European Review for Medical and Pharmacological Sciences 2020; 24: 6237-6244

MiR-942-5p alleviates septic acute kidney injury by targeting FOXO3

N. LUO, H.-M. GAO, Y.-Q. WANG, H.-J. LI, Y. LI

Department of Intensive Care Unit, Tianjin First Central Hospital, Tianjin, China

Abstract. – OBJECTIVE: Sepsis refers to the systemic inflammatory response caused by infection. Acute kidney injury (AKI) in sepsis is very common, and there are many complicated mechanisms for the occurrence of septic AKI. This article aimed to study the role of miR-942-5p in inflammation and apoptosis of septic AKI and its potential mechanism.

MATERIALS AND METHODS: Real Time-quantitative Polymerase Chain Reaction (RT-qPCR) was performed to detect the expression of RNAs. The protein expression was detected using Western blot. The contents of inflammatory factors in the cell supernatant were detected using commercial enzyme-linked immunosorbent assay (ELISA) kits. Cell Counting Kit-8 (CCK-8) assay was utilized to compare the cell viability of each group. Terminal dexynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) staining and flow cytometry were used to observe cell apoptosis.

RESULTS: MiR-942-5p expression was reduced in lipopolysaccharide (LPS)-treated HK-2 cells. MiR-942-5p mimic could observably increase miR-942-5p expression. The overexpression of miR-942-5p dramatically inhibits the expression of inflammatory factors and Bax, but increase Bcl-2 expression. MiR-942-5p overexpression greatly reversed the LPS-induced decrease in viability of HK-2 cells. In addition, we observed that LPS can markedly increase the number of apoptosis, while miR-942-5p mimic can reduce it.

CONCLUSIONS: Taken together, our results demonstrated that miR-942-5p expression was reduced in the LPS-treated HK-2 cells, and miR-942-5p overexpression can inhibit LPS-induced inflammation and apoptosis of HK-2 cells via targeting FOXO3.

Key Words:
Sepsis, Acute kidney injury, MiR-942-5p, FOXO3.

Introduction

Sepsis is a life-threatening organ dysfunction disease caused by an imbalanced host response caused by infection[1]. Sepsis is one of the most important diseases currently leading to death in clinically critical patients; its incidence has increased from 8.27/10,000 to 24.04/10,000 in the past 20 years, and the prevalence of severe sepsis has increased by an average of 13.0% to 13.3% per year. The global mortality rate of sepsis is as high as 28%, and its mortality is increasing year by year at a rate of 5.6%[2-4]. The pathophysiology of sepsis is very complex. Critical patients often develop multiple organ dysfunction syndrome, of which the kidney is one of the most commonly affected organs[5,6]. The morbidity rate for acute kidney injury (AKI) is 64% during the first twenty-four hours after sepsis occurs. The mortality rate for sepsis doubles due to coincidence of AKI. The morbidity and mortality rates for septic AKI are high[7,8]. To date, there has been mostly anti-infectious and supportive therapies and specific therapy is absent because the pathogenesis of septic AKI is now still unclear. The current research indicates that the pathogenesis of septic AKI is related to multiple factors, including renal ischemia-reperfusion injury caused by hemodynamic changes during sepsis, renal cell apoptosis, and complex inflammatory responses induced by endotoxin, and immune network responses, endothelial dysfunction, necrotic cell-induced renal tubular obstruction, and extensive formation of renal microvascular microthrombus due to coagulation dysfunction[9-12].

Some small non-coding RNAs are involved in the pathogenesis of septic AKI[13,14]. Non-coding RNA is an important life-regulating element that regulates DNA, including "house-keeping" ncRNA, piwi-interacting RNA (piRNA), microRNA (miRNA) and long-chain non-coding RNA (lncRNA). MiRNA is a non-coding RNA of about 22 nucleotides in length, which binds to the 3'UTR of the target mRNA, thereby inhibiting gene expression at the post-transcriptional level[15]. MiR-942-5p is involved in the pathophysiological

Corresponding Author: Yin Li, MM; e-mail: liyin0711@126.com

regulation of diseases, including cell proliferation and apoptosis[16,17]. However, little is known about the regulatory role of miR-942-5p in septic AKI.

Therefore, this report aims to study the role of miR-942-5p in inflammation and apoptosis of septic AKI.

