

Original Article

MiR-339 inhibited lipopolysaccharide-induced CXCL2 and CCL2 expression by targeting SIRT2 in renal tubular epithelial cells HK-2

Haitao Zhang^{1,2}, Zhengbo Pan¹, Hairong Cai¹

¹Department of Urology Surgery, Taizhou Municipal Hospital, Taizhou University Medical School, Taizhou, Zhejiang Province, China; ²Department of Urology Surgery, Sir Run Run Shaw Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang Province, China

Received February 10, 2018; Accepted October 12, 2018; Epub March 15, 2019; Published March 30, 2019

Abstract: Acute kidney injury (AKI) is characterized as a sudden decline of kidney function and requires renal replacement therapy and has high mortality. Although numerous studies have identified microRNAs (miRNAs) served as biomarkers for the detection of acute kidney injury, the effects of miRNAs in renal tubule epithelial cell during AKI pathogenesis and the underlying molecular mechanism still remain unknown. Here, we found that miR-339 expression was down-regulated and inversely correlated with SIRT2 expression in AKI patients. Additionally, overexpression of miR-339 downregulated LPS-induced inflammatory chemokines CXCL2 and CCL2 expression, while knockdown of miR-339 upregulated CXCL2 and CCL2 expression in human proximal tubule cell line (HK-2) cells. Moreover, SIRT2 was the direct target of miR-339 and miR-339 suppressed the effects of LPS on CXCL2 and CCL2 expression in HK-2 cells via targeting SIRT2. Furthermore, results also indicated that overexpression of SIRT2 reversed the miR-339-imposed inhibitory effects on LPS-induced up-regulation of the phosphorylation of JNK and p38 in HK-2 cells. Taken together, these findings suggested that miR-339 may be an important therapeutic target for AKI.

Keywords: Acute kidney injury, lipopolysaccharide, renal tubule epithelial cell, miR-339, SIRT2

Introduction

Acute kidney injury (AKI), defined as the rapid loss of renal function, is known as a common and significant problem in clinical and lead to the dysregulation of volume and electrolyte homeostasis [1-3]. Accordingly, various factors are responsible for AKI formation. And LPS is one of the most common cause of AKI [4, 5]. It has been shown that LPS could induce AKI via prompting inflammatory response, stimulating the secretion of pro-inflammatory cytokines, which is linked to the activation of I κ B α /NF- κ B and MAPKs (p38, ERK1/2, and JNK1/2) signaling pathways [6-8]. Therefore, inhibiting LPS-induced inflammation might be an effective method to prevent and treat AKI.

MicroRNAs (miRNAs), a family of small noncoding RNAs, regulate gene expression by base-pairing to the 3' untranslated region (3'UTR) of their target genes. As microRNAs can control

the expression of multiple proteins through hundreds or thousands of targets via influencing multiple signaling pathways, the number of studies on the functions of microRNAs in AKI progression is increasing. MiR-21 is now considered a novel biomarker when diagnosing and treating AKI [9] and has effects to protect kidney injury induced by sepsis [10]. MicroRNA-34a suppresses autophagy in tubular epithelial cells in AKI [11]. Moreover, miR-146a is essential for lipopolysaccharide (LPS)-induced cross-tolerance against kidney ischemia/reperfusion injury in mice [12]. However, the effects of miRNAs in renal tubule epithelial cell during AKI pathogenesis still need to be illustrated.

In this study, we found that miR-339 expression was down-regulated and inversely correlated with SIRT2 expression in AKI patients. Then, a lipopolysaccharide (LPS)-induced AKI experimental model was established in human kidney epithelial (HK-2) cells. Finds indicated that miR-

miR-339 inhibited LPS-induced CXCL2 and CCL2 by targeting SIRT2

339 regulated LPS-induced inflammatory chemokines CXCL2 and CCL2 expression in human proximal tubule cell line (HK-2) cells. Moreover, SIRT2 was the direct target of miR-339 and miR-339 suppressed the effects of LPS on CXCL2 and CCL2 expression in HK-2 cells via targeting SIRT2. Furthermore, our results indicated that overexpression of SIRT2 reversed the miR-339-imposed inhibitory effects on LPS-induced up-regulation of the phosphorylation of JNK and p38 in HK-2 cells. These findings prompt us to understand miR-339 may be a potential target for treating LPS-induced sepsis AKI.

Materials and methods

Samples collection

Eight critical patients who developed acute kidney injury (AKI), defined as more than 1.5-fold increase in serum creatinine in compliance with RIFLE-Acute Kidney Injury Network criteria. Control samples were collected from 5 healthy volunteers. Patients and healthy volunteers received a percutaneous renal biopsy guided by B ultrasound. These samples were collected from October of 2016 to August of 2017. All experiments were approved by the research ethics committee of Sir Run Run Shaw Hospital, College of Medicine, Zhejiang University. The informed consents were obtained from all patients. All samples were frozen at -80°C.

Cell culture

Human proximal tubule epithelial cell line HK-2 was obtained from Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China). The cells were cultured in Keratinocyte Serum Free Medium (K-SFM) (Invitrogen, USA) containing 10% fetal calf serum (Gibco, USA), 100 IU/ml penicillin and 100 mg/ml streptomycin (Sigma, USA), and incubated at 37°C in humidified air containing 5% CO₂. HK-2 cells were stimulated with LPS (1 µg/ml) (Sigma, USA) for 24 h.

