

Original Article

Differential expression profiles assay of miRNAs in rat serum after traumatic hemorrhagic shock

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Abstract: Traumatic hemorrhagic shock (THS) as one of the clinical common disease characterized by the pathogenesis of complex, long duration and high mortality, has been raised much more attention by people, and however the early diagnose marker was not still clearly illuminated. To address it, rat THS model was established based on an acute mechanical injury approach with the detection of serum inflammatory cytokines, and serum differential expression miRNAs were identified using Illumina HiSeq4000 with bioinformatics analysis, and validated by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Wherein, the consistent miRNAs were performed gene ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway clustering. As expected, with the trauma time prolonging, the expression level of inflammatory factors, including TNF- α and IL-6, was significantly increased, and that of anti-inflammatory factors, including IL-2 and IL-10, was significantly decreased, and 4-hr-trauma was an optimal time. For miRNA high throughput sequencing, a total of 18 up-regulated miRNAs, and a total of 21 down-regulated miRNAs were indentified, and the expression level of rno-miR-34c-3p and rno-miR-194-5p was consistent with the results of high throughput sequencing, and involved in several biological process as GO clustering, and rno-miR-34c-3p was directly involved in the regulation of TLR4 pathway, and rno-miR-194-5p was directly involved in the regulation of GOT2 pathway. By high throughput sequencing, several differential expression miRNAs were identified after THS, and not only provided as significant reference on the mechanism study of THS, and also provided a potential early diagnosis biomarker for THS treatment in clinic.

Keywords: Traumatic hemorrhagic shock, inflammatory cytokines, high-throughput sequencing, miRNA, differential expression

Introduction

Traumatic hemorrhagic shock (THS) is a type of shock complications caused by severe trauma with acute circulatory insufficiency, and accompanied with the hypovolemic, hypoxemia, low-flow issue perfusion, organ injuries, microcirculatory disturbance and cellular hypoxia [1-4]. Previous study has well documented that the generation of several cytokines in the early stage of THS may lead to inflammatory reaction, and further promote the healing of wound [5-7]. Nevertheless, the increasing of cytokines could enhance local reactions by blood circulation and aggravate the cascade effect of systemic inflammatory response syndrome (SIRS) [2, 8, 9]. Furthermore, the symptom of multiple organ dysfunction syndrome (MODS) was caused by constant high levels of cytokines

[10-12]. In clinical, the successful ratio of resuscitation in the treatment of THS was gradually increased with the development and the improvement of emergency mode [12-15]. However, the mortality was still high due to the SIRS and MODS caused by the uncontrolled release of inflammatory cytokines, and therefore to search a rapid and precise biomarker in the early diagnose of THS had to be settled urgently, especially MicroRNA (miRNA).

miRNA is a kind of small non-coding RNA containing 20-25 nucleotides, and broadly expressed in the cell of eukaryote and virus, and functioned by base-pairing with complementary sequences of the mRNA of target gene, and regulated the transcription and translation of it [16-18]. As a key regulating factor, miRNA involved in multiplex biological function, such

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as developmental, hemopoiesis, organogenesis, cell proliferation and apoptosis [17, 19-22]. Recently, serum miRNA is gradually becoming an auxiliary diagnose marker due to the characteristic of stable expression, non-invasive, easily collection, unsuitable degradation, high repeatability and specificity [23-25]. A variety of serum miRNAs have been well documented as a early diagnose biomarker of muscle injury, liver injury, brain injury, acute infarction and tumor, etc [23, 26-30], and however the differential expression profiles of serum miRNA after THS was still not fully elucidated so far. Based on it, the optimal trauma time was initially conformed with the altering of inflammatory factors, including TNF- α and IL-6, and anti-inflammatory factors, including IL-2 and IL-10, and high-throughput sequencing was performed followed with bioinformatics analysis, and expected to identify the differential expression miRNAs for the auxiliary diagnosis of THS in the early stage.

Materials and methods

Animals and grouping

A total of 80 Wistar (male, 260-300 g) rats were purchased and raised in the Chinese PLA General Hospital Animal Center with the house temperature of $25 \pm 2^\circ\text{C}$, the humidity of 40-60%, and 12 hrs light/dark cycle, and randomly divided into 8 groups, including 0 hrs, 1 hrs, 2 hrs, 4 hrs, 8 hrs, 16 hrs, 24 hrs, 48 hrs, each group 10. All animal experiments were approved by the Institutional Animal Care and Use Committee of the Chinese PLA General Hospital and conformed to the current guidelines for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Establishment of rat traumatic hemorrhagic shock

The rat traumatic hemorrhagic shock model was established based on an acute mechanical injury method as following. For one week adaptive feeding, rats were intraperitoneally injected sodium pentobarbital (80 mg/kg) to anesthetize, and fixed. Subsequently, a No. 22 arteriovenous indwelling needle was used to perform the catheterization of cervical artery and vein with heparin sodium (25 U/ml) antico-

agulation, and the blood pressure was monitored by a two channel physiological recorder (type LMS2B) to stable the blood pressure between 80 mmHg to 100 mmHg. Then, when the blood pressure was stabled for 10 min at least, rat left leg was fixed on the chassis of a man-made bracket, and a total of 300 g iron was freely fall from the height of 25 cm to cause the comminuted fracture of the middle section of the femur. After 30 min, artery intubation and quick bleeding, and simultaneously monitored the blood pressure, when blood pressure dropped to 40-50 mmHg, maintaining 1 hrs, and rapidly venous reinfusion two time volume of liquids with a speed of 20 ml/hrs, including autologous anticoagulated blood and ringer, and sutured skin after disinfection, and regulate feeding. After modeling, the rat was killed by dislocation method, and the whole blood was collected for further using.