Materials and Methods

Cell Culture

HK-2 cells were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA). The cells were cultured in an incubator containing 5% CO_2 in 37°C. The cell culture medium was composed of Dulbecco's Modified Eagle's Medium F-12 (DMEM/F-12; Gibco, Rockville, MD, USA) and 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA) and 1% penicillin/streptomycin (Gibco, Rockville, MD, USA). MiR-942-5p mimic (RibiBio, Guangzhou, China) was transfected into HK-2 cells to increase miR-942-5p expression. LPS (500 ng/mL) (Sigma, St. Louis, MO, USA) was utilized to construct a cellular model of septic AKI.

Real Time-Quantitative Polymerase Chain Reaction (RT-qPCR)

Total RNA of HK-2 cells was extracted by TRIzol kit (Invitrogen, Carlsbad, CA, USA). The cDNA of miRNA was synthesized using the miRNA 1st Strand complementary deoxyribose nucleic acid (cDNA) Synthesis Kit (Vazyme, Nanjing, China) and the cDNA of mRNA was synthesized using HiScript II Q Select RT SuperMix for qPCR (Vazyme, Nanjing, China). RT-qPCR was performed using AceQ Universal SYBR qPCR Master Mix (Vazyme, Nanjing, China). We used U6 to normalize the expression of miR-942-5p and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) to normalize the expression of mRNA. All the primers were listed in Table I.

Enzyme-Linked Immunosorbent Assay (ELISA)

The levels of inflammatory factors (TNF-α, IL-6, IL-1β) in the cell supernatant were detected by commercial ELISA detection kits (Elabscience, Wuhan, China) in accordance with the protocols.

Western Blot

The cells were first lysed using radioimmunoprecipitation assay (RIPA) lysate (Beyotime, Shanghai, China) containing protease inhibitors and phosphatase inhibitors, and then, the cells were repeatedly pipetted with a pipette and transferred to a 1.5 mL centrifuge tube. After sufficient lysis, the cell lysate was centrifuged at 4°C and a centrifugal force of 12000 g for 30 minutes. Then, we transferred the supernatant to a new centrifuge tube, added 1/4 of the supernatant volume of 5 × sodium dodecyl sulphate (SDS) loading buffer (Beyotime, Shanghai, China), and cooked in 100°C boiling water for 10 min.

The proteins were then loaded in equal mass, subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) electrophoresis, and then, transferred to polyvinylidene difluoride (PVDF; EpiZyme, Shanghai, China) membranes. The PVDF membranes were then placed in 5% skim milk and blocked for 2 h at about 22°C. After that, the membranes were placed into primary antibodies (Bax, Abcam, Cambridge, MA, USA, Rabbit, 1:1000; Bcl-2, Abcam, Cambridge, MA, USA, Rabbit, 1:1000; TNF-α, Abcam, Cambridge, MA, USA, Rabbit, 1:1000; IL-6, Abcam, Cambridge, MA, USA, Rabbit, 1:1000; IL-1β, Abcam, Cambridge, MA, USA, Rabbit, 1:1000; FOXO3, Abcam, Cambridge, MA, USA, Rabbit, 1:1000; GAPDH, Abcam, Cambridge, MA, USA, Rabbit, 1:1000) and incubated at 4°C overnight. Then, we washed the membranes three times using Tris-Buffered Saline and Tween-20 (TBST), and we transferred the membrane to the sec-

Table I. Real time PCR primers.

Gene name	Forward (5′>3′)	Reverse (5′>3′)
Bax	CAGTTGAAGTTGCCATCAGC	CAGTTGAAGTTACCATCAGC
Bcl-2	GACTGAGTACCTGAACCGGCATC	CTGAGCAGCGTCTTCAGAGACA
FOXO3	CTCTCGCCCATGCTCTAC	TTCAGTCAGCCCATCATTC
miR-942-5p	AGGGTCTTCTCTGTTTTGGC	GTTGTGGTTGGTTGGTTTGT
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
GAPDH	ACAACTTTGGTATCGTGGAAGG	GCCATCACGCCACAGTTTC

Real Time-quantitative Polymerase Chain Reaction (RT-qPCR).

