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MiR-205 influences renal injury in sepsis rats through HMGB1-PTEN signaling pathway

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Abstract. – OBJECTIVE: To investigate the influences of micro ribonucleic acid (miR)-205 on renal injury in sepsis rats through the high-mobility group box 1 (HMGB1)-phosphatase and tensin homolog deleted on chromosome ten (PTEN) signaling pathway.

MATERIALS AND METHODS: A rat model of sepsis-induced renal injury was established by cecal ligation and perforation. The rats were randomly divided into 3 groups, namely the Sham group, the Model group, and the miR-205 group. Hematoxylin and eosin (HE) staining was applied to examine the pathological renal morphology. The enzyme-linked immunosorbent assay (ELISA) was adopted to measure the serum levels of Caspase-3 and Bcl-2-associated X protein (Bax) in rats. Cell apoptosis rate in the renal tissues was detected via terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay. Finally, the protein levels of phosphorylate-HMGB1 (p-HMGB1) and p-PTEN in the renal tissues were determined using the Western blotting (WB) assay.

RESULTS: Compared with those in the Sham group, the pathological morphology of the renal tissues was poor in Model group. The serum levels of Caspase-3 and Bax, the apoptosis rate, and the protein levels of p-HMGB1 and p-PTEN were remarkably enhanced in the Model group compared to the Sham group. In comparison with those in Model group, the pathological changes in renal morphology, apoptosis-related indexes, and protein levels of p-HMGB1 and p-PTEN were alleviated in the miR-205 group.

CONCLUSIONS: MiR-205 agonist can improve the pathological morphology in the sepsis rats with renal injury, improve renal cell apoptosis, and inhibit the protein levels of HMGB1 and PTEN in renal tissues. MiR-205 alleviates sepsis-induced renal injury through the HMGB1-PTEN signaling pathway.

Key Words:
MiR-205, HMGB1, PTEN, Sepsis.

Introduction

Sepsis is a systemic inflammatory response syndrome induced by infections or highly suspicious foci of infection, mainly including bacteria, fungi, and viruses[1-3]. Characterized by serious conditions, sepsis is primarily a common complication of severe traumas, infections after surgical procedures, or burns. It is recognized as a major cause of death for patients in the intensive care unit, posing a difficulty and emphasis in the field of critical care medicine. The incidence rate of sepsis is on the rise year by year, and the pathogenesis is fairly complex[4,5]. The therapeutic methods for early-stage sepsis is dominated by fluid resuscitation or drug application, such as glucocorticoid and antibiotics[6,7]. However, the therapeutic efficacy of sepsis is not satisfactory. Hence, exploring the pathogenesis of sepsis is of importance for seeking more efficacious therapeutic measures.

The pathogenesis of sepsis is complex, mainly involving inflammatory factors, immune functions, and coagulation disorders. It is clinically believed that the inflammatory response in the body is the most important pathogenesis, of which the imbalanced inflammatory system will manifest increases in the release and expressions of cytokines in the early stage, including tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). Moreover, cytokines in the late stage have close correlations with the mortality rate of sepsis. Clinical studies have discovered that the high-mobility group box 1 (HMGB-1) affects activities of transcription, replication, and differentiation in cells. The release of HMGB-1 into the extracellular space will result in the aggravation of sepsis. In addition, immunodepression and coagulation dis-

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orders induced by the enhanced anti-inflammatory response can affect the condition and prognosis of the disease[8-11].