Measurement of the expression level of rat serum inflammatory cytokines by enzyme linked immunosorbent assay (ELISA), including TNF- α , IL-2, IL-6, IL-10

The above-collected rat whole blood was incubated for 30 min at room temperature, and centrifuged at 4°C , 4,000 rpm for 10 min to collect serum sample. Subsequently, the serum TNF- α , IL-2, IL-6, and IL-10, expression level was detected by rat TNF- α ELISA kit (No. KRC3021, Thermo, USA), rat IL-2 ELISA kit (No. KRC0022, Thermo, USA), rat IL-6 ELISA kit (No. ER3IL65, Thermo, USA) and rat IL-10 ELISA kit (No. ERIL10, Thermo, USA) according to the manufacturer's instructions. After detection, data was recorded at 450 nm using a microplate reader during 15 min, and analyzed by SPSS software (version 21.0, <http://spss.en.softonic.com/>; Chicago, IL, USA), and histogram analysis was performed using Origin 9.5 software (<http://www.originlab.com/>).

miRNA extraction

The above-collected rats whole blood was incubated for 30 min at room temperature, and centrifuged at 4°C , 4,000 rpm for 10 min to collect serum sample, and a total of 200 μL serum was prepared to extract the miRcute miRNA extraction kit (DP501, TIANGEN Biotech (Beijing) CO., LTD, Beijing, China) according to the manufacturer's instructions. The concen-

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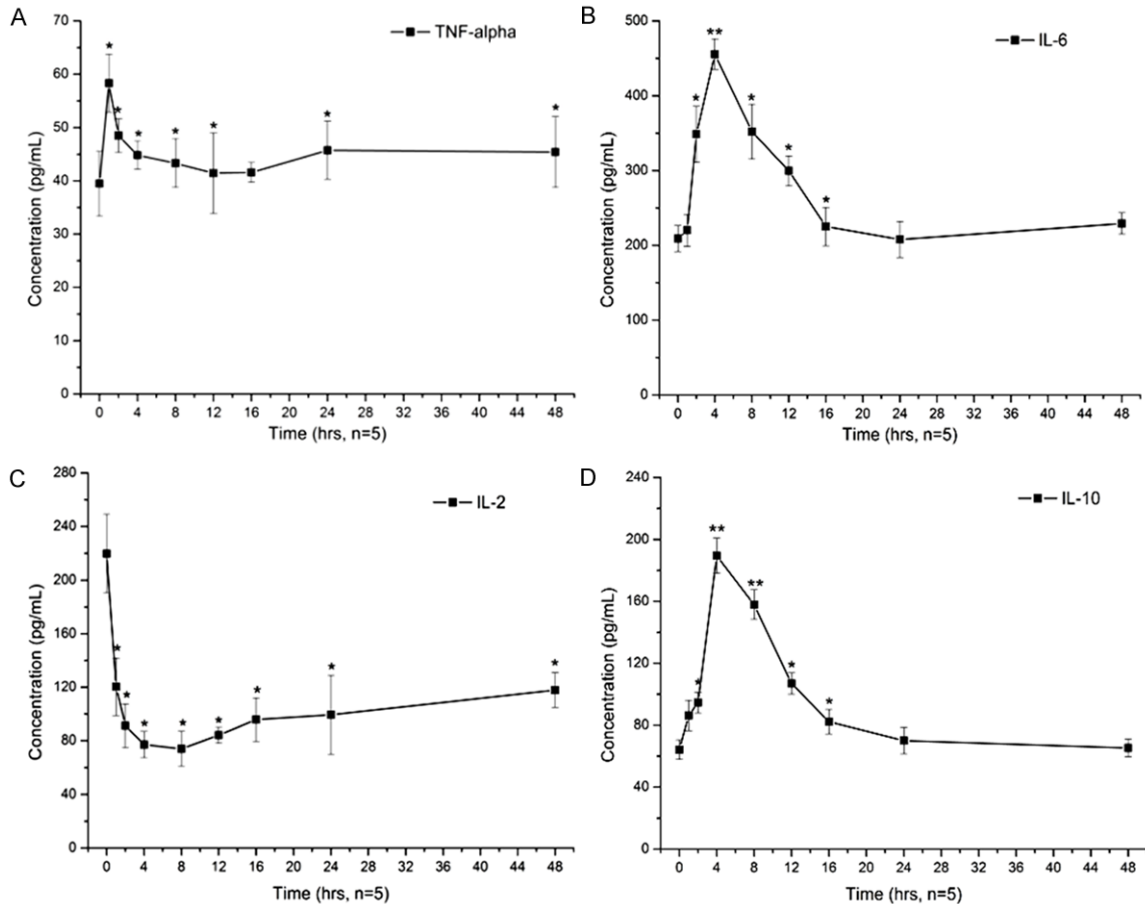


Figure 1. The expression level assay of TNF- α , IL-6, IL-2, and IL-10 in serum after different time traumatic hemorrhagic shock by ELISA. A. The expression level assay of TNF- α in serum after different time traumatic hemorrhagic shock by ELISA; B. The expression level assay of IL-6 in serum after different time traumatic hemorrhagic shock by ELISA; C. The expression level assay of IL-2 in serum after different time traumatic hemorrhagic shock by ELISA; D. The expression level assay of IL-10 in serum after different time traumatic hemorrhagic shock by ELISA. The image indicated that with the trauma time prolonging, the expression level of TNF- α and IL-6 was significantly increased to the peak, and slightly decreased, and similarly the expression level of IL-2 and IL-10 in serum was significantly decreased to the valley, and slightly increased (*: $P < 0.05$; **: $P < 0.01$, when compared to control).

tration and the purity of miRNA was validated by ultraviolet spectrophotometry.

