Title

Rotator Cuff Tear Size Regulates Fibroadipogenic Progenitor Number and Gene Expression Profile in the Supraspinatus Independent of Patient Age

Journal

The American Journal of Sports Medicine, 50(1)

ISSN

0363-5465

Authors

Davies, Michael R
Chi, Hannah
Kaur, Gurbani
et al.

Publication Date

2022

DOI

10.1177/03635465211054512

Peer reviewed

HHS Public Access

Author manuscript

Am J Sports Med. Author manuscript; available in PMC 2022 July 14.

Published in final edited form as:

Am J Sports Med. 2022 January ; 50(1): 208–215. doi:10.1177/03635465211054512.

Rotator Cuff Tear Size Regulates Fibroadipogenic Progenitor Number and Gene Expression Profile in the Supraspinatus Independent of Patient Age

Michael R. Davies, MD[*,†],

Hannah Chi, BS[†],

Gurbani Kaur, BS[†],

Mengyao Liu, BS[†,‡],

C. Benjamin Ma, MD[†],

Hubert T. Kim, MD, PhD[†,‡],

Xuhui Liu, MD[†,‡],

Brian T. Feeley, MD[†,‡]

Investigation performed at University of California, San Francisco, San Francisco, California, USA

Abstract

Background: Fatty infiltration of rotator cuff muscle is a limiting factor in the success of repairs. Fibroadipogenic progenitors (FAPs) are a population of stem cells within the rotator cuff that can differentiate into white adipocytes, fibroblasts, and beige adipocytes. The effects of patient age and rotator cuff tendon tear size on the number, differentiation patterns, and gene expression profiles of FAPs have not yet been analyzed.

Purpose: To determine if patient age and rotator cuff tear size independently regulate FAP number, differentiation patterns, and gene expression profiles.

Study Design: Controlled laboratory study.

Methods: Supraspinatus muscle samples were collected from 26 patients between the ages of 42 and 76 years with partial- or full-thickness rotator cuff tears. FAPs were quantified using fluorescence-activated cell sorting. Gene expression analysis was performed across a custom 96-gene panel using NanoString. In vitro differentiation assays of FAPs were conducted using adipogenic, fibrogenic, and beige-inducing (amibegron-treated) media, and quantitative polymerase chain reaction was used to assess gene expression differences between adipogenic and amibegron media conditions. Multivariable linear regressions were performed using Stata to independently analyze the effects of age and rotator cuff tear size on FAP number, differentiation, and gene expression.

[*]Address correspondence to Michael R. Davies, MD, Department of Orthopaedic Surgery, University of California, San Francisco, 1700 Owens St, San Francisco, CA 94158, USA (michael.davies@ucsf.edu).

[†]Department of Orthopaedic Surgery, University of California, San Francisco, San Francisco, California, USA.

[‡]San Francisco Veteran Affairs Health Care System, San Francisco, California, USA.

Results: Increasing age and tear size were independently correlated with increased FAP number (β_{age} = 0.21, P = .03; $\beta_{tear\ size}$ = 3.86, P = .05). There was no clear association between age and gene expression of freshly sorted FAPs. Under adipogenic and fibrogenic media conditions, increasing age and tear size were independently associated with increased adipogenic and fibrogenic differentiation of FAPs. Under amibegron treatment conditions, age positively correlated with increased beige differentiation (β = 1.03; P < .0001), while increasing tear size showed a trend toward decreased beige differentiation (β = −4.87; P = .1). When gene expression patterns between adipogenic and amibegron media conditions were compared, larger tear size strongly inhibited beige gene expression, while advanced age did not.

Conclusion: Patient age and rotator cuff tear size independently regulated FAP number, differentiation, and gene expression. Age and tear size were positively correlated with increased FAP number and fibrogenic/adipogenic differentiation. Advancing patient age did not limit FAP beige differentiation and gene expression, while increasing rotator cuff tear size strongly inhibited these processes.

Keywords

rotator cuff; fatty infiltration; fibroadipogenic progenitors; stem cells

Rotator cuff tears are the most common musculotendinous injury treated by orthopaedic surgeons.[27] The prevalence of rotator cuff tears increases with age, with approximately 50% of patients aged >65 years presenting with a tear.[19,20] Muscle quality remains an important factor in determining outcomes of attempted rotator cuff repair, and long-term changes such as fatty infiltration and fibrosis are associated with worse postoperative functional outcomes in patients who undergo rotator cuff repair.[5,9,22,23] Based on our current understanding of rotator cuff muscle degeneration, the atrophy and fatty degeneration of rotator cuff muscle that occur in the setting of chronic tendon tears appear to be irreversible even after successful repair.[7,12]

