Epimuscular Fat Infiltration and Muscle Residing PDGFRβ^{+} Progenitors Contribute to Fatty Degeneration Following Massive Rotator Cuff Tears in a Mouse Model

Madhavan Anbuchelvan[1], Angela Li[1], Maya S. Abu-Zahra[1], Amir Fathi[1], *Frank A. Petrigliano[1], *Ayelet Dar[1]
[1]University of Southern California, Los Angeles, CA, USA
Email of Presenting Author: anbuchel@usc.edu
*Co-senior authors

Disclosures: Madhavan Anbuchelvan (N), Angela Li (N), Maya S. Abu-Zahra (N), Amir Fathi (N), Frank A. Petrigliano (N), Ayelet Dar (N).

INTRODUCTION: Massive rotator cuff tears (RCT) are often associated with progressive muscle degeneration alongside muscle atrophy, scar formation, and adipose tissue accumulation. However, the source of pathological muscle adipose tissue is still not fully defined. While previous studies demonstrate that intracellular fat originates from muscle-residing PDGFRb^{+}PDGFRa^{+} fibro-adipogenic progenitor cells[1], little is known about the role of epimuscular fat in fatty degeneration of the RC. Therefore, we conducted studies aimed to define the source of degenerative adipose tissue in a chronically injured RC using a mouse model of massive RCTs.

METHODS: We performed tendon transection and denervation (TTDN) of mouse RC. TTDN-operated C57BL/6 mice were transplanted with an epimuscular fat graft (FG) derived from transgenic PDGFRβ-Cre x mTmG mice immediately post TTDN; FGs were placed on top of the injured supraspinatus and infraspinatus and engrafted RCs were harvested at 6 weeks post TTDN. The contribution of FG-derived progenitors as well as mature adipocytes to the accumulation of fluorescently labeled fat in recipient RC was analyzed by measurement of Tomato Red and GFP signals by fluorescent microscopy. Additionally, we used inducible PDGFRβ-Cre x mTmG mice to trace the contribution of GFP^{+}PDGFRb^{+} progenitors to the development of intracellular fat by screening tissue sections of non-injured and injured RCs for the presence of GFP^{+} adipocytes at 6 weeks following TTDN.

RESULTS: MRI reading and histopathology analysis of human RCT supported the notion that RC fatty degeneration originates from two different sources: active migration of epimuscular fat into RC muscles or, alternatively, development of adipose tissue near large interstitial blood vessels. However, these interpretations are limited by the angle of the examined sections. Therefore, we assessed the potential contribution of epimuscular fat to fatty infiltration in a combined mouse model of chronic RC injury and fluorescently labeled epimuscular fat grafts (Figure 1A). Fluorescent microscopy analysis revealed that the FG infiltrated into the injured host RC within 6 weeks post-transplantation (Figure1B) and the FG appeared viable, as indicated by the presence of host-anastomosed blood vessels within the graft. Remodeling of grafts was associated with the proliferation of GFP^{+}PDGFRβ^{+} cells that also migrate into adjacent recipient injured muscle (Fig. 1B). Histological examination of RC sections of PDGFRβ-Cre x mTmG mice at 6 weeks post-TTDN revealed the presence of GFP^{+} adipocyte clusters residing between atrophied myofibers (Figure 1C), demonstrating the direct contribution of GFP^{+}PDGFRβ^{+} to degenerative intramuscular adipogenesis. Confirming previous observations using non-inducible PDGFRβ-Cre x mTmG mice[1], inducible GFP^{+}PDGFRb^{+} progenitors also populated fibrotic scars in injured RC.

DISCUSSION: Altogether, these findings demonstrate that fatty degeneration originates from two different sources: epimuscular fat and injury-mediated adipogenic differentiation of muscle-residing PDGFRβ^{+} fibro-adipogenic progenitors. The presence of graft-derived fat cells in injured muscles indicates that these cells are motile and might actively undertake local functions needed to compensate for the loss of functional skeletal tissue.

SIGNIFICANCE: This research contributes to a deeper understanding of RC injury in the clinical setting; the regeneration of RC muscle tissue following injury is hindered by the infiltration of surrounding fat tissue and proliferation of stromal cells. This research can be used to better diagnose and strategize treatment plans for patients suffering from RC injury by assessing the degree of fatty infiltration. Treatments can be developed to target the rate of epimuscular fatty infiltration.

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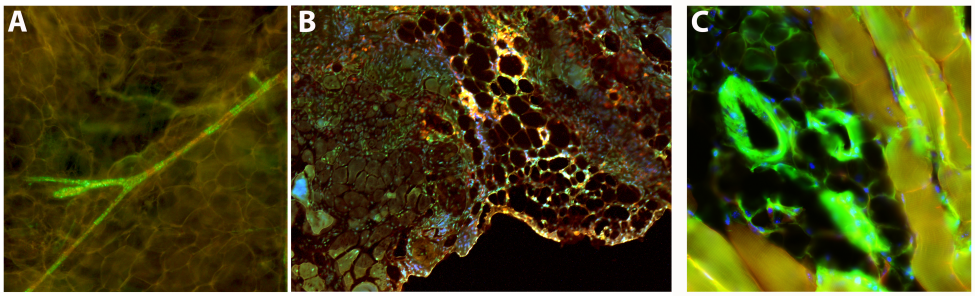

Figure 1. Origin of fatty degeneration in a mouse model of massive rotator cuff tears. (**A**) Gross appearance of epimuscular fat graft (FG) derived from donor PDGFRβ-Cre x mTmG mice. GFP is co-expressed by PDGFRβ^{+} cells and seen predominantly in perivascular cells. (**B**) Infiltration of fluorescently labeled FG into unlabeled injured RC muscle of C57/BL6 at 6 weeks post TTDN. FG-derived GFP^{+} cells are seen localized at muscle scar tissue (**C**) PDGFRβ^{+} cells localize to the supraspinatus interstitium and blood vessels contribute to pathologic adipogenesis following massive rotator cuff tear.