Plasmids and cell transfection

SIRT2 cDNA were purchased from Genewiz (Suzhou, China). Expression plasmid for SIRT2 was generated by inserting cDNA coding for SIRT2 into pCDNA3 vectors by Gateway cloning (Invitrogen). The miR-339 mimic, inhibitor and

their control were purchased from GenePharma (Shanghai, China), which were chemically synthesized fragments. HK-2 cells were seeded in six-well plates at 2×10^5 cells/well and then transfected with different concentration of miR-339 mimic, miR-339 inhibitor, pCDNA3-SIRT2 plasmids or their control via using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. Finally, the cells were collected for further analysis.

Real-Time PCR

Total RNA was isolated from AKI patient's samples and cultured HK-2 cells using Trizol reagent (Invitrogen, USA). Reverse Transcription for cDNA was performed using TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, USA) or iScript™ cDNA Synthesis Kit (Bio-Rad, USA). TaqMan Human MiRNA Assay Kit (Applied Biosystems) was used to evaluate the relative expression of miR-339, U6 was used as the internal control for miR-339. Platinum SYBR Green qPCR Supermix UDG kit (Invitrogen) was used to evaluate the relative level of SIRT2, CXCL2 and CCL2, and GAPDH was used as the internal control.

Western blot analysis

Cellular protein was extracted from HK-2 cells by using RIPA lysis buffer (Thermo Pierce, USA) and quantified by using a BCA Protein Assay Kit (Thermo Pierce). Protein samples were separated by SDS-PAGE, transferred onto a PVDF membrane (Bio-Rad, USA), and incubated with SIRT2, CXCL2, CCL2, JNK, p-JNK, P38, p-P38 and GAPDH antibodies (Santa Cruz, USA) for overnight at 4°C. After washing with TBS-T three times, the membranes were then probed with the appropriate secondary antibodies (Santa Cruz) for 2 h at room temperature, and enhanced chemiluminescence was performed according to the manufacturer's instructions (Beyotime, China). All the experiments were repeated in triplicate.

Immunofluorescent staining

After transfection of pCDNA3-SIRT2, miR-339 mimic, inhibitor or control (GenePharma, Shanghai, China) for 12 h and then treatment of LPS (1 µg/ml) for 12 h, HK-2 cells were cultured on sterile glass coverslips and fixed in 4% paraformaldehyde for 30 min. After washing

miR-339 inhibited LPS-induced CXCL2 and CCL2 by targeting SIRT2

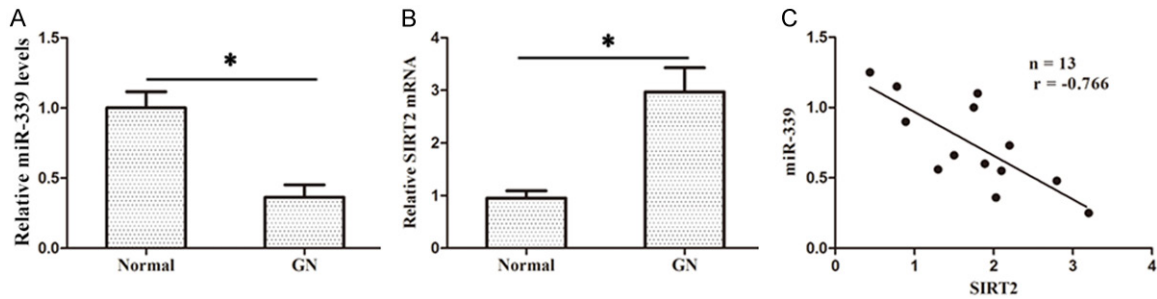


Figure 1. MiR-339 expression was decreased and inversely correlated with SIRT2 expression in AKI patients. A. miR-339 mRNA levels were examined by real-time PCR in acute kidney injury (AKI) (n = 8) patients and healthy controls (n = 5). Data are shown as mean \pm SD. $P < 0.05$ by Student's t test. B. SIRT2 mRNA levels were examined by real-time PCR in acute kidney injury (AKI) (n = 8) patients and healthy controls (n = 5). Data are shown as mean \pm SD. $P < 0.05$ by Student's t test. C. The association between miR-339 expression and the SIRT2 mRNA levels were evaluated by using Spearman's correlation analysis (n = 13, $r = -0.766$, $P < 0.05$).

with cold PBS three times, the coverslips were permeabilized with 0.1% Triton X-100 for 5 min, blocked in 5% bovine serum albumin (BSA), and then incubated with the primary antibodies (CXCL2, CCL2, JNK, p-JNK, P38 and p-P38, Santa Cruz) at 4°C overnight. The slips were next washed and incubated with anti-rabbit secondary IgG (Proteintech Group) at room temperature for 1 h. Cells were observed using a Nikon (Melville, NY, USA) Eclipse TE300 fluorescence microscope.

Luciferase assay

Firstly, the miRBase software was used to predict the binding sites of SIRT2 in the 3'UTR with the miR-339, and the wild type and mutated SIRT2 3'-UTR sequence were inserted into the pmirGLO (Promega, USA), which were named pmirGLO-SIRT2-WT and pmirGLO-SIRT2-MUT. When co-transfected miR-339 mimics with pmirGLO-SIRT2-WT or pmirGLO-SIRT2-MUT into HK-2 cells with LPS treatment, luciferase activity was measured using the dual luciferase assay (Promega) according to the manufacturer's instructions and normalized against the Renilla luciferase activity.

