Tendon-Cartilage Crosstalk in a Proximal Biceps Tenotomy Mouse Model

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INTRODUCTION: Many patients with massive rotator cuff tears progress to rotator cuff arthropathy, a condition characterized by severe osteoarthritis of the glenohumeral (GH) joint in the setting of rotator cuff degeneration. It is difficult to determine the role that local tissue crosstalk plays in progression of this disease because altered biomechanics secondary to rotator cuff tears also contribute to humeral head articular cartilage degeneration. Surgical animal models of rotator cuff pathology have been used to characterize the effect of tendon injury on the tendon and adjacent bone. The shortcoming of these models is that they require substantial soft tissue trauma, precluding isolation of loading, trauma, and paracrine effects. To allow for direct examination of crosstalk between tendon and cartilage in isolation of biomechanical and trauma effects, we developed a novel proximal biceps tenotomy mouse model. In this model, we create an isolated extraarticular tenotomy to the biceps to unload the tendon without any tissue trauma near the joint. Notably, tenotomy of the biceps in humans and rodents has been shown to *not* alter glenohumeral joint biomechanics. This model therefore produces a pathologic tendon near the joint and allows us to isolate the effects of tendon-cartilage crosstalk from the effects of joint loading and trauma. We hypothesized that extraarticular biceps tenotomy would lead to (1) intraarticular biceps tendon inflammation and degeneration, and (2) adjacent humeral head cartilage pathology, (3) without concurrent changes to shoulder biomechanics.

METHODS: Animal experiments were conducted with approval by the Columbia University Institutional Animal Care and Use Committee (IACUC). All mice were 12-weeks-old at the time of surgery. *Surgical technique:* In Surgery mice, a subpectoral tenotomy of the long head of the biceps was performed just proximal to the myotendinous junction of the left forelimb, and a sham surgery was performed on the right forelimb. No surgeries were performed on Control mice. *Functional assessment:* Gait analysis was performed on Surgery and age-matched Control mice using a high-speed camera and illuminated footprint technology to detect gait metrics at 28 days following surgery. All animals were sacrificed following gait analysis. *Histology:* Proximal biceps tendon specimens attached to the glenoid and humeral head specimens from 28-day postop (16-week-old) mice were fixed in 4% PFA, decalcified with formic acid, serially dehydrated in ethanol, and embedded in paraffin. Representative tendon sections were stained with hematoxylin & eosin (H&E) and Alcian blue, and humeral head sections were stained with H&E, Alcian blue and safranin O. Sections from each sample group were blinded and evaluated using QuPath. The Bonar score was used to score tendon specimens [1]. The murine shoulder arthritis scoring (MSAS) system was used to assess mouse humeral head cartilage [2]. *Gene Expression:* Immediately following mouse sacrifice and dissection at 28 days following surgery, tissues were flash frozen and subsequently pulverized using a tissue homogenizer. RNA extraction was performed using guandinium thiocyanatephenol-chloroform and interphase separation. RNA cleanup was performed using spin columns. RNA quantity and quality were determined using a spectrophotometer, and then RNA was reverse-transcribed into cDNA using a High-Capacity cDNA Reverse Transcription Kit. Tendon specimens were combined (2 tendons per sample analyzed) to obtain adequate RNA for analysis. For cartilage specimens, cartilage-, bone-, mechanics- and OA-related genes (*Acan, Runx2, Dkk1, Col2A1, Col10A1, BGLAP, ALPL, BMP2, MMP13, Piezo1, Piezo 2*) were assessed, and for tendon specimens, inflammation-related genes (*TGFbeta1, IL1beta, IL6)* were assessed. All RNA samples were evaluated by SYBR Green based quantitative reverse transcription-polymerase chain reaction. Glyceraldehyde 3-phosphate dehydrogenase served as a housekeeping gene, and relative mRNA expression level for each target gene was determined at $2^{-\Delta\Delta Ct}$. *Statistical analysis:* A one-way ANOVA was used to assess all groups and paired t-tests were used to compare the right and left forepaws of surgery mice.