Micro ribonucleic acids (miRNAs) are a category of small non-coding RNA molecules with 21-23 nucleotides in length, exerting vital regulatory roles in the growth and development of the organisms and participating in the occurrence and development of tumors as oncogenes or anti-oncogenes. MiR-205 has been proven to be involved in the pathology of multiple tumors, such as proliferation, apoptosis, and infiltration. However, in-depth investigations which provide adequate data supporting the specific mechanism of sepsis are lacked[12,13]. HMGB1 is a pro-inflammatory factor in the pathogenesis of sepsis that induces the synthesis and secretion of inflammatory factors in the case of acute stress damage to the body, thus participating in the inflammatory response process[14]. Besides, phosphatase and tensin homolog deleted on chromosome ten (PTEN) is regarded as a tumor-suppressor gene, which is involved in transcription and differentiation through phosphorylation[15]. In this research, a rat model of sepsis-induced renal injury was established using cecal ligation and perforation. We mainly investigated the mechanism of miR-205 in the renal injury of sepsis rats through the HMGB1-PTEN signaling pathway, thereby providing a scientific basis for clinical study.

Materials and Methods

Reagents

The reagents used were: enzyme-linked immunosorbent assay (ELISA) kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), bovine serum albumin (BSA; Invitrogen, Carlsbad, CA, USA), phosphorylate-HMGB1 (p-HMGB1) and p-PTEN primary antibodies (Beijing Bioss Biological Technology Co., Ltd., Beijing, China), radio immunoprecipitation assay (RIPA, Beyotime, Shanghai, China) lysis buffer, diaminobenzidine (DAB) kit and hematoxylin and eosin (HE) staining kit (Shanghai YuanMu Biological Technology Co., Ltd., Shanghai, China), and miR-205 agonist (Guangzhou RiboBio Co., Ltd., Guangzhou, China).

Instruments

The instruments used were: Gel imager (Invitrogen, Carlsbad, CA, USA), electrophoresis apparatus and transmembrane apparatus (Bio-Rad, Hercules, CA, USA), microplate reader (Molecular Devices, USA), and ultra-low-temperature refrigerator (Thermo Fisher Scientific, Waltham, MA, USA).

Animals

The male Sprague-Dawley (SD) rats (animal certification number: SCXK 20170329, Beijing Laboratory Animal Research Center) weighing (220 ± 10) g were habituated for a week. The rats were given free access to food and water, and housed in the environment with the humidity of 40-60% and temperature of 22-25°C. This study was approved by the Animal Ethics Committee of Nanjing Medical University Animal Center.

Establishment of Rat Model of Sepsis-Induced Renal Injury[16]

The rats were randomly divided into the Sham group (n=10), the Model group (n=10) and the miR-205 agonist (MiR-205) group (n=10). The rat model of sepsis-induced renal injury was established via cecal ligation and perforation in the Model group and MiR-205 group. Briefly, the rats were anesthetized with 10% chloral hydrate and fixed on a surgical plate in the supine position. Then a 1 cm-long incision was made on the right side of the midline of the lower abdomen with a pair of surgical scissors. The cecum was found, and the terminal cecum was dissected using a blunt separator. Next, the cecum was ligated at 1 cm from the ileocecal valve and then perforated. The intestinal contents were extruded out. After that, the peritoneal membrane was sutured. In the Sham group, the rats were only subjected to laparotomy without cecal ligation and perforation. Each rat was raised in separate cages after resuscitation, followed by HE staining for detection of pathological morphology.

Detection of Blood Urea Nitrogen (BUN) and Creatinine (Cr) Levels in Rat Serum Via Biochemical Method

The serum levels of BUN and Cr in each group of rats were quantitatively measured through double-antibody sandwich ELISA in strict accordance with the instructions.

Measurement of Caspase-3 and Bcl-2-Associated X Protein (Bax) Levels in Rat Serum

According to the kit instructions, the standard curves were plotted to determine the concentrations. 100 μL of sample diluent and 100 μL of reference substance were added into each blank well for incubation. Then 100 μL of biotin-labeled

antibody working solution was applied and incubated for 1 h, followed by washing with Phosphate-Buffered Saline (PBS). After that, 100 μL of avidin-horseradish peroxidase (HRP)-labeled working solution was added into each well and incubated at 37°C for 30 min, and the reaction was terminated using 50 μL of termination buffer. The absorbance at the wavelength of 450 nm was measured using the microplate reader.