Fibroadipogenic progenitors (FAPs) are a recently described population of multipotent resident muscle stem cells whose role in muscle degeneration and regeneration is not yet fully understood.[10,21] While the persistence of FAPs in chronically injured muscle results in adipogenic and fibrogenic differentiation patterns,[14,15] other studies have found that a subset of FAPs play an essential role in the regenerative response to muscle injury.[25,26,28] In the setting of rotator cuff tears, FAPs have been identified as the major source of adipocytes in rotator cuff fatty infiltration.[14] FAPs are heterogeneous and have the potential to differentiate and give rise to multiple populations of cells, including white fat and fibroblasts.[15] White fat is the major adipose tissue type present in adult muscle and likely drives the deleterious effects of fatty infiltration.[1] However, FAPs have also been shown to possess promyogenic beige fat potential, as demonstrated by the expression of the thermogenic marker uncoupling protein 1 (UCP1) in conjunction with promyogenic growth factors including insulin-like growth factor 1 and follistatin.[3] In small animal models, induction of beige adipogenesis in injured rotator cuff muscle, either through the use of the β3 agonist amibegron or via transplantation of UCP1 + beige FAPs, has been shown to be effective in treating fatty degeneration in murine rotator cuff tear and repair models and to be correlated with improved animal gait.[13,24]

The effects of muscle tear size on rotator cuff FAP infiltration have been demonstrated. More severe full-thickness rotator cuff tears have significantly more FAPs present when compared with partial-thickness tears.[3] Tear size also affects FAP differentiation capacity and gene expression profile.[3] However, the effect of patient age, independent of tear size, on FAP cell number and gene expression profile has not yet been investigated. Furthermore, while β agonist–induced beige adipogenic differentiation of FAPs has been demonstrated in muscle from both partial-thickness and full-thickness rotator cuff tears,[3] it is not yet known whether FAPs in the muscle of elderly patients maintain the full ability to undergo directed beige adipogenesis and in turn exhibit the associated expression of promyogenic growth factors previously noted in both murine and human FAPs.[3,13,24] Characterizing the regenerative capacity of FAPs in the muscle of older adult patients would have implications for treatment decisions, including determining the utility of induced beige adipogenesis as a therapeutic approach in elderly patients.

The purpose of this study was to investigate how age might affect the characteristics of FAPs in human rotator cuff muscle, including their number, gene expression patterns, and beige regenerative potential. We hypothesized that patient age would independently affect FAP cell number, differentiation capacity, and gene expression profile. However, we expected amibegron treatment of human FAPs would continue to induce UCP1 + beige differentiation regardless of patient age.

METHODS

Patient Population and Surgical Biopsy

This study received institutional review board approval before commencement (IRB No. 18-26000). Patients were recruited from the University of California, San Francisco, Sports Medicine Clinic from 2 surgeons (B.T.F., C.B.M.) between June 2019 and December 2020. Inclusion criteria and tear thickness categorizations were as previously described by Feeley et al.[3] Patients gave consent in the clinic or on the day of surgery to the research coordinator or attending surgeon. Patient data are shown in Table 1. Note that a discrete time interval between initial rotator cuff injury or symptoms and surgery was not available for all patients but is provided for patients for whom there was clear documentation of this interval.

After the rotator cuff repair procedure was completed, the biopsy specimen was identified and obtained 2 to 3 cm from the muscle-tendon junction from the superior aspect of the supraspinatus muscle, as previously described.[3] Samples weighed approximately 5 to 7 mg each, and a sample of deltoid muscle was also obtained from each patient as an internal control.

Muscle Sample Preparation and Flow Cytometry

Muscle was digested with 0.2% collagenase for 90 minutes followed by 0.4% dispase treatment for 30 minutes. Human FAPs were isolated from the muscle specimens using the Beckton Dickinson Aria II with propidium iodide live and dead staining. To select for FAPs, we used CD31−, CD45−, CD184−, CD29−, CD56−, CD34 +, and PDGFRα + markers.[21] See Appendix Figure A1 (available in the online version of this article) for

fluorescence-activated cell sorting gating strategy. Cell numbers of FAPs were reported as the percentage of total live cells sorted.

Cell Differentiation Assay

Freshly sorted FAPs were cultured in 24-well plates at an initial density of 5000 cells per well in F10 + 20% fetal bovine serum + 1% antibiotics (standard FAP media) and were grown to a density of 0.5×10^6 cells per well before being passaged. Cells were passaged twice before treatment with terminal differentiation medium, which included fibrogenic media (+ 10 ng/mL TGF-β1), adipogenic media (StemPro adipogenic differentiation kit; ThermoFisher), or beige differentiation media (StemPro adipogenic differentiation kit + 10 μM amibegron) for 2 weeks. No substantial proliferation was observed after cells were switched to differentiation medium conditions. Three technical replicates were performed for each patient-derived sample. Cells were then fixed in 4% paraformaldehyde and stained for α-SMA (fibrogenic marker), perilipin (adipogenic marker), or UCP-1 (beige adipogenic marker). Expression indices for each treatment condition were quantified using ImageJ (National Institutes of Health) as previously described.[3]

RNA Isolation, NanoString Transcriptomic Analysis, and Quantitative Polymerase Chain Reaction

The samples were treated with TRIzol reagent (Thermo Fisher Scientific) to extract total RNA according to the manufacturer's instructions. For initial gene expression analysis of FAPs from freshly harvested human muscle samples, we used NanoString nCounter technology (NanoString), a molecular barcode-based gene expression array used to directly count RNA transcripts from a predetermined array of genes without the need of a reverse transcription reaction.[4,8,11] For NanoString analysis, a custom-designed nCounter gene expression code set including 96 predefined genes was used for analysis of total RNA extracts (Appendix Table A1, available online). For each hybridization reaction, at least 100 ng of RNA in a 5-μL volume was used in the probe set–target RNA hybridization reactions, which were performed according to the manufacturer's protocol. The resulting purified probe set–RNA complexes were then immobilized on nCounter Cartridges and quantified on the Digital Analyzer. The data were then analyzed using nCounter software, which was used to generate heat maps of normalized gene expression, perform principal component analysis, and create differential gene expression plots. To analyze patient age independent of tear size, we performed principal component analysis and differential gene expression analysis on patient rotator cuff samples from partial-thickness tears and the corresponding deltoid control samples.

