Single-cell RNA-sequencing of herniated human intervertebral discs reveal small populations of macrophages and fibroblasts as dominant drivers for the pro-inflammatory environment and fibrosis

Jennifer Gansau1, Levon Rodriguez1,2, Nadine Schrode1, Kennedy Salamat1, Saad Chaudhary1, Andrew C. Hecht1, Robert Sebra2, James C. Iatridis1

1 Leni and Peter W. May Department of Orthopaedics, Icahn School of Medicine at Mount Sinai, New York, NY
2 Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY

Email: jennifer.gansau@mssm.edu

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INTRODUCTION: Back and neck pain are major global healthcare challenges causing tremendous socioeconomic burden and costing >$134.5B in annual US health care costs1. Intervertebral disc (IVD) degeneration (IVDD) and herniation are strongly associated with back and neck disability. Disease-modifying treatments are needed to address the extensive cellular and structural changes in the complex IVDD conditions. Improved understanding of cells involved in IVDD from back pain patients can give insights into pathophysiology and inform new treatments. Single-cell RNA-sequencing (scRNA-Seq) with bioinformatics can decipher the heterogeneous cell populations and their functions. There remains little clarity on the cells responsible for propagating pro-inflammatory and painful processes in human herniated IVDs, even though scRNA-Seq in human IVDs has been performed and identified IVD cells in healthy and degenerated stages2-4. Furthermore, scRNA-Seq is a new and dynamic field requiring replication, validation, and integration of multiple studies to better understand roles of human IVD cells. This study used scRNA-Seq on human IVDs collected from subjects undergoing anterior disc fusion (ADF) surgery for painful IVDD and herniation and identified cell populations responsible for pain-driving and pro-inflammatory processes.

METHODS: IVD herniation tissue from 3 subjects (1 male, 2 female) from ADF surgery were collected under an IRB-approved protocol and digested for 12 hours (400U/mL collagenase II). Single-cell suspensions were processed using the 10X Genomics Chromium 3rd Gene Expression V3 Kit and sequenced using an Illumina S1 NovaSeq chip. Cell Ranger software v7.1 mapped reads to the human GRCh38-2020-A reference genome. Data processing via Seurat v4.3 included quality control filtering, normalization, dataset integration, and unsupervised graph-based clustering. Cell clusters were visualized with uniform manifold approximation and projection (UMAP) and annotated with UniCell, a semi-automated deep learning annotation model comprising 28M annotated cells from 898 studies. Gene set enrichment analysis (GSEA) through EnrichR identified functions of clusters using gene ontology (GO) and MSigDB Hallmark 2020 pathways. Cytokines in ADF of 9 subjects (6 male, 3 female) were measured with multiplex assay on conditioned media from herniated IVD tissues6.

RESULTS: IVD tissues from 3 ADF subjects enabled isolation and sequencing of 13,259 cells, which were divided into 14 clusters (Fig. 1A). UniCell annotated specific cell clusters based on their probability of association with previously reported cell types like chondrocytes (Fig. 1B). The close proximity of all IVD populations and overlapping gene signatures suggests limited phenotypic distinction between annulus fibrosus (AF) and nucleus pulposus (NP) cells. Furthermore, markers related to vascularization and sensitization, especially VEGFA and NGF, were expressed broadly in most IVD cells (Fig 1C, D). Cluster 6 showed high expression of pain-related markers BDNF, TAC1 and CALCA (CGRP) that had faint expression by other clusters (Fig 1D). Cluster 11 was identified as fibroblasts using DotPlot and angiogenesis using GSEA revealed it to have involvement in vascularization and fibrotic remodeling (Fig 2B). Multiplex protein assay of herniated IVD conditioned media identified many pro-inflammatory cytokines and chemokines including IL-6, TNFα and MCPs (Fig. 3A). Importantly, Cluster 13 alone expressed the majority of these cytokines (Fig 3B) and was annotated as macrophages by UniCell (Fig 3C). GSEA showed Cluster 13 was enriched for TNFa signaling and inflammatory responses (Fig 3D), and volcano plot (not shown) confirmed upregulated differentially expressed genes IL1B, CCL3 and CCL4 supporting their role in pro-inflammatory conditions.

DISCUSSION: IVD tissues from spine surgery subjects with painful IVDD and herniation were collected and analyzed with scRNA-Seq to identify 14 cell populations and distinguish 3 novel cell clusters found to play roles in driving pain and pro-inflammatory responses. The majority of AF and NP clusters had relatively little distinction in expression patterns, and most cells expressed VEGF and NGF, consistent with prior studies showing IVD cells can promote angiogenesis and neuritis growth5. Fibroblast Cluster 11 expressed genes implicated in vascularization, matrix remodeling and inflammation, and suggesting a role in fibrotic scarring6. Fibroblast Cluster 11 also had close proximity on the UMAP to macrophage Cluster 13 further supporting involvement in scarring. Importantly, macrophage Cluster 13 expressed the majority of pro-inflammatory cytokines found within the herniated tissue, which emphasizes their importance for IVDD progression and provides a potential target for therapies. Macrophages were present in surprisingly small numbers in this study, yet macrophages were shown to interact with NP cells in discoscopy NP cells4, supporting the concept that a small number of cells can play pivotal roles in orchestrating the responses of much larger cell populations. Cluster 6 expressed highest neuropetepeptide levels suggesting a role in pain. UniCell identified Cluster 6 as lymphocytic and also identified clusters with characteristics of T cells and fat stem cells that warrant further analysis to elucidate their roles in IVD-specific contexts. Ongoing studies are integrating this dataset with healthy IVD cell data to clarify how IVDD cells differ from healthy IVD cells to inform pathobiology and identify therapeutic targets.

SIGNIFICANCE: Small populations of fibroblasts and macrophages in IVDD herniation tissue were identified that may play a role orchestrating painful processes and propagating IVDD.


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