

Chronic contusion spinal cord injury in rats

Abstract

In this study, publicly available microarray data generated following spinal cord injury in adult rats was analyzed to identify the genes affected by this chronic injury. The data was generated by a study examining gene expression changes resulting from chronic injury using tissue samples 35 days post-injury (Aimone et al., 2004). These injured samples were compared to laminectomy-only controls. This dataset was analyzed with GeneSifter® (VizX Labs, Seattle, WA). This microarray data analysis system was used to discover differentially regulated genes, and map them to candidate biological terms and pathways. Several clear patterns of gene expression were discovered which correlated with genes involved in lipid metabolism and inflammation.

Introduction

The GeneSifter microarray data analysis system was used to analyze data from rat spinal cord samples. Tissue from control rats (laminectomy-only) was compared with samples 35 days post-injury. The analysis methodology can be broken down into two major steps: identification of differentially expressed genes, and the determination of biological significance of both individual genes and groups of genes. GeneSifter accomplishes this by creating Gene Ontology (GO), and KEGG pathway reports. Both reports make use of z-score statistics to identify terms or pathways that are significantly over- or under-represented than expected by chance. This study describes the use of these methods to identify biological themes resulting from chronic spinal cord injury.

Microarray data

Gene expression profiles were measured in rat spinal cord tissue samples from the epicenter of injury using Affymetrix® GeneChip® Rat Genome U34A array containing probe sets for ~7000 genes, and ~1000 ESTs. Three biological replicates were prepared for each time point. Data for each array was downloaded from the NCBI Gene Expression Omnibus (GEO), GSE2599. The MAS5-derived signal and

detection calls were used for subsequent analysis.

Identification of differentially expressed genes

Using filtering criteria of at least 1.5 fold change, all present calls in at least one group, and a Welch's t-test where $p < 0.05$, the raw data was reduced to a list of 707 genes. Applying the method of Benjamini and Hochberg (Reiner, et al., 2003) to estimate a false discovery rate of 5% reduced the list to 619 (analysis parameters are summarized in Table 1). Of this set, 253 genes were upregulated, while the remainder, 366, were downregulated.

Statistical test	Welch's t-test $p < 0.05$
Fold change	1.5
Quality	All present
Multiple testing correction	Benjamini-Hochberg (FDR)

Table 1. Analysis methodology. A summary of the analysis parameters used to filter the raw data. Application of these methods resulted in a list of 619 genes, which were analyzed further for biological significance.

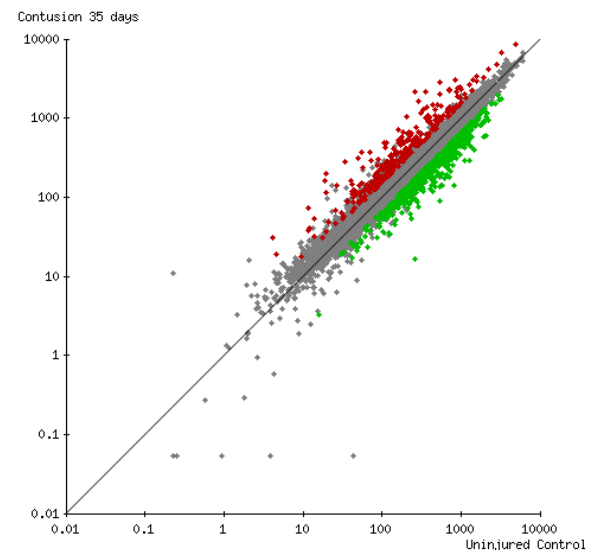


Figure 1. Scatter plot of all data points. Genes that did not pass the filtering criteria are displayed in gray, upregulated genes are shown in red, while downregulated genes are shown in green.

Biological significance

The biological process GO terms and KEGG pathways were examined using a z-score report. The z-score report identifies ontology terms or pathways that are significantly over- or under-represented (Doniger et al., 2003). Among the

A.

Biological Process Ontology	Up	Array	z-up
antigen presentation	4	7	6.61
humoral immune response	7	24	5.73
immune response	23	191	5.02
complement activation	5	18	4.68
neutrophil chemotaxis	3	10	3.82
lipid transport	5	26	3.55
oxygen and reactive oxygen species metabolism	6	41	3.06
lipoprotein metabolism	3	14	2.99
enzyme linked receptor protein signaling pathway	9	80	2.85
lymphocyte proliferation	4	24	2.81
response to oxidative stress	5	35	2.73
proteolysis and peptidolysis	14	155	2.67
chemotaxis	5	40	2.38
angiogenesis	4	29	2.35
induction of apoptosis	5	41	2.31

B.