For quantitative polymerase chain reaction (qPCR), cDNA was then synthesized using the Transcriptor First Strand cDNA Synthesis Kit (Roche Applied Bioscience Inc). To quantify gene expression, we performed reverse transcription PCR using SYBR Green Detection (Bio-Rad Laboratories Inc) and an Applied Biosystems Prism 7900HT detection system (Applied Biosystems Inc) on a panel of 18 genes (Appendix Table A2, available online). The housekeeping gene S26 was used to normalize expression levels (average CT across all samples, 18.3 ± 2.7), and fold changes in supraspinatus samples relative to control muscle samples were calculated using ΔΔCT as previously described.[3]

Statistical Analysis

Statistical analyses were performed using Stata (Version 16.1; StataCorp LP). Descriptive statistics including mean and standard deviation were calculated. The Pearson correlation coefficient was used to assess the relationship between pairs of continuous variables: patient age and percentage of FAPs sorted. One-way analysis of variance with post hoc Tukey honestly significant difference test was used to assess the relationship between rotator cuff tear size and percentage of FAPs sorted. Point biserial correlation test was used to assess patient sex and percentage of FAPs sorted. Multivariable linear regressions were then performed to assess the association between the independent variables of age and tear size and the dependent variables of FAP percentage, differentiation markers, and gene expression markers. For assessing the differential effects of patient age and tear size on FAP cell number and differentiation capacity, partial and small tears were compared with medium and large tears. For qPCR, given a subset of samples used for analysis (n = 6), full-thickness tears were compared with partial-thickness tears. Statistical significance was defined as $P < .05$. For Multivariable linear regressions, data are presented as β coefficient and P value.

RESULTS

The average number of FAPs isolated from injured supraspinatus muscle was significantly larger than that isolated from the uninjured deltoid (73,053 ± 45,548 vs 10,141 ± 9981; $P < .001$) (Figure 1A). There was no significant difference in total live cells sorted between cuff and deltoid samples. Univariate linear regression of patient age and percentage of FAPs sorted demonstrated a strong positive correlation, with older patients having significantly larger numbers of FAPs compared with younger patients (Pearson $r = 0.66$; $P = .005$) (Figure 1B). One-way analysis of variance with post hoc Tukey honestly significant difference test demonstrated a significant increase in FAP percentage between patients with large tears and partial tears ($P = .012$) (Figure 1C). Patient sex was additionally evaluated using the point biserial correlation test and was not found to be correlated significantly with FAP percentage ($r = −0.033$; $P = .898$). Body mass index (BMI) was likewise not found to have a significant univariate correlation with sorted FAP percentage (average BMI, 26.3 ± 4.2; $r = 0.29$; $P = .08$). Therefore, patient sex and BMI were not included as independent variables for subsequent analysis. Multivariable linear regression analysis including patient age and tear size demonstrated that these 2 variables are independently associated with an increase in the percentage of sorted FAPs (Table 2).

NanoString gene transcriptional profiling of freshly harvested FAPs from rotator cuff and deltoid muscle confirmed that FAPs were heterogeneous in their overall gene expression profiles (Figure 2A), as has been previously reported.[6,15,17] Muscle type and patient age were then analyzed as univariate predictors of differences in gene expression for each gene in the panel, and it was found that while muscle type (cuff compared with deltoid) resulted in many significant differences in gene expression, patient age did not appear to significantly alter overall gene expression profile in the univariate analysis (Figure 2B). Principal component analysis of gene expression as a function of patient age, controlling for muscle type and tear size, likewise showed heterogeneous clustering of patient samples indicative of a poor correlation between overall gene expression and patient age (Figure

2C). Differential gene expression analysis demonstrated 2 genes in the panel that were significantly regulated in FAPs as a function of patient age (C/EBPd: fold change, 0.88; P = .022; and SMAD3: fold change, 1.1; P = .029), with the remaining genes not displaying significant differential gene expression (Figure 2D).

FAPs from patients of different ages and with tear sizes were then cultured in adipogenic, fibrogenic, or beige media and assessed for corresponding differentiation markers (Figure 3). Multivariable linear regression analysis demonstrated that increasing age was significantly correlated with increased adipogenic (β = 1.92, P < .0001), fibrogenic (β = 1.68, P = .001), and beige differentiation (β = 1.03, P < .0001), while increasing tear size was significantly correlated with fibrogenic differentiation (P = .045). Increasing tear size also demonstrated a trend toward increased adipogenic differentiation (β = 817, P = .096) and decreased beige differentiation (β = −4.87, P = .10), although these relationships were not statistically significant when controlling for patient age.

