Muscle Progenitor Cell Regenerative Capacity in the Torn Rotator Cuff

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ABSTRACT: Chronic rotator cuff (RC) tears affect a large portion of the population and result in substantial upper extremity impairment, shoulder weakness, pain, and limited range of motion. Regardless of surgical or conservative treatment, persistent atrophic muscle changes limit functional restoration and may contribute to surgical failure. We hypothesized that deficits in the skeletal muscle progenitor (SMP) cell pool could contribute to poor muscle recovery following tendon repair. Biopsies were obtained from patients undergoing arthroscopic RC surgery. The SMP population was quantified, isolated, and assayed in culture for its ability to proliferate and fuse in vitro and in vivo. The SMP population was larger in muscles from cuffs with partial tears compared with no tears or full thickness tears. However, SMPs from muscles in the partial tear group also exhibited reduced proliferative ability. Cells from all cuff states were able to fuse robustly in culture and engraft when injected into injured mouse muscle, suggesting that when given the correct signals, SMPs are capable of contributing to muscle hypertrophy and regeneration regardless of tear severity. The fact that this does not appear to happen in vivo helps focus future therapeutic targets for promoting muscle recovery following rotator cuff repairs and may help improve clinical outcomes. © 2015 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 33:421–429, 2015.

Keywords: muscle; progenitor; rotator cuff; shoulder; regeneration

Rotator cuff (RC) injuries are common causes of shoulder weakness, pain, and limited mobility in humans. It has been estimated that 30% of the population over 60 years of age has a full thickness tear of at least one rotator cuff tendon.[1] Frequently these injuries are chronic and remain untreated for years, which complicates surgical repair of the tear site. The success of surgical intervention is highly variable with re-tear rates ranging from 20 to 90% depending on tear size and chronicity.[2,3]

One reason for the large outcome range may be the maladaptation of RC musculature in response to chronic tears, including atrophy, fibrosis, and fatty degeneration.[4–6] In cases of chronic tendon tears with retraction, muscle volume may be nearly entirely replaced by fat.[6,7] Furthermore, MRI-based muscle cross-sectional areas may overestimate the more functionally relevant value of physiological cross-sectional area (PCSA). PCSA is a calculated value that estimates the total muscle cross sectional area from muscle volume, fiber length, and pennation angle. Increase in intramuscular fat and connective tissue, increased pennation angle, and sarcomere shortening from muscle retraction would all lead to reduced PCSA, but these changes would not be captured by MRI-based measurements of muscle area. This poses a significant challenge for surgical repair as the degree of atrophy and fatty degeneration is highly correlated with negative clinical outcomes and high re-tear rates.[4,7,8] Furthermore, fatty degeneration and muscle atrophy are observed to persist following repair.[4,7] This is surprising since muscular atrophy due to unloading is completely reversible in rodents and humans following limb casting.[9,10] Similar chronic ruptures to the achilles or the distal biceps tendon with muscle retraction (which remain untreated for >1 year) can be surgically repaired with good recovery of strength, muscle volume, and low re-tear rates.[11,12] This evidence suggests that there may be a number of possible defects in the recovery of rotator cuff muscles following tendon tear.

In response to sudden load increase, skeletal muscle will typically undergo rapid growth to increase fiber size and number, utilizing progenitors that surround the muscle fiber.[13,14] These skeletal muscle progenitor (SMP) or "satellite" cells respond to signals for muscle growth or repair by activating, proliferating, and fusing with each other or with existing myofibers.[15] In this way, muscle is able to adapt to loading changes for optimal use. Experimental interventions in mice have demonstrated that later stages of hypertrophy, muscle repair, and the formation of nascent muscle fibers are all severely blunted if SMPs are unable to proliferate or fuse.[13,14,16] Thus, we hypothesized that poor rotator cuff muscle recovery following restoration of functional loading could be due to SMP population defects.

To test this hypothesis, muscle biopsies were obtained from patients undergoing arthroscopic rotator cuff surgery and the population of skeletal muscle progenitor cells was quantified and isolated. Interestingly, both the size and the proliferation rates of these populations varied as a function of cuff tear state. However, the ability to fuse and contribute to new myotubes in vitro and myofibers in vivo was unchanged. These findings provide further insight into a

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cellular pathology that may underlie muscle changes seen following tears to the rotator cuff and suggest SMPs as a therapeutic target.

METHODS

Muscle biopsies were obtained from the distal third of the supraspinatus (SS) and infraspinatus (IS) muscles from 17 patients undergoing arthroscopic RC surgery (Table 1). Biopsies of the deep surface of the deltoid muscle were also obtained in a subset of 10 of these patients as a non-rotator cuff (NRC) control. RC muscles were classified as having no tear (NT), a partial thickness tear (PT), or a full thickness tear (FT) by the operating surgeon. Patients classified as PT had torn one or more tendons partially but not completely through the sagittal plane of the tendon. Conversely, patients classified as FT had completely torn through the sagittal plane of the tendon but do not have muscle retraction. The study was approved by UC San Diego's IRB and signed written consent obtained from all participants. See Supplemental Methods for a detailed description of all techniques used.

Cell Isolation and Quantification

Muscle biopsies of approximately 15–25 mg were obtained using an arthroscopic rongeur. Tissue was digested and cells isolated as described in the Supplemental Methods.

