Matrix Biology xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Matrix Biology

journal homepage: www.elsevier.com/locate/matbio

The injury response of aged tendons in the absence of biglycan and decorin

Andrew A. Dunkman [a,1], Mark R. Buckley [a,1], Michael J. Mienaltowski [b], Sheila M. Adams [b], Stephen J. Thomas [a], Akash Kumar [a], David P. Beason [a], Renato V. Iozzo [c], David E. Birk [b], Louis J. Soslowsky [a,*]

[a] The McKay Orthopaedic Research Laboratory, University of Pennsylvania, 424 Stemmler Hall, 3450 Hamilton Walk, Philadelphia, PA 19104, USA
[b] Department of Molecular Pharmacology & Physiology, University of South Florida Morsani College of Medicine, 12901 Bruce B. Downs Blvd, MDC 8, Tampa, FL 33612, USA
[c] Department of Pathology, Anatomy & Cell Biology, Thomas Jefferson University, 1020 Locust Street, Jefferson Alumni Hall, Suite 249, Philadelphia, PA 19107, USA

ARTICLE INFO

Available online xxxx

Keywords:
Tendon
Injury
Aging
Proteoglycan
Biglycan
Decorin

ABSTRACT

Recent studies have demonstrated that the small leucine-rich proteoglycans (SLRPs) biglycan and decorin impact tendon development, aging and healing in mature mice. However, despite the increased risk of tendon injury in the elderly, the role of SLRPs in tendon repair has not been investigated in aged animals. Therefore, our objective was to elucidate the influences of bigylcan and decorin on tendon healing in aged mice to relate our findings to previous work in mature mice. Since the processes of aging and healing are known to interact, our hypothesis was that aging mediates the role of biglycan and decorin on tendon healing. Patellar tendons from wild-type, biglycan-null and decorin-null mice were injured at 270 days using an established model. At 3 and 6 weeks post-surgery, structural, mechanical and biochemical analyses were performed and compared to uninjured controls. Early stage healing was inferior in biglycan-null and decorin-null mice as compared to wild type. However, tendons of all genotypes failed to exhibit improved mechanical properties between 3 and 6 weeks post-injury. In contrast, in a previous investigation of tendon healing in mature (i.e., 120 day-old) mice, only biglycan-null mice were deficient in early stage healing while decorin-null mice were deficient in late-stage healing. These results confirm that the impact of SLRPs on tendon healing is mediated by age and could inform future age-specific therapies for enhancing tendon healing.

1. Introduction

Tendon is a highly durable connective tissue comprised of a water-saturated network of uniaxially-aligned collagen fibrils. This specialized structure endows tendon with specific mechanical and structural properties that allow for proper transmission of force from muscle to bone. Thus, mechanical alterations produced by tendon injury adversely impact muscle performance and can lead to substantial pain and disability (Carroll et al., 2008). Unfortunately, advanced age is a risk factor for tendon injury (Yamamoto et al., 2010), and wound healing is impaired by the aging process (Kletsas et al., 2000). Therefore, understanding the constituent molecules involved in tendon repair in aged animals is an important step in the development of treatment strategies for tendon injury in the elderly.

Recent investigations have implicated the class I small leucine-rich proteoglycans (SLRPs), biglycan and decorin, as important regulators of tendon development, aging and healing. Decorin, the predominant SLRP found in tendon, is comprised of a horseshoe-shaped decorin core protein and a sulfated chondroitin sulfate/dermatan sulfate (CS/DS) glycosaminglycan (GAG) (Scott et al., 2004; Orgel et al., 2009) while biglycan consists of a core protein with two sulfated (CS/DS) GAGs (Scott et al., 2006) and is generally expressed in tendon at lower levels (Zhang et al., 2006). The absence of either SLRP during development results in the formation of tendons with abnormal collagen fibril structures and altered mechanical properties (Robinson et al., 2004, 2005; Zhang et al., 2006; Dourte et al., 2012, 2013). Moreover, in mature (120 day-old) mice, the absence of decorin significantly impairs the long-term injury response of tendon while the absence of biglycan is detrimental only for early-stage healing (0–3 weeks post-injury) (Dunkman et al., 2013b). However, the post-developmental effects of SLRPs are not age-independent. In fact, the absence of decorin inhibits structural and mechanical alterations associated with the aging process (Dunkman et al., 2013a). Thus, the impact of SLRPs on tendon healing may also be influenced by the interacting process of aging. Nevertheless, the effects of biglycan and decorin on healing in aged mice have not yet been investigated. Therefore, the objective of this study was to investigate the impacts of bigylcan and decorin on the injury response of tendons in aged mice and to compare with previous work in mature mice. We hypothesized that the aging process mediates the specific effect of SLRPs on the repair response and that the deleterious effects of aging on tendon healing will be exacerbated by the absence of SLRPs.

