Microarray-based gene expression analysis of an animal model for closed head injury

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ABSTRACT

Objective: Traumatic brain injury (TBI) is a major cause of death and disability in both children and the elderly. Mortality from TBI is said account for 1–2% of all deaths. One-third to one-half of all traumatic deaths is due to head injury. Of those who survive, the majority is left with significant disabilities, including 3% who remain in a vegetative state and only approximately 30% who make a good recovery. Microarray studies and other genomic techniques facilitate the discovery of new targets for the treatment of diseases, which aids in drug development, immunotherapeutics and gene therapy. Gene expression profiling or microarray analysis enables the measurement of thousands of genes in a single RNA sample. Methods: In this study, adult Wistar-albino rats underwent TBI using a trauma device. Brain tissues and blood samples were taken for gene expression at 1, 12 and 48 h post-trauma and were then analysed via microarray. Total RNA was isolated using an RNeasy Mini Kit (QIAGEN-Sample & Assay Technologies, Hilden, Germany) and tested using a 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). Overall changes in gene expression were evaluated using Agilent Whole Rat Genome 4 × 44 K oligonucleotide arrays and analysed with GeneSpring (GeneSpring 6.1, Silicon Genetics, Redwood City, CA) software. Only genes with a signal-to-noise ratio of above 2 in the experiments were included in the statistical analysis. Results: ANOVA (p < 0.05) was performed to identify differentially expressed probe sets. Additional filtering (minimum 2-fold change) was applied to extract the most differentially expressed genes based on the study groups (Control vs. 1st hour, Control vs. 12th hour, Control vs. 48th hour). Differentially expressed genes were detected via microarray analysis. A gene interaction-based network investigation of the genes that were identified via traditional microarray data analysis describes a significantly relevant gene network that includes the C1ql2, Cbml, Sdc1, Bdnf, MMP9, and Cd47 genes, which were differentially expressed compared with the controls. Conclusions: In this study, we will review the current understanding of the genetic susceptibility of TBI with microarrays. Our results highlight the importance of genes that control the response of the brain to injury as well as the suitability of microarrays for identifying specific targets for further study.

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Introduction

Traumatic brain injury (TBI) is a major cause of death and disability in both children and the elderly. In the literature, TBI is commonly classified as mild, moderate or severe based on the Glasgow Coma Score (GCS) upon first examination.1–4 Mild and severe TBI is considered to have the same underlying neuropathology, namely diffuse axonal and vascular injury occurring to a variable extent in the parasagittal deep white matter, spreading from the cortex to the brainstem.5 Mild traumatic brain injury (MTBI) can be accompanied by facial and skull fractures. The presence of a skull fracture in MTBI implies an increased risk of intracranial injuries.6 Small vessel injuries can lead to haemorrhagic diffuse axonal injury lesions predominantly in grey matter, owing to better vascularisation in this tissue. Injury to larger blood vessels results in intracerebral haemorrhages.7 Following a contusion or haemorrhage, blood extends into the adjacent cortex where neurons undergo secondary necrosis due to ischaemia.8

Cerebral ischaemia can occur following MTBI and may be an influential factor in determining the neurological outcome. Nabika et al. reported ischaemia of the internal capsule after mild head injury in a 1-year-old boy.9 In an experimental study of Jenkins...
et al., it was proposed that mild mechanical injury could potentiate selective ischaemic hippocampal neuronal necrosis in the absence of overt axonal injury.\textsuperscript{10} There appears to be an interaction between ischaemia and neuronal injury following MTBI. Son et al. measured the N-acetylaspartate/creatine ratio and lactate signal during in vivo proton magnetic resonance spectroscopy to define metabolic brain changes associated with MTBI in patients with regional brain contusion and an initial GCS of 13–15. Cell loss and ischaemic damage have been indicated in the region of interest at early stages.\textsuperscript{4,11}