Construction of miRNA library and high throughput sequencing

The above-validated miRNA was separated by polyacrylamide gel to collect the bands of 18-30 nt, and then added adapter on the 5'- and 3'-terminal by polymerase chain reaction (PCR), and the sample was performed high throughput sequencing using Illumina HiSeq 4000 system in BGI company.

Bioinformatics analysis of the differential expression miRNA in serum

The raw data was filtered to remove the redundant joints, uncorrelated and low quality

sequences, and performed sequence alignment to miRBase database, and the differential expression miRNAs was analyzed using t-test combing with the Fold Change (FC), and required $P < 0.05$, the $\log_2(\text{FC}) > 2$ of up-regulated miRNAs, and the $\log_2(\text{FC}) < 2$ of down-regulated miRNAs, and according to the differential expression miRNAs to perform heatmap and volcano analysis using R (<https://www.R-project.org/>).

Gene ontology (GO) clustering

The GO database (<http://geneontology.org/>) includes three functional categories: biological process, cellular component, and molecular function. Genes could be further organized by directed acyclic graph according to their scope.

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Table 1. The significantly up-regulated and down-regulated miRNAs of rat serum after different time traumatic hemorrhagic shock

ID	Base mean	Base meanA	Base meanB	Fold change	log2 Fold change	p-value	p-adj
rno-miR-34c-3p	14.5	2	27	13.5	3.754887502	0.001561784	0.295000897
rno-miR-3560	92	13	171	13.2	3.717412797	0.000000003	0.000001093
rno-miR-375-3p	11	2	20	10	3.321928095	0.012006025	0.863233203
rno-miR-29c-5p	9.5	2	17	8.5	3.087462841	0.028590648	1
rno-miR-325-3p	18.5	4	33	8.3	3.044394119	0.001759142	0.295000897
...
rno-miR-493-5p	5.5	9	2	0.22	-2.169925001	0.255522657	1
rno-miR-544-5p	6.5	11	2	0.18	-2.459431619	0.151899555	1
rno-miR-582-5p	7.5	13	2	0.15	-2.700439718	0.028324205	1
rno-miR-133b-5p	7.5	13	2	0.15	-2.700439718	0.018832420	1
rno-miR-194-5p	10	18	2	0.11	-3.169925001	0.021438929	1

