The predict value of serum miRNA150 on sepsis prognosis

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Abstract: Sepsis is a systemic inflammatory response syndrome mainly caused by various types of infection. MiR-150 plays an important role in the inflammatory response by inhibiting the transcription of target genes. This study established rat sepsis model and detected miR-150 level in serum to analyze its clinical value for the prognosis of sepsis. SD rats in SPF grade were used to establish sepsis model by cecum ligation. ELISA was performed to detect levels of IL-6, CRP, and TNF-α in serum. Real time PCR was applied to test miR-150 expression. Our result showed that the rats appeared shortness of breath, sleepiness, dysphoria, stop eating, stop drinking, and dullness hair at 30 min after modeling. The symptom gradually aggravated over time. Sepsis rats showed significantly increased levels of WBC, IL-6, CRP, and TNF-α in serum at 6 h, 12 h, and 24 h, compared with those in sham operation group (P < 0.05). The expression of miR-150 in sepsis rats obviously decreased from 6 h compared with that in sham operation group and control group (P < 0.05). MiR-150 level in sham operation group presented a slight decline compared with control (P < 0.05). Additionally, our data indicated a negative correlation between miR-150 expression and levels of WBC, IL-6, CRP, and TNF-α (P < 0.05). In conclusion, we propose that MiR-150 is a valuable tool for the prognosis of sepsis, and the development of novel drug targeting against miR-150 may provide leads for the sepsis therapy.

Keywords: miR-150, sepsis, IL-6, CRP, TNF-α

Introduction

Sepsis is a type of infection that induced systemic inflammatory response syndrome. Due to the increase of various kinds of microorganism and toxins in the blood and tissue, a series of systemic diseases, including multiple organ dysfunction, acute respiratory distress, acute or chronic infection, and shock, occur, which have a significant negative impact on the health-related quality of life [1]. It has been demonstrated that approximately 600,000 sepsis patients were diagnosed in North America every year with mortality as 30%-50% and medical costs were up to $17 billion. Moreover, the morbidity and mortality from sepsis shows an increasing trend year by year. It has been estimated that the population of sepsis patients may reach 1.1 million by 2020 [2]. Recent survey revealed that in Mainland China, sepsis morbidity, mortality, and cost in ICU were 8.68%, 48.7%, and $11,390 [3]. The major reasons for severe morbidity and mortality of sepsis are trauma and aging and more effective treatments for sepsis remain to be further developed in spite of the certain role of complicated and high-risk surgeries [4]. MicroRNA (miRNA) is a kind of non-coding RNAs that can bind with target mRNA to exert regulatory role in inflammation, metabolism, and tumor process through a series of signaling pathways [5]. Early diagnosis, severity evaluation, prognosis, and intervention can reduce sepsis aggravation and decline the morbidity rate. Recent progresses obtained further knowledge about miRNAs and demonstrated that they could be treated as new indicators in early diagnosis and prognosis of sepsis [6]. So far miRNA investigation has been mainly focused on miR-155, miR-125b, miR-146, miR-132, and miR-223. MiR-150 remains much to be learnt. To clarify whether miR-150 has indication role in sepsis occurrence and development, this study established sepsis rat model and detected miR-150 level in serum to analyze its clinical value in prognosis.
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Materials and methods

Experimental animal

45 male SD rats in SPF grade weighted 220-250 (205±15) g were provided by the animal experiment center of Zhejiang University School of Medicine. Rats were used for all experiments, and all procedures were approved by the Animal Ethics Committee of The First People’s Hospital of Hangzhou.

Reagents

IL-6, CRP, and TNF-α ELISA kits were purchased from Keygentec (Nanjing, China). DMEM, fetal calf serum, restriction enzyme, DNA ligase, plasmid DNA kit, and PCR kit were bought from Dalian Green Bamboo Biotech Company.

Grouping

SD rats were randomly and equally divided into three groups including sepsis group, sham operation group, and normal control. Cecum ligation was used to establish sepsis model. The rats in sham operation group only received laparotomy but not cecum ligation. The rats in each group were feed with same food and water.

