Histol Histopathol (2023) 38: 443-452

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORIGINAL ARTICLE **Open Access**

Downregulation of miR-527 alleviates sepsis-induced acute kidney injury via targeting Beclin1

Ke Xu[1,*], Xiaojun Mo[2,3,*], Yijun Wang[2,3], Zhenhua Zeng[4], Ziqiang Xu[2], Dongyou Yue[2], Guicheng Li[1], Tao Li[1], Junhong Liu[2] and Jiemin Yuan[2]

[1]Department of Critical Care Medicine, [2]Department of Emergency, First People's Hospital of Chenzhou, Chenzhou, Hunan, [3]The First School of Clinical Medicine and [4]Department of Critical Care Medicine, Nanfang Hospital, Southern Medical University, Guangzhou, Guangdong, China

*These authors contributed equally to this research

Summary. Background. Sepsis-induced acute kidney injury (AKI) is known to result from the inflammatory responses. MiRNAs participate in the development of sepsis-induced AKI. Nevertheless, the function of miR-527 in sepsis-induced AKI remains unclear.

Methods. Cell viability was evaluated by CCK8 assay, and TUNEL staining was applied to assess cell apoptosis. Pro-inflammatory cytokine (TNF-α, IL-6 and IL-1β) levels were evaluated by ELISA. Meanwhile, the relation among miR-527 and Beclin1 was detected by dual luciferase report assay. Western blot and RT-qPCR were used to examine the protein and mRNA levels, respectively. Furthermore, an *in vivo* model was constructed to assess the function of miR-527 in sepsis-induced AKI.

Results. MiR-527 downregulation significantly alleviated the symptoms of sepsis-induced AKI in mice. MiR-527 level in HK-2 cells was significantly upregulated by LPS, and downregulation of miR-527 notably reversed LPS-induced inhibition of HK-2 cell viability by inhibiting apoptosis. In addition, LPS greatly increased TNF-α, IL-6 and IL-1β levels in supernatant of HK-2 cells, while miR-527 inhibitor partially restored this phenomenon. Meanwhile, Beclin1 was found to be the downstream mRNA of miR-527, and miR-527 inhibitor notably upregulated the level of LC3. MiR-527 downregulation reversed LPS-induced HK-2 cell injury through suppression of TGF-β pathway.

Conclusion. Downregulation of miR-527 alleviated sepsis-induced AKI via targeting Beclin1. Thus, miR-527 might act as a vital mediator in sepsis-induced AKI.

Key words: Sepsis, AKI, miR-527, Beclin1

Corresponding Author: Yijun Wang, Department of Emergency, First People's Hospital of Chenzhou, No. 102 Luojiajing Road, Chenzhou, Hunan 423000, China. e-mail: wangyijun_12@126.com

DOI: 10.14670/HH-18-531

Introduction

Sepsis usually results from unbalanced response from the host to infection. (Yu et al., 2022) The hallmark of sepsis is organ failure, and it has become a major public health burden worldwide due to high morbidity and mortality (Sun et al., 2021c). It was reported that approximately 35% of sepsis patients could develop into AKI (Sun et al., 2021a). In addition, sepsis can induce apoptosis in kidney tissues, and this phenomenon might aggravate the progression of AKI (Zhou et al., 2021; Behal et al., 2022). Although much work has been done to study sepsis-induced AKI, the outcomes remain limited. Hence, it is urgent to explore novel ideas for inhibiting the progression of sepsis-induced AKI.

MicroRNAs (miRNAs) are a group of small noncoding RNAs which are known to be a focal research object for their key role in regulation of post-transcription (Wang et al., 2021b; Zheng et al., 2021). Studies have shown that miRNAs participate in the development of sepsis-induced AKI. For instance, Wei et al. found that upregulation of miR-21-5p was able to reverse the therapeutic effect of hsa_circ_0068,888 on sepsis-induced AKI (Wei et al., 2021); Liu et al. indicated that miR-452 downregulation was able to suppress the development of sepsis-induced AKI (Liu et al., 2020b); Fan et al. demonstrated that overexpression of miR-424-5p was able to reverse the effect of lncRNA CASC9 on LPS-induced HK-2 cell injury through targeting TXNIP (Fan et al., 2021). The data of our preliminary experiments (RNA sequencing and RT-qPCR) suggested that miR-527 was significantly upregulated in kidney tissues of sepsis mice (unpublished observation, Fig. 1). However, the role of miR-527 in sepsis-induced AKI remains unclear.

Autophagyis an ability of cells to prevent their own death (Liu et al., 2020a), which can also protect cells against apoptosis (Sang et al., 2021). In addition, under some conditions, cells will switch to an autophagy

©The Author(s) 2023. **Open Access.** This article is licensed under a Creative Commons CC-BY International License.

pathway when apoptosis is inhibited (Zhao et al., 2020). Meanwhile, it has been reported that upregulation of Beclin1 and LC3 can contribute to the progression of autophagy, while activation of p62 might inhibit autophagy (Zhao et al., 2018; Zhang et al., 2019b). It has been previously reported that autophagy was closely associated with sepsis-induced AKI (Kaushal and Shah, 2016). For example, p53 deacetylation might alleviate sepsis-Induced AKI through inducing autophagy (Sun et al., 2021b). Therefore, autophagy may play a key role during the progression of sepsis-induced AKI.

Based on the above background, we decided to evaluate the function of miR-527 in sepsis-induced AkI. In addition, this research sought to study the function of miR-527 in cell autophagy during sepsis-induced AKI. We hope this work will shed new light on discovering novel strategies for the treatment of sepsis.

Materials and methods

Animal study

C57BL/6 mice (n=18, 6-8 weeks) were obtained from Beijing River Laboratories. The mice were kept in conditions of SPF. All procedures were carried out as per the guidelines of laboratory animals. The Ethics Committee of First People's Hospital of Chenzhou approved this study.

In this work, the mice were randomly divided into sham, CLP and CLP + LINC00963 shRNA1 group (n=6/group). For constructing the model of sepsis-induced ALI, mice in CLP and CLP + miR-527 inhibitor group underwent CLP surgery according to the previous report (Luo et al., 2021). Then, mice in CLP+ miR-527 inhibitor were injected with 50 µl miR-527 inhibitor (Beyotime) via the tail vein 48h before CLP surgery. In addition, mice in Sham and CLP group were administrated with saline through the tail vein 48h before CLP surgery. Meanwhile, the survival percent of mice was recorded after CLP surgery. Finally, serum, plasma and kidney tissues of mice were collected for further analysis.

Blood urea nitrogen (BUN) and creatinine detection

The concentrations of BUN and creatinine in serum of mice were detected by biochemical detection kits (Jiancheng Bioengineering) in line with the manufacturer's instructions.

Hematoxylin-eosin (H&E) staining

Paraformaldehyde (4%) was used to fix the kidney tissues of mice for 24h, paraffin-embedded and sectioned (4 µm). Next, gradient alcohol was used to dehydrate the sections, and then sections were cleared and mounted. Then, hematoxylin and eosin solution (Beyotime) was applied to stain the sections. After that, the injury of kidney tissues was observed under a light microscope.

