Bioinformatics Analysis of MiRNA in Peripheral Blood of Patients with Severe Traumatic Brain Injury

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Abstract

Objective: To analyze the target genes and functions of miRNA in peripheral blood of patients with severe traumatic brain injury (sTBI) by bioinformatics.

Methods: Gene microarray data from peripheral blood of sTBI patients and control group were retrieved from the GEO database. The differentially expressed miRNA were screened by bioinformatics method. The target genes were predicted, the biological functions and signal pathways were analyzed, and the miRNA and target gene regulatory networks were constructed.

Results: The microarray GSE21854 was obtained.145 differentially expressed miRNA were screened out and a total of 580 target genes were predicted. The functions of these target genes mainly included negative regulation of cell proliferation and negative regulation of transforming growth factor - β receptor signal pathway, which were mainly distributed in Ras signal pathway and transforming growth factor - β signal pathway, etc. The regulatory network map of miRNA and target genes showed that hsa-miR-125a-5p, hsa-miR-760, hsa-miR-217, hsa-miR-199a-3p and hsa-miR-543 were the core of regulation.

Conclusion: There are differentially expressed miRNA in peripheral blood of patients with sTBI. Hsa-miR-125a-5p, hsa-miR-760, hsa-miR-217, hsa-miR-199a-3p, and hsa-miR-543 are closely related to the progression of sTBI.

Keywords: Severe traumatic brain injury; MiRNA; Peripheral blood; Bioinformatics

Background

Traumatic brain injury (TBI) has a high fatality rate and disability rate, especially for the severe TBI (sTBI)[1]. Several genes have been shown to be involved in the pathophysiology of sTBI[2], so understanding the role of these genes has implications for the treatment of sTBI. MiRNA inhibits translation of target mRNA[3] and can serve as a biological marker for some diseases[4]. Recent studies have shown that peripheral blood miRNA expression is elevated after injury[5]. We searched the GEO database for microarray reports of peripheral blood associated with TBI[6], but these studies only performed differential expression analyses on microarray datasets. In order to deeply dig the value of these data, we used RStudio and

BioConductor software to screen differentially expressed miRNA with the bioinformatics method, predict the target genes, and analyze the biological function and signal pathway enrichment of the predicted results. In the end, we constructed a regulatory network for differentially expressed miRNA and target genes to reveal the miRNA and functions closely related to sTBI.

1. **Data and Methods**

1.1. **Data source:** MiRNA expression microarray data from sTBI patients were retrieved from the GEO database. The dataset needs to meet 3 requirements: ① The whole genome miRNA expression microarray; ② The patients with sTBI and the normal control; ③ The data contain the original data or the data after standardization

1.2. **Differential expression:** MiRNA was screened under the environment of RStudio software (version 3.5.3). The background correction, standardization and log2 conversion of microarray data were performed using limma

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software package in Bioconductor (version 3.9). Screening was based on the threshold of $P < 0.05$ and fold change in ploidy > 1.5.

1.3. MiRNA target gene prediction: MiRanda website and Tar-getScan website were used for target gene prediction analysis of differentially expressed miRNA, and the results were subsequently analyzed.

1.4. Biological function analysis and signal pathway analysis: Differentially expressed miRNA were submitted to DAIVD (https://david.ncifcrf.gov/) website for biological function (Gene Ontology, GO) analysis. The signal pathways were analyzed by using Kyoto Encyclopedia of Genes and Genomes (KEGG) website.

1.5. Regulatory analysis of miRNA and target genes: It was analyzed by using the Graph function in the miRNA module of miRWalk website[7]. The interaction network between miRNA and target genes with core regulatory role was obtained, and the importance of the node was indicated by the degree of connectivity between the nodes.

2. Results

2.1. GSE microarray data features: GSE21854 microarray dataset was obtained by retrieving GEO database, which was used to detect the expression level of miRNA in peripheral blood of sTBI patients. A total of 80 samples were collected. 40 peripheral blood samples were collected from patients with sTBI and healthy controls. LC_MRA-1001_miRHuman_13.0_090309 was used. Of the 40 sTBI cases, 30 were male and 10 were female; the age ranged from 18 to 42 years, the mean age was 26 years, and the median age was 24 years; the sampling time was (68 ± 8) h after the injury. The healthy control group consisted of 30 males and 10 females, aged 19-43 years, with an average age of 27.8 years and a median age of 25.5 years.

2.2. Differentially expressed miRNA: It was detected by RStudio software. After log2 correction and quality control, 145 differentially expressed miRNA were obtained after minus substandard sample results, of which 79 were up-regulated and 66 were down-regulated. The first five differentially expressed miRNA were: hsa-miR-574-3p up, hsa-miR-574-5p up, hsa-miR-1246 up, H sa-miR-605 down, and hsa-miR-145 * up.

2.3. MiRNA target genes prediction: The differentially expressed miRNA were predicted through TargetScan and miRanda, respectively, and 580 target genes were obtained after taking the intersection of the predicted results. See Table 1.