Sepsis model establish

Food and water were fasted before 12 h and 4 h, respectively, before experiments. Rats received 0.3% pentobarbital sodium at 1 ml/100 g intraperitoneal injection for anesthesia. Then they were fixed in the supine position and received conventional abdominal skin disinfection. Longitudinal incision via ventral midline line at 2 cm long was made to expose the cecum. After ligating the end of cecum at 5 mm from ileocecal valve, we made three holes at ligation and 1/2 of cap end to form appendix leakage. The rats in sham operation group only received laparotomy and cecum exposure. The clinical manifestation was observed at 30 min after modeling to determine the success of the implementation.

Serum specimen collection

The rats were euthanized at 6 h, 12 h, and 24 h after operation. 6 ml inferior vena venous blood was extracted with EDTA anticoagulation and kept at room temperature for 1 h. After centrifuged at 3000 r/min for 10 min, the supernatant was collected and stored in -20°C.

Enzyme linked immunosorbent assay (ELISA)

Standard substance: 0.75 ml distilled water was added to the bottle containing different amounts of CRP for 15 min. Washing buffer: 40 ml thick lotion was diluted by 960 ml distilled water or deionized water. Incubation buffer: 12 ml biotin was added to 600 μl Tracer. Plate washing: Wash the microplate coated by streptavidin by washing buffer. 25 μl standard substance or sample was added to each well, together with 100 μl incubation buffer. The plate was vibrated at room temperature for 1 h and washed for 6 times. 100 μl TMB-HRP substrate was added and the plate was vibrated under room temperature for 30 min. After added by 100 μl HRP stop buffer, the plate was then vibrated for 1 min and read at 405 nm on microplate reader for OD value within 15 min. OD value and CRP concentration were used to draw the standard curve for IL-6, CRP, and TNF-α content calculation.

qPCR

Total RNA was extracted by TRIlzol according to the manual. RNA concentration was calculated by A260/A280. 200 ng total RNA was used to synthetize poly A tail and cDNA. qPCR amplification was performed based on cDNA by using the kit of SYBR green supermix (Biorad, USA). The reaction contained 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 30 s. U6 was selected as internal reference. Primers used were listed in Table 1.

Statistical analysis

All data was treated by SPSS17.0 software. Enumeration data was presented as mean ± standard deviation and calculated by Chi-square test. Measurement data was calculated by ANOVA or Spearman correlation analysis. P < 0.05 was considered as statistically significant.

Table 1. The primers for the detection of miR-150 and U6

<table>
<thead>
<tr>
<th>Gene</th>
<th>Source</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-150</td>
<td>S: TGCCCAACAAGGTCAAGA</td>
<td>Rat 56</td>
</tr>
<tr>
<td></td>
<td>A: GACAGGTAGTGCGGGAT</td>
<td></td>
</tr>
<tr>
<td>U6</td>
<td>S: GTTGGACCTTCACACCCC</td>
<td>Rat 56</td>
</tr>
<tr>
<td></td>
<td>A: GTGGCCATCTTGCTGAAGCT</td>
<td></td>
</tr>
</tbody>
</table>

The rats were euthanized at 6 h, 12 h, and 24 h after operation. 6 ml inferior vena venous blood was extracted with EDTA anticoagulation and kept at room temperature for 1 h. After centrifuged at 3000 r/min for 10 min, the supernatant was collected and stored in -20°C.

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Statistical analysis

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Results

General condition of rats model

General condition was observed after sepsis model was established. The rats in control group showed normal appetite, breath, and activity, while the rats in sepsis model group appeared shortness of breath, sleepiness, dysphoria, stop eating, stop drinking, and dullness hair at 30 min after modeling.

Changes of WBC, IL-6, CRP, and TNF-α levels in serum at different time points

Sepsis rats showed significantly increased levels of WBC, IL-6, CRP, and TNF-α in serum at 6 h, 12 h, and 24 h compared with sham operation group and control group (P < 0.05). In sham operation group, WBC, IL-6, CRP, and TNF-α levels slightly elevated at 12 h compared with 6 h, whereas they showed no statistical difference at 24 h compared with control group (Table 2).

Expression analysis of miR-150

Real time PCR showed that the expression of miR-150 in rats without the operation maintained a high level at each time point, while significantly decreased level in sepsis rats were observed from 6 h to 24 h (P < 0.05). MiR-150 level in sham operation group, on the other hand, presented a slight decline compared with control (P < 0.05) (Table 3).

