scRNA-seq Analysis Identifies Prosaposin (PSAP) Signaling between Intervertebral Disc, Dorsal Root Ganglion, Spinal Cord, and Inflammatory Cells in a Rat *In-Vivo* Chronic Discogenic Pain Model

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Disclosures: None

INTRODUCTION: Chronic low back pain affects >600 million people globally and is projected to impact ~850 million by 2050, representing a leading and growing cause of disability and socioeconomic burden [1]. Intervertebral disc (IVD) degeneration (IVDD) accounts for ~40% of back pain cases and is often termed discogenic pain [2]. Disease-modifying therapies for discogenic pain remain unavailable, underscoring a need to better understand disease mechanisms. Increasing evidence points to the IVD interacting dynamically with neural systems, particularly the dorsal root ganglion (DRG) and spinal cord (SC) in painful conditions, with immune cell involvement which enhances nociception and pain chronicity in discogenic pain [3]. While much is known on discogenic pain, there remains an important gap identifying factors implicated in the cellular and molecular crosstalk between the neuronal tissues involved in discogenic pain. We therefore analyzed IVD, DRG and SC using single cell RNA sequencing (scRNA-Seq) in a rat chronic discogenic pain model to determine the underlying signaling networks crucial in the injury response. Our Aims were to: i) determine the cells most injury-affected by chronic IVDD in the IVD, DRG and SC; ii) determine which signaling pathway is responsible for cross-talk between these most injury-affected cell types; and iii) determine signaling pathways important in immune cells found in all 3 tissue types.

METHODS: With IACUC approval, IVDD was induced in skeletally mature (5-6 month old) male rats (n=10) via annulus fibrosus injury that involved a triple needle puncture (26G needle to 3 mm depth) at midline and anterolateral sites and PBS injection to the L3-4, L4-5, and L5-6 IVDs. This procedure had been shown to induce severe and reproducible IVDD with behavioral changes indicating back pain, as previously described [4]. Sham-operated rats (IVD located and exposed but not injured) served as controls. At 8 weeks' post-surgery, L3-4, L4-5, and L5-6 lumbar IVDs, L2 DRGs, and lumbar SC segments adjacent to the injury sites were isolated, digested and processed for single-cell RNA sequencing (37,538 total cells across all tissues). To determine cells important in IVDD, data were processed and integrated in Seurat with Unicell-machine-learning assisted cell annotations refined to identify spinal-specific cells, as previously described [5]. Differential gene expression analysis was performed using Seurat with the FindMarkers() function to identify transcriptionally altered cells in IVD, DRG, and SC tissues in IVDD, applying a threshold of log2FC > 1 and an adjusted p-value <0.05. To determine important signaling pathways for cellular crosstalk, we used CellChat to model ligand-receptor interactions between IVD-DRG and DRG-SC cells, and immune cells with interactions validated through permutation-based significance testing.

RESULTS: Unsupervised clustering identified 16 transcriptionally distinct cell types including an immune cell cluster across IVD, DRG, and SC tissues with and without IVDD (Fig. 1A). The most injury-affected (relative change in cell #) cell types were the Fibrochondrocytes, Schwann-like cells, and Oligodendrocytes in IVD, DRG and SC, respectively (Fig. 1B). CellChat revealed prosaposin (PSAP) as the most significant inter-tissue signaling pathway mediating crosstalk from IVD Fibrochondrocytes to DRG Schwann-like cells, and from DRG Schwann-like cells to SC Oligodendrocytes (Fig. 2). Immune cells were sub-clustered, exhibiting heterogeneous immune cell types that clustered together for IVD, DRG and SC (Fig. 3A & 3B). Several immune cell populations expressed PSAP receptors, suggesting a contribution to inter-tissue signaling networks in IVDD, with macrophage 1, neutrophil 3, and microglia populations most increased with IVDD (Fig. 3C & 3D).

