Single-Cell RNA Aging Clock Reveals Accelerated Biological Aging of Fibroadipogenic Progenitors in Post-Injury Rotator Cuff

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Introduction: Fibroadipogenic progenitor (FAP) cells have been identified as key mediators of muscle fatty infiltration post rotator cuff (RC) tears [1]. Aging is known to impair muscle regeneration, in part by diminishing FAP function and number [2]. Aged FAPs secrete fewer pro-regenerative factors, contributing to poor muscle stem cell activity. However, it remains unknown whether RC injury in patients induces a premature aging phenotype in FAPs at the single-cell RNA level. Recently, transcriptomic aging clocks based on single-cell RNA sequencing have been developed to quantify cellular biological age in the nervous tissue of mice [3]. Applying a similar approach, we hypothesize that the RC injury environment accelerates the biological aging of FAP cells compared to uninjured deltoid muscle in the same patients.

Methods: One male and four female patients (ages 42-79) undergoing RC repair provided muscle samples from their torn rotator cuff and healthy deltoid. Single-cell RNA sequencing (scRNA-seq) was performed, and FAP cells were subsetted based on the $PDGFRA^+$ $CD34^+$ double marker. 7,822 FAP cells were obtained from deltoid samples and 11,380 from RC samples. Cell-cycle phase for each FAP cell (G0/G1, S, or G2/M) was determined from transcriptomic signatures. We defined each sample's biological age as 100 – 100 × proliferative fraction, where the proliferative fraction was the percentage of FAPs in S or G2/M phase [3]. We then generated 100 pseudocells per sample, each with 15 randomly selected FAP transcriptomes. The log-normalized gene expression from 26,808 common genes was used to train a regularized elastic net regression model in order to predict biological age. We used leave-one-sample-out cross-validation to evaluate model performance across the 10 samples.

Results: The cell cycle phase distribution (Figure 1, bottom row) showed a visibly higher proportion of FAPs in the proliferative phases (S and G2/M) in deltoid compared to RC samples. The single-cell transcriptomic aging clock generated for this study (Figure 2) predicted FAP biological age in both deltoid and RC with high accuracy, achieving a Pearson correlation of r = 0.796 between predicted and actual biological age, with a mean absolute error of ±6.63 years. On average, the injured RC FAP population shifted toward an older biological state (Figure 3A), with a medium effect size (Cohen's d = 0.62) for the biological age difference between the injured RC and uninjured deltoid muscle. Figure 3B demonstrates that deltoid samples maintained a strong positive correlation between chronological and biological age (r = 0.70), while RC samples showed only a weak correlation (r = 0.20), suggesting that injury-induced aging occurs independently of patient chronological age.

Discussion: Our data demonstrate that FAP cells in injured RC muscle exhibit transcriptomic profiles of accelerated biological aging when compared to FAPs from healthy muscle in the same individuals. This premature aging may help explain the impaired regeneration and fatty degeneration seen after RC tears that are not reversible following successful repair. Additionally, we demonstrate the first application of a single-cell RNA aging clock on FAPs in a clinically relevant scenario, adapting approaches previously applied in mice and other organ systems [3]. The disrupted correlation between chronological and biological age in injured tissue indicates that injury overrides normal aging patterns. As such, the strong performance of our transcriptomic clock highlights its potential use for quantifying biological age changes in tissue-specific cell populations.

Significance/Clinical Relevance: Reducing the biological age of FAPs by promoting a proliferative phenotype could mitigate fibro-fatty muscle degeneration [4]. Therapeutic strategies that prevent this premature aging FAP signature may reverse aspects of muscle degeneration and improve healing outcomes after rotator cuff tears, substantially enhancing the quality of life of patients.

References: [1] Subhash et al. Current Tissue Microenvironment Reports. 2022. [2] Lukjanenko L. et al. Cell Stem Cell. 2019. [3] Buckley M.T. et al. Nature Aging. 2023. [4] Liu L. et al. J Cachexia Sarcopenia Muscle. 2022.

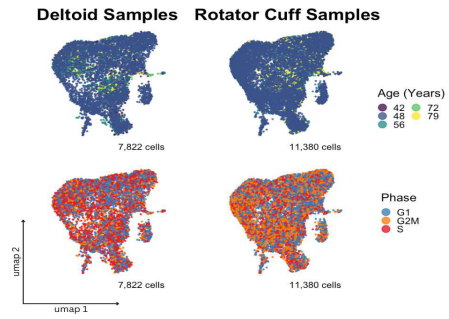

Figure 1: UMAPs of Deltoid (left) and RC (right) Samples. Total cell counts were 7,822 and 11,380 respectively. Top row color coded by patient age and bottom row by cell cycle phase.

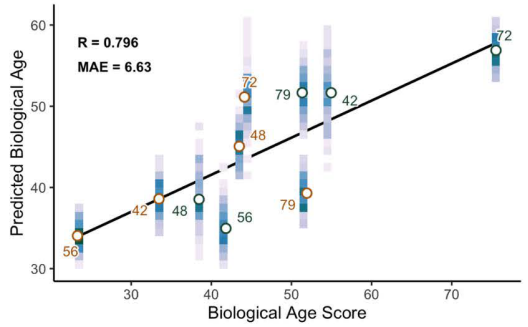

Figure 2: Performance of the elastic-net transcriptomic aging clock on RC (green) and Deltoid (brown) samples. Predicted biological ages correlated strongly with true biological age (r = 0.796) with mean absolute error of 6.63 years

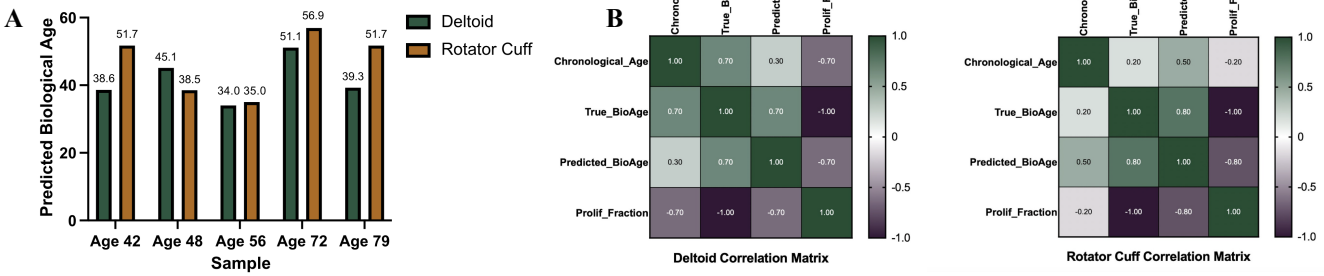

Figure 3: (A) Predicted biological age of paired patient samples (green = RC, brown = deltoid) ordered by chronological age. **(B)** Correlation Matrix of Deltoid (left) and RC (right) samples. Deltoid had strong positive correlation (r = 0.70) between chronological and true biological age while RC samples had a weak correlation (r = 0.20)