To further assess the effects of age and tear size on FAP gene expression under different media conditions, we performed qPCR on FAPs cultured using either adipogenic or beige-inducing media (Table 3). Fold changes were calculated relative to uninjured control muscle samples, and multivariable linear regressions were then used to correlate patient age and tear size with gene expression levels. Under adipogenic media conditions, tear size was found to be consistently correlated with increased expression of markers of white adipogenesis when controlling for age, while age did not display as strong a correlation with increased adipogenic gene expression (Table 3). When cells were cultured in amibegron-treated media, age was found to positively correlate with increased expression of beige adipogenesis genes, while increasing tear size was strongly negatively correlated with beige adipogenic gene expression (Table 3). These data suggested that increasing tear size, but not age, hindered the ability of amibegron to promote beige adipogenesis in FAPs.

DISCUSSION

In this study, we have assessed the effects of patient age and tear size on the number, differentiation patterns, and gene expression patterns of FAPs harvested from patient rotator cuff muscle samples. We have determined that increasing age as well as increasing tear size were independently correlated with increasing FAP number in the rotator cuff but had differential effects on gene expression and targeted differentiation. Increasing patient age did not appear to inhibit the ability of FAPs to undergo directed beige adipogenesis. However, larger tear size was strongly correlated with inhibition of this process at the level of gene expression. This finding highlights the important goal of diagnosing and addressing rotator cuff tears before they become too large in size, regardless of patient age, to preserve the proregenerative beige potential of FAPs.

Over the past decade, FAPs have been found to play a central role in mediating the response to muscle injury and regeneration[1,21,25,26]; however, few studies have studied the role and function of this cellular population in orthopaedic patients. In the setting of patients with rotator cuff tears, we have previously shown that increasing cuff tear size was correlated with an increase in FAPs in injured muscle.[3] In this study, we found that increasing patient

age was also correlated with increasing FAP number independent of tear size, although with a smaller β coefficient than that of tear size (0.21 compared with 3.86, respectively). Previous reports have found that increasing age was associated with greater fatty infiltration of the rotator cuff.[2,16] Our findings in this study suggested that FAP accumulation in the muscles of older patients may be a factor underlying this pathology, although clinically the effect of increasing tear size on FAP accumulation in injured muscle appears to be greater.

We next sought to broadly characterize the transcriptional profiles of FAPs obtained from patient muscle samples. NanoString gene expression analysis allows for efficient and accurate transcriptional profiling to aid in this characterization.[4,8,11] In our analysis, we found that across the predesigned 96-gene code set, there was marked heterogeneity in FAP gene expression among freshly sorted FAPs, as evidenced by a lack of clear hierarchical clustering of samples by age, tear size, or muscle type seen on heat maps. This was consistent with the findings of several other studies suggesting that FAPs likely consist of a spectrum of different mesenchymal stem cell subtypes within muscle.[6,15,17] When analyzing age and controlling for muscle type and tear size, we found that age did not highly influence the clustering of patient samples seen with principal component analysis and did not affect the expression of most genes included in the custom panel. The 2 genes that were significantly different in expression as a function of age, SMAD3 and C/EBPd, did not show fold changes that would be expected to be of clinical significance (fold changes, 1.1 and 0.88, respectively). Thus, from a transcriptomic perspective, it does not appear that patient age globally influences FAP gene expression patterns in freshly sorted cells across the 96 genes that were selected for analysis.

To supplement this higher-level overview of gene expression patterns, we next examined the differentiation potential of FAPs as a function of age and tear size when cultured under different conditions. We found that increasing age consistently resulted in increased perilipin-positive white adipocytes, α-SMA–positive fibroblasts, and UCP1-positive beige adipocytes when controlling for tear size. While the findings of increased white adipogenic and fibrogenic differentiation capacity correlated with previous observations of increased and more severe fibrofatty degeneration of the rotator cuff in older patients,[2,3,5,7] the effect of age on β3 adrenergic-mediated beige adipogenesis has not previously been evaluated. Our findings suggested that age alone was not a limiting factor in the potential of FAPs to undergo beige differentiation with amibegron treatment. Conversely, increasing tear size was found to have a negative correlation with beige adipogenic differentiation potential, although this was not statistically significant. Taken together, these findings suggested that patient age and tear size may affect beige differentiation of FAPs via different mechanisms. Given a lack of clear age-based differences in gene expression profiles of freshly sorted FAPs seen using NanoString, it is possible that these functional differences were regulated at the epigenetic level or involved signaling pathways that were not evaluated using the custom gene panel. Saccone et al[18] previously demonstrated that in patients with late-stage muscular dystrophy compared with those with early-stage disease, increased epigenetic regulation of FAPs via histone deacetylases ultimately resulted in increased fibroadipogenic differentiation of FAPs and a loss of promyogenic function. It is possible that a similar epigenetic process occurs in the FAPs isolated from the muscles of older patients as well as those with larger, chronic

cuff tears, as such epigenetic changes would not be detectable via a standard gene expression panel. Thus, future studies are needed to evaluate this potential mechanism.

A positive correlation was noted between tear size and white adipogenic as well as fibrogenic differentiation, although only the correlation with fibrogenesis was found to be statistically significant. Although fibrosis is a common histological marker of chronically injured muscle, it is not currently evaluated clinically in the setting of rotator cuff degeneration since it cannot be detected using standard imaging modalities. Given that advanced age and increasing tendon tear size independently promoted fibrogenic differentiation of FAPs, muscle fibrosis may be a useful marker of overall muscle health in the rotator cuff once the requisite diagnostic imaging protocols are readily available for its quantification.