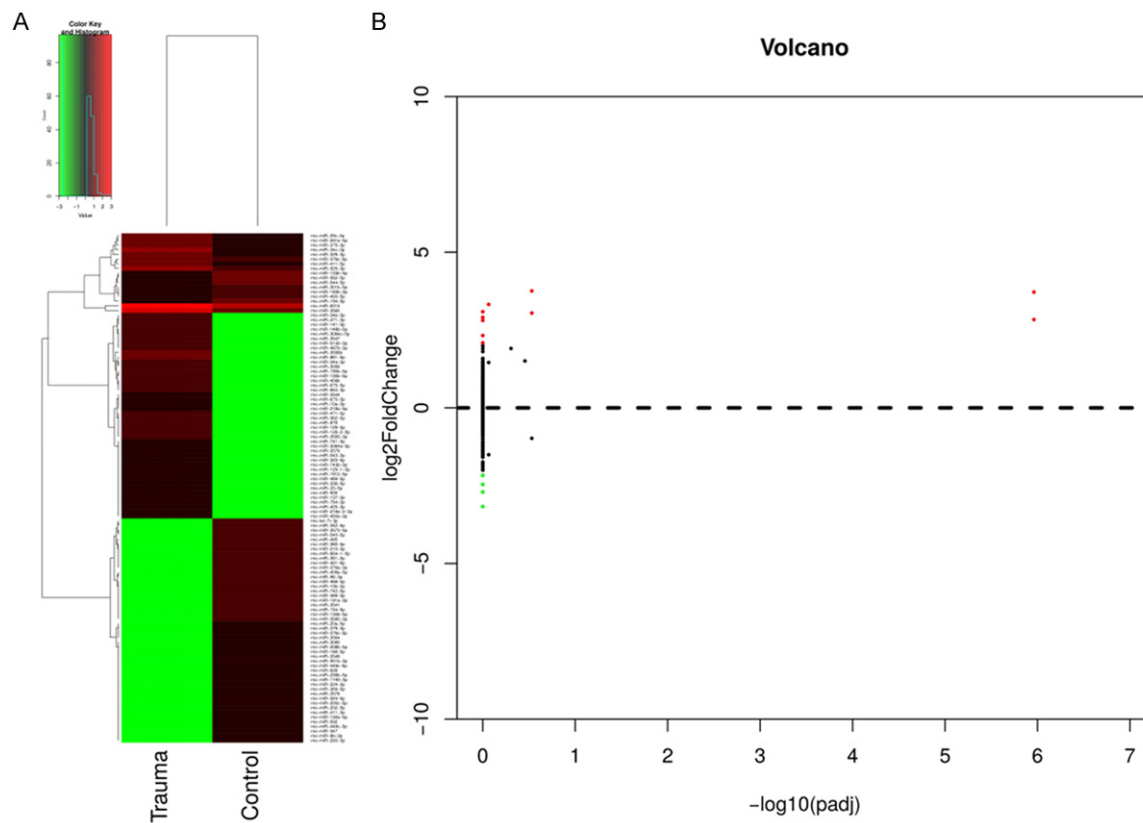


Figure 2. The heatmap and volcano analysis of the differential expression miRNAs in rat serum. A. The heatmap analysis of the differential expression level miRNAs in rat serum; B. The volcano analysis of the differential expression level miRNAs in rat serum.

In GO clustering, genes are considered significantly enriched based on the ratio of the observed GO term for all genes/GO term for a single gene set. First, each gene that was assigned a particular GO term was broadly

noted in the upper father node, then the p-value of each enriched GO term was determined using a hypergeometric distribution, and the p-value was adjusted using the false discovery rate (FDR), with P = 0.05 selected as the thresh-

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Table 2. The primers used for amplification of miRNAs in this study

miRNA	Sequence of primers (5'→3')
rno-miR-34c-3p-frs	ACACTCCAGCTGGGAATCACTAACCACA
rno-miR-34c-3p-rvs	CTCAACTGGTGTGCTGGAGTCGGCAATTCAGTTGAGCCTGGCTG
rno-miR-3560-frs	ACACTCCAGCTGGG CAAATCCTTGCCCG
rno-miR-3560-rvs	CTCAACTGGTGTGCTGGAGTCGGCAATTCAGTTGAGAATGCACC
rno-miR-375-3p-frs	ACACTCCAGCTGGGTTTGTTCGTTCCGGC
rno-miR-375-3p-rvs	CTCAACTGGTGTGCTGGAGTCGGCAATTCAGTTGAGTCACGCGA
rno-miR-582-5p-frs	ACACTCCAGCTGGG TACAGTTGUTTCAAC
rno-miR-582-5p-rvs	CTCAACTGGTGTGCTGGAGTCGGCAATTCAGTTGAGAGTAACTG
rno-miR-133b-5p-frs	ACACTCCAGCTGGGGCTGGTCAAACGG
rno-miR-133b-5p-rvs	CTCAACTGGTGTGCTGGAGTCGGCAATTCAGTTGAGACTTGTT
rno-miR-194-5p-frs	ACACTCCAGCTGGGTGTAACAGCAACTC
rno-miR-194-5p-rvs	CTCAACTGGTGTGCTGGAGTCGGCAATTCAGTTGAGTCCACATG
U6-frs	CTCGCTTCGGCAGCACA
U6-rvs	AACGCTTCACGAATTTGCGT

old value. Subsequently, redundant GO terms were removed, and the hierarchy chart's terminal nodes were selected as the final significantly enriched GO terms.

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway clustering

The KEGG database (<http://www.genome.jp/kegg/pathway.html>) is used to systematically analyze gene function and genomic information from biological pathways, and to further group biological pathways according to metabolism, enzyme, biochemical reaction, gene regulation, and protein-protein interaction. Here, KEGG signaling pathway analysis was applied, followed by hypergeometric distribution analysis and the FDR method to give an adjusted *P*-value ($P = 0.05$ as a threshold value).

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) assay of miRNA

The above-extracted miRNA was used as a template in a reverse transcription reaction using a kit (TOYOBO, Japan), according to the manufacturer's instructions. The miRNA reverse transcription reaction mixture included 10 μ L 2 \times loading buffer, 1.2 μ L miRNA RT primer/U6 small nuclear RNA primer, 2 μ L miRNA template, 0.2 μ L MMLV reverse transcriptase, and 6.6 μ L DEPC-treated H₂O. The reaction was incubated at 26°C for 30 min, followed by 42°C for 30 min, and then by 85°C for 10 min. For qRT-PCR, 100 ng cDNA was used as the tem-

plate in a reaction mixture that included 10 μ L 2 \times Master Mix, 0.08 μ L forward primer, 0.08 μ L reverse primer, 2 μ L cDNA template, 0.4 μ L Taq DNA polymerase, and 7.44 μ L ddH₂O. The qPCR amplification conditions were as follows: one cycle of 95°C for 3 min; 40 cycles of 95°C for 12 s, 62°C for 30 s, and 72°C for 30 s. The results were analyzed using the SDS 1.4 software (Applied Biosystems) based on 2^{- $\Delta\Delta$ Ct}, and histogram analysis using the Origin 9.5 software (<http://www.originlab.com/>).

Statistical analysis

All data expressed as the mean \pm standard deviation (SD). Statistical analysis was performed with one-way ANOVA using SPSS software (version 21.0, <http://spss.en.softonic.com/>; Chicago, IL, USA), and Student's *t*-tests were performed in a group of two sample, and $P < 0.05$ and $P < 0.01$ were considered to indicate significant differences and highly significant differences, respectively.

