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The effect of myocardial infarction-associated transcript 2 (Mirt2) and miR-101 on sepsis-induced myocardial injury in rats

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Abstract. – OBJECTIVE: The aim of this study was to explore the effect of myocardial infarction associated transcript 2 (Mirt2) and miR-101 on sepsis-induced myocardial injury in rats and its mechanism.

MATERIALS AND METHODS: Sprague Dawley (SD) rats were divided into sham group, CLP group, CLP + adeno-associated virus (AAV)-lncRNA Mirt2 group, CLP + AAV-NC group and CLP + AAV-lncRNA Mirt2 + agomiR-101 group. Rat CLP model was constructed. PanoViewb1500 was used to detect left ventricular systolic blood pressure (LVSP), left ventricular end diastolic blood pressure (LVEDP), ejection fraction (EF) and fraction shortening (FS) values. Thereafter, Mirt2 and miR-101 expression levels were detected by real-time polymerase chain reaction (RT-PCR), myocardial pathological damage was detected by hematoxylin-eosin (HE) staining, and Interleukin-1β (IL-1β) expression was by immunohistochemical staining. Next, enzyme linked immunosorbent assay (ELISA) was performed to detect the levels of serum inflammatory factors, tumor necrosis factor α (TNF-α), IL-1β, Interleukin-6 (IL-6), myeloperoxidase (MPO), cardiac troponin I (cTn I) and creatine kinase isoenzyme (CK-MB). After that, Western blot was used to detect the expression of inflammatory factors and Phosphatidylinositol-3-kinases (PI3K)/protein-serine-threonine kinase (AKT) signaling pathway. Finally, Dual-Luciferase reporter gene assay was conducted to detect the relationship between Mirt2 and miR-101.

RESULTS: Compared with those in the sham group, the cardiac function of the rats in the CLP group was significantly deteriorated, the cardiac structure was disordered, and the expression of pro-inflammatory related factors was significantly increased. Compared with those in CLP group, the cardiac function of the CLP + AAV-lncRNA Mirt2 group was alleviated, the PI3K/AKT signaling pathway was activated, the cardiac structure was slightly disordered, and the expression of pro-inflammatory related factors was reduced. To some extent, miR-101 could directly inhibit the effect of Mirt2.

CONCLUSIONS: Mirt2 can silence miR-101 and inhibit myocardial inflammatory response in sepsis rats through the PI3K/AKT signaling pathway, thus improving cardiac structure and function.

Key Words:
MiRNA-106, Pediatric osteosarcoma, PI3K/AKT signaling pathway.

Abbreviations

Mirt2: Myocardial infarction associated transcript 2; LVEDP: left ventricular end diastolic blood pressure; LVSP: left ventricular systolic blood pressure; EF: ejection fraction; FS: fraction shortening; IL-1β: Interleukin-1β; TNF-α: tumor necrosis factor α; IL-6: Interleukin-6; MPO: myeloperoxidase; cTn I: cardiac troponin I; CK-MB: creatine kinase isoenzyme; AAV: adeno-associated virus; SSC: Surviving Sepsis Campaign; lncRNAs: Long noncoding RNAs; LPS: lipopolysaccharide; CLP: cecal ligation puncture; DEPC: diethyl pyrocarbonate: RIPA: radioimmunoprecipitation assay; BCA: bicinchoninic acid; SDS-PAGE: sodium dodecyl sulphate-polyacrylamide gel electrophoresis; PVDF: polyvinylidene difluoride; ECL: enhanced chemiluminescence: ICUs: intensive care units; TLRs: Toll like receptors; NF-kB: nuclear factor-k-gene binding; MAPK: mitogen-activated protein kinase; PI3K: Phosphatidylinositol-3-kinases; AKT: protein-serine-threonine kinase; Th17: T helper cell 17; HMGB-1: high mobility group protein 1; Th2: T Helper cells 2; Treg: Regulatory T cells; ncRNAs: Non-coding RNAs; ICAM-1: intercellular adhesion molecule-1; iNOS: inducible nitric oxide synthase; WB: Western Blot; ELISA: Enzyme-Linked Immunosorbent Assay; HE: Hematoxylin-Eosin; RT-PCR: Real-Time Polymerase Chain Reaction.

Introduction

Sepsis is one of the common serious complications in the clinic, which is caused by microbial infection, including shortness of breath,

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tachycardia, fever, and abnormal white blood cell count. A series of symptoms, such as multiple organ dysfunction syndrome, can lead to septic shock[1]. In septic shock, half of the patients are accompanied by septic myocardial inflammation. Inflammatory cytokines play an important role in it, among which tumor necrosis factor α (TNF-α) can inhibit the contractile function of the heart muscle and cause cardiac dysfunction[2]. About 40-60% of patients with sepsis are associated with myocardial injury, with the mortality rate of 70-90%, while that of patients without myocardial injury is 20%. Sepsis-induced myocardial injury (SMI) places a huge burden on the healthcare industry and the national economy[3]. Surviving Sepsis Campaign (SSC)'s international guidelines for the treatment of sepsis and septic shock have rejected many of the previously recommended treatment options. Despite nearly 100 clinical trials, there is no optimal treatment[4]. It means that the current diagnosis and treatment of SMI still faces many problems, such as complex pathophysiology, generation and treatment of widely drug-resistant bacteria, iatrogenic damage, and high medical expenses. Therefore, it is of great significance to research the pathogenesis of SMI and its evolution, and to find effective therapeutic drugs.