Given these observed differences in cultured FAP differentiation patterns, we next analyzed the effect of age and tear size on white and beige adipogenic gene expression. We found that increasing tear size was strongly correlated with increased expression of white adipose markers and decreased expression of beige adipose markers. While age was significantly correlated with several white adipogenic and beige adipogenic markers, the strength of the correlation was not as strong as that of increasing tear size. Under beige-inducing media conditions, tear size again demonstrated a strong negative correlation with beige gene expression, while age demonstrated a significant positive association with beige gene expression when controlling for tear size, although the β coefficients associated with age were 100-fold to 500-fold less in magnitude than those associated with tear size. Taken together, we found that while age and tear size were independently correlated with FAP gene expression, tear size may play a stronger role in regulating this process.

This study had several limitations. FAPs represent a heterogeneous population of mesenchymal stem cells. It is possible that the heterogeneity observed among freshly sorted FAPs analyzed using NanoString transcriptomal analysis was due to the effect of capturing diverse gene expression patterns among multiple subpopulations of FAPs.[17] Future studies may utilize single-cell RNA sequencing technology to better distinguish among subpopulations of FAPs, although NanoString allows for efficient and focused gene expression analysis of numerous tissue samples simultaneously and presents a less resource-intensive option for focused analysis. Next, patient sample size for this study was limited, but the study was powered to detect differences in FAP cell number between rotator cuff and deltoid muscle samples, as previously described.[3] Additionally, given the chronic nature of symptoms and lack of an exact time frame of cuff injury in the majority of patients, we were unable to ascertain the exact time interval between rotator cuff tear and surgery for many patients and therefore cannot accurately comment on the true chronicity of tears in many patients. This may have implications for the attenuation of beige adipogenesis noted in patients with larger tears, as tear size is likely to be linked with tear chronicity. Finally, we recognize that age and tear size are variables that are partially related, as studies have previously determined a positive correlation between increasing age and tear size[2,9]; however, the purpose of performing a multivariable linear regression of these 2 variables was to be able to assess the independent contributions of their effects.

CONCLUSION

We have determined that patient age and rotator cuff tear size independently predicted an increase in FAP cell number in human muscle biopsy samples. We have found that while both age and tear size led to an increase in directed adipogenic and fibrogenic differentiation of FAPs, increasing age had a positive correlation with β3 agonist–directed beige differentiation capacity and increasing tear size impaired the process of beige differentiation in FAPs. Similarly, increasing tear size was strongly positively correlated with an increase in white adipogenic gene expression and impaired beige adipogenic gene expression in response to a β3 agonist compared with uninjured muscle, while increasing age had a weaker positive contribution to both of these processes. These findings have important implications for future cell-based therapies that may utilize the regenerative potential of FAPs, as we have shown that tear size, but not age, may be a limiting factor in the regenerative capacity of these cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

One or more of the authors has declared the following potential conflict of interest or source of funding: M.D. received grant funding for this study from the AOSSM (YIG-2020-B) and the Orthopaedic Research and Education Foundation (A135210), and B.T.F., X.L., and H.T.K. received grant funding from the National Institutes of Health (R01-AR072669). C.B.M. has received research support from Aesculap, Samumed, and Zimmer; consulting fees from Conmed, Stryker, Tornier, Wright Medical Technology, and Zimmer Biomet; royalties from Conmed and SLACK; and hospitality payments from Arthrex. B.T.F. has received research support from Orthofix and hospitality payments from Zimmer Biomet; he also holds stock options in Biomiks and Kaliber. H.T.K. has received educational support from Intuitive Surgical. AOSSM checks author disclosures against the Open Payments Database (OPD). AOSSM has not conducted an independent investigation on the OPD and disclaims any liability or responsibility relating thereto.

REFERENCES

1. Arrighi N, Moratal C, Clément N, et al. Characterization of adipocytes derived from fibro/adipogenic progenitors resident in human skeletal muscle. Cell Death Dis. 2015;6(4):e1733. [PubMed: 25906156]
2. Barry JJ, Lansdown DA, Cheung S, Feeley BT, Ma CB. The relationship between tear severity, fatty infiltration, and muscle atrophy in the supraspinatus. J Shoulder Elbow Surg. 2013;22(1):18–25. [PubMed: 22541866]
3. Feeley BT, Liu M, Ma CB, et al. Human rotator cuff tears have an endogenous, inducible stem cell source capable of improving muscle quality and function after rotator cuff repair. Am J Sports Med. 2020;48(11):2660–2668. [PubMed: 32730704]
4. Fortina P, Surrey S. Digital mRNA profiling. Nat Biotechnol. 2008;26(3):293–294. [PubMed: 18327237]
5. Gerber C, Schneeberger AG, Hoppeler H, Meyer DC. Correlation of atrophy and fatty infiltration on strength and integrity of rotator cuff repairs: a study in thirteen patients. J Shoulder Elbow Surg. 2007;16(6):691–696. [PubMed: 17931904]
6. Giuliani G, Vumbaca S, Fuoco C, et al. SCA-1 micro-heterogeneity in the fate decision of dystrophic fibro/adipogenic progenitors. Cell Death Dis. 2021;12(1):1–24. [PubMed: 33414393]
7. Gladstone JN, Bishop JY, Lo IK, Flatow EL. Fatty infiltration and atrophy of the rotator cuff do not improve after rotator cuff repair and correlate with poor functional outcome. Am J Sports Med. 2007;35(5):719–728. [PubMed: 17337727]