Assessing MTBI is problematic for a number of reasons. First, the standard protocol used to assess TBI severity and plan rehabilitation is dominated by CT, MRI and neurobehavioral procedures. Whilst these procedures may be effective for moderate to severe injury, they may be less useful for the assessment of MTBI, as CT, MRI, EEG and other routine neurological evaluations may appear normal.\textsuperscript{12,13}

There appears to be an interaction between post-traumatic cerebral ischaemia and secondary neuronal injury. The early identification of ischaemia is an important predictor of long-term outcome. Therefore, a rapid blood test to diagnose post-MTBI ischaemia may be essential.\textsuperscript{5}

The aim of this study is to describe differential time-dependent gene expression by whole genome microarray analysis following a designed mild traumatic brain injury event. Accordingly, this study consists of two main steps: first, the application of an MTBI device to induce brain injury and, second, to determine the time-dependence (0, 24 and 48 h) of gene expression via whole genome microarray analysis.

**Material and methods**

To investigate ischaemic events following MTBI, we used an animal model of closed head injury. The severity of brain injury is easily controlled in this model through the adjustment of the height and mass of the weight used for injury. 30 Adult Wistar-albino rats were provided by the Experimental Medical Research Centre of the Medical Faculty of Kocaeli University, and the Animals Ethics Committee approved the experimental protocol. In this study, closed traumatic brain injury was induced by a decrease in weight according to an experimental model that was initially described by Marmarou et al. and further refined by Ucar et al.\textsuperscript{14,15}

**Animals**

In the present study, 30 male adult Wistar-albino rats (200–250 g) were housed individually under controlled environmental conditions (12-h light/12-h dark cycle). Animals were divided into two groups: 15 rats underwent MTBI, and 15 rats served as control animals. Initially, the rats were anesthetised with ether. Animals were able to breathe spontaneously throughout the procedures. In control animals, all experimental procedures were performed with the exception of MTBI induction.\textsuperscript{4}

**Trauma device and induction of mild traumatic brain injury**

The trauma device consisted of a single column of a plexiglass tube containing a steel weight fixed to a metal helmet and attached to the skull vertex of the rat by bonewax. The steel weight freely falls through a 1 m vertical section of the plexiglass tube held in place with a ring stand\textsuperscript{15} (Fig. 1).\textsuperscript{4} We used an inflexible rope to restrict the movement of the weight after the first impact, as recommended by Ucar et al.\textsuperscript{15} The tube was perforated at 3 cm intervals to minimise the effect of frictional forces. The animal was placed in the prone position on a foam bed. A 3 mm thick stainless-steel disc served as the helmet. Before the force was applied, a midline skin incision was performed, and the skin was reflected to expose the skull. The metallic disc was fixed to the central portion of the skull. A cylindrical weight was placed in the tip of the tube, and the opening of the tube was centred over the helmet directly on the rat’s skull. A 300 g weight was then dropped from the top of the tube from a height of exactly 1 m. The animals were moved from the device after impact and returned to their cage. The rats were observed for 1 h. Then, all of the animals were anaesthetised again, and blood samples were collected from the right atrium of the heart.\textsuperscript{4}

**Light microscopic examinations**

Frontal and parietal brain tissues were sampled for histopathological investigation. Brains were removed, fixed in 4% paraformaldehyde overnight at 4 °C, embedded in paraffin wax, and serially sectioned. Serial coronal 5-mm sections were subjected to standard haematoxylin and eosin (HE) staining. Briefly, the sections were de waxed with xylene and rehydrated through a series of graded alcohols. They were mounted on slides, stained with haematoxylin for 3 min, and then rinsed in tap water and stained with eosin for 2 min. The sections were dehydrated and cleared with xylene. A pathologist examined the slides via light microscopy.\textsuperscript{4}

Dulbecco’s Modified Eagle’s Medium (DMEM), penicillin-streptomycin, and trypsin-EDTA were purchased from Gibco-BRL (Grand Island, NY, USA), Foetal calf serum (FCS) was purchased from Hyclone (Logan, Utah, USA). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was purchased from Sigma (St. Louis, MO).

