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MicroRNA-21-3p/Rcan1 signaling axis affects apoptosis of cardiomyocytes of sepsis rats

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Abstract. Accumulating evidence has reported the role of microRNA-21-3p (miR-21-3p) in sepsis, and our objective was to discuss the effect of the miR-21-3p/regulator of calcineurin 1 (Rcan1) axis on cardiomyocyte apoptosis of septic rats. miR-21-3p and Rcan1 expression in myocardial tissues of lipopolysaccharide (LPS)-treated rats and LPS-treated H9c2 cardiomyocytes was determined. The influences of downregulating miR-21-3p or upregulating Rcan1 in cardiomyocyte apoptosis of LPS-treated rats and LPS-treated H9c2 cardiomyocytes were then evaluated. The target relation between Rcan1 and miR-21-3p was verified. It was observed that miR-21-3p was elevated and Rcan1 was reduced in LPS-treated rats and LPS-treated H9c2 cardiomyocytes. Downregulating miR-21-3p or upregulating Rcan1 could suppress cardiomyocyte apoptosis of LPS-treated rats and LPS-treated H9c2 cardiomyocytes. Based on the dual-luciferase activity assay, miR-21-3p was directly targeted Rcan1 in H9c2 cardiomyocytes. In the rescue experiment, the LPS+miR-21-3p inhibitor+si-Rcan1 group enhanced the apoptosis of H9c2 cardiomyocytes in comparison to the LPS+miR-21-3p inhibitor+si-NC group. Together, our findings identify that the miR-21-3p/Rcan1 axis may affect apoptosis of cardiomyocytes in sepsis, which provides a new idea for understanding the potential mechanism of sepsis.

Key words: Sepsis — microRNA-21-3p — Regulator of calcineurin 1 — Cardiomyocytes — Apoptosis

Introduction

Sepsis is a life-threatening organ dysfunction induced by a dysregulated host response to infection (He et al. 2019). Sepsis is characterized by systemic inflammatory response syndrome and is frequently induced by trauma, hemorrhage, and abdominal surgery (Yu et al. 2017). The symptoms of sepsis patients vary according to fever, shock, and organ dysfunction (Chen et al. 2019). The morbidity of sepsis is on the rise due to aging populations with impaired immunity on account of immunosenescence (Hotchkiss et al. 2013). Excessive inflammatory responses, excess tissue damage (proinflammatory), or immunosuppression induced by sepsis increase susceptibility to secondary infections (anti-inflammatory) (Fang et al. 2018). One of the most frequently affected organs by sepsis dysfunction is the heart, and sepsis-associated heart dysfunction appears under several types of heart diseases (Wasyluk et al. 2021). Septic cardiomyopathy is an essential feature of sepsis-related cardiovascular failure, and patients often present reduced ventricular contractility, ventricular dilatation, as well as ventricular dysfunction (Martin et al. 2019). Therapy of severe sepsis includes early identification, rapid and appropriate therapy with antimicrobial agents, microbial source control, and goal-directed ventilatory, hemodynamic, and metabolic therapies (Aygun et al. 2019). Therefore, prognosis prediction and early rec-

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ognition are important for the treatment of sepsis (Dai et al. 2017). Thus, it is imperative to search for effective intervention and therapy for sepsis.

In physiological conditions, apoptosis and autophagy play essential roles in cell renewing (Li et al. 2016). Impairment of autophagy has been demonstrated to contribute to apoptotic cardiomyocyte death and contractile dysfunction in sepsis (Wu et al. 2020). Apoptosis has been regarded as a determining process in myocardial depression induced by sepsis (Neviere 2001). MicroRNAs (miRNAs) can inhibit or degrade genetic targets (Liu et al. 2019). Evidence has revealed the diagnostic or prognostic values of many circulating miRNAs in sepsis patients (Wang et al. 2016). miRNA-21 (miR-21) is expressed in various organs, including the heart and kidney in mammals (Chuppa et al. 2018). Additionally, miR-21 has been found to correlate with cardiac injury and cardioprotection (Kura et al. 2020), participates in lipopolysaccharide (LPS)-induced myocardial injury (Li et al. 2022), as well as sepsis-associated cardiac dysfunction (Zhang et al. 2021). Xue et al. have stated that miR-21-5p impedes the LPS-induced sepsis progression in H9c2 cells (Xue et al. 2021). Especially, miR-21-3p has been disclosed to be the key modulator at the intersection of inflammation, redox biology, energy metabolism, as well as cancer biology (Gomez et al. 2015; Song et al. 2018). In addition, miR-21-3p from fibroblasts-derived exosomes serving as a potent paracrine-acting RNA molecule, induces cardiomyocyte hypertrophy (Bang et al. 2014). As reported, miR-21-3p serves a significant role in metabolism changes of renal tubular epithelial cells during sepsis-related acute kidney injury (Lin et al. 2019). It is revealed that miR-21-3p controls sepsis-linked cardiac dysfunction (Wang et al. 2016). Moreover, the latest research has stated that miR-21-3p could promote the development of diabetic cardiac fibrosis (Shi et al. 2021). However, the concrete mechanism of miR-21-3p still needs verification. The regulators of calcineurin (Rcan) proteins are crucial endogenous regulators, interacting with calcineurin and altering its function through interfering with calcineurin/cytosolic nuclear factor of activated T-cells binding (Hattori et al. 2019). Rcan1 is a multifunctional protein, and it can repress the expression of calcineurin and modulates mitochondrial function (Peiris and Keating 2018). Rcan1 exerts functions in the progression of tubulointerstitial fibrosis via modulating the mitochondrial quality (Sang et al. 2020). The cardiomyocyte-specific Rcan1 overexpression impedes cardiac hypertrophy induced by pathological or physiological stimuli in mice (Corbalan and Kitsis 2018). The miR-21-3p/Rcan1 axis acts as a therapeutic target for the innate circadian changes in cardiac protection (Rotter et al. 2014). Moreover, there is an article highlighting that the epigenetic inhibition of Rcan1 aggravates podocyte injury under the conditions of HIV infection and diabetic nephropathy (Li et al. 2018). Nevertheless, the effect of the miR-21-3p/Rcan1 signaling axis in sepsis remains to be elucidated. Thus, the central objective of the present study was to investigate the effect of the miR-21-3p/Rcan1 signaling axis in autophagy and apoptosis of cardiomyocytes in sepsis rats.

