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Original Article

Inhibition of microRNA-155 alleviates lipopolysaccharide-induced kidney injury in mice

Yuqian Ren*, Yun Cui*, Xi Xiong, Chunxia Wang, Yucai Zhang

*Department of Critical Care Medicine, Shanghai Children's Hospital, Shanghai Jiao Tong University, Shanghai 200062, P. R. China. *Equal contributors.*

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Abstract: Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. Accumulated evidences suggest that microRNAs (miRNAs) are related with inflammation-associated diseases. The aim of this study is to investigate whether miR-155 is involved in lipopolysaccharide (LPS)-induced kidney injury, and to explore the underlying mechanisms. Mice were intraperitoneally injected with LPS to construct endotoxemia mice model, and miR-155 inhibitor was injected via tail vein to suppress the expression of miR-155 in kidney. The results indicated that the expression of miR-155 was markedly increased in renal tissues of LPS-treated mice. And miR-155 inhibitor protected mice from LPS-induced kidney injury associated with the lower levels of TNF-α and IL-6 in renal tissues. Furthermore, inhibition of miR-155 increased the expression of suppressor of cytokine signaling 1 (SOCS1), a target gene of miR-155 and a negative regulator of Janus activated kinase (JAK)-signal transducer and activator of transcription (STAT) signaling pathway. Consistently, inhibition of miR-155 suppressed the expression of JAK2, STAT3 and phosphorylated STAT3 (p-STAT3). All these results indicated that inhibition of miR-155 protects mice from LPS-induced kidney injury possibly through regulating SOCS1-JAK2/STAT signaling pathway, which suggested that miR-155 might be an important and potential target in developing therapy for preventing sepsis-associated kidney injury.

Keywords: miR-155,LPS-induced kidney injury, SOCS1,JAK/STAT pathway

Introduction

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection [1], which is the leading cause of death in pediatric intensive care unit (PICU). Nearly 50% of all patients with sepsis develop acute kidney injury (AKI) and impair renal function [2], which contribute to about 50% in AKI patients [3]. Despite advances in management strategies, the mortality rate of pediatric sepsis-associated severe AKI is 64% [4]. Renal inflammation, microvascular dysfunction and endothelial cell injury are attributable to nitric oxide pathway activation, leukocyte adhesion, reactive oxygen species (ROS), and inflammation [5]. Therefore, it is urgent to develop new agents to effectively control the inflammatory response to improve sepsis-associated kidney injury.

MicroRNAs (miRNAs) are non-coding endogenous RNAs, ranging from 18 to 25 nucleotides in length, which target messenger RNA (mRNA) and effect translational repression in post-transcriptional level [6]. miRNAs regulate immune response and inflammatory response, which plays a vital role in sepsis [7]. Recent studies have revealed that microRNA-155 (miR-155), miR-150, miR-125b and miR-146 are involved in inflammatory regulation [6, 8, 9]. miR-155 induces pro-inflammatory activity of normal liver cells [10]. miR-155 also contributes to the pathological process of kidney damage via regulating innate and adaptive immune responses [11]. Pre-treatment with antagomir-155 reduced vascular leak induced by human cerebral malaria (CM) sera in an ex vivo endothelial microvessel model via regulation of endothelial activation, microvascular leak and blood brain barrier dysfunction in CM [12].

Lipopolysaccharide (LPS) stimulated the expression of miR-155 and suppressed the expression of SOCS1 in RAW264.7, and resveratrol protects macrophages from LPS-induced

Table 1. The scale used to evaluate the grade of mice

Indicator score	Breath	Mental reaction	Appetite	Weight	Secretion	Stool property	Activity
0 score	Normal	Normal	Normal	Normal	Normal	Normal	Normal
1 score	Faster > 5%	Indifference	Reduced	Reduced > 5%	little	Frequently	slower
2 score	Faster > 10%	Drowsiness	Reduced obviously	Reduced > 10%	little	Diarrhea	Less dynamic
3 score	Faster > 15%	Weak	Apastia	Reduced > 15%	Much	Watery diarrhea	Move after stimulation