RESULTS: *Proximal biceps tenotomy did not alter shoulder biomechanics:* Gait analysis performed on Surgery mice (n=19, 10M, 9F) 28 days after surgery and age-matched Control mice (n=12, 6M, 6F) showed no differences in gait metrics between groups ($p>0.05$). Specifically, there were no differences in forepaw (FP) stance width, hindpaw (HP) stance width, relative intensity of stance in the FP compared to HP, length of time standing on a single paw, paw print area, time length of FP swing and FP stride length between groups. There were also no differences between the right and left forepaws of Surgery mice ($p>0.5$). *Proximal biceps tenotomy resulted in tendinopathy:* Surgery tendons (n=5) had significantly higher histology scores for tenocytes, ground substance, collagen and vascularity compared to control (n=6, $p<0.05$) and sham (n=5, $p<0.05$) tendons. Surgery tendons also had significantly higher total Bonar scores (mean 5.6) compared to control (mean 0.7, $p=0.0008$) and sham (mean 0.4, $p=0.02$) tendons (Figure 1). *TGFbeta1* expression was significantly greater in surgery (n=2) compared to control (n=2, mean 4.9-fold change, $p=0.04$) and sham tendons (n=2, $p=0.02$). *Biceps tendinopathy led to early signs of humeral head degeneration:* Surgery humeral heads (n=4) were significantly less spherical when compared to control (n=5, $p=0.0006$) and sham (n=5, $p=0.04$) shoulders (Figure 2). The MSAS score was also significantly greater in Surgery humeral heads when compared to Control humeral heads ($p=0.01$) (Figure3). There were no significant differences in *Piezo1* and *Piezo2* expression amongst groups ($p>0.05$).

DISCUSSION: There were no significant differences in gait between surgery and control mice, and no significant differences amongst groups in *Piezo* gene expression, suggesting that early signs of tendinopathy and cartilage degeneration following proximal biceps tenotomy were not driven by biomechanical changes to the joint. Surgery tendons exhibited higher Bonar scores on histology and signs of inflammation in gene expression when compared to controls, suggesting that extraarticular biceps tenotomy led to tendon degeneration and inflammation. In surgery shoulders, the adjacent humeral head cartilage exhibited subtle signs of degeneration on histology, as reflected by higher MSAS scores, implying crosstalk between the pathologic tendon and the adjacent cartilage. Additional work is necessary to characterize regional differences in the extraarticular and intraarticular portions of tendon following tenotomy, to identify changes in gene expression in the cartilage, and to characterize the nature of the communication between pathologic tendon and the cartilage.

SIGNIFICANCE/CLINICAL RELEVANCE: This study presents evidence of tendon-cartilage crosstalk in the setting of intraarticular tendon degeneration and inflammation, in isolation of biomechanical effects. These findings suggest that therapeutic targets may be identified in tendon-cartilage paracrine signaling to prevent cartilage damage and osteoarthritis progression after intraarticular tendon injury such as a rotator cuff tear. These findings also have clinical implications regarding potential long-term side effects following the treatment of proximal biceps tendinopathy with tenotomy.

REFERENCES: [1] Maffulli et al. “Movin and Bonar Scores Assess the Same Characteristics of Tendon Histology.” Clin Orthop Rel Res (2008). [2] Zingman et al. “Shoulder arthritis secondary to rotator cuff tear: a reproducible murine model and histopathologic scoring system.” J Orthop Res (2017).

IMAGES AND TABLES:

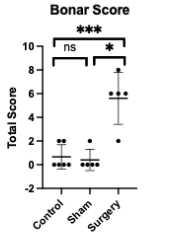

Figure 1: Histologic assessment of tendon

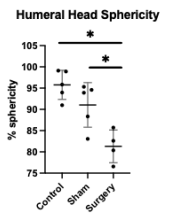

Figure 2: Histologic assessment of humeral head sphericity

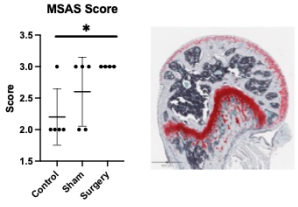

Figure 3: Histologic assessment of humeral heads. Representative section of a humeral head status post tenotomy.