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Bioinformatics analysis of gene expression profiles in the rat cerebral cortex following traumatic brain injury

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Abstract. – BACKGROUND: Traumatic brain injury (TBI) is a serious neurodisorder commonly caused by sports related events or violence. It is the leading cause of disability in people under 40.

AIM: In order to elucidate the molecular mechanism of the secondary injury after TBI.

MATERIALS AND METHODS: In this study, we downloaded gene expression profile on TBI model with sham controls for gene set enrichment analysis and pathway analysis.

RESULTS: At a q-value of 5%, 361 genes were up-regulated and 373 were down-regulated in samples obtained at 48 hours after TBI. Functional analyses revealed that steroid biosynthesis, cell cycle, metal ion transport, inflammation and apoptosis were significantly dysregulated during the late period after trauma. In addition, MAPK3 (mitogen-activated protein kinase 3), was identified as the hub node in the protein-protein interaction (PPI) network constructed by the differentially expressed genes (DEGs).

CONCLUSIONS: Further elucidation of genes and proteins in our study may reveal their potential as novel therapeutic targets.

Key Words:
Traumatic brain injury, Molecular mechanism, Gene expression profile, Bioinformatics analysis.

Introduction

Traumatic brain injury (TBI) is a serious neurodisorder commonly caused by sports related events or violence[1]. It is the leading cause of disability in people under 40, and severely disables 150-200 people per million annually[2,3]. TBI is primarily characterized by impaired neurological function and severe neurostructural damage[4]. TBI survivors often suffer from a wide range of post-traumatic deficits, including impairments in behavioral, cognitive, and motor function[5]. So, many researchers studied on TBI from different points of view to find a good therapy; however, little success clinically has achieved.

Our understanding of the molecular mechanisms of TBI has improved over the last decades. There is a complex cascade of cellular inflammatory response following TBI which propagates secondary brain damage. Major molecules, such as growth factors, catecholamines, neurokinins, cytokines and chemokines, involve in this cascade[6]. Studies in experimental TBI model using cDNA microarray hybridization technique have shown differential regulation of hundreds of genes which take part in the physiological and pathological response to TBI[7-11], such as interleukin-1 (IL-1), IL-6, tumor necrosic factor (TNF)-α, caspase-1 and -3, and haemoxygenase-1 (HO-1). However, molecular research into TBI is still needed because of the high complexity of the brain and its neuronal circuits.

In this study, we downloaded the microarray data on TBI model with sham controls from Gene Expression Omnibus (GEO) database and analyzed the altered gene expression profile and abnormal biological processes. Further elucidation of genes and proteins in our study may reveal their potential as novel therapeutic targets.

Materials and Methods

Microarray Data

The microarray data on TBI (GSE24047) were downloaded from Gene Expression Omnibus

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(GEO) database. This dataset was deposited by Shojo et al[8]. Rats were transcardially perfused with physiological saline under general anesthesia at 3, 6, 12 and 48 h after moderate fluid percussion (n = 3 per group; total = 12) or the sham operation (n = 1; total = 4). Microarray analyses were performed using GeneChip rat genome 230 2.0 platform. We downloaded the raw data and normalized the gene expression intensities by using robust multi-array average (RMA) algorithm[12].

Identification of Differentially Expressed Genes (DEGs)

The gene expression levels of each time point were compared with those of its preceded time points. As a result, the following pair-wised comparisons were generated: 3 h versus sham; 6 h versus 3 h; 12 h versus 3 and 6 h; and 48 h versus 3, 6 and 12 h. The *t*-test was used to compute the probability of genes being differentially expressed for each comparison. The resulting *p*-values were submitted to q-value package[13] in R to estimate the q-value (false discovery rate). Genes with q-value < 5% were considered as significantly differentially expressed.

Gene Set Enrichment and Pathway Analysis

DAVID (The Database for Annotation, Visualization and Integrated Discovery) is a program that checks for an enrichment of genes with specific Gene Ontology (GO), Kyoto Encyclopedia Genes and Genomes (KEGG), and SwissProt terms[14]. To understand the biological significance of DEGs, we inputted the up-regulated and down-regulated genes into DAVID respectively for GO and KEGG term enrichment analyses. Redundant/similar/hierarchical annotation terms were grouped into functional annotation clusters by fuzzy clustering.