inflammatory response via suppressing the expression of miR-155 and the signal transducer and activator of transcription (STAT)1/STAT3 [13]. Moreover, Janus activated kinase (JAK)/STAT signaling pathway is a principal signaling pathway for the signal transduction of many pivotal cytokines involved in sepsis [14]. It is initiated when a peptide ligand binds to its corresponding homodimeric or heterodimeric receptors, then, receptor-associated JAKs are brought into close proximity through receptor oligomerization. Promoters of cytokines such as IFN-γ, TNF-α and ILs located on STATs binding sites are stimulated by signals [15]. This prompts a large number of inflammatory cytokines released, participating in the body's pro-inflammation and anti-inflammation processes. SOCSs, a negative regulatory protein family, can complete a negative feedback loop in the JAK/STAT pathway resulting in cardioprotective effects via anti-apoptosis, ROS scavenging and up-regulating angiogenic factors; however, the excessive activation of this pathway induces excessive NO production and oxidative stress and contributes to the maladaptive response [16]. Meanwhile, SOCS1 is one of the target genes of miR-155. Inhibited miR-155 can directly promote the expression of SOCS1 and suppress its downstream genes expression including IL-1β, IL-6, TNF-α and other proinflammatory factors, which protects from inflammation-induced organ damage [17, 18]. Recent studies indicated that inhibition of miR-155 protects mice from sepsis-induced acute lung injury and septic liver injury [19, 20]. Our previous study proved that inhibition of miR-155 attenuated the LPS-induced liver injury through downregulating the JAK/STAT signaling pathway [21]. However, the role of miR-155 in sepsis-associated kidney injury mediated by inflammatory cytokines is still unknown.

In present study, we speculated that inhibition of miR-155 could regulate JAK/STAT signal pathway via influencing the expression of SOCSs, which results in reduction of the downstream inflammatory factors and mediators, thus alleviating kidney injury from uncontrolled inflammation. Here, endoxemia model was induced by intraperitoneal injection of LPS in BALB/c mice. To investigate the effect of inhibition of miR-155 on LPS-induced kidney injury, miR-155 inhibitor were intravenously injected into mice through the tail vein to suppress the expression of miR-155 in kidney. The aim of this study is to identify whether inhibition of miR-155 could protect mice from LPS-mediated AKI and to explore the underlying mechanisms possibly related to SOCS1-JAK/STAT signaling pathway.

Materials and methods

Experimental animals

A total of 190 male BALB/c mice (weight, 20 ± 2 g; 4-week-old) were purchased from the Department of Animal science of Fudan University in Shanghai (Shanghai, China). The mice were kept in cages with a 12 h light-dark cycle and accessed to dry pellets and sterile water ad libitum. All experiments were conducted in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The present study was approved by the ethics committee of the Children's Hospital Affiliated to Shanghai Jiaotong University (Shanghai, China).

LPS dose gradient experiment

Thirty mice were used to investigate the appropriate dose of LPS used to construct the endoxemia model. These mice were randomly divided into 5 groups (n=6 per group). After anesthetizing with pentobarbital (0.3 mg·kg^{-1}), LPS (*E. coli* 0111:B4, Sigma-Aldrich, St. Louis, MO, USA) was intraperitoneally injected with different dose of 5, 10, 20, 40, 60 mg·kg^{-1} in each group, respectively. Then, we observed general status of mice in each group after 12 h, 24 h and 48 h, and recorded the grade of mice using evalu-

Table 2. Primers for *Socs1, Stat1, Gapdh*, *miR-155* and *5S* in RT-qPCR used in this work

Gene	Forward (F)/ reverse (R)	Primer
Socs1	F	5'-TCCGATTACCGGCGCATCACG-3'
	R	5'-CTCCAGCAGCTCGAAAAGGCA-3'
Stat1	F	5'-ATTTCTCCTTCTGGCCTTG-3'
	R	5'-AGGAACGTCCCTGGCTG-3'
miR-155	F	5'-CGGCGGTTAATGCTAATTGTGAT-3'
	R	5'-GTGCAGGGTCCGAGGT-3'
5S	F	5'-TCGTCTGATCTCGGAAGCTA-3'
	R	5'-AAGCCTACAGCACCCGGTAT-3'
Gapdh	F	5'-TGCACCACCAACTGCTTAGC-3'
	R	5'-GCATGGACTGTGGTCATGAG-3'

Table 3. The score of mice injected with different dose of LPS at indicated time point

Scores / Group	12 h		24 h		48 h	
	Death	Scores	Death	Scores	Death	Scores
5 mg·kg^{-1}	0	0	0	0	0	0
10 mg·kg^{-1}	0	1	0	0.67	0	0.33
20 mg·kg^{-1}	1	10.5	1	9	0	4
40 mg·kg^{-1}	2	13	1	9.75	0	6.33
60 mg·kg^{-1}	3	16.33	1	13.67	0	9

ation indicators including breath, mental reaction, appetite, weight, secretions, stool property and activity, which were shown in **Table 1**.