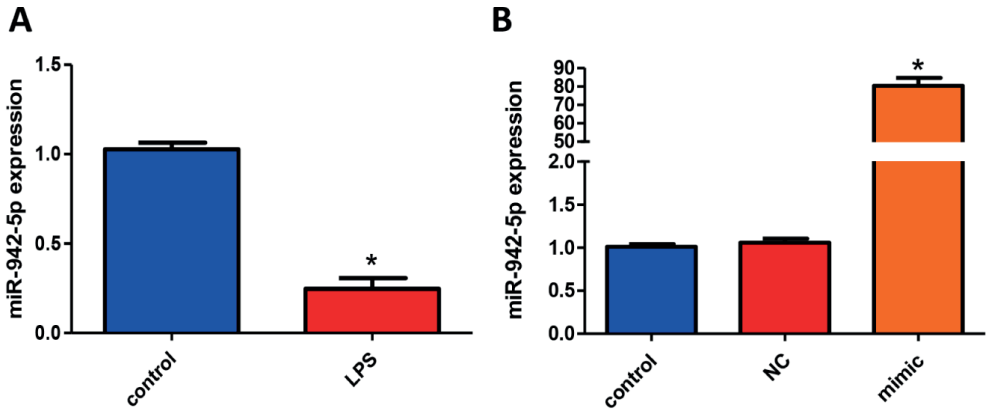

Figure 1. MiR-942-5p expression was reduced in LPS-treated HK-2 cells. **A,** The results of RT-qPCR suggested that miR-942-5p was reduced in HK-2 cells treated with LPS ("*" $p<0.05$ *vs.* control, n = 3). **B,** MiR-942-5p mimic significantly increase the expression of miR-942-5p in HK-2 cells ("*" $p<0.05$ *vs.* control, n = 3).

ondary antibody and incubated the membrane for 2 h. Finally, a fluorescence imaging system was used for exposure photography.

Flow Cytometry

Cells were collected using trypsin digestion, washed using phosphate-buffered saline (PBS), and resuspended in 300 μL of binding buffer. Fluorescein isothiocyanate (FITC)-Annexin V/Propidium Iodide (PI) dual staining method was used. We added 5 μL of AnnexinV-FITC (KeyGen, Nanjing, China) and mixed. The mixture was protected from light and incubated for 15 min. We added 5 μL of PI (KeyGen, Nanjing, China) 5 minutes before detection and mixed, and then, detected apoptosis by flow cytometry.

Terminal Dexynucleotidyl Transferase (TdT)-Mediated dUTP Nick End Labeling (TUNEL) Staining

The cells were washed three times using PBS, and then, fixed using 4% paraformaldehyde for 30 to 60 min. The cells were then washed three times with PBS, and added with 0.1% Triton X-100 (Solarbio, Beijing, China) and incubated for 20 min. Then, the cells were washed three times again using PBS. After that, we added 50 μL of TdT solution (Roche, Basel, Switzerland) to the sample and incubated at 37°C for 60 min. After washing three times with PBS, nuclear staining was performed with 4',6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich, St. Louis, MO, USA). TUNEL staining was showed by a fluorescence inverted microscope.

Cell Counting Kit-8 (CCK-8) Assay

CCK-8 (MCE, Monmouth Junction, NJ, USA) was utilized to detect the cell viability in line with the protocol. Finally, the optical density (OD) value at 450 nm was obtained using a microplate reader.

Luciferase Activity Assay

The Luciferase vectors which contained wild-type (WT) or mutant (MUT) 3′-UTR of FOXO3 were constructed by RibiBio (Guangzhou, China). HEK293T cells were co-transfected with the vectors combined with miR-942-5p mimic or negative control (NC). The Luciferase activity was measured by Dual-Glo® Luciferase Assay System.

Statistical Analysis

Data were expressed as $\chi \pm s$ and were plotted using GraphPad Prism 5 software (La Jolla, CA, USA). The differences between the two groups were analyzed by using the Student's *t*-test. Comparison between multiple groups was done using One-way ANOVA test followed by Post-Hoc Test (Least Significant Difference). $p<0.05$ indicated the significant difference.

Results

MiR-942-5p Expression Was Reduced In LPS-Treated HK-2 Cells

First, we treated HK-2 cells with LPS and detected miR-942-5p expression in the cells 24 hours later. We found that miR-942-5p expression was dramatically reduced in the LPS group com-

