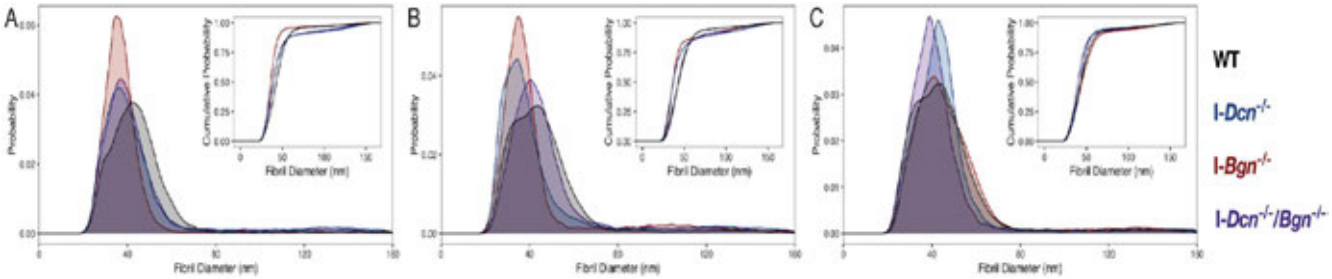

Figure 2. Probability density and cumulative distributions (insets) plots for **(A)** TM5-3wk, **(B)** TM5-6wk, and **(C)** TM21-6wk.

Figure 3. Gene expression summary table. Genes listed are significantly increased in the column group relative to the row group.

distribution of fibrils at TM5 compared to WT, the lack of difference at TM21 suggests that the improved mechanical properties previously observed at both TM5 and TM21 are not due to changes in collagen fibril size distributions. Instead, we speculate that superior healing in these groups is due to changes in the non-collagenous tendon matrix, which then influences matrix synthesis, deposition, and organization. This is supported by observed increases in gene expression for non-collagenous matrix components and matrix remodeling proteins in these groups.

Significance

This study investigated the roles of the SLRPs decorin and biglycan during the early proliferative and remodeling phases of tendon healing. This data indicates that *Bgn* knockdown increases non-collagenous matrix and matrix remodeling gene expression following injury, which is consistent with improved mechanical properties previously observed with knockdown of *Bgn* in healing tendons.

References

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