Defining the Role of Fibro-Adipogenic Progenitor Cells in Fibrosis and Fatty Infiltration of Muscle After Rotator Cuff Tears

Anne Y. Ning, B.A.¹, Bharat Ravishankar, B.A.¹, Mengyao Liu², Hubert Kim¹², Xuhui Liu¹², Brian T. Feeley, MD¹².
¹UCSF, San Francisco, CA, USA, ²San Francisco VA Medical Center, San Francisco, CA, USA.


Introduction: Rotator cuff (RC) tears are the most common shoulder injury among orthopedic patients. While small tears are amenable to surgical repair, large and massive tears are associated with irreversible muscle atrophy, fibrosis, and fatty infiltration (FI). Muscle atrophy and fatty infiltration have been identified as independent factors responsible for failure of attempted rotator cuff repairs. However, to date, the origin of adipocytes and fibroblasts in RC muscles after tendon tears remains unknown. Thus, there is currently no effective treatment for fibrosis and fatty infiltration after RC tears. Recent studies have shown that a newly discovered population of fibro-adipogenic progenitor (FAP) cells, identifiable by the marker PDGFRα, can differentiate into both fat and fibrotic tissue (1). These cells are also believed to stimulate myogenic activity for muscle regeneration after damage. However, excessive proliferation and fibro- and adipogenesis of FAP cells causes muscle fibrosis and fatty infiltration after muscle injury. To date, the role of FAP cells in rotator cuff muscle pathology remains undefined. In this study, we investigated the fate of FAP cells in rotator cuff muscles after massive tendon tears using PDGFRα-GFP reporter mice. Our results suggest that FAP cells are the main source of adipocytes and a major source of fibroblasts seen in rotator cuff muscles after massive tendon tears.

Methods: Surgical Procedure: Adult female Sprague-Dawley rats at 200-250g were divided into two groups. Both were subjected to a unilateral transection of the infraspinatus and supraspinatus tendons along with a 5 mm resection of the suprascapular nerve (2). No surgery was performed on the contralateral side to serve as an internal control. The same procedure was repeated on a separate group of female PDGFRα-GFP reporter mice. All procedures were approved by the San Francisco Veterans Affairs Medical Center (SFVAMC) Institutional Animal Care and Use Committee (IACUC). Rats were sacrificed 2 and 6 weeks post-operatively and reporter mice were sacrificed at 6 weeks.

Histology: Rat supraspinatus muscles from both control and surgical sides were harvested and flash frozen in liquid nitrogen-cooled methylbutane for histology analysis. Muscle samples were cryosectioned at a thickness of 10 µm, then fixed in 4% paraformaldehyde. Only the bellies of the muscles were used for analysis. A double immunohistochemistry (IHC) assay was conducted using antibodies for PDGFRα (1:10 dilution) and either PPARγ (1:400), adiponectin (1:100), vimentin (1:300), or αSMA (1:80). Alexa-Fluor® conjugated secondary antibodies were used at a dilution of 1:5000. Sections were mounted on slides using VectaShield with DAPI and visualized using AxioVision software. Supraspinatus and infraspinatus muscles from the PDGFRα-GFP reporter mice were harvested and probed according to the same protocol. Oil-Red-O staining was performed as previously described (2).

Results: Effects of the RC tear were analyzed in rat models at both two week and six week timepoints. Analysis of muscle histology demonstrated an increase in the FAP cell population as indicated by the
PDGFRα marker across samples compared to controls at all timepoints after surgery compared to their respective controls (Figure 1). IHC also showed increases in PPARγ, adiponectin, vimentin, and αSMA staining in surgery groups compared to controls. In vivo expression of endogenous PDGFRα was also seen to overlap with adipocytes found in muscles six weeks post-operation, suggesting transformation of the FAP cells into adipocytes (Figure 2). Furthermore, in vivo cell tracing of PDGFRα also shows that FAP cells are the main source for adipocyte formation in muscle and a major source for fibrosis in rotator cuff muscles after massive tendon tears (Figure 3).

**Discussion:** There are many muscle resident progenitor cell populations that have the potential to contribute to fibrogenesis and adipogenesis. In the past, we have examined the fate of Tie-2(+) endothelial lineage cells in RC muscle atrophy and FI. Our results from Tie-2-GFP reporter mice showed that endothelial lineage cells do not differentiate into adipocytes in fatty infiltration of RC muscles (3). FAP cells are an appealing cellular source for the detrimental changes that occur in muscle after RC tears. In this study, we determined that FAP cells differentiate into fat and fibrotic cells after massive RCT injury in both rat and mouse models. Cell tracing with PDGFRα-GFP reporter mice showed that FAP cells are the main source for adipocyte formation in muscle and a major source for fibrosis in RC muscles after massive tendon tears. Future directions involve using flow cytometry to isolate the FAP cell population from RC muscles (4). Analysis of their genetic and epigenetic profiles will allow us to identify key pathways that may lead the way to development of new treatments for RC fibrosis and FI through the inhibition of FAP cell proliferation and differentiation.

**Significance:** The study shows the role of the newly discovered FAP cell population in FI and fibrosis of muscle after RC tears. Understanding the regulation of these cells after RC injury will allow for treatments to better functional outcomes after surgical repair of RC injuries.
Figure 1. Double immunohistochemistry. Sections of rat supraspinatus muscles at 2 and 6 weeks post-op were stained for PDGFRα in the FITC channel and (a) PPARγ; (b) adiponectin; (c) vimentin; and (d) αSMA in the rhodamine channel. Images taken at 20x.

Figure 2. In vivo cell tracing. Sections of PDGFRα-GFP reporter mouse infraspinatus muscles 6 weeks post-operatively were analyzed using (a) Oil Red O stain, (b) expression of endogenous PDGFRα-GFP, and (c) merging images from (a) and (b). Images taken at 40x.
Figure 3. *In vivo* cell tracing. Sections of reporter mouse supraspinatus muscles 6 weeks post-operation showing (a) endogenous PDGFRα-GFP, (b) adiponectin, (c) αSMA, and (d) nuclei stained with DAPI, 40x. (e) Merged image; from left to right, arrows point to FAP cells differentiated into (1) fibrocytes and (2) adipocytes.