

Impaired Healing of Rotator Cuff Correlates with Altered MMP and Collagen Expression of Supraspinatus Tendon

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INTRODUCTION

Rotator cuff disease is a common cause of shoulder pain and dysfunction, especially in older and sedentary people [1-3]. A spectrum of pathology exists with painful but low-grade partial thickness tears at one end and massive rotator cuff tears causing pseudo-paralysis at the other. Many extrinsic (impingement, demographic factors) and intrinsic (age-related degeneration, inflammation, hypovascularity, oxidative stress, etc) factors have been proposed as possible etiologies to explain why the disease advances rather rapidly in some patients but little or no progression in others [3-6]. Despite technical improvements in rotator cuff repair such as double row constructs, stronger suture anchors, etc, failure rates remain high (20-80%) after rotator cuff repair depending on the tear type. We hypothesized that impaired healing of rotator cuff was correlated with increased joint inflammation and decreased matrix production.

METHODS:

Forty five patients presenting for arthroscopic rotator cuff repair were prospectively enrolled and 37 patients completed all required testing. Samples of the torn supraspinatus tendon at the tear margin, subscapularis tendon, synovial tissue, and bursal tissue were harvested according to IRB approved protocols. Samples were taken under sterile conditions from the operating room, frozen, and later isolated for mRNA isolation using a mirVana miRNA isolation kit (Ambion) before reverse transcribed into cDNA (iScript, BioRad). Real time PCR was performed (BioRad) for selected pro-inflammatory factors (IL-1, IL-6, TNF- α , COX2), angiogenesis factors (VEGF) and tissue-remodeling genes (MMP-1, MMP-9, MMP-13, TIMP-1, COL1A1, type III collagen, SMA and Biglycan). GAPDH was used as a housekeeping gene for reference. All the primers were designed and tested for real-time PCR based on a human cell-line stimulated by IL-1. The identification and sequence of the samples were blinded and randomized according to HIPPA guidelines. For histological analysis, tissue from each group was fixed, sectioned and stained with H & E. microRNA analysis was performed by Exiqon, Inc. A standard targeted ultrasound was performed at an average of 6 months postoperatively. Linear correlation and Student's t-Test were performed with significance set at 0.05.

RESULTS:

There were 29 healed (78.4%) rotator cuff repairs based on postoperative ultrasound and 8 defects (21.6%). Both Healed and Defect groups showed significantly improved of should function (ASES score, Figure 1). The Defect group had statistically significant increased levels of MMP-1 and MMP-9 (Figure 2). Histomorphological changes (H&E) shows increased pro-inflammatory cells, vascularity, surface irregularity and synovial thickening in the Defect group as compared to the Healed group (Figure 3). This is consistent with increased expression of miR146b, a NF-kB regulatory microRNA, in the Defect group (Figure 3). H&E stains of supraspinatus tendon shows a decreased fibroblast population and less organized collagen fibrils in (B) the defect group as compared to (A) the Healed group. This agrees with significant increased COL1:COL3 ratio as shown in Figure 4. This suggests that the intrinsic regulation of newly formed collagen is critical for better clinical outcome of rotator cuff repair.

DISCUSSION:

Our findings from histology, mRNA and miRNA analyses support our hypothesis that impaired healing of rotator cuff is closely associated with increased joint inflammation and mis-regulation of collagen expression. Since MMP-1 and MMP-9 is highly effective in cleaving collagen and upregulated by pro-inflammatory cytokines. It is important for regulating synovial inflammation. Our finding in matrix integrity also highlights the importance of collagen expression types, the base for biomechanical properties, which are altered in failed repairs. These results provide increased insight into the biology of rotator cuff healing and potential targets for future therapeutics.

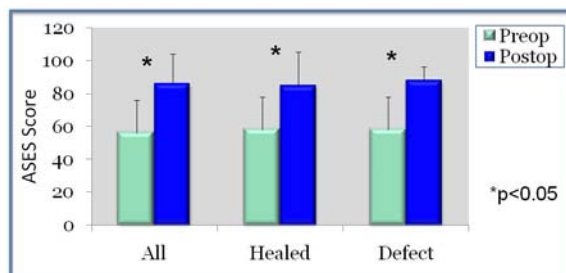


Figure 1. ASES Shoulder Scores were collected at baseline and follow up and showed improvement in both groups ($p < 0.05$).

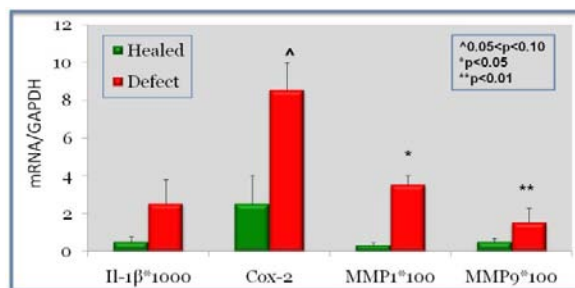


Figure 2. Increased expression of MMP-1& MMP-9 implies greater collagen degradation and inflammation in the Defect group.

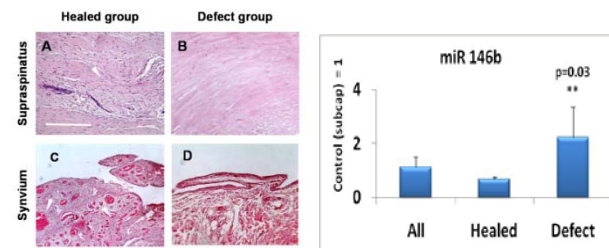


Figure 3. Histomorphological changes (H&E, bar=200 μ m) shows a decreased cell population with less organized collagen fibrils in supraspinatus and increased pro-inflammation and synovial thickening in the Defect group. This is consistent with increased expression of miR146b in the Defect group.

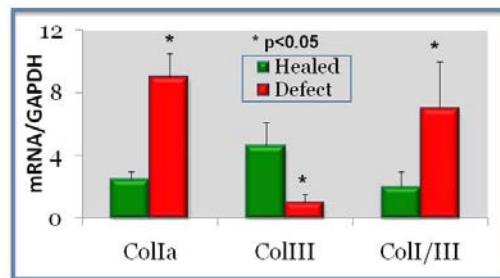


Figure 4. Increased expression of ColIA1 and decreased expression of ColIII in the Defect group suggests impaired healing of rotator cuff is closely related to the integrity of newly formed collagen.

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