**RNA isolation, quality and quantity determination**

Total cell RNA was prepared using RNeasy columns (Qiagen, Valencia, CA). The quality and quantity of total RNA was
determined with an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). The samples were selected for microarray or for real-time PCR experiments provided that they had an >8.0 RNA integrity number, a clear gel image and no DNA contamination as observed on the histogram.

Quantitative real-time PCR (Q-RT-PCR)

cDNA was synthesised using DNase-I-treated total RNA and a First Strand cDNA Synthesis Kit for RT-PCR (Roche Diagnostic Corp., Indianapolis, IN, USA) according to the manufacturer’s instructions. Quantitative polymerase chain reactions to determine Sdc1, Bdnf and MMP9 gene expression were performed using a LightCycler (Roche Diagnostic Corp., Indianapolis, IN, USA), and the G6PD gene was used as internal control.

Microarray measurements

A total of 1000–1000 ng quality-checked total cellular RNA was reverse transcribed by the Low-input RNA Linear Amplification Kit (Agilent Technologies, Palo Alto, CA) and then transcribed to Cy3-labelled cRNA according to the manufacturer. The labelled cRNA was purified (RNeasy Kit, Qiagen, Valencia, CA), and the dye content (>9.0 pmol dye/µg cRNA) and concentration of cRNA were measured by a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). A total of 1650 ng of Cy3-labelled cRNA was hybridised to Whole Human Genome Oligo 4 × 44 K microarrays overnight at 65°C, and then the slides were washed and treated with Stabilising and Drying Solution (Agilent Technologies, Palo Alto, CA) and scanned by Agilent Microarray Scanner. All steps were performed according to the manufacturer (Agilent Technologies, Palo Alto, CA).

Statistical analysis

Data normalisation and analysis

The data were normalised by the Feature Extraction software, version 7.5, with default parameter settings for one-colour oligonucleotide microarrays and then transferred to GeneSpring 9.02 program (Agilent Technologies, Palo Alto, CA) for further statistical evaluation. In GeneSpring, we applied normalisation and data transformation steps for one-colour data as recommended by Agilent Technologies. The data were analysed using GeneSpring, and genes with a >2.0-fold differential expression were further analysed by statistical tests. An unpaired T-test was used to compare groups. A multiple testing correction method by Benjamini–Hochberg was applied with a p < 0.05 cut-off in our statistical tests.

Gene network analysis

For gene network analysis, gene symbols (HUGO), Agilent probe IDs and the fold change of the differentially regulated genes (curcumin treated samples versus the control) were imported into the IPA 5.0 software (Ingenuity Systems, Redwood City, CA). In IPA, the analysis was conducted with p < 0.05 as the cut-off point. The genes with known gene symbols (HUGO) and their corresponding expression values were uploaded into the software. Each gene symbol was mapped to its corresponding gene object in the Ingenuity Pathways Knowledge Base. Networks of these genes were algorithmically generated based on their connectivity and assigned a score. The score is a numerical value used to rank networks according to how relevant they are to the genes in the input dataset, but it may not be an indication of the quality or significance of the network. The score considers the number of focus genes in the network and the size of the network to approximate how relevant this network is to the original list of genes. The network identified is then presented as a graph indicating the molecular relationships between genes/gene products. Genes are represented as nodes, and the biological relationship between two nodes is represented as an edge (line). The intensity of the node colour indicates the degree of up- or down-regulation. Genes in uncoloured notes were not identified as differentially expressed in our experiment and were integrated into the computationally generated networks based on the evidence stored in the IPA knowledge memory that indicates relevance to this network.