Statistical analysis

Data are presented as means \pm SD of three independent experiments. The one-way analysis of variance (ANOVA) test or student's t test were used for statistics. The correlation between miR-339 and SIRT2 expression levels was determined by Spearman's correlation analysis. Values of $P < 0.05$ were considered statistically significant.

Results

The miR-339 expression was downregulated and inversely correlated with SIRT2 expression in AKI patients

To explore whether miR-339 was involved in modulating renal inflammation, we firstly studied miR-339 expression in samples from AKI patients. Our results displayed that miR-339 expression was significantly downregulated in AKI patients compared with healthy control (**Figure 1A**). Then, we hypothesized that the expression of SIRT2 was regulated by miR-339 in AKI. Therefore, we detected the expression of SIRT2 by performing real-time PCR in samples from AKI patients and healthy control. As expected, our findings displayed that SIRT2 expression was significantly upregulated in AKI patients (**Figure 1B**), and SIRT2 levels was inversely negative correlated with miR-339 expression in AKI patients (Spearman's correlation, $r = -0.766$ and $P = 0.0022$) (**Figure 1C**).

MiR-339 suppressed the proinflammatory effects of LPS on renal tubule epithelial cell

LPS plays a critical role in renal inflammatory injury during sepsis AKI. Renal inflammatory injury induced inflammatory cell infiltration through the up-regulation of chemokines, such as CXCL2 and CCL2 [13, 14]. Therefore, we next verified whether miR-339 suppressed the CXCL2 and CCL2 expression induced by LPS in HK-2 cells. As shown in **Figure 2A-F**, we performed real-time PCR, Western blot, and Immunofluorescent (IF) assays, the results showed that miR-339 overexpression significantly