TUNEL staining

Paraffin sections were washed and permeabilized. Subsequently, TUNEL mixtures (50 µl) were used to incubate the sections at 37°C with no light. POD was used to incubate the sildes at 37°C for signal conversion. Then, slides were rinsed and treated with DAB at room temperature for 10 min. Finally, the result was observed by using an optical microscope.

Cell culture and treatment

HK-2 cells were obtained from ATCC. Cells were maintained in DMEM containing 10% FBS (Gibco) in conditions of 5% CO_2 and 37°C. To mimic sepsis-

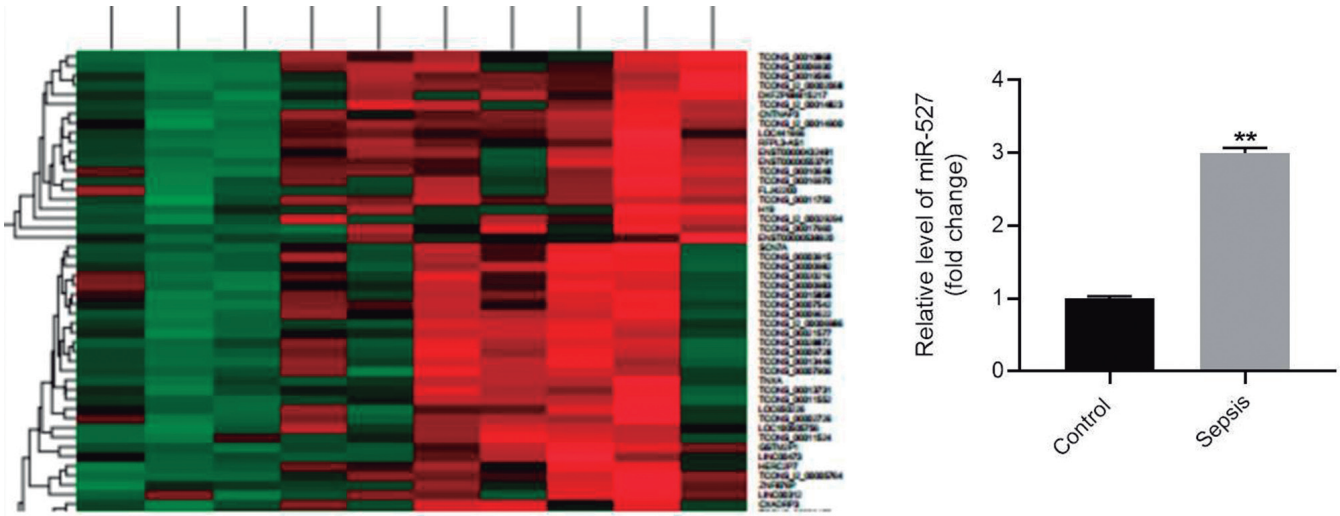

Fig 1. RNA sequencing and RT-qPCR for miR-527 in kidney tissues of sepsis mice (unpublished observations).

induced AKI *in vitro*, HK-2 cells were treated with LPS (100 ng/ml) for 48h.

Cell transfection

HK-2 cells were transfected with miR-527 inhibitor or NC (Genepharma) by Lipofectamine 2000 (Invitrogen) for 24h. RT-qPCR was performed to verify the efficiency of cell transfection. Meanwhile, miR-527 inhibitor is a chemically-modified sequence-specific nucleic acid was designed for down-regulation of miR-527 activity.

Reagents

Rapamycin (1 nM) was obtained from Sigma.

CCK8 assay

HK-2 cells (5×10^3 per well) were seeded overnight. Next, cells were exposed to LPS or LPS + miR-527 inhibitor or LPS + miR-527 inhibitor + Rapamycin for 24, 48 or 72h. Afterwards, cells were exposed to CCK8 (10 μl). After 2h of further incubation, the absorbance of HK-2 cells (450 nm) was assessed by a microplate reader.

ELISA

IL-6, TNF-α and IL-1β levels in PMVEC supernatants or plasma of mice were assessed by ELISA kits (Jiancheng Bioengineering).

LDH and SOD detection

The levels of LDH and SOD in supernatants of HK-2 cells were assessed by using commercial kits (Jiancheng Bioengineering Co. Ltd).

Dual luciferase reporter assay

3'-UTR of Beclin1 including the binding sites of miR-527 were synthetized from Genepharma. Afterwards, they were cloned into the vectors (pmirGLO, Promega, Madison, WI, USA) for establishing reporter vectors Beclin1 (WT/MT), respectively. The Beclin1 (WT/MT) were transfected into HK-2 cells by Lipofectamine 2000. Dual-Glo System was applied to assess the luciferase activity.

RT-qPCR

TRIzol was applied to extract total RNA from cells or tissues. The PrimeScript RT kit (TaKaRa, Ver.3.0) was applied to synthesize cDNA. SYBR kit was used in RT-qPCR detection. The conditions were as follows: 94°C (2 minutes), followed by 35 cycles (94°C for 30s and 55°C for 45s). The sequences were as follows: miR-527 forward, 5'-TGGGTACTTCAGGGATCCAG-3' and reverse, 5'-TGAGGCTCTTCCCTCACTGT-3'; Beclin1 forward, 5'-GGACTATGTATTGGTCCCTACCG-3' and reverse 5'-TCGATGGTTGCAATGGTGTC-3'; β-actin forward, 5'-CCAGGTGGTCTCCTCTGA-3' and reverse, 5'-TGCTGTCACCTTCACCGTTC-3'. $2^{-\Delta\Delta t}$ method was applied to quantify the data.