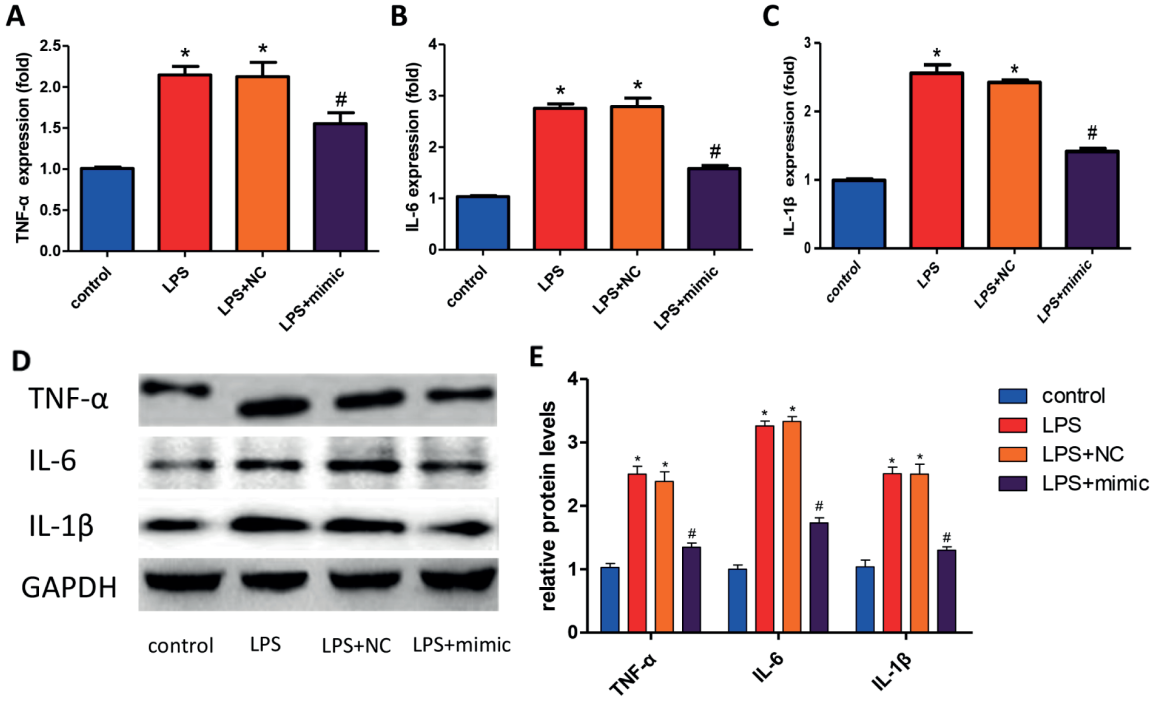

Figure 2. MiR-942-5p overexpression inhibited LPS-induced inflammation in HK-2 cells. **A-C,** The content of inflammatory factors (TNF-α, IL-6, IL-1β) in the cell supernatant was detected using ELISA kits ("*" $p<0.05$ *vs.* control, "#" $p<0.05$ *vs.* LPS+NC, n=3). **D,** The expression of TNF-α, IL-6 and IL-1β in each group was detected using Western blot. GAPDH is an internal reference. **E,** Statistical results of expression of proteins ("*" $p<0.05$ *vs.* control, "#" $p<0.05$ *vs.* LPS+NC, n=3).

pared to the control group (Figure 1A). For subsequent functional experiments, we overexpressed miR-942-5p by transfecting miR-942-5p mimic in HK-2 cells (Figure 1B).

MiR-942-5p Overexpression Suppressed LPS-Induced Inflammation In HK-2 Cells

After transfecting miR-942-5p mimic or NC in HK-2 cells and adding LPS, we examined the content of inflammatory factors (TNF-α, IL-6, IL-1β) in the cell supernatant in each group. LPS significantly induced the inflammatory response of HK-2 cells. The overexpression of miR-942-5p can inhibit the content of inflammatory factors in the cell supernatant (Figure 2A-C). In addition, we also detected the expression of inflammatory factors in cells by Western blot (Figure 2D and 2E). The results were similar to the above.

MiR-942-5p Overexpression Suppressed LPS-Induced Apoptosis In HK-2 Cells

It can be observed from Figure 3A that miR-942-5p overexpression can reverse the decrease of cell viability caused by LPS. Through RT-qPCR and Western blot, we found that LPS can promote Bax expression and inhibit Bcl-2 expression in HK-2 cells at the transcription and translation levels (Figure 3B-3D). In addition, the results of flow cytometry showed that LPS can significantly induce cell apoptosis, which could be prevented by miR-942-5p mimic (Figure 3E). The results of TUNEL staining were consistent with this finding (Figure 3F).