miR-339 inhibited LPS-induced CXCL2 and CCL2 by targeting SIRT2

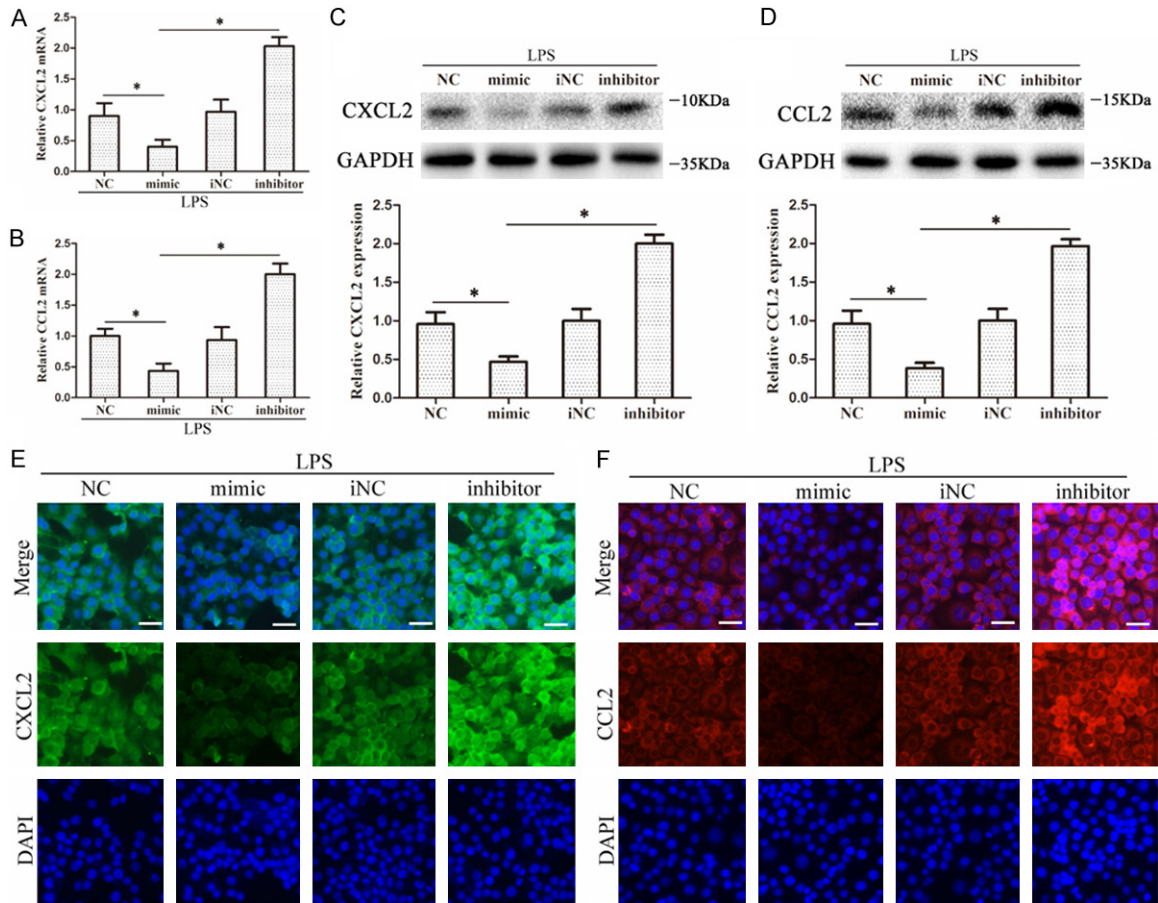


Figure 2. MiR-339 regulated LPS-induced CXCL2 and CCL2 expression in renal tubule epithelial cell HK-2. A and B. Relative CXCL2 and CCL2 mRNA levels were evaluated by real-time PCR after transfection with miR-339 mimic, inhibitor, or control in HK-2 cells. Then cells were stimulated with LPS for 12 h after transfection for 12 h. Data are shown as mean \pm SD. $n = 4$, $P < 0.05$ by ANOVA. C and D. The HK-2 cells were transfected with miR-339 mimic, inhibitor, or control and then stimulated with LPS for 12 h after transfection for 12 h. Relative CXCL2 and CCL2 protein levels were evaluated by Western blot. Data are shown as mean \pm SD. $n = 4$, $P < 0.05$ by ANOVA. E and F. The HK-2 cells were transfected with miR-339 mimic, inhibitor, or control and then stimulated with LPS for 12 h after transfection for 12 h. Relative CXCL2 and CCL2 protein levels were evaluated by immunofluorescence. Data are representative of three independent experiments.

repressed LPS-induced CXCL2 and CCL2 expression at the mRNA and protein levels, while down-regulation of miR-339 levels obviously increased LPS-induced CXCL2 and CCL2 expression in HK-2 cells. Taken together, our findings confirmed that miR-339 could suppress the *proinflammatory* effects of LPS on HK-2 cells.

SIRT2 was the direct target of miR-339

TargetScan (<http://www.targetscan.org>) and miRanda (<http://www.microrna.org>) were used to identify potential targets of miR-339. A binding site for miR-339 in the SIRT2 3'UTR was identified (**Figure 3A**). The luciferase reporter

assay implied that transfection with miR-339 mimic significantly reduced the luciferase activity of SIRT2-WT when compared with that of negative control group, while the change in the luciferase activity of SIRT2-MUT was not significantly (**Figure 3B**). We further performed real-time PCR and Western blot analysis to explore the association between miR-339 and SIRT2. The results showed that SIRT2 mRNA (**Figure 3C**) and protein (**Figure 3D and 3E**) levels were decreased in the miR-339 overexpressing group compared with negative control group, whereas SIRT2 mRNA and protein levels were substantially increased in miR-339 downregulating group. Collectively, these data indicated that SIRT2 was a direct target of miR-339.

miR-339 inhibited LPS-induced CXCL2 and CCL2 by targeting SIRT2

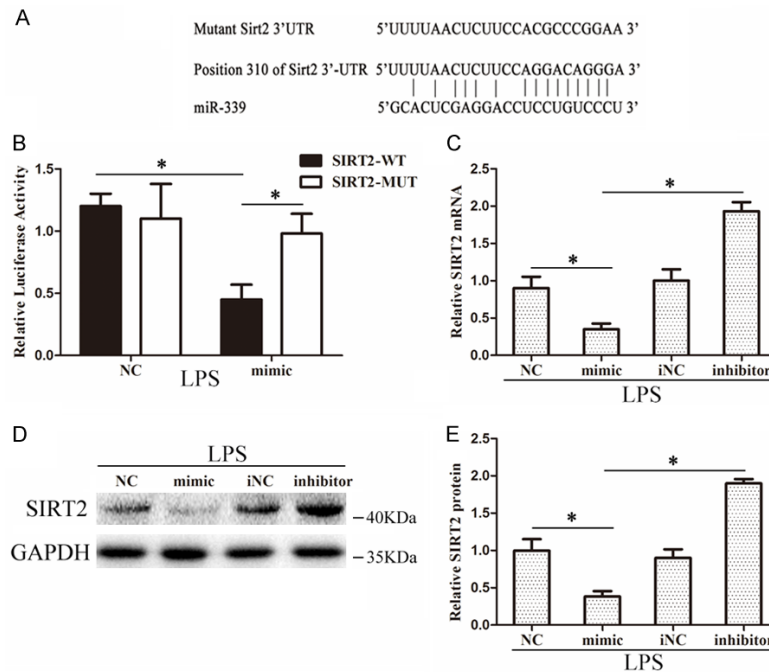


Figure 3. MiR-339 reduced the expression of SIRT2 via directly targeting 3'UTR of SIRT2. **A.** The wild-type (SIRT2-WT) and miR-339-binding site deletion mutation (SIRT2-MUT) of SIRT2 3'UTR. **B.** HK-2 cells were co-transfected with miR-339 mimic and pmirGLO-SIRT2-WT, pmirGLO-SIRT2-MUT, or empty pmirGLO plasmid vectors, and then stimulated with LPS for 12 h after transfection for 12 h. Relative Luciferase activity was measured after transfection for 24 h. Data are shown as mean \pm SD. $n = 3$, $P < 0.05$ by ANOVA. **C.** The HK-2 cells were transfected with miR-339 mimic, inhibitor, or control, and then stimulated with LPS for 12 h after transfection for 12 h. Relative SIRT2 mRNA levels were evaluated by real-time PCR. Data are shown as mean \pm SD. $n = 5$, $P < 0.05$ by ANOVA. **D.** The HK-2 cells were transfected with miR-339 mimic, inhibitor, or control, and then stimulated with LPS for 12 h after transfection for 12 h. Relative SIRT2 protein levels were evaluated by Western blot. Data are representative of three independent experiments. **E.** Relative intensity measurements provided a quantitative means of examining protein bands on the Western blot. GAPDH was used as a control. Data are shown as mean \pm SD. $n = 3$, $P < 0.05$ by ANOVA.