Construction of endoxemia model and study design

One hundred and sixty mice were divided into four groups (n=40 per group): Blank group, LPS group, scramble control plus LPS groups (scramble group) and miR-155 inhibitor plus LPS groups (miR-155 inhibitor group). After anesthetizing with pentobarbital (0.3 mg·kg^{-1}), mice of blank groups were intraperitoneally injected with sterile saline (Blank group), with LPS at the dose of 20 mg·kg^{-1} in LPS group (LPS group), respectively. To investigate the role of miR-155 in LPS-induced kidney injury, mice were intravenously injected with 80 mg·kg^{-1} miR-155 inhibitor through the tail vein twice per day for 3 days (miR-155 inhibitor group) before LPS treatment, using scrambler as control (scramble group). Rat miR-155 inhibitor (5'-ACCCCUAUCACAAUUAGCAUUAA-3', 2'-O-methyl modification, glucosinolates skeleton modification and 30-cholesterol modification) and its scrambled control (5'-CAGUACUUUUGUGUAGUACAA-3',2'-O-methyl modification, glucosinolates skeleton modification and 30-cholesterol modification) were synthesized by Genepharma (Shanghai, China). Subsequently, mice of miR-155 inhibitor group and scramble group were injected with 20 mg·kg^{1} LPS. Mice in LPS, miR-155 inhibitor and scramble groups were sacrificed at 6, 12, 24 and 48 h after LPS exposure, respectively (n=10 per time point). One side kidney samples were frozen in liquid nitrogen for western blotting and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis, and the other side kidney samples were fixed in 4% paraformaldehyde overnight and stained with haematoxylin and eosin as previously described [22].

ELISA assays

The levels of TNF-α and IL-6 in kidney were measured according to the manufacturer's instructions with ELISA kit (R&D Systems), which were normalized by protein concentration determined using bicinchoninic acid (BCA) protein measurement kit (R&D Systems, Minneapolis, MN, USA). And the results of the levels of TNF-α and IL-6 in kidney were presented as pg/mg protein.

Histological analysis of renal tissues

Renal tissues were fixed in 4% paraformaldehyde overnight at room temperature and then transferred to 70% ethanol before paraffin embedding. After that, samples were embedded using paraffin, and then paraffin-embedded samples were sectioned at 4-μm thickness. For pathological analysis, paraffin sections were stained with hematoxylin and eosin (H&E) [22]. The sections were observed under an optical microscope (CKX31SF; Olympus Corporation, Tokyo, Japan).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA of the kidney samples was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA). We used ultramicro ultraviolet spectrophotometer to determine the value of A260/280, and the A260/280 value is between 1.8-2.0 suggested

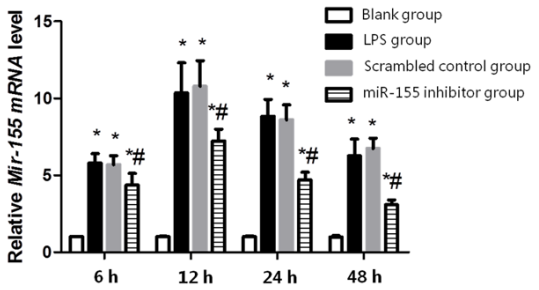

Figure 1. miR-155 expression in renal tissues of mice. LPS administration induces the expression of miR-155, and miR-155 inhibitor effectively suppresses the miR-155 levels in renal tissues. *indicates $P < 0.05$ *vs.* blank group. #indicates $P < 0.05$ *vs.* the LPS group.

a high purity, and recorded RNA concentration. Then, 1 μg total RNA was reverse-transcribed using commercially available kits (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. The primers of *miR-155*, *Stat1* and *Socs1* used in qPCR were shown in **Table 2**. We performed qPCR analyses for miR-155 using TaqMan miRNA assays (Ambion) in Universal PCR Master mix (Applied Biosystems),with the following parameters: 95°C for 10 min, followed by 40 cycles of 15 sec at 95°C, 1 min at 60°C. The melting curves were acquired by heating the samples to 95°C for 1 min, cooling to 55°C for 1 min and then slowly increasing the temperature from 65 to 95°C at a rate of 0.5°C/30 sec. 5 S and GAPDH mRNA were used as an endogenous control for data normalization and calculated the relative expression. miR-155 was adjusted by standardization based on the 5S mRNA levels, *Socs1* and *Stat1* were adjusted by standardization based on the *Gapdh* mRNA levels. Samples for each experimental condition were run in triplicate. The relative expression of target gene was calculated by formula $2^{-\Delta\Delta t}$.

