TRPV4 activation promotes intervertebral disc regeneration following injury

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INTRODUCTION: Injury to the intervertebral disc (IVD) causes degeneration and leads to the development of low back pain. IVD degeneration is characterized by loss of structure, impaired mechanical function, and a chronic inflammatory environment [1]. Despite this prevalent susceptibility for degeneration, the IVD possesses limited regenerative capacity and the degeneration often progresses to painful mechanical impairments. We have previously shown that the Ca^{2+} permeable ion channel Transient receptor potential vanilloid-type 4 (TRPV4) in the IVD that can be activated by mechanical loading, and its repeated activation in an intact IVD promotes matrix synthesis [2,3]. We hypothesized that TRPV4 can be leveraged for regeneration of the IVD following an injury. Using a combination of in vitro and in vivo models, we performed gain-of-function and loss-of-function experiments to investigate the role of TRPV in mediating IVD degeneration and regeneration.

METHODS: We first screened for the relevant intervertebral disc cell type that expressed TRPV4 using single-cell RNA-sequencing (scRNA-seq). Five levels (Co5/6-Co9/10) were injured with a bilateral stab using a 30G needle in 12-wk old, female C57BL6 mice (n=3). Seven days post injury, single-cell RNA-sequencing evaluated the IVD response to injury compared to controls (Co12/13-Co16/17). 15 healthy and 15 injured IVDs were pooled, rendered into cell suspension, sequenced, data filtered and normalized, reduced by PCA, and analyzed using Seurat. After identify the annulus fibrosus (AF) cells as the most transcriptionally active for TRPV4, we performed a loss of function experiment in AF cells using the same tail IVD injury model in transgenic $TRPV4^{lox/lox}$;Col2a1-$CreER^{T2}$ mice to selectively ablate TRPV4 in the Col2a1-expressing AF population. All mice received (WT, cKO) received two doses of tamoxifen (1mg/10g) two weeks prior to injury. Functional KO of TRPV4 was confirmed through Ca^{2+} imaging. Bulk RNA-seq compared WT and cKO in either healthy or injured IVDs (n=5). Finally, we performed in vitro gain and loss of function experiments using small molecule modulators of TRPV4. Lumbar functional spine units (FSUs) were extracted from NF-κB -luciferase reporter transgene (FVB. Cg-Tg(HIV-EGFP,luc)8Tb/J; JAX) mice and placed in culture media. A bilateral stab with a 30G needle was made to half of all IVDs and IVDs were administered either DMSO (vehicle), TRPV4 agonist GSK101 (2μM), or TRPV4 antagonist GSK205 (10μM) for 3 hours each day for 7 consecutive days. Media was collected for ELISA and IVDs were processed for histology. IVDs were independently graded by two blinded graders using a standardized histopathology scoring system [4].

RESULTS: scRNA-seq identified 4 of 9 cell clusters as AF clusters using the markers *Acan*, *Col2*, and *Col1*. 3 of 4 AF clusters showed a significant increase in TRPV4 following injury (Figure 1A). Bulk RNA-seq showed injury in WT mice resulting in 118 DEGs (112 up, 6 down) compared to just 29 DEGs in the cKO (7 up, 22 down), including the downregulations of *Col2a1*, *Lox*, and *Prg4* (Figure 1B). Comparing the WT with cKO injured IVDs, extracellular matrix genes *Col2a1* and *Col6a1/2/3* were downregulated (Figure 1C). Histology from organ culture showed injury significantly increased IVD degeneration and inhibition of TRPV4 worsened injury-induced degeneration (Figure 1D/E). Repeated activation of TRPV4 after injury was mildly protective against degeneration. TRPV4 activation also resulted in increased IL-6 and LIF, both of which are proinflammatory cytokines crucial to regeneration (Figure 1F/G).

DISCUSSION: While TRPV4 has been widely implicated in having a role in tissue response to mechanical loading, its increased expression following injury indicates it may also have function in tissue injury and repair [2,3]. TRPV4 activation in the IVD has previously been shown to activate the IL-6 pathway, promote GAG production, and suppress chemokine secretion. Following injury, cKO of TRPV4 resulted in a significant decrease in extracellular matrix (ECM) genes. TRPV4 may thus be necessary for tissue remodeling following injury. IL-6 and LIF, both downstream of TRPV4, increase ECM production in several musculoskeletal tissues, and were significantly increased following repeated activation of TRPV4 in an in vitro injury model. [5] This activation provided protection against injury-induced degeneration. As TRPV4 activation promotes matrix synthesis, these data suggest that TRPV4 activation may accelerate the recovery of the IVD following injury through ECM anabolism. With the absence or inhibition of TRPV4, the IVD loses some remodeling capabilities and deteriorate significantly due to injury.

SIGNIFICANCE/CLINICAL RELEVANCE: The IVD has poor regenerative capability and the mechanisms that drive this limited regeneration are not well understood. This study investigates TRPV4 activation as a potential pathway to be exploited to improve regenerative therapeutics for IVD degeneration.

REFERENCES: [1] Risbud et al. 2014. [2] Easson et al. 2023 [3] Easson et al. 2023. [4] Melgoza et al. 2021. [5] Xiao et al. 2019.

Figure 1: A) scRNA revealed increased expression (p<0.0001) of TRPV4 after injury in 3 of 4 AF cell clusters. B) Comparing the two cKO groups (healthy and injured), injury decreased expression (p<0.001) of ECM genes *Lox*, *Prg4*, *Col2a1*, and *Col6a3*. C) Comparison of the two injured groups revealed KO of TRPV4 decreased *Col2a1*, *Col6a1/2/3*, and *Fgfr3* expression. D/E) Histology of in vitro injury showed TRPV4 activation by GSK101 provided mild protection against degeneration. F/G) Pro-regenerative cytokines, IL-6 and LIF, were increased (p<0.05) downstream of TRPV4 activation.