MiR-942-5p Inhibited Inflammation and Apoptosis In HK-2 Cells by Targeting FOXO3

Through bioinformatics analysis, we found that the 3'UTR of FOXO3 has a binding site for miR-942-5p (Figure 4A). Through Western blot, we found that LPS can promote FOXO3 expression, but after miR-942-5p overexpression, FOXO3 expression was greatly suppressed (Figure 4B and 4C). The results of RT-qPCR are consistent with this (Figure 4D). To further prove whether there is a binding site between miR-942-5p and FOXO3, we performed a Luciferase reporter gene experiment. In the WT group, miR-942-5p mimic greatly inhibited the fluorescence activity, while the fluorescence activity in the MUT group did not significantly change (Figure 4E).

Discussion

Sepsis is a severely dysregulated inflammatory response caused by an infection, which can cause dysfunction of organs far from the infection site. When AKI occurs, the patient's mortality rate increases, the time spent in ICU increases, and a large amount of medical resources are consumed. When compared with AKI caused by non-sepsis, septic AKI has special pathophysiology characteristics, so different intervention methods are needed[5,18,19].

The pathophysiology of sepsis kidney injury is complicated and involved in many factors, including changes in intrahepatic hemodynamics, infiltration of inflammatory cells, endothelial dysfunction, glomerular blood vessel thrombosis, necrotic tubular obstruction of renal tubules, etc. Some scholars[20] have proved that inflammation-induced immune response, inflammatory response are involved. Many cytokines are activated, of which IL-1, TNF-α and IL-6 are the most important. Eventually, a waterfall of inflam-