Detection of Cell Apoptosis in Rat Renal Tissues Via Terminal Dexynucleotidyl Transferase (TdT)-Mediated dUTP Nick End Labeling (TUNEL) Assay

The sections were fixed with 4% paraformaldehyde and permeabilized for 15 min, and TdT solution was added dropwise onto each piece of tissue for reaction at 37°C in the dark, followed by washing with PBS for three times and incubation with 50 μL of streptavidin-TRITC-labeled solution in the dark for 30 min. The nucleus was counterstained with DAPI (Beyotime, Shanghai, China) staining solution, and washed with PBS repeatedly. After that, the staining results were observed under a microscope.

Comparison of Renal Injury Score of Sepsis Rats

HE staining was performed to observe the pathological changes in the renal tissues, including the occurrence of renal tissue edema, hyperemia, leukocyte infiltration in the renal glomerulus, and vacuolar degeneration of the epithelial cells. The renal injury was scored as 0-4 points, with 0 point for normal kidney, 1 point for minimal renal injury (0-5%), 2 points for mild renal injury (5-25%), 3 points for moderate renal injury (25-75%), and 4 points for severe renal injury (75-100%).

Detection of p-HMGB1 and p-PTEN Protein Levels in Rat Renal Tissues Via Western Blotting (WB)

The rat renal tissues were lysed in 1 × radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, China) and centrifuged to harvest the supernatant. The protein concentration was detected via Bradford method. Subsequently, the protein sample was loaded using dodecyl sulfate, sodium salt-polyacrylamide gel electrophoresis (SDS-PAGE) at 120 V. Then, the electrophoresis products were transferred onto a polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA) under a constant current, followed by blocking with 5% albumin from bovine serum (BSA) for 2 h and incubation again with primary antibodies for p-HMGB1 (1:1000) and p-PTEN (1:1000). After washing with Tris-Buffered Saline and Tween-20 (TBST), the horseradish peroxidase (HRP)-labeled secondary antibodies were added. Finally, the DAB developer was utilized for color development.

Statistical Analysis

The Statistical Product and Service Solutions (SPSS) 17.0 software (SPSS, Chicago, IL, USA) was used for data processing and analysis. The measurement data was presented as ($\overline{\chi}\pm s$), and the *t*-test was performed to compare the intergroup differences. The enumeration data were expressed by case (n), and a Chi-square test was conducted to compare the inter group differences. $p<0.05$ suggested that the difference was statistically significant.

Results

MiR-205 Agonist Ameliorated the Pathological Morphology of Renal Tissues in Sepsis Rats With Renal Injury

In the Sham group, there were no apparent changes in the pathology of renal tissues, showing clear structure without edema and hyperemia. The renal tubule was intact in the structure, without evident cell injury and necrosis. There was prominent renal tissue injury, renal cell necrosis, and detachment, as well as edema in a large number of cells in the Model group, in which the brush border disappeared, and the lumen became smaller. In miR-205 group, the renal tissue injury was alleviated, and the pathological morphology was significantly improved, suggesting that miR-205 agonist can ameliorate the symptoms of renal injury in sepsis rats (Figure 1).

MiR-205 Agonist Reduced Serum Levels of BUN and Cr in Sepsis Rats With Renal Injury

The serum levels of Cr and BUN in the Model group were remarkably higher than those in the Sham group ($p<0.01$). In comparison with those in the Model group, the serum levels of Cr and BUN in sepsis rats evidently declined in miR-205 group after application of miR-205 agonist ($p<0.01$) (Table I).

