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Screening of key genes associated with contused rat spinal cord with DNA microarray

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Abstract. – OBJECTIVES: To identify key genes and novel potential therapeutic targets for contused spin cord injury through analyzing microarray data.

MATERIALS AND METHODS: Gene expression data set GSE2599 was downloaded from Gene Expression Omnibus, including 3 rat spinal cord injury (SCI) samples and 3 healthy controls. Data pre-treatment and differential analyses were performed with packages of R. Cluster analysis was done with gene expression values to globally present the difference between the two states. Functional enrichment analysis was performed for all the DEGs with DAVID tools. The most up- and down-regulated genes were picked out and their interactors were predicted with String. Pathway enrichment analysis was done with GENECODIS for all the genes in the network.

RESULTS: A total of 227 DEGs were screened out, 132 up-regulated genes and 145 down-regulated genes. Response to wounding, response to organic substance and defense response was the top 3 significant functional terms. APOBEC1 was the most up-regulated gene while HPD was the most down-regulated one. Their interactors were obtained and network was constructed. Pathway enrichment analysis revealed that tyrosine metabolism and other metabolism-related pathways were significantly over-represented.

CONCLUSIONS: A range of DEGs were revealed in present study, which could deepen the understandings about the mechanisms of SCI and guide future researches on treatment development.

Key Words:
Contused rat spinal cord, Differentially expressed gene, Function enrichment analysis, Interaction network, Enrichment analysis.

Abbreviations

BH method = Benjamini and Hochberg method; FDR = False discovery rate; DAVID = Database for Annotation, Visualization and Integrated Discovery; STRING = Search Tool for the Retrieval of Interaction Genes; GO = Gene Ontology; KEGG = Kyoto Encyclopedia of Genes and Genomes; SwissProt: Swiss Protein Database; APOBEC1 = Apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1; A2M = Alpha-2-macroglobulin; HPD = 4-hydroxyphenylpyruvate dioxygenase.

Introduction

Spinal cord injury (SCI) is a severe trauma in central nervous system, which usually results in loss of feeling and motor function. Common SCI comes from traumatic spinal fracture or fracture dislocation, but tumors, vascular lesions of the spine, spinal inflammation can also contribute to it[1]. It presents poor prognosis and high morbidity and, thus, becomes the focus and difficulty in medical research. In order to study its pathogenesis, pathological changes and treatment measures, people develop animal models with good clinical relevance, repeatability, standardized operation, i.e. rat model[2,3].

Many studies have been carried out to elucidate the underlying mechanisms. Neuronal and glial apoptosis after traumatic SCI has been found to contribute to the neurological dysfunction[4,5]. Up-regulation of pro-inflammatory cytokines like IL-1beta, TNF-alpha and IL-6 are also observed, suggesting a role of inflammation in SCI[6]. At the same time, people have been trying to develop treatment methods, which bring hopes to patients with this disease[7]. Implantation of autologous Schwann cells into sites of spinal cord injury to support and guide axonal growth is a preferred choice[8]. Neurotrophic factors can benefit axonal growth[9]. Moreover, other types of cells are also

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taken into consideration. Ankeny et al[10] suggest marrow stromal cells as a potential SCI repair strategy. Biernaskie et al[11] report that skin-derived precursors generate myelinating Schwann cells which promote remyelination and functional recovery after contusion spinal cord injury.

Nevertheless, more studies are needed to deepen the knowledge about the molecular mechanisms, from which gene therapy may be developed[12]. Microarray technology is a powerful tool to explore the global changes in the incidence and development of cancer[13]. Therefore, in present study, gene expression profiles for rat SCI samples were compared with healthy controls to screen out differentially expressed genes (DEGs), which might play important roles in development of SCI. Then bioinformatic tools, like functional enrichment analysis and network analysis, were adopted to further disclose key genes, which held potential for treatment of SCI.

Materials and Methods

Microarray data

Microarray data set GSE2599[14] was downloaded from Gene Expression Omnibus (GEO), including 3 SCI samples and 3 normal controls. Data was collected using GPL85 [RG_U34A] Affymetrix Rat Genome U34 Array. Probe annotation files were also acquired.

