Regional FDL Tendon Development Involves Differential Pericellular Matrix Expression and Presence

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Introduction

Tendon requires highly aligned collagen I fibrils to aid in mechanical strength in response to tensile loading. Regions of tendon that wrap around bones or joints, however, experience additional compressive loading and display a complex, fibrocartilaginous tissue phenotype[1]. Fibrocartilage and resident chondrogenic cells rely heavily on the collagen VI-rich pericellular matrix (PCM) for proper mechanosensation and homeostasis[2]. While the fibrocartilage matrix within wrap-around tendon regions has been characterized[3], the development of this unique tissue region and its PCM content remains unknown. Therefore, the objective of this study was to define differential PCM expression and presence within the highly aligned (tensile) tendon matrix and the wrap-around (compressive) fibrocartilage matrix during murine FDL tendon development. We hypothesized that PCM expression and synthesis is increased in the compressive region compared to the tensile region as early as two-weeks postnatally due to compressive joint loads from ambulation at this age.

Methods

Animals

Wild-type P7 (n = 9), P14 (n = 7/sex) and P21 (n = 7/sex) mice were used in this study (IACUC approved). At sacrifice, mouse hindlimbs were harvested, fixed in 4% RNAse-clean PFA for 3hr, then embedded and flash frozen in OCT. Embedded limbs were cryosectioned at 20μm for gene expression or at 8μm for histological staining.

Gene Expression

Tensile and compressive regions of sectioned FDL tendon were microdissected and separated using 25G needles. Regional samples were digested with proteinase K, and RNA was extracted with Zymo Quick-RNA MicroPrep kits. cDNA was reverse transcribed and preamplified for 15 cycles with *Col6a(1-3), Bgn,* and *Abl1* Taqman assays. RT-qPCR was performed on preamplified cDNA for those target genes. ΔCt values for each gene were calculated based on corresponding *Abl1* Ct values.

Histology

After fixation, samples were decalcified with EDTA for 4-5 days prior to embedding and sectioning. Sections were stained with rabbit anti-collagen VI antibody (Fitzgerald, 70R-CR009x) and Hoechst nuclear stain prior to being imaged on a Zeiss Axio Scan.Z1. The tensile and compressive tendon regions were segmented, and mean antibody intensity was quantified for each region using FIJI.

Statistics

Paired t-tests were used to compare ΔCt values for measured genes and mean staining intensity between the tensile and compressive region. Significance was set at $p \leq 0.05$ and trends at $p \leq 0.1$.

Results

In P7 FDL tendons, the compressive region showed increased expression of *Col6a1*, with trending increases in *Col6a2* and *Bgn*, compared to the tensile region (Figure 1). At P14, the compressive region exhibited increased expression of all measured *Col6* genes and of *Bgn* compared to that of the tensile region. At P21, *Col6a3* and *Bgn* had trending expression increases in the compressive region compared to the tensile region. Quantification of collagen VI antibody staining revealed no regional differences in P7 FDL tendons (Figure 2). However, mean staining intensity was increased in the compressive region compared to the tensile region at P14 and P21.

Discussion

Results demonstrate that regional differences in PCM begin at early postnatal ages in the FDL tendon. The tendon PCM is comprised of collagen VI α-chains[4], and evidence suggests that biglycan helps organize the tendon PCM[5]. While the compressive region exhibited some increases in PCM gene expression compared to the tensile region at P7, this increased expression was consistently observed across all measured

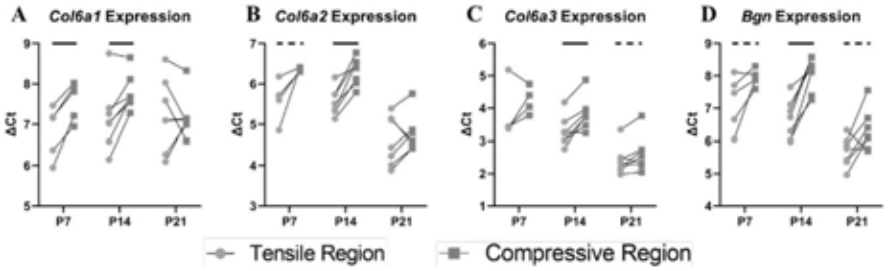

Figure 1. Region-dependent PCM gene expression peaks at P14. Expression of Col6 genes **(A-C)** and of Bgn (D) was higher in the compressive region compared to the tensile region by P14. Most of these regional differences were not present at P21. Solid lines denote $p < 0.05$, dotted lines denote $p < 0.1$.