Long noncoding RNAs (lncRNAs) are a kind of universal eukaryotic cell with more than 200 nt of RNA transcripts in length, but they do not encode protein and exist in the nucleus and cytoplasm in the form of RNA. Besides, they can regulate protein-coding genes, the mechanism and regulation of gene expression in multiple level, and has important functions in the inflammatory response, apoptosis, oxidative stress[5]. Of note, the lncRNA IL17R moiety inhibits the inflammatory response induced by bacterial endotoxin lipopolysaccharide (LPS)[6]. In addition, lncRNA Mirt2 regulates the inflammatory responses through interaction with microRNAs[7] and inhibits TNF-α-induced SH-Sy5y cell inflammation[8]. It has also been found that lncRNA Mirt2 can inhibit the inflammatory response of LPS-induced HK-2 cell damage[9]. In the past few decades, researchers have explored the pathogenesis of sepsis and found that the occurrence and development of sepsis are closely related to immune system dysfunction and gene expression in addition to endotoxin and pathogens. Sepsis causes excessive inflammation, immunosuppression, or excessive tissue damage, increasing the susceptibility to secondary infections[10,11]. Therefore, exploring the immune response mechanism related to sepsis is very important for the diagnosis and treatment of sepsis.

The objective of this research was to investigate the effect of lncRNA Mirt2 on inflammatory response and cardiac function in sepsis rats, and to find possible therapeutic targets for sepsis and related complications.

Materials and Methods

Animal Model Preparation

Forty healthy male Sprague Dawley (SD) rats weighing 250-300 g were purchased from Shanghai Jiesijie Experimental Animal and were fed in an environment of constant temperature and humidity and alternating light and dark (12 h) for 48 h. A cecal ligation puncture (CLP) was used to prepare a rat sepsis model. Rats underwent aseptic laparotomy under ether inhalation anesthesia. The sham group was opened to find the cecum and then returned to the abdominal cavity and closed abdomen layer by layer. Except for the sham group, the other groups were opened under anesthesia. The cecum was ligated with 4-0 silk (Ke Hui, Henan) thread at a distance of 1 cm from the end of the cecum. The intestinal canal was wet, the abdominal cavity was closed and the abdomen was closed layer by layer. After operation, rats in each group were injected with 2 mL of normal saline, and repeated 12 h later. This investigation was approved by the Animal Ethics Committee of Rizhao People's Hospital Animal Center.

Echocardiography and Sample Collection

After the preparation of the animal model, rats in the CLP + AAV-NC group, CLP + AAV-lncRNA Mirt2 group, and CLP + AAV-lncRNA Mirt2 + agomir-101 group were injected by tail vein with AAV-NC group, AAV-lncRNA Mirt2 group and agomir-101 group respectively. Adenovirus and agomir were provided by Genechem (Shanghai, China). On Day 1 and 2 after treatment, an appropriate amount of blood was collected to detect the CK-MB and cTnI content. On Day 5, the small animal ultrasound imaging system PanoViewb1500 (Nuohai Life Science, Shanghai, China) was used to detect LVSP, LVEDP, EF and FS of rat myocardium. The rats were sacrificed after the end of echocardiography, and an ap-

propriate amount of blood was centrifuged, and the serum and myocardial tissues were stored at -80°C for subsequent use.

Real-Time Polymerase Chain Reaction (RT-PCR)

After grouping according to the test method (tissue samples need to be mashed by a homogenizer), TRIzol (Thermo Fisher Scientific, Waltham, MA, USA) and chloroform reagent (Camilo Biological, Nanjing, China) were added in order. After centrifugation, the supernatant and isopropyl alcohol (Camilo Biological, Nanjing, China) were added. After sufficient shaking, the supernatant was centrifuged and discarded. The precipitate was washed with 75% ethanol (Camilo Biological, Nanjing, China), and the RNA concentration was measured by dissolving the RNA precipitate with diethyl pyrocarbonate (DEPC) water (Thermo Fisher Scientific, Waltham, MA, USA). Continuous wavelength microplate reader was used to determine the ratio of RNA: A260 / A280 between 1.8 and 2.0, indicated that RNA was qualified, and the average concentration of the two duplicate wells was recorded. The complementary deoxyribose nucleic acid (cDNA) was synthesized according to the reverse transcription kit (Thermo Fisher Scientific, Waltham, MA, USA), and then the PCR was performed using a PCR instrument (Bedford, UK). The primer sequences were shown in Table I. The mRNA level of each group was detected by SsoFast EvaGreen Supermix kit (Bio-Rad, Hercules, CA, USA), and the results were calculated by $2^{-\Delta\Delta Ct}$ method.

