Poster 130: Tendon-to-bone Healing in a CCR2 Knockout Mouse Model of Delayed Rotator Cuff Repair

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Objectives: Rotator cuff tears are one of the most prevalent musculoskeletal injuries with over 250,000 rotator cuff repairs performed annually in the Unites States. While surgical techniques have advanced, incomplete/failed healing after rotator cuff repair is still relatively common with rates ranging from 11-95%. Extrinsic factors such as excessive early activity and patient comorbidities such as smoking and diabetes have been previously identified as factors which may contribute to decreased rates of rotator cuff repair healing. However, there has been a recent focus on the intrinsic biologic factors which affect repair healing at the tendon-bone interface. CCR2 is a chemokine receptor that has been linked to the recruitment of monocytes into wound sites in early inflammatory stages and is associated with an increase in "pro-inflammatory" macrophages. The purpose of this study was to evaluate the role of CCR2 on rotator cuff tendon healing by evaluating relative gene expression and biomechanics following rotator cuff repair in wildtype (WT) and CCR2-/- knockout (KO) mice in a chronic rotator cuff injury model. We hypothesized that CCR2 KO would lead to decreased inflammatory cell recruitment and increased biomechanical properties after rotator cuff repair compared to WT mice.

Methods: All procedures were approved by our Institutional Animal Care and Use Committee (Protocol# 2019-0021). A total of 28 12-week-old male mice were utilized for this study and divided into 2 groups (wildtype C57BL/6J and CCR2 KO). All mice underwent unilateral supraspinatus tendon detachment at the initial surgery, followed by delayed supraspinatus repair 2 weeks following the initial procedure. They were sacrificed at the 4-week time point following the second procedure (Fig 1). The primary outcome measures included biomechanical testing and gene expression analysis. Biomechanical testing was performed on the repaired rotator cuff tendons on a custom-designed materials testing system. Contralateral intact tendons were also tested as controls. Specimens were prepared and loaded to failure at a rate of 1mm/min. Load-to-failure data were recorded and stiffness was calculated from the load-deformation curves. Following total RNA isolation from both the control and repaired tendons (50ng) and muscles (100ng), gene expression was measured using the NanoString nCounter® Fibrosis Panel. Genes were assessed based on fold change and significance level, determined by adjusted p-values using the Benjamini-Yekutieli procedure. The significance level was set at $p=0.05$ for all statistical analyses.

Results: All repaired tendons remained intact at the time of sacrifice 4 weeks following repair. The CCR2 KO group had thicker, more robust tendons on gross inspection than the WT controls. Biomechanical analysis demonstrated a significantly increased load-to-failure and stiffness of the supraspinatus tendon repair in the CCR2 KO group compared to the WT group. Mean load-to-failure in the WT group was 1.64 N ± 0.41 N versus 2.50 N ± 0.42 N in the CCR2 KO group ($p<0.001$) (Fig 2A). Mean stiffness in the WT group was 1.43 ± 0.66 N/mm versus 3.00 ± 0.95 N/mm in the CCR2 KO group ($p=0.001$) (Fig 2B). There were significant differences in both load-to-failure and stiffness between the repaired tendons and the intact contralateral tendons in both the WT and

CCR2 KO groups (p<0.001). There were no significant differences in load-to-failure or stiffness between the WT and CCR2 KO controls. The site of failure was in the tendon midsubstance for all of the rotator cuff repair samples in both groups. NanoString analysis comparing WT repaired tendons with WT controls revealed 42 differentially expressed genes (see Fig 3 for relative pathway scores). There was significantly increased expression of Ccr2 in the WT repair group compared to the WT control group (log2 fold ratio: 3.28, 95% CI 2.63 to 3.94, p=0.003). NanoString analysis of the repaired tendon samples confirmed lower expression of Ccr2 in the repaired tendons of the CCR2 KO mice compared to WT tendons. There were no significant differences in expression ratios of the supraspinatus muscle samples between the CCR2 KO repair group and the WT repair group. There were also no significant differences in gene expression between the CCR2 KO repair group and the CCR2 KO control group muscles.

Conclusions: In this study, we utilized a delayed rotator cuff repair model, which is more clinically relevant than previously established models of acute tendon transection and repair. Utilizing CCR2 KO mice, we found that there were significant differences in biomechanical properties – both load-to-failure and stiffness – between the WT mice and the CCR2 KO mice. Given the role of CCR2 in recruitment and accumulation of pro-inflammatory macrophages, these data suggest that excessive or unresolved inflammation may hinder tendon healing. Our results suggest that CCR2 KO leads to improved biomechanical properties in a mouse model of delayed rotator cuff repair. Additional studies are necessary to further elucidate the role of CCR2 in inflammation in the setting of chronic rotator cuff disease; however, CCR2 may be a promising target for novel therapeutics which aim to improve tendon healing and decrease re-tear rates following rotator cuff repair.

