U·P·O·J

The Differential Roles of Decorin and Biglycan in the Early Proliferative and Remodeling Phases of Tendon Healing

Thomas Leahy, BS[1]
Ashley Fung, BS[1]
Stephanie Weiss, BS[1]
David Birk, PhD[2]
Louis Soslowsky, PhD[1]

[1]McKay Orthopaedic Research Laboratory University of Pennsylvania

[2]Department of Molecular Pharmacology and PhysiologyUniversity of South Florida

Introduction

Tendon matrix consists of highly organized collagen fibrils with small leucine rich proteoglycans (SLRPs) bound to the fibril surface. SLRPs decorin and biglycan play a critical role in regulating tendon healing processes. Specifically, using conventional $Bgn^{-/-}$ and $Dcn^{-/-}$ mice, the absence of biglycan diminished initial tendon healing following injury while the absence of decorin reduced late tendon healing.[1] However, these studies have confounding effects due to the absence of decorin and biglycan during development[2] and do not allow the roles of these SLRPs to be defined at specific phases (inflammation, proliferation, remodeling) of healing. Therefore, the objective of this study was to define the roles of decorin and biglycan in specific healing phases using inducible knockouts. We hypothesized that a complete knockout (i.e., a Bgn-Dcn double knockout) earlier in the healing process would have the greatest negative effect since both SLRPs would be absent throughout the proliferative and remodeling phases. Further, we hypothesized that decorin knockout would result in more pronounced negative effects on healing compared to biglycan knockout regardless of time of induction and that decorin knockout would reduce tendon healing similarly when knocked out during early or late stage healing due to its known role in the later remodeling phase.

Methods

Study Design

Female wildtype (WT, n=48), $Dcn^{flox/flox}$ (I-$Dcn^{-/-}$ n = 48), $Bgn^{flox/flox}$ (I-$Bgn^{-/-}$, n = 48), and compound $Dcn^{flow/flox}/Bgn^{flox/flox}$ (I-$Dcn^{-/-}$/$Bgn^{-/-}$, n = 48) 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 tamoxifen (2 mg/40g body weight). WT mice received tamoxifen injections at 120 days and were evenly divided between the uninjured control group, which were sacrificed at 150 days, and surgery groups which were sacrificed at 3 or 6 weeks post-injury, representing the early remodeling and mid-remodeling phases of tendon healing, respectively. Mice from knockout genotypes underwent surgery and were evenly divided between Cre-induction during the early proliferative period (tamoxifen injections beginning at 5 days, termed TM5) or during the remodeling period (tamoxifen injections beginning at 21 days, termed TM21). TM5 animals were sacrificed at 3 or 6 weeks post-injury, while TM21 mice were sacrificed at 6 weeks post-injury (n = 16/genotype/induction timepoint/sacrifice timepoint).

Mechanical Testing Protocol

The patellar tendon-bone complex from one limb of each animal was dissected and prepared for mechanical testing. Tendons were then subjected to mechanical testing consisting of preconditioning followed by a quasi-static ramp to failure. Material properties of the tendons were calculated from the load-displacement data via optical tracking of stain lines on the tendon using MATLAB. Throughout mechanical testing, dynamic collagen fiber realignment was quantified using cross-polarization imaging, and regional fiber alignment data was interpolated with a polynomial fit as a function of strain from the load-displacement data between 0 and 4% tendon strain.

Statistics

Comparisons were made between genotypes and the relevant uninjured and WT controls at each induction timepoint-sacrifice timepoint combination (TM5-3wk, TM5-6wk, and TM21). Mechanical properties were compared with three separate one-way ANOVAs with Bonferroni post-hoc corrections with significance at $p \leq 0.05$ and trends at $p \leq 0.1$. Fiber realignment properties were compared between strain levels and genotypes with a two-way ANOVA. If the effect of strain level or genotype was significant, a one-way ANOVA with Bonferroni post hoc corrections was performed (if strain had a significant effect, multiple comparisons were made from 0% strain to all strain levels and between adjacent strain levels) with significance set at $p \leq 0.05$.