Results

Establishment of rat traumatic hemorrhagic shock

To elucidate the expression profile of miRNA in rat serum of traumatic hemorrhagic shock, the rat traumatic hemorrhagic shock model was correctly established, and 12 rats were died due to the excessive blood loss and cardiac arrest, and the surplus rats could free eat and

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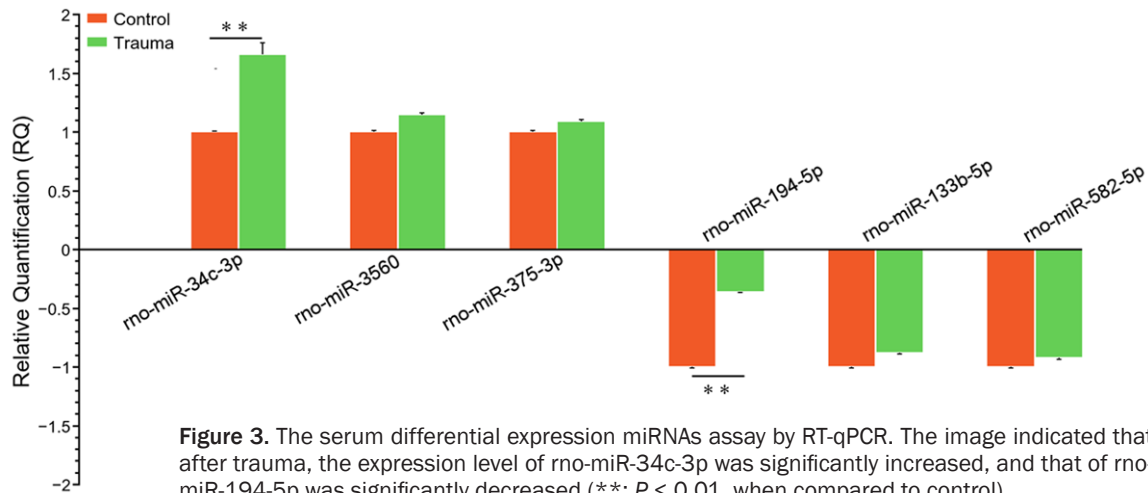


Figure 3. The serum differential expression miRNAs assay by RT-qPCR. The image indicated that after trauma, the expression level of rno-miR-34c-3p was significantly increased, and that of rno-miR-194-5p was significantly decreased (**: $P < 0.01$, when compared to control).

drink, and did not free move, large blood loss, poor spirit, and also combined with chills and tremor, etc.

The expression level of TNF- α and IL-6 was significantly increased to the peak after 4 hrs trauma, and that of IL-2 and IL-10 was significantly decreased to the valley after 4 hrs trauma

As exhibiting of **Figure 1A**, when compared to 0 hrs trauma, the TNF- α expression level was significantly increased with trauma time prolonging, and reached to peak after 2 hrs trauma, and then slightly decreased for 48 hrs trauma (*: $P < 0.05$, **: $P < 0.01$). Similarly, the expression level of IL-6 also exhibited the same trend, and the perk was appeared at 4 hrs trauma (**Figure 1B**, *: $P < 0.05$, **: $P < 0.01$). In addition, the expression level of IL-2 and IL-10 was significantly decreased with trauma time prolonging, and the valley was appeared at 4 hrs trauma (**Figure 1C** and **1D**, *: $P < 0.05$, **: $P < 0.01$).

A total of 18 up-regulated miRNAs and 21 down-regulated miRNAs were identified

After high throughput sequencing and bioinformatics analysis, a total of 18 up-regulated miRNAs and 21 down-regulated miRNAs were identified, and the five significantly up-regulated miRNAs, including rno-miR-34c-3p, rno-miR-3560, rno-miR-375-3p, rno-miR-29c-5p, and rno-miR-325-3p, and the five significantly down-regulated miRNAs, including rno-miR-194-5p, rno-miR-133b-5p, rno-miR-582-5p, rno-miR-544-5p, and rno-miR-493-5p, were exhib-

ited in **Table 1**. In addition, the heatmap and volcano of differential expression miRNAs was analyzed as shown in **Figure 2A** and **2B**, and manifested as significant different in control and trauma group although limitation of the amount of serum miRNA.

The expression of rno-miR-34c-3p was significantly up-regulated, and that of rno-miR-194-5p was significantly down-regulated

The expression level of three significantly up-regulated miRNAs, including rno-miR-34c-3p, rno-miR-3560, and rno-miR-375-3p, and three significantly down-regulated miRNAs, including rno-miR-194-5p, rno-miR-133b-5p, and rno-miR-582-5p, was validated by RT-qPCR, and the selected miRNA sequences and the primers used for amplification were shown in **Table 2**. As exhibiting of **Figure 3**, in three selected significant up-regulated miRNAs, the expression level of rno-miR-34c-3p was significantly increased in 4 hrs trauma group when compared to that of control (**: $P < 0.01$). Similarly, in three selected significant down-regulated miRNAs, the expression level of rno-miR-194-5p was significantly decreased in 4 hrs trauma group when compared to that of control (**: $P < 0.01$). Therefore, the rno-miR-34c-3p and rno-miR-194-5p were chosen for further analysis.

