Claude 3.5 Haiku

Results

Single-Nucleus RNA-Sequencing Reveals Neuronal Heterogeneity Following Spinal Cord Injury

To characterize the transcriptional dynamics of neuronal cells after spinal cord injury, we performed single-nucleus RNA-sequencing on neurons sampled from the mid-thoracic spinal cord at multiple time points: uninjured control, and 1, 4, 7, 14 days, 1 month, and 2 months post-injury (Fig. 1). Uniform Manifold Approximation and Projection (UMAP) dimensional reduction revealed distinct clustering patterns that differentiated injured and uninjured neuronal populations (Fig. 1, left panel) and captured the temporal progression of transcriptional changes across injury time points (Fig. 1, right panel).

Differential Expression Analysis Uncovers Time-Dependent Transcriptional Shifts

Using a linear model implemented in Limma, we identified genes with significantly altered expression patterns over time in injured versus uninjured neurons. The top differentially expressed genes demonstrated consistent downregulation across injury progression (Table 1). Notably, the most significantly altered genes included:

1. **Lysosomal Enzymes**: Psap (Prosaposin), Ctsb (Cathepsin B), and Ctsl (Cathepsin L) showed markedly reduced expression, suggesting potential disruptions in cellular degradation and autophagy mechanisms.
2. **Membrane and Trafficking Proteins**: Genes like Sdcbp (Syntenin-1), Rrbp1 (Ribosome Binding Protein 1), and Itm2b (Integral Membrane Protein 2B) exhibited decreased expression, indicating potential alterations in membrane dynamics and intracellular trafficking.
3. **RNA Processing**: Pabpc1 (Poly(A) Binding Protein Cytoplasmic 1) showed reduced expression, potentially impacting mRNA stability and translation.

These consistent expression changes suggest a coordinated transcriptional response to spinal cord injury, characterized by downregulation of genes involved in protein processing, membrane dynamics, and cellular maintenance.

Functional Pathway Enrichment Reveals Molecular Mechanisms

Gene Ontology (GO) enrichment analysis of differentially expressed genes provided deeper insights into the biological processes modulated after spinal cord injury:

Upregulated Pathways:

- Regulation of membrane potential
- Dendrite development
- Vesicle-mediated transport in synapses
- Regulation of synaptic structure and activity

- Postsynaptic organization

Downregulated Pathways:

- Regulation of innate immune response
- Apoptotic signaling pathway
- Alcohol metabolic process
- Response to endoplasmic reticulum stress

The upregulated pathways suggest active neuronal remodeling and synaptic plasticity, while downregulated pathways indicate suppression of inflammatory and stress-related processes.

Biological Implications

Our findings reveal a complex, time-dependent transcriptional response to spinal cord injury. The consistent downregulation of lysosomal enzymes and membrane-associated proteins suggests potential mechanisms of neuronal adaptation and potential vulnerability. The enriched pathways highlight active synaptic remodeling alongside suppression of inflammatory responses, providing a nuanced view of neuronal response to injury.

Limitations: While this analysis provides comprehensive transcriptional insights, further functional validation will be crucial to definitively establish the mechanisms underlying these observed changes.