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MiR-150 predicts survival in patients with sepsis and inhibits LPS-induced inflammatory factors and apoptosis by targeting NF-κB1 in human umbilical vein endothelial cells

Yuezhen Ma [a], Yanni Liu [a], Huihui Hou [a], Yanfen Yao [a], Hua Meng [b, *]

[a] Department of Intensive Care Unit, Shandong Provincial Third Hospital, No.12, Central Wuying Hill Road, Jinan 250031 Shandong, China
[b] Department of Orthopedics, The Second Hospital of Shandong University, No.247, Beiyuan Avenue, Jinan 250033, Shandong, China

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ABSTRACT

MiR-150 is involved into some pathological processes, such as tumorigenesis and autoimmune diseases. However, little is known about the involvement of miR-150 in human sepsis. In this study, plasma miR-150 level had a diagnostic and independent prognostic value in patients with sepsis, and negatively correlated with renal dysfunction and 28-day survival as well as plasma levels of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). MiR-150 expression was also significantly decreased in human umbilical vein endothelial cells (HUVECs) and C57BL/6 mice with sepsis after lipopolysaccharides (LPS) treatment. In-vitro, miR-150 over-expression protected HUVECs from LPS-induced apoptosis and the expressions of nuclear factor-κB1 (NF-κB1), IL-6, TNF-α, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin. Furthermore, NF-κB1 was identified as a direct target of miR-150. Restored NF-κB1 expression antagonized the protective effects of miR-150, while suppression of NF-κB1 enhanced these protective effects. Our findings indicate miR-150 predicts survival in patients with sepsis and inhibits LPS-induced inflammatory factors and apoptosis by targeting NF-κB1 in human umbilical vein endothelial cells. Thus, miR-150 may be a useful biomarker or target in the diagnosis, prognosis and treatment of patients with sepsis.

1. Introduction

Sepsis and subsequent multiple organ failure remain the major cause of morbidity and mortality in the intensive care units (ICU) [1,2]. In spite of the development of some new therapeutic compounds, sepsis/septic shock is still accompanied by high mortality rates up to 50% in the ICU [3,4]. Therefore, it is essential to find novel targets or biomarkers to significantly improve therapeutic effects and prognostic assessment in patients with sepsis admitted to ICU.

MiRNAs are endogenous, non-coding single-stranded RNAs (19–23 nucleotides) that regulate the expression of target genes according to sequence complementarity principles [5,6]. Varieties of miRNAs have been demonstrated to be involved in the inflammation progression by the NF-κB pathway [7,8]. A specific deregulation of miR-150 was described in different diseases, such as bacterial infections or autoimmune diseases [9–11]. Most studies suggested that miR-150 alleviated inflammatory responses to promote angiogenesis or prevent tissue injury [12–15]. However, the mechanisms underlying miR-150-involved sepsis are rarely investigated.

In the present study, we firstly explored the role of miR-150 in the diagnosis and prognosis in septic patients. Then, we investigated the biological functions of miR-150, and then clarified its potential mechanisms in human sepsis.

2. Materials and methods

2.1. Study design

In this work, 120 patients with sepsis (67 male, 53 females; a median age of 48 years, range 39–59 years) were prospectively recruited in the ICU of Shandong Provincial Third Hospital. 50 healthy blood donors (30 male, 20 female, median age 47 years, and range 29–68 years) with normal values for blood counts, C-reactive protein (CRP), liver enzymes and creatinine were recruited from the

* Corresponding author.
E-mail address: menghuasdu@163.com (H. Meng).

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Abbreviations

3′-UTR	3′-untranslated region
AUC	area under the curve
CLP	cecal ligation and puncture
HEK293T	human embryonic kidney-293T
HUVECs	human umbilical vein endothelial cells
ICAM-1	intercellular adhesion molecule-1
IL-6	interleukin-6
LPS	lipopolysaccharides
miR-150	miRNA-150
miR-NC	negative control miRNAs
NF-κB1	nuclear factor-κB1
ROC	receiver operating characteristic
TNF-α	tumor necrosis factor-α
VCAM-1	vascular cell adhesion molecule-1
WBC	white blood cell count

blood donation center as healthy controls. Blood samples were collected prior to therapeutic interventions, centrifuged for 10 min at 2000 g, and serum samples were stored at −80 °C until use. Patients were included in the study upon providing written informed consent. The study protocol conformed to Declaration of Helsinki and was approved by the ethics committee of Shandong Provincial Third Hospital. All patients were treated in accordance with current guidelines for treatment of sepsis (Surviving Sepsis Campaign) and specific guidelines of the respective boards.