Myogenic Hydrogel Preparation and Chemical Induction

Polyacrylamide matrices of muscle-mimicking stiffness were prepared as described in the Supplemental Methods. To chemically induce differentiation, cells were exposed to a myogenic induction media. Cultures were allowed to differentiate for 4 days, then cells were either prepared for RNA isolation or immunostaining.

Cell Proliferation

Cell proliferation was measured using a Click-iT EdU assay (Life Technologies, Carlsbad, CA) with an 18 h pulse. EdU positive nuclei were identified using a detection algorithm designed in Matlab, and the percentage of dividing cells was calculated as EdU positive nuclei divided by total nuclei.

Quantification of Myogenic Gene Expression

RNA was isolated from confluent cultures using Trizol reagent (Life Technologies, Carlsbad, CA) according to the manufacturer's protocol. cDNA was generated and gene expression quantified as described in the Supplemental Methods.

Immunostaining

Fixed and permeabilized cultures were stained with anti-Myosin Heavy Chain and counterstained with DAPI. Nuclei inside myosin heavy chain positive myotubes were counted using a custom algorithm designed in Matlab.

Table 1. Patient Demographic Information

Patient Number	Demarcation	Class	Sex	Age	BMI	SS Class	IS Class	Notes
21	square	NT	M	39	45.8	0	0	Bursitis
22	diamond	NT	M	42	24.4	0	0	Prior RCR with adhesions and immobile arthrofibrosis
34	down triangle	NT	M	38	28.9	0	0	Small labra tear (no repair needed)
37	up trianlge	NT	F	54	26.8	0	0	Impingement with bursitis and biceps tenosynovitis
62	star	NT	M	56	28.5	0	0	Biceps rupture, tenodesis (meds: Lipitor)
63	circle	NT	F	53	23.9	0	0	Adhesions
27	circle	PT	M	59	33.0	1	0	Partial thickness SS tear with adhesions, Smoker
32	square	PT	F	54	26.6	1	0	Partial thickness SS tear, type I labral tear
57	up triangle	PT	M	57	27.4	1	0	Partial thickness SS tear, bursitis, Smoker
82	down triangle	PT	M	60	31.6	1	0	Partial thickness SS tear, bursitis, Hypertension
86	diamond	PT	M	56	31.0	1	0	Partial thickness SS tear, intact IS (meds: Omeprazole, Tramado)
33	circle	FT	F	62	34.7	2	2	2 cm full thickness SS and IS tears with some retraction, (meds: sulfasalazine and steroids)
47	diamond	FT	F	57	24.6	2	0	Large full thickness SS tear with retraction to humeral head
64	star	FT	M	43	29.8	2	0	Full thickness SS 2 cm tear, superior labral tear
68	up triangle	FT	M	53	38.0	2	2	Full thickness bursal sided tear of SS and IS with retraction, bursitis, labral tear, (meds: Lipitor)
80	down triangle	FT	M	62	22.8	2	2	Full thickness SS and IS tears, partial subscapularis tear, bursitis
83	square	FT	F	59	23.0	2	1	8 mm full thickness SS tear, partial IS tears, subscapularis fraying, (meds: hormone replacement)

The symbol used for each patient in the Figures is listed in the Demarcation column (square ■, diamond , down triangle ▼, up triangle ▲, star ★, circle ●). The classification used for each patient is listed in the column labeled Class (NT no tear, PT partial thickness tear, FT full thickness tear). The sex (M male, F female), age at surgery (years), and body mass index (BMI m2/kg) are also provided in similarly labeled columns. SS (supraspinatus) and IS (infraspinatus) tears are classified in SS Class and IS Class columns (0 = no tear, 1 = partial thickness tear, 2 = full thickness tear). Finally, surgical notes on the health and shoulder status of each patient are provided in the column labeled Notes.