Biological Process Ontology	Down	Array	z-down
isoprenoid biosynthesis	5	5	9.08
sterol biosynthesis	9	16	8.72
cholesterol biosynthesis	8	15	7.95
lipid biosynthesis	18	75	6.89
negative regulation of microtubule depolymerization	3	4	5.96
steroid biosynthesis	10	39	5.38
cell-cell signaling	26	189	4.9
glycolysis	7	25	4.81
synaptic transmission	21	150	4.47
lipid metabolism	24	209	3.71
regulation of protein kinase activity	3	9	3.57
perception of pain	3	9	3.57
exocytosis	6	33	3.09
carboxylic acid biosynthesis	5	27	2.87
neuropeptide signaling pathway	6	38	2.68
neurogenesis	19	199	2.4
regulation of neurotransmitter levels	7	55	2.25

Table 2. Significant gene ontology terms affected by spinal cord injury. Part A contains the GO terms most prevalent in the 253 upregulated genes. Part B displays the z-scores associated with the downregulated genes. Up/Down column contains the number of genes in each ontology term, "Array" is the number of genes on the array (U34A) that are in the specified ontology. The last column, z-up or z-down, displays the z-score statistic.

upregulated genes, the most significant terms were those associated with immune response, particularly relating to neutrophil function. Also highly over-represented were genes involved in induction of apoptosis and angiogenesis. Several GO terms were also significantly over-represented amongst the downregulated genes including: lipid biosynthesis (cholesterol and other steroids), and regulation of protein kinase activity. A selected list of significant GO terms

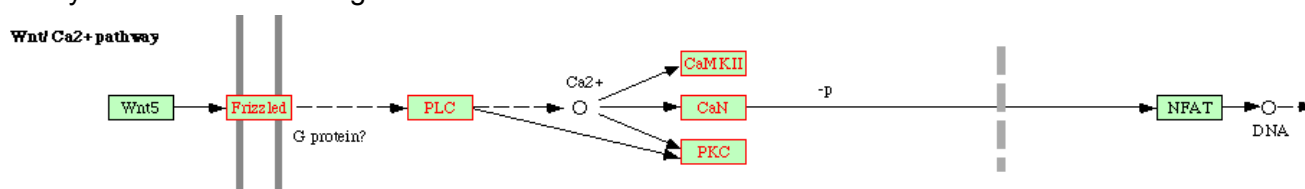


Figure 2. Wnt/Ca²⁺ signaling pathway. Differentially regulated genes in the 35 days post-injury sample are highlighted in red.

for both groups of genes can be found in Table 2 (for a complete list, see supplemental material).

We also examined pathway information for these results using data from the Kyoto Encyclopedia of Genes and Genomes (KEGG). Of particular interest was the Wnt/Ca²⁺ signaling pathway, which showed broad regulation (Figure 2). A complete list of the affected KEGG pathways can be found in the supplemental material.

Conclusions

Microarray analysis of the contused rat spinal cord identified 619 genes that were differentially expressed. The time point examined, 35 days post-injury, suggests that these are chronic changes in response to injury. The GO terms associated with immune response, with several terms implicating neutrophil function, were over-represented among the upregulated genes. Cholesterol synthesis and metabolism was over-represented significantly in the downregulated genes, as was extracellular signaling. These findings help to clarify the complex gene expression changes resulting from chronic spinal injury.

Supplemental Material

Raw data is available from the Gene Expression Omnibus (GSE2599). Data can also be viewed and analyzed using the GeneSifter Data Center (www.genesifter.net/web/DC).

References

- Aimone et al., 2004. Spatial and temporal gene expression profiling of the contused rat spinal cord. *Experimental Neurology* 189(2): 204-221
- Doniger et al., 2003. MAPPfinder: using Gene Ontology and GenMAPP to create a global gene-expression profile from microarray data. *Genome Biology* 4(1): R7
- Reiner et al., 2003. Identifying differentially expressed genes using false discovery rate controlling procedures. *Bioinformatics* 19(3): 368-375