Materials and Methods

Ethics statement

All animal experiments were approved by the Institutional Animal Care Use Committee of the Third Affiliated Hospital of Chongqing Medical University (Jeer Hospital). The ARRIVE guidelines were utilized to evaluate the quality of *in vivo* experiments.

Experimental animals

Specific pathogen-free grade healthy adult male Sprague Dawley (SD) rats with a body weight of 250–280 g were bought from the animal center of Chongqing Medical University (Chongqing, China). Rats were adaptively fed at 20–23°C with a 12 h light-dark cycle for 1 week. Six-hour starvation was required before induction of sepsis.

SD rats were divided into several groups ($n = 8$ *per* group): control group, LPS group, LPS+antagomir NC group, and LPS+miR-21-3p antagomir group. The sepsis model in rats was induced by intraperitoneal (i.p.) injection of LPS at 10 mg/kg (a single dose) (Fodor et al. 2015). The control rats were i.p. injected with an equal volume of normal saline, and the experimental rats were injected with antagomir NC or miR-21-3p antagomir (30 mg/kg/day) through the tail vein for 3 days. Antagomir NC (miR3N0000001-4-5) and miR-21-3p antagomir (miR30004711-4-5) were composed by RiboBio (Guangdong, China). Forty-eight hours after the successful induction of sepsis, the rats were euthanized. The blood samples were harvested for enzyme-linked immunosorbent assay (ELISA). Then, the myocardial tissues were collected for histological staining and biological analysis. The experimental flow chart is exhibited in Supplementary material, Figure S1.

Detection of related indexes in serum

The blood samples of rats were centrifuged for 15 min at 1500 rpm. The cardiac function indices such as aspartate aminotransferase (AST) (C010-2-1), lactate dehydrogenase (LDH) (A020-2-2), and cardiac troponin-I (CTnI) (E019-1-1) were measured concerning commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The concentration of interleukin (IL)-6 (H007-1-1) and IL-1β (H002) in serum were tested following commercial kits (Nanjing Jiancheng Bioengineering Institute).

Hematoxylin-eosin (HE) staining

Rat myocardial tissues were collected from rats of each group, fixed in 4% paraformaldehyde for 24 h, dehydrated until permeabilization, embedded in paraffin and sectioned (4 μm), and subjected to HE staining. Pathological changes in myocardial tissues were observed under a light microscope.

Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end labeling (TUNEL) staining

TUNEL apoptosis detection kit (C1098, Beyotime, Shanghai, China) was implemented to analyze the apoptosis in the myocardial tissues of rats. The paraffin sections were deparaffinized, hydrated, and added with 20 mg/ml DNase-free proteinase K. Then, the sections were incubated with 3% hydrogen peroxide solution and reacted with 50 ml of TUNEL working solution in each well. Then the reaction was terminated by 0.3 ml of labeled reaction termination solution. Next, the sample was added with 50 ml of streptavidin-horseradish peroxidase solution, developed by 0.5 ml of diaminobenzidine, and re-stained with hematoxylin for observation under an optical microscope (Zhang et al. 2020).

Treatment of H9c2 cardiomyocytes

Rat H9c2 cardiomyocytes (ATCC, VA, USA) were cultivated in the medium (Dulbecco's modified Eagle's medium (DMEM), 10% fetal bovine serum, 1% penicillin-streptomycin). To induce sepsis, H9c2 cardiomyocytes were treated with LPS at a concentration of 10 μg/ml) (Yu et al. 2019).

miR-21-3p mimic (miR10004711-1-5), miR-21-3p inhibitor (miR20004711-1-5), overexpression (oe)-Rcan1, si-Rcan1 and the corresponding NCs (RiboBio, Guangdong, China) were transfected into H9c2 cardiomyocyte through Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA), and grouped as following: LPS+mimic NC group, LPS+miR-21-3p mimic group, LPS+inhibitor NC group, LPS+miR-21-3p inhibitor group, LPS+oe-NC group, LPS+oe-Rcan1 group, LPS+miR-21-3p inhibitor+si-NC group and LPS+miR-21-3p inhibitor+si-Rcan1 group. H9c2 cells were collected 48 h later (Wang et al. 2017).

Flow cytometry

H9c2 cardiomyocytes were harvested and resuspended in 500 μl binding buffer and incubated with 5 μl fluorescein isothiocyanate (FITC) and 5 μl propidium iodide according to Annexin V-FITC Apoptosis Detection Kit I (556547, BD Biosciences, NJ, USA). Afterwards, cell apoptosis was tested on the flow cytometer (Beckman Coulter, CA, USA).

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

The total RNAs in tissues and cells were extracted by Trizol reagent (Invitrogen). With a Mir-X miRNA First-Strand Synthesis kit (638315, Takara, Dalian, China) and PrimeScript RT reagent kit (RR047A, Takara), RNA was reverse transcribed into cDNA. The cDNA was implemented for qPCR with the SYBR Premix Ex Taq kit (RR047A, Takara) by using an ABI Prism 7500 Fast Real-Time PCR system (Applied Biosystems, StepOnePlus, USA). Gene relative expression was calculated by the $2^{-\Delta\Delta Ct}$ method. All primers were synthesized by Sangon Biotech (Shanghai, China). Primer sequences are exhibited in Supplementary materials, Table S1.