Screening of differentially expressed genes (DEGs)

Raw data was converted into recognizable format and missing values were filled up[15]. After data normalization[16], package multtest[17] was chosen for differential analysis. Multiple testing correction was applied with BH method[18]. FDR (false discovery rate) < 0.05 and |logFC| > 1 were set as the cut-offs to screen out DEGs.

Cluster analysis

To globally present the difference in gene expression pattern between SCI and healthy control, cluster analysis was conducted for all the samples[19].

Functional enrichment analysis for the DEGs

Functional enrichment analysis is able to reveal disturbed biological functions based upon DEGs[20]. Therefore, DAVID[21] was chosen in present study and FDR <0.05 was selected as the threshold. DEGs were divided into up- and down-regulated genes before analysis.

Construction of interaction network

Proteins usually interplay with each other to display certain functions[22]. The most up- and down-regulated gene were picked out and then interactors were predicted with String[23], followed by construction of interaction network. String connects major databases and predicts interactions based upon experiments, text mining and sequence homology.

Pathway enrichment analysis for genes in the network

GENECODIS is a web-based tool for functional annotation, which integrates information from GO, KEGG, SwissProt, etc[24]. Therefore, it was selected for pathway enrichment analysis for all the genes in the network and adj $p < 0.05$ was set as the cut-off.

Results

Differentially expressed genes

Normalized gene expression data are shown in Figure 1A and a good normalization performance was obtained. A total of 227 DEGs were screened out, 132 up-regulated genes and 145 down-regulated genes.

Cluster analysis result

The cluster analysis result is shown in Figure 1B. SCI samples could be easily distinguished from the healthy controls as obvious differences existed in the gene expression pattern.

Functional enrichment analysis result

The results are shown in Figure 2. Response to wounding, response to organic substance and defense response were the top 3 significantly over-represented terms. There terms were closely related with SCI, proving the confidence of our findings.

Interaction network

APOBEC1 and HPD were the most up- and down-regulated gene, respectively (Figure 3). Interactors of these two genes were retrieved by String and then network was constructed (Figure 4), comprised of 73 interactions among 20 genes. Details were listed in Table I.

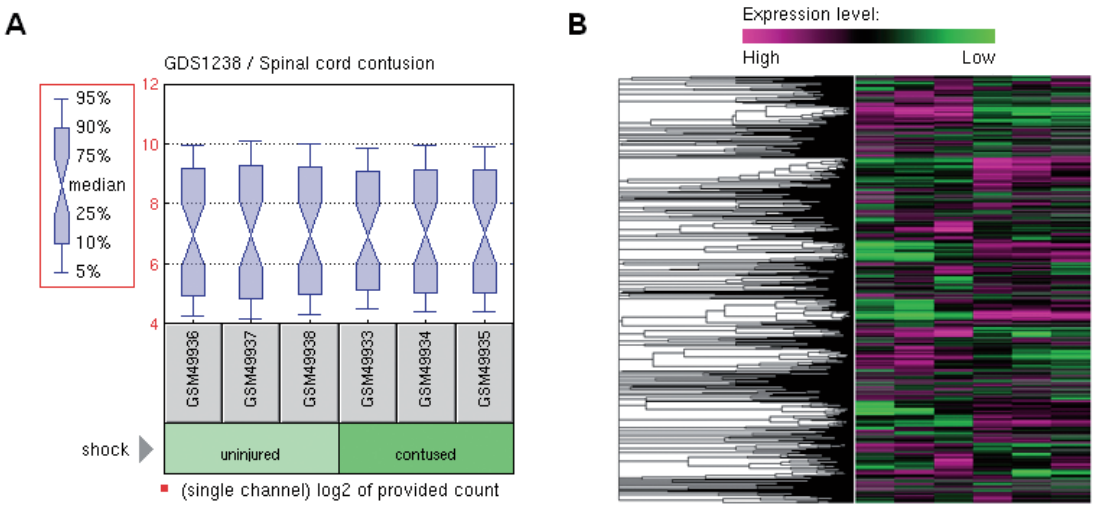

Figure 1. Box plot for normalized gene expression data and cluster analysis result. ***A,*** Box plot for gene expression data. The medians are almost at the same level, indicating a high performance of normalization. ***B,*** Cluster analysis result for gene expression data. The expression values Clustering Figure Red indicates over-expression while green for under-expression.