Gene Set Enrichment Analysis (GSEA)

The GSEA was performed by GSEA v2.0 software. For the GSEA, a collection of canonical pathway gene sets representing 639 pathways was downloaded from the Molecular Signatures Database (MSigDB) website. The pathway gene sets are curated from the Kyoto Encyclopaedia of Genes and Genomes (KEGG), BioCarta and GenMAPP online pathway databases. These gene sets are canonical representations of biological processes that were compiled by domain experts. The probes were represented by their Human Gene Organization (HUGO) gene names. Gene sets were assessed as to whether they scored high individually when compared with the other possible choices of gene sets. This provided an unbiased means of assessing pathways and tested gene lists with respect to enrichment or the degree of representation of highly regulated genes. A positive normalised enrichment score (NES) indicates a correlation with the control, whilst negative values indicate a correlation with curcumin treatment. The nominal p-value estimates the statistical significance of the enrichment score for a single gene set. We drew conclusions from the top gene sets that had a false discovery rate of less than 5% and a p-value less than 0.05, both of which are acceptable cut-offs for the identification of biologically relevant gene sets.

Results

In the trauma group, examination by light microscopy revealed neuron damage in the inner layer of the cortex and neuronal necrosis in the outer layers of the cortex of rat brains following cerebral hypoxia. Red neurons were seen at an early stage of ischaemia by haematoxylin and eosin staining (Fig. 2).

![Fig. 2. Red neurons (ischemic neurons) were seen in early stage of ischemia by haematoxylin and eosin staining (HE 200×).](image-url)
Microarray gene expression analysis

Whole genome microarray analysis showed that after trauma, the expressions of 279 genes were changed compared to controls. One hour after trauma (first experimental group), 35 genes were down-regulated, and 32 genes were up-regulated (Fig. 3). Twelve hours after trauma (second experimental group), 118 genes were down-regulated, and 73 genes were up-regulated. Forty-eight hours after trauma (third experimental group), 83 genes were down-regulated, and 75 genes were up-regulated. We observed that most of the genes with altered expression levels after trauma normally function in cell signalling pathways, cell proliferation, cell differentiation and the regulation of transcription.

The expression of 14 genes was analysed in each experimental group. The expression levels of Bdnf, C1ql2, Cbnl, Cd47, Mmp9, Sdc1, Slc27a2, and Tnnt3 were higher in the first hour of trauma than either the control group or at other experimental time points. A stepwise and significant decrease in the expression levels of these genes was observed after 1-h post-trauma. In contrast, the expression levels of Dmkn, Fzr12, Hal, Htr2a, Pilra, and Slc22a25 were decreased in the first hour of trauma, increasing gradually after 1-h (Fig. 4).

Whole genome microarray analysis data also identified 33 genes whose expression changed in only one of the experimental time points compared to the control. The expression levels of 7 genes after 48 h, and another 26 genes were comparable to the controls after 1-h of trauma. The names and the functions of these genes are shown in Table 1.

Verification of selected genes by quantitative real-time PCR

To substantiate the results of the microarray studies, quantitative real-time PCR was performed to assess the mRNA expression...
of Sdc1, Bdnf and MMP9. Microarray and real-time PCR resulted in similar gene expression changes for these three genes, confirming the reliability of our microarray results at the mRNA level.

### Discussion

The aim of this study is to understand the biological background of MTBI. For this purpose, we generated MTBI rats injured by an MTBI device. We isolated brain samples from injured rats and analysed gene expression in the tissue by whole genome microarray analysis. Traditional gene expression analyses using large-scale microarray data focus on statistically differentially expressed genes, which may lead to the oversight of potentially important disease-related genes. The Gene Set Enrichment Analysis (GSEA) addresses this problem by focusing on gene sets rather than individual genes. Our GSEA focused on predefined gene sets from pathway databases. We drew conclusions only from the top genes with a false discovery rate less than 5% and a p-value of less than 0.05.

We applied this analysis to the microarray data and generated a list of 279 genes that were differentially expressed at various time points. We were able to classify the data at distinct time points into the following categories: genes that were up-regulated 1 h, 12 h and 48 h after the injury; genes that were down-regulated in 1 h, 12 h and 48 h after the injury; genes that were expressed at only 12 h and 48 h; and genes that were expressed at all three time points.