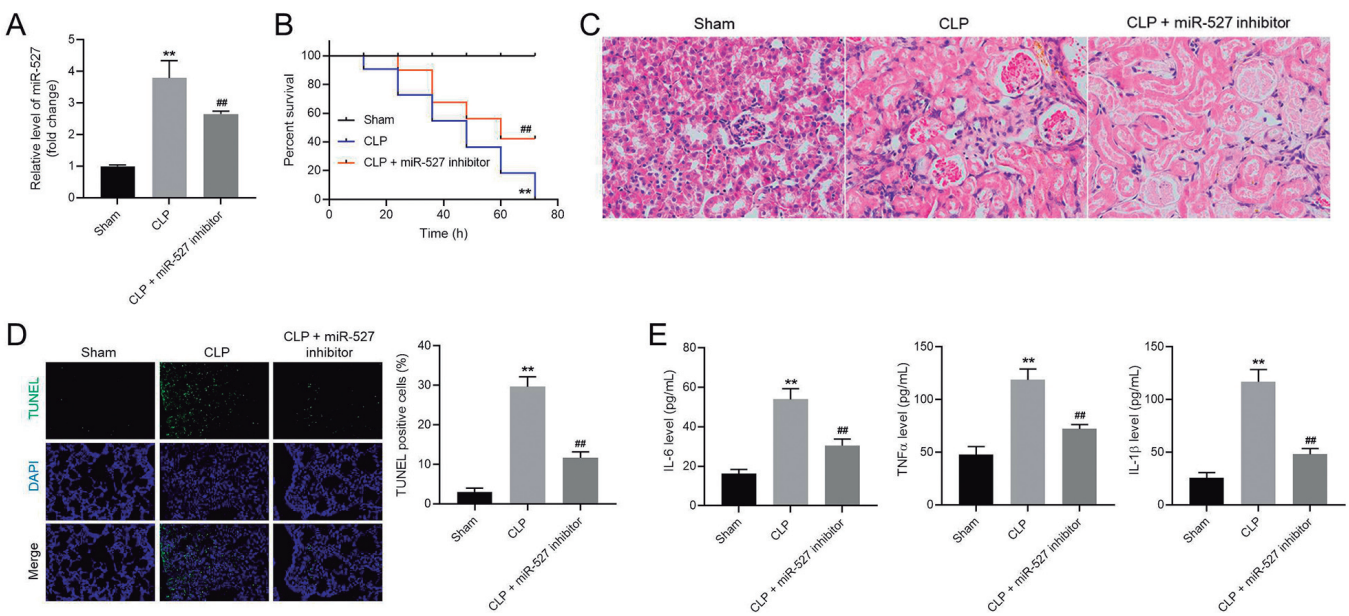

Fig. 2. MiR-527 downregulation notably inhibited the symptoms of sepsis-induced AKI in mice. **A.** The level of miR-527 in tissues of mice was assessed by RT-qPCR. **B.** The percentage of survival in mice was recorded. **C.** The injury of kidney tissues in mice was observed by H&E staining. **D.** The apoptosis of kidney tissues in mice was evaluated by TUNEL staining. **E.** The levels of IL-1β, IL-6 and TNF-αin plasma of mice were detected by ELISA. **P<0.01 compared with sham. ##P<0.01 compared with CLP.

Immunofluorescence

HK-2 cells were fixed with 4% paraformaldehyde, penetrated and blocked at room temperature. Then, HTFs were incubated with anti-LC3 antibody (Abcam; 1:1000) at 4°C overnight. After that, cells were incubated with secondary antibody (IgG, Abcam; 1:5000) for 1h. The nuclei were stained with DAPI (Beyotime). Finally, cells were observed under a fluorescence microscope.

Western blot

RIPA was applied to isolate proteins, and the protein was quantified by using BCA kit. The proteins (40 μg per lane) were divided by SDS-PAGE (10%). Subsequently, proteins were transferred onto PVDF membranes. Then, the primary antibodies were used to incubate the membranes overnight after blocking with skimmed milk (5%) for 1h. Afterwards, secondary antibodies (HRP-conjugated, 1:5,000) were applied to incubate the membranes for 1h. The antibodies were as follows: anti-Bax (1:1,000), anti-Bcl-2 (1:1,000), anti-active caspase 3 (1:1,000), anti-p62 (1:1,000), anti-LC3 (1:1,000), anti-Beclin1 (1:1,000), anti-Smad2 (1:1,000), anti-p-Smad2 (1:1,000), anti-Smad3 (1:1,000), anti-p-Smad3 (1:1,000) and anti-β-actin (1:1,000). ECL kit was applied to measure the protein bands. All antibodies were obtained from Abcam. IPP 6.0 was applied for analyzing the densitometry.

Statistical analysis

Three independent experiments were performed in each group. Mean ± standard deviation (SD) was applied to represent all data. Student's t-test was applied to analyze the comparisons between two groups, and one-way analysis of variance (ANOVA) followed by Tukey's test (Graphpad Prism7) were applied to analyze the differences between multiple groups (more than 2 groups). $P<0.05$ suggests a large difference.

Results

MiR-527 downregulation notably inhibited the symptoms of sepsis-induced AKI in mice by suppression of TGF-β/Smad signaling

To further confirm the function of miR-527 in sepsis-induced AKI, an *in vivo* model of sepsis-induced AKI was established. The data showed that CLP significantly upregulated the level of miR-527 in mice, while miR-527 inhibitor partially restored this phenomenon (Fig. 2A). CLP notably inhibited the percentage of survival of mice, which was significantly restored by downregulation of miR-527 (Fig. 2B). In addition, CLP-induced inflammatory infiltration in mice was obviously reduced by miR-527 inhibitor (Fig. 2C).

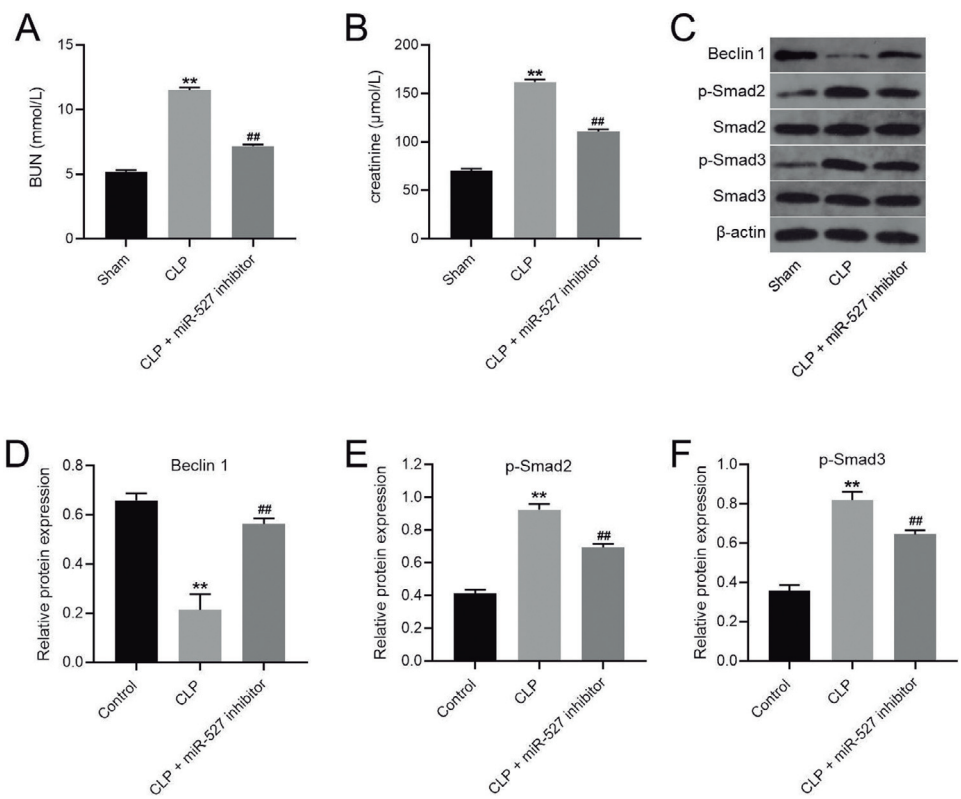

Fig. 3. Downregulation of miR-527 alleviated the progression of sepsis-induced AKI by inactivating TGF-β pathway. **A, B.** The concentrations of BUN and creatinine in serum of mice were detected by biochemical detection kits. **C.** The levels of Beclin1, p-Smad2, Smad2, Smad3 and p-Smad3 in HK-2 cells were detected by western blot. **D-F.** The relative level of Beclin1 was quantified via normalization to β-actin. The relative levels of p-Smad2 and p-Smad3 were quantified via normalization to total proteins. **P<0.01 compared with sham. ##P<0.01 compared with CLP.