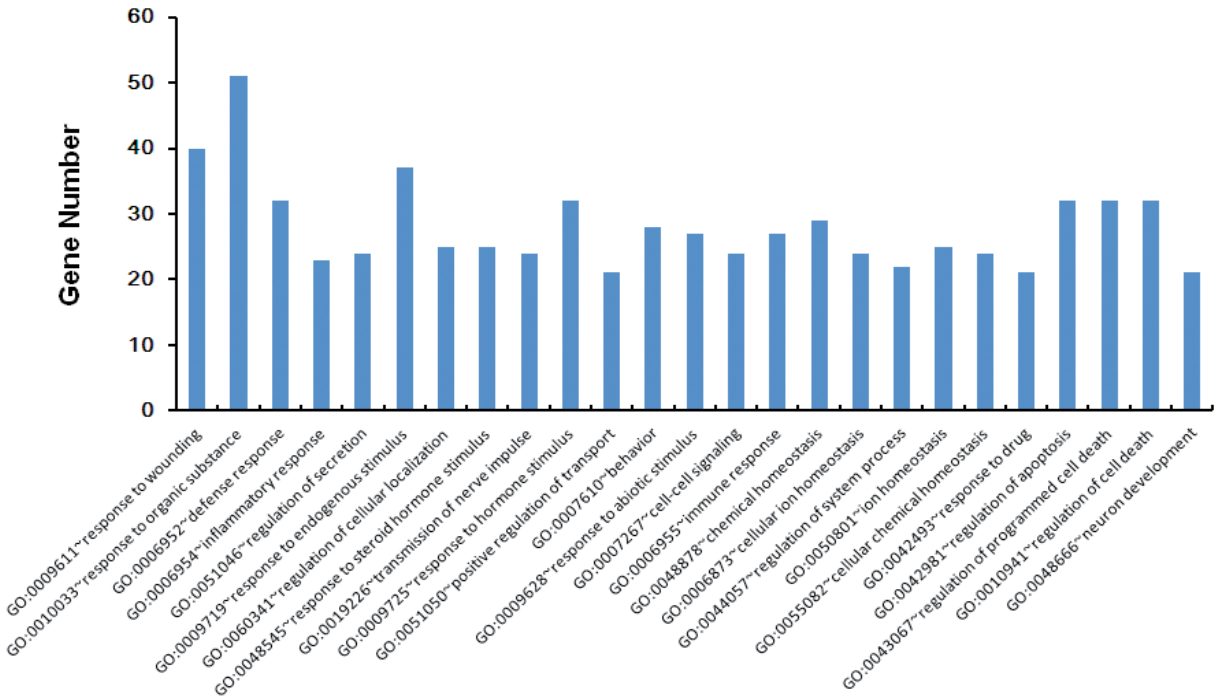

Figure 2. Functional enrichment analysis result for DEGs.

Pathway enrichment analysis result

Four functional terms were over-represented in the network and all of them were associated with metabolism. Tyrosine metabolism was the most significant one and HPD was also included in this term.

Discussion

In present study, gene expression data for SCI samples were compared with those for healthy controls and a range of DEGs were identified. Functional enrichment analysis showed that re-