Western blot analysis

The tissue and cells were lysed using RIPA supplemented with 0.1 mM PMSF of lysis buffer (Thermo Fisher Scientific, Inc., Waltham, MA, USA), and total protein was extracted. Electrophoresis was performed after protein quantification using the bicinchoninic acid method, and then the protein was transferred to the PVDF membrane (Millipore, Billerica, MA, USA) and blocked with 5% skimmed milk. The membrane was added with primary antibodies β-actin (1:1000, ab8227, Abcam, MA, USA), Rcan1 (1:500; H00010231-B01P, Novus Biologicals, Littleton, CO, USA), Bax (1:2000, ab182733, Abcam), Bcl-2 (1: 2000, ab182858, Abcam) and Cleaved caspase-3 (1:500, ab32042, Abcam), mixed with appropriate secondary antibodies, and developed with enhanced chemiluminescence solution. After exposure, the gray value was analyzed by Fluor Chem 8900 software.

Dual luciferase reporter gene assay

The wild-type (WT) 3'UTR or mutated (MUT) 3'UTR of Rcan1 containing the putative miR-21-3p binding site was inserted into the psiCHECK2 plasmid (Promega, WI, USA). H9c2 cardiomyocytes were transfected with WT or MUT plasmid by Lipofectamine 2000 (Invitrogen), and transfected with miR-21-3p mimic or NC mimic, respectively. The firefly and renilla luciferase activities were gauged by a dual luciferase reporter gene assay kit at 48 h post-transfection.

Statistical analysis

SPSS 22.0 software (IBM Corp. Armonk, NY, USA) and GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA) were used for data analysis. Measurement data were indicated as mean ± standard deviation.

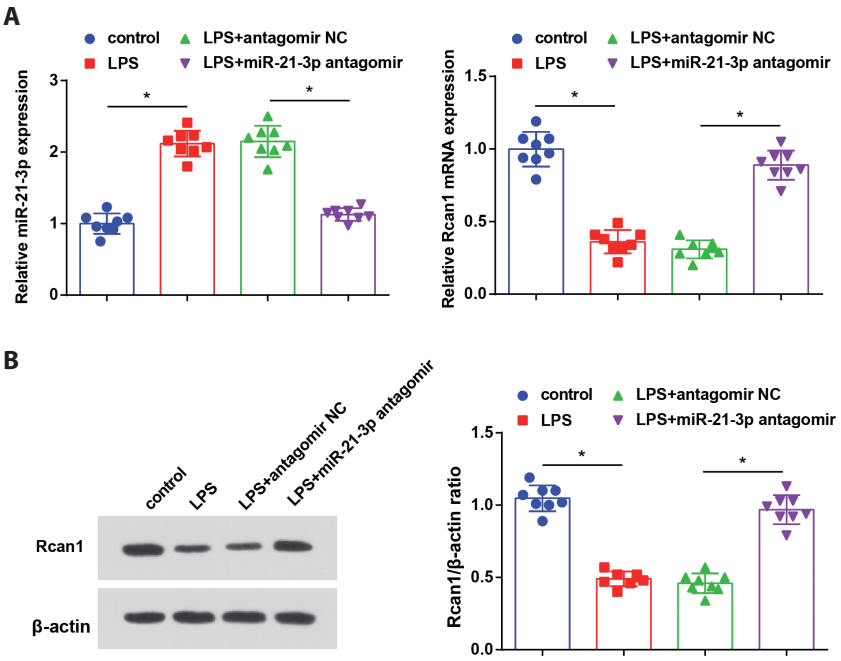


Figure 1. miR-21-3p level increases and Rcan1 level decreases in septic rats. miR-21-3p and Rcan1 expression in heart tissues of rats was tested by RT-qPCR (**A**) and Western blot (**B**). Measurement data were indicated as mean ± SD. * $p < 0.05$. LPS, lipopolysaccharide; NC, negative control; miR-21-3p, microRNA-21-3p; Rcan1, calcineurin 1.

Comparisons between the two groups were performed using the *t*-test, and comparisons among multiple groups were analyzed by one-way analysis of variance (ANOVA) with Tukey's *post hoc* test. $p < 0.05$ was considered to be statistically significant.

Results

miR-21-3p expression level increases and Rcan1 expression level decreases in septic rats

To assess the expression of miR-21-3p and Rcan1 in sepsis, RT-qPCR and Western blot analysis were performed, eventually presenting that miR-21-3p expression was enhanced and Rcan1 expression was depressed in the LPS group compared with the control group (both $p < 0.05$). miR-21-3p expression was suppressed and Rcan1 expression was elevated in the LPS+miR-21-3p antagomir group *versus* the LPS+antagomir NC group (both $p < 0.05$) (Fig. 1A,B).

Depleted miR-21-3p ameliorates myocardial injury induced by LPS

In the control group, the cardiomyocytes were well arranged, the morphology of the nucleus was normal, and no inflammatory infiltration appeared. In the LPS group, cardiomyocytes were swollen and disordered, and the nuclei were abnormal, inflammatory infiltration and fluid exudation appeared in intercellular substance. Interstitial inflammatory cell infiltration and fluid exudation were reduced in the LPS+miR-21-3p antagomir group (Fig. 2A).

The contents of AST, LDH, CTnI, IL-6, and IL-1β in serum were examined (Fig. 2B–F), and the results reported that all of their contents were raised in the LPS group compared with the control group (all $p < 0.05$), but their contents were diminished in the LPS+miR-21-3p antagomir group *versus* the LPS+antagomir NC group (all $p < 0.05$).