MiR-339 suppressed the proinflammatory effects of LPS on HK-2 via targeting the SIRT2

Finally, based on our observations that SIRT2 was the direct target of miR-339, we investigated whether miR-339 played a suppressive role in LPS-induced CXCL2 and CCL2 expression via targeting the SIRT2. As shown in **Figure 4A-F**, we also performed real-time PCR, Western blot, and Immunofluorescent (IF) assays, overexpression of miR-339 by transfecting its mimic decreased LPS-induced the CXCL2 and CCL2 expression at both mRNA and protein levels in HK-2 cells, while overexpression of SIRT2 blocked these suppressive effects. The findings demonstrated that SIRT2 was the target of

miR-339 and contributed to the suppressive effects of miR-339 on LPS-exposed HK-2 cells.

MiR-339 suppressed SIRT2/JNK/p38 signaling in LPS-exposed HK-2

LPS activates JNK/p38 pathway and phosphorylation of JNK and p38 are required for LPS-induced gene expression. Following, we further examined the phosphorylation of JNK and p38 by Western blot and IF assays. Findings displayed that overexpression of SIRT2 reversed the miR-339-imposed inhibitory effects on LPS-induced up-regulation of the phosphorylation of JNK and p38 in HK-2 cells (**Figure 5A-D**). Taken together, these results indicated that miR-339 inhibited LPS-mediated the secretion of pro-inflammatory cytokines via targeting the SIRT2/JNK/p38 signaling in HK-2 cells.

Discussion

The biological role of majority miRNAs in the pathophysiology of AKI still remains ambiguous [15, 16]. Therefore, investigation for the

functions and underlying mechanisms of miRNAs in AKI may contribute to understand the pathogenesis of AKI and provide novel therapeutic targets. Here, we focus on the role of miR-339 in AKI pathogenesis. In this study, we showed that miR-339 expression was decreased and inversely correlated with SIRT2 in AKI patient's samples.

LPS is one of the potent pro-inflammatory stimulants via inducing the expression of pro-inflammatory cytokines in AKI pathogenesis, such as CXCL2 and CCL2 [13, 14, 17]. Our data revealed that miR-339 overexpression significantly inhibited LPS-induced CXCL2 and CCL2 expression at the mRNA and protein level in