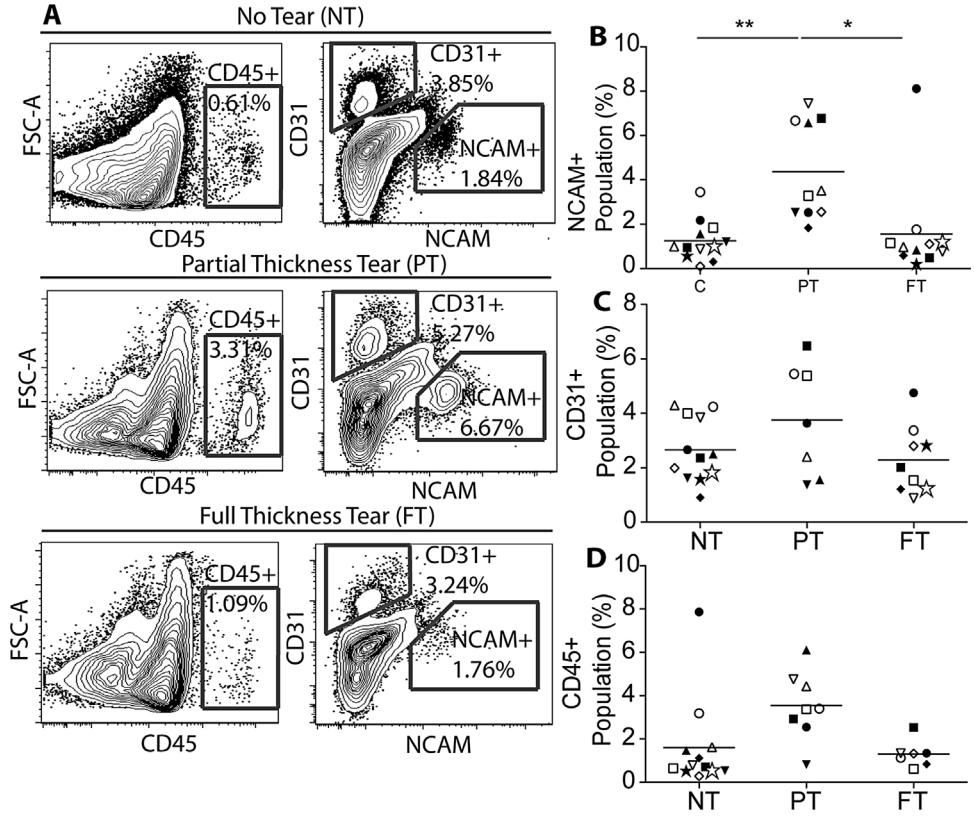

Figure 1. Quantification of three cell populations in rotator cuff biopsies. (A) Representative flow cytometry contour plots of isolated cells labeled with three population specific fluorescent antibodies: NCAM (skeletal muscle progenitor cells), CD31 (endothelial cells), and CD45 (inflammatory cells). (B) Quantification of the percentage of the NCAM+ (skeletal muscle progenitor) cell population relative to all single cells. (C) Quantification of the percentage of CD31+ (endothelial) cells relative to all single cells. (D) Quantification of the CD45+ (hematopoietic) cells relative to all single cells. Quantification is based on the gray gates shown in panel A and in Supplementary Figure 1 A. Within each category, individual patient data are indicated by different symbols. Closed symbols indicate supraspinatus data and open symbols indicate infraspinatus data. $^{**}p < 0.01$, $^{*}p < 0.05$.

Cell Transplantation

Tibialis anterior (TA) muscles of $Rag1^{tm/Mom}$ were injected bilaterally with 10 μL notexin to induce muscle degeneration. 24 h following notexin injection, either 100,000 SMPs, suspended in saline, or saline alone was injected into individual TA muscles. All procedures were performed in accordance with NIH's Guide for the Use and Care of Laboratory Animals and approved by UC San Diego.

Histology

One week following injection, mice were euthanized and TA muscles were dissected bilaterally. Muscles were flash frozen in liquid nitrogen cooled isopentane, embedded in OCT and 10 μm sections were cut through the cross section of the muscle midbelly. Sections were stained with anti-human lamin A and anti-laminin b2 and co-stained with DAPI.

Statistical Analysis

For comparisons across tear states, a one-way ANOVA with repeated measures was performed with a significance level (α) set at 0.05. Where appropriate, a multiple corrections Bonferroni post-hoc test was done. Significance levels were also assessed by Mann-Withney tests as indicated. Whenever possible, data were averaged from experimental technical duplicates across passages 3 and 4. Results in the text are presented as mean ± standard error.

RESULTS

Seventeen patients were recruited; six patients with no tear (NT), five with a partial thickness (PT) tear, and six with a full thickness (FT) tear of at least one tendon, respectively (Table 1). All three groups contained a mixture of male and female patients with no significant difference in age (NT: 47.0 ± 3.3 years, PT: 57.2 ± 1.1 years, FT: 56.0 ± 3.2 years) or body mass index (NT: 29.7 ± 3.3 m^2/kg, PT: 29.9 ± 1.2 m^2/kg, FT: 28.8 ± 2.9 m^2/kg) between groups as assessed by non-parametric analysis. All patients in the PT group had a partial tear to the supraspinatus tendon only while patients in the FT group had either a full thickness tear to the supraspinatus tendon only or to both the supraspinatus and infraspinatus tendons.

RC Tear State Influences Cell Population Dynamics

SMP cells were identified by their unique expression of the surface protein NCAM[17] and lack of expression of the hematopoietic marker CD45 and endothelial marker CD31. Contour plots of fluorescence intensity show a distinct population of higher fluorescence intensity on the NCAM axis in biopsies from all tears states (Fig. 1A), but that population was a significantly larger

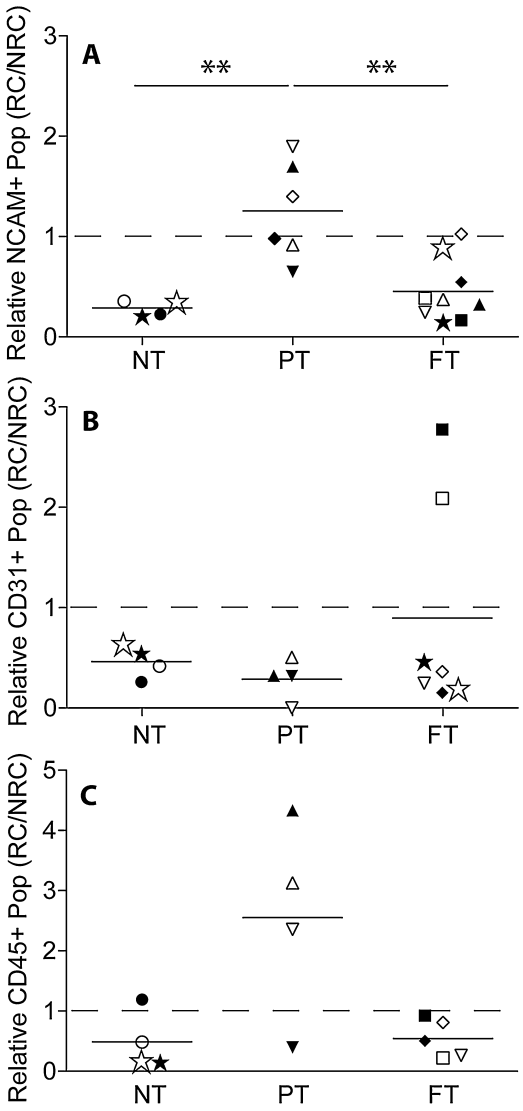

Figure 2. Ratiometric comparison of patient-matched cell populations. Ratio of patient-matched rotator cuff (RC) to non-rotator cuff (NRC) muscles for the NCAM+ (A), CD31+ (B), or CD45+ (C) populations in the no tear (NT), partial thickness (PT) tear, and full thickness (FT) tear groups. $^{**}p < 0.01$.