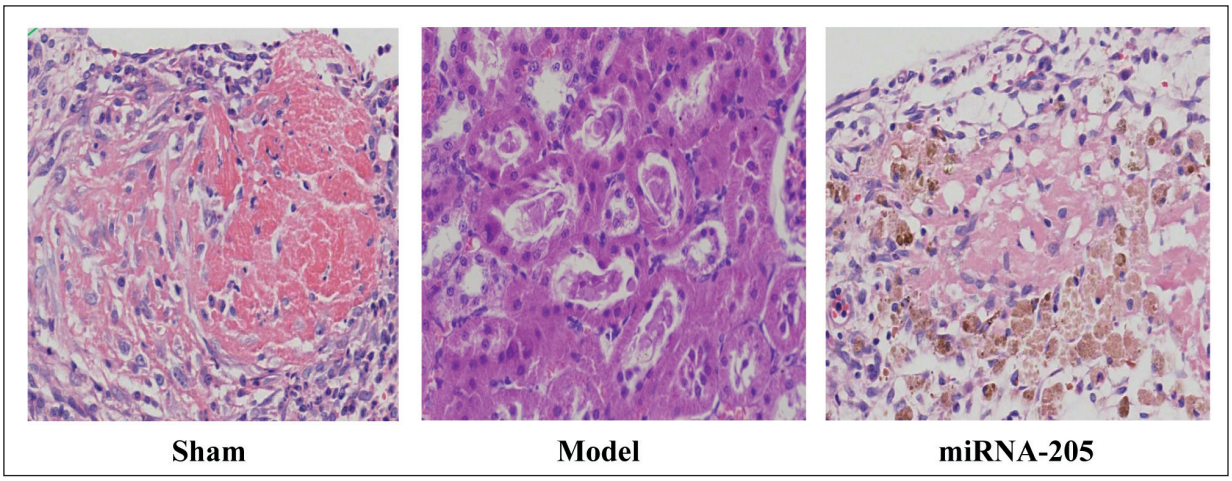

Figure 1. Pathological morphology of renal tissues in each group of rats detected via HE staining (20×).

MiR-205 Agonist Lowered the Content of Serum Caspase-3 and Bax in Sepsis Rats With Renal Injury

In the Model group, the levels of Caspase-3 and Bax were remarkably higher than those in the Sham group (p<0.05). After the application of miR-205 agonist in miR-205 group, the levels of Caspase-3 and Bax in sepsis rats markedly decreased compared with those in the Model group (p<0.05) (Table II).

MiR-205 Agonist Affected the Cell Apoptosis in Renal Tissues in Sepsis Rats With Renal Injury

The apoptosis rate in the Model group was signficantly higher than that in the Sham group (p<0.01), and it was notably lowered in the sepsis rats in the miR-205 group treated with miR-205 agonist in comparison with that in the Model group (p<0.05) (Figure 2).

MiR-205 Agonist Affected Renal Injury in Sepsis Rats With Renal Injury

The renal injury score was elevated in the Model group compared with that in the Sham group (p<0.01). The renal injury in the sepsis rats was ameliorated after the treatment with miR-205 agonist, (that was,) indicating that miR-205 group had a lower renal injury score than the Model group (p<0.05) (Figure 3).

Table I. Levels of serum BUN and Cr in rats.

Group	Cr (μmol/L)	BUN (mmol/L)
Sham group	29.32 ± 4.09	5.23 ± 0.65
Model group	92.78 ± 9.99*	20.98 ± 2.97*
MiR-205 group	43.67 ± 5.09#	8.99 ± 1.09#

Note: *Model group *vs.* Sham group, p<0.01. #MiR-205 group *vs.* Model group, p<0.01.

Table II. Levels of serum Caspase-3 and Bax in rats.

Group	Caspase-3 (pg/L)	Bax (pg/L)
Sham group	20.74 ± 2.67	32.26 ± 3.52
Model group	46.25 ± 5.98*	66.35 ± 7.62*
MiR-205 group	29.43 ± 2.84#	33.77 ± 3.58#

Note: *Model group *vs.* Sham group,p<0.05. # MiR-205 group *vs.* Model group, p<0.05.

