J Shoulder Elbow Surg (2018) ■■, ■■–■■

ELSEVIER

Journal of Shoulder and Elbow Surgery

www.elsevier.com/locate/ymse

ORIGINAL ARTICLE

Neer Award 2018: Platelet-derived growth factor receptor α co-expression typifies a subset of platelet-derived growth factor receptor β–positive progenitor cells that contribute to fatty degeneration and fibrosis of the murine rotator cuff

Andrew R. Jensen, MD, MBE[a,*], Benjamin V. Kelley, MD[a], Gina M. Mosich, MD[a], Allison Ariniello, BS[a], Claire D. Eliasberg, MD[b], Brandon Vu, BS[a], Paras Shah, BS[a], Sai K. Devana, BS[a], Iain R. Murray, MBChB, PhD[c], Bruno Péault, PhD[a,c,1], Ayelet Dar, PhD[a,1], Frank A. Petrigliano, MD[a,1]

[a]*Orthopaedic Hospital Research Center, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, USA*
[b]*Hospital for Special Surgery, New York, NY, USA*
[c]*BHF Centre for Vascular Regeneration, Scottish Centre for Regenerative Medicine, University of Edinburgh, Edinburgh, UK*

Background and hypothesis: After massive tears, rotator cuff muscle often undergoes atrophy, fibrosis, and fatty degeneration. These changes can lead to high surgical failure rates and poor patient outcomes. The identity of the progenitor cells involved in these processes has not been fully elucidated. Platelet-derived growth factor receptor β (PDGFRβ) and platelet-derived growth factor receptor α (PDGFRα) have previously been recognized as markers of cells involved in muscle fibroadipogenesis. We hypothesized that PDGFRα expression identifies a fibroadipogenic subset of PDGFRβ$^+$ progenitor cells that contribute to fibroadipogenesis of the rotator cuff.

Methods: We created massive rotator cuff tears in a transgenic strain of mice that allows PDGFRβ$^+$ cells to be tracked via green fluorescent protein (GFP) fluorescence. We then harvested rotator cuff muscle tissues at multiple time points postoperatively and analyzed them for the presence and localization of GFP$^+$ PDGFRβ$^+$ PDGFRα$^+$ cells. We cultured, induced, and treated these cells with the molecular inhibitor CWHM-12 to assess fibrosis inhibition.

This study was presented at the 80th Annual Meeting of the Western Orthopaedic Association; September 29, 2016; Indian Wells, CA, USA.
This study received ethical approval from the University of California, Los Angeles Institutional Animal Care and Use Committee (ARC No. 2012-042-11C).

*Reprint requests: Andrew R. Jensen, MD, MBE, Department of Orthopaedic Surgery, University of California, Los Angeles, 1250 16th St, Ste 2100a, Santa Monica, CA 90404, USA.
E-mail address: ajensen@mednet.ucla.edu (A.R. Jensen).
[1]These authors contributed equally to this work.

https://doi.org/10.1016/j.jse.2018.02.040

Results: GFP$^+$ PDGFRβ$^+$ PDGFRα$^+$ cells were present in rotator cuff muscle tissue and, after massive tears, localized to fibrotic and adipogenic tissues. The frequency of PDGFRβ$^+$ PDGFRα$^+$ cells increased at 5 days after massive cuff tears and decreased to basal levels within 2 weeks. PDGFRβ$^+$ PDGFRα$^+$ cells were highly adipogenic and significantly more fibrogenic than PDGFRβ$^+$ PDGFRα$^-$ cells in vitro and localized to adipogenic and fibrotic tissues in vivo. Treatment with CWHM-12 significantly decreased fibrogenesis from PDGFRβ$^+$ PDGFRα$^+$ cells.
Conclusion: PDGFRβ$^+$ PDGFRα$^+$ cells directly contribute to fibrosis and fatty degeneration after massive rotator cuff tears in the mouse model. In addition, CWHM-12 treatment inhibits fibrogenesis from PDGFRβ$^+$ PDGFRα$^+$ cells in vitro. Clinically, perioperative PDGFRβ$^+$ PDGFRα$^+$ cell inhibition may limit rotator cuff tissue degeneration and, ultimately, improve surgical outcomes for massive rotator cuff tears.
Level of evidence: Basic Science Study; Molecular and Cell Biology; Animal Model