miR-339 inhibited LPS-induced CXCL2 and CCL2 by targeting SIRT2

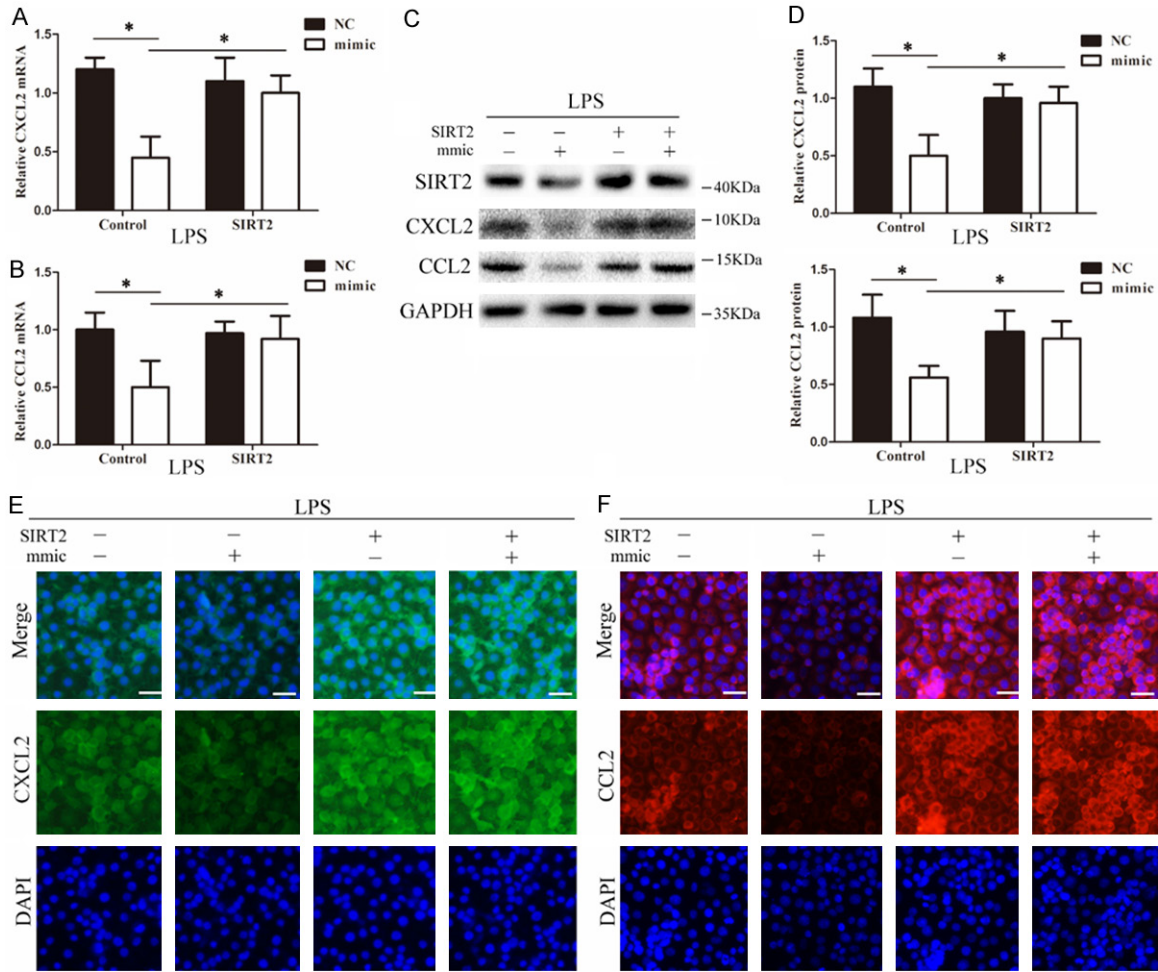


Figure 4. SIRT2 acted as the functional target of miR-339 in HK-2 cells. A and B. The HK-2 cells were transfected with control, miR-339 mimic and miR-339 mimic + pCDNA3-SIRT2, and then stimulated with LPS for 12 h after transfection for 12 h. Relative SIRT2 mRNA levels were evaluated by real-time PCR. Data are shown as mean \pm SD. $n = 5$, $P < 0.05$ by ANOVA. C and D. The HK-2 cells were transfected with control, miR-339 mimic and miR-339 mimic + pCDNA3-SIRT2, and then stimulated with LPS for 12 h after transfection for 12 h. Western blot assay was performed to detect the levels of CXCL2 and CCL2 protein. Blots are representative of three independent experiments. Data are shown as mean \pm SD. $n = 3$, $P < 0.05$ by ANOVA. E and F. The HK-2 cells were transfected with control, miR-339 mimic and miR-339 mimic + pCDNA3-SIRT2, and then stimulated with LPS for 12 h after transfection for 12 h. Immunofluorescence assay was performed to detect the levels of CXCL2 and CCL2 protein. Data are representative of three independent experiments.

HK-2 cells, while lack of miR-339 had the opposite effect. CXCL1/CXCL2 regulates the early phase of neutrophil recruitment in LPS-induced inflammation [18]. CCL2 also plays a critical role in inflammatory renal injury via recruitment and activation of monocytes and macrophages [19, 20]. Together, these data suggested that miR-339 was involved in modulating the early stages of LPS induced AKI.

Recent studies have shown that microRNAs are involved in the pathogenesis of acute kidney injury via regulating their target gene expres-

sion [21-23]. MiR-339 is shown to suppress gastric carcinogenesis through targeting NOVA1 [24], and luciferase assay implies that SIRT2 was a direct target of miRNA-339 in SH-SY5Y cells [25]. Here, we not only discovered that SIRT2 expression was inversely correlated with miR-339, but also SIRT2 was the target of miR-339.

Sirtuin 2 (SIRT2), a NAD-dependent histone deacetylase, regulates LPS-induced renal tubular CXCL2 and CCL2 expression via activating p38/JNK pathway [26]. To confirm whether the