Correlation analysis between serum miR-150 expression and WBC, IL-6, CRP, and TNF-α levels

Serum WBC, IL-6, CRP, and TNF-α levels in sepsis rats gradually upregulated after surgery and reached peak at 24 h. Correlation analysis showed that miR-150 expression was negatively correlated with serum WBC, IL-6, CRP, and TNF-α levels at 24 h (r=-0.712, P=0.001; r=-0.701, P=0.001; r=-0.736, P=0.001; r=-0.728, P=0.001) (Figure 1).

Discussion

Sepsis is the systemic inflammatory response syndrome caused by infection, leading to septic shock and multiple organ dysfunction syndrome. Some severe cases even appear an outcome endpoint of death. Especially after the complicated surgery, main postoperative complications commonly included trauma, burn, critical disease, and infection. The invasion of pathogenic microorganisms, as one of the critical factors, along with the components, causes infection and aggravates inflammatory responses and organ damage, which injures the. For instance, lymphocyte apoptosis were in-

Table 2. Levels of WBC, IL-6, CRP, and TNF-α in serum at different time point

<table>
<thead>
<tr>
<th>Group</th>
<th>WBC (10^9/L)</th>
<th>IL-6 (ng/ml)</th>
<th>TNF-α (ng/ml)</th>
<th>CRP (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sepsis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 h</td>
<td>10.05±4.27</td>
<td>347.34±56.25</td>
<td>420.31±52.27</td>
<td>6.65±0.29</td>
</tr>
<tr>
<td>12 h</td>
<td>12.52±5.56</td>
<td>384.27±56.44</td>
<td>484.13±58.04</td>
<td>9.24±1.34</td>
</tr>
<tr>
<td>24 h</td>
<td>14.02±6.13</td>
<td>392.85±57.38</td>
<td>464.35±51.32</td>
<td>9.45±1.28</td>
</tr>
<tr>
<td>Sham operation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 h</td>
<td>6.04±1.01</td>
<td>28.12±3.27</td>
<td>28.36±3.86</td>
<td>1.24±0.43</td>
</tr>
<tr>
<td>12 h</td>
<td>6.27±1.04</td>
<td>31.32±3.81</td>
<td>31.75±4.03</td>
<td>1.48±0.45</td>
</tr>
<tr>
<td>24 h</td>
<td>6.31±1.12</td>
<td>31.21±3.27</td>
<td>31.15±3.66</td>
<td>1.72±0.41</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 h</td>
<td>5.95±0.84</td>
<td>28.01±3.27</td>
<td>28.24±38.11</td>
<td>1.24±0.32</td>
</tr>
<tr>
<td>12 h</td>
<td>6.02±0.96</td>
<td>29.32±3.81</td>
<td>29.15±38.32</td>
<td>1.28±0.35</td>
</tr>
<tr>
<td>24 h</td>
<td>6.07±1.02</td>
<td>29.81±3.47</td>
<td>29.41±38.53</td>
<td>1.42±0.42</td>
</tr>
</tbody>
</table>

*P < 0.05, compared with sham operation group; #P < 0.05, compared with control; P < 0.05, compared with at 6 h; &P < 0.05, compared with at 12 h.

Table 3. MiR-150 expression changes at different time point

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>miR-150</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 h</td>
<td>12 h</td>
</tr>
<tr>
<td>Sepsis</td>
<td>15</td>
<td>8.02±0.22*</td>
</tr>
<tr>
<td>Sham operation</td>
<td>15</td>
<td>10.51±1.61*</td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
<td>11.13±1.02*</td>
</tr>
</tbody>
</table>

*P < 0.05, compared with sham operation group; #P < 0.05, compared with control.
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duced in patients with sepsis, resulting in further dysregulation of immune function as well as a weakened immune system [7, 8]. As endogenous non-coding small RNA, miRNA can mediate mRNA cracking, restrain the translation process, posttranscriptionally regulate mRNA, and inhibit gene expression [9]. The miRNA was firstly found in caenorhabditis elegans. Advances occur as tens of thousands of miRNAs had been discovered by researchers [10]. MiRNAs can accurately regulate the immune reaction from multiple aspects, such as regulate the immune cell differentiation, proliferation, and signaling pathway, and strengthen the innate immune and adaptive immune response. MiR-150 is able to adjust immune system diseases including RA and systemic lupus erythematosus, and participates in inflammation [11]. It has been found that miRNA regulates inflammatory response imbalance through a series of antigen-antibody reaction and the receptor pathway, including antigen presentation, cytokines, toll-like receptor, and lymphoid cell receptor. MiRNA is of great significance in sepsis occurrence, development and prognosis [12].