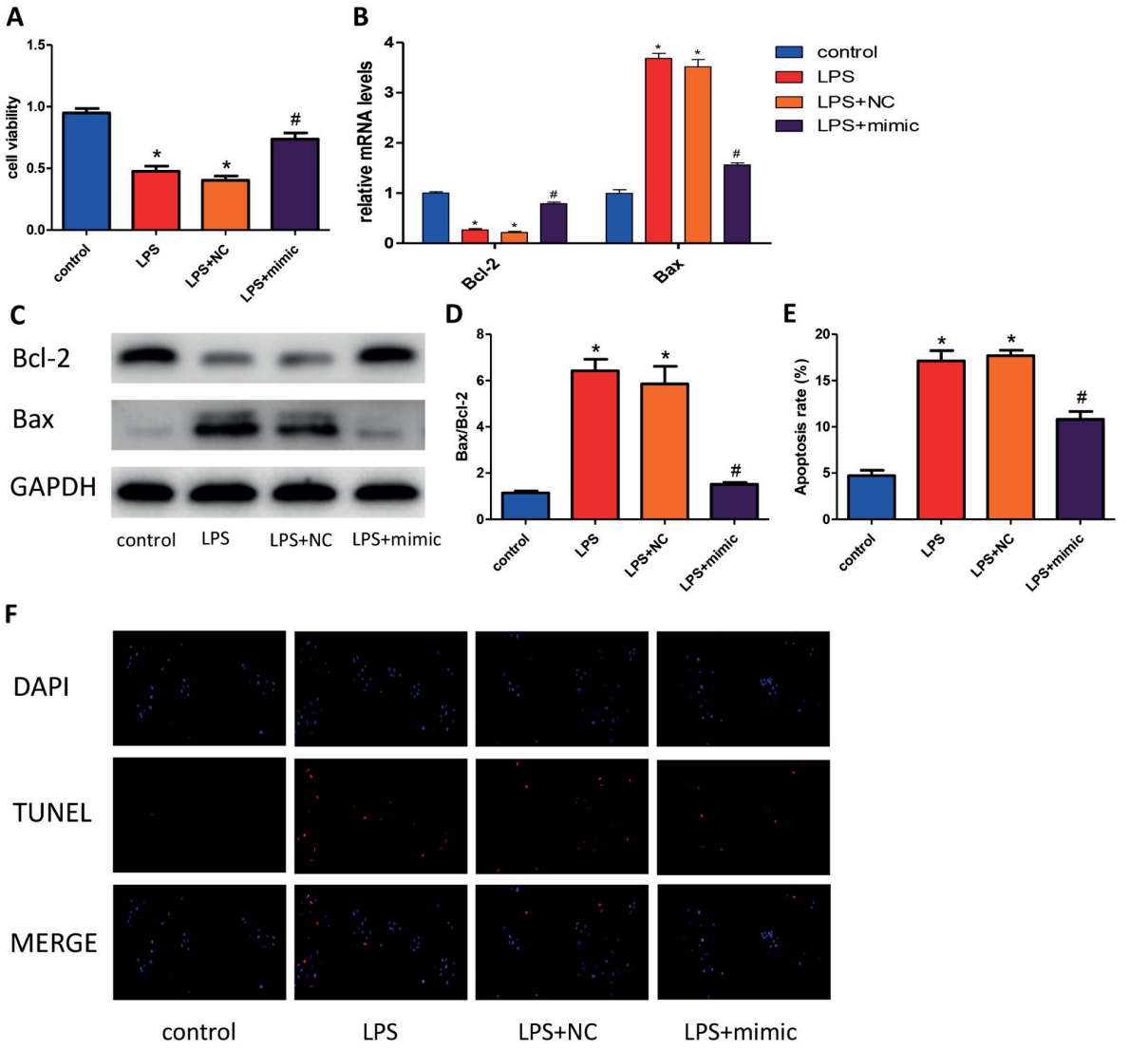

Figure 3. MiR-942-5p overexpression inhibited LPS-induced apoptosis in HK-2 cells. **A,** Cell viability was tested by CCK-8 assay ("$_{*}$" $p<0.05$ *vs.* control, "$_{\#}$" $p<0.05$ *vs.* LPS+NC, n=3). **B,** The expression of Bax mRNA and Bcl-2 mRNA were detected by RT-qPCR ("$_{*}$" $p<0.05$ *vs.* control, "$_{\#}$" $p<0.05$ *vs.* LPS+NC, n=3). **C,** The expression of Bax and Bcl-2 in each group was detected using Western blot. **D,** Statistical results of Bax/Bcl-2 ("$_{*}$" $p<0.05$ *vs.* control, "$_{\#}$" $p<0.05$ *vs.* LPS+NC, n=3). **E,** Apoptosis rate was detected by flow cytometry ("$_{*}$" $p<0.05$ *vs.* control, "$_{\#}$" $p<0.05$ *vs.* LPS+NC, n=3). **F,** Apoptosis was observed by TUNEL staining, (magnification: 400×).

