# Rotator Cuff Injury Leads to Age Acceleration of Skeletal Muscle Stem Cells

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**Introduction:** Rotator cuff tears (RCTs) are the leading cause of shoulder pain and disability in aging adults. Despite superior surgical techniques, the rate of RCT retear after surgical repair remains unacceptably high,and is confounded by patient intrinsic factors—including age, sex, and fatty infiltration.

**Materials and Methods:** Intraoperative human supraspinatus muscle biopsies were tested and compared with ipsilateral deltoid muscle sample controls. Muscle specimens were evaluated across multiple histologic markers and variables and categorized based on rotator cuff pathology on preoperative imaging and intraoperative evaluation, representing a range from fully degenerated to healthy. Additionally, human muscle satellite cells (SCs) were isolated from samples and tested with pharmacologic agents that could enhance their proliferation in culture, and then evaluated for engraftment potential and repair in mice.

**Results:** We found that despite SC pool preservation in human supraspinatus muscle with higher Goutallier grades, the majority of SCs were senescent and incapable of activation (Figure 1). Furthermore, while aging appeared to be a strong driver of senescence and fibrotic atrophy, there remains an increase in SC senescence with increasing Goutallier grade that is independent of age and sex. We observed that the atrophy associated with aging and higher Goutallier grades is not due to selective myofiber type composition or metabolic changes. Interestingly, treatment of SCs with n-acetylcysteine (NAC), an antioxidant precursor, resulted in significant expansion of aged SCs *in vitro*. To demonstrate *in vivo* functionality, NAC-treated human SCs resulted in enhanced engraftment and myofiber repair in mice compared to aged SCs alone (Figure2).

**Discussion:** *These novel data show that age-related rotator cuff SC senescence predisposes older adults to impaired muscular repair and fibrofatty atrophy following tendon injury. It also shows that Goutallier grade is independently associated with SC senescence and suggests that SC oxidative stress balance may be a target for cellular rejuvenation.* A major strength of our study was the evaluation of human supraspinatus and control deltoid muscle biopsies. Through this comparison, we demonstrate key tissue-level age-related hallmarks of heterogeneity, implying that not all tissues age at the same rate and that our bodies are a mosaicism of ages. This is the first time that age mosaicism, the non-uniform turnover of cells and proteins, has ever been reported with rotator cuff pathology. Furthermore, the chronic inflammatory state due to injury may induce SC aging in younger adults, while adjuvant therapies to enhance redox balance in tissues may be targets to mitigate cellular age acceleration/tissue degeneration. Future work investigating the biological clock of SCs in different muscles including from rotator cuff muscle would further address mechanisms of mosaic aging across tissues and direct adjuvant treatment to improve patient outcomes.

**Figure 1**: Schematic showing the methodology behind obtaining human biopsies, where the Pax7+ SCs are located, and how they were stained and imaged for senescence ($p16^{ink4a}$) and DNA damage (y-H2AX)

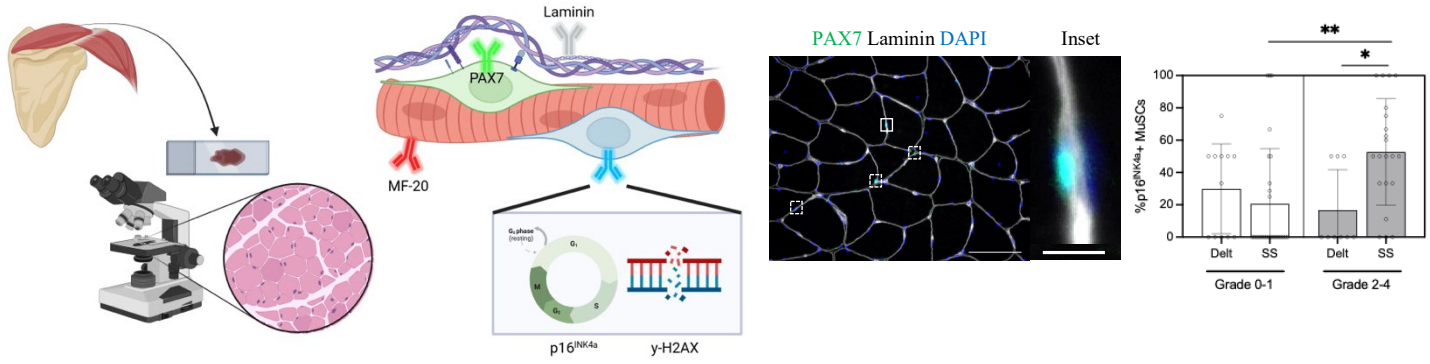


**Figure 2:** Schematic showing the methodology for demonstrating NAC-treated human SCs resulted in enhanced engraftment and increased myofiber repair in mice compared to aged SCs alone