Gene set enrichment analysis (GSEA) focus on groups of genes that share common biological function, chromosomal location, or regulation[15]. To find biological processes or pathways which cause global mRNA perturbation, GSEA was performed using all genes that were ranked by their difference in expression between the 48 h group and the remaining groups. Entrez gene IDs (identifiers) for rat were first mapped to human Entrez gene IDs according to orthologous relationships from MGD (The Mouse Genome Database), which is the international community resource for integrated genetic, genomic and biological data about the laboratory mouse[16]. Random gene sets were generated for estimating the q-value. As recommended, only gene sets with q-value < 25% were considered as significantly enriched.

Protein-Protein Interactions (PPIs) Analysis

STRING (Search Tool for the Retrieval of Interacting Genes) is an online database which provides uniquely comprehensive coverage and ease of access to both experimental as well as predicted PPI information[17]. To identify protein-protein interactions that may play roles in post-injury brain biology, we submitted the DEGs to STRING. In our analysis, we only extracted those experimentally validated interactions with a confidence score > 0.4.

Results

Identification of Differentially Expressed Genes

At a q-value of 5%, few genes were differentially expressed in the 3 h versus sham group, 6 h versus 3 h group as well as 12 h versus 3 h and 6 h group. In fact, only CCL was identified as significantly up-regulated gene in the 3 h versus sham group.

On the other hand, 361 genes were significantly up-regulated in the 48 h group compared the pooled 3, 6 and 12 h group and 373 genes were down-regulated. Given the significant difference in gene expression profile of 48 h group compared with other groups, our further analysis focused on the 48 h group. We denoted samples obtained at 48 hours after TBI as the late post-TBI group and remaining samples as early post-TBI group.

GSEA and Pathway Analysis

At a q-value of 5%, the up-regulated genes in 48 h group were significantly enriched in 1 KEGG pathway and 4 GO terms (Table I). These pathway and biological processes were all associated with steroid biosynthesis. The down-regulated genes in 48 h group were significantly enriched in cellular component of organelle membrane and envelope (Table II).

At a q-value of 25%, GSEA analysis re-captured the up-regulation of inflammatory response and apoptosis (Table III) in early post-TBI group as identified by the original study[8]. Strikingly,

Table I. DAVID functional analysis for the up-regulated genes in 48 h post-TBI.

ID	Term	Count	Benjamini
rno:00100	Steroid biosynthesis	7	1.09E-04
GO:0016126	Sterol biosynthetic process	8	0.001860765
GO:0006695	Cholesterol biosynthetic process	7	0.00479652
GO:0008610	Lipid biosynthetic process	18	0.012005463
GO:0006694	Steroid biosynthetic process	9	0.03060858

Table II. DAVID functional analysis for the down-regulated genes in 48 h post-TBI.

ID	Term	Count	Benjamini
GO:0005739	Mitochondrion	55	2.51E-04
GO:0031090	Organelle membrane	40	0.008817702
GO:0031975	Envelope	28	0.009349932
GO:0031966	Mitochondrial membrane	21	0.010189885
GO:0031967	Organelle envelope	28	0.011111585
GO:0005740	Mitochondrial envelope	21	0.021022629
GO:0005743	Mitochondrial inner membrane	17	0.025873751
GO:0019866	Organelle inner membrane	17	0.03926025

Table III. Gene set enrichment analysis of early post-TBI group.

