Analysis of key genes and modules during the courses of traumatic brain injury with microarray technology

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ABSTRACT. Gene expression data acquired at different times after traumatic brain injury (TBI) were analyzed to identify differentially expressed genes (DEGs). Interaction network analysis and functional enrichment analysis were performed to extract valuable information, which may benefit diagnosis and treatment of TBI. Microarray data were downloaded from Gene Expression Omnibus and pre-treated with MATLAB. DEGs were screened out with the SAM method. Interaction networks of the DEGs were established, followed by module analysis and functional enrichment analysis to obtain insight into the molecular mechanisms. A total of 39 samples at six time points (30 min, 4, 8, 24, 72 h, and 21 days) were analyzed and generated 377 DEGs. Eight modules
were identified from the networks and network ontology analysis revealed that cell surface receptor-linked signaling pathway, response to wounding and signaling pathway were significantly overrepresented. Altered risk genes and modules in TBI were uncovered through comparing the gene expression data acquired at various time points. These genes or modules could be potential biomarkers for diagnosis and treatment of TBI.

**Key words:** Traumatic brain injury; Time series; Risk genes; Network modules

**INTRODUCTION**

Traumatic brain injury (TBI), also known as intracranial injury, is a type of brain injury caused by external pressure, such as shock, explosion (Rehman et al., 2008) infection, or stroke. It can be divided into closed injury and open injury (Maas et al., 2008). Due to limitations of current technologies, it is not possible to detect temporary or permanent loss of function as well as structural damage (Parikh et al., 2007). Therefore, discovery of biomarkers indicating the progression of TBI is rather necessary.

A number of studies have been carried out to describe or disclose the changes. Long et al. (2003) found 253 differentially expressed genes (DEGs) in traumatically injured mouse hippocampus. Michael et al. (2005) analyzed the gene expression profile of TBI in humans and identified 104 DEGs. Besides, microRNA expression profile is also taken into consideration (Lei et al., 2009). Clear progression has also been made in the treatment of TBI. To date, neuroprotection has been regarded as an important part of the treatment of TBI (Faden, 2002). Di Giovannì et al. (2005) reported that cell cycle inhibition is an effective way to decrease both neuronal cell death and reactive gliosis after experimental TBI.

However, the process of TBI is dynamic, which involves a range of genes working at different times. Therefore, microarray time-course experiment is rather favorable and necessary in this field. It can not only detect thousands of genes, but also reflects the dynamic changes in gene expression. There are several methods to analyze the data, such as STEM (short time-series expression miner) (Ernst et al., 2005; Ernst and Bar-Joseph, 2006) GeneTS (Schafier and Strimmer, 2005; Opgen-Rhein and Strimmer, 2007), TANOVA (time-course analysis of variance) (Zhou et al., 2010) and GQL (graphical query language) (Costa et al., 2005), and these methods have their advantages and disadvantages. Here, we propose a modular analysis method, combining protein-protein interaction information and DEGs, to identify significant disturbed modules associated with TBI, followed by functional enrichment analysis to reveal their biological functions and uncover the underlying mechanisms in the progression of TBI. Our findings can help in developing new ways of diagnosis and treatment of TBI.

**MATERIAL AND METHODS**

**Microarray data**

The microarray data set GSE2392 (Natale et al., 2003) was obtained from Gene Ex-
expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/). Raw data were collected with information of the platform GPL85 (Affymetrix Rat Genome U34 Array). Totally, 39 mouse samples were included at six time points: 30 min, 4, 8, 24, 72 h, and 21 days after moderate levels of lateral fluid percussion-induced brain injury in rats. MATLAB was chosen for data pre-treatment and the average value was given for probes corresponding to one gene. Missing values were filled in with the K-means method.

**Screening of DEGs**

Differential analysis was conducted using the SAM (significance analysis of microarrays) method (Tusher et al., 2001) in MEV (MultiExperiment Viewer) (Saeed et al., 2003). Delta of 2 was set as the cut-off criterion to determine DEGs.

**Establishment of interaction networks**

Collaborations between genes and pathways are the basis of biological functions. Similarly, pathogenesis of disease can be attributed to interactions between a number of genes. Therefore, interaction networks of DEGs were established, from which global alterations were observed and investigated. First, information about protein-protein interaction was downloaded from I2D (http://ophid.utoronto.ca/ophidv2.204/) (Brown and Jurisica, 2005, 2007). Next, self-interactions and duplicates were removed and UniProtKB was converted to Entrez Gene ID with ID mapping in Uniprot (Apweiler et al., 2004). To extensively mine disease-related modules, loose criteria were applied in the present study to filter interactions.