Our results reveal that several pathways related to cell signalling, proliferation, cell cycle and cell junctions have the strongest correlation amongst our study groups. Our goal was to identify genes that play important roles in neuroprotection, inflammation, nerve repair, and osteogenic and epithelial differentiation. With this in mind, we focused our attention on genes that were related to those pathways. Gene interaction-based network investigations of the differently expressed genes that were obtained by traditional microarray data analysis identified a relevant gene network that included the C1qL2, Cbn, Sdc1, Bdnf, MMP9 and Cd47 genes.

The complement component (C1qL1) subfamily is highly expressed in the CNS and essential for synapse regulation. In the adult brain, C1qL1, 2 and 3 mRNAs are primarily expressed in different parts of neurons. C1qL2 mRNA is strongly expressed in the dentate gyrus of the hippocampus. Recent studies report that C1qL mRNA expression may reflect the depolarised state of immature neurons in certain brain regions. Indeed, C1qL subfamily proteins are secreted and form both homomeric and heteromeric complexes. C1qL proteins are known to form hexameric and higher-order complexes via their N-terminal cysteine residues. Yuzuki indicates that biochemical properties of C1qL proteins are similar to those of the Cbn subfamily and speculate that similar to Cbn, C1qL subfamily members may play diverse roles by forming homomeric and heteromeric complexes in the CNS. In our study, the observed expression levels of these two genes post-MTBI were quite similar. Both genes were highly expressed within the first 12 h post-MTBI, following which their expression was dramatically down-regulated. The C1qL subfamily is both structurally and evolutionarily related to the Cbn family; however, the distinct spatial and temporal expression patterns suggest that this family also has discrete functions. The levels of Cbn1 mRNA closely parallel synapse formation between granule cells and Purkinje