GO clustering of rno-miR-34c-3p and rno-miR-194-5p regulated target genes

As exhibiting of **Figure 4A**, in order to obtain some credible target genes of rno-miR-34c-3p, three miRNA target gene prediction software

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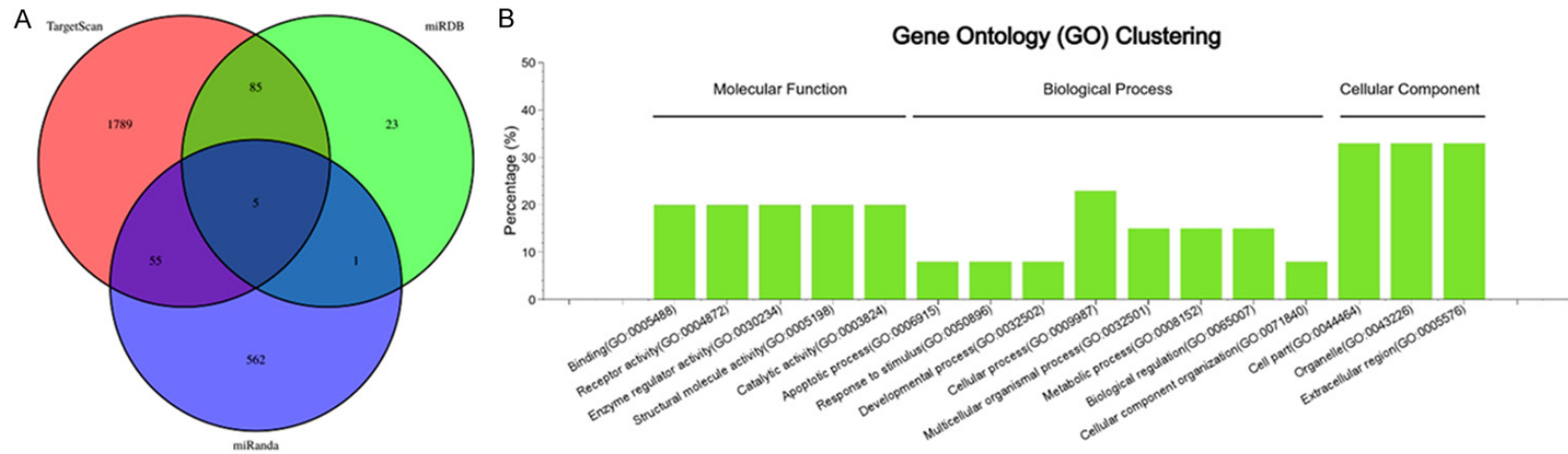


Figure 4. The cross-over analysis rno-miR-34c-3p regulated target genes by venn image, and GO clustering of it. A. The cross-over analysis rno-miR-34c-3p regulated target genes by venn image; B. The GO clustering of rno-miR-34c-3p regulated target genes. The images indicated that in three databases of TargetScan, miRDB, and miRanda, a total of five cross-over genes were identified, and involved in several significant molecular function, biological process, and cellular component.

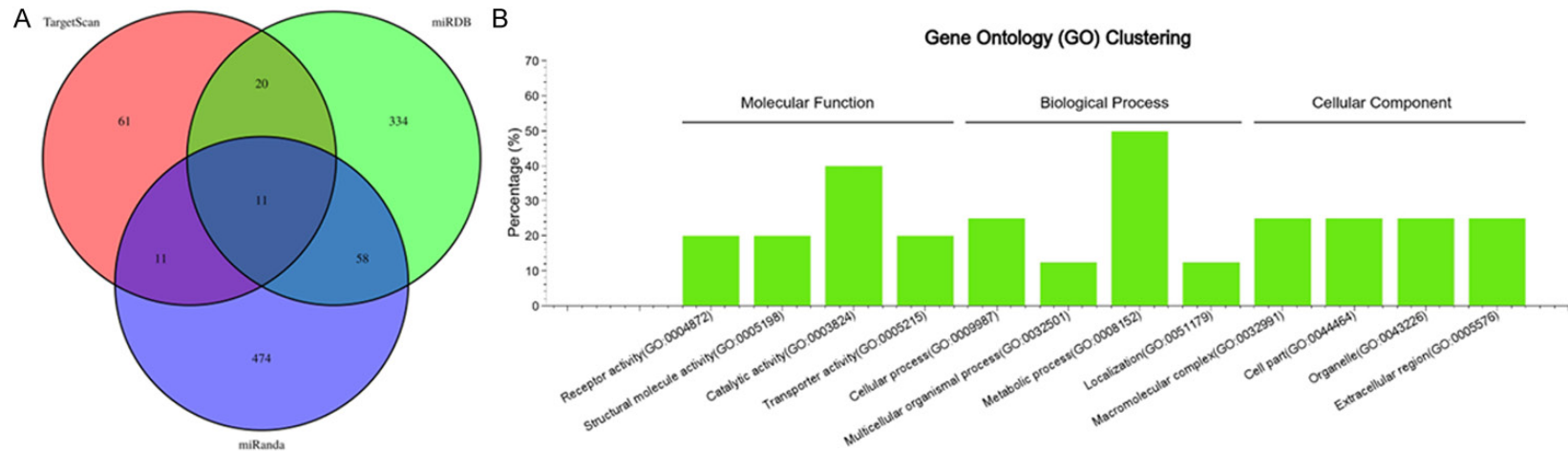
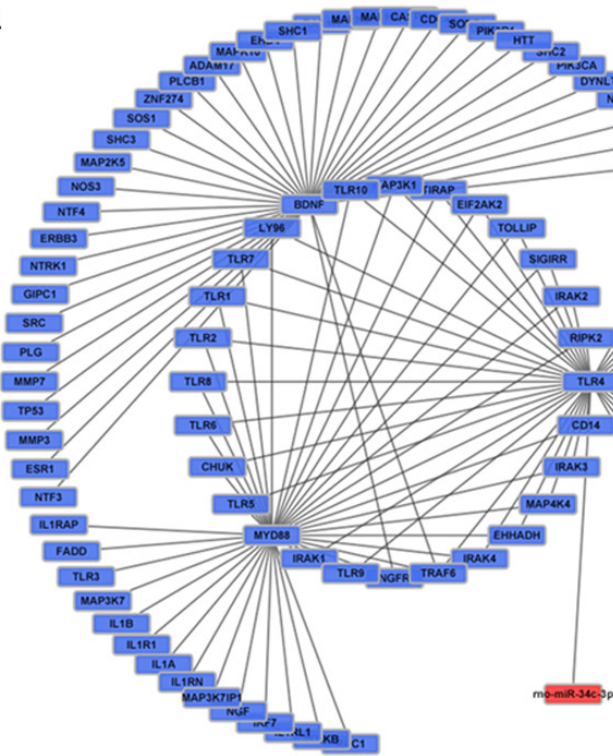


