Multimodal expression analysis of spinal cord injury in rat model

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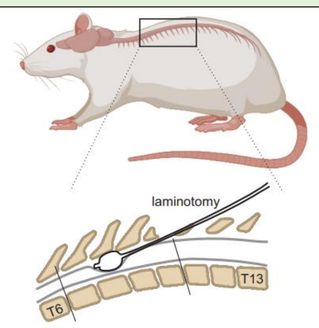
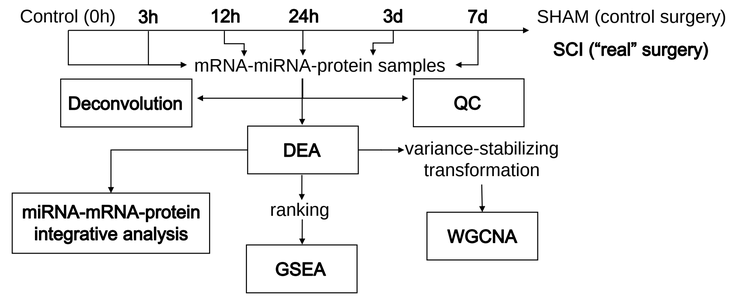
Background & Experimental design

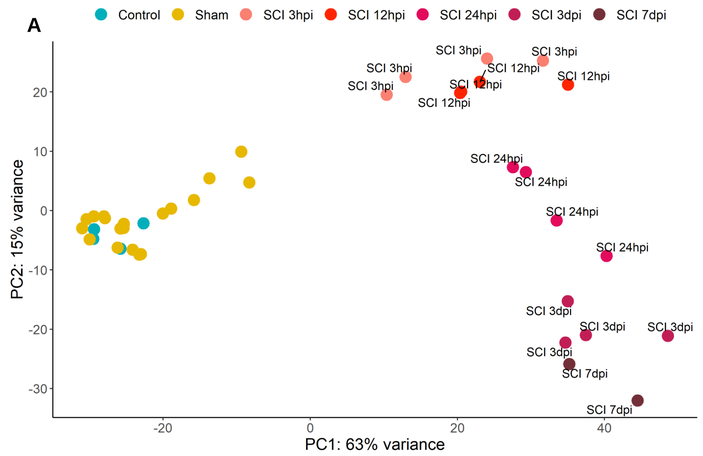
Spinal cord injury (SCI) is a severe and devastating neurological disease with no effective treatment strategy. SCI involves complex interaction of wide spectrum of molecules governing biochemical and physiological processes during the progression of the injury. Although, some progress has been made in characterization of molecular networks underlying pathophysiological changes in SCI, a deeper understanding of these processes is still missing. Here, we performed complex **multiomics analysis** of experimental contusion model of SCI. We collected **RNA-seq**, **smallRNA-seq** and **proteomics** data on *Rattus norvegicus* with experimental SCI at different time intervals (**3, 12, 24 hours** after injury and **3, 7 days after injury**) and compared them with SHAM surgery controls. To analyze genome-wide expression changes, we applied various methods, such as **DEA**, **WGCNA**, **GSEA**, **multimodal network analysis**, as well as analysis of the **cell type proportion**.