It is suggested that the low expression of miR-21-3p improves the myocardial structure destruction and myocardial dysfunction induced by LPS in rats.

Down-regulated miR-21-3p abolishes the apoptosis of cardiomyocytes induced by LPS

The apoptosis of cardiomyocytes in the myocardial tissues of LPS-treated rats was tested by TUNEL staining (Fig. 3A,B) and RT-qPCR and western blot assay (Fig. 3C,D), and the results revealed that the apoptosis was raised, Bax and Cleaved caspase-3 expression increased and Bcl-2 expression decreased in the LPS group compared with the control group (all $p < 0.05$). The apoptotic situation was attenuated in the LPS+miR-21-3p antagomir group in comparison to the LPS+antagomir NC group (all $p < 0.05$).

In vitro test was used to further verify the results obtained from *in vivo* experiment. miR-21-3p expression after cell transfection was detected, and the results showed (Fig. 3E) that miR-21-3p expression in cardiomyocytes was decreased in the LPS+miR-21-3p inhibitor group while it increased in the LPS+miR-21-3p mimic group in contrast to their respec-

tive NC group ($p < 0.05$). Flow cytometry, RT-qPCR and Western blot assay were implemented to assess the effect of miR-21-3p on apoptosis of LPS-stimulated H9c2 cardiomyocytes. It was presented that the apoptosis of cardiomyocytes was depressed, Bcl-2 expression was heightened while Bax and Cleaved caspase-3 expression was suppressed in the LPS+miR-21-3p inhibitor group *versus* the LPS+inhibitor NC group (all $p < 0.05$), whereas the LPS+miR-21-3p mimic group promoted apoptosis of cardiomyocytes compared with the LPS+mimic NC group (all $p < 0.05$) (Fig. 3F–H).

miR-21-3p directly targets to Rcan1 3'UTR

To further elucidate the mechanism of miR-21-3p in apoptosis of H9c2 cardiomyocytes, the target gene of miR-21-3p was predicted by the TargetScan website, and it was found that Rcan1 might be a target of miR-21-3p. Thus, miR-21-3p inhibitor was transfected into H9c2 cardiomyocytes, and changes in Rcan1 expression were determined. The results unveiled that in H9c2 cardiomyocytes, Rcan1 expression was reduced in the miR-21-3p mimic group, while its expression was elevated in the miR-21-3p inhibitor group in contrast to their respective NC group ($p < 0.05$) (Fig. 4A,B).

Additionally, the dual luciferase reporter gene assay was implemented to verify that miR-21-3p was directly targeted Rcan1 3'UTR in H9c2 cardiomyocytes (Fig. 4C). It was observed that up-regulating miR-21-3p suppressed the luciferase activity of Rcan1 WT 3'UTR in H9c2 cardiomyocytes. The mutation of miRNA binding sites showed no response to miR-21-3p targeting (Fig. 4D), suggesting that Rcan1 was a target gene of miR-21-3p in H9c2 cardiomyocytes.

Highly expressed Rcan1 reduces apoptosis of cardiomyocytes treated by LPS

In order to discuss the biological functions of Rcan1 in H9c2 cardiomyocytes, oe-Rcan1 was transfected into H9c2