8. Goytain A, Ng T. NanoString nCounter technology: high-throughput RNA validation. Methods Mol Biol. 2020;2079:125–139. [PubMed: 31728967]
9. Jeong HY, Kim HJ, Jeon YS, Rhee YG. Factors predictive of healing in large rotator cuff tears: is it possible to predict retear preoperatively? Am J Sports Med. 2018;46(7):1693–1700. [PubMed: 29595993]
10. Joe AW, Yi L, Natarajan A, et al. Muscle injury activates resident fibro/adipogenic progenitors that facilitate myogenesis. Nat Cell Biol. 2010;12(2):153–163. [PubMed: 20081841]
11. Kulkarni MM. Digital multiplexed gene expression analysis using the NanoString nCounter system. Curr Protoc Mol Biol. 2011;chapter 25:unit25B.10.
12. Lansdown DA, Lee S, Sam C, Krug R, Feeley BT, Ma CB. A prospective, quantitative evaluation of fatty infiltration before and after rotator cuff repair. Orthop J Sports Med. 2017;5(7):2325967117718537.
13. Lee C, Liu M, Agha O, Kim HT, Feeley BT, Liu X. Beige FAPs transplantation improves muscle quality and shoulder function after massive rotator cuff tears. J Orthop Res. 2020;38(5):1159–1166. [PubMed: 31808573]
14. Liu X, Ning AY, Chang NC, et al. Investigating the cellular origin of rotator cuff muscle fatty infiltration and fibrosis after injury. Muscles Ligaments Tendons J. 2016;6(1):6–15. [PubMed: 27331027]
15. Malecova B, Gatto S, Etxaniz U, et al. Dynamics of cellular states of fibro-adipogenic progenitors during myogenesis and muscular dystrophy. Nat Commun. 2018;9(1):3670. [PubMed: 30202063]
16. Melis B, Wall B, Walch G. Natural history of infraspinatus fatty infiltration in rotator cuff tears. J Shoulder Elbow Surg. 2010;19(5):757–763. [PubMed: 20363160]
17. Oprescu SN, Yue F, Qiu J, Brito LF, Kuang S. Temporal dynamics and heterogeneity of cell populations during skeletal muscle regeneration. iScience. 2020;23(4):100993. [PubMed: 32248062]
18. Saccone V, Consalvi S, Giordani L, et al. HDAC-regulated myomiRs control BAF60 variant exchange and direct the functional phenotype of fibro-adipogenic progenitors in dystrophic muscles. Genes Dev. 2014;28(8):841–857. [PubMed: 24682306]
19. Sher JS, Uribe JW, Posada A, Murphy BJ, Zlatkin MB. Abnormal findings on magnetic resonance images of asymptomatic shoulders. J Bone Joint Surg Am. 1995;77(1):10–15. [PubMed: 7822341]
20. Tempelhof S, Rupp S, Seil R. Age-related prevalence of rotator cuff tears in asymptomatic shoulders. J Shoulder Elbow Surg. 1999;8(4):296–299. [PubMed: 10471998]
21. Uezumi A, Fukada S, Yamamoto N, et al. Identification and characterization of PDGFRα + mesenchymal progenitors in human skeletal muscle. Cell Death Dis. 2014;5(4):e1186. [PubMed: 24743741]
22. Valencia AP, Lai JK, Iyer SR, et al. Fatty infiltration is a prognostic marker of muscle function after rotator cuff tear. Am J Sports Med. 2018;46(9):2161–2169. [PubMed: 29750541]
23. Wang Z, Liu X, Davies MR, Horne D, Kim H, Feeley BT. A mouse model of delayed rotator cuff repair results in persistent muscle atrophy and fatty infiltration. Am J Sports Med. 2018;46(12):2981–2989. [PubMed: 30198747]
24. Wang Z, Liu X, Liu M, et al. β3-Adrenergic receptor agonist treats rotator cuff fatty infiltration by activating beige fat in mice. J Shoulder Elbow Surg. 2021;30(2):373–386. [PubMed: 32599287]
25. Wosczyna MN, Konishi CT, Carbajal EE, et al. Mesenchymal stromal cells are required for regeneration and homeostatic maintenance of skeletal muscle. Cell Rep. 2019;27(7):2029–2035. [PubMed: 31091443]
26. Wosczyna MN, Rando TA. A muscle stem cell support group: coordinated cellular responses in muscle regeneration. Dev Cell. 2018;46(2):135–143. [PubMed: 30016618]
27. Yamamoto A, Takagishi K, Osawa T, et al. Prevalence and risk factors of a rotator cuff tear in the general population. J Shoulder Elbow Surg. 2010;19(1):116–120. [PubMed: 19540777]
28. Yao L, Tichy ED, Zhong L, et al. Gli1 defines a subset of fibroadipogenic progenitors that promote skeletal muscle regeneration with less fat accumulation. J Bone Miner Res. 2021;36(6):1159–1173. [PubMed: 33529374]