Hematoxylin-Eosin (HE) Staining

After grouping according to the test method, the myocardium was fixed in a 10% formaldehyde solution (Jian Cheng, Nanjing, China), paraffin-embedded, sectioned, and then dewaxed with xylene (Jian Cheng, Nanjing, China) and washed with water. Then, they were stained with hematoxylin staining solution (Jian Cheng, Nanjing, China) for 5 minutes, washed with running water for 1 minute, differentiated with 75% hydrochloric acid ethanol solution (Jian Cheng, Nanjing, China) for several seconds, washed with running water for 2 minutes until the nucleus became blue, and re-stained with 0.5% eosin staining solution (Jian Cheng, Nanjing, China) for 3 minutes. After dehydration by gradient ethanol (Jian Cheng, Nanjing, China) and transparency of xylene, the sections were sealed with resin and observed under a light microscope (LEICA, Wetzlar, German).

Immunohistochemical Staining

After grouping according to the test method, the sections of each group were dewaxed with xylene and washed with running water. The antigen was repaired with citrate buffer (PH 6.0). The sections were incubated with H_2O_2 reagent (Jian Cheng, Nanjing, China) for 30 minutes, and then blocked with 10% sheep serum (Jian Cheng, Nanjing, China) for 1 h. Then, the primary antibody (IL-1β, Abcam, Cambridge, MA, USA, Rabbit, 1:1000) was then selected for incubation overnight at 4°C. On the second day, the sections were incubated with secondary antibody (Yifei Xue, Nanjing, China, 1:500) for 1 h. The DAB color reagent (Jian Cheng, Nanjing, China) was added dropwise, and the degree of staining was observed under a microscope. After staining the nucleus with hematoxylin, the tissues were differentiated with 75% hydrochloric acid ethanol solution for several seconds, washed with the running water for 2 minutes until the nucleus became blue, and counterstained by 0.5% eosin

Table I. RT-PCR primers.

Gene name	Forward (5′>3′)	Reverse (5′>3′)
IL-1β	GCAACTGTTCCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
IL-6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
TNF-α	CCTCTCTCTAATCAGCCCTCTG	GAGGACCTGGGAGTAGATGAG
IL-4	GGTCTCAACCCCCAGCTAGT	GCCGATGATCTCTCTCAAGTGAT
IL-10	GCTCTTACTGACTGGCATGAG	CGCAGCTCTAGGAGCATGTG
MPO	AGTTGTGCTGAGCTGTATGGA	CGGCTGCTTGAAGTAAAACAGG
LncRNA Mirt2	TCAACACTTTCCATAGGT	ATTGTGAGGTCCAGATAG
MiR-101	GCTGTCAACGATACGCTA	CAGTACTGTG ATAACTGAA
U6	GTCGGAGTCAACGGATT	AAGCTTCCCGTTCTCAG
GAPDH	ACAACTTTGGTATCGTGGAAGG	GCCATCACGCCACAGTTTC

RT-PCR, Real-Time Polymerase Chain Reaction.

staining solution for 3 minutes. After dehydration by gradient ethanol and transparency of xylene, the sections were sealed with resin and observed under a light microscope.

Enzyme-Linked Immunosorbent Assay (ELISA)

Double antibody sandwich method was used to measure inflammatory factors and myocardial injury indicators. According to the kit (Elabscience, Wuhan, China) instructions, the working solution was first configured, and then added to the standard and sample wells with serum. After color development, the reaction was stopped with a stop solution, and the absorbance (OD value) of each well was detected at a wavelength of 450 nm. The regression equation of the standard curve was calculated according to the concentration and OD values, and ELISA Calc was used for calculation. The logistic curve was used as the fitting model, and the blank well was used as the background.

Western Blot (WB)

After the tissues of each group were processed separately according to the test method (tissue samples need to be mashed by a homogenizer), the tissues were fully lysed with radioimmunoprecipitation assay (RIPA) lysate (Camilo Biological, Nanjing, China), the supernatant was extracted after centrifugation, and bicinchoninic acid (BCA) kit (Camilo Biological, Nanjing, China) was used to detect the total protein concentration. The protein concentration of each group was adjusted to be consistent. 20 μg proteins were taken from each group and separated with 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Then, the proteins were transferred to polyvinylidene difluoride (PVDF, Ye Sen, Shanghai, China) membrane by semi-dry transfer method, and blocked with 5% skim milk for 1 h. Then, the appropriate concentration of primary antibody (IL-1β, Abcam, Cambridge, MA, USA, 1:2000, TNF-α, Abcam, Cambridge, MA, USA, 1:1000; IL-4, Abcam, Cambridge, MA, USA, 1:1000, PI3K, Abcam, Cambridge, MA, USA, 1:2000, p-PI3K, Abcam, Cambridge, MA, USA, 1:2000, AKT Abcam, Cambridge, MA, USA, 1:1000, p-AKT, Abcam, Cambridge, MA, USA, 1:1000, β-actin, Abcam, Cambridge, MA, USA, 1:2000) was added, for incubation overnight at 4°C. On the second day, the secondary antibody (Yifei Xue, Nanjing, China, 1:2000) was added for incubation at room temperature for 2 h, and then the enhanced chemiluminescence (ECL, Jian Cheng, Nanjing, China) was added dropwise