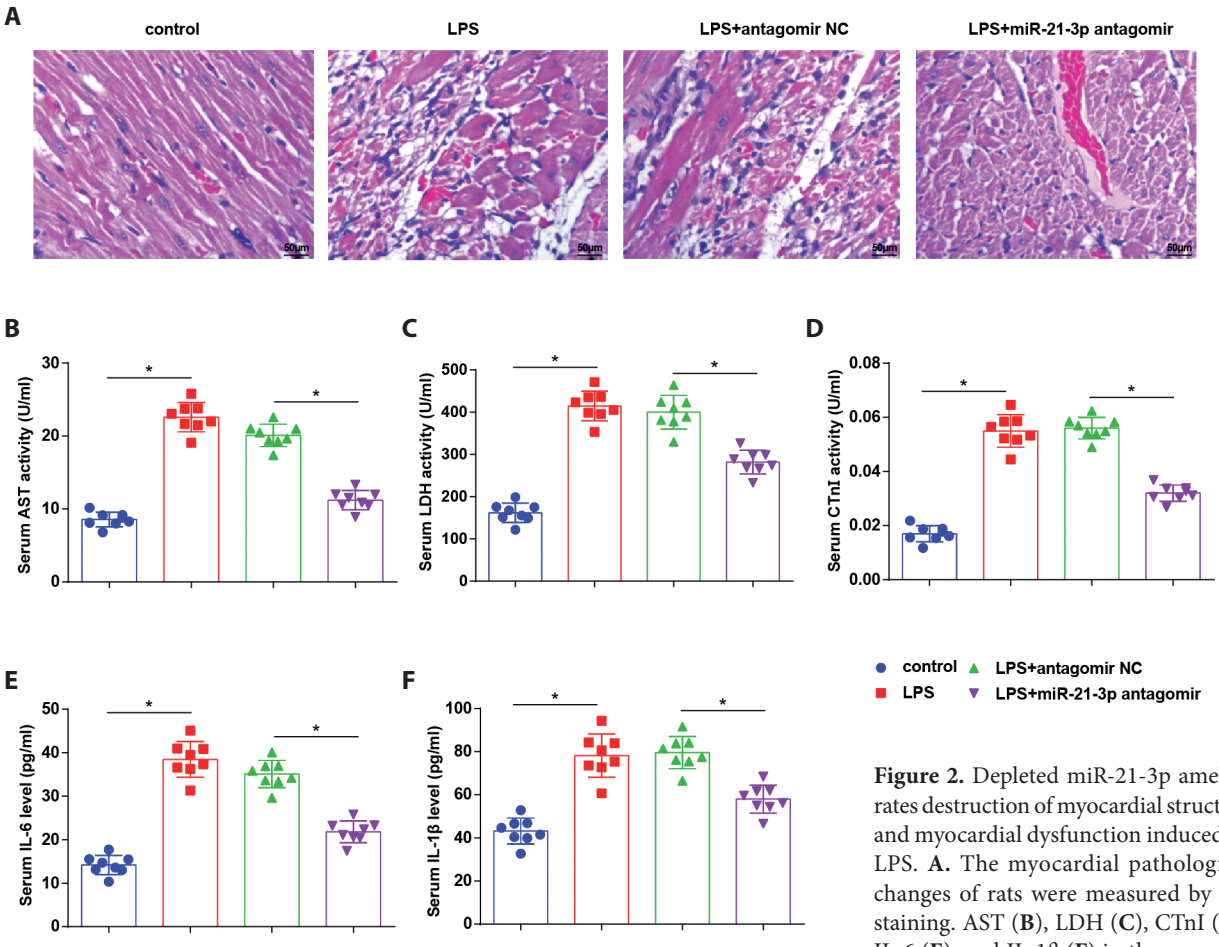


Figure 2. Depleted miR-21-3p ameliorates destruction of myocardial structure and myocardial dysfunction induced by LPS. **A.** The myocardial pathological changes of rats were measured by HE staining. AST (**B**), LDH (**C**), CTnI (**D**), IL-6 (**E**), and IL-1β (**F**) in the serum of rats were assessed by ELISA. Measurement data were indicated as mean ± SD. * $p < 0.05$. LPS, lipopolysaccharide; NC, negative control; miR-21-3p, microRNA-21-3p; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; CTnI, cardiac troponin-I; IL, interleukin.

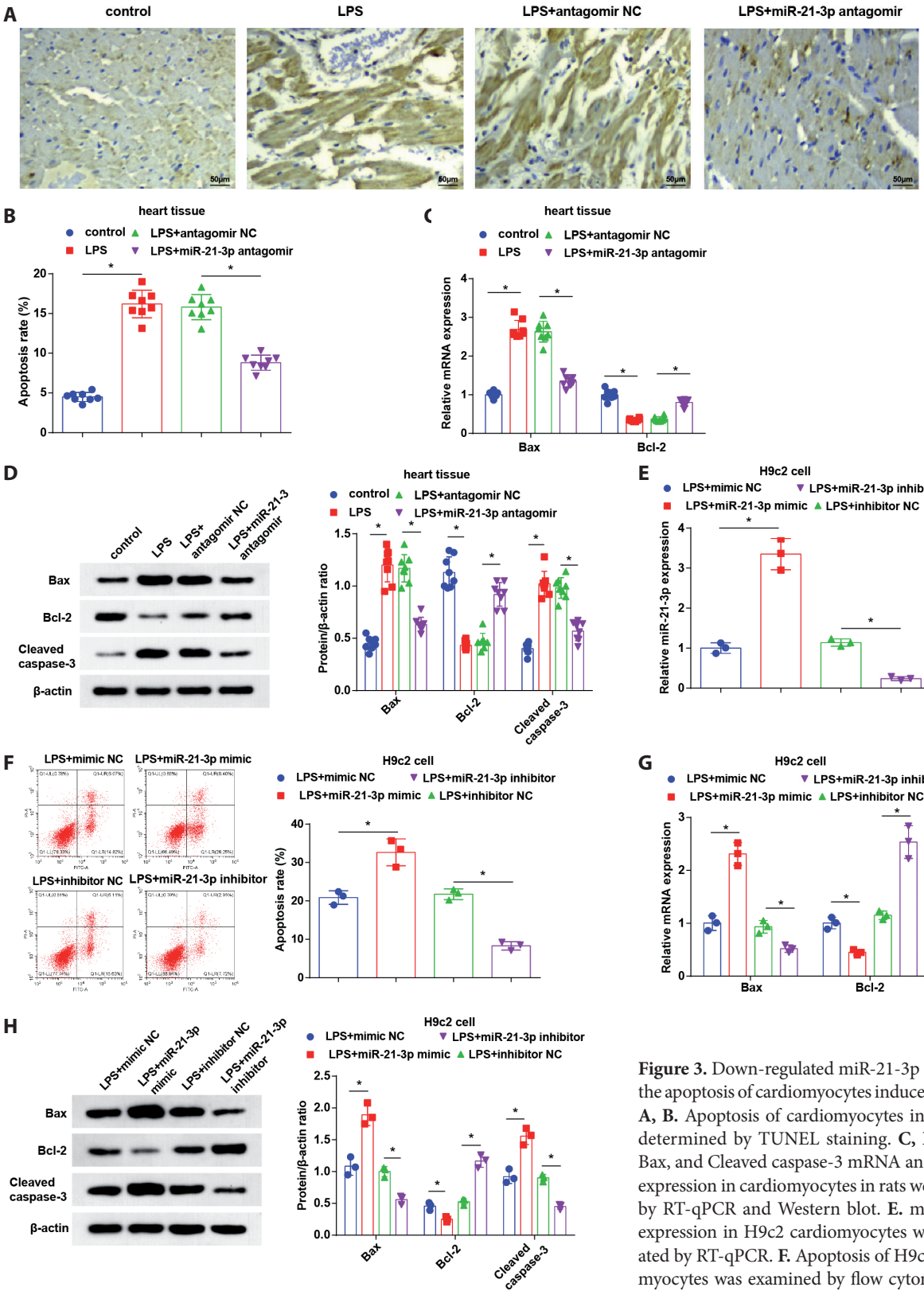


Figure 3. Down-regulated miR-21-3p abolishes the apoptosis of cardiomyocytes induced by LPS. **A, B.** Apoptosis of cardiomyocytes in rats was determined by TUNEL staining. **C, D.** Bcl-2, Bax, and Cleaved caspase-3 mRNA and protein expression in cardiomyocytes in rats were tested by RT-qPCR and Western blot. **E.** miR-21-3p expression in H9c2 cardiomyocytes was evaluated by RT-qPCR. **F.** Apoptosis of H9c2 cardiomyocytes was examined by flow cytometry. **G, H.** Bcl-2, Bax, and Cleaved caspase-3 mRNA and protein expression in H9c2 cardiomyocytes were tested by RT-qPCR and Western blot. Measurement data were indicated as mean ± SD. * $p < 0.05$. LPS, lipopolysaccharide; NC, negative control; miR-21-3p, microRNA-21-3p.