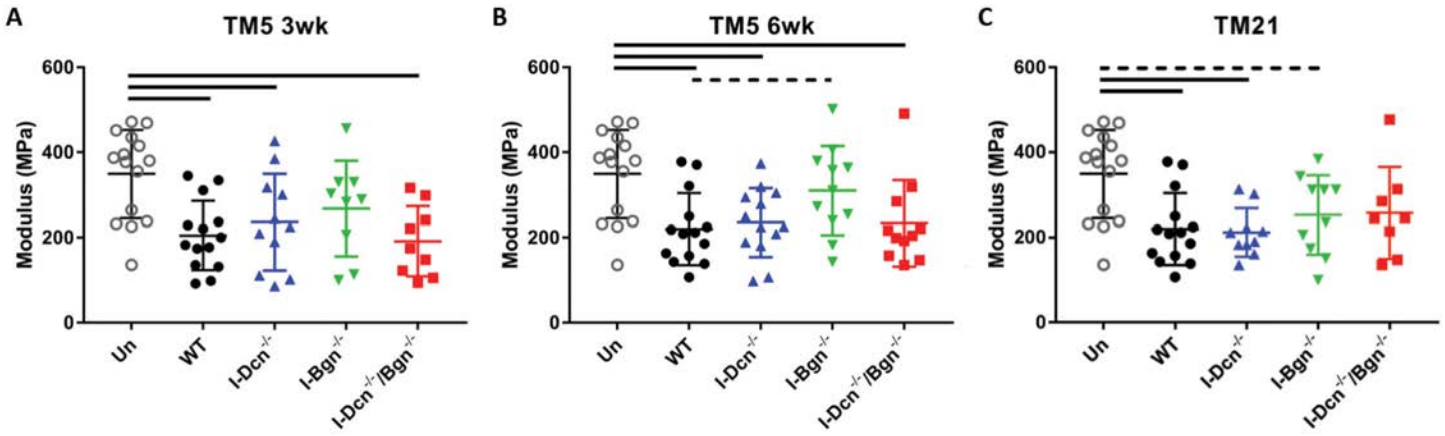

Figure 1. Tendon modulus comparisons at TM5-3wk **(A)**, TM5-6wk **(B)**, and TM21 **(C)**. The TM5 I-Bgn$^{-/-}$ group abolished differences with injury, while TM5 and TM21 I-Dcn$^{-/-}$groups showed consistent effects on mechanics. Solid bars represent significance ($p \leq 0.05$) and dotted bars represent trends ($p \leq 0.1$).

Results

Injury significantly reduced modulus at both 3 and 6 weeks in the WT tendons (Fig 1). Decreased modulus was maintained in both the TM5 I-*Dcn*$^{-/-}$ and TM5 I-*Dcn*$^{-/-}$/*Bgn*$^{-/-}$ groups but not in the TM5 I-*Bgn*$^{-/-}$ group at either 3 or 6 weeks. In the TM21 groups, a decreased modulus was maintained in the I-*Dcn*$^{-/-}$ group with a trending decrease in the I-*Bgn*$^{-/-}$ group and no difference between the uninjured and I-*Dcn*$^{-/-}$/*Bgn*$^{-/-}$ groups. There were no changes in max stress with injury or between genotypes, except for a trending decrease in max stress at 6 weeks in the I-*Dcn*$^{-/-}$ group (data not shown). All tendons showed significantly increased fiber realignment with increasing strain in both the insertion and midsubstance regions (Fig 2, insertion data not shown). There were no differences in realignment between genotypes, except in the midsubstance region of the TM5 I-*Dcn*$^{-/-}$ tendons at 6 weeks, which showed significantly less realignment relative to the uninjured control.

