PDGFRα+ Progenitor Cells Contribute to Muscle Fibroadipogenesis Following Massive Rotator Cuff Tears in a Mouse Model

Andrew R. Jensen MD MBE, Ayelet Dar PhD, Benjamin Kelley BS, Claire Eliasberg BS, Sai K. Devana BS, David R. McAllister MD, Frank A. Petriglino MD

University of California, Los Angeles, Department of Orthopaedic Surgery

Introduction
Rotator cuff tears affect an estimated 10% of patients over the age of 60, leading to significant activity related pain and decreased quality of life [1]. After sustaining a massive tear, the rotator cuff often undergoes muscle atrophy and fatty degeneration, leading to poor surgical outcomes. The cellular processes underlying these fibroadipogenic changes remain unknown, although PDGFRα+ cells have previously been implicated [2]. Recently, it has been demonstrated in the hind limb that following acute, reversible transectional injury, PDGFRα+ cells are responsible for fibrotic and adipogenic muscle degeneration [3]. We hypothesized that the PDGFRα+ PDGFRβ+ cell population contributes to fibroadipogenesis following massive rotator cuff tears.

Methods
We performed the previously validated model surgery for massive rotator cuff tear (Fig. 1), tenotomy and denervation (TT+DN), on the right supraspinatus of 8-10 week-old PDGFRβ-Cre x tm1GFP mice (n=18). These mice express GFP in cells co-expressing PDGFRβ. At 5-days, 2-, 4- and 6-weeks postop, these mice and sham surgery controls were sacrificed and their supraspinatus muscles were harvested. Tissue sections were stained with Oil Red O and Sirius Red for adipogenesis and fibrosis analysis. Muscle tissues were immuno-labeled with anti-PDGFRα and anti-PDGFRβ to assess differences in PDGFRα+ PDGFRβ+ cell localization. Dissociated cells from supraspinatus tissue were either analyzed by flow cytometry or sorted and cultured in vitro differentiation experiments to assess the fibroadipogenic potential of these cell populations. Our institutional review board approved all animal experiments.

Results
All mice underwent progressive muscle atrophy, fibrosis and adipogenesis following TT+DN procedure, as demonstrated by H&E, Pineo Stains, and Oil Red O staining. These changes were greatest at 6 weeks post TT+DN. Immunohistochemistry, we identified PDGFRα+ cells, GFP+ fibrotic tissue, and GFP+ adipocytes in supraspinatus interstitial scar tissue and adipogenic tissue following TT+DN (Fig. 2B-D). This indicated a significant contribution of PDGFRα+ PDGFRβ+ cells to fibroadipogenesis of the rotator cuff following massive tendon tear.

Immunohistochemistry demonstrated a significant increase in the density of PDGFRα+ PDGFRβ+ cells at 5-days following TT+DN (Fig. 3). Similarly, flow cytometry analyses revealed that the frequency of this PDGFRα+ sub-population increased from baseline at 5 days following TT+DN and subsequently decreased to baseline levels within 2 weeks (Fig. 3).

On in vitro cell culture analysis, freshly isolated PDGFRα+ PDGFRβ+ cells had no myogenic activity but were both adipogenic and fibrogenic without induction. This contrasted with PDGFRβ+ pericytes, which had significant myogenic but no fibroadipogenic potential (data not shown).

Figure 1. TT+DN. Adapted from Ichinose et al [4].

Figure 2. PDGFRα+ cells localize to the supraspinatus interstitial (A) and are involved in fibrosis (B) and adipogenesis (C) following massive rotator cuff tear. The PDGFRα+ PDGFRβ+ subpopulation is present in the perivascular space (D) following massive rotator cuff tear.

Figure 3. The tissue density of PDGFRα+ GFP+ cells increases from pre-injury (CT1) to 5 days post injury (5 days). FACS analysis shows that this cell population increases significantly within 5 days of injury and returns to basal level by 2 weeks (n=3 per group).

Conclusions
Our study demonstrates that PDGFRα+ PDGFRβ+ fibroadipogenic progenitor cells directly contribute to fibroadipogenesis of the rotator cuff following a massive tear in the murine model. Pharmacological inhibition of the PDGFRα+ PDGFRβ+ cell population or depletion of PDGFRα+ cell fraction prior to cell transplantation in a regenerative treatment model may diminish fatty degeneration following massive rotator cuff tears. These strategies could be used clinically to decrease fatty infiltration and improve outcomes following rotator cuff repair surgery.

Acknowledgements
This research was supported in part by the H&H Lee Research Program

Bibliography