Consistently, CLP significantly promoted the apoptosis in tissues, while the effect of CLP was largely abolished by miR-527 inhibitor (Fig. 2D). Furthermore, IL-1β, TNF-α and IL-6 levels in plasma of mice were markedly upregulated by CLP, which were largely reversed by downregulation of miR-527 (Fig. 2E). Then, the kidney function of mice was examined. The results indicated that CLP significantly upregulated the concentrations of BUN and creatinine in serum of mice, while miR-527 downregulation partially restored this phenomenon (Fig. 3A,B).

Meanwhile, western blot was performed to verify the mechanism by which miR-527 downregulation reverses CLP-induced tissue injury in mice. The data demonstrated that CLP greatly upregulated the levels of p-Smad2, p-Smad3 and inhibited the expression of Beclin1 in mice, while these phenomena were markedly abolished in the presence of miR-527 inhibitor (Fig. 3C-F). Based on the above data, it could be suggested that miR-527 inhibitor notably inhibited the progression of

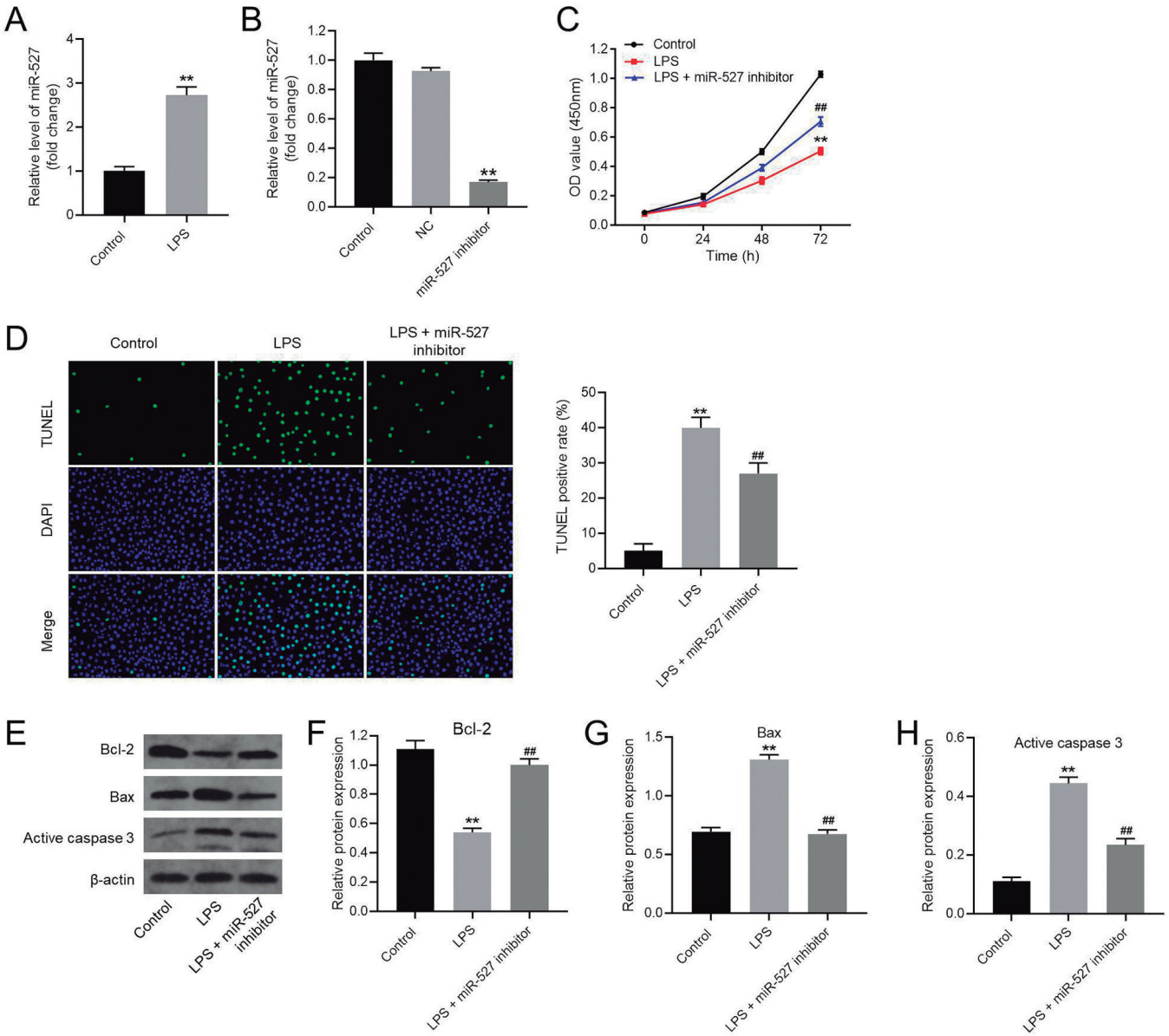

Fig. 4. Downregulation of miR-527 significantly reversed LPS-induced apoptosis in HK-2 cells. **A.** HK-2 cells were exposed to LPS (100 ng/ml) for 48 h. The level of miR-527 in HK-2 cells was investigated by RT-qPCR. **B.** HK-2 cells were transfected with NC or miR-527 inhibitor. The level of miR-527 in HK-2 cells was tested by RT-qPCR. **C.** HK-2 cells were treated with LPS or LPS + miR-527 inhibitor for 24, 48 or 72h. The absorbance (450 nm) of HK-2 cells was measured by microreader after cells were treated with CCK8 reagent. **D.** The apoptosis of HK-2 cells was assessed by TUNEL staining. **E-H.** The levels of Bcl-2, Bax and active caspase 3 in HK-2 cells were detected by western blot. The relative levels were normalized to β-actin.**P<0.01 compared with control. ##P<0.01 compared with LPS.

sepsis-induced AKI in mice by suppression of TGF-β/Smad signaling.

Downregulation of miR-527 significantly reversed LPS-induced apoptosis in HK-2 cells

To investigate the role of miR-527 in sepsis-induced AKI, HK-2 cells were exposed to 100 ng/ml LPS, and then RT-qPCR was performed. As indicated in Figure 4A, miR-527 level in HK-2 cells was increased by LPS. Meanwhile, the level of miR-527 in HK-2 cells was markedly reduced by miR-527 inhibitor (Fig. 4B). LPS greatly decreased the viability of HK-2 cells, while this phenomenon was largely rescued by miR-527 inhibitor (Fig. 4C). Furthermore, LPS-induced HK-2 cell apoptosis was significantly restored by miR-527 inhibitor (Fig. 4D). Mechanistically, miR-527 was able to reverse LPS-induced apoptosis in HK-2 cells through mediation of Bcl-2, Bax and active caspase 3 (Fig. 4E-H). Altogether, miR-527 inhibitor significantly reversed LPS-induced apoptosis in HK-2 cells.