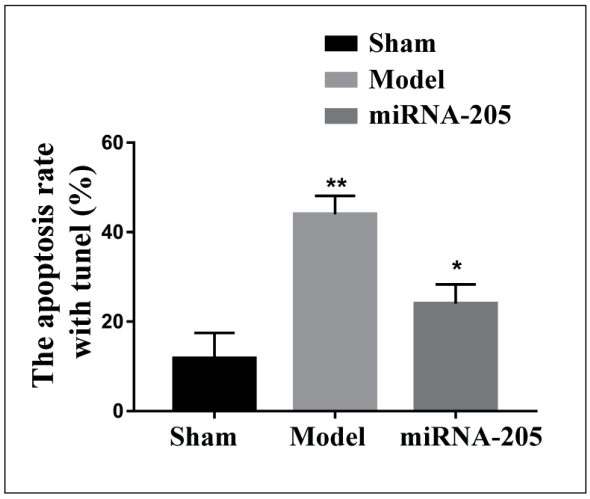

Figure 2. Comparison of cell apoptosis in rat renal tissues. Note: *MiR-205 group *vs.* Model group, p<0.05. **Model group *vs.* Sham group, p<0.01.

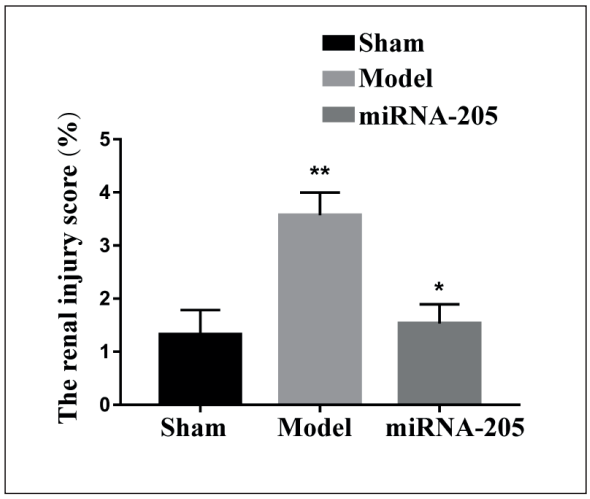

Figure 3. Comparison of renal injury score in each group. Note: *MiR-205 group *vs.* Model group, $p<0.05$. **Model group *vs.* Sham group, $p<0.01$.

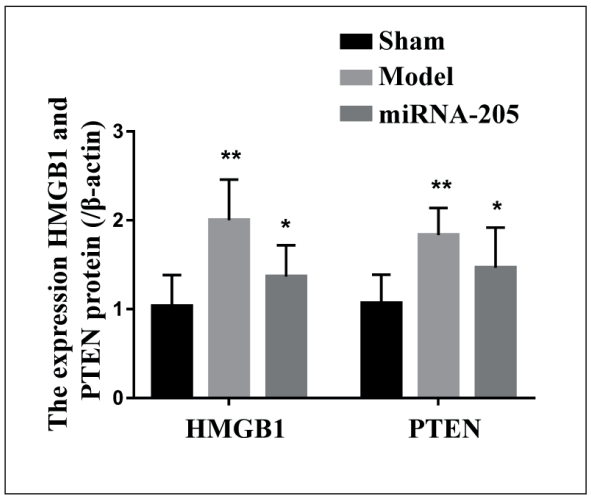

Figure 4. Protein levels of HMGB1 and PTEN in each group. Note: *MiR-205 group *vs.* Model group, $p<0.05$. **Model group *vs.* Sham group, $p<0.01$.

MiR-205 Agonist Inhibited the Protein Levels of HMGB1 and PTEN in Renal Tissues in Sepsis Rats With Renal Injury

The protein levels of HMGB1 and PTEN in the Model group were higher than those in the Sham group ($p<0.01$). After the application of miR-205 agonist in the miR-205 group, the protein levels of HMGB1 and PTEN were prominently lower than those in the Model group ($p<0.05$) (Figure 4).

