**Mechanosensitive Pathway Activation Following Intervertebral Disc Injury**

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**Introduction:** The intervertebral disc (IVD) supports spine load and flexibility, playing a substantial role in everyday mobility. Among musculoskeletal tissue diseases, low back pain (LBP) is the leading cause of disability globally, where a growing prevalence and limited therapeutic interventions drives an annual U.S. economic burden of over $100 billion.[1,2] Injurious mechanical stimuli from spinal motions lead to degenerative changes in the IVD.[5] IVD herniations, where the nucleus pulposus (NP) extrudes beyond the annulus fibrosus (AF), result in expansive cell death and catabolic tissue remodeling.[5] Understanding the cellular processes associated with disc herniation is crucial for treatment development. Mechanosensing of resident IVD cells within an injured or diseased environment is believed to play a key role in propagating disease pathology, specifically in triggering IVD extracellular matrix (ECM) degradation and inflammation.[4,6] We aimed to evaluate mechanoactivation in an in vivo murine model of AF injury, to identify possible mechanosensing mediators following injury. ***We hypothesized Rho/ROCK and YAP/TAZ mechanosensning pathways would be increasingly activated within IVDs that had undergone annular puncture, compared to healthy IVDs.***

**Methods:** *Puncture model:* Caudal IVDs of skeletally mature (C57BL/6) female mice were punctured percutaneously with a 26G needle inserted through 2/3 of disc thickness using fluoroscopic guidance. IVDs were collected 2-weeks post injury for analysis (N=5). Adjacent IVDs and IVDs from non-injured mice (baseline) served as controls. *Histology:* Grading of SafO/Fast green (cartilage, mucin) stained sections was carried out using a mouse specific grading scale (higher scores: increased degeneration). *Disc Height:* Disc height was measured by averaging 3 measurements taken between endplates throughout the IVD using the Aperio imaging software (Leica Biosystems). *Gene Expression:* Samples were snap-frozen immediately following sacrifice, and expression of mechanosensing genes and those indicative of immune cell infiltration was conducted using RT-qPCR. Statistical differences were analyzed by conducting a t-test ($p<0.05$: significant, $p<0.1$: trend).

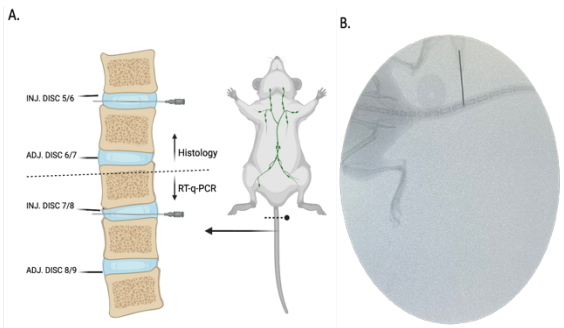


*Figure 1:* ***(A) Experimental Design Schematic.*** *(B) Fluoroscopic image of a mouse tail during the needle puncture procedure, showing placement of the 26G needle into the IVD.*

**Results:** Histopathologic analysis of IVDs, from an uninjured mouse showed an intact AF and NP with dense staining indicating healthy cartilage matrix (Fig. 2A). IVDs adjacent to injury demonstrated a healthy structural composition, similar to baseline IVDs, with minimal to no degenerative changes (Fig. 2A). Injured IVDs exhibited significant degeneration with a loss of the NP cellularity and structure. Interestingly, discs adjacent to injury ($p=0.0078$) and injured discs ($p=0.0016$) exhibited significant decreases in disc height compared to baseline (Fig. 2B). Gene expression analysis revealed significant increases in *Rock* ($p=0.0336$) and *Taz* ($p=0.0431$) expression in injured IVDs compared to baseline IVDs (Fig. 2C). An additional

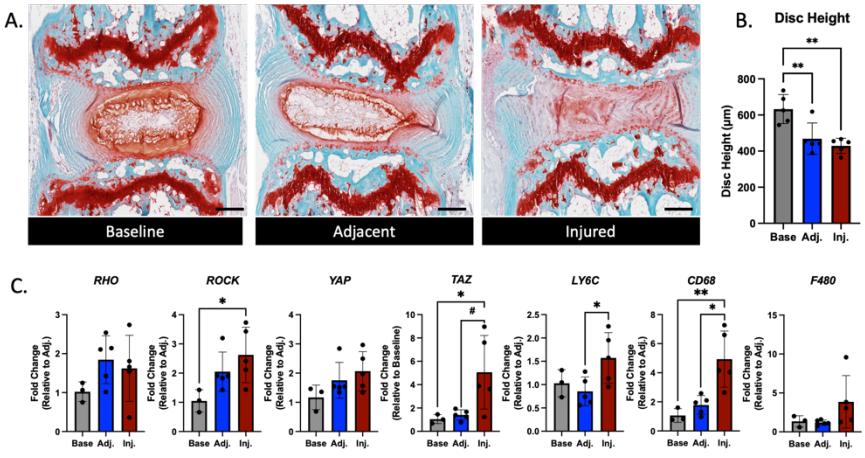


*Figure 2:* ***Histological and Gene Expression Analysis of Intervertebral Disc (IVD) Samples Post-Injury.*** *(A) Representative Safranin O/Fast Green-stained histological images of baseline, adjacent, and injured IVDs. Scale bar = 200μm. (B) Disc height (μm) measured from histological sections. (C) Gene expression (fold change relative to baseline).*

analysis of phagocytic innate immune cell markers revealed a significant increase in the *Ly6c* (monocyte marker) within injured IVDs compared to adjacent IVDs (p=0.0366), and a significant increase in *CD68* (macrophage marker) within injured IVDs compared to adjacent (p=0.0348) and baseline (p=0.0045) IVDs. The increased expression in injured discs underscores the activation of these pathways in response to AF injury, contributing to the observed histological degeneration and inflammation.

**Discussion:** This study investigated mechanosensitive pathway activation in IVDs following AF injury using a murine needle puncture model. Gene expression analysis showed significant upregulation of mechanosensitive genes, particularly YAP/TAZ and Rho/ROCK, in severely degenerated IVDs 2 weeks post injury. Future work will evaluate the localization of pathway activation, and downstream targets, throughout tissue microstructures via methods in IHC. Additionally, the disc height analysis suggests a significant reduction within both adjacent and injured IVDs. Further investigation will evaluate how adjacent discs respond at later time points, as altered spinal mechanics may lead to additional inflammation and mechanopathway activation. Ultimately, this study demonstrates mechanoactivation and possible downstream inflammation within an AF injury model. This provides the groundwork for future exploration of therapeutic strategies to modulate these pathways and mitigate disease.

**Acknowledgments**

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