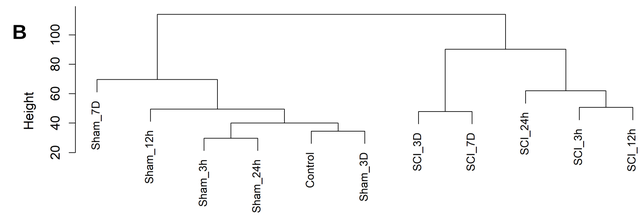


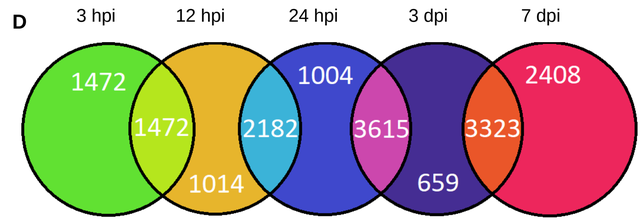


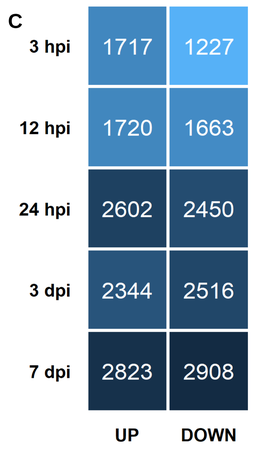
For each time point, 4 samples were taken from each group (SHAM and SCI). Qualitative data analysis and hierarchical clustering of samples were made. After excluding deleted samples, a quantitative analysis was carried out where many unique genes with differential expression were recognized ($\log_2$(fold change) > 0.58; adjusted P-value < 0.05). Computer deconvolution was also performed to display the relative changes in cell types at different time points after SCI. Finally, the analysis of the correlation between miRNA and mRNA expression levels was performed.

Differential Expression Analysis & Quality Control



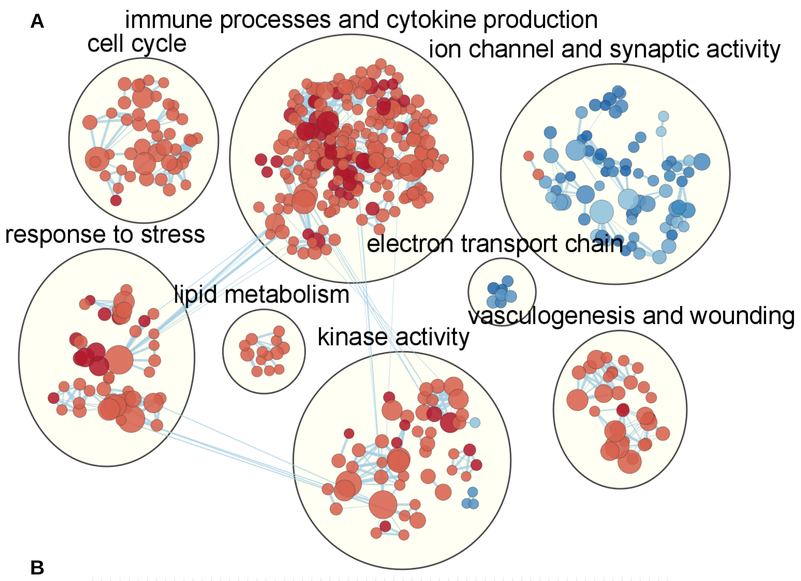


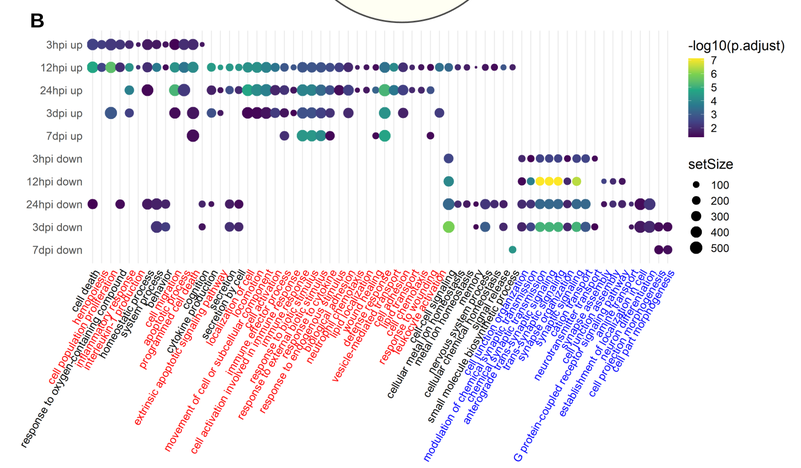




PCA plot (A) obtained as a result of qualitative analysis of data before DEA and **hierarchical clustering (B)** of samples. These two plots show how the samples from the early time points (3, 12, 24 hours) separate from the samples from the later time points (3, 7 days). **The number of differentially expressed genes (C) and their distribution between time points (D)** are shown in the following figures. These data reflect an increase in the total number of DE genes over the time post injury, but there is also a trend towards an increase in the number of genes shared between subsequent time points, with a decrease in the number of unique genes for a specific time point, except for 7 dpi.