cardiomyocytes, and the impact of Rcan1 on apoptosis of cardiomyocytes was detected. The LPS+oe-Rcan1 group exhibited elevated Rcan1 levels and suppressed apoptosis of LPS-stimulated cardiomyocytes in H9c2 cardiomyocytes in comparison to the LPS+oe-NC group (both $p < 0.05$) (Fig. 5A–E).

To further address the effect of the miR-21-3p/Rcan1 axis on the apoptosis of cardiomyocytes in sepsis rats, we co-transfected the LPS+miR-21-3p inhibitor+si-NC and the LPS+miR-21-3p inhibitor+si-Rcan1 into LPS-treated H9c2 cardiomyocytes, respectively. Data analysis disclosed that Rcan1 expression was decreased in the LPS+miR-21-3p inhibitor+si-Rcan1 group compared to LPS+miR-21-3p inhibitor+si-NC group ($p < 0.05$) (Fig. 5F,G). Then, experimental data exhibited that the effect of the LPS+miR-21-3p inhibitor+si-NC group on reducing apoptosis of LPS-treated H9c2 cardiomyocytes was better than the LPS+miR-21-3p inhibitor+si-Rcan1 group ($p < 0.05$) (Fig. 5H–J).

Discussion

Sepsis, which is featured by severe systemic inflammation, is a global disease burden with rising morbidity and severity (Zeng et al. 2019). Depletion of miR-21-3p may be a protective method for treating cardiac dysfunction induced by sepsis (Wang et al. 2016). The current study was designed to explore the effect of the miR-21-3p/Rcan1 signaling axis on the apoptosis of cardiomyocytes in sepsis rats.

As previously described, miR-21 results in the pathogenesis of diseases in diverse organs, such as the kidneys, potentially through silencing metabolic pathways (Gomez et al. 2015). In kidney disease, miR-21 participates in the pathogenic responses, and both the genetic deletion and pharmacologic suppression of miR-21 could attenuate disease progression (Chau et al. 2012). Evidence has shown that miR-21 has been found to correlate with cardiac injury and cardioprotection (Kura et al. 2020), participates in LPS-induced myocardial injury (Li et al. 2022), as well as sepsis-associated cardiac dysfunction (Zhang et al. 2021). Our investigation unveiled that miR-21-3p expression was enhanced in the myocardial tissues of sepsis rats and in LPS-stimulated cardiomyocytes. The functional analysis further disclosed that depletion of miR-21-3p could improve the myocardial dysfunction and myocardial structure destruction, and reduce inflammation and apoptosis in the myocardial tissues of LPS-treated rats. Also, *in vitro* experiment verified the anti-apoptotic property of down-regulated miR-21-3p for LPS-stimulated cardiomyocytes. There are some articles that have stressed the same functions of miR-21-3p in human diseases. In a study by Lin et al., miR-21-3p expression is raised during sepsis and the associated acute kidney injury,