Downregulation of miR-527 notably reversed LPS-induced inflammatory responses in HK-2 cells

To assess the function of miR-527 in inflammatory response of HK-2 cells, ELISA was used. The data revealed the levels of IL-6, TNF-α and IL-1βin HK-2 cell supernatants were significantly enhanced by LPS, which were largely abolished by miR-527 downregulation (Fig. 5A). Consistently, LPS-induced upregulation of LDH and SOD in HK-2 cells was significantly rescued by miR-527 inhibitor (Fig. 5B). Taken together, downregulation of miR-527 notably reversed LPS-induced inflammatory responses in HK-2 cells.

MiR-527 directly targets Beclin1

To explore the downstream mRNAs of miR-527, targetscan database was performed. As shown in Figure 6A, Beclin1 was identified to be the downstream target of miR-527, and the luciferase activity in WT-Beclin1 was decreased by miR-128-3p upregulation (Fig. 6B). However, miR-527 did not influence the luciferase activity in MT-Beclin1 (Fig. 6B). In addition, upregulation of miR-527 significantly inhibited the level of Beclin1 in HK-2 cells (Fig. 6C). It has been reported that Beclin1 and LC3 were key mediators in cell autophagy as upregulation of Beclin1 and LC3 might promote the formation of autophagosomes (Chen et al., 2017). Thus, the level of LC3 was investigated. The data showed that LPS notably inhibited the level of LC3 in HK-2 cells, while the effect of LPS on LC3 expression

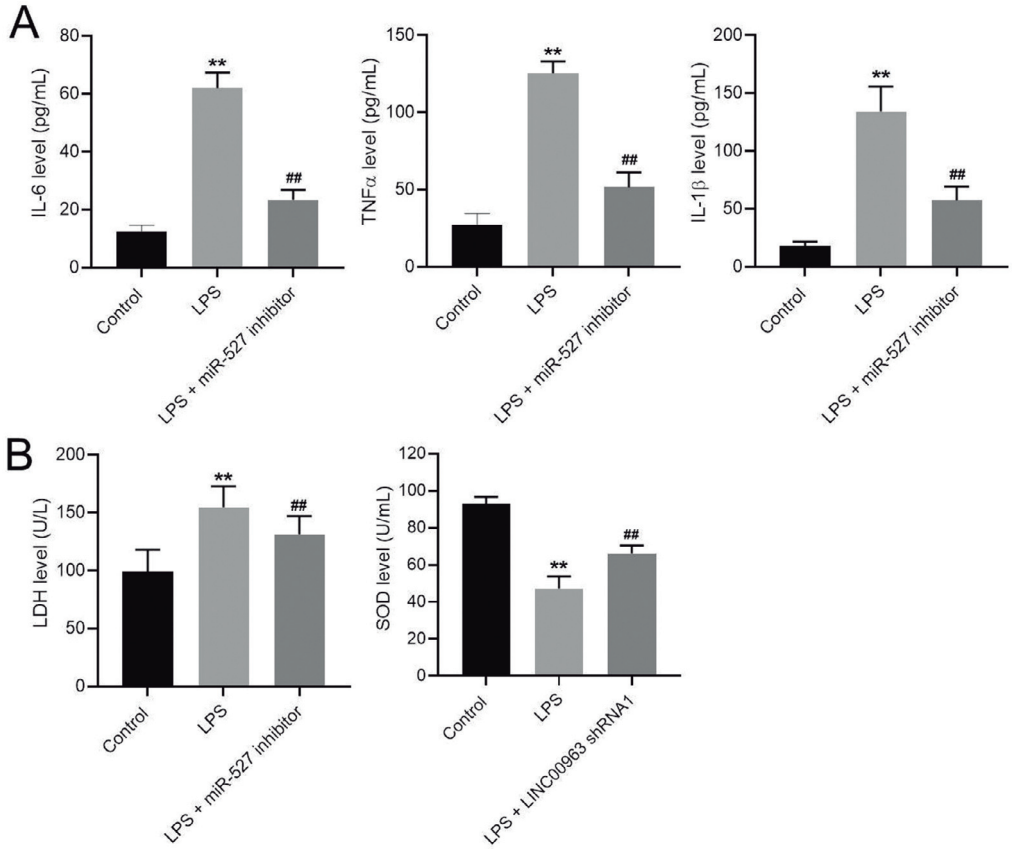

Fig. 5. Downregulation of miR-527 notably reversed LPS-induced inflammatory responses in HK-2 cells. **A.** The levels of IL-1β, IL-6 and TNF-α in HK-2 cell supernatants were assessed by ELISA. **B.** The levels of LDH and SOD in HK-2 cells were investigated by commercial kits. **P<0.01 compared with control. ##P<0.01 compared with LPS.

was rescued by miR-527 inhibitor (Fig. 6D). Furthermore, LPS notably inhibited the ratio of LC3 II/LC3 I in HK-2 cells, which was restored by miR-527 inhibitor (Fig. 6E). In summary, MiR-527 directly targets Beclin1.

MiR-527 inhibitor significantly reversed LPS-induced HK-2 cell growth inhibition by inducing autophagy

In order to further investigate the mechanism by which miR-527 mediates the growth of LPS-treated HK-2 cells, western blot was used. As revealed in Figure 7A-E, LPS significantly inhibited the level of Beclin-1 and upregulated the expressions of p62, p-Smad2 and p-Smad3 in HK-2 cells, while miR-527 inhibitor reversed these phenomena. In addition, miR-527 inhibitor reversed LPS-induced inhibition of HK-2 cell proliferation by inhibiting apoptosis, while the effect of miR-527 inhibitor was partially rescued by Rapamycin (Fig. 7F,G). To sum up, miR-527 downregulation significantly reversed LPS-induced HK-2 cell growth inhibition by inducing autophagy.

Discussion

It was reported that miRNAs are involved in the progression of sepsis-induced kidney injury. For instance, Sang et al. found that upregulation of miR-214 might induce PTEN/Akt/mTOR-mediated autophagy to ameliorate sepsis-induced kidney injury (Sang et al., 2021); Yang et al. suggested that downregulation of miR-125a-5p was able to aggravate sepsis-caused kidney injury by upregulation of TRAF6 (Yang et al., 2021). In this research, we found miR-527 was upregulated in