Western blotting

Proteins (20 μg) were separated by 10% SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes (Amersham, Little Chalfont, UK). To detected the target proteins, we incubated membranes with primary antibodies blocked by nonfat dry milk (2.5%). Then, the antibodies for SOCS1, STAT3, p-STAT3, JAK2 and β-actin purchased from Cell Signalling Technology were incubated at 4°C overnight. After that, the goat anti-rabbit secondary antibody was used to incubate at room temperature for 1 hour. Subsequently, the membranes were detected by SuperSignal West Femto Maximum Sensitivity Substrate kit (ThermoPierce) to get image and then analyzed. The levels of target proteins were normalized by GAPDH.

Statistical analysis

All data were analyzed with SAS software. The results are presented as the mean ± standard deviation, except for specific indication. Significant differences were assessed either by two-tailed Student's *t* test or one-way ANOVA followed by the Student-Newman-Keuls (SNK) test. A *P* value less than 0.05 was considered statistically significant.

Results

The appropriate dose of LPS for construction of endoxemia mice model is 20 mg·kg^{-1}

To explore the appropriate dose of LPS to construct endoxemia mice model, the LPS dose gradient experiment were done using 5, 10, 20, 40 and 60 mg·kg^{-1} in each group, respectively. Through comparing the grade of mice using evaluation indicators including breath, mental reaction, appetite, weight, secretions, stool property and activity (**Table 3**), the mice of 60 mg·kg^{-1} group had the highest scores and displayed obvious toxic symptoms resulting in the highest mortality with half deaths within 12 h after LPS treatment. However, there were no dead mice in 5 mg·kg^{-1} and 10 mg·kg^{-1} group with low scores in each time point and unobvious endoxemia symptoms, suggesting that these two dosages might be too lower for LPS-induced endoxemia model. Furthermore, mice in 20 mg·kg^{-1} group displayed endoxemia symptoms in each time point. And there was one mouse died within 12 h and another mice died within 24 h after LPS treatment, which suggested that 20 mg·kg^{-1} LPS was the right dose to construct endoxemia model.

miR-155 inhibitor effectively suppresses the expression of miR-155 in kidney

To investigate the role of miR-155 inhibition in LPS-induced endoxemia model, miR-155 inhibitor was intravenously injected via tail vein twice one day for 3 days. Firstly, the expression of

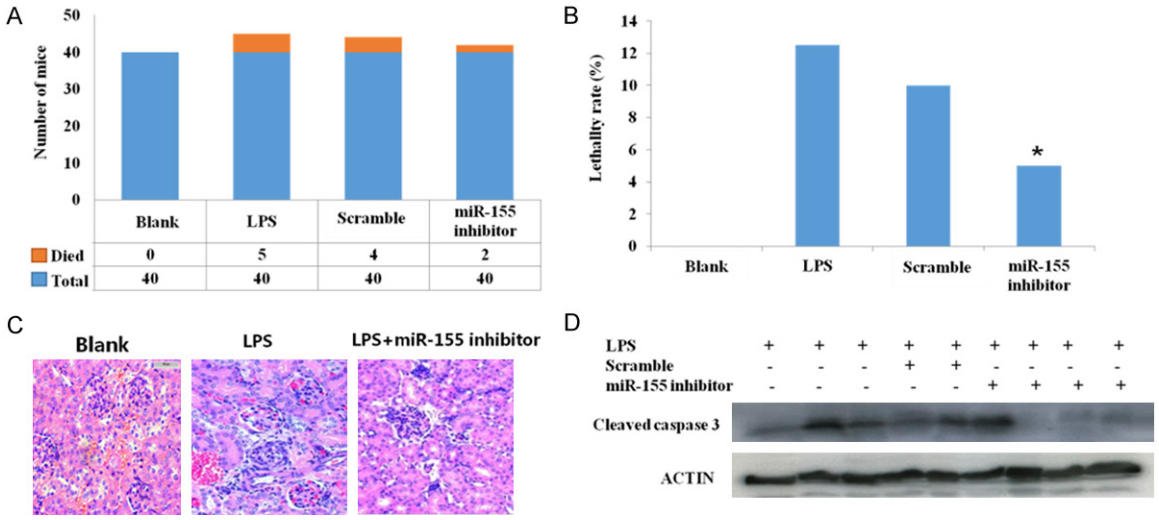

Figure 2. Inhibition of miR-155 alleviates LPS-induced kidney injury. A: Mice used in this study and died mice in control (Blank), LPS-treated (LPS), LPS-treated plus scramble control (Scramble), and LPS-treated plus miR-155 inhibitor (miR-155 inhibitor) groups; B: The mortality rate of mice in indicated groups at 24 h after LPS treatment; C: Renal sections of mice in indicated groups at 24 h after LPS treatment (hematoxylin and eosin staining, × 400); D: Cleaved caspase 3 expression in renal tissues.

