

Tenascin C Deletion Impairs Tendon Healing and Functional Recovery after Rotator Cuff Repair in a Murine Model

Robert Z. Tashjian¹, Jared Zitnay¹, Nikolas H. Kazmers¹, Shivakumar R. Veerabhadraiah¹, Antonio C. Zelada¹, Matthew Honeggar¹, Peter N. Chalmers¹, Heath B. Henninger¹, Mick J. Juryne^{1,2*}

¹Dept. of Orthopaedics and ²Human Genetics, University of Utah, Salt Lake City, UT

*mjjuryne@genetics.utah.edu - juryneclab.org

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INTRODUCTION: Failure of healing after rotator cuff repair (RCR) is common. Genetic variants in the extracellular matrix protein Tenascin C are associated with impaired tendon healing and it is expressed in rotator cuff tendon tissue after injury, suggesting it may have a role in the repair process. The purpose of the current study was to evaluate the effect of deletion of the Tenascin C gene in a murine rotator cuff repair model on tendon healing after RCR.

METHODS: The supraspinatus tendon was transected and repaired with 6-0 Prolene suture on the left shoulder of 24 12-14 week old male mice. The mice included in the repairs were Wild-Type (WT) and Tenascin C null mice (Tnc⁻). Histological, activity testing and RNA-seq analyses were performed on operated (WT-RCR, Tnc⁻-RCR) mice. The unoperated, contralateral shoulder of WT-RCR and Tnc⁻-RCR mice was used for histological controls. WT-RCR (n=3) and Tnc⁻-RCR (n=3) were sacrificed at 8 weeks postoperative and used for histological analysis. WT-RCR (n=3) and Tnc⁻-RCR (n=3) were sacrificed at 2 weeks postoperative and used for RNA-seq. Finally, WT-RCR (n=4) and Tnc⁻-RCR (n=8) underwent activity testing at 8 weeks postoperative.

RESULTS: Tnc⁻ mice have severe bone and tendon defects following rotator cuff repair. Tnc⁻-RCR animals have a high degree of uncalcified fibrocartilage, severe disorganization of the organizing fibers, and increased bone remodeling compared to unoperated contralateral WT and TNC shoulders (Figure 1). Tnc⁻ mice have reduced activity after rotator cuff repair including reduced wheel rotations, wheel duration and wheel episode average velocity compared to WT-RCR (Figure 2). Loss of Tnc following rotator cuff repair alters gene expression in the shoulder in comparison to WT-RCR with upregulation of the Hedgehog pathway and downregulation of the p53 pathway.

DISCUSSION: Deletion of Tenascin C results in a disorganized enthesis after rotator cuff repair, reduces activity and alters gene expression when compared to repairs in control animals. The data supports that variant genes identified in clinical studies of *TNC* that impair healing likely have a direct causative effect on rotator cuff repair failure. The Hedgehog pathway promoted mineralization of fibrocartilage after tendon repair is required for normal enthesis healing although upregulation can lead to abnormal ossification and altered enthesis site biomechanics. Inhibition of p53 function during tendon repair has been shown to delay the inflammatory process and delay tendon healing. Further research is required to evaluate tissue specific alterations of the gene, the interactions of Tnc and the Hedgehog and p53 pathways as well as possible adjuvants to improve enthesis healing in the setting of mutations.

SIGNIFICANCE: Identification of individuals with variants in *TNC* may help inform clinical decisions to repair a rotator cuff tear.

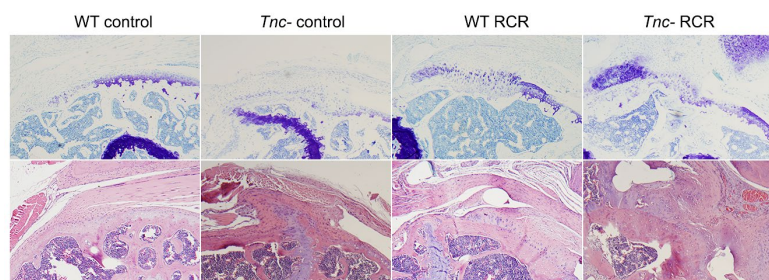


Figure 1. Tenascin C null (Tnc⁻) mice have severe bone and tendon defects following rotator cuff repair. Histological analysis of shoulder tissue sections 8 weeks post-rotator cuff repair. Tnc⁻-RCR animals have a high degree of uncalcified fibrocartilage, severe disorganization of the organizing fibers, and increased bone remodeling compared to controls and WT RCR animals. Sections stained with Toluidine Blue (top row) and H&E (bottom row).

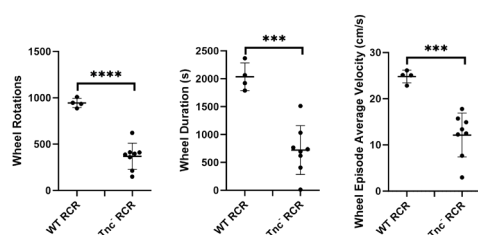


Figure 2. Tenascin C null (Tnc⁻) mice have reduced wheel activity after rotator cuff repair. 7 weeks post-rotator cuff repair animals were acclimated to the activity cages with wheels for 4 days. Following 2 days' rest, they were re-tested for 4 days. Graphs represent 4-day averages for each animal. Normality was assessed using the Shapiro-Wilk test and outliers were identified using Grubb's test (alpha = 0.05). Error bars represent ±SD and statistically significant differences of $P \leq 0.001$ (***), and $P \leq 0.0001$ (****) were determined by two-way ANOVA with Tukey's multiple comparisons test.

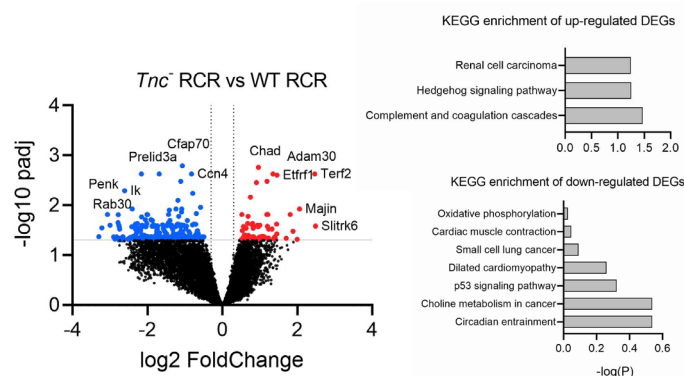


Figure 3. Loss of Tenascin C following rotator cuff repair alters gene expression in the shoulder. RNA-seq analysis of RNA isolated from WT and Tnc⁻ shoulders 14 days post-RCR. Red dots indicate genes upregulated and blue dots indicate genes downregulated in Tnc⁻-RCR compared to WT RCR shoulders. KEGG analysis indicates pathways that are up- or down regulated in Tnc⁻-RCR vs WT RCR shoulders.