SINGLE CELL RNA-SEQUENCE ANALYSES REVEAL UNIQUELY EXPRESSED GENES AND HETEROGENOUS CELLS IN THE RAT MODEL OF INTERVERTEBRAL DISC DEGENERATION

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INTRODUCTION

- Intervertebral disc (IVD) degeneration is a common contributor to back pain, affecting ~ 85% of people[1].
- Multiple cell types exist within healthy IVDs[2], including chondrocyte-like nucleus pulposus cells and annulus fibrosus cells.
- With degeneration, non-resident cells infiltrate the IVD and exacerbate pathobiological processes[3].
- Single-cell RNA sequencing (scRNA-seq) can determine distinct cell populations and transcriptional profile in the healthy and the degenerating IVD.

OBJECTIVE

Define the identities and functions of unique cell populations that infiltrate the IVD following the lumbar disc puncture (LDP) in a rat model.

METHODS

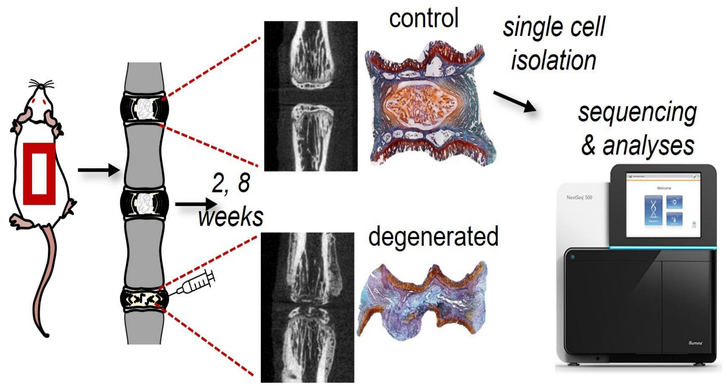


Figure 1. 10X single-cell sequencing workflow

- Rats (n=8, male Sprague-Dawley, 8 weeks) underwent surgery for unilateral lumbar disc puncture at L4-5 & L5-6 IVDs via 27G needle (LDP group), with the L2-3 and L3-4 serving as uninjured, control IVDs (CON group) in the same animal.
- This LDP model reproducibly induces low back pain behavior and sensory neuronal adaptation[4].
- Rats were allowed to recover postoperatively for either 2 (n=4) or 8 weeks (n=4).
- Rats were euthanized, IVD tissues were isolated, and pooled tissues were digested via pronase/collagenase (<4h total) to obtain IVD cells.
- Cell data was reduced via quality control filtering (<10% mitochondrial features, gene counts 500-3,000, unique molecular identifier 500-30,000) for a total of 42,560 and 51,365 cells for CON and LDP samples, respectively.
- cDNA libraries were immediately obtained for each group (LDP/CON) in each animal.
- Each sample was sequenced (10x Genomics, 3' v3.1; Illumina NovaSeq S4) and analyzed as described with use of Partek Flow (Partek, Inc.).

DATA ANALYSES/RESULTS

- Principal components analysis (PCA) was used with K-means clustering to identify optimal separation of cell clusters for each sample.
- Cell clusters were visualized using uniform manifold approximation and projection (UMAP, Figure 2).

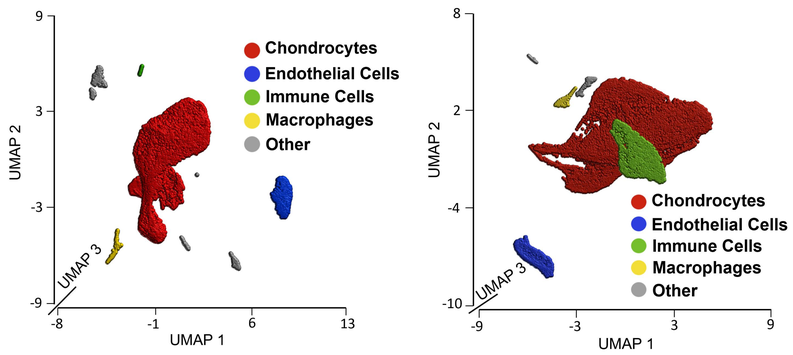


Figure 2. UMAP representation of five clusters for pooled CON (left) and LDP (right) IVDs 2 weeks postoperatively

- For CON and LDP samples, the cell type of each cluster was identified with gene markers from prior scRNA-seq studies of the rat IVD, revealing four distinct cell populations, roughly corresponding to chondrocytes, endothelial cells, macrophages, and immune cells (Table 1)[5].

Cell type	CON	LDP	Gene marker
Chondrocytes	87.74%	82.43%	ACAN, COL2A1, SOX9, FMOD, COMP
Endothelial cells	7.29%	6.41%	CDH5, PLVAP, IFITM3, FLT1
Macrophages	1.01%	1.41%	CD14, MRC1, CD68, LYZ2
Immune cells	0.21%	8.2%	CD79B, BLNK, PTPRC, MYB

Table 1. Summary of genes examined for definition of cell subtypes. Percentages of total cells assigned to each cluster is shown averaged across four rats for CON and LDP samples 2 weeks postoperatively.