Fig 1. Experimental Flow Chart

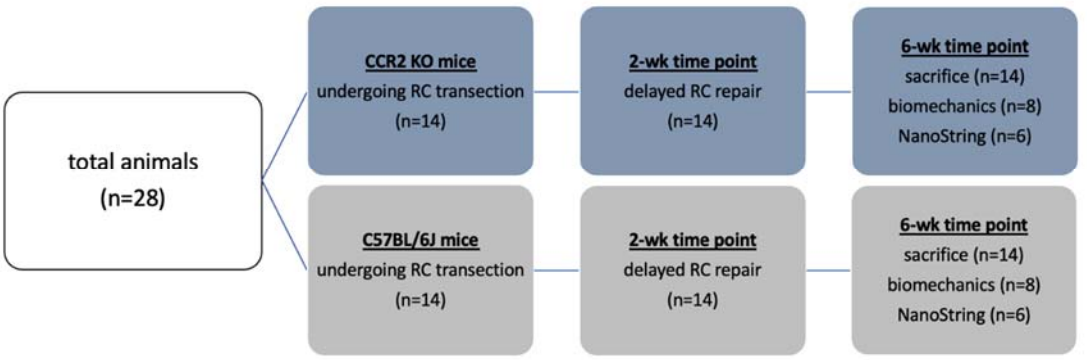
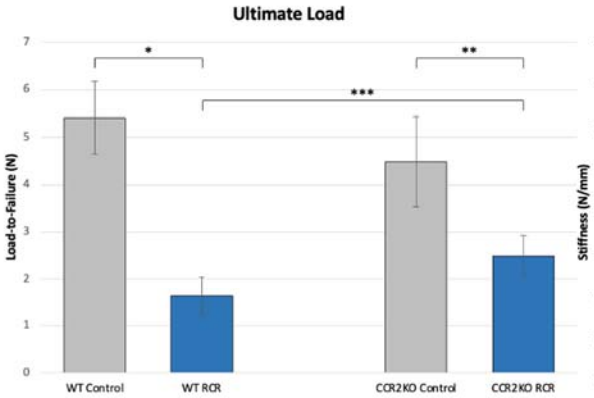
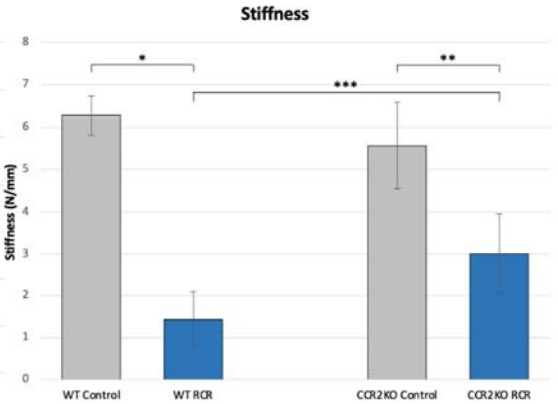


Fig 2A. Biomechanical Testing – Ultimate Load for Tendon Samples



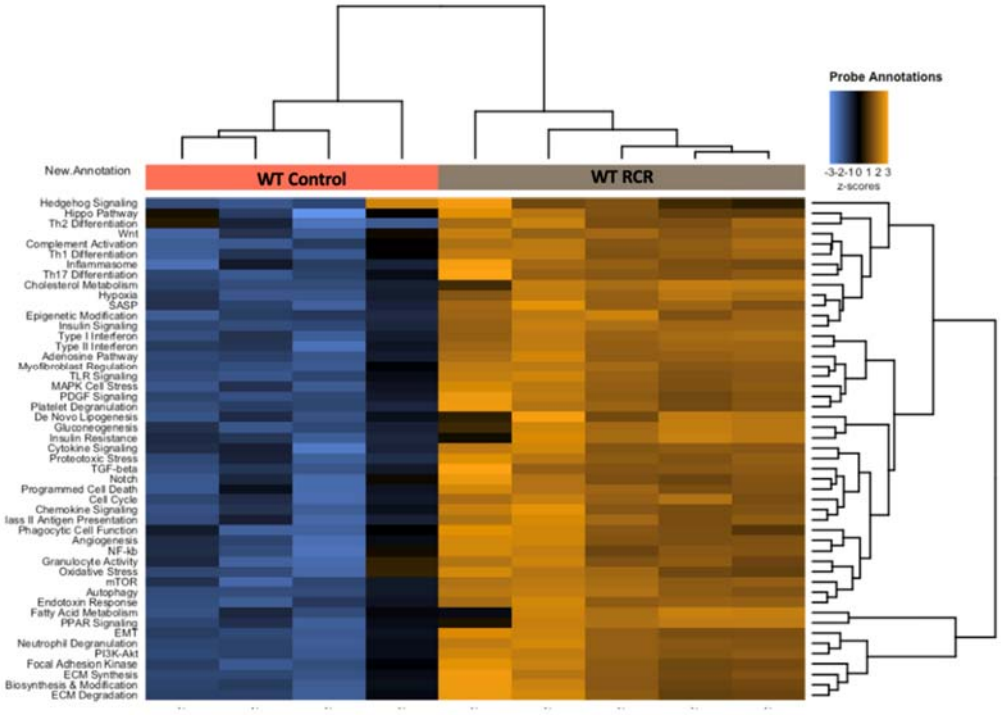
No significant difference between WT control and CCR2KO control groups

Fig 2B. Biomechanical Testing – Stiffness for Tendon Samples



No significant difference between WT control and CCR2KO control groups

Fig 3. Heatmap of pathway scores for WT control vs. WT rotator cuff repair tendons



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