

A Comparative Analysis of Tie2-Positive IVD Progenitor Cells in Single-Cell and Bulk Transcriptomics

Introduction:

Back pain and disability are often attributed to intervertebral disc (IVD) degeneration¹, where current treatments are limited by an incomplete understanding of IVD biology. This study focuses on the Angiopoietin-1 receptor Tie2, which marks a progenitor cell subset in the nucleus pulposus (NP)², crucial for repair and regeneration^{3,4}. We aim to elucidate the transcriptomic profile of these Tie2-positive NP progenitor cells (NPPCs) to understand their role in IVD homeostasis and repair.

Methods:

We utilized single-cell and bulk RNA sequencing to characterize the transcriptomic profiles of Tie2-positive NPPCs from bovine and human IVDs.

For single-cell sequencing, reanalysis was conducted on the dataset published by Calìo et al. (2021)⁵, which included samples comprising pooled cells from three adjacent discs of either distal or proximal region of the bovine coccygeal tail of two biological replicates.

Single-cell suspension was obtained through enzymatic dissociation using Collagenase Type II. Library preparation followed the single-cell 3'-version-3-protocol⁶ and sequencing was performed on the Illumina NovaSeq platform. The sequencing data were aligned to the *Bos taurus* reference genome (UMD-v3.1 Release-92) from Ensembl. Post-sequencing analysis involved cell cluster identification using the Seurat package.

In parallel, bulk RNA sequencing was conducted on human IVD samples. Cells were isolated, and Tie2-positive cells were obtained by subsequent fluorescent activated cell sorting (FACS). These cells were subsequently expanded for one week. We compared Tie2-enriched samples from four healthy individuals, to Tie2-negative samples from three healthy individuals using the NovaSeq system.

Differential expression analysis was carried out using DESeq2 to pinpoint marker genes specific to Tie2-enrichment. Furthermore, we performed Pearson correlation analysis contrasting the expression profiles of each identified single-cell cluster against the average profile of the Tie2-enriched samples, which facilitated the delineation of potential NPPC clusters.

Results:

We identified 14 distinct cell clusters (Fig. 1), underscoring NP cellular heterogeneity. Although not expressing a significant higher expression of the TEK gene, the gene for Tie2, Tie2-positive NPPCs exhibited a unique gene expression profile when compared to Tie2-negative cells, with specific marker genes identified. Through correlation analysis, these NPPCs were associated with specific clusters, suggesting a specialized "niche" in the IVD. Enrichment analysis indicated their involvement in tissue homeostasis and regeneration.