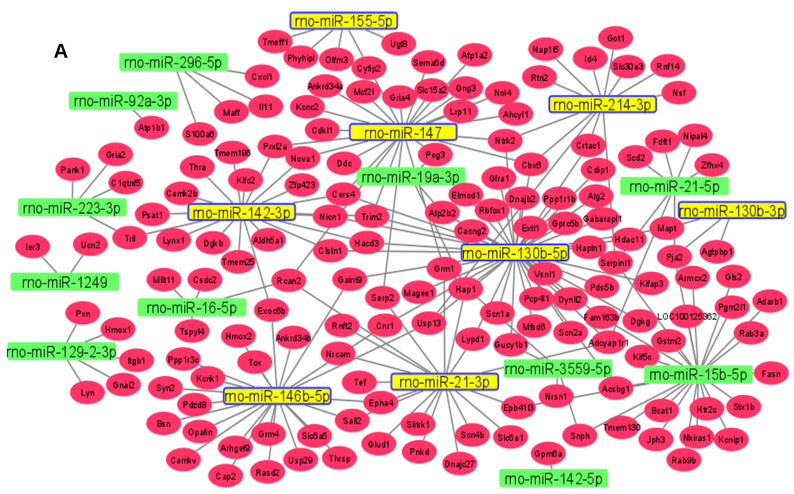
Gene Set Enrichment Analysis

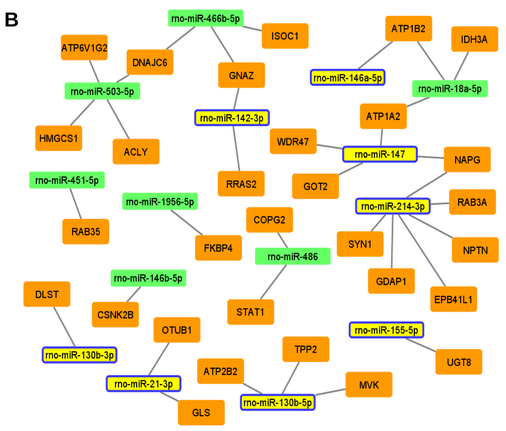




Gene set enrichment analysis reveals biological processes altered upon spinal cord injury in rats. Enriched GO terms are visualized as **network (A)**, with nodes representing enriched GO terms and lines between nodes representing overlapping gene sets. Most upregulated cellular processes include metabolic pathways, extracellular matrix remodeling, activation of cell cycle and process associated with immune response such as cytokine production. Most downregulated genes are associated with transmembrane ion channel transport, synaptic activity and electron transport channel. The processes with the highest significance values are displayed in the **figure (B)**, where the color of the process on the x-axis symbolized the direction of the changes (red - up-regulated, blue - down-regulated, black - in both).

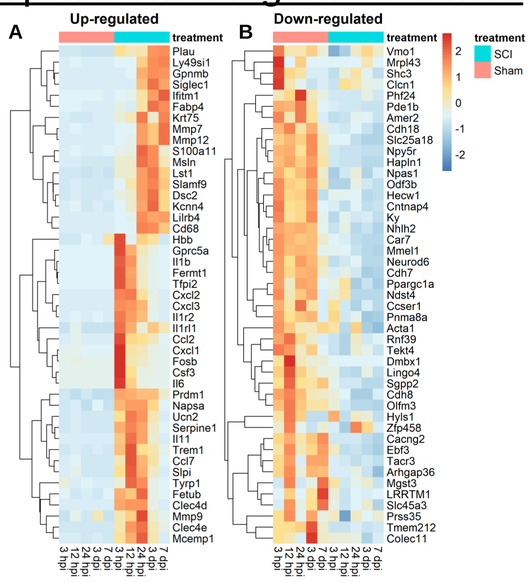
miRNA-mRNA-proteomic Integrative Analysis