miR-339 inhibited LPS-induced CXCL2 and CCL2 by targeting SIRT2

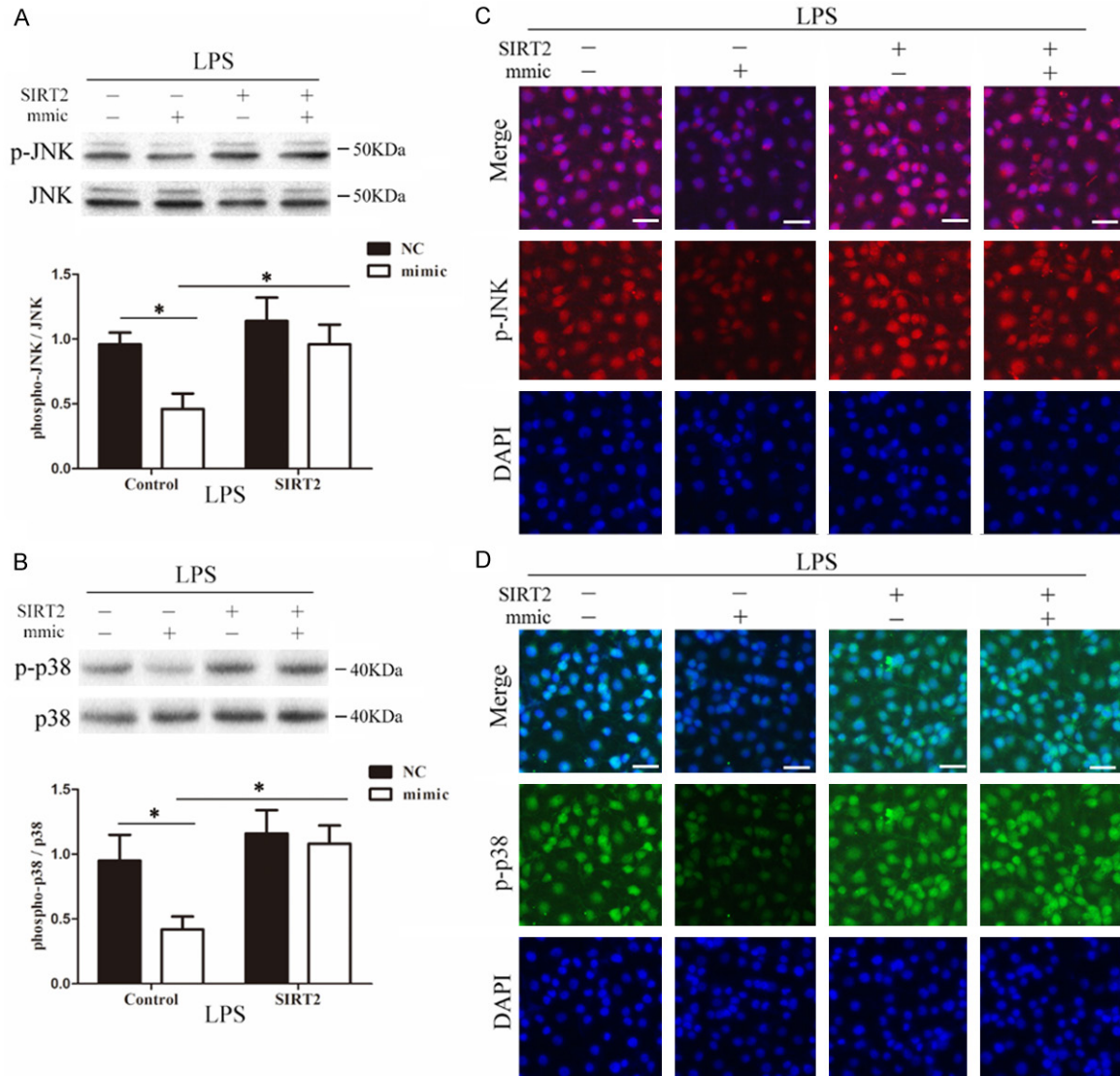


Figure 5. Overexpression of SIRT2 reversed the miR-339-imposed suppressive effects on LPS-induced activation of the JNK and p38 pathway in HK-2 cells. A and B. The HK-2 cells were transfected with control, miR-339 mimic and miR-339 mimic + pCDNA3-SIRT2, and then stimulated with LPS for 12 h after transfection for 12 h. Western blot assay was performed to detect phosphorylation of JNK and p38 levels. Blots are representative of three independent experiments. Data are shown as mean \pm SD. $n = 3$, $P < 0.05$ by ANOVA. C and D. The HK-2 cells were transfected with control, miR-339 mimic and miR-339 mimic + pCDNA3-SIRT2, and then stimulated with LPS for 12 h after transfection for 12 h. Immunofluorescence assay was performed to detect phosphorylation of JNK and p38 levels. Data are representative of three independent experiments.

SIRT2 is the functional target of miR-339 in AKI pathogenesis, we performed further experiments on HK-2 cell by co-transfecting with SIRT2 plasmid and miR-339 mimic in response to LPS. Our data shown that overexpression of SIRT2 reversed the miR-339-imposed inhibitory effects on LPS-induced up-regulation of CXCL2 and CCL2 expression. Moreover, miR-339 inactivated the p38/JNK pathway, while exogenous overexpression of SIRT2 reversed

this suppressive effect in HK-2 cells stimulated with LPS. In conclusion, this study provides novel evidence that may be useful for future studies exploring therapeutic approaches for AKI by targeting miR-339.

Acknowledgements

This work was supported by Laboratory of Research Center of Zhejiang University.

Disclosure of conflict of interest

None.

Address correspondence to: Hairong Cai, Department of Urology Surgery, Taizhou Municipal Hospital, Taizhou University Medical School, 381 Zhongshan Road East, Taizhou 318000, Zhejiang Province, China. Tel: +86-0576-88858000; Fax: +86-0576-88858024; E-mail: caihairongzj@foxmail.com