Keywords: Rotator cuff; massive rotator cuff tear; rotator cuff repair; fatty degeneration; fibroadipogenic progenitor cell; PDGFRα; PDGFRβ

Rotator cuff tears affect an estimated 10% of the population over the age of 60 years, leading to significant activity-related pain and decreased quality of life.[11] Over 75,000 rotator cuff repair surgical procedures are performed annually in the United States alone.[45]

While the outcomes of rotator cuff repair surgical procedures are generally positive, surgical failures lead to significant morbidity.[18] Failures often require costly revision surgery or salvage procedures such as reverse total shoulder arthroplasty.[38] Tendon retears are the leading cause of surgical failure, and the rate of retear or persistent tear is strongly associated with muscle atrophy and fatty degeneration.[7] Massive rotator cuff tears are associated with significant fatty degeneration and the highest rates of tendon retear, with reported incidences of up to 94%.[36]

After rotator cuff tears, the severity of fibroadipogenesis, which is the pathologic process characterized by fatty degeneration and fibrotic scar formation, correlates with increasing tear size, patient age, and duration of the tear.[34] Fatty degeneration in particular has been associated with poor surgical outcomes and is the basis of the Goutallier rotator cuff tear staging system.[5,24] These degenerative processes lead to decreased muscle strength and tissue compliance, making surgical repair and biological healing challenging.[34]

The cellular origin of these fibroadipogenic degenerative changes noted in massive rotator cuff tears remains unclear. Researchers have identified a candidate mesenchymal stem cell population in skeletal muscle that possesses both fibrotic and adipogenic potential on differentiation.[22,25,42] A number of different antigen combinations—including CD31$^-$, CD45$^-$, integrin α7$^-$, and stem cells antigen-1$^+$[22]; CD31$^-$, CD45$^-$, and platelet-derived growth factor receptor α (PDGFRα) positive[43]; and CD31$^-$, CD45$^-$, integrin α7$^-$, stem cells antigen-1$^+$, and PDGFRα$^+$—have characterized these progenitor cells, which are sometimes referred to as "fibroadipogenic progenitor (FAP) cells."[25] Researchers have identified PDGFRα, in particular, as a reliable marker of FAP cells,[19] while platelet-derived growth factor receptor β (PDGFRβ)–positive cells have been identified in muscle tissue as a marker of some progenitor cells that are implicated in pathologic tissue fibrosis.[15] Researchers have found that PDGFRα$^+$ cells are involved in fibrotic and adipogenic muscle degeneration following toxin-mediated injury to the hindlimb or injury to the rotator cuff of animals with muscular dystrophy, as well as in age-associated sarcopenia.[1,19,30,41,43] Dulauroy et al[9] demonstrated that a subset of PDGFRα$^+$ cells expressing a disintegrin and metalloproteinase protein–12 possess increased fibrogenic potential and that ablation of these cells decreases pathologic fibrosis.

Through targeting the α_v subunit of integrins in fibrogenic myofibroblasts by the administration of CWHM-12, a small α_v integrin inhibitor, researchers have effectively reduced transforming growth factor β1 (TGF-β1)–mediated fibrosis in the liver, kidney, and lung.[17] Researchers have identified PDGFRα$^+$ cells in human skeletal muscle tissue as well, although the role of these cells in human pathology, including rotator cuff muscle degeneration, remains largely undefined.[41]

To establish whether co-expression of PDGFRα and PDGFRβ identifies a population of profibrotic and proadipogenic cells involved in muscle tissue degeneration following massive rotator cuff tears, we examined the capacity for purified populations of these cells to differentiate into adipogenic and fibrotic cell lineages in culture. Furthermore, we used this validated model of massive rotator cuff tears in a transgenic mouse to evaluate the contribution of these cells to fibrosis and fatty degeneration in vivo. Last, to demonstrate the potential for inhibiting degradative tissue processes from PDGFRβ$^+$ PDGFRα$^+$ cells, we examined the ability of CWHM-12 to inhibit fibrogenesis after massive rotator cuff tears in vitro. We show that rotator cuff–derived PDGFRβ$^+$ PDGFRα$^+$ cells have fibrogenic and adipogenic potentials in vitro, directly contribute to fibroadipogenesis after massive rotator cuff tears in vivo, and can be inhibited through treatment with CWHM-12 in vitro.

Materials and methods

This is a laboratory study using transgenic mice to evaluate the etiology of rotator cuff fibrosis and fatty degeneration following massive rotator cuff tears.