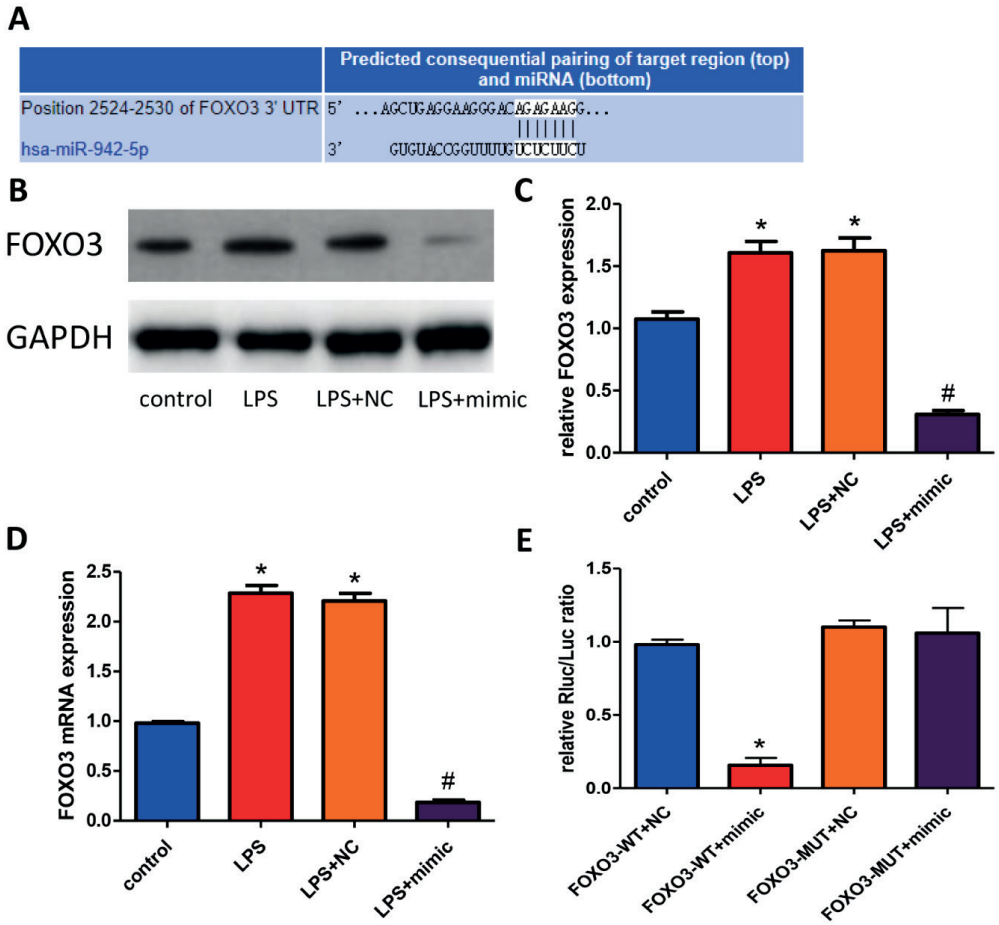

Figure 4. MiR-942-5p directly targets FOXO3. **A,** Binding site predicted by the TargetScan database. **B,** The expression of FOXO3 was detected using Western blot. **C,** Statistical results of FOXO3 ("*" $p<0.05$ *vs.* control, "#" $p<0.05$ *vs.* LPS+NC, n=3). **D,** The expression of FOXO3 mRNA was detected by RT-qPCR ("*" $p<0.05$ *vs.* control, "#" $p<0.05$ *vs.* LPS+NC, n=3). **E,** MiR-942-5p overexpression significantly decreased the Luciferase activity in WT group, but did not decrease the Luciferase activity in mutant group ("*" $p<0.05$ *vs.* WT+NC, n = 3).

mation is formed, the circulation is unstable, and organ dysfunction and septic shock eventually appear. When the inflammatory response and compensatory anti-inflammatory response occur, the body is in an immunosuppressive state. At this time, cytokine secretion and monocyte antigen presentation change, lymphocyte proliferation decreases, and apoptosis increases[21-23].

MiRNAs are widely involved in the physiological and pathological regulation of many diseases, such as tumors, cardiovascular diseases, and kidney diseases. The regulation of miRNAs in biological processes is mainly achieved by binding the target mRNA, thereby reducing its expression[24]. The forkhead box (FOX) family is divided into 19 groups (FOXA-FOXS). FOXO belongs to the O subfamily of the forkhead box family. FOXO3 is one of the largest and most important transcriptional regulators of cellular activity in the FOXO family. FOXO3 is widely expressed in various tissues and organs, including skeletal muscle, heart muscle, kidney, liver, pancreas, thymus and nervous system. And FOXO3 is implicated in various biological regulation including cell proliferation, differentiation, senescence, apoptosis, oxidative stress, inflammation and so on[25-28]. In our study, miR-942-5p can inhibit LPS-induced inflammation and apoptosis in septic AKI by targeting FOXO3.

In this paper, we elaborated the regulatory role of miR-942-5p in the pathophysiology of septic AKI *in vitro* from both aspects of inflammation and apoptosis. We revealed, for the first time, that the expression of miR-942-5p is reduced in

LPS-treated HK-2 cells, and upregulation of miR-942-5p could inhibit inflammation and apoptosis of HK-2 cells *via* targeting FOXO3. However, due to the complexity of the pathogenesis of septic AKI, the regulatory role of miR-942-5p still needs to be further explored, including studies on oxidative stress, proliferation, and autophagy in renal tubular epithelial cells. Gain-of-function and loss-of-function experiments of FOXO3 and *in vivo* experiments also need to be further improved.