Discussion

As an infection-induced systemic inflammatory response, sepsis may aggravate into septic shock and even multiple-organ dysfunction syndrome if timely and effective treatments are lacked. The mortality rate of sepsis is extremely high and displays an increasing trend year by year. Significant achievements have been made in the diagnosis and treatment of sepsis. Fluid resuscitation and fluid therapy applied as early as possible can prominently improve severe infection, hypotension, hypovolemia, and organ perfusion. In addition, vasoactive agents such as norepinephrine and epinephrine are the preferred drugs. Infections should be timely controlled during the treatment of sepsis. For example, the infection foci can be eliminated from the source by empirical treatment with antibiotics and control of infection source in the early stage. It is proposed that insulin can be utilized to control the blood glucose concentration after the sepsis reached the stability, thus effectively reducing the prevalence rate of complications. In recent years, growing attention has been paid to the application of the activated protein C with the efficacy of repressing thrombus and resisting inflammation, which is widely used for critically ill patients[17]. However, the clinical efficacy of the above-mentioned therapeutic measures is unsatisfactory, and the complex pathogenesis brings difficulties to the treatment of sepsis.

Some studies have discovered that the primary pathogenesis of sepsis is the destruction of the inflammatory system balance and the dysregulated pro-inflammatory and anti-inflammatory factors. As a category of non-coding RNAs with 23 nucleotides in length, miRNAs exert crucial function in various diseases such as tumor, diabetes, and nephropathy. They can control the expressions and release of pro-inflammatory and anti-inflammatory factors to some extent. According to clinical findings, miRNAs participate in inflammatory pathways and fine-tune multiple targets involving in the pathway, to ameliorate the inflammatory responses. MiR-205 is newly discovered, but its mechanism in regulating sepsis progression has not been clarified. HMGB1, a highly conserved nuclear protein, is an important inflammatory factor for sepsis. The serum level of HMGB1 is positively correlated to the mortality of sepsis. As an independent traumatogenic factor for sepsis, HMGB1 is secreted by immune cells and induces many cells to be secret and release inflammatory mediators in the case of acute stress damage, thereby regulating the inflammatory process. Phosphorylated PTEN is a vital type of anti-oncogene involved in

cellular behaviors[18,19]. Zhou et al[13] pointed out that the HMGB1-PTEN signaling pathway accelerates the Tregs differentiation by activating the TGF-β signaling pathway in macrophages, and they found that an elevated expression level of Tregs suggests a lower degree of lung injury. It is also indicated that the HMGB1-PTEN axis can repress the inflammatory responses in sepsis and significantly improve inflammatory response.

In this research, the rat model of sepsis-induced renal injury was established to explore the regulatory role of miR-205 in sepsis through the HMGB1-PTEN signaling pathway. It was discovered that miR-205 agonist could prominently improve the damage to pathological morphology of rat renal tissues and markedly decrease the renal injury score ($p<0.05$). Meanwhile, it could reduce BUN and Cr levels. The level of BUN increases in the case of renal insufficiency in decompensation period, and the release of Cr is enhanced in serious renal function impairment. According to these data, the relative levels of BUN and Cr in miR-205 group were significantly lower than those in the Model group, manifesting that miR-205 agonist was capable of ameliorating renal function impairment. It was also shown that miR-205 group had markedly lower levels of Caspase-3 and Bax than the model group, illustrating that miR-205 agonist can notably reduce the degree of inflammations in renal tissues and can display strong anti-inflammatory effects. The cell apoptosis was observed via TUNEL assay, and it was shown that the apoptosis rate in miR-205 group was markedly lower than that in the Model group, suggesting that miR-205 agonist was able to inhibit the cell apoptosis in renal tissues. Moreover, the WB assay was adopted to detect the protein expressions of p-HMGB1 and p-PTEN in three groups. The results manifested that the protein levels remarkably declined in miR-205 group compared with those in the Model group ($p<0.05$), indicating that miR-205 can suppress the renal cell apoptosis in rats with sepsis-induced renal injury through the HMGB1-PTEN signaling pathway.

Conclusions

MiR-205 inhibited the HMGB1-PTEN signaling pathway, exerted strong anti-inflammatory effects and played an important role in protecting the kidney of sepsis rats, thereby providing effective and authentic scientific data for clinical treatment.

Conflict of Interests

The Authors declared that they have no conflict of interests.

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