Supraspinatus Muscle Cellular Composition in Degenerating and Aging Human Rotator Cuff Tears

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INTRODUCTION: Rotator cuff tears (RCTs) are a common cause of shoulder pain and weakness in adults, resulting in significant disability. They account for an estimated 2 million physician visits each year in the United States alone, and while the incidence increases with age (up to an estimated 30% of individuals over the age of 60 and 80% of individuals over 80) young people also suffer from RCTs. Although there have been tremendous strides in optimizing surgical and medical management of these injuries, the rate of failure is approximately 20% depending on tear size. Secondary muscle atrophy, fatty infiltration, and fibrosis are critical factors for clinical outcomes; however, mechanisms driving these changes remain unclear. Previous animal studies have shown an age-related depletion, and a decrease in functionality and regenerative capacity of muscle specific stem cells (MuSC)—perhaps caused by inflammation-induced senescence and paracrine signaling. Furthermore, the regenerative cascade is altered in aged muscle and there is a shift from muscle repair to extracellular matrix deposition. Although previous studies have shown slower functional recovery in females compared to males, the role of sex in muscle regeneration has not been well investigated. As MuSC integrity, fatty atrophy, and fibrosis are important factors in the context of clinical outcomes after RCT repair, here we characterize differences in MuSC density, myofiber size, and collagen deposition after rotator cuff injury across Goutallier grade, age, and sex.

METHODS: After receiving IRB approval, supraspinatus muscle biopsies (n=11, age range 24-79 years) were obtained from patients undergoing standard of care surgery. Samples were cryosectioned and immunostained with Pax7, MF20, and laminin to identify MuSCs, myofibers, and basal lamina, respectively. Pax7+DAPI+ MuSCs were counted and normalized to myofiber number to yield MuSC density (MuSCs/100 myofibers), and myofiber cross-sectional areas (CSA) were generated using Imaris software. Samples were also immunostained with Collagen type I/II/III and MF20 to quantify fibrosis content using ImageJ. Finally, a subset of samples were immunostained with Pax7, p16ink4a, H2AX, MF20, and Myh7 to investigate the proportion of senescent MuSCs (Pax7+p16ink4a+), MuSCs with DNA damage (Pax7+H2AX+), and type-1 myofibers (Myh7+). Statistical analyses comparing MuSC density, myofiber CSA, and collagen content by Goutallier grade and age were performed using one-way ANOVA, whereas differences by sex were assessed with unpaired t-tests on Prism GraphPad, with significance level set to $\alpha < 0.05$.

RESULTS: Histologic analysis of human supraspinatus muscle samples revealed myofiber CSA was most significantly affected by age and sex. Myofiber CSA was reduced in middle-aged and old muscle compared to young muscle, and reduced in females compared to males. Myofiber CSA was also significantly reduced in Goutallier grade 1-2 muscle compared to grade 0. The proportion of type-1 endurance myofibers (%Myh7+ myofibers/total myofibers) ranged from 40-70% with trending increases of type-1 myofibers with age but was comparable between males and females of similar ages and Goutallier grade. MuSC density per 100 myofibers was similar between all Goutallier grades, but the proportion of senescent MuSCs (%Pax7+p16ink4a+) or MuSCs with DNA damage (%Pax7+H2AX+) trended to increase with higher Goutallier grade. Collagen content as a proportion of total CSA was also greater in higher Goutallier grade samples, in which we additionally found tissues infiltrated by CD10+ fibroblasts and CD68+ macrophages. Furthermore, where myofibers had been replaced by non-muscle tissue, we found increases in the extracellular matrix protein fibronectin and an increased subset of DLK1+ myofibroblasts.

DISCUSSION: This work set out to identify whether degeneration of rotator cuff musculature is accelerated by cells of the microenvironment or by cellular senescence. Our data show that after RCTs, myofiber atrophy increases with age and is associated with changes in the ratio of type-1 and type-2 myofibers, where smaller myofibers tended to be type 1. This suggests that preservation of type-2 myofibers may have protective effects, whereas age-associated increases in type-1 myofibers may be associated with worse outcomes. Although we show no significant differences in MuSC density across Goutallier grade, there were greater proportions of senescent MuSCs with DNA damage at intermediate Goutallier grades, suggesting that chronic degeneration could affect MuSC function. Higher Goutallier grade was found to be associated with greater collagen content and a subset of these samples contained CD10+ human fibroblasts. Future work seeks to measure how extracellular collagen and fibroblasts affect MuSC state, and to further identify hallmarks of MuSC functional ability to proliferate and differentiate.

SIGNIFICANCE/CLINICAL RELEVANCE: This study serves as a basis for understanding the intrinsic and extrinsic defects in skeletal muscle repair. We aim to shed light on both predicting outcomes of rotator cuff tear surgery and identifying new targets for adjuvant treatments to improve muscle regeneration in degenerating and aging RCTs.

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IMAGES AND TABLES:

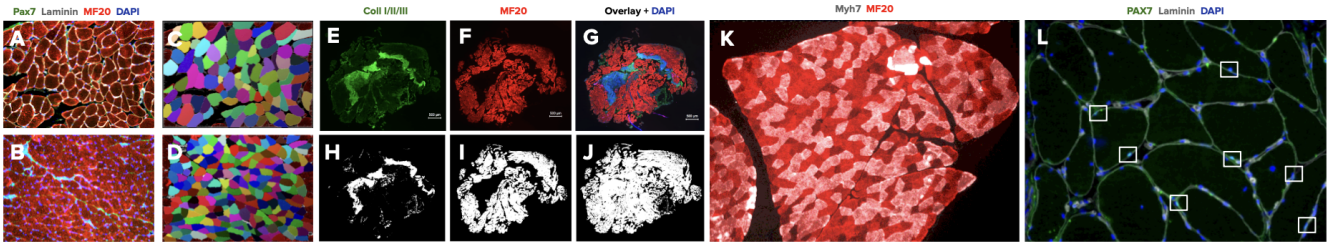

Figure 1. **Histological parameters used for evaluation of rotator cuff tear pathology. Left.** Immunofluorescent staining of supraspinatus muscle from female Goutallier grade 4 (A) and Goutallier grade 0 (B) used to quantify myofiber CSA with Imaris (C, D). **Mid Left.** Total collagen CSA (E) and myofiber CSA (F) to calculate the proportion of collagen to total sample area (G) using binary intensity thresholds on ImageJ (H, I, J). **Mid Right.** Representative images of Myh7+ type 1 myofibers (pink) and MF20+ type 2 myofibers (red) (K). **Right.** Representative image of Pax7+DAPI+ MuSCs (boxes) used to calculate MuSC density per 100 myofibers (L).