Figure 5. The cross-over analysis rno-miR-194-5p regulated target genes by venn image, and GO clustering of it. A. The cross-over analysis rno-miR-194-5p regulated target genes by venn image; B. The GO clustering of rno-miR-194-5p regulated target genes. The images indicated that in three databases of TargetScan, miRDB, and miRanda, a total of eleven cross-over genes were identified, and involved in several significant molecular function, biological process, and cellular component.

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A



B

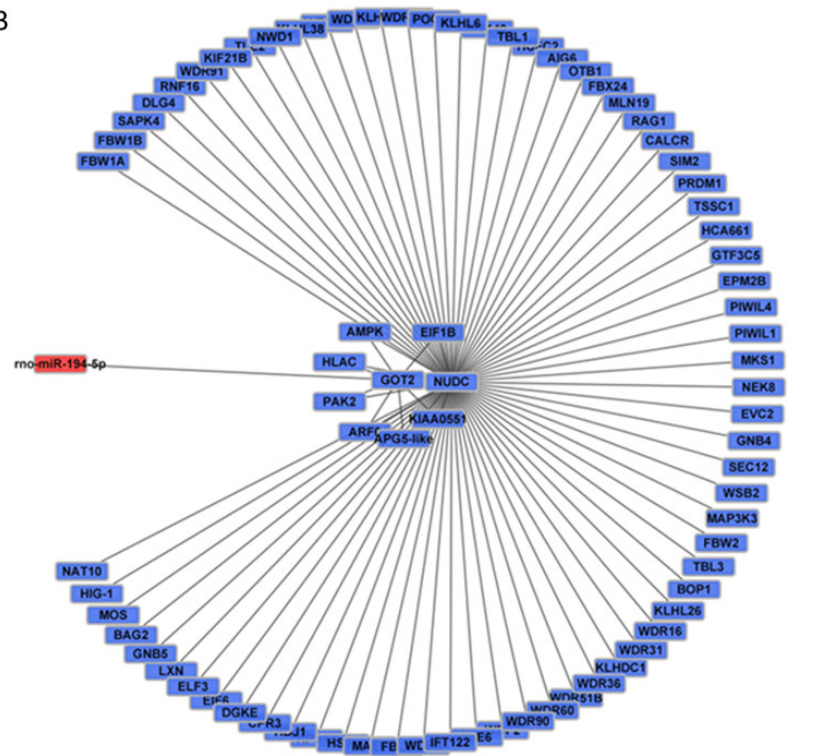


Figure 6. KEGG pathway clustering of rno-miR-34c-3p and rno-miR-194-5p regulated target genes. A. KEGG pathway clustering of rno-miR-34c-3p regulated target genes. B. KEGG pathway clustering of rno-miR-194-5p regulated target genes.

was chosen, including TargetScan, miRDB, and miRanda, and the cross-over target genes was analyzed, and exhibited by Venn image, and a total of five cross-over genes was obtained. Based on it, as exhibiting of **Figure 4B**, rno-miR-34c-3p regulated genes involved in the molecular function of binding, receptor activity, enzyme regulator activity, structural molecule activity, and catalytic activity, and the biological process of apoptotic process, response to stimulus, developmental process, cellular process, multicellular organism process, metabolic process, biological regulation, and cellular component organization, and the cellular component of cell part, organelle, and extracellular region. Similarly, a total of eleven cross-over genes was obtained as Venn image shown in **Figure 5A**, rno-miR-194-5p regulated genes involved in the molecular function of receptor activity, structural molecule activity, catalytic activity, and transporter activity, and the biological process of cellular process, multicellular organism process, metabolic process, and localization, and the cellular component of macromolecular complex, cell part, organelle, and extracellular.

KEGG pathway clustering of rno-miR-34c-3p and rno-miR-194-5p regulated target genes

As exhibiting of **Figure 6A**, rno-miR-34c-3p could directly involve in the regulation of Toll-like receptor 4 (TLR4) gene in the complex pathway, and similarly that of rno-miR-194-5p could directly involved on the regulation of GOT2 gene in the complex pathway (**Figure 6B**).