### Table 1

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Expression level</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fkbp6</td>
<td>12 h down–48h down</td>
<td>FK506 binding protein 6 (Fkbp6)</td>
</tr>
<tr>
<td>Cpa5</td>
<td>12 h down–48h up</td>
<td>Carboxypeptidase A5</td>
</tr>
<tr>
<td>Gpr6</td>
<td>12 h down–48h up</td>
<td>G protein-coupled receptor 6</td>
</tr>
<tr>
<td>lkbk1</td>
<td>12 h down–48h up</td>
<td>Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase epsilon</td>
</tr>
<tr>
<td>Neurod1</td>
<td>12 h down–48h up</td>
<td>Rattus norvegicus neurogenin differentiation 1</td>
</tr>
<tr>
<td>Oprk1</td>
<td>12 h down–48h up</td>
<td>Kappa-type opioid receptor</td>
</tr>
<tr>
<td>Prss22</td>
<td>12 h down–48h up</td>
<td>Protease, serine, 22</td>
</tr>
<tr>
<td>Ptpn1</td>
<td>12 h down–48h up</td>
<td>Protein tyrosine phosphatase, receptor type, V</td>
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<td>Runx2</td>
<td>12 h down–48h up</td>
<td>Runt related transcription factor 2</td>
</tr>
<tr>
<td>Sncb4b</td>
<td>12 h down–48h up</td>
<td>Sodium channel, voltage-gated, type IV, beta</td>
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<td>Tbc1d10c</td>
<td>12 h down–48h up</td>
<td>TBC1 domain family, member 10C</td>
</tr>
<tr>
<td>Tbc1d5</td>
<td>12 h down–48h up</td>
<td>TBC1 domain family, member 5</td>
</tr>
<tr>
<td>Tinag</td>
<td>12 h down–48h up</td>
<td>Tubulointerstitial nephritis antigen</td>
</tr>
<tr>
<td>Tnn1</td>
<td>12 h down–48h up</td>
<td>Troponin I type 3 (cardiac)</td>
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<tr>
<td>Tnn1</td>
<td>12 h down–48h up</td>
<td>Troponin T type 1 (skeletal, slow)</td>
</tr>
<tr>
<td>Cd36</td>
<td>12 h down–48h down</td>
<td>CD3 molecule, epsilon polypeptide</td>
</tr>
<tr>
<td>Chrme</td>
<td>12 h up–48h down</td>
<td>Cholinergic receptor, nicotinic, epsilon</td>
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<td>Foxe1</td>
<td>12 h up–48h down</td>
<td>Forkhead box E1 (thyroid transcription factor 2)</td>
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<td>Gng7</td>
<td>12 h up–48h down</td>
<td>Rattus norvegicus guanidine nucleotide binding protein (G protein), gamma 7</td>
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<td>Homer1</td>
<td>12 h up–48h down</td>
<td>GLGF-domain protein Homer</td>
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<td>Hsbp1</td>
<td>12 h up–48h down</td>
<td>Heat shock protein 1</td>
</tr>
<tr>
<td>Mmp28</td>
<td>12 h up–48h down</td>
<td>Matrix metalloproteinase 28</td>
</tr>
<tr>
<td>Nr4a3</td>
<td>12 h up–48h down</td>
<td>Nuclear receptor subfamily 4 group A member 3</td>
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<td>Nnxr2</td>
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<td>Nucleoredoxin-like 2</td>
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<td>12 h up–48h down</td>
<td>Podoplanin</td>
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<td>Zdhhc18</td>
<td>12 h up–48h down</td>
<td>Zinc finger, DHHC-type containing 18</td>
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<td>Acdy8</td>
<td>1 h down–12h down</td>
<td>Rattus norvegicus adenylate cyclase 8</td>
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<tr>
<td>Rgs9</td>
<td>1 h down–12h up</td>
<td>Regulator of G-protein signalling 9</td>
</tr>
<tr>
<td>Gzmc</td>
<td>1 h down–12h up</td>
<td>Granzyme C</td>
</tr>
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<td>Megf10</td>
<td>1 h down–12h up</td>
<td>Multiple EGF-like domains 10</td>
</tr>
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<td>Gucal1a</td>
<td>1 h up–12h down</td>
<td>Guanylate cyclase activator 1a (retina)</td>
</tr>
<tr>
<td>Cd28</td>
<td>1 h up–12h down</td>
<td>Rattus norvegicus Cd28 molecule</td>
</tr>
<tr>
<td>Gper2</td>
<td>1 h up–12h down</td>
<td>G protein-coupled receptor 6B</td>
</tr>
</tbody>
</table>
cells. The Cbln1 gene encodes cerebellin, a small polypeptide that is preferentially expressed in the cerebellum but also present at variable concentrations elsewhere in the CNS, notably in the hypothalamus. We speculate that Cbln1 regulates the precise formation of new synaptic connexions during the restitution of damaged neurons in the striatum. A member of the C1q family is also expressed in developmental CNS synapses, functioning in normal and pathological synapse elimination processes. These findings suggest that C1qL2 and Cbln expression is critical in the CNS. We speculate that these two genes may play important roles in synaptic organisation and disorganisation in damaged brain regions.

We also found four genes that were up-regulated or down-regulated at the same time point. The first one of these was Bdnf, which encodes another neurotrophin, such as Cbln. An important neurotrophic factor, BDNF, plays a role in improving the pathological state of neurons, promoting the regeneration of injured neurons, and reducing neuronal apoptosis. The second identified gene was MMP9. MMPs belong to a family of zinc-dependent endopeptidases, which are secreted as inactive proenzymes and activated by partial proteolytic cleavage. MMP-9 is the dominant MMP released by most endothelial cells and appears to play an important role in the degradation of basement membrane type VI collagen and other matrix proteins. In the central nervous system, all CNS cells, including neurons and glia, produce MMPs. In addition, MMPs are implicated in several important physiological events, including promoting the migration of neural precursors to injury sites and in the initial step of oligodendrocyte myelination. The third identified gene was cd47. CD47 shows a very broad cell and tissue distribution amongst neuronal cells. CD47 mediates apoptotic and oxidative cell death in neurons. Activation of CD47 induces cytotoxicity in brain endothelial cells and is known to contribute to inflammation and platelet aggregation, which could exacerbate ischaemia. Syndecan-1 (sdc1) was the fourth gene that was observed to be highly expressed during the 1 h and 12 h time periods. Syndecans are cell surface co-receptors that have been implicated in both normal and pathological events, presumably through their regulation of cell–cell and cell–matrix interactions, cell migration, development, neovascularisation, and tumorigenesis. The best understood member is syndecan-1, which interacts with many effector molecules, such as fibroblast growth factor and extracellular matrix proteins, including fibrillar collagens. Indeed, the induction of syndecan-1 activity by growth factors is a key step in tissue regeneration.