DISCUSSION: This study demonstrated chronic IVDD in rats alters cell populations and transcriptomes of IVD, DRG, and SC cells, with PSAP as the only pathway identified in CellChat involved in communications between IVD-DRG and DRG-SC. Further, immune cell populations expressing similar transcriptomes across tissue types, also expressed PSAP receptors that were increased with IVDD conditions. Several scRNA-seq studies in the IVD have highlighted heterogeneous structural cell populations, progenitors, and shifts in inflammatory and neural marker expression following degeneration [5-7]. Complementary analyses in DRG and SC revealed injury-induced glial and neuronal subsets that drive sensitization and pain pathways [8]. This study extends these observations by integrating all three tissues into a single atlas and identifying PSAP as a novel signaling pathway and potential mediator of cross-tissue communication. PSAP has been shown to promote neuroprotection, glial cell activation, and inflammatory resolution in neural injury and disease [9], and therefore could play a role in neuropathologies associated with IVDD and chronic back pain. These transcriptomic results are being validated with ongoing protein measurements in animals of both sexes, and future mechanistic studies in multiple species are warranted.

SIGNIFICANCE: This scRNA-seq atlas integrates IVD, DRG, and SC cells to provide insight into multi-tissue mechanisms of back pain. We identified PSAP as a novel signaling pathway in tissue-specific and immune cells and a potential future IVDD therapeutic target.

REFERENCES: [1] Ferreira+ Lancet Rheum 2023; [2] Fujii+ JBMR Plus 2019; [3] Lai+ Int J Mol Sci 2024; [4] Mosley+ Sci Rep, 2020; [5] Panebianco+ FASEB J 2021; [6] Rohanifar+ Appl Sci 2022; [7] Cherif+ Int J Mol Sci 2022 [8] Jung+ Nat Comm 2023; [9] Meyer+ Brian Res 2014

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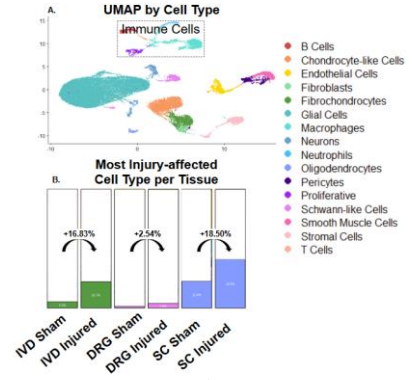

Figure 1 UMAP identified 16 distinct cell types in the combined IVD, DRG, and SC atlas. Fibrochondrocytes, Schwann-like cells, and Oligodendrocytes were the most injury-affected cells in each tissue.

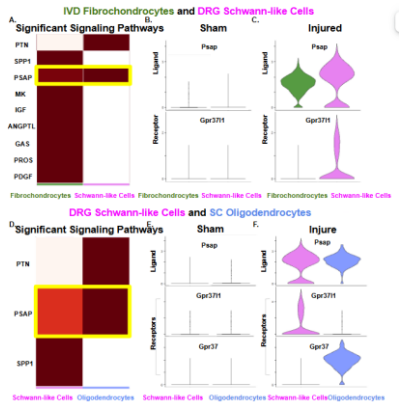

Figure 2 CellChat identified PSAP signaling as the only pathway involved in inter-tissue communications between IVD-DRG and DRG-SC (yellow boxes) in chronic IVDD.

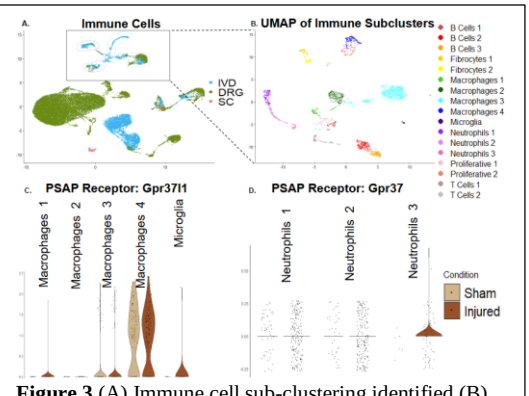

Figure 3 (A) Immune cell sub-clustering identified (B) 17 cell types. (C) Macrophages 1 and Microglia populations, which had an M1-phenotype, expressed the PSAP receptor with injury. (D) Neutrophil 3, which exhibited and immature and proliferative phenotype, also express the PSAP receptor with injury.