- The LDP group revealed similar cell clusters as the CON group. However, the proportion of immune cells increased greatly in LDP samples (8.20%) compared to CON samples (0.210%) 2 weeks postoperatively (Figure 3).

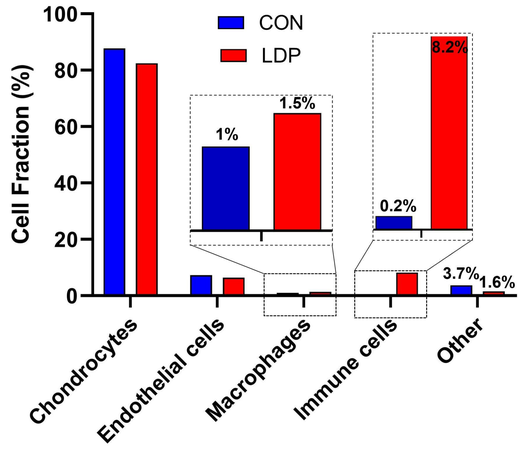
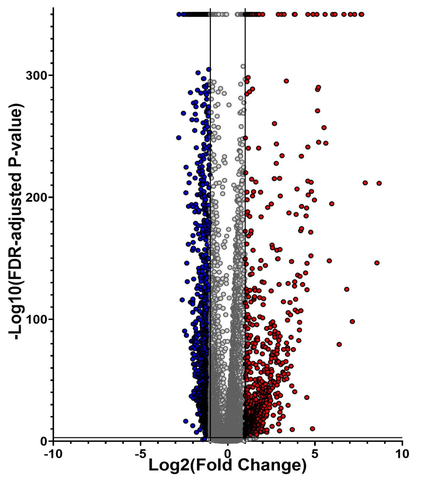


Figure 3. Comparison of distributions of identified cell populations between CON and LDP samples 2 weeks postoperatively.

DATA ANALYSES/RESULTS

- Differential gene expression was analyzed to detect differences between CON and LDP cell populations for each of four rats (Figure 4).
- While the number of genes differentially expressed between LDP and CON differed across rat samples, 60 common genes were found to be upregulated in LDP compared to CON (FDR p-value<0.05, 2 rat samples, 2 weeks postoperatively).
- Common genes were largely associated with the immune cell cluster (Figure 5).
- Gene enrichment analysis (GEA) confirm that the upregulated genes belonged to critical immune processes.

Figure 4. Representative volcano plot depicting differentially expressed genes in LDP vs CON samples from one rat



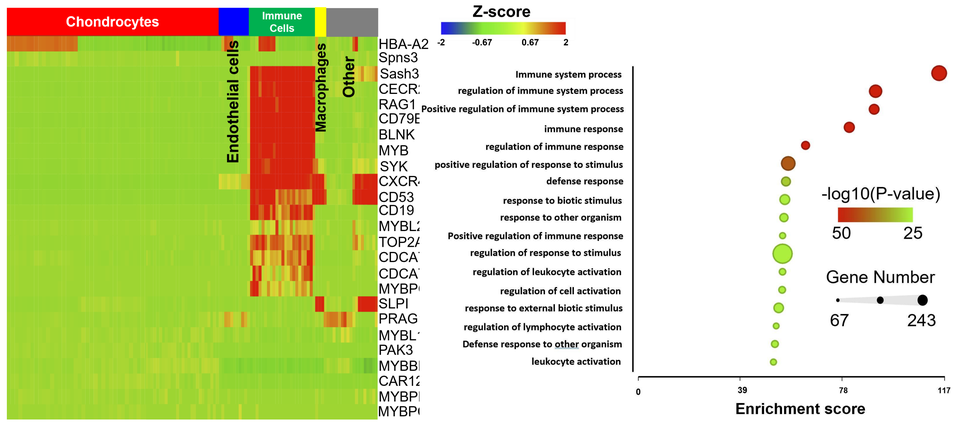


Figure 5. Heat map (left) indicating the source cell clusters and expression levels for a select set of genes commonly upregulated in LDP vs CON samples 2 weeks postoperatively. Representative bubble map (right) indicating gene enrichment analysis of upregulated genes LDP vs CON for one rat.

DISCUSSION

- K-means clustering of five clusters was sufficient to identify four major cell populations in the IVD, namely chondrocytes, endothelial cells, macrophages, and immune cells.
- Phenotypic markers of IVD degeneration were identified and analyzed to be mainly related to immune cell processes.
- The upregulated immune responses are associated with lymphocytes (SASH3), macrophages (CXCR4, CD53) and innate immunity (SLPI).
- Future work will identify critical immune processes that contribute to IVD degeneration and the causal mechanisms for low back pain.

REFERENCES

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