Discussion

In this study, the optimal trauma time of THS was confirmed based on the altering of inflammatory factors, including TNF- α and IL-6, and anti-inflammatory factors, including IL-2 and IL-10, and manifested as the significant increasing of TNF- α and IL-6, and the significant decreasing of IL-2 and IL-10 with trauma time prolonging. Based on it, numerous differential expression miRNAs with 18 up-regulated miRNAs and 21 down-regulated miRNAs were identified. Wherein, the expression level of rno-miR-34c-3p and rno-miR-194-5p was consistent with the results of high-throughput sequencing, and involved in several significant biological process.

As one of the leading causes of death in the global healthy individuals, THS severely threat-

en the social development of human beings due to its high disability and lethality [31-33]. After trauma, acute circulatory insufficiency was occurred with the tissue ischemia and hypoxia [3, 12, 34]. THS is generally accompanied with the occurrence of inflammation, serious systemic inflammation including SIRS and MODS, and further damaged to distal vital organs [3, 35]. In other to deeply explore the mechanism of THS, and found an effective intervention strategy, it is a key factor to establish a rapid and a stable THS model to simulate its clinical symptom, and therefore an effective animal model to simulate the THS trauma eagerly needed to establish. As a common experimental model, acute mechanical injury method was widely used in the trauma study, and had a lot of advantages, such as the simplicity of operation, the better repeatability and the maneuverability, and therefore rat THS trauma model was established based on acute mechanical injury method using a man-made bracket as an experimental device in this study. After modeling, a total of 12 rats were died due to due to the excessive blood lose and cardiac arrest, and exhibited a significant application value to simulate THS trauma.

Inflammatory cytokines are a kind of biological molecular, and forms the organism immune response, including interleukin, interferon, tumor necrosis factor, chemotactic factor and colony stimulating factor, etc, and plays a key role on the regulation of organ immune response, differentiation and development of immune cells [36-38]. Wherein, TNF- α is a signaling cytokine involving in systemic inflammation in the acute phrase, and is one of the main culprit behind that caused cascade effect of SIRS and MODS [39, 40]. IL-6 is a potent pleiotropic cytokine that regulates cell growth and differentiation and plays an important role in immune response [41-43]. The expression of IL-6 in blood is a key signal to reflect the degree of severity of THS. Under normal conditions, the concentration of IL-6 in blood circulation was lower. Once in stress conditions, the expression of IL-6 was significantly increased with inflammation degree aggravated [6, 44]. IL-2 is a heterotrimeric protein expressed on the surface of certain immune cells, such as lymphocytes, and could activate the proliferation of T cells, NK cells, and the activation of B cells and the immune of response monocyte/macrophage [43]. IL-10 is a key anti-inflammatory

cytokine, and plays a key role on the balance of anti-inflammatory reaction and pro-inflammatory reaction [43]. When the pro-inflammatory reaction occupied the dominant advantages, the body presented the symptom of SIRS. Oppositely, when the anti-inflammatory reaction occupied the dominant advantages, the body presented as immunosuppression [40, 43]. In this study, we systematic measured the expression level of TNF- α , IL-6, IL-2 and IL-10 in serum at different trauma time, and exhibited that the expression level of inflammatory cytokines after THS was significantly altered, and mainly manifested as the increasing of TNF- α and IL-6, and the decreasing of IL-2 and IL-10. After acute mechanical injury, the wounding leg appeared seriously inflammatory reaction, and the production of cytokines entered the blood circulation. The expression of TNF- α significantly increased after 1 hrs trauma, and subsequently returned to the normal level. The expression of IL-2 significantly decreased after 4 hrs trauma, and continuously decreased with time prolonging after 48 hrs. The expression of IL-6 was significantly increased after 4 hrs, and gradually returned to the normal level after 16 hrs. The expression of IL-10 was significantly increased after 4 hrs, and gradually returned to the normal level after 20 hrs, and therefore the 4 hrs trauma was selected as a optimal trauma time.

Blood examination method have advantageous aspects of convenient, rapidly and non-invasive [45-47]. It is a significantly index to evaluate the trauma severity degree via detecting the expression of specific marker [47, 48]. Recently study demonstrated that it was urgent need to seek a rapid and stable marker instead of slow reaction biomarker. miRNA remained stable in serum, and quickly changed under different expression profiles of diseases. A great deal of research have been carried out that serum miRNA was a novel therapy target to evaluate the occurrence and development of diseases [47, 49]. It was crucial to deeply understand the mechanism of miRNA which involved in the inflammatory reaction on the course of THS, and found a novel miRNA which was related to inflammatory factor. With the development of gene sequencing, next generation sequencing technology have been preferred by more and more researchers on account of high throughput, high efficiency and high reliability [50-52].

In this study, a total of 39 significant differential expression miRNAs was identified after high throughput sequencing, including 18 up-regulated miRNAs and 21 down-regulated miRNAs. qRT-PCR results demonstrated that the expression of rno-miR-34c-3p was significantly up-regulated, and that of rno-miR-194-5p was significantly down-regulated, and was consistent with the results of high throughput sequencing, and involved in several significant biological process as GO and KEGG pathway clustering.

Although several differential expression miRNAs were identified in serum after THS in this study, and however that several additional miRNAs and its biological function need to be further evaluated in the future due to the limitation of sample size, the variability and the instability of data.

The present study identified several differential expression miRNAs in serum after THS trauma, and two significant differential expression miRNAs, including rno-miR-34c-3p and rno-miR-194-5p, were confirmed involving in several biological process, and exhibited a significant reference on the study of the mechanism of THS, and also provided a potential early diagnosis biomarker of THS.

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Disclosure of conflict of interest

None.

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