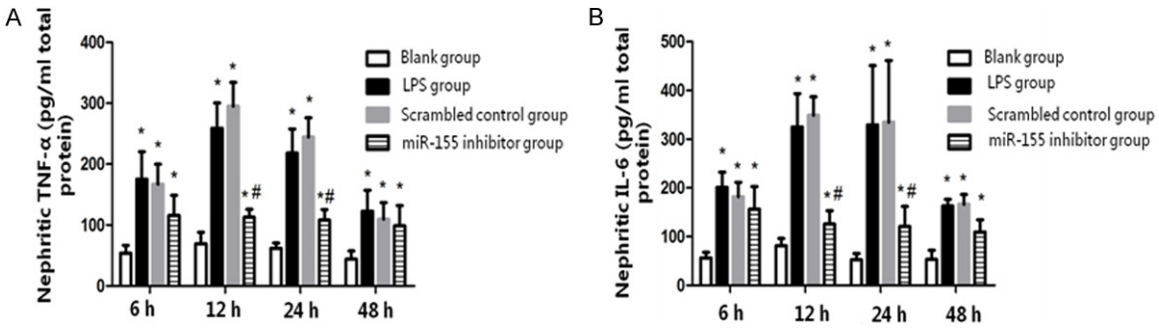

Figure 3. Inhibition of miR-155 significantly reverses the higher level of TNF-α and IL-6 at 12 h and 24 h after LPS treatment in renal tissues. The levels of TNF-α and IL-6 in renal tissues were detected by ELISA (n=6 per group). A: The level of TNF-α; B: The level of IL-6. *indicates $P < 0.05$ *vs.* blank group. #indicates $P < 0.05$ *vs.* the LPS group.

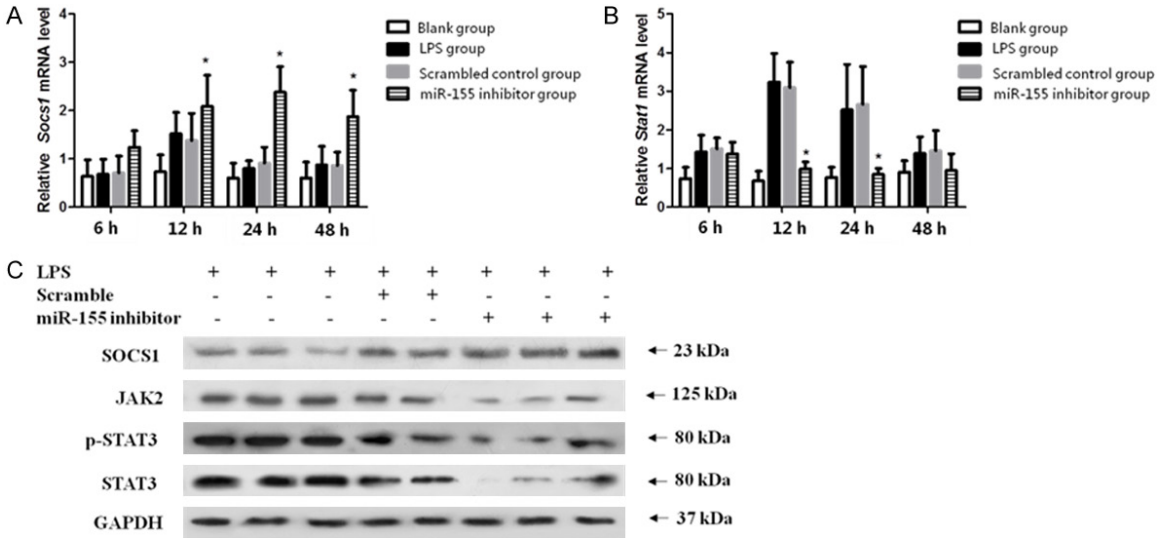

Figure 4. Inhibition of miR-155 increases the expression of SOCS1 and suppresses the expression of JAK, STAT1, STAT3 and phospho-STAT3 (p-STAT3). The mRNA levels of *Socs1* and *Stat1* were determined by RT-PCR at indicated time point after LPS treatment, and the protein levels of SOCS1, JAK2, STAT3, p-STAT3 were detected at 24 h after LPS administration by western blotting (n=6 per group). A: *Socs1* mRNA; B: *Stat1* mRNA; C: The protein levels of SOCS1, JAK2, STAT3, p-STAT3. *indicates $P < 0.05$ *vs.* blank group. #indicates $P < 0.05$ *vs.* the LPS group.