References

- [1] Hoste EA, Bagshaw SM, Bellomo R, Cely CM, Colman R, Cruz DN, Edipidis K, Forni LG, Gomersall CD, Govil D, Honore PM, Joannes-Boyau O, Joannidis M, Korhonen AM, Lavrentieva A, Mehta RL, Palevsky P, Roessler E, Ronco C, Uchino S, Vazquez JA, Vidal Andrade E, Webb S, Kellum JA. Epidemiology of acute kidney injury in critically ill patients: the multinational AKI-EPI study. *Intensive Care Med* 2015; 41: 1411-1423.
- [2] Cerda J, Baldwin I, Honore PM, Villa G, Kellum JA, Ronco C. Role of technology for the management of aki in critically ill patients: from adoptive technology to precision continuous renal replacement therapy. *Blood Purif* 2016; 42: 248-265.
- [3] Ma YF, Fu J, Qian L, Tao TT. Serum miRNA expression and correlation with clinical characteristics in acute kidney injury. *Int J Clin Exp Pathol* 2017; 10: 8721-8726.
- [4] Kim DH, Jung YJ, Lee AS, Lee S, Kang KP, Lee TH, Lee SY, Jang KY, Moon WS, Choi KS, Yoon KH, Sung MJ, Park SK, Kim W. COMP-angiopoietin-1 decreases lipopolysaccharide-induced acute kidney injury. *Kidney Int* 2009; 76: 1180-1191.
- [5] Zarjou A, Agarwal A. Sepsis and acute kidney injury. *J Am Soc Nephrol* 2011; 22: 999-1006.
- [6] Hocherl K, Schmidt C, Kurt B, Bucher M. Inhibition of NF-kappaB ameliorates sepsis-induced downregulation of aquaporin-2/V2 receptor expression and acute renal failure in vivo. *Am J Physiol Renal Physiol* 2010; 298: F196-204.
- [7] An X, Shang F. RA-XII exerts anti-oxidant and anti-inflammatory activities on lipopolysaccharide-induced acute renal injury by suppressing NF-kappaB and MAPKs regulated by HO-1/Nrf2 pathway. *Biochem Biophys Res Commun* 2018; 495: 2317-2323.
- [8] Chen Y, Song RP, Zhang Y. Triptolide reduces podocytes injury through blocking ERK and JNK pathways in passive Heymann nephritis (PHN) model. *Int J Clin Exp Med* 2017; 10: 692-699.
- [9] Li YF, Jing Y, Hao J, Frankfort NC, Zhou X, Shen B, Liu X, Wang L, Li R. MicroRNA-21 in the pathogenesis of acute kidney injury. *Protein Cell* 2013; 4: 813-819.
- [10] Fu D, Dong J, Li P, Tang C, Cheng W, Xu Z, Zhou W, Ge J, Xia C, Zhang Z. MiRNA-21 has effects to protect kidney injury induced by sepsis. *Biomed Pharmacother* 2017; 94: 1138-1144.
- [11] Liu XJ, Hong Q, Wang Z, Yu YY, Zou X, Xu LH. MicroRNA-34a Suppresses autophagy in tubular epithelial cells in acute kidney injury. *Am J Nephrol* 2015; 42: 168-175.
- [12] Dai Y, Jia P, Fang Y, Liu H, Jiao X, He JC, Ding X. miR-146a is essential for lipopolysaccharide (LPS)-induced cross-tolerance against kidney ischemia/reperfusion injury in mice. *Sci Rep* 2016; 6: 27091.
- [13] Galliera E, Corsi MM, Bonecchi R, Locati M, Mantovani A. Chemokines as pharmacological targets. *Mini Rev Med Chem* 2008; 8: 638-646.
- [14] Chung AC, Lan HY. Chemokines in renal injury. *J Am Soc Nephrol* 2011; 22: 802-809.
- [15] Zou YF, Zhang W. Role of microRNA in the detection, progression, and intervention of acute kidney injury. *Exp Biol Med (Maywood)* 2018; 243: 129-136.
- [16] Xie Y, Han JR, Chen F, Chang JY, Wang B, Li FZ, Sun XT, Jiang DY. MicroRNA expression patterns of ankle synovial tissues in rats with monosodium urate crystal-induced gouty arthritis. *Int J Clin Exp Pathol* 2016; 9: 1127-1137.
- [17] Xu X, Yin P, Wan C, Chong X, Liu M, Cheng P, Chen J, Liu F, Xu J. Punicalagin inhibits inflammation in LPS-induced RAW264.7 macrophages via the suppression of TLR4-mediated MAPKs and NF-kappaB activation. *Inflammation* 2014; 37: 956-965.
- [18] De Filippo K, Dudeck A, Hasenberg M, Nye E, van Rooijen N, Hartmann K, Gunzer M, Roers A, Hogg N. Mast cell and macrophage chemokines CXCL1/CXCL2 control the early stage of neutrophil recruitment during tissue inflammation. *Blood* 2013; 121: 4930-4937.
- [19] Anders HJ, Vielhauer V, Schlondorff D. Chemokines and chemokine receptors are involved in the resolution or progression of renal disease. *Kidney Int* 2003; 63: 401-415.
- [20] Lu QM, Lu Y, Zhang Y, Li ZH, Xie XH. Establishment and evaluation of rat trauma hemorrhagic liver injury model. *Int J Clin Exp Pathol* 2017; 10: 7340-7349.
- [21] Lan YF, Chen HH, Lai PF, Cheng CF, Huang YT, Lee YC, Chen TW, Lin H. MicroRNA-494 reduces ATF3 expression and promotes AKI. *J Am Soc Nephrol* 2012; 23: 2012-2023.
- [22] Ranganathan P, Jayakumar C, Tang Y, Park KM, Teoh JP, Su H, Li J, Kim IM, Ramesh G. MicroRNA-150 deletion in mice protects kidney from myocardial infarction-induced acute kidney injury.

miR-339 inhibited LPS-induced CXCL2 and CCL2 by targeting SIRT2

- jury. *Am J Physiol Renal Physiol* 2015; 309: F551-558.
- [23] Wang S, Zhang Z, Wang J, Miao H. MiR-107 induces TNF-alpha secretion in endothelial cells causing tubular cell injury in patients with septic acute kidney injury. *Biochem Biophys Res Commun* 2017; 483: 45-51.
- [24] Shen B, Zhang Y, Yu S, Yuan Y, Zhong Y, Lu J, Feng J. MicroRNA-339, an epigenetic modulating target is involved in human gastric carcinogenesis through targeting NOVA1. *FEBS Lett* 2015; 589: 3205-3211.
- [25] Wang JY, Li H, Ma CM, Wang JL, Lai XS, Zhou SF. Acupuncture may exert its therapeutic effect through microRNA-339/Sirt2/NFkappaB/FOXO1 axis. *Biomed Res Int* 2015; 2015: 249013.
- [26] Jung YJ, Lee AS, Nguyen-Thanh T, Kim D, Kang KP, Lee S, Park SK, Kim W. SIRT2 regulates LPS-induced renal tubular CXCL2 and CCL2 expression. *J Am Soc Nephrol* 2015; 26: 1549-1560.