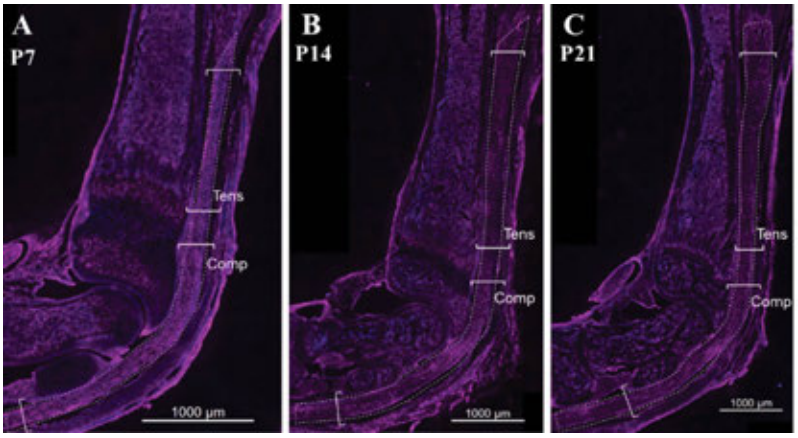

Figure 2. Region-dependent PCM presence is apparent at P14. Collagen VI staining (pink) reveals no differences in intensity between the tensile (Tens) and compressive (Comp) regions of the FDL tendon at P7 **(A)**. By P14 **(B)**, the compressive region contains higher intensity staining compared to the tensile region, which persists at P21 **(C)**. Signal intensity quantification shows that these changes are significant (D). Solid lines denote $p < 0.05$.

genes by P14. This expression pattern was supported by elevated PCM content in the compressive region at P14, which persisted at P21. Supporting our hypothesis, this result suggests that increased PCM expression and synthesis in the compressive region is driven by complex joint loads during murine gait. Mice begin walking quickly by two weeks of age[6], which would lead to increased joint flexion and loading on wrap-around tendons. Tendon cells respond to these forces by producing a fibrocartilaginous, GAG-rich matrix[7] with thickened PCM staining (Figure 2). As a result, the tendon PCM is likely a critical regulator of tendon cell phenotype. A limitation of this study is the inability to precisely define tensile and compressive tendon regions, as there are likely no regions that experience purely compressive or tensile loading. However, prior studies demonstrate that regional differences in wrap-around tendons are exacerbated by compressive loading[7,8], supporting our definitions of tensile and compressive tendon regions. Anatomical markers were used to segment these regions, making them consistent across samples and age groups. Future work will analyze the differential response of these FDL tendon regions to knockout of PCM molecules.

Significance

This work defines temporal regional development of the PCM within the murine FDL tendon. Understanding the differential development of these tendon regions provides insight into how tendon cells respond to physical cues, which is critical for treatment paradigms.

References

1. Benjamin M, Ralphs JR. Fibrocartilage in tendons and ligaments—an adaptation to compressive load. *Journal of Anatomy*. 1998;193 (Pt 4)(Pt 4):481-494.

2. Sanchez-Adams J, Wilusz RE, Guilak F. Atomic force microscopy reveals regional variations in the micromechanical properties of the pericellular and extracellular matrices of the meniscus. *Journal of Orthopedic Research*. 2013;31(8):1218-1225.

3. Perez-Castro, Ana V., and Kathryn G. Vogel. In situ expression of collagen and proteoglycan genes during development of fibrocartilage in bovine deep flexor tendon. *Journal of Orthopaedic Research* 1999;17(1):139-148.

4. Ritty T, Robyn R, Heuser J. Tendon cell array isolation reveals a previously unknown fibrillin-2-containing macromolecular assembly. *Structure*. 2003;11(9): 1179-1188.

5. Wiberg C, Heinegård D, Wenglén C, et al. Biglycan organizes collagen VI into hexagonal-like networks resembling tissue structures. *Journal of Biological Chemistry*. 2002;277(51):49120-6.

6. Clarke K, Still J. Development and consistency of gait in the mouse. *Physiology & Behavior*. 2001;73(1-2) 159-164.

7. Evanko S, Kathryn V. Proteoglycan synthesis in fetal tendon is differentially regulated by cyclic compression in vitro. *Archives of Biochemistry and Biophysics*. 1993;307(1) 153-164.

8. Malaviya P, Bultler D, Boivin G, et al. An in vivo model for load-modulated remodeling in the rabbit flexor tendon. *Journal of Orthopaedic Research*. 2000;18(1): 116-125.