miR-155 in kidney was detected to prove the effect of miR-155 inhibitor injection. Interestingly, LPS treatment significantly stimulates the expression of miR-155 in kidney from 6h to 48 h after treatment, and the expression of miR-155 reaches peak value at 12 h after LPS treatment. And miR-155 inhibitor significantly suppresses the expression of miR-155 in kidney at 6 h, 12 h, 24 h and 48 h after LPS treatment (**Figure 1**).

miR-155 inhibitor decreases the mortality of LPS-treated mice and improves the LPS-induced kidney injury

As expected, mice in LPS group performed obvious toxic symptoms compared with the blank control group, for example fatigue, increased heart rate and reduced activity and food intake. The visual study indicated that mice in miR-155 inhibitor group had less severe symptoms compared with LPS group. There were totally 11 mice died at the end of experiment. In LPS group, three mice died within 12 h and 2 died between 12 h-24 h with the mortality of 12.5%. As a contrast, 2 died within 12 h in miR-155 inhibitor group with the mortality of 5%, but no mice died between 12-24 h. In scramble group, 2 mice died within 12 h and 2 mice died within 24 h with the mortality of 10% (**Figure 2A**, **2B**).Furthermore, the histology analysis of renal tissues showed that histologic evaluation of the kidney in the LPS group showed marked pathological lesions characterized by numerous inflammatory cell infiltrations in the renal interstitium, focal neutrophil infiltration in glomerulus, cloudy swelling of renal tubular epithelial, degeneration and necrosis of glomerular and renal tubular necrosis. Fortunately, treatment with miR-155 inhibitor could attenuate the severity of pathological damages in a certain degree with reduction of inflammatory cell infiltrations. And kidney tissue had a normal structure in blank group (**Figure 2C**). The expression of cleaved caspase 3 is higher in renal tissues of mice in LPS group, which were significantly reversed by miR-155 inhibitor (**Figure 2D**).

Inhibition of miR-155 decreases the level of TNF-α and IL-6 in renal tissues

In order to further explore the role of miR-155 in inflammatory response, we determined the level of TNF-α, IL-6 in Blank group, LPS group, scramble group and miR-155 inhibitor group. The levels of TNF-α in renal tissues of mice injected with LPS were significantly increased from 6 h to 48 h after LPS treatment with the peak value at 12 h ($P < 0.05$, **Figure 3A**). Similarly, the levels of IL-6 in renal tissues of mice injected with LPS were significantly increased from 6 h to 48 h after LPS treatment with higher value at 12 h and 24 h ($P < 0.05$, **Figure 3B**). Interestingly, inhibition of miR-155 significantly suppressed the higher level of both TNF-α and IL-6 induced by LPS treatment ($P < 0.05$) (**Figure 3A**, **3B**). Furthermore, the levels of TNF-α and IL-6 in renal tissues of mice in scramble group were similar to the mice in the LPS group, which suggested that miR-155 inhibitor plays a key role in inhibiting the inflammatory response induced by LPS.

miR-155 inhibitor suppresses the expression of JAK/STAT signaling pathways associated with upregulating the expression of SOCS1

Given that miR-155 directly targets to SOCS1, a key negative regulators of JAK/STAT1 signaling pathway, the effect of miR-155 inhibitor on the expression of SOCS1-JAK/STAT1 signaling pathway were analyzed in mice of Blank, LPS, scramble and miR-155 inhibitor group. The mRNA expressions of *Socs1* were significantly increased in renal tissues of mice of miR-155 inhibitor groups at 12 h, 24 h and 48 h after LPS treatment ($P < 0.01$, **Figure 4A**). Consequently, the *Stat1* mRNA levels were significantly decreased in renal tissues of mice in miR-155 inhibitor group at 12 h and 24 h after LPS treatment ($P < 0.01$, **Figure 4B**). Further study indicated that the protein levels of SOCS1 were obviously increased in renal tissues of mice of miR-155 inhibitor group (**Figure 4C**). Consistently, the protein levels of JAK2, STAT3 and its activation form p-STAT3, as important

proteins in JAK/STAT signaling pathways, were significantly decreased in renal tissues of mice in miR-155 inhibitor group (**Figure 4C**).

