Transforming Growth Factor Beta Is Overexpressed In A Rat Model Of Rotator Cuff Tears

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Disclosures:

Introduction: Muscle changes after rotator cuff injuries have been implicated as an important factor in determining outcome of surgical rotator cuff repairs. Patients with muscle atrophy and fatty infiltration have been found to have worse outcomes and higher rerate rates than those that do not have atrophy and fatty infiltration. Previous studies have implicated the Akt/mTOR pathway as a potential regulator of muscle atrophy in multiple models. Currently the upstream regulation of this pathway in the RCT injury model has not been defined.

TGF-B is a cytokine that has a role in diverse pathological processes including immunity, cancer, asthma, heart disease, aging, and muscular disorders. TGF-B exists in three isoforms called TGF-B1, TGF-B2, and TGF-B3. It is part of a superfamily of factors that includes myostatin and the bone morphogenetic proteins (BMPs). In skeletal muscle processes, TGF-B has been implicated in its role in fibrosis, regulation of muscle size, aging, and after denervation.

TGF-B functions through both a canonical and non-canonical pathway. In the canonical pathway, TGF-B binds to its receptor, and phosphorylates SMAD-2 and SMAD-3, allowing these transcription factors to enter the nucleus and increase transcriptional activity. This pathway has been implicated in control of muscle change after injury in multiple studies. TGF-B has been found to activate the mTOR complex 1 (mTORC1), and mTORC2 through activation of the PI3 kinase. This has been shown to regulate Akt1 and Akt2, both of which can regulate gene expression and regulate atrophy. Through our previous studies, we have found that this pathway is important in both muscle atrophy and fatty infiltration in a RCT model. In previous studies, we found that the Akt/mTOR pathway was important in the regulation of muscle size after RCT, and regulated the amount of fatty infiltration after RCT(1). Thus, the canonical pathway may be an important regulator of muscle atrophy and fatty infiltration.

Thus, TGF-B is an appealing target for a central regulator of the downstream consequences of RCT as it appears to have the ability to control fibrosis as well as muscle fiber size through its activation. In this study, we sought to determine if there was an increase in TGF-B expression after a simulated massive rotator cuff tear in a rat model.

Methods: Adult male Sprague-Dawley rats at 250 g weight were randomly divided into two groups. The first group underwent a complete supraspinatus and infraspinatus tendon transection as well as a suprascapular nerve injury where a 5 mm long segment of supraspinalus nerve was removed after it branches from the superior trunk of the brachial plexus. This procedure allowed for supraspinatus/infraspinatus muscle denervation while leaving the rotator cuff intact. Sham surgery was performed on the opposite side to serve as internal control. All procedures were approved by our Institutional Animal Care and Use Committee. A power analysis based on our previous results of SS and IS muscle weight loss at 2 weeks after TT showed that 6 animals per group would be needed to see a significant difference with α = 0.05 and β = 0.80. Eight animals were performed for each group in order to account for any unexpected variability.

Rats were sacrificed at 2 and 6 weeks after surgery. Supraspinatus muscles from both surgical and control sides were harvested and preserved. Muscles were sectioned in half and used to measure fibrosis with histology. The remainder of the tissue was utilized to evaluate expression of TGF-B and its downstream products with RT-PCR and Western Blot Analysis. We evaluated TGF-B1 and B2 expression. We also evaluated Col1a, Col III and alpha-SMA as downstream products of TGF-B canonical expression. SMAD 2, 3 as well as 1/5/8 were evaluated with Western blot activity to look for activation via phosphorylation. For western blotting, fifty µg of protein from muscle samples was loaded on 10% NUPAGE Bis-Tris gels and transferred to PVDF membranes. Membranes were blocked and incubated in LC3B primary antibody overnight (1:1000 dilution). A HRP conjugated goat-anti-rabbit secondary antibody was used at a dilution of 1:10,000. Bands of developed blots were quantified using ImageJ Software (NIH).

A paired T-test was used for data analysis. Significance was defined as a p value of less than 0.05. Data is presented as the mean ± standard deviation.

Results: We evaluated the effects of a combination of TT+DN in 16 rats at both 2 and 6 weeks after rotator cuff injury. There was a 4-fold increase in TGF-B1 expression at both 2 and 6 weeks, and a 3-fold increase in TGF-B2 expression at these time-points when quantifying with both PCR and western blot analysis. At both 2 weeks and 6 weeks, we saw a significant increase in fibrosis, as well as an increase in Col-Ia, Col III, and a-SMA as markers of fibrotic development. Thus, there appears to be an increase in TGF-B1 and B2, with a concomitant increase in fibrotic markers and fibrosis when evaluated by histology.
We next evaluated the downstream expression of the canonical pathway (SMAD-2 and SMAD-3) and non-canonical (SMAD 1/5/8) at 2 and 6 weeks. There was significant upregulation of SMAD-2 activity (as assessed via expression of phosphorylated SMAD) at both timepoints, but no increase in activity of SMAD 3. There was also a marked increase in activity of SMAD 1/5/8 (6 fold) compared to sham surgery. Myostatin expression was not significantly altered at either timepoint. There was also no significant increase in TGF-B receptor expression.

**Discussion:** The results of this study demonstrate that TGF-B is overexpressed at both early and later timepoints after a massive RCT in a small animal model. TGF-B expression may be a central regulator of the changes seen in rotator cuff injuries. Upregulation of TGF-B resulted in an increase of pro-fibrotic collagen genes that are activated by SMAD 2/3 transcriptional regulation. Although fibrosis is not quantified via MRI in a clinical setting, it may be responsible in part for the reduced compliance of the muscle-tendon unit when attempting a repair. Further studies evaluating the effects of TGF-B inhibition are warranted.

**Significance:** The study shows the potential role of TGF-B in the regulation of muscle changes seen after rotator cuff tears.

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**References:**