percentage of cells in PT biopsies compared with NT or FT groups (Fig. 1B). Interestingly, NCAM populations in both the supraspinatus (Fig. 1B, closed symbols) and infraspinatus (Fig. 1B, open symbols) biopsies were elevated in this group despite the partial tear occurring in the supraspinatus tendon; these data suggest that local tears may induce remodeling in neighboring muscles. Endothelial cells and inflammatory cells, indicated by expression of CD31 and CD45, respectively (Fig. 1A), showed no significant differences in expression as a function of tear state (Fig. 1C and D). There were no significant correlations between any of the cell populations and the patient age or BMI.

To control for quantification differences driven by other patient-specific, non-cuff state related variables, we compared rotator cuff (RC) quantification to a NRC, patient-matched control biopsy of the deltoid muscle. Even when normalized to NRC samples, biopsies from PT cuffs had elevated NCAM positive populations compared to the NT and FT groups (Fig. 2A). To ensure the accuracy of the NCAM positive population identification and persistence of the population expression profile over multiple passages, sorted and cultured SMPs were re-evaluated for NCAM expression. Cultures from each RC state were >95% positive for NCAM, indicating a highly pure population of SMPs (Supplementary Fig. 1). These data suggest that the NCAM positive SMP population expands in response to a partial but not full tendon tear.

SMPs From Cuffs With Partial Thickness Tears Have Impaired Proliferation

Upon injury, SMPs must expand in vivo.[18] To assess their proliferative capacity in vitro, SMPs were grown on muscle stiffness-mimicking hydrogels and exposed to a division-marking label (EdU). Fewer SMPs from PT biopsies divided in the experimental time-frame, as indicated by the deficit of red EdU+ nuclei in the partial tear compared to the NT cultures (Fig. 3A). EdU signal quantification showed a nearly two-fold reduction in the percentage of EdU positive cells in the PT cultures compared with the NT cultures. SMPs from FT cultures also exhibited slower proliferation, albeit not as reduced as from PT patients (Fig. 3B). Interestingly, when these values were normalized to the patient-matched NRC cultures, there were no significant differences in the relative EdU positive fraction between groups (Fig. 3C). This implies that there is a deficit in proliferation in the NRC muscles from PT cuffs as well.

SMPs are Able to Fuse Robustly in Culture Regardless of Cuff State

SMPs must also be able to robustly fuse in vivo as a precursor to mature muscle fiber development.[19,20] Myotube formation can be identified in SMP cultures by expression and striated organization of the muscle specific isoform of myosin heavy chain (MHC). Following fusion induction, many myotubes containing a large number of nuclei and striated MHC were visible in SMP cultures from each tear state (Fig. 4A, green). Quantification of the percentage of the culture that participated in fusion events, i.e., the percentage of total nuclei contained within MHC positive structures, was not significantly different between groups (Fig. 4B). Additionally, overall MHC expression was not significantly different between cultures from the different cuff states (Fig. 4C). When these data were normalized to the patient-matched NRC cultures, there was still no difference detected between groups (Fig. 4D and E). Similarly, expression of two earlier stage indicators of myogenesis, MyoD and MEF2C, was unchanged between groups (Supplementary Fig. 2). These data suggest that despite proliferative differences, cells are equally capable of contributing to myotubes independent of cuff state.