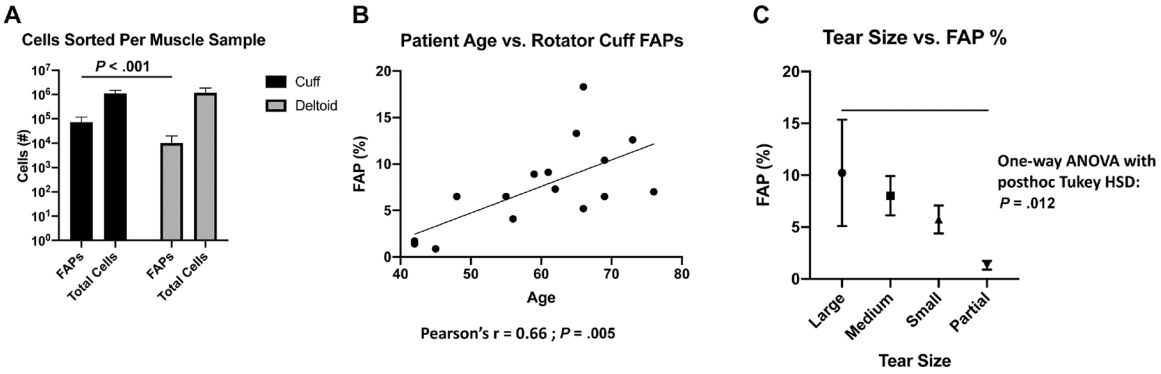


Figure 1.

(A) Average number of fibroadipogenic progenitors (FAPs) sorted per muscle sample compared with total live cells sorted via fluorescence-activated cell sorting. (B) Pearson correlation was performed between patient age and FAP percentage of sorted cells. (C) One-way analysis of variance (ANOVA) with post hoc Tukey honestly significant difference (HSD) test performed between patient rotator cuff tear size (partial, small, medium, or large) and FAP percentage. Solid bar indicates $P < .05$.

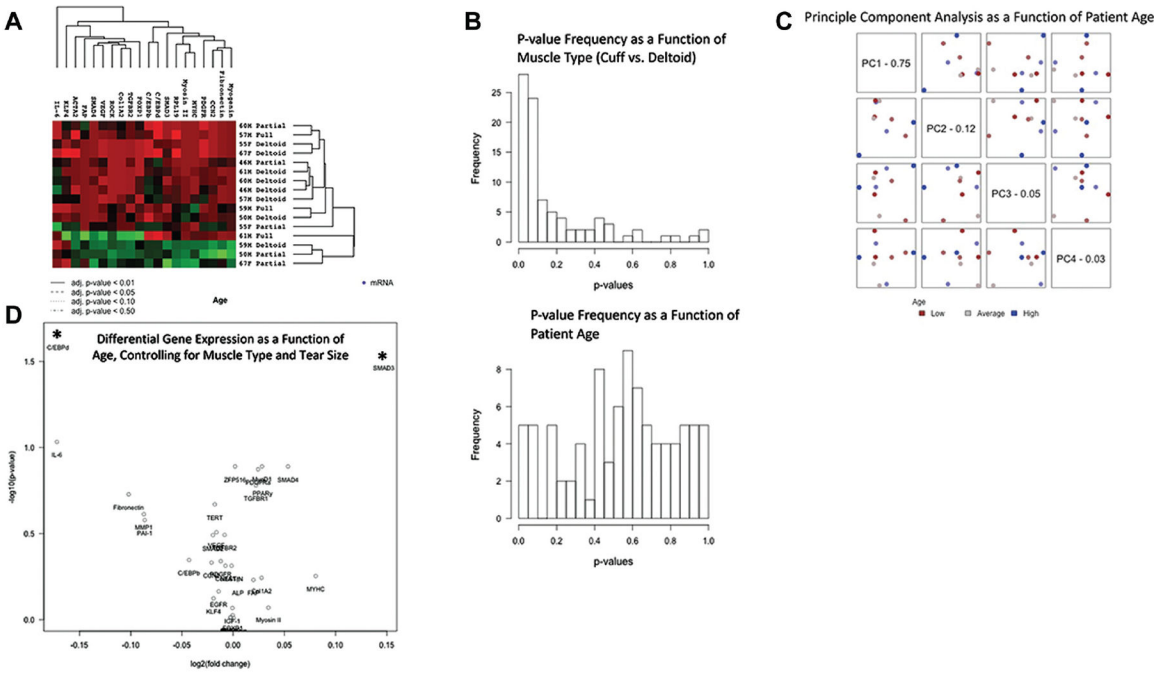
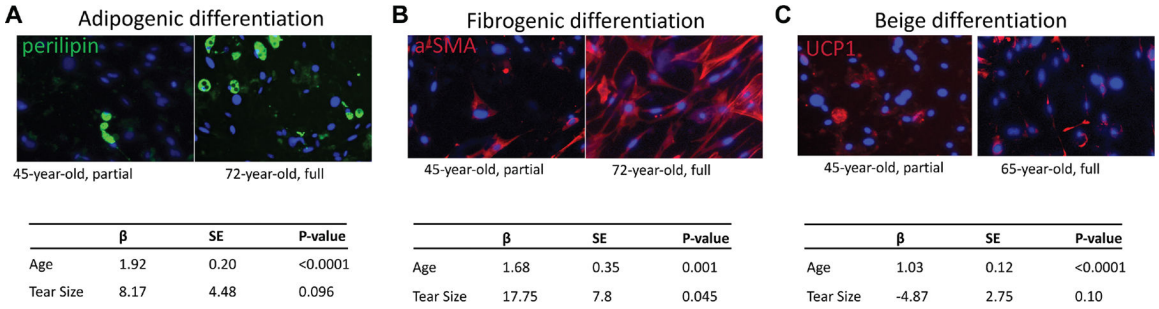


Figure 2.