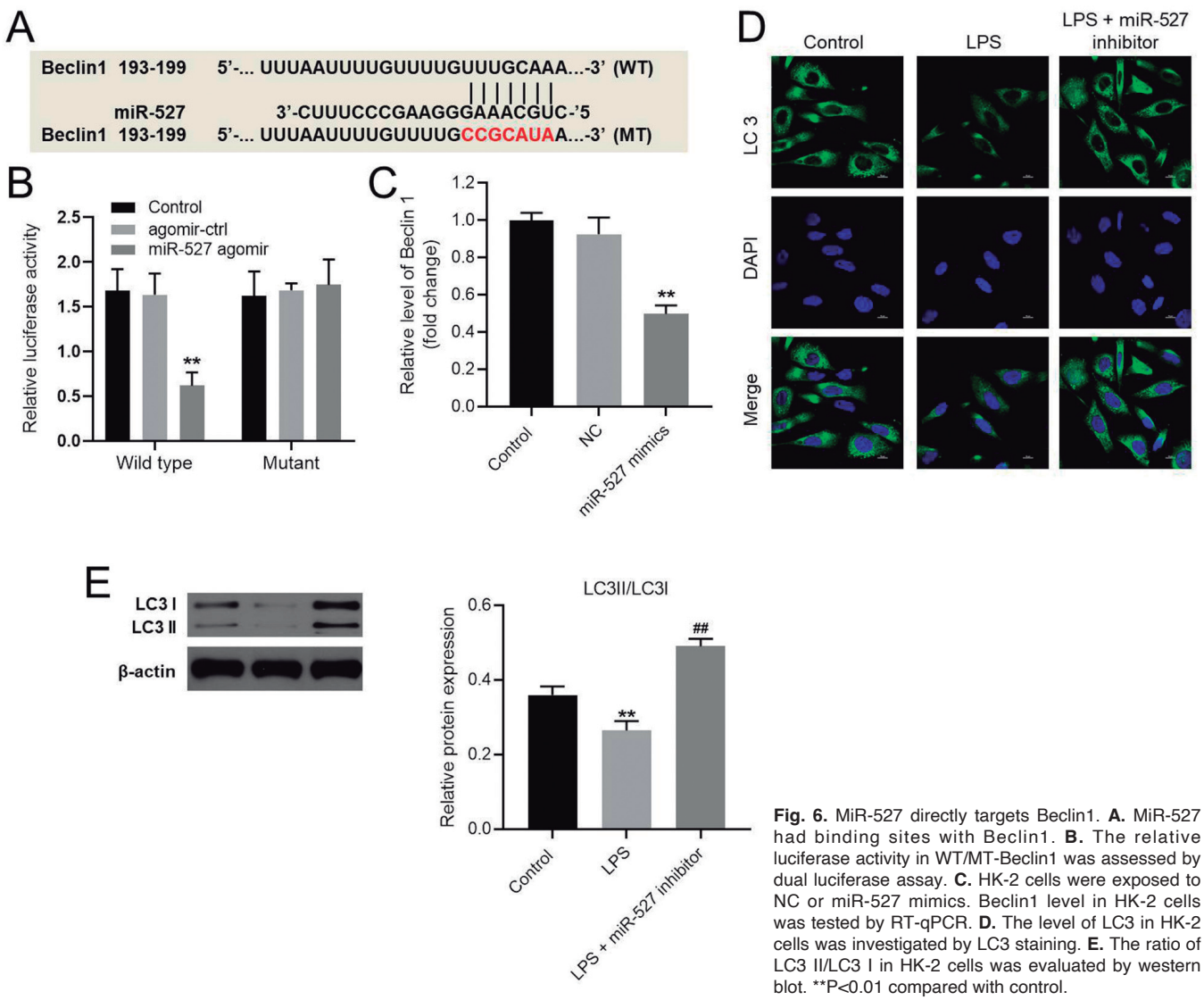

Fig. 6. MiR-527 directly targets Beclin1. **A.** MiR-527 had binding sites with Beclin1. **B.** The relative luciferase activity in WT/MT-Beclin1 was assessed by dual luciferase assay. **C.** HK-2 cells were exposed to NC or miR-527 mimics. Beclin1 level in HK-2 cells was tested by RT-qPCR. **D.** The level of LC3 in HK-2 cells was investigated by LC3 staining. **E.** The ratio of LC3 II/LC3 I in HK-2 cells was evaluated by western blot. **P<0.01 compared with control.

sepsis-induced AKI. In addition, downregulation of miR-527 might reverse LPS-induced injury in HK-2 cells through targeting Beclin1. Thus, our study firstly explored the function of miR-527 in sepsis-induced AKI, suggesting that miR-527 might act as a key mediator in sepsis.

It has been revealed that miR-527 can exert its function through targeting mRNAs. For example, Wu et al. found miR-527 inhibited the tumorigenesis of liver cancer through targeting FBXW7 (Wu et al., 2021); Feng et al. indicated that miR-527 was able to inhibit the malignant behavior of bladder cancer cells through targeting Robo1 (Feng et al., 2021). This work found miR-527 may target Beclin1 in sepsis-induced AKI. Beclin1 was known to be the crucial mediator in cell autophagy, and its upregulation promoted the process of autophagy (Xie et al., 2021). In addition, LC3 was confirmed to be the crucial modulator in cell autophagy (Song et al., 2022). Our finding was consistent with the recent studies, further suggesting that miR-527 can act as a key mediator of cell autophagy.

It has been reported that autophagy played a crucial role in sepsis-induced cell injury (Ho et al., 2016; Sun et al., 2018). Although the mechanisms and functions of apoptosis and autophagy are distinct, they are closely correlated. More importantly, autophagy is essential for the onset of apoptosis, and it can triggers the progression of apoptosis (Wang, 2015; Liu et al., 2019). Therefore, it can be suggested that miR-527 inhibitor reverses LPS-induced HK-2 cell growth inhibition via inducing autophagy. Meanwhile, LC3 and Beclin 1 were reported to act as vital players during autophagy (Luo et al., 2019). Consistent with these data, our finding confirmed that downregulation of miR-527 induced autophagy in LPS-treated HK-2 cells via mediation of these proteins. On the other hand, the interaction of Beclin1 and Bcl-2 has been confirmed to play a key role in the crosstalk between autophagy and apoptosis. According to the previous references (Maiuri et al., 2010; Prerna and Dubey, 2022), the dissociation of Beclin1 from Bcl-2 is essential for its autophagic activity, and Bcl-2 only inhibits autophagy when it is present in the endoplasmic reticulum (ER) (Maiuri et al., 2010). In addition, NAF-1 (nutrient-deprivation autophagy factor-1) might bind to Bcl-2 at the ER, and NAF-1 is a component of the inositol-1,4,5 trisphosphate (IP3) receptor complex