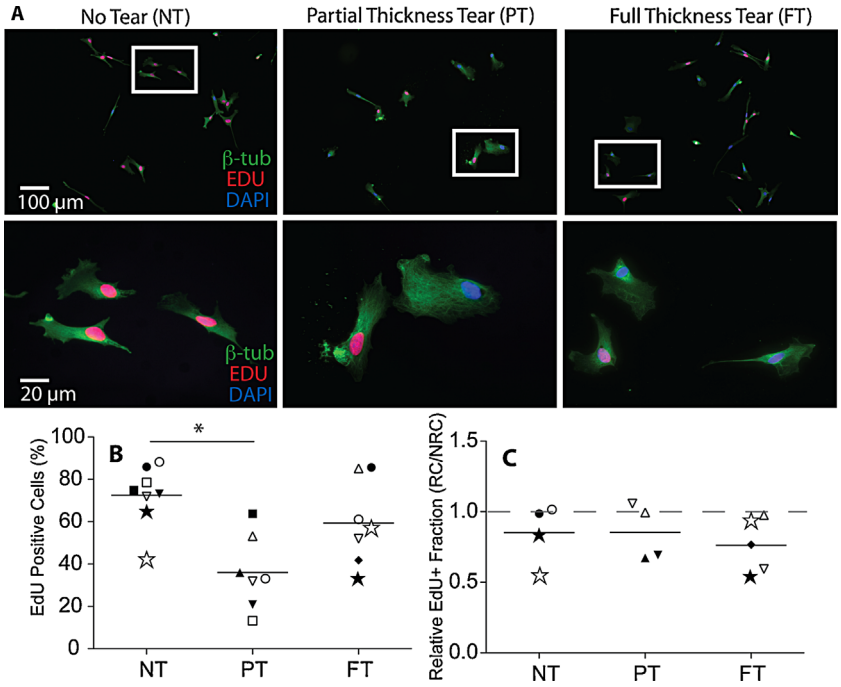

Figure 3. Proliferation rates of skeletal muscle progenitor cell populations, quantified using an EdU assay. (A) Immunofluorescence images of EdU pulsed cultures counter-stained with DAPI (blue) and β tubulin (green). Cells that have divided during the 18 h pulse can be identified by the red EdU tag incorporated into the nucleus. (B) Quantification of the percentage of EdU positive cells as a function of tear state. (C) Quantification of the percentage of EdU positive populations from rotator cuff (RC) muscles normalized to a patient-matched non-rotator cuff (NRC) Deltoid control. $^{*}p < 0.05$.

SMPs From All Cuff States Are Able to Fuse with Regenerating Fibers In Vivo

While SMPs appear to have equal fusion capacity in vitro regardless of cuff state, their ability to fuse in vivo with host muscle is unclear. SMPs from each cuff state were injected into notexin injured mouse *tibialis anterior* muscles to determine if they were capable of participating in host regeneration. Engrafted human cells derived from each cuff state were identified histologically by positive staining for a human-specific isoform of Lamin A (red), a nuclear protein, which co-localized with DAPI (blue) in the center of a laminin (green) outlined fiber (Fig. 5). Examples are marked with white boxes and shown at higher magnification in the right column of Fig. 5. These data show that SMPs are capable of differentiation and fusion in an in vivo model of regeneration as well as in vitro independent of cuff state.

DISCUSSION

Chronic rotator cuff tears are frequently characterized by persistent muscle atrophy and fatty degeneration,[4–6] which is thought to contribute to poor clinical outcomes.[4,8] Much of this failure has been attributed to the inability of RC muscle to properly recover in response to restored functional loading.[4,7] Muscle regeneration and hypertrophy should normally rely on SMP cells to add fiber volume and sarcomeres, but limited RC muscle recovery following repair suggests a potential defect with SMPs. We thus hypothesized that the insensitivity of these muscles to growth cues, e.g., increased loading and fiber damage,[21] could be due to negative and persistent changes to the progenitor cells following chronic RC tears. This alteration could arise from a depletion of SMPs, a defect in their ability to proliferate or differentiate in response to stimuli or a combination thereof.

Impaired SMP Population Size and Proliferation But Not Fusion May Contribute to Poor RC Repair Outcomes

First, if chronic RC tears result in a depletion of the muscle progenitor pool, the reduced SMP population could be unable to meet the muscle's recovery needs. Animal studies have shown that disease, unloading, and aging all decrease the progenitor cell population and negatively effect the ability of the muscle to regenerate and hypertrophy,[22,23] For RC muscle, in addition to atrophying, chronically retracted muscles subtract sarcomeres.[24] During surgical repair, the shortened muscle is stretched to its original length, potentially forcing sarcomeres to operate at distances and velocities that exceed normal function and potentially inducing muscle injury.[21] The sudden increase in loading coupled with the increased potential for injury following repair, suggests that RC muscles may need to activate their resident SMPs.

Contrary to clinical outcomes that suggest a potential problem with this cell population, we found no decrease in the SMP population from the muscles of the fully torn rotator cuff compared with cuffs without tears, which suggests that SMP population size may not be the limiting factor. In line with the recent