Discussion

In the present study, we performed a LPS-induced endoxemia model to explore the effects of miR-155 inhibitor on sepsis-associated AKI in mice. The appropriate dose of LPS for construction of endoxemia mouse model was 20 mg· kg^{-1} body weight used in this work. Inhibition of miR-155 via injected miR-155 inhibitor in LPS-treated mice effectively alleviated LPS-induced kidney injury, and increased the mRNA and protein levels of SOCS1, as well as suppressed the expression of STAT1, JAK2, STAT3 and p-STAT3. Meanwhile, the higher levels of TNF-α and IL-6 in renal tissues of mice treated with LPS were suppressed by miR-155 inhibitor administration. All these results demonstrated that inhibition of miR-155 protects mice from LPS-induced kidney injury possibly through regulating SOCS1-JAK/STAT signaling pathway.

Kidney is the important target organ of inflammation response caused by infection. Severe infection can lead to AKI and ARF (acute renal failure) [23]. Septic AKI is the most common AKI syndrome in intensive care unit (ICU), which was associated with high risk of in-hospital mortality [24]. Our understanding of the pathogenesis of septic AKI is limited. Animal models of septic AKI have been developed to enable sophisticated and invasive measurements that cannot be performed in humans. Renal hemodynamic changes, endothelial dysfunction, renal interstitial inflammatory cell infiltration induced by LPS, micro thrombus in glomerular and renal tubular congestion and so on are main mechanism of infection-related kidney injury [5, 25]. LPS administration was used to construct the endoxemia model that mimics the clinical characteristics of sepsis, but the appropriate dose of LPS is controversial. To mimic the sepsis-associated AKI, the 6-8 weeks old ICR mice intraperitoneally injected with LPS at 3 mg·kg^{-1} with a high mortality (50%, 11/22) [26], 8 week-old CD-1 mice were injected with LPS at 10 mg·kg^{-1} [27], and the female 8-12 weeks C57BL/6 mice were injected intraperitoneally with 10 mg·kg^{-1} of LPS [28]. All these results indicated that the dose of LPS for mimicking sepsis-associated AKI was different, which might depend on the species of mice. In present study, we examined the effects of different dose (5, 10, 20, 40, 60 mg·kg^{-1}) of LPS on 8-week-old BALB/c mice mortality and scores indicating mice activity. And the results indicated that 20 mg·kg^{-1} is appropriate dose to construct the endoxemia model that mimic sepsis associated AKI. The 20 mg·kg^{-1} LPS-induced endoxemia model with AKI displays with inflammatory cell infiltration in renal tissues and increased expression of cleaved caspase 3 in renal suggesting more apoptosis.

LPS administration induces kidney injury presented as numerous inflammatory cell infiltrations in the renal interstitium. In present study, miR-155 inhibitor alleviates LPS-induces kidney injury presented as the normal glomerulus and renal tubular in mice of miR-155 inhibitor group with a lower mortality than LPS group (5% VS. 12.5%), suggesting that miR-155 might be potential drug target for treatment of sepsis-associated kidney injury. Septic AKI often is mediated by a concomitant pro- and anti-inflammatory state that is activated in response to various pathogen-associated molecular patterns, such as endotoxin, as well as damage-associated molecular patterns. LPS, as endotoxin produced by pathogen, could be identified by TLR4 (Toll-Like Receptor 4) found in the kidney to promote the important transcription factors NF-κB into nucleus to regulate gene expression of the primary inflammatory mediums TNF-α, IL-1, IL-6 and IL-8, which further stimulates caspase-dependent or -independent apoptosis pathway contributing to the pathogenesis of septic AKI [29]. In present study, miR-155 inhibitor significantly decreases the levels of TNF-α and IL-6 in kidney tissue, and suppressed the expression of cleaved caspase 3 in kidney tissue. All these results suggested that inhibition of miR-155 plays a key role in blocking the inflammatory response and related apoptosis in kidney of mice injected with LPS. It is important to control the levels of inflammatory factors involved in the pathology of septic AKI. For example, astragaloside IV (AS-IV) was proved to decrease LPS-stimulated production of blood TNF-α and IL-6 in the kidneys in a rodent model of endotoxinemia [27], eupafolin nanoparticle improved LPS-induced renal injury through anti-inflammatory activities [30], and Xuebijing injection could beneficial for

improvement of clinical symptoms in septic AKI patients via decreasing the levels of blood IL-6 [31]. All these results suggested the control of inflammatory response is critical for improving septic AKI. Consequently, inhibition of miR-155 improves the pathogenic change of kidney tissue and suppresses the apoptosis indicated as decreased the expression of cleaved caspase 3. It still need further study to explore the relationship between inflammatory response and apoptosis suppressed by miR-155 inhibitor administration.