In this study, we selected SD rats in SPF grade to establish sepsis model through cecum ligation centesis. The rats in control group presented normal appetite, respiration, and activities, while the rats in sepsis group appeared shortness of breath, sleepiness, dysphoria, stop eating, stop drinking, and dullness hair at 30 min after modeling. Equal numbers of rats were killed at 6 h, 12 h, and 24 h after modeling. Sepsis rats showed significantly increased levels of WBC, IL-6, CRP, and TNF-α in serum at 6 h, 12 h, and 24 h compared with sham operation group. Their levels were obviously higher than that in sham operation group and control group at each time point. In sham operation group, the expressions of WBC, IL-6, CRP, and TNF-α in serum slightly elevated at 12 h compared with 6 h, whereas they showed no statistical difference at 24 h compared with control group. It revealed that WBC, IL-6, CRP, and

Figure 1. Correlation between serum miR-150 expression and WBC, IL-6, CRP, and TNF-α.
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TNF-α levels gradually upregulated in sepsis rats, suggesting that the sepsis rat model was successfully established. CRP had been discovered by Tillet and Francis in early 1930. CRP is mainly synthetized by cytokines-stimulated liver cells. The over expression of reactive protein primarily occurs in the acute phase of tissue injury. As a kind of inflammatory reaction repair factor, it can be used in the detection of tissue damage [13]. TNF-α is earliest factor released into the blood among various cytokines, especially those participate in the inflammatory response. It is therefore an important indicator for timely and accurately reflecting the intensity of inflammation response, so as to evaluate the severity [14]. IL-6 is an anti-inflammatory factor mainly secreted by CD4+ Th2 cells. During late stage of sepsis, the body was in immunosuppressive state and gave priority to generate Th2 cells. Thus, IL-6 kept in high level in blood [15], which is consistent with our results.

This study detected miR-150 expression at different time point and found it with no alternation but maintained at high level in normal control group. It significantly decreased in sepsis rats from 6 h to 24 h. MiR-150 level in sham operation group gradually declined compared with control. Recent findings showed that except miR-150, levels of miR-342 and miR-146b appeared reduction in sepsis patients. On the contrary, miR-182, miR-486, and miR-143 presented different degrees of overexpression [16]. Studies reported that miR-150 showed the most obvious difference in inflammation among multiple miRNAs [17], which was consistent with our results.

Correlation analysis indicated that miR-150 expression was negatively correlated with serum WBC, IL-6, CRP, and TNF-α levels. As cytokines such as IL-6, CRP, and TNF-α can promote inflammation and lead to sepsis, miR-150 may have predictive effect on sepsis occurrence, development, and prognosis. Pioneering work also proposed that miR-150 level can assess the severity of patients with sepsis [18]. Previous studies showed that the sequence of miR-150 was complementary to TNF-α and IL-10 coding sequences, manifesting a regulatory role in the balance between proinflammatory and anti-inflammatory factors [19]. The change of MiR-150 expression may reflect inflammation severity to a certain extent, and provide guidance for sepsis occurrence, development, and prognosis [20].

To sum up, the levels of WBC, IL-6, CRP, and TNF-α in serum gradually elevated in sepsis rats, while miR-150 expression persistently reduced. Our data illustrated a negative correlation between MiR-150 and levels of WBC, IL-6, CRP, and TNF-α. MiR-150 performed predictive function for sepsis occurrence, development, and prognosis. Our result highlights the potential role of novel drug target against miR-150 for sepsis therapy, which provides a future basis for fundamental research and clinical application.

Disclosure of conflict of interest

None.

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References

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