**Module mining and functional annotations**

The network was visualized with Cytoscape (Shannon et al., 2003; Smoot et al., 2011). The topological property was analyzed with its plug-in Network Analysis. The modules were then mined with MCODE and functional annotations were given by network ontology analysis (NOA, http://app.aporc.org/NOA/index.html) (Wang et al., 2011).

**RESULTS**

**Identification of DEGs**

Differential analysis was conducted with the SAM method for gene expression profiles obtained at 6 time points in TBI, and Delta = 2 was chosen as the threshold (Figure 1). A total of 377 DEGs were identified, 235 upregulated and 142 downregulated (Table 1). Only a few DEGs were obtained at the first and last time points, while the number of DEGs was the highest at 8 h (Figure 2). This was in accordance with clinical observation, suggesting that our results were highly reliable.
Biomarker screening for traumatic brain injury

**Figure 1.** Differentially expressed genes (DEGs) screened out by SAM method. Downregulated DEGs were in green, upregulated in red and non-DEGs in black.

**Figure 2.** Interaction network for DEGs at 4-h circles represent genes and edges represent interactions = upregulated in red and downregulated in blue.

**Table 1.** Number of DEGs at different time points of TBI.

<table>
<thead>
<tr>
<th>Time point</th>
<th>Upregulated</th>
<th>Downregulated</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 h</td>
<td>10</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>4 h</td>
<td>25</td>
<td>3</td>
<td>28</td>
</tr>
<tr>
<td>8 h</td>
<td>145</td>
<td>103</td>
<td>248</td>
</tr>
<tr>
<td>24 h</td>
<td>35</td>
<td>23</td>
<td>58</td>
</tr>
<tr>
<td>72 h</td>
<td>20</td>
<td>13</td>
<td>33</td>
</tr>
<tr>
<td>21 days</td>
<td>10</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>235</td>
<td>142</td>
<td>377</td>
</tr>
</tbody>
</table>

DEGs = differentially expressed genes; TBI = traumatic brain injury.
Interaction networks for DEGs

On the basis of the information collected from I2D, 6 interaction networks were established for DEGs at various time points. The topological properties were calculated and are shown in Table 2.

<table>
<thead>
<tr>
<th>Time point</th>
<th>No. of nodes</th>
<th>No. of edges</th>
<th>No. of upregulated edges</th>
<th>No. of downregulated edges</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 h</td>
<td>317</td>
<td>358</td>
<td>358</td>
<td>0</td>
</tr>
<tr>
<td>4 h</td>
<td>244</td>
<td>252</td>
<td>220</td>
<td>32</td>
</tr>
<tr>
<td>8 h</td>
<td>2066</td>
<td>4542</td>
<td>3059</td>
<td>1483</td>
</tr>
<tr>
<td>24 h</td>
<td>579</td>
<td>1031</td>
<td>903</td>
<td>124</td>
</tr>
<tr>
<td>72 h</td>
<td>512</td>
<td>616</td>
<td>402</td>
<td>214</td>
</tr>
<tr>
<td>21 days</td>
<td>270</td>
<td>281</td>
<td>281</td>
<td>0</td>
</tr>
</tbody>
</table>

Results of module mining

Modules in the network were mined with MCODE of Cytoscape and several significant modules were acquired at 8 and 72 h. Two typical modules obtained from time point 8 h are shown in Figure 3.

Functional enrichment analysis results for modules

The functions of modules were analyzed with NOA and P less than 0.05 was set as the cut-off. For module 1, cell surface receptor-linked signaling pathway (GO:0007166), response to wounding (GO:0009611) and signaling pathway (GO:0023060) were significantly overrepresented.

DISCUSSION

Changes in gene expression and subsequently in pathways occur during the progression of TBI. Systematic evaluation of the alterations is helpful in disclosing the mechanisms as well as identifying biomarkers. GSE2392 was first used to compare gene expression patterns...
of lateral fluid percussion-induced brain injury in rat and lateral controlled cortical impact injury in mice in 2003 (Natale et al., 2003), and the main results were the identification of candidate molecular functions and pathways induced by brain injury. In the present study, we combined differential analysis and module mining of the DEG interaction networks to analyze the microarray data of lateral fluid percussion-induced TBI in rats. Several modules were obtained at time points 8 and 72 h. Functional enrichment analysis of the mined modules revealed that cell surface receptor-linked signaling pathway, response to wounding and signaling pathway were significantly changed. A range of interesting DEGs were also uncovered, which may be potential biomarkers.

Signal transducer and activator of transcription 3 (STAT3) is a member of the STAT protein family. It acts as a transcription activator in response to cell stimuli and thus plays a key role in many cellular processes such as cell growth and apoptosis (Grandis et al., 2000; Abell et al., 2005). Inhibition of STAT3 expression induces apoptosis in certain cancers, such as breast cancer (Kunjigal et al., 2009) and prostate cancer (Mora et al., 2002). It’s involvement in the protection and regeneration of neurons has been reported (Xi et al., 2003). Kretz et al. (2005) found that erythropoietin promotes regeneration of adult neurons via the activation of the Jak2/Stat3 and PI3K/AKT pathways. It also regulates astrogliosis and scar formation after spinal cord injury (Herrmann et al., 2008). We found that it was upregulated at 8 h, suggesting that apoptosis was repressed at that time. Maybe it can be used to monitor the renewal process of neurons.

Signal transducer and activator of transcription 5B (STAT5B) is another member of the STAT protein family and it was also upregulated at 8 h after TBI. It exhibits similar functions as STAT3 and is implicated in diverse biological processes, such as TCR signaling, apoptosis and adult mammary gland development. It is believed that constitutive activation of Stat5b contributes to carcinogenesis in vivo (Xi et al., 2003). Inhibition of STAT5b suppresses proliferation of human glioblastoma multiforme cells, and thus, it may serve as a therapeutic target (Liang et al., 2009). It can be activated by erythropoietin and thus mediates proliferation signaling (McPherson and Juul, 2007).

Chemokine (C-C motif) receptor 5 (CCR5) is a member of the beta chemokine receptor family. It is involved in inflammation (Qin et al., 1998; Bing et al., 2006) and thus plays a role in the pathogenesis of brain injury and neurodegenerative disorders (Whitney et al., 2009). It has been found to be upregulated in TBI (Schmidt et al., 2004) and this was confirmed in the present study. Galasso et al. (1998) reported that excitotoxic brain injury stimulates expression of the chemokine receptor CCR5 in neonatal rats. Its ligands include monocyte chemoattractant protein 2 (MCP-2) macrophage inflammatory protein 1 alpha (MIP-1 alpha), macrophage inflammatory protein 1 beta (MIP-1 beta), and so on. Besides, it can mediate the migration of mesenchymal stem cells to the impaired site in the brain (Ji et al., 2004a). It has been found that chemokine receptors are widely expressed in neural progenitor cells (Ji et al., 2004b). Carbonell et al. (2005) reported that pharmacological blockade of CCR5 reduces the migration velocity of perilesional microglia (Carbonell et al., 2005).

Janus kinase 2 (JAK2) is a protein tyrosine kinase. Its upregulation may be beneficial to the injured cortex; for example, it interacts with STAT3 and mediates the neuroprotection by erythropoietin (Kretz et al., 2005; Grasso et al., 2007).

Moreover, functional enrichment analysis was also conducted for DEGs at 4 h. It showed that upregulated genes were enriched in terms of negative regulation of protein amino acid phosphorylation and phosphorus metabolic process, and downregulated genes were en-
riched in the epoxygenase P450 pathway and several metabolic processes. Previous studies have indicated the close relationship between TBI and changes in amino acid neurotransmitters. Schumann et al. (2008) found that inhibition of NR2B phosphorylation restores alterations in N-methyl-D-aspartate (NMDA) receptor expression and improves functional recovery following TBI in mice. Spaethling et al. (2012) reported that NMDA receptor-mediated phosphorylation of GluR1 subunits contributes to the appearance of calcium-permeable AMPA receptors after mechanical stretch injury. Bhardwaj et al. (2000) concluded that P-450 epoxygenase and NO synthase pathways are involved in the local cerebral blood flow response to NMDA receptor activation. We found here that the epoxygenase P450 pathway was repressed, which is in accordance with previous findings.

CONCLUSION

Overall, differential analysis combined with module mining was adopted in the present study to identify TBI-related key modules. Functional enrichment analysis further described their biological functions in the progression of TBI. A number of novel and known significantly altered genes and pathways were identified. These findings provide directions for future research, and some of them can be developed into biomarkers for diagnosis or treatments.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES


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