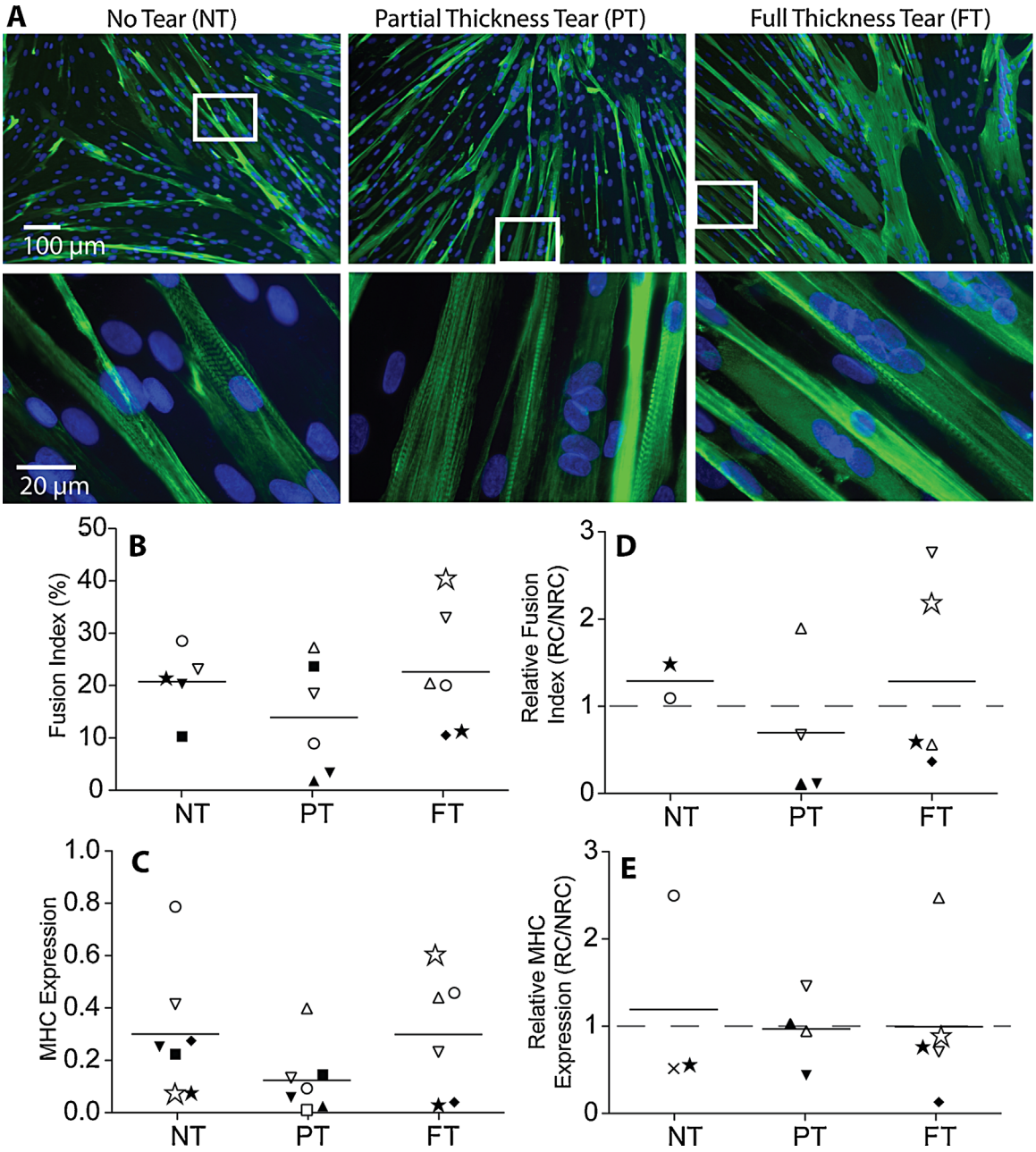

Figure 4. Quantification of fusion rates in skeletal muscle progenitor cell populations as a function of tear state. (A) Immunofluorescence images of myotubes formed from the fusion of SMPs identified by myosin heavy chain (MHC, green). Striations of MHC are visible in higher magnification images. (B) Quantification of the fusion index (% nuclei in myotubes) as a function of tear state. (C) MHC gene expression quantified by qPCR as a function of tear state. (D) Fusion index from rotator cuff (RC) muscles normalized to a patient-matched non-rotator cuff (NRC) Deltoid control. (E) MHC expression from rotator cuff (RC) muscles normalized to a patient-matched non-rotator cuff (NRC) deltoid control.

histological findings of Lundgreen et al., we found an elevated SMP population in muscles from cuffs with partial thickness tears,[25] compared with full thickness tears. The increase in SMP population in PT tears may be a consequence of activation and proliferation in response to dynamic changes in loading at the fiber level, though other factors such as inflammation and altered kinematics cannot be excluded and should be investigated in future studies. However, rotator cuff biopsies generally had a lower percentage of SMPs than the patient matched non-rotator cuff deltoid control or other human muscles with SMP fractions between 9 and 11%.[17,26] Thus, it is possible that rotator cuff muscles simply have an inherently smaller number of progenitor cells and an associated lower regenerative potential regardless of tear state.

Secondly, regeneration could be blunted by defective SMP proliferation. Animal studies have demonstrated that muscle injury, inflammation, and atrophy negatively effect progenitor cells' ability to prolifer-