* Corresponding author. Tel.: +1 215 898 8653; fax: +1 215 573 2133.
E-mail address: soslowsk@upenn.edu (L.J. Soslowsky).
[1] Co-first authors A.A. Dunkman and M.R. Buckley contributed equally to this work.

0945-053X/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.matbio.2013.10.008

2. Results

2.1. Biomechanical properties

In aged wild type (WT), decorin null ($Dcn^{-/-}$) and biglycan null ($Bgn^{-/-}$) mice, the dynamic modulus |E*| of injured tendons was significantly lower than in uninjured controls (Fig. 1a, Supplemental Table 1). Thus, as expected, injured tendons produced lower stress in response to an induced cyclic strain and therefore exhibited inferior mechanical properties. However, improvements in this parameter during the healing process were not detected—that is, |E*| did not significantly increase from 3 to 6 weeks post-injury in any genotype group.

In aged WT mice, tanδ was generally unchanged following injury, although tanδ increased slightly at low frequencies and low strains (Fig. 1b, Supplemental Table 1). In contrast, aged $Bgn^{-/-}$ and $Dcn^{-/-}$ tendon became more fluid-like (increased tanδ) after injury. In other words, these tendons became more dissipative and were less able to store and release elastic energy during cyclic loading. During the healing process, tanδ decreased slightly at high strains and low frequencies in the mutant genotypes.

Comparing across genotypes, |E*| in uninjured tendons was generally highest in the $Bgn^{-/-}$ group (Fig. 1, Supplemental Table 2). However, at 3 weeks post-injury, |E*| in WT tendons was significantly higher than both $Dcn^{-/-}$ and $Bgn^{-/-}$. Similarly, tanδ in uninjured tendons was highest in the WT group. But at 3 weeks post-injury, tanδ was lowest in the WT group. At 6 weeks post-injury, |E*| was highest in the WT group while no differences among genotypes were found in tanδ. However, as mentioned previously, |E*| did not change from 3 to 6 weeks in any genotype group. Taken together, these findings demonstrate that WT tendons exhibited superior mechanical properties compared to the null genotypes (increased |E*| and decreased tanδ) at 3 weeks post-injury but not prior to injury. That is, improvements in mechanical properties from 0 to 3 weeks post-injury were greater in WT tendons than in the mutant genotypes.

2.2. SLRP expression

Aged, uninjured $Dcn^{-/-}$ mouse tendons expressed more biglycan than uninjured WT mouse tendons (Fig. 2a). Biglycan expression was upregulated after injury in aged WT tendons while expression of biglycan in $Dcn^{-/-}$ mice was constant at all pre- and post-injury time points. Aged, uninjured biglycan-null mice exhibited a trend towards higher decorin expression than WT (Fig. 2b). Differences in decorin expression after injury were not detected in any genotype groups. No changes in fibromodulin expression between injury states or genotype groups were found (data not shown).

2.3. Tenocytes and fiber alignment

Uninjured $Bgn^{-/-}$ tendons were significantly more cellular than WT (Fig. 3a). After injury, across all genotypes, there was a pronounced and significant increase in cellular density marking proliferation and repair (Fig. 3a,d–l). Similarly, more rounded cells dominated in all genotypes after injury (Fig. 3b,d–l). Moreover, at 6 weeks after injury, $Dcn^{-/-}$ tendons were less cellular than WT (Fig. 3a). Finally, changes in collagen fiber alignment following injury were not detected (Fig. 3c).