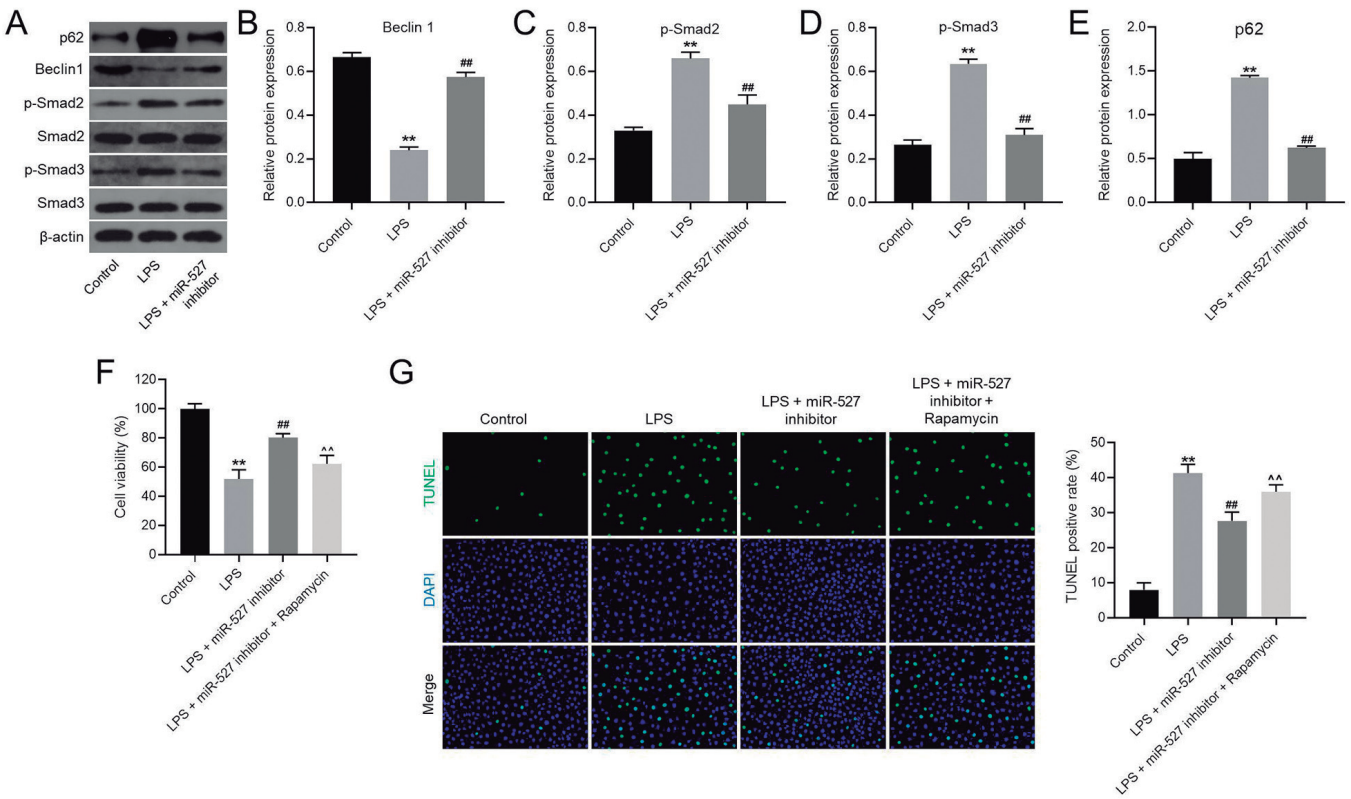

Fig. 7. MiR-527 inhibitor greatly reversed LPS-induced HK-2 cell growth inhibition by inducing autophagy. **A.** The levels of p62, Beclin1, p-Smad2, Smad2, Smad3 and p-Smad3 in HK-2 cells were detected by western blot. **B-E.** The relative levels of Beclin1 and p62 were quantified via normalization to β-actin. The relative levels of p-Smad2 and p-Smad3 were normalized to total proteins. **F.** HK-2 cells were treated with LPS, LPS + miR-527 inhibitor or LPS + miR-527 inhibitor + Rapamycin. The absorbance (450 nm) of HK-2 cells was measured by microreader after cells were treated with CCK8 reagent. **G.** The apoptosis of HK-2 cells was investigated by TUNEL staining. **P<0.01 compared with control. ##P<0.01 compared with LPS. ^^P<0.01 compared with LPS + miR-527 inhibitor.

which can lead to the interaction of Bcl-2 with Beclin1 (Prerna and Dubey, 2022). These functions enable Bcl-2 to functionally antagonize Beclin1-mediated autophagy. In this study, we found that the levels of Bcl-2 and Beclin1 in LPS-treated HK-2 cells were upregulated by miR-527 inhibitor. Thus, our study was consistent with these previous references, and the function of miR-527 in the interation between Beclin1 and Bcl-2 will be studied in future.

The TGF-β pathway is known to be involved in the progression of sepsis, and its activation can lead to the phosphorylation of Smad2 and Smad3 (Sun et al., 2021a; Wang et al., 2021a). Moreover, induction of autophagy can lead to the inactivation of the TGF-β pathway (Cheng et al., 2021). In our study, miR-527 downregulation was found to inhibit the expressions of p-Smad2 and p-Smad3 in LPS-treated HK-2 cells. In addition, a previous study found activation of TGFBR2/Smad signaling can lead to the progression of sepsis (Zhang et al., 2019a). Based on the above contents, it could be suggested that downregulation of miR-527 can inactivate TGF-β signaling during the progression of sepsis-induced AKI through inducing autophagy.

Indeed, there are some shortcomings in this work as follows: 1) more mRNAs targeted by miR-527 in sepsis-induced AKI remain to be further explored; 2) more mechanisms by which miR-527 regulates sepsis-induced AKI need to be detected; 3) the function of miR-527 in the interation between Beclin1 and Bcl-2 remains unexplored. Hence, more investigations are essential in future.

In summary, downregulation of miR-527 attenuates sepsis-induced AKI via targeting Beclin1. Thus, this study might supply new ideas on exploring strategies against sepsis-induced AKI.

Acknowledgements. Not applicable.

Conflict of interests. The authors declare no competing interests in this research.

Funding. This study was supported by the Scientific Research Projects of Hunan Health Commission (No. 20201105), the Scientific and Technological Development Project of Chenzhou Municipal Science and Technology Bureau (No. zdyf201926), the Scientific and Technological Development Project of Chenzhou Municipal Science and Technology Bureau (No. ZDYF2020052) and Natural Science Project of Xiangnan University (No. 2019XJ70).

References

Behal M.L., Nguyen J.L., Li X., Feola D.J., Neyra J.A. and Flannery A.H. (2022). Azithromycin and major adverse kidney events in critically ill patients with sepsis-associated acute kidney injury. Shock 57, 479-485.

Chen J., Yu Y., Li S., Liu Y., Zhou S., Cao S., Yin J. and Li G. (2017). MicroRNA-30a ameliorates hepatic fibrosis by inhibiting Beclin1-mediated autophagy. J. Cell. Mol. Med. 21, 3679-3692.

Cheng R., Xu H. and Hong Y. (2021). miR221 regulates TGF-beta1-induced HSC activation through inhibiting autophagy by directly targeting LAMP2. Mol. Med. Rep. 24, 777.

Fan H.P., Zhu Z.X., Xu J.J., Li Y.T., Guo C.W. and Yan H. (2021). The lncRNA CASC9 alleviates lipopolysaccharide-induced acute kidney injury by regulating the miR-424-5p/TXNIP pathway. J. Int. Med. Res. 49, 3000605211037495.

Feng L., Fu D., Gao L., Cheng H., Zhu C. and Zhang G. (2021). Circular RNA_0001495 increases Robo1 expression by sponging microRNA-527 to promote the proliferation, migration and invasion of bladder cancer cells. Carcinogenesis 42, 1046-1055.

Ho J., Yu J., Wong S.H., Zhang L., Liu X., Wong W.T., Leung C.C., Choi G., Wang M.H., Gin T., Chan M.T.V. and Wu W.K.K (2016). Autophagy in sepsis: Degradation into exhaustion? Autophagy 12, 1073-1082.

Kaushal G.P. and Shah S.V. (2016). Autophagy in acute kidney injury. Kidney Int. 89, 779-791.