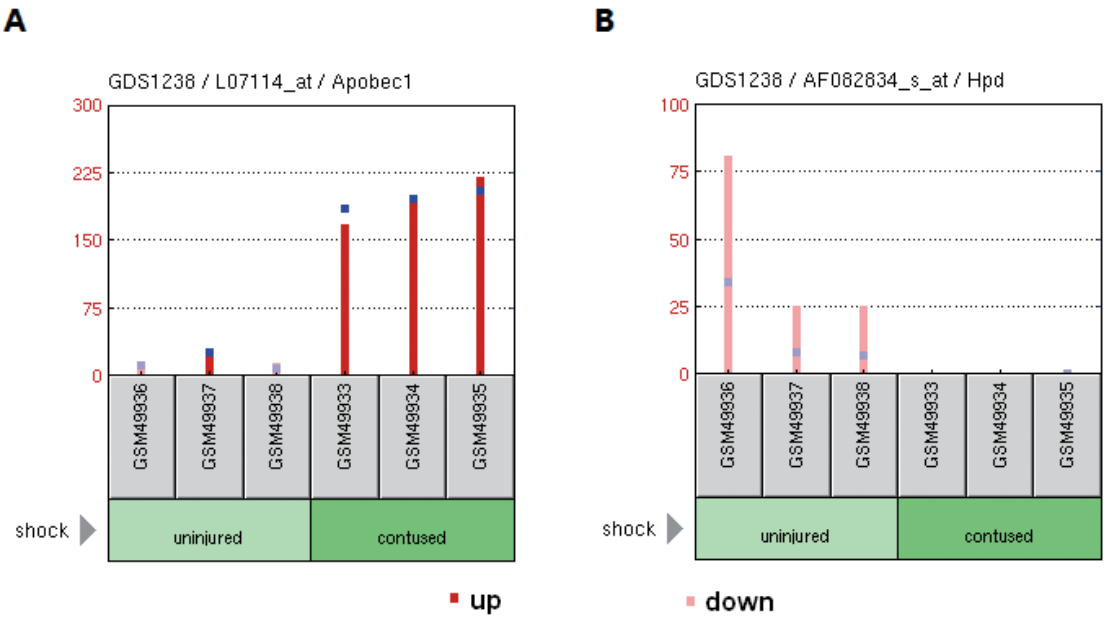

Figure 3. Gene expression values in each sample for APOBEC1 ***(A)*** and HPD ***(B)***.

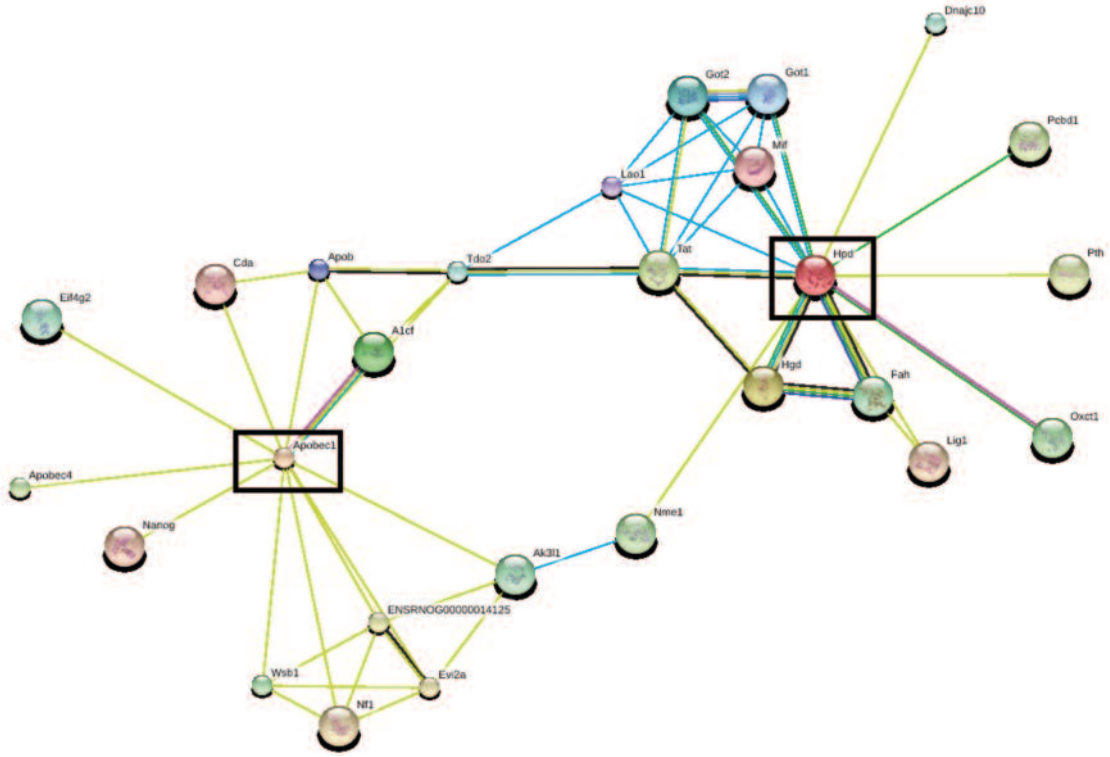

Figure 4. Interaction network for APOBEC1 and HPD.

sponse to wounding was most significantly over-represented. A considerable number of genes were associated with inflammatory response, which was in accordance with previous studies[25,26]. Klusman and Schwab find that administration of pro-inflammatory cytokines (IL-1 beta, IL-6 and INF alpha) to mice after SCI can reduce the amount of tissue loss[27], indicating the role of inflammation in

Table I. Pathway enrichment analysis result for genes in the network.