Table 1. **Differential Expression of Target Gene in Peripheral Blood MiRNA of sTBI**

Entrez code	Gene marker	Description
387509	GPR153	Human G protein-coupled receptor 153, mRNA
9563	H6PD	Human hexose-6-phosphate dehydrogenase, transcription variant X3, mRNA
26099	SZRD1	Human SUZ RNA binding domain contains 1 SZRD1 protein, transcription variant 1, mRNA
126917	IFFO2	Human intermediate filament family orphanin 2 protein, IFFO2 transcription variant X1, mRNA
440574	MINOS1	Human mitochondrial intimal assembly system 1 protein, transcriptional variant 5, mRNA

2.4. MiRNA target gene function analysis and signal pathway analysis: The functional analysis of 580 target genes showed that the function was mainly focused on the cell pathway number hsa04014 hsa04350 hsa05214 hsa04218 hsa04070.

Table 2. **Biological Functions of Differentially Expressed Target Genes in Peripheral Blood MiRNA of sTBI**

GO number	Biological function
GO: 0008285	Negative regulation of cell proliferation
GO: 003051	Negative regulation of transforming growth factor - β receptor signal pathway
GO: 0006198	Cyclic adenosine phosphate catabolism
GO: 0031054	Precursor miRNA processing
GO: 0045740	Positive regulation of DNAreplication

2.5. Analysis of miRNA target gene signal pathway: Analysis of the signal pathway of 580 target genes through KEGG database showed that the signal pathway was mainly distributed in Ras signal pathway, transforming growth factor - β signal pathway and so on. See Table 3.

Table 3. **Signal Pathway of Differentially Expressed Target Gene in Peripheral Blood MiRNA of sTBI**

Path number	Signal pathway
hsa04014	Ras signal pathway
hsa04350	Transforming growth factor - β signal pathway
hsa05214	Tumor signal pathway
hsa04218	Cell aging
hsa04070	Positive regulation of DNA replication

2.6. Analysis of miRNA and target gene network regulation: The interaction network was established by constructing a targeted regulatory relationship between differentially expressed miRNA and target genes. The results showed that hsa-miR-125a-5p, hsa-miR-760, hsa-miR-217, hsa-miR-199a-3p and hsa-miR-543 were the core of regulation.

3. Discussion

The case fatality rate of sTBI is more than 20%, and the prognosis of more than half of the patients is poor[9]. This is related to the pathogenesis of sTBI, not only acute primary injury caused by mechanical external force, but also secondary injury, including neuron death, blood-brain barrier destruction and neurological dysfunction. In view of the unpredictability and uncontrollability of acute primary injury, current study focuses on the mechanism of secondary injury[10]. In brain development, miRNA affects the process of brain development[11] and is involved in synaptic formation, neuron formation, differentiation, and maturation[12]. The results showed that the expression level of miRNA in brain tissue changed after sTBI in mice[13], and involved in the processes of post-injury inflammation, neuron apoptosis and cognitive impairment[10]. However, in clinical work, the detection of miRNA in patients' brain tissue is not only technically difficult, but also may cause more harm to patients. Recent studies have found changes in cerebrospinal fluid and blood miRNA in patients with sTBI[6,14]. We searched the peripheral blood gene microarray data of patients with sTBI in GEO database, and obtained 145 differentially expressed miRNA by Rstudio software analysis. Based on the preset criteria, we got 79 miRNA up-regulated and 66 down-regulated; 580 target genes were screened by bioinformatics, and the further biological function and signal pathway analysis showed that these target genes were mainly concentrated in negative regulation of cell proliferation, negative regulation of transforming growth factor - β receptor signal pathway, and so on. The signal pathway is mainly distributed in Ras signal pathway, transforming growth factor - β signal pathway and so on. By constructing the differentially expressed miRNA - target gene regulatory network, hsa-miR-125a-5p, hsa-miR-760, hsa-miR-217, hsa-miR-199a-3p, hsa-miR-543 were the core of the regulatory network. Previous studies have found elevated serum levels of hsa-miR-199a-3p in mild and moderate TBI patients[14], suggesting that hsa-miR-199a-3p may be associated with the degree of TBI injury.

It is important to note that sTBI patients are often associated with maxillofacial trauma and may have an impact on the results of the analysis. Studies have shown that miRNA can differentiate sTBI patients from patients with simple maxillofacial trauma[6]. In addition, in patients with sTBI, GCS scores of 3-5 and 6-8 had significantly different treatment outcomes, and patients with other systemic diseases may also have an impact on the outcome of the analysis. Previous studies have found that the same miRNA exhibit opposite trends in mild TBI and sTBI patients[6]. These evidences suggest that miRNA have both potential and limitations in the diagnosis and treatment of diseases and need to be further explored and identified in future studies.

To sum up, this paper used bioinformatics to screen out differentially expressed miRNA in peripheral blood in sTBI patients, further analyzed the target genes of these miRNA, as well as the biological function and signal pathway of the target genes, and found out the miRNA with core regulatory function, which provided the basis and foundation for the follow-up exploration, and also provided a new direction for understanding the pathogenesis and treatment of sTBI.

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