NanoString nCounter gene expression analysis of freshly sorted fibroadipogenic progenitors from rotator cuff and deltoid muscle samples. (A) Heat map demonstrating transcriptome heterogeneity of rotator cuff (n = 8) and deltoid (n = 8) samples across the 20 genes with the highest average expression. (B) *P* value distribution charts demonstrating significant univariate association between muscle type (cuff vs deltoid) and overall gene expression but not with patient age. (C) Principal component (PC) analysis map of patient age as a continuous variable demonstrating weak association between age and overall gene expression. (D) Differential gene expression volcano plot of patient age as an independent variable, controlling for muscle type (cuff vs deltoid) and tear size (all partial tear samples). **P* < .05.



	β	SE	P-value
Age	1.92	0.20	<0.0001
Tear Size	8.17	4.48	0.096

	β	SE	P-value
Age	1.68	0.35	0.001
Tear Size	17.75	7.8	0.045

	β	SE	P-value
Age	1.03	0.12	<0.0001
Tear Size	-4.87	2.75	0.10

Figure 3.

Sorted fibroadipogenic progenitors (FAPs) grown under (A) adipogenic, (B) fibrogenic, (C) and beige-inducing treatment conditions. Expression indices for (A) perilipin, (B) α-SMA, and (C) UCP1 were calculated for FAPs from each patient sample as previously described. Multivariable linear regressions using age and tear size as independent variables and expression index as the dependent variable are shown for each treatment condition. UCP1, uncoupling protein 1.

TABLE 1

Characteristics of Patients[a]

Characteristic	Value
Age, y, mean (range)	59.1 (42–76)
Sex, n	
Male	19
Female	7
BMI, mean ± SD	26.3 ± 4.2
Tear size, n	
Partial	8
Small	7
Medium	3
Large	8
Time to surgery, mo, mean ± SD	26.2 ± 42.7

[a]BMI, body mass index.

TABLE 2

Effect of Patient Age and Tear Size on Fibroadipogenic Progenitor Percentage

	β Coefficient	SE	*P* Value
Age	0.21	0.087	.03
Tear size	3.86	1.8	.05

TABLE 3

Differential Gene Expression as a Function of Patient Age and Tear Size[a]

Gene Expression in Adipogenic Medium			Gene Expression in Beige-Inducing Medium		
Gene	**Age β Coefficient; *P* Value**	**Tear Size β Coefficient; *P* Value**	**Gene**	**Age β Coefficient; *P* Value**	**Tear Size β Coefficient; *P* Value**
Adiponectin	0.19; .11	−0.68; .7	Adiponectin	0.081; .4	−1.15; .53
PPARy	0.22; .17	**36.67; .001**	PPARy	0.058; .39	−1.55; .26
FABP4	**0.89; .04**	−4.7; .42	***FABP4***	**0.86; .023**	1.98; .65
CEBP	0.096; .48	**25.58; .002**	CEBP	0.1; .051	−1.49; .099
Leptin	0.04; .58	**9.24; .004**	Leptin	0.025; .53	0.75; .36
CD36	0.059; .53	**6.45; .029**	CD36	0.0018; .513	0.35; .531
UCP1	0.136; .21	**9.71; .011**	***UCP1***	**5.14; .019**	**−1921.83; <.0001**
PRDM16	**1.13; .022**	**−33.74; .0007**	***PRDM16***	**5.1; .019**	**−1930.57; <.0001**
MTUS1	0.24; .15	−2.48; .38	***MTUS1***	**4.36; .026**	**−922.28; <.0001**
EVA1	−0.41; .7	16.1; .45	***EVA1***	**4; .039**	**−1145.19; <.0001**
ZIC1	**0.45; .018**	**−5.95; .048**	ZIC1	0.11; .18	**−7.91; .007**
KCNK3	0.0057; .052	−0.083; .1	KCNK3	1.22; .54	−15.98; .13
Col1A	0.0035; .098	−0.041; .248	Col1A	0.0095; .52	0.56; .12
Col3A	0.00043; .64	0.0077; .67	Col3A	0.0056; .48	0.196; .25
ACTA2	0.012; .093	−0.25; .39	***ACTA2***	**0.0045; .022**	**0.48; <.0001**
F288	0.13; .078	−2.5; .074	***F288***	**1.89; .043**	**−216.54; <.0001**
F315	0.031; .1	**−1.46; .012**	***F315***	**4.51; .024**	**−805.85; <.0001**
IGF1	**0.05; .017**	**−1.09; .012**	***IGF1***	**1.97; .041**	**−219.25; <.0001**

[a]Bold values indicate $P < .05$.