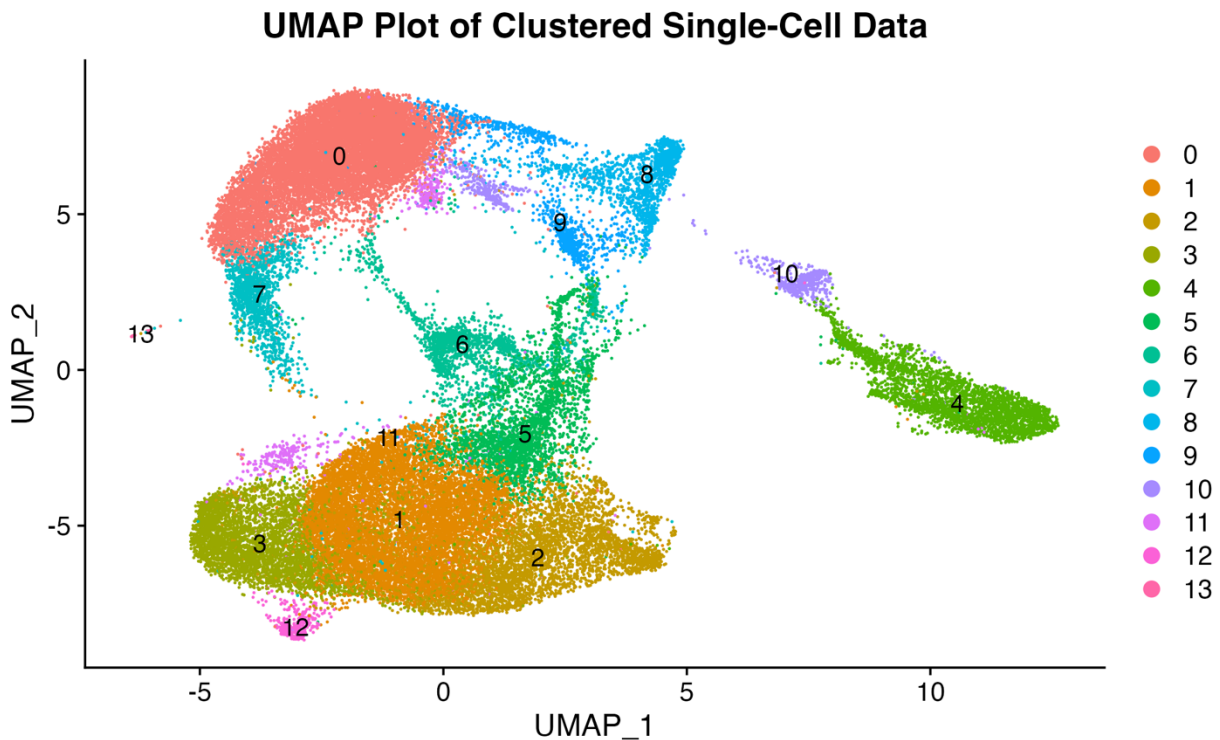


Figure 1: UMAP Plot of Clustered Single-Cell Data. This UMAP plot illustrates 14 distinct cell clusters from integrated single-cell RNA sequencing data of bovine NP cells, highlighting cellular diversity within the NP. Each point represents a single cell, color-coded to indicate cluster assignment.

Discussion:

Our comparative transcriptomic approach has advanced the understanding of Tie2-positive NPPCs in IVD biology. The identified gene signatures and pathways offer insights into their potential roles in IVD repair and interactions necessary for tissue integrity. These findings hold promise for developing targeted therapies to harness progenitor cells for IVD regeneration and could lead to clinical applications pending further validation.

Acknowledgements:

This work was supported by the Swiss National Science Bridge Discovery Project # 40B2-0_211510/1 (<https://data.snf.ch/grants/grant/211510>).

We thank the Functional Genomics Center Zurich (FGCZ) for their expertise and assistance with the RNA sequencing.

References:

1. Brinjikji, W. *et al.* MRI Findings of Disc Degeneration are More Prevalent in Adults with Low Back Pain than in Asymptomatic Controls: A Systematic Review and Meta-Analysis. *Am. J. Neuroradiol.* **36**, 2394–2399 (2015).
2. Lyu, F., Cheung, K. M. C., Zheng, Z., Wang, H., Sakai, D., & Leung, V. (2019). IVD progenitor cells: a new horizon for understanding disc homeostasis and repair. *Nature Reviews Rheumatology*, *15*(2), 102–112. <https://doi.org/10.1038/s41584-018-0154-x>

3. Sakai, D. *et al.* Successful fishing for nucleus pulposus progenitor cells of the intervertebral disc across species. *JOR SPINE* **1**, e1018 (2018).
4. Sakai, D. *et al.* Exhaustion of nucleus pulposus progenitor cells with ageing and degeneration of the intervertebral disc. *Nat. Commun.* **3**, 1264 (2012).
5. Caliò, M., Gantenbein, B., Egli, M., Poveda, L., & Ille, F. (2021). The cellular composition of bovine coccygeal intervertebral discs: A Comprehensive Single-Cell RNASEQ analysis. *International Journal of Molecular Sciences*, *22*(9), 4917.
<https://doi.org/10.3390/ijms22094917>
6. Hagemann-Jensen, M. (2020, February 19). *Smart-seq3 Protocol protocol references*. protocols.io. <https://www.protocols.io/view/smart-seq3-protocol-36wgq5rjxgk5/v3/references>

Keywords:

Intervertebral disc regeneration research, Nucleus pulposus progenitor cells, Angiopoietin-1 receptor Tie2, Single-cell transcriptomics, Bulk transcriptomics

Authors:

Stirnimann A. ^{1,2,3,4}, Schlagenhof L. ^{2,3,4}, Gantenbein B. ^{2,3}, Ille F. ¹

¹ Center of Competence in Aerospace Biomedical Science and Technology, Lucerne University of Applied Sciences and Arts, Hergiswil, Switzerland.

² Tissue Engineering for Orthopaedics and Mechanobiology, Bone and Joint Program, Department for BioMedical Research (DBMR) of the Medical Faculty, University of Bern, Bern, Switzerland.

³ Department of Orthopaedic Surgery and Traumatology, Inselspital, Bern University Hospital, Medical Faculty, University of Bern, Bern, Switzerland

⁴ Graduate School for Cellular and Biomedical Sciences (GCB), University of Bern, Bern, Switzerland