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) degeneration[1], 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 Caliò 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-protocol[6] 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.

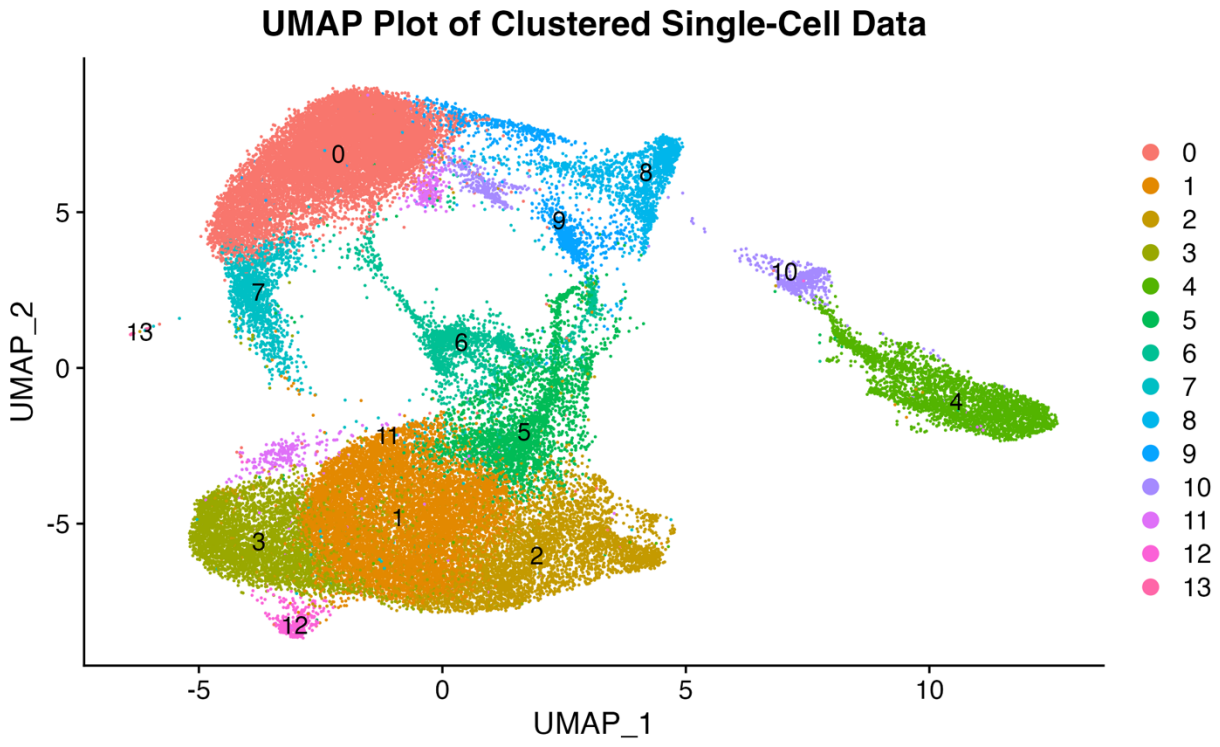

Figure 1: UMAP Plot of Clustered Single-Cell Data. This UMAP plot illustrates 14 distinct cell clusters from integrated single-cell RNA sequencing data of bovine NP cells, highlighting cellular diversity within the NP. Each point represents a single cell, color-coded to indicate cluster assignment.

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.

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Keywords:

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

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