Pathway	Test	Genes
Tyrosine metabolism	O = 8; adj p = 1.99e-18	MIF, GOT1, LAO1, TAT, HGD, FAH, HPD, GOT2
Phenylalanine metabolism	O = 6; adj p = 1.58e-15	MIF, GOT1, LAO1, TAT, HPD, GOT2
Phenylalanine, tyrosine and tryptophan biosynthesis	O = 4 ; adj p = 1.94e-12	GOT1, LAO1, TAT, GOT2
Cysteine and methionine metabolism	O = 4; adj p = 1.20e-08	GOT1, LAO1, TAT, GOT2

O: number of genes in the pathways; adj *p*: *p* values after multiple testing correction.

SCI is rather complex. More and more evidence show that early inflammatory responses may participate in secondary injury processes while delayed inflammatory events may be reparative[28]. Our findings might provide molecular targets to modulate the inflammatory response.

Alpha-2-macroglobulin (A2M) is a protease inhibitor and cytokine transporter, i.e. IL-6[29]. It has been confirmed to be a modulator of hemostatic and inflammatory reactions[30]. It might stimulate the inflammatory response as it was up-regulated in SCI. However, according to Bai et al[31], it can bind to nerve growth factor and, thus, neutralize neuroprotection during glaucoma. Therefore, its expression should be accurately controlled. Serpin peptidase inhibitor, clade G (C1 inhibitor), member 1 (SERPING1) is a highly glycosylated plasma protein involved in the regulation of the complement cascade[32]. Fibronectin 1 (FN1) is involved in cell adhesion and migration processes such as wound healing. It exists in a soluble dimeric form in plasma and diffuses into lesions as intramedullary hemorrhages occur[33,34]. It may work as an indicator to reflect the degree of injury. However, Sroga et al[35] indicate that rats and mice exhibit distinct inflammatory reactions after spinal cord injury, therefore, it should be cautious to interpret these findings.

Apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1 (APOBEC1) was the most up-regulated gene while 4-hydroxyphenylpyruvate dioxygenase (HPD) was the most down-regulated gene. APOBEC1 is a member of the cytidine deaminase enzyme family[36] and HPD is an enzyme in the catabolic pathway of tyrosine[37]. Pathway enrichment analysis indicated that genes in their interaction network were involved in metabolisms like tyrosine metabolism and phenylalanine metabolism.

Secondary injury is featured by a series of alterations in intracellular metabolism and gene expression, such as release of excitatory amino acids[38] and lipid peroxidation[39], which eventually lead to apoptosis of nerve cells[40]. Liu et al[41] indicate that excitatory amino acids rise to toxic levels upon impact injury to the rat spinal cord. Accordingly, we found that metabolisms about amino acids like tyrosine, phenylalanine, tryptophan, cysteine and methionine were enriched in the DEGs included in the interaction network. Tyrosine nitration can serve as an indicator for nitric oxide (NO)-mediated oxidative inflammatory reactions. Spinal ischemia, hypoxia-induced oxidative stress and inflammatory responses lead to a number of superoxide anion and it reacts with NO and then generate ONOO-ion, which further react with tyrosine and finally generate nitrotyrosine[42]. It could be speculated that tyrosine nitration results in conformational changes in proteins and thus contributes to the disturbation of normal biological functions. Therefore, HPD might be a potential target to modulate these metabolisms and thus reduce the degree of injury.

Conclusions

Taken together, a range of DEGs were obtained through comparing gene expression profiles of rat SCI with those of healthy controls. These genes might play important roles in the development of SCI according to the functional enrichment analysis. Of course, more researches are needed to exploit their potentials in clinical applications.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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