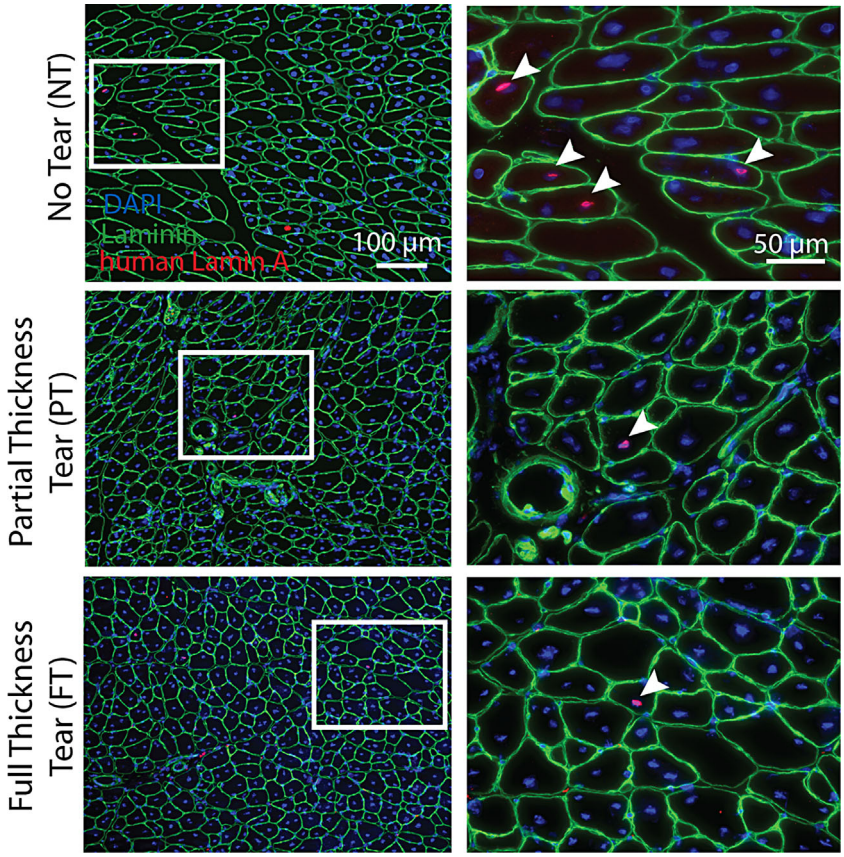

Figure 5. Engraftment of rotator cuff muscle progenitor cells into regenerating mouse muscle. Images in the left-hand column show histological sections based on tear state. Nuclei derived from human SMPs are indentified by a human-specific lamin A antibody (red). Counterstaining with laminin (green) and DAPI (blue) demonstrate human nuclei centrally located in muscle fibers indicating that they were incorporated during regeneration (arrows). White box (left) indicates where images were magnified in right-hand column.

ate,[10,18,27] which along with reduced cell activation lead to poor muscle regeneration.[16] We found significant reduction in SMP proliferation from partially but not from fully torn cuffs relative to untorn cuffs. Tear-dependent differences between expansion in vivo and in vitro suggest that the altered loading among other changes in the SMP niche in PT tears may precondition cells to respond to mechanically active environments; conversely, the niche change for untorn RCs and FT tears must similarly activate signals within SMPs that regulate the cell cycle in vitro when passive but not active loading is present. Overall these data suggest that although there are more SMPs in the PT condition, they may be less able to activate, proliferate, and meet the regenerative need. However, when normalized to a non-rotator cuff control, this effect disappeared indicating that deltoid SMP proliferation was similarly reduced in partial thickness tears. It has been suggested that tears to one rotator cuff tendon more globally alter loading and affect adjacent muscles[28]; our data appear consistent with this suggestion.

Finally, impaired muscle regeneration could result from poor SMPs differentiation and fusion into new or hypertrophying muscle fibers. While there is not yet consensus about whether progenitor cells are required for muscle hypertrophy, there is general agreement that SMPs are necessary for injury repair and the formation of new fibers.[13] We found that cells from all cuff states were able to differentiate and fuse in culture and could contribute to muscle regeneration in a mouse injury model in vivo. Though this model is limited in representing a torn RC, i.e., chemically induced injury vs. mechanical reloading, it does show that RC SMPs are capable of fusing into fibers should they get the appropriate signals.

It is worth noting that SMPs are not the only muscle-resident cell population potentially contributing to muscle hypertrophy and repair, including PW1+ interstitial cells, muscle-derived stem cells (MDSCs), mesangioblasts, and pericytes.[15,29] Though SMPs are required for muscle regeneration, these other populations could supplement the response when SMPs are unable to fully meet the regenerative need. Future studies should investigate these population dynamics as well.

These experiments have several important limitations that warrant discussion. First, although patients in the NT group did not have a cuff tear, they did have another RC pathology (bursitis, tendonitis etc.). This means that their cuff muscles likely had confounding factors such as inflammation and decreased use,

limiting their power as true "controls." Future studies might use biopsies from patients with instability, though loading might be altered in this cohort as well. Second, though there was no difference in age between groups in this study, the patient population as a whole was relatively older. As the SMP population has been shown to decline in both numbers and proliferative capacity with increasing age,[30,31] muscles from all cuff states could have impaired regenerative capacity at this age. Finally, biopsy size was a limiting factor in this study as relatively few SMPs (on the order of 1,000) could be isolated per patient requiring significant expansion in culture and limiting the number of experiments that could be run. Future studies will benefit from elimination of confounding factors known to influence SMP function such as age and gender,[31,32] larger biopsies, NRC controls distant from the shoulder, and a NT group without shoulder dysfunction.

Our data suggest that rehabilitating RC muscles will likely require the (re) activation of SMPs, and to date, our poor understanding of how SMPs are affected during chronic tears has created a critical knowledge gap. This study suggests that poor clinical outcomes in full thickness RC repairs are potentially due to the microenvironment rather than SMP's ability to differentiate or participate in fusion; in the absence of promyogenic cues, this niche could influence SMPs (or other vessel derived myogenic populations) to contribute to the disease state through transdifferentiation into myofibroblasts.[33] Yet these data at least suggest that in addition to surgical repair, activation strategies (*i.e.,* pharmacological or mechanical) for SMPs to push them into the regenerative cycle may be required so they can remodel and strengthen the muscle after tendon repair.

