

Muscle Histological Difference Between Tendon Injuries of Rotator Cuff and Tibialis Anterior

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INTRODUCTION

Rotator cuff (RC) tears/injuries are common, age-related degenerative musculoskeletal disorders, characterized by the development of muscle atrophy, fibrosis, and fatty infiltration (FI), which compromise muscle quality and function (1). The mechanism of FI has not been determined. Fibro-adipogenic progenitors (FAPs) are a population of resident muscle stem cells that cause FI and fibrosis in injured muscle (2). Dr. Feeley's group has reported that RC muscle FAPs have the highest concentration, proliferative capacity, and adipogenic potential across other muscle groups (3). FI is known to interfere with the healing of RC tears, but this patterned pathology may not be as prevalent across other types of musculotendinous injuries (3). In this study, we compared the muscle histology between RC and tibialis anterior (TA) tendon injuries of aged mice. We hypothesize there are intrinsic differences in muscle histology between TA and RC tendon injuries. Another aim for this study is to find an alternative animal model to study FI and fibrosis in the muscle after tendon injury.

METHODS

Animals: C57BL-6J (WT) mice were purchased from Jackson laboratory and bred at Colorado State University's (CSU) animal facility. Male and female mice at 9-10 months old were used for this study. The right-side RC and TA tendons were injured, and the left-side tendons were used for a non-injured control. All animal protocols used within were approved by CSU's Animal Care and Use Committee. **RC injury:** The RC injury was performed following a published protocol (4). Briefly, after general anesthesia (2% isoflurane) a 2-3 cm skin incision was made on the anterior side of the shoulder with a scalpel. A deltoid-splitting transacromial approach exposed the supraspinatus and infraspinatus tendons which were then finely detached from their humeral footprint. Tendons were also transected just distal to the myotendinous junction, allowing for removal of the tendon, and preventing spontaneous reattachment of the tendon to the surrounding fascia. The wounds were then sutured. **TA tendon injury:** Similarly to the RC injury, the TA tendon was transected and 2-3mm tendons were removed to prevent spontaneous tendon reattachment. Six weeks post injury, the RC and TA muscles were harvested and flash-frozen in liquid nitrogen-cooled 2-methylbutane for cry-sectioning (10µm sections). **Histochemistry:** Both HE and picro-sirius red staining were performed according to the manufacturer's instructions to assess skeletal muscle-related atrophy and fibrosis, respectively. **Immunohistochemistry:** The muscle cryosections were fixed with 4% PFA, blocked with 10% donkey serum and then incubated with a primary antibody for perilipin (adipocyte marker) to determine levels of FI. Alexa fluor 594 conjugated donkey anti-rabbit IgG was used as a secondary antibody. The cross-sectional area (CSA) of myofibers, percentage of collagen deposit, and percent of perilipin+ area were analyzed using ImageJ software. **Statistical analysis:** All results are presented as mean ± standard deviation (SD). Means from the RC and TA tendons, injured and non-injured were compared using Student's t-test with pairwise comparisons; significance is indicated by a p value < 0.05.

RESULTS

Tendon injury-related muscle atrophy varied between tendon injury models. To assess muscle atrophy at the myofibril level, HE staining was performed and analyzed by measuring the CSA of individual muscle fibers. The HE staining and CSA analysis showed significant myofibril-atrophy in the TA tendon injury. Interestingly, we did not find significant muscle atrophy after RC tendon injury (Fig. 1A-B). **Variations in collagen deposition denoted significant deviations between tendon injury models.** Fibrosis is an adverse outcome commonly known to impair muscle function after musculotendinous injuries (3). To determine if the RC and TA tendon injuries resulted in muscle fibrosis, picro-sirius red staining was completed and analyzed by measuring the percentage of collagen deposit. While both the RC and TA tendon injuries developed fibrotic tissue (red-stained collagen), the RC tendon injury model showed elevated levels of fibrosis when compared to the TA tendon injury model (Fig. 2A and B, $p < 0.0158$). **FI remained significantly higher in the RC tendon injury compared to TA tendon injury.** FI can be observed by HE staining and selectively targeted by perilipin for precise analysis. In our study we performed both HE and perilipin staining to compare FI in the RC and TA tendon injuries. Selective staining revealed substantial FI in the RC muscle after injury and minimal FI in the TA muscle after injury (Fig. 3A-B). Quantification of the perilipin+ area also confirmed that FI is significantly increased in the RC tendon injury model compared to the TA tendon injury model (Fig. 3C, $p < 0.0037$). These results indicate that histological alterations such as injury-related muscle atrophy, injury induced fibrosis, and FI after injury vary significantly between different musculotendinous injuries and that alternative animal models cannot be easily substituted when studying FI and fibrosis in the muscle after tendon injury due to these indicated variations.

DISCUSSION

FI, fibrosis, and muscle atrophy are physiological changes that take place within the skeletal muscle tissue after musculotendinous injuries (3). In an attempt to study the mechanism behind these musculoskeletal disorders, we have found that the pathological patterns of these disorders present differently based on the type/location of musculotendinous injuries. Our results revealed that RC tendon injuries have increased fibrosis and FI but may not be subjected to injury-related muscle atrophy. Our results also indicated that while TA tendon injuries do not demonstrate high levels of FI, they still have slight levels of fibrotic tissue buildup and are subjected to injury-related muscle atrophy. The collected data from our study is consistent with current research and allows us to safely conclude that intrinsic differences in muscle histology do exist between different tendon injury models. Although the reason for these histological variations is not currently understood they may be related to differences in BMP and TGF- β expression. RC tendon injuries have been found to overexpress BMP that may inhibit muscle atrophy while simultaneously promoting FI (5). TGF- β has been linked to fibrosis and is also overexpressed in RC tendon injuries explaining the increased fibrotic tissue buildup indicated in the RC model versus the TA model (6). Future applications of this study will include the incorporation of our overexpressed estrogen related receptor gamma (ERR- γ) model to investigate if the known alterations in BMP and TGF- β gene signaling pathways play a major role in these musculoskeletal disorders resulting after musculotendinous injuries.

SIGNIFICANCE. The intrinsic differences between musculotendinous injuries requires additional animal models and further insight into FI mechanisms so new heights in skeletal muscle injury-specific rehabilitation can be reached.

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