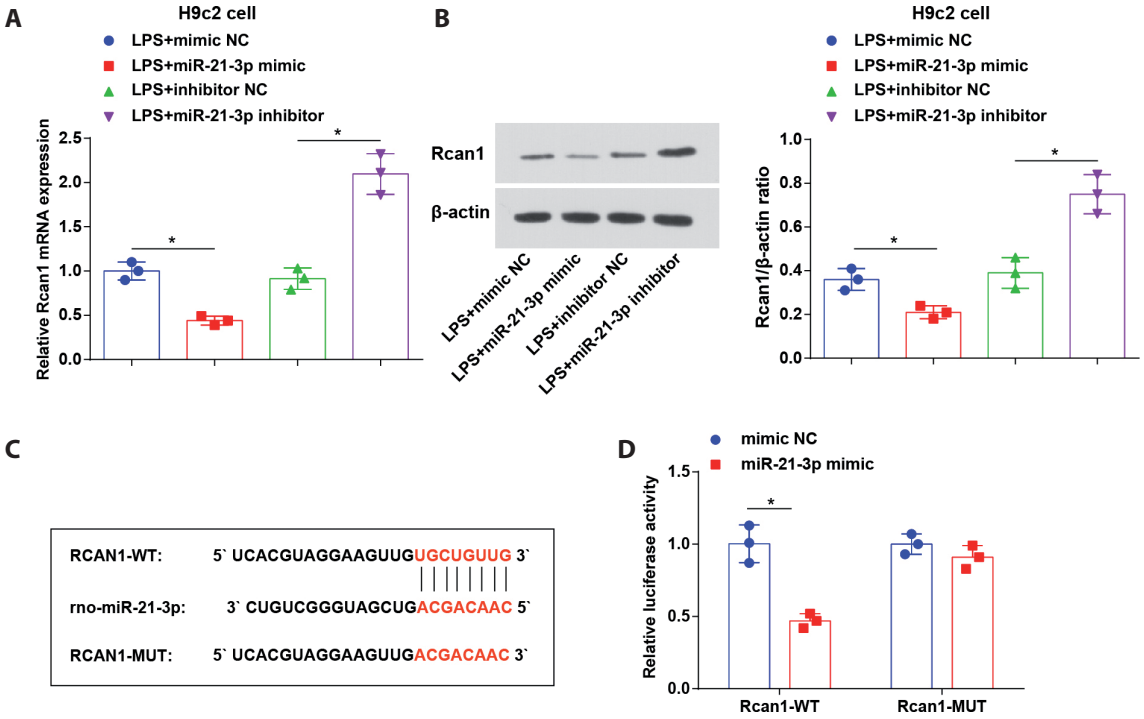
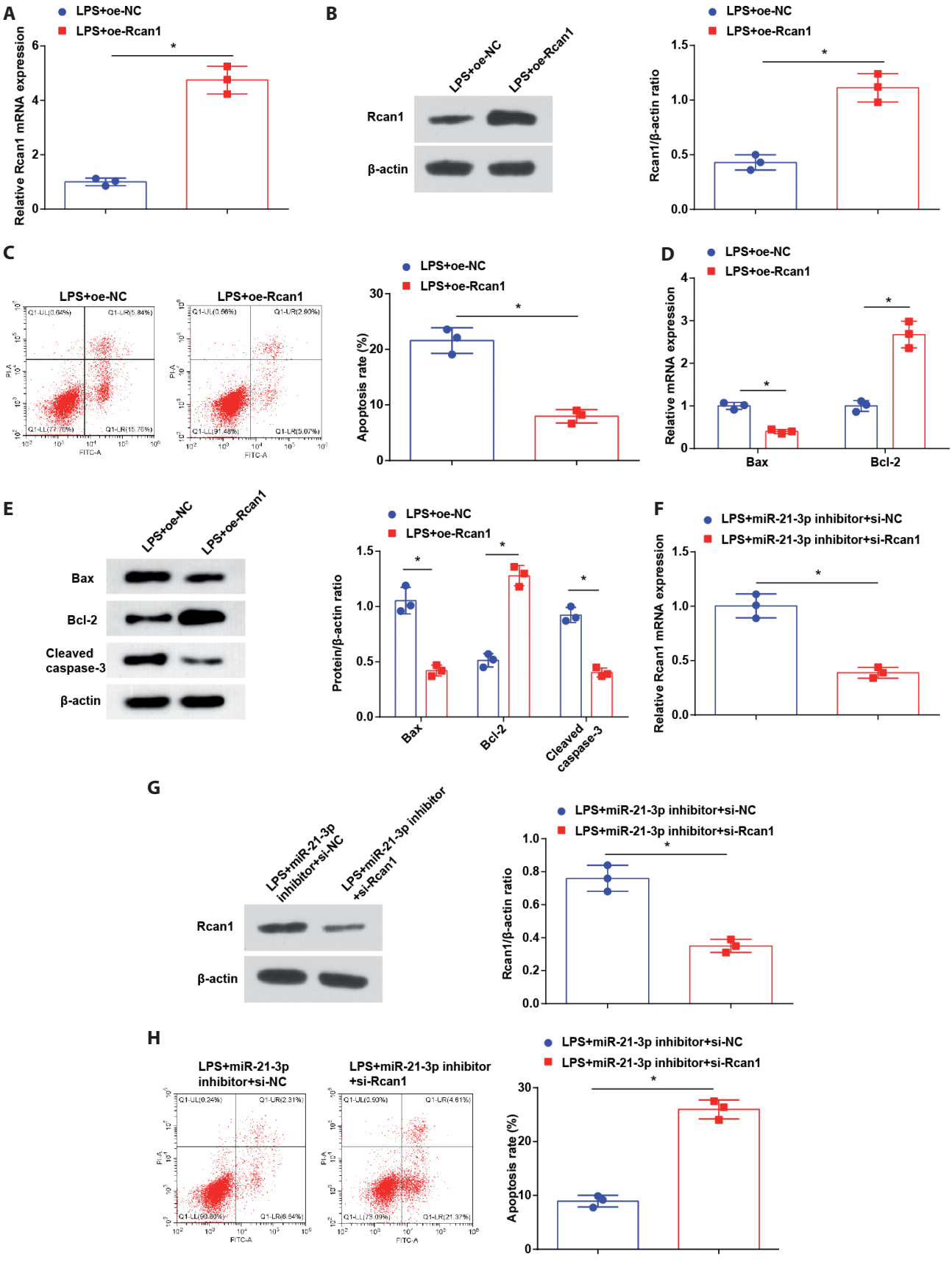


Figure 4. miR-21-3p directly targets Rcan1 3'UTR. **A, B.** Rcan1 expression in H9c2 cardiomyocytes transfected with miR-21-3p inhibitor or mimic. **C, D.** Targeting relation between miR-21-3p and Rcan1. Measurement data were indicated as mean ± SD. * $p < 0.05$. LPS, lipopolysaccharide; NC, negative control; miR-21-3p, microRNA-21-3p; Rcan1, calcineurin 1; WT, wild type; MUT, mutant type.