Recent study indicated that LPS treatment induces the expression of miR-155 in splenocyte, monocytes and macrophages [32]. Furthermore, LPS-induced miR-155 in macrophages is involved in activation of TLR and apoptosis signaling pathways, as well as promotes the translation of TNF-α [33]. Our present study confirmed that the expression of miR-155 significantly increased in renal tissues of mice in LPS group, and miR-155 inhibitor could significantly suppress the expression levels of TNF-α and IL-6 in renal tissues. Furthermore, previous study indicated that inhibition of miR-155 improves the prognosis of patients with sepsis by suppressing the levels of proinflammatory cytokines via regulating the expression of SOCS1 [34]. Consistently, the expression of SOCS1 in kidney of mice injected with miR-155 inhibitor was significantly increased in our study. SOCS1 is a negative regulatory protein for the JAK/STAT pathway [16]. Accumulated evidences indicated that JAK/STAT signaling pathway had participated in organ damage and other dysfunctions in sepsis models. JAK/STAT pathway can be treated as a potential target in sepsis early intervention [14]. Our previous study proved that inhibition of miR-155 attenuated the LPS-induced liver injury through down-regulating the JAK/STAT signaling pathway [21]. In the nucleus, dimerized STATs initiate transcription of target genes, then promote the release of pro-inflammatory, anti-inflammatory cytokines and inflammatory medium [15]. Among them, JAK2, STAT1 and STAT3 areclosely related to sepsis. STAT1 is extensively activated in important organs such as liver, lung, kidney and intestine [35]. In present study, the expression of STAT1, JAK2, STAT3 and p-STAT3 were increased in kidney of mice in LPS group, which was associated with the higher levels of TNF-α and IL-6 in renal tissue. Moreover, miR-155 inhibitor increased the expression of SOCS1 resulting in negatively regulating the expression of STAT1, JAK2, STAT3 and p-STAT3, which was associated with the lower level of TNF-α and IL-6 in renal tissue. Given that AG490 (a JAK2 inhibitor) blocking the JAK/STAT signaling pathway reduces the expression of TNF-α and IL-10 in liver, lung and kidney tissues, resulting in improved liver functional indexes and kidney damage [36, 37], we speculated that miR-155 inhibitor suppresses the levels of TNF-α and IL-6 partially through suppressing the activity of JAK/STAT signaling pathway.

An extensive body of research has proved that miR-155 is closely related to inflammation and immune regulation mainly though regulating the SOCS1-JAK/STAT signaling pathways [21, 38, 39]. Moreover, SOCS1 is a target gene of miR-155 [34, 40]. So, miR-155 inhibitor protecting mice from LPS-induced AKI possibly depends on the regulation of SOCS1-mediated JAK/STAT signaling pathway. Otherwise, miR-155 directly suppresses SOCS1 and induces the production of IL-12 and other cytokines [41], and miR-155 can directly target to transforming growth factor-beta-activated kinase-1-binding protein 2 (TAB2) to regulate inflammation in septic lung injury [19]. To further investigate the possible mechanisms of miR-155 inhibitor involved in protecting mice from LPS-induced kidney injury, it needs further study to explore the effect of miR-155 inhibitor on the levels of cytokines besides of TNF-α and IL-6, and to enclose other potential target genes such as TAB2. In addition, miR-155 is involved in regulation of T-cell responses [17, 42]. Given that miR-155 inhibitors were injected via tail vein, it might affect T-cell responses, which contributes to LPS-induced inflammatory responses in renal tissue. All these need further study in the future.

In conclusion, inhibition of miR-155 protects mice from LPS-induced kidney injury associated with the higher expression of SOCS1 and suppressed activation of the JAK/STAT signaling pathway. These results suggested that miR-155 might be a novel and potential therapeutic target for treatment of sepsis-associated kidney injury.

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Disclosure of conflict of interest

None.

Address correspondence to: Drs. Yucai Zhang and Chunxia Wang, Department of Critical Care Medicine, Shanghai Children's Hospital, No.355 Luding Road, Putuo District, Shanghai 200062, P. R. China. E-mail: zyucai2018@163.com (YCZ); karencx0465@163.com (CXW)

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