In our study, we demonstrate that these six genes are up-regulated at the same time point and down-regulated after 48 h. These six proteins may contribute to the regeneration of injured neurons and the elimination of the pathologic molecular organisation after brain injury.

Studies demonstrate that BDNF and MMP9 may interact. In Sun et al., the role of BDNF and MMP9 in angiogenesis is discussed, and it has been shown that BDNF induces the expression and activation of MMP-9. This activation of MMPs may be a pro-angiogenic mechanism of BDNF. Angiogenesis may play an important role in mediating functional recovery after TBI. Nearly all of the neurorestorative agents that improve functional outcome after TBI also increase angiogenesis and neurogenesis. Our findings concerning BDNF and MMP9 highlight the importance of the interaction between BDNF and MMP9 in the functional recovery of the brain after injury events. These findings highlight that the manipulation of angiogenesis may be a potential therapeutic alternative for brain injury. Previous research has also suggested a correlation between MMP9 and Syndecan-1. An alteration in syndecan-1 expression would be expected to affect cell survival and proliferation, as syndecan-1 may help injured cells modulate their ability to respond to injury. Oh et al. demonstrated that syndecan-1 overexpression leads to an increase in MMP-9 expression. In our results, these two genes had the same temporal expression pattern. Our findings are supported by a recent publication demonstrating synd1–dependent MMP9 expression. These results suggested that MMPs, which are up-regulated following CNS injury, can contribute to the recovery of CNS function. According to these findings, following injury, MMP expression leads to neuronal cell death, turnover and remodelling of the extracellular matrix to initiate structural and functional recovery.

Recently, it has been shown that activation of the integrin-associated protein CD47 mediates the development of blood–brain barrier injury and oedema after cerebral ischaemia. CD47 is a widely expressed molecule in brain tissue, including endothelial cells, macrophages and neurons. Activation of CD47 induces cytotoxicity in brain endothelial cells and is known to contribute to inflammation and platelet which could worsen ischaemia. Koshimizu et al. suggests that CD47 is involved in caspase-dependent apoptosis in CNS neurons and that the CD47-induced death of cultured cortical neurons may be regulated by the interaction with BDNF. et al. reported that CD47 knockout mice had less oedema, inflammation and secondary brain injury after transient focal cerebral ischaemia. Jin et al. also showed that MMP-9 levels were markedly lower in CD47 knockout brains compared to wild-type brains, and they concluded that CD47 is broadly involved in neuroinflammation and that this integrin-associated protein plays a role in promoting MMP-9 up-regulation, neutrophil extravasation, brain swelling and the progression of acute ischaemic brain injury.

Interestingly, our data suggest that there are some gene expression similarities between CD47, MMP9 and BDNF. As a result, we conclude that MMP9 and BDNF have multiple incompatible effects that are the result of brain injury. At the same time, MMP9 interacts with both brain injury protective and cytotoxic molecules. One approach that avoids the interaction of BDFN and MMP9 with cytotoxic proteins, such as CD47, may aid in the recovery from brain injury. These results led us to hypothesise that CD47 might be an important anti-inflammatory target for brain injury therapy.

In summary, we have shown that several genes encoding molecules that promote neuroprotection, inflammation, nerve repair, and epithelial differentiation (C1qL2, Cbln, Sdc1, Bdnf, MMP9, and Cd47) are significantly up-regulated in injured rat brain tissue compared with their wt counterparts. These results may help identify genes with an important role in the molecular and cellular events that regulate neural survival and repair in the injured nervous system.

**Conflict of interest statement**

There is no conflict of interest.

**Acknowledgments**

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**References**