Liu J., Liu W. and Yang H. (2019). Balancing apoptosis and autophagy for Parkinson's disease therapy: Targeting BCL-2. ACS Chem. Neurosci. 10, 792-802.

Liu Y., Xiao J., Sun J., Chen W., Wang S., Fu R., Liu H. and Bao H. (2020a). ATG7 promotes autophagy in sepsisinduced acute kidney injury and is inhibited by miR526b. Mol. Med. Rep. 21, 2193-2201.

Liu Z., Yang D., Gao J., Xiang X., Hu X., Li S., Wu W., Cai J., Tang C., Zhang D. and Dong Z. (2020b). Discovery and validation of miR-452 as an effective biomarker for acute kidney injury in sepsis. Theranostics 10, 11963-11975.

Luo Z., Xu X., Sho T., Zhang J., Xu W., Yao J. and Xu J. (2019). ROS-induced autophagy regulates porcine trophectoderm cell apoptosis, proliferation, and differentiation. Am. J. Physiol. Cell Physiol. 316, C198-C209.

Luo S., Ding X., Zhao S., Mou T., Li R. and Cao X. (2021). Long non-coding RNA CHRF accelerates LPS-induced acute lung injury through microRNA-146a/Notch1 axis. Ann. Transl. Med. 9, 1299.

Maiuri M.C., Criollo A. and Kroemer G. (2010). Crosstalk between apoptosis and autophagy within the Beclin 1 interactome. EMBO J. 29, 515-516.

Prerna K. and Dubey V.K. (2022). Beclin1-mediated interplay between autophagy and apoptosis: New understanding. Int. J. Biol. Macromol. 204, 258-273.

Sang Z., Dong S., Zhang P. and Wei Y. (2021). miR214 ameliorates sepsisinduced acute kidney injury via PTEN/AKT/mTORregulated autophagy. Mol. Med. Rep. 24, 683.

Song J., Hou L., Quan R., Wang D., Jiang H. and Liu J. (2022). Synergetic contributions of viral VP1, VP3, and 3C to activation of the AKT-AMPK-MAPK-MTOR signaling pathway for Seneca Valley virus-induced autophagy. J. Virol. 96, e0155021.

Sun J., Ge X., Wang Y., Niu L., Tang L. and Pan S. (2021a). USF2 knockdown downregulates THBS1 to inhibit the TGF-beta signaling pathway and reduce pyroptosis in sepsis-induced acute kidney injury. Pharmacol. Res. 176, 105962.

Sun M., Li J., Mao L., Wu J., Deng Z., He M., An S., Zeng Z., Huang Q. and Chen Z. (2021b). p53 deacetylation alleviates sepsis-Induced acute kidney injury by promoting autophagy. Front Immunol. 12, 685523.

Sun R., Zhao N., Wang Y., Su Y., Zhang J., Wang Y., Yu Y., Wang G., Wang Z. and Xie K. (2021c). High concentration of hydrogen gas alleviates lipopolysaccharide-induced lung injury via activating Nrf2 signaling pathway in mice. Int. Immunopharmacol. 101, 108198.

Sun Y., Yao X., Zhang Q.J., Zhu M., Liu Z.P., Ci B., Xie Y., Carlson D.,

Rothermel B.A., Sun Y., Levine B., Hill J.A., Wolf S.E., Minei J.P. and Zang Q.S. (2018). Beclin-1-dependent autophagy protects the heart during sepsis. Circulation 138, 2247-2262.

Wang K. (2015). Autophagy and apoptosis in liver injury. Cell Cycle 14, 1631-1642.

Wang B., Sun Q., Ye W., Li L. and Jin P. (2021a). Long non-coding RNA CDKN2B-AS1 enhances LPS-induced apoptotic and inflammatory damages in human lung epithelial cells via regulating the miR-140-5p/TGFBR2/Smad3 signal network. BMC Pulm. Med. 21, 200.

Wang M., Wei J., Shang F., Zang K. and Zhang P. (2021b). Down-regulation of lncRNA SNHG5 relieves sepsis-induced acute kidney injury by regulating the miR-374a-3p/TLR4/NF-kappaB pathway. J. Biochem. 169, 575-583.

Wei W., Yao Y., Bi H., Xu W. and Gao Y. (2021). Circular RNA circ_0068,888 protects against lipopolysaccharide-induced HK-2 cell injury via sponging microRNA-21-5p. Biochem. Biophys. Res. Commun. 540, 1-7.

Wu Y., Fan T., Zhao Y., Hu R., Yan D., Sun D., Gao L., Qin L., Xue X. (2021). Circular RNA hsa_circ_0001306 functions as a competing endogenous RNA to regulate FBXW7 expression by sponging miR-527 in hepatocellular carcinoma. J Cancer 12, 6531-6542.

Xie S., Jiang X., Qin R., Song S., Lu Y., Wang L., Chen Y. and Lu D. (2021). miR-1307 promotes hepatocarcinogenesis by CALR-OSTC-endoplasmic reticulum protein folding pathway. iScience 24, 103271.

Yang C., Yang C., Huang Z., Zhang J., Chen N., Guo Y., Zahoor A. and Deng G. (2021). Reduced expression of MiR-125a-5p aggravates LPS-induced experimental acute kidney injury pathology by targeting TRAF6. Life Sci. 288, 119657.

Yu S., Song S.A., Jun K.R., Park H.Y. and Lee J.N. (2022). Clinical performance of monocyte distribution width for early detection of sepsis in emergency department patients: A prospective study. Ann. Lab. Med. 42, 286-289.

Zhang C., Li J., Qiu X., Chen Y. and Zhang X. (2019a). SUMO protease SENP1 acts as a ceRNA for TGFBR2 and thus activates TGFBR2/Smad signaling responsible for LPS-induced sepsis. Biomed. Pharmacother. 112, 108620.

Zhang Y., Wang L., Meng L., Cao G. and Wu Y. (2019b). Sirtuin 6 overexpression relieves sepsis-induced acute kidney injury by promoting autophagy. Cell Cycle 18, 425-436.

Zhao W., Zhang L., Chen R., Lu H., Sui M., Zhu Y. and Zeng L. (2018). SIRT3 protects against acute kidney injury via AMPK/mTOR-Regulated autophagy. Front. Physiol. 9, 1526.

Zhao Y., Feng X., Li B., Sha J., Wang C., Yang T., Cui H. and Fan H. (2020). Dexmedetomidine protects against lipopolysaccharide-Induced acute kidney injury by enhancing autophagy through inhibition of the PI3K/AKT/mTOR pathway. Front. Pharmacol. 11, 128.

Zheng C., Wu D., Shi S. and Wang L. (2021). miR-34b-5p promotes renal cell inflammation and apoptosis by inhibiting aquaporin-2 in sepsis-induced acute kidney injury. Ren. Fail. 43, 291-301.

Zhou Y., Qing M. and Xu M. (2021). Circ-BNIP3L knockdown alleviates LPS-induced renal tubular epithelial cell injury during sepsis-associated acute kidney injury by miR-370-3p/MYD88 axis. J. Bioenerg. Biomembr. 53, 665-677.

Accepted October 6, 2022