Conclusions

To sum up, this study illustrated that miR-942-5p expression was decreased in an *in vitro* model of septic AKI, and miR-942-5p overexpression can inhibit LPS-induced inflammation and apoptosis of HK-2 cells *via* targeting FOXO3.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Rello J, Valenzuela-Sanchez F, Ruiz-Rodriguez M, Moyano S. Sepsis: a review of advances in management. Adv Ther 2017; 34: 2393-2411.
2) Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. N Engl J Med 2003; 348: 1546-1554.
3) Gaieski DF, Edwards JM, Kallan MJ, Carr BG. Benchmarking the incidence and mortality of severe sepsis in the United States. Crit Care Med 2013; 41: 1167-1174.
4) Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med 2001; 29: 1303-1310.
5) Poston JT, Koyner JL. Sepsis associated acute kidney injury. BMJ 2019; 364: k4891.
6) Chertow GM, Burdick E, Honour M, Bonventre JV, Bates DW. Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. J Am Soc Nephrol 2005; 16: 3365-3370.
7) Mayeux PR, MacMillan-Crow LA. Pharmacological targets in the renal peritubular microenvironment: implications for therapy for sepsis-induced acute kidney injury. Pharmacol Ther 2012; 134: 139-155.
8) Bagshaw SM, Lapinsky S, Dial S, Arabi Y, Dodek P, Wood G, Ellis P, Guzman J, Marshall J, Parrillo JE, Skrobik Y, Kumar A. Acute kidney injury in septic shock: clinical outcomes and impact of duration of hypotension prior to initiation of antimicrobial therapy. Intensive Care Med 2009; 35: 871-881.
9) Skube SJ, Katz SA, Chipman JG, Tignanelli CJ. Acute kidney injury and sepsis. Surg Infect (Larchmt) 2018; 19: 216-224.
10) Wan L, Bagshaw SM, Langenberg C, Saotome T, May C, Bellomo R. Pathophysiology of septic acute kidney injury: what do we really know? Crit Care Med 2008; 36: S198-S203.
11) Langenberg C, Wan L, Egi M, May CN, Bellomo R. Renal blood flow in experimental septic acute renal failure. Kidney Int 2006; 69: 1996-2002.
12) Langenberg C, Bagshaw SM, May CN, Bellomo R. The histopathology of septic acute kidney injury: a systematic review. Crit Care 2008; 12: R38.
13) Huo R, Dai M, Fan Y, Zhou JZ, Li L, Zu J. [Predictive value of miRNA-29a and miRNA-10a-5p for 28-day mortality in patients with sepsis-induced acute kidney injury]. Nan Fang Yi Ke Da Xue Xue Bao 2017; 37: 646-651.
14) Lin Y, Ding Y, Song S, Li M, Wang T, Guo F. Expression patterns and prognostic value of miR-210, miR-494, and miR-205 in middle-aged and old patients with sepsis-induced acute kidney injury. Bosn J Basic Med Sci 2019; 19: 249-256.
15) Vaschetto LM. MiRNA activation is an endogenous gene expression pathway. RNA Biol 2018; 15: 826-828.
16) Ouyang Z, Tan T, Zhang X, Wan J, Zhou Y, Jiang G, Yang D, Guo X, Liu T. CircRNA hsa_circ_0074834 promotes the osteogenesis-angiogenesis coupling process in bone mesenchymal stem cells (BMSCs) by acting as a ceRNA for miR-942-5p. Cell Death Dis 2019; 10: 932.
17) Liu N, Zuo C, Wang X, Chen T, Yang D, Wang J, Zhu H. MiR-942 decreases TRAIL-induced apoptosis through ISG12a downregulation and is regulated by AKT. Oncotarget 2014; 5: 4959-4971.
18) Gomez H, Kellum JA. Sepsis-induced acute kidney injury. Curr Opin Crit Care 2016; 22: 546-553.
19) Prowle JR, Bellomo R. Sepsis-associated acute kidney injury: macrohemodynamic and microhemodynamic alterations in the renal circulation. Semin Nephrol 2015; 35: 64-74.
20) Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. N Engl J Med 2003; 348: 138-150.
21) Monneret G, Venet F, Pachot A, Lepape A. Monitoring immune dysfunctions in the septic patient: a new skin for the old ceremony. Mol Med 2008; 14: 64-78.
22) Keir I, Kellum JA. Acute kidney injury in severe sepsis: pathophysiology, diagnosis, and treatment recommendations. J Vet Emerg Crit Care (San Antonio) 2015; 25: 200-209.
23) Zarbock A, Gomez H, Kellum JA. Sepsis-induced acute kidney injury revisited: pathophysiology, prevention and future therapies. Curr Opin Crit Care 2014; 20: 588-595.

24) Lu TX, Rothenberg ME. MicroRNA. J Allergy Clin Immunol 2018; 141: 1202-1207.

25) Pan Q, Xie X, Guo Y, Wang H. Simvastatin promotes cardiac microvascular endothelial cells proliferation, migration and survival by phosphorylation of p70 S6K and FoxO3a. Cell Biol Int 2014; 38: 599-609.

26) Qi XF, Chen ZY, Xia JB, Zheng L, Zhao H, Pi LQ, Park KS, Kim SK, Lee KJ, Cai DQ. FoxO3a suppresses the senescence of cardiac microvascular endothelial cells by regulating the ROS-mediated cell cycle. J Mol Cell Cardiol 2015; 81: 114-126.

27) Furukawa-Hibi Y, Kobayashi Y, Chen C, Motoyama N. FOXO transcription factors in cell-cycle regulation and the response to oxidative stress. Antioxid Redox Signal 2005; 7: 752-760.

28) Eijkelenboom A, Burgering BM. FOXOs: signalling integrators for homeostasis maintenance. Nat Rev Mol Cell Biol 2013; 14: 83-97.