2.2. Cell culture

HUVECs and human embryonic kidney-293T (HEK293T) cells were obtained from the Cell Resource Center of Shanghai Institute of Life Science (Shanghai, China). The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS, Invitrogen), 100 U/mL penicillin (Invitrogen) and 100 mg/mL streptomycin (Invitrogen) at 37 °C in a humidified and 5%-CO2 atmosphere.

2.3. Cell transfection

MiR-150 mimics, miRNA negative control (miR-NC), small interfering RNAs (siRNAs) targeting NF-κB1 (si-NF-κB1) and negative control siRNA (si-control) were chemically produced by GenePharma Co., Ltd. (Shanghai, China). The NF-κB1 overexpressing pcDNA3.1-NF-κB1 plasmids and empty pcDNA3.1 plasmids were acquired from GeneCopoeia (Guangzhou, China). For the transfection assays, cells were inoculated into six-well plates at 60%–70% confluence. The transfection was performed using Lipofectamine® 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

2.4. RNA isolation, qRT-PCR, and enzyme linked immunosorbent assay (ELISA)

RNA was extracted using TRIzol (Invitrogen) and RNeasy Mini Kit (Qiagen), and reverse transcribed to cDNA using SuperScript Reverse Transcriptase III following the manufacturer's protocol (Invitrogen). Quantitative reverse transcription-PCR (qRT-PCR) was performed with iCycler thermal cycler (Bio-Rad) using iQ SYBR Green supermix. U6 or GAPDH was used as an internal standard. ELISA was conducted using specific Quantikine ELISA kit (R&D). Serum protein levels were quantified using Inflammatory Cytokine and Chemokine Multi-Analyte ELISArray and specific single analyte ELISA kits (Qiagen) with manufacturer's protocol.

2.5. Western blot analysis

Total protein lysates were resolved by 10% SDS-PAGE and transferred to polyvinyldifluoride membranes (Millipore, Bedford, MA). After blocking in Tris buffered saline containing 0.1% Tween-20 (TBS-T) with 5% nonfat dry milk for 30 min, membranes were washed 4 times in TBS-T and incubated with primary antibodies overnight at 4 °C. Primary antibodies were all obtained from Abcam (Cambridge, MA). After extensive washing, membranes were incubated with horseradish peroxidase-linked goat polyclonal anti rabbit IgG secondary antibodies at a dilution of 1:2000 for 1 h at room temperature. Immunoreactivity was detected by enhanced chemiluminescence (ECL kit, Pierce Biotechnology) and exposure to radiography film. GAPDH served as the loading control.

2.6. Apoptotic assay

Apoptosis analysis was performed to identify and quantify the apoptotic cells by using Annexin V-FITC/PI apoptosis detection kit (BD Biosciences, Franklin Lakes, NJ, USA) followed by flow cytometry.

2.7. Dual luciferins activity assay

The constructed pMiR-luciferase report vector (Promega, Madison, WI, USA) with 3′UTR sequence of NF-κB1 carrying the putative miR-150 binding sites was used to assess binding effect between miR-150 and NF-κB1. Cells were co-transfected with the constructed vector and miR-150 mimic by using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). The empty vector and scramble control were used as negative control, respectively. Reporter analyses were performed by using the dual-luciferase assay system (Promega, Madison, WI, USA) according to the manufacturer's instructions.

2.8. Animal experiments

C57BL/6 mice (25–30 g, 8–12 weeks of age) were purchased from Experimental Animal Center of Shandong University (Jinan, China). All animals were maintained in animal rooms at 22 °C in a 12-h light/dark cycle and received food and water ad libitum. All animal experiments were approved by the Ethical Committee on Animal Research at Shandong Provincial Third Hospital. The mouse model of cecal ligation and puncture (CLP) as a well-established model for polymicrobial sepsis was used. C57BL/6 mice were subjected to CLP surgery as described previously [16]. The mice were intratracheally administered with LPS (Sigma-Aldrich St.Louis, MO, USA) at the dose of 10 mg/kg body mass, and the control group received equal amount of phosphate buffer saline (PBS). Blood was taken before and 24 h after surgery and serum was stored in −80 °C until use.

2.9. Statistical analysis

Data were presented as mean ± standard deviation (SD). Two-tailed student's t-test was applied to compare the differences between two groups. One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison was employed to compare the differences among three independent groups. Correlation was determined using Pearson's correlation analysis. Survival curves were plotted using the Kaplan-Meier curves and compared using the log-rank test. 28-day survival data was evaluated using