

CRISPRi Editing of TNFR1 and IL6ST Reduces Pain Sensitivity in Disc Degeneration

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INTRODUCTION: Chronic low back pain (LBP) is the leading cause of disability worldwide. Among the prominent causes of LBP is degeneration of the intervertebral disc (IVD), a condition known as degenerative disc disease (DDD). DDD is characterized by the breakdown of the extracellular matrix, loss of disc height, inflammation, and pain. While many treatment strategies for disc degeneration and its associated pain have been proposed, the underlying mechanisms remain largely unknown, making it challenging to develop therapeutics effectively targeting this pain. Our strategy utilizes lentiviral-delivered CRISPR epigenome editing of IL6ST and TNFR1 expression to the IVD in an *in vivo* model of lumbar DDD, building off of previous work by our lab that has demonstrated the ability to lessen disc degeneration via *in vivo* CRISPR-editing of TNFR1 (**Figure 1A**).

METHODS: *Rat Luciferase Injections:* This study was approved by the University of Utah IACUC. Lentiviral-packaged vectors containing the luciferase gene were delivered using a novel, non-surgical, image-guided approach to the lumbar spine of rats (n=5/group) (**Figure 1B**). After 1 week, DRGs (**Figure 1C**), lumbar motion segments (**Figure 1D**), and major organs were harvested and imaged for luminescence levels. *Rat Cadaver Practice Injections:* Practice injections of blue dye using our novel approach (**Figure 1F**) were performed in 9 lumbar IVDs to ensure our ability to properly deliver to the IVDs (**Figure 1G**). After injections, injected IVDs were immediately harvested and imaged (**Figure 1G**). *Intradiscal CRISPR Lentiviral Injections:* Following practice injections, *in vivo* experiments were performed (**Figure 1H**). Under anesthesia, rat lumbar IVDs received intradiscal injections (**Figure 1F**) via 30-gauge needles of PBS, TNFR1, or IL6ST CRISPRi epigenome editing systems (n=7/group) (**Figure 1I**). *Lumbar IVD Puncture:* Two weeks following initial injections, under anesthesia, intervertebral disc injury was induced in previously injected rat lumbar IVDs via image-guided annular puncture (AP) with 25-gauge needles. *Behavioral Pain Assays:* Prior to injections, animals were acclimated to the handler, and behavioral assays and pre-procedure Von Frey and Hargreaves baseline data were collected. Each behavioral data collection session allowed time for animals to acclimate to the collection equipment and the experimenter. The experimenter was blinded for the duration of data collection. Data were collected 3 days post-annular puncture and continued weekly for four weeks (**Figure 1J-M**). *Statistical Analysis:* One-factor ANOVAs with Tukey-Kramer post-hoc tests were performed on the behavioral data, $\alpha=0.05$.

RESULTS: The luciferase injection experiments (**Figure 1B**) demonstrate our ability to deliver lentiviral-packaged vectors to the lumbar spine that express in both the DRGs (**Figure 1C**) and IVD (**Figure 1D**). We also performed luciferase imaging on major organs with no expression seen, indicating that the vectors remain localized. To establish our ability to deliver vectors and an annular puncture to the lumbar spine IVD with our novel approach (**Figure 1F**), practice injections on rat cadavers were performed before beginning our CRISPR epigenome editing experiments and subsequent behavioral assays. In our practice dye injections, we successfully injected blue dye into the nucleus pulposus at the center of the IVD in 9 out of 9 lumbar IVDs (**Figure 1G**). After the safety and efficacy of our method of delivery were established by these experiments, intradiscal delivery of CRISPR epigenome editing vectors targeting the TNFR1 or IL6ST promoter regions was performed, followed two weeks later by an annular puncture to induce IVD degeneration. Von Frey and Hargreaves behavioral data showed successful initiation of degeneration in the PBS control group. These data also showed significantly reduced normalized paw withdrawal threshold (PWT) and normalized paw withdrawal latency (PWL) in both the TNFR1 and IL6ST groups when compared to the PBS control group (**Figure 1J & 1L**). These significant changes show a reduction in thermal and mechanical pain sensitivity in both groups (**Figure 1K & 1M**).

DISCUSSION: Previously, we have shown the ability of CRISPR interference (CRISPRi) editing of TNFR1 to modulate cellular responses to inflammation *in vitro* and in an *in vivo* rat tail model of DDD (**Figure 1A**). In this study, we established the safety and efficacy of a novel, non-surgical, image-guided approach to deliver to the lumbar spine, utilizing *ex vivo* luminescent imaging. We then demonstrated a decrease in mechanical and thermal pain sensitivity with CRISPRi of TNFR1 and IL6ST in this model using Von Frey and Hargreaves behavioral assays. Additional studies into the simultaneous editing of TNFR1 and IL6ST and other potential targets should be explored in order to affect redundant or synergistic pain signaling associated with DDD and to explore the specific mechanisms by which these changes in sensitization are seen.

SIGNIFICANCE/CLINICAL RELEVANCE: These results demonstrate the safety and efficacy of our novel, non-surgical, image-guided delivery approach to the lumbar spine IVD and our ability to deliver lentivirus to the lumbar spine and have expression in the DRG and IVD. Most importantly, these results establish CRISPR epigenome editing of TNFR1 and IL6ST as a potential treatment strategy for lessening pain due to degeneration of the IVD.