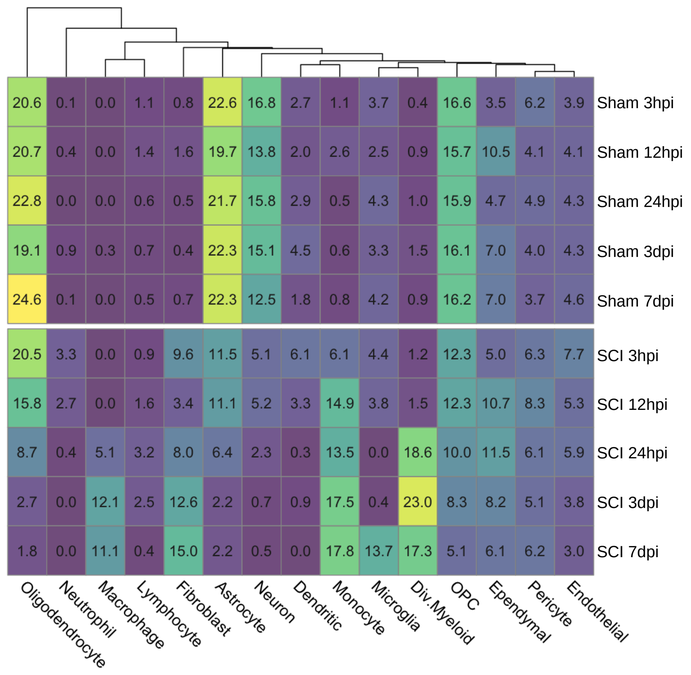
The results of the integration of miRNA-mRNA (A) and miRNA-proteins (B) with each other based on predictions of miRNA targets obtained in **miRWalk.** The results of DEA were used for integration. The threshold parameters for miRNA-mRNA correlation analysis were set as adjusted P-value < 0.05 and r < -0.85. The threshold for miRNA-protein correlation was lower (adjusted P-value <0.05; r - negative). The negative correlation value means that an increase in the amount of miRNA leads to a decrease in its mRNA and/or protein targets. In both figures, miRNAs that are common to both mRNA and proteins are shown in yellow.

Up & Down Regulated Genes



The top-9 genes after DEA for each time point are shown in this figure. The heatmaps were divided using hierarchical clustering into two parts - early and later genes in the spinal cord injury.

Computational Deconvolution



Estimated proportion changes for major cell types between sample groups. Computational deconvolution was performed using CibersortX algorithm to estimate the proportion changes for major cell types based on transcriptomic data from Milich *et al.*, 2021. Plot shows between-group comparisons. Spinal cord injury is accompanied by marked proliferation of microglia and dividing myeloid cells along with neuronal and oligodendrocytes death.

Conclusion and next steps

- SCI is manifested by massive changes in gene expression, resulting in over 9000 differentially expressed genes.
- Transcriptome response is divided into early response (up to 24 hours after injury) and late response (3 and 7 days after injury).
- The early response is associated with cell death, cell migration, angiogenesis. Activation of immune cells begins 12 hours after injury and reaches its peak in 3-7 days. The processes associated with synaptic activity are suppressed up to 3 days after the injury. Starting from 3 days from injury, there is a change in the organism response, which is often referred as a sub-acute stage of injury in the literature (James *et al.*, 2011).
- Transcriptomic changes are partly explained by decrease of oligodendrocytes, astrocytes, and neuronal cells. After injury in the late phase, the level of fibroblasts, macrophages, monocytes, microglia and dividing myeloid cells increases.
- Further, we plan to to find, with the help of other databases and available literature, the key miRNAs playing the role in SCI, their potential targets and characterized processes involved.

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