A
LPS+oe-NC
LPS+oe-Rcan1
Relative Rcan1 mRNA expression
B
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Rcan1
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C
LPS+oe-NC
LPS+oe-Rcan1
Apoptosis rate (%)
D
Relative mRNA expression
Bax
Bcl-2
E
Bax
Bcl-2
Cleaved caspase-3
β-actin
Protein/β-actin ratio
F
LPS+miR-21-3p inhibitor+si-NC
LPS+miR-21-3p inhibitor+si-Rcan1
Relative Rcan1 mRNA expression
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LPS+miR-21-3p inhibitor +si-Rcan1
Rcan1
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Apoptosis rate (%)

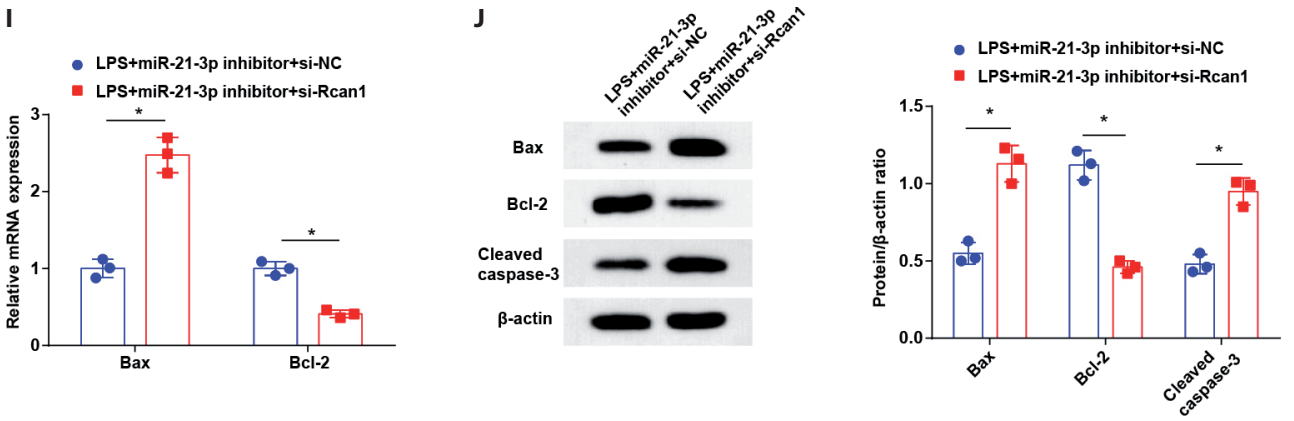

Figure 5. Highly expressed Rcan1 suppresses apoptosis of cardiomyocytes treated by LPS. **A, B.** Rcan1 expression in H9c2 cardiomyocytes was tested by RT-qPCR and Western blot. **C.** Apoptosis of H9c2 cardiomyocytes was measured by flow cytometry. **D, E.** Bcl-2, Bax, and Cleaved caspase-3 mRNA and protein expression in H9c2 cardiomyocytes was determined by RT-qPCR and Western blot. **F, G.** Rcan1 expression in H9c2 cardiomyocytes after co-transfection was examined by RT-qPCR and Western blot. **H.** Apoptosis of H9c2 cardiomyocytes after co-transfection was measured by flow cytometry. **I, J.** Bcl-2, Bax, and Cleaved caspase-3 mRNA and protein expression in H9c2 cardiomyocytes after co-transfection was tested by RT-qPCR and Western blot. Measurement data were indicated as mean ± SD. * $p < 0.05$. LPS, lipopolysaccharide; NC, negative control; miR-21-3p, microRNA-21-3p; Rcan1, calcineurin 1; oe, overexpression.

and depleting miR-21-3p dramatically weakens the apoptosis rate of tubular epithelial cells (Lin et al. 2019). Wang et al. have supported that miR-21-3p is elevated in cardiomyocytes after LPS induction, and lowly expressed miR-21-3p diminishes the cell size of cardiomyocytes, myocardial autophagy, and cardiac dysfunction, as well as facilitates survival of mice induced by LPS (Wang et al. 2016). Shi et al. have witnessed the up-regulation of miR-21-3p in diabetic cardiac fibrosis, and miR-21-3p overexpression induces pyroptosis and collagen deposition of cardiac fibroblasts under high glucose (Shi et al. 2021). As for the correlation of miR-21 with inflammation-related factors, it is revealed that the plasma level of miR-21 is positively associated with IL-6 level in sepsis patients (Zhang 2019). Another study has also demonstrated that miR-21 deficiency leads to a reduction of IL-1β production and relieves septic shock caused by LPS (Xue et al. 2019). All these studies have further convinced the findings of our research.

Next, data collected from our research highlighted the targeting relation between miR-21-3p and Rcan1, which was for the first time discussed in the study of sepsis-induced apoptosis of cardiomyocytes. In actuality, Rcan1 expression was observed to be downregulated in the *in vivo* experiments, as well as in the *in vitro* models of sepsis. Rcan1 plays a role in the pathophysiologies of various cardiovascular diseases, neurological disorders, as well as pancreatic dysfunction (Ermak et al. 2011; Peiris and Keating 2018). Nevertheless, the action of Rcan1 in sepsis is still poorly understood. Notably, research has addressed that Rcan1 expression is downregulated in the glomerular podocytes of human immunodeficiency virus-related nephropathy and diabetic nephropathy (Li et al. 2018). It is also suggested that Rcan1 loss contributes to the deteriorating podocyte injury and glomerular disease, further validating the role of Rcan1 in maintaining the functions of normal podocytes (Li et al. 2018). Furthermore, Rcan1 overexpression impedes cardiac hypertrophy induced by pathological or physiological stimuli in mice (Corbalan and Kitsis 2018). However, we need more research to further confirm the effect of Rcan1 on sepsis.

In conclusion, we find that the miR-21-3p/Rcan1 axis may link to apoptosis in cardiomyocytes with sepsis. Therefore, targeting miR-21-3p may provide a therapeutic approach to sepsis. These results may help to illuminate a better recognition of the concrete mechanisms of the miR-21-3p/Rcan1 axis, and offer a basis for novel strategies for further effective regimens of sepsis. However, the downstream signaling pathways involved in the network of the miR-21-3p/Rcan1 in sepsis-induced apoptosis of cardiomyocytes are not investigated in our study, which could be a future research direction.

Conflict of interest. The authors have no conflicts of interest.

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