2.4. Fibril structure

In uninjured tendons, fibril diameter distributions appeared bimodal for all genotypes (Fig. 4a–c). In $Bgn^{-/-}$ tendons, the large diameter subpopulation exhibited a distinct peak that was more than twice as high as the peak of the small diameter subpopulation (Fig. 4b). Thus, large diameter fibrils were substantially more frequent than smaller diameter fibrils. In contrast, the large diameter subpopulations in uninjured WT and $Dcn^{-/-}$ tendons were only slightly larger than the corresponding small diameter subpopulations (Fig. 4a,c). Moreover, the larger diameter subpopulation in $Dcn^{-/-}$ tendons was shifted to smaller diameters.

After injury, there was a marked increase in the number of smaller diameter fibrils, attributable to the assembly of new fibrils (Fig. 4d–i). At both 3 and 6 weeks post-injury, WT and $Dcn^{-/-}$ tendons exhibited bimodal fibril diameter distributions with little overlap between subpopulations. However, the large diameter subpopulation in the $Dcn^{-/-}$ group was shifted to larger diameters (Fig. 4f,i). In contrast, both the small and large diameter subpopulations in $Bgn^{-/-}$ tendons were shifted to smaller diameters and substantial overlap between subpopulations was apparent at 3 weeks post-injury (Fig. 4e). However,

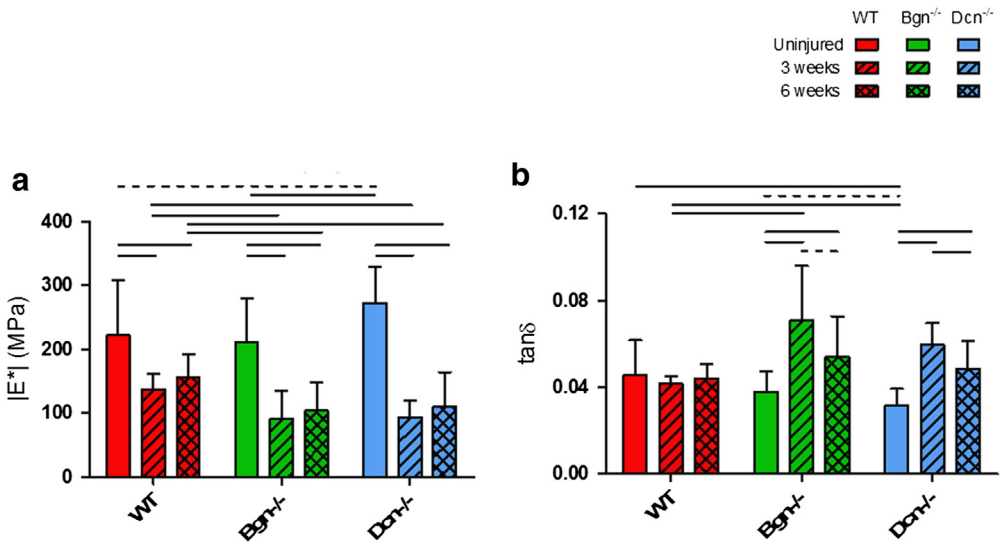

Fig. 1. Dynamic modulus (|E*|) and tangent of the phase angle (tanδ) (a–b) as a function of injury state for each genotype group and (c–d) as a function genotype for each injury state (n = 12–19 specimens per genotype per injury state, mean ± standard deviation). Results are shown for a 1 Hz deformation at a strain level of 8%, but data were similar at other frequencies and strain levels. Improved mechanical properties (increased |E*| and decreased tanδ) were not measured for any genotype group between 3 and 6 weeks post-injury. However, |E*| was highest and tanδ was lowest in the WT group at 3 weeks-post injury, indicating that these tendons exhibited superior early-stage healing. Solid horizontal bars denote $p < 0.05/2$ while dashed bars denote $p < 0.01/2$.

Please cite this article as: Dunkman, A.A., et al., The injury response of aged tendons in the absence of biglycan and decorin, Matrix Biol. (2013), http://dx.doi.org/10.1016/j.matbio.2013.10.008