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REFERENCES

1. Tempelhof S,Rupp S, Seil R. 1999. Age-related prevalence of rotator cuff tears in asymptomatic shoulders. J Shoulder Elbow Surg 8:296–299.
2. Galatz LM, Ball CM, Teefey SA, et al. 2004. The outcome and repair integrity of completely arthroscopically repaired large and massive rotator cuff tears. J Bone Joint Surg Am 86-A:219–224.
3. Bishop J, Klepps S, Lo IK, et al. 2006. Cuff integrity after arthroscopic versus open rotator cuff repair: a prospective study. J Shoulder Elbow Surg 15:290–299.
4. Gerber C, Schneeberger AG, Hoppeler H, et al. 2007. Correlation of atrophy and fatty infiltration on strength and integrity of rotator cuff repairs: a study in thirteen patients. J Shoulder Elbow Surg 16:691–696.
5. Fuchs B, Weishaupt D, Zanetti M, et al. 1999. Fatty degeneration of the muscles of the rotator cuff: assessment by computed tomography versus magnetic resonance imaging. J Shoulder Elbow Surg 8:599–605.
6. Goutallier D, Postel JM, Bernageau J, et al. 1994. Fatty muscle degeneration in cuff ruptures. Pre- and postoperative evaluation by CT scan. Clin Orthop Relat Res 304: 78–83.
7. Gladstone JN, Bishop JY, Lo IKY, et al. 2007. 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 35:719–728.
8. Harryman DT, Mack LA, Wang KY, et al. 1991. Repairs of the rotator cuff. Correlation of functional results with integrity of the cuff. J Bone Joint Surg Am 73:982–989.
9. Jones SW, Hill RJ, Krasney PA, et al. 2004. Disuse atrophy and exercise rehabilitation in humans profoundly affects the expression of genes associated with the regulation of skeletal muscle mass. FASEB J. 18:1025–1027.
10. Mitchell PO, Pavlath GK. 2004. Skeletal muscle atrophy leads to loss and dysfunction of muscle precursor cells. Am J Physiol Cell Physiol 287:C1753–C1762.
11. Wapner KL, Pavlock GS, Hecht PJ, et al. 1993. Repair of chronic Achilles tendon rupture with flexor hallucis longus tendon transfer. Foot Ankle 14:443–449.
12. Sanchez-Sotelo J, Morrey BF, Adams RA, et al. 2002. Reconstruction of chronic ruptures of the distal biceps tendon with use of an achilles tendon allograft. J Bone Joint Surg Am 84-A:999–1005.
13. Fry CS, Lee JD, Jackson JR, et al. 2014. Regulation of the muscle fiber microenvironment by activated satellite cells during hypertrophy. FASEB J 28:1654–1665.
14. Salleo A, La Spada G, Falzea G, et al. 1983. Response of satellite cells and muscle fibers to long-term compensatory hypertrophy. J Submicrosc Cytol 15:929–940.
15. Yin H, Price F, Rudnicki MA. 2013. Satellite cells and the muscle stem cell niche. Physiol Rev 93:23–67.
16. Ruffell D, Mourkioti F, Gambardella A, et al. 2009. A CREBC/EBPbeta cascade induces M2 macrophage-specific gene expression and promotes muscle injury repair. Proc Natl Acad Sci USA 106:17475–17480.
17. Dellavalle A, Sampaolesi M, Tonlorenzi R, et al. 2007. Pericytes of human skeletal muscle are myogenic precursors distinct from satellite cells. Nat Cell Biol 9:255–267.
18. Schultz E, Jaryszak DL, Valliere CR. 1985. Response of satellite cells to focal skeletal muscle injury. Muscle Nerve 8:217–222.
19. Moss FP, Leblond CP. 1971. Satellite cells as the source of nuclei in muscles of growing rats. Anat Rec 170:421–435.
20. Lepper C, Partridge TA, Fan CM. 2011. An absolute requirement for Pax7-positive satellite cells in acute injury-induced skeletal muscle regeneration. Development 138:3639–3646.
21. Ward SRS, Sarver JJJ, Eng CMC, et al. 2010. Plasticity of muscle architecture after supraspinatus tears. J Orthop Sports Phys Ther 40:729–735.
22. Jejurikar SS, Kuzon WM. 2003. Satellite cell depletion in degenerative skeletal muscle. Apoptosis 8:573–578.
23. Mozdziak PE, Pulvermacher PM, Schultz E. 2000. Unloading of juvenile muscle results in a reduced muscle size 9 wk after reloading. J Appl Physiol 88:158–164.
24. Tomioka T, Minagawa H, Kijima H, et al. 2009. Sarcomere length of torn rotator cuff muscle. J Shoulder Elbow Surg 18:955–959.
25. Lundgreen K, Lian OB, Engebretsen L, et al. 2013. Lower muscle regenerative potential in full-thickness supraspinatus tears compared to partial-thickness tears. Acta Orthop 84:565–570.

26. Smith LR, Chambers HG, Lieber RL. 2013. Reduced satellite cell population may lead to contractures in children with cerebral palsy. Dev Med Child Neurol 55:264–270.
27. Langen RC, Schols AM, Kelders MC, et al. 2001. Inflammatory cytokines inhibit myogenic differentiation through activation of nuclear factor-kappaB. FASEB J 15:1169–1180.
28. Cheung S, Dillon E, Tham SC, et al. 2011. The presence of fatty infiltration in the infraspinatus: its relation with the condition of the supraspinatus tendon. Arthroscopy 27:8–8.
29. Chen CW, Corselli M, Péault B, et al. 2012. Human blood-vessel-derived stem cells for tissue repair and regeneration. J Biomed Biotechnol 2012:597439.
30. Renault V, Thornell LE, Eriksson PO, et al. 2002. Regenerative potential of human skeletal muscle during aging. Aging Cell 1:132–139.
31. Corbu A, Scaramozza A, Badiali-DeGiorgi L, et al. 2010. Satellite cell characterization from aging human muscle. Neurol Res 32:63–72.
32. Deasy BM, Schugar RC, Huard J. 2008. Sex differences in muscle-derived stem cells and skeletal muscle. Crit Rev Eukaryot Gene Expr 18:173–188.
33. Li Y, Huard J. 2002. Differentiation of muscle-derived cells into myofibroblasts in injured skeletal muscle. Am J Pathol 161:895–907.

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