Discussion

Contrary to our hypothesis, the TM5 I-*Dcn*$^{-/-}$/*Bgn*$^{-/-}$ and TM21 I-*Dcn*$^{-/-}$/*Bgn*$^{-/-}$ groups did not exhibit significantly reduced tendon healing at 3 or 6 weeks relative to the WT control. Moreover, decorin knockout did not show a significant negative effect on tendon mechanics compared to biglycan knockout at either TM5 or TM21. Interestingly, biglycan knockout appeared to reduce the negative effects of tendon injury, as there were no negative mechanical effects of injury at 3 or 6 weeks in the TM5 I-*Bgn*$^{-/-}$ tendons. Further, these results suggest that biglycan plays a negative role in early healing, as TM21 I-*Bgn*$^{-/-}$ had negative changes with injury not present in TM5 I-*Bgn*$^{-/-}$. Finally, while TM5 I-*Dcn*$^{-/-}$ and TM21 I-*Dcn*$^{-/-}$ had similar effects on tendon mechanics as hypothesized, TM5 I-*Dcn*$^{-/-}$, but not TM21 I-*Dcn*$^{-/-}$ showed altered fiber realignment behavior at 6 weeks, suggesting that decorin plays a role in regulating fiber organization in early stage tendon healing. These results contrast with previous studies using conventional knockout mice that suggested that *Bgn*$^{-/-}$ mice had impaired early tendon healing and *Dcn*$^{-/-}$ mice had clearly diminished late stage tendon healing,[1] underscoring the importance of using inducible animals to distinguish the specific temporal roles of these SLRPs in tendon healing. Future work will elucidate the underlying mechanisms behind altered tendon healing with temporal deletion of decorin and biglycan by investigating gene expression, matrix composition, and fibril structure in these tendons.

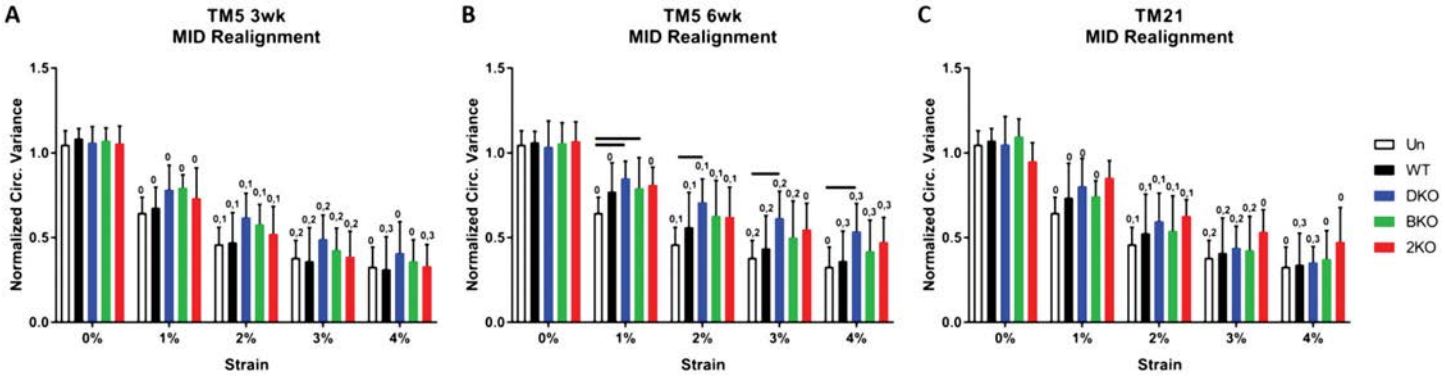

Figure 2. Midsubstance realignment data for TM5-3wk **(A)**, TM5-6wk **(B)**, and TM21 **(C)**. All groups showed increased alignment with increasing strain. TM5 I-Dcn$^{-/-}$ group showed altered fiber realignment behavior relative to uninjured. Solid bars represent significance ($p \leq 0.05$) between genotypes. Numbers above indicate significance from noted strain values given the comparisons performed (see methods).

Significance

This study used novel inducible knockout mice for decorin and biglycan to investigate the temporal roles of these SLRPs during the early proliferative and remodeling phases of tendon healing. This data suggests that biglycan plays a significant negative role in tendon healing, particularly in the early proliferative phase, while decorin does not play a drastic temporal role in tendon healing.

Acknowledgements

We acknowledge financial support fromNIH/NIAMS (R01AR068057 and P30AR069619).

References

1. Dunkman, *et al.* *Ann. Biomed. Eng.* 42, 201.
2. Dourte, *et al.* *J. Orthop. Res.* 31, 2013.
3. Dunkman, *et al.* *Matrix Biol.* 35, 2014.