RAT SUPRASPINATUS TENDON EXPRESSES CARTILAGE MARKERS WITH OVERUSE

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INTRODUCTION

Rotator cuff tendinopthy represents a common condition in the young and old alike, and is increased in certain athletic and occupational environments. The mechanism by which tendinopathy is induced through overuse activity is not well understood because only end-stage tissue is typically available for analysis. The use of an animal model provides an opportunity to induce the injury in a controlled manner, so that the early events in this condition can be evaluated. A rat model of tendon overuse has been developed that generates reproducible and significant changes in the histology, geometry, and mechanical properties of the supraspinatus tendon that are consistent with an overuse injury by 4 weeks of exercise [1]. Although some inflammatory and angiogenic markers have been shown to be altered in this model [2], a broader approach is necessary to fully understand the complex processes responsible for the overuse injury. The objective of this study was to investigate the response of the supraspinatus tendon to overuse at a molecular level using transcriptional profiling, and to identify potential markers for tendinopathy. We hypothesized that markers associated with injury, remodeling and degeneration would be upregulated, while inflammatory markers would not.

METHODS

Male Sprague-Dawley rats (400-450 g) were used in this study, which was approved by the IACUC. Twenty-four rats were subjected to a supraspinatus tendon overuse protocol, for 1 week (n=8), 2 weeks (n=8), and 4 weeks (n=8). The protocol consists of downhill running (10% grade) at 17 m/min for 1 hour/day, 5 days/week [1]. An additional six rats were used as cage-activity controls (time 0).

At necropsy, the supraspinatus tendons from each shoulder and the patellar tendons from each knee were carefully removed. The patellar tendon serves as an internal control for the effect of exercise, as it does not show gross signs of overuse injury with this protocol [3]. The harvested tendons were weighed and then snap frozen in liquid nitrogen. The tissues were freeze-fractured and then extracted using TRIzol reagent (Invitrogen). RNA was isolated from the aqueous phase of the extract using an RNeasy kit (QIAGEN). RNA concentrations were determined using a spectrophotometer.

Transcriptional profiling to monitor the expression level of greater than 30,000 transcripts was performed with an Affymetrix rat genome 230 2.0 array. All array images were visually inspected for defects and quality. Arrays with high background, low signal intensity, or major defects were eliminated from further analysis. Signal values were determined using Gene Chip Operating System 1.0 (GCOS, Affymetrix). For each array, all probe sets were normalized to a mean signal intensity value of 100. The default GCOS statistical values were used for all analyses. A gene was considered detectable if the mean expression in any tissue was greater than 100 signal units and the percentage of samples with a Present (P) call as determined by GCOS default settings was greater than or equal to 66%. Normalized signal values were transformed to the log base10. A gene was considered to be differentially expressed if the p-value from an ANOVA test was <0.01 and the difference between running and control was at least 2 fold at any time point.

RESULTS

More than 400 genes were regulated in the supraspinatus tendon after overuse. Of the top genes upregulated consistently across multiple time points, many are highly expressed in cartilage tissue, including col2a1 (Figure 1), aggrecan, versican (Figure 2), sox9 and cartilage glycoprotein 39. These genes were not upregulated in the patellar tendons of the same animals, indicating that the increased expression is likely due to the overuse stimulus, not just the exercise. Previous analysis showed an increased expression of the inflammatory markers COX2 and FLAP and angiogenesis markers VEGF and VWF using RT-PCR [2]. In the current study, a more comprehensive list of genes involved in inflammation and angiogenesis was examined. Based on GO ontology annotation, 213 genes on the Affymetrix rat genome 230 2.0 array are part of an inflammatory response and 35 genes are involved in angiogenesis. Only two inflammatory genes (MX2 and complement C4) and one angiogenesis response gene (SERPINE1) were upregulated in the overuse tendons after 4 weeks, suggesting that there is little inflammation or angiogenesis occurring at the time points examined. No significant regulation of any MMPs, ADAMs, ADAMTSs or serine proteases was detected. Furthermore, injury and repair genes, such as Type III collagen, were not regulated. Very few genes were upregulated in both the patellar and supraspinatus tendons of the rats, suggesting that there was not a general response to exercise in these animals.

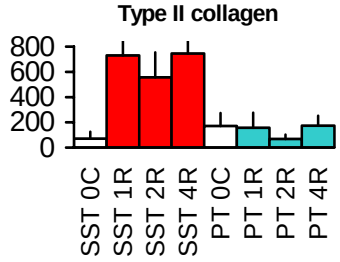

Figure 1: Normalized expression level of Type II collagen in rat supraspinatus tendon (SST) and patellar tendon (PT) at 0, 1, 2 and 4 weeks. Type II collagen mRNA levels are significantly higher in runners (R) than the time 0 control (C) in the SST but not in the PT.

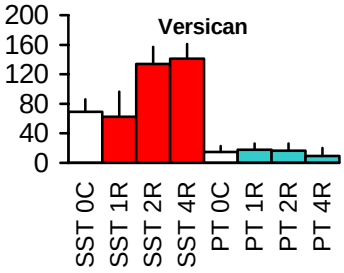

Figure 2: Normalized expression level of Versican in rat supraspinatus tendon (SST) or patellar tendon (PT) at 0, 1, 2 and 4 weeks. Versican mRNA levels are significantly higher in runners (R) than the time 0 control (C) in the SST but not in the PT.

DISCUSSION

Using transcriptional profiling, this study investigated the response of the supraspinatus tendon to overuse at a molecular level. The most striking finding was the increased expression of genes that are highly expressed in cartilage, but found in low levels in normal tendon. This suggests that the tendon may be converting toward a fibrocartilage phenotype as a result of the overuse. The change in phenotype could be due to the compression of the tendon that occurs as it repeatedly passes through the acromial arch, supporting a potentially important role for this anatomic feature. An increase in the synthesis of large proteoglycans such as aggrecan and versican could result in excessive levels of glycosaminoglycans that have been shown to exist in end-stage human pathology [4]. However at this stage, the pattern of mRNA expression in this animal model suggests a unique response to the overuse condition. There was a general absence of markers of inflammation, which lends further support to the use of the term “tendinosis” rather than “tendonitis” in describing tendon overuse conditions. Our findings at the mRNA level must be confirmed at the protein level through further analysis. In addition, age-matched non-running controls should be included in the analysis.

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