Name	Es	Nes	q-value
Toll-like receptor signaling pathway	-0.5803414	-2.1635337	0.001098113
Nod like receptor signaling pathway	-0.60602033	-2.021938	0.012609651
Potassium ion transport	-0.54066473	-1.8819821	0.064536385
Cellular response to stimulus	-0.61996955	-1.7730696	0.17085575
Insulin receptor signaling pathway	-0.65840626	-1.751397	0.17789559
Feeding behavior	-0.59518164	-1.744424	0.16024052
Apoptosis	-0.45920566	-1.7390779	0.14574444
Carbohydrate biosynthetic process	-0.5276914	-1.711318	0.16781057
RIG-I-like receptor signaling pathway	-0.49522346	-1.6931274	0.17735606
Inflammatory response	-0.4156208	-1.6583182	0.22416341
Spliceosome	-0.41450658	-1.6430942	0.23489085
Metal ion transport	-0.4043716	-1.625672	0.24987301
Calcium ion transport	-0.55174595	-1.6232235	0.2358638
Glutamate signaling pathway	-0.609504	-1.6191143	0.22635508
Developmental maturation	-0.609256	-1.6167685	0.21581021
Adipocytokine signaling pathway	-0.43701494	-1.5947909	0.24254861
Activation of protein kinase activity	-0.5653822	-1.5927136	0.23175448
tRNA metabolic process	-0.5747668	-1.5899161	0.22404608
Chemokine signaling pathway	-0.368447	-1.5553997	0.24925287
Regulation of transcription from RNA polymerase II promoter	-0.34898534	-1.5523543	0.24396327
Viral genome replication	-0.5727582	-1.5505344	0.23695989
Negative regulation of transcription from RNA polymerase II promoter	-0.4179912	-1.5380859	0.24682745

*ES: enrichment score; NES: normalized enrichment score.

cell cycle genes were found tending to be up-regulated in the late post-TBI group (Table IV), which was sample obtained at 48 hours after TBI. Interestingly, GSEA identified the up-regulation of calcium and potassium ion transport in early post-TBI group (Table III). Glutamate signaling pathway was also found up-regulated in early post-trauma group (Table III).

PPI Network Construction

By submitting the up-regulated genes and down-regulated genes into STRING respectively,

Table IV. Gene set enrichment analysis of late post-TBI group.

Name	Es	Nes	q-value
Lysosome	0.6999008	2.53668	0
Regulation of mitosis	0.72846955	2.0845277	8.83e-04
M phase	0.582502	2.0745482	0.001163896
DNA replication	0.69444	2.0539799	0.001518343
M phase of mitotic cell cycle	0.6028164	2.0300019	0.002103742
Lysine degradation	0.66550756	1.979894	0.005676726
Mitosis	0.58409715	1.9215467	0.009911417
Antigen processing and presentation	0.60644805	1.9002243	0.011812491
Systemic lupus erythematosus	0.5774859	1.8852803	0.013015609
Tryptophan metabolism	0.64335126	1.8580505	0.017409803
Cell cycle process	0.48407272	1.8357767	0.02270431
Double strand break repair	0.71664715	1.8321775	0.02147199
Cell cycle checkpoint_go_0000075	0.6102876	1.7878711	0.036072627
Drug metabolism cytochrome p450	0.5809512	1.7818928	0.035922572
Cell cycle phase	0.47507733	1.7624816	0.043162234
Intestinal immune network for IGA production	0.5984467	1.7542166	0.044223256
Complement and coagulation cascades	0.52860105	1.7504798	0.043567095
Electron transport_go_0006118	0.56729895	1.7489855	0.04186573
Asthma	0.64221454	1.7339602	0.046619214
Autoimmune thyroid disease	0.6081052	1.7315193	0.04542683

*ES: enrichment score, NES: normalized enrichment score.

we obtained PPIs associated with the differentially expressed genes. Figure 1 shows the PPI relationships among the down-regulated genes in late post-TBI group. No hub node was detected in these relationships. Figure 2 shows the PPI network constructed by the up-regulated genes. MAPK3 (mitogen-activated protein kinase 3) is the hub node in the network.

Discussion

TBI is a serious insult that frequently leads to neurological dysfunction or death. The molecular mechanisms of TBI are complicated. There is no suitable therapy method for it until now. This present study attempts to expand our understanding of the molecular mechanism of TBI. Results showed that gene expression were significantly altered in the late period after TBI. GSEA and pathway analyses suggested that biological processes of steroid biosynthesis, cell cycle, metal ion transport inflammation and apoptosis, were significantly dysregulated.

From Table I, pathway of steroid biosynthesis was significantly dysregulated in TBI. Steroids are known to play neuroprotective roles following TBI or during neurodegenerative processes[1]. For example, the administration of progesterone as a neuroprotective agent following TBI has recently entered phase III clinical trials[18]. The application of 17^2-estradiol/progesterone during the first 48h after stroke revealed neuroprotection after two weeks[19].

From Table II, we could find that genes located in mitochondrion or organelle membrane were significantly differentially expressed after TBI. Our result agrees with a previous report which found that genes involved in mitochondrial enzymes for oxidative phosphorylation were dysregulated in injured brains[20]. Luo et al[21] suggested that injured neurons have undergone mitochondrial and lysosomal membrane permeability damage, and the mechanism can be exploited with pharmacological interventions. Typically described as "permanently postmitotic", central nervous system (CNS) neurons must constantly keep their cell cycle in check[22]. There is evidence that neurons which are at risk of neurodegeneration are also at risk of re-initiating a cell cycle process that involves the expression of cell cycle proteins and DNA replication[22]. In response to neurotoxic signals, post-mitotic neurons relax the vigilance and make attempts to reenter the cell cycle. β-amyloid peptide could cause aberrant activation of mitogen-activated kinase (MAK)-

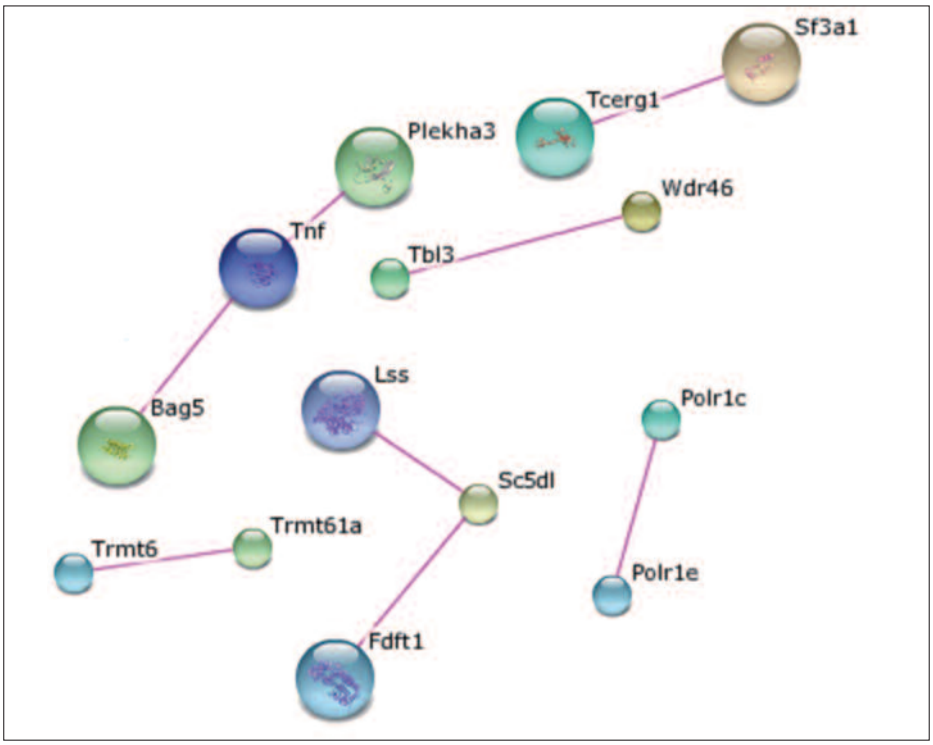

Figure 1. Protein-protein interactions among genes down-regulated in late post-TBI group. No protein has more than two interacted partners.

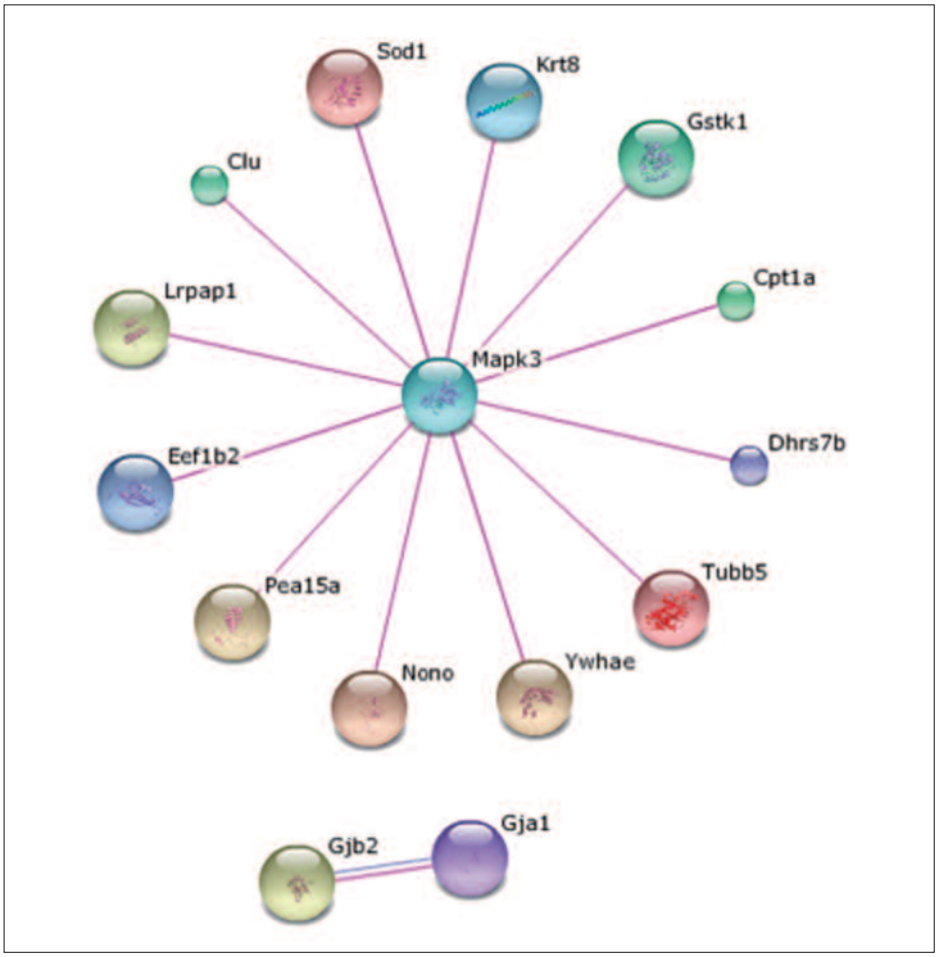

Figure 2. Protein-protein interactions among genes up-regulated in late post-TBI group. Mapk3, with 12 interacted partners, emerged as a hub protein/gene.

extracellular signal-regulated kinase (ERK) signaling, which promoted the entry of neurons into the cell cycle, resulting in their apoptosis[23].

GSEA showed that glutamate signaling pathway was dysregulated in TBI model. Glutamate is one of the most prominent neurotransmitter in human body. Glutamate signaling in the CNS plays a pivotal role in the acute pathophysiology of TBI[5]. The blood–brain barrier and cellular membranes can be compromised by primary mechanical forces of the initial injury, which allowing increased glutamate release into the extracellular space[24]. Matsushita et al[25] reported that several fold increases in the extracellular concentration of glutamate initiated by a TBI, which subside within 2hours.

TBI could cause a de-regulated flux of ions. To restore ionic balance, increasing activities in membrane pumps would be required which enhanced glucose consumption in consequence. GSEA results also suggested metal ion transport were altered after TBI, mainly related to potassium ion (K^+) and calcium ion (Ca^{2+}) transport. Potassium ion release into the extracellular space has been detected after TBI[26]. High levels of extracellular K^+ may disrupt energy homeostasis after brain injury[27]. Besides, K^+ stimulates oxygen uptake in glial cells and deprives traumatized neurons of their oxygen supply[28]. Several studies have documented the involvement of calcium ion-dependent cysteine protease calpain after TBI[29-31].

MAPK3 is the hub node of the PPI network, suggesting it play important roles in TBI. Mori et al[32] provided *in vitro* and *in vivo* evidences that perturbations in MAPK signaling pathway are involved in the pathophysiology of TBI. Zhao et al[33] suggested that the interactions between sirtuin 1 (SIRT1) and MAPK/ERK pathway regulate neuronal apoptosis induced by mechanical trauma injury *in vitro* and *in vivo*. The original study[8] has also suggested MAPK pathway as one of the major pathways after TBI. Our analysis further depicted the interactions of MAPK3, which may be worth of in-depth investigation.

Conclusions

Our results suggested gene expression profiles were significantly altered in the late period after TBI. These altered genes were mainly involved in steroid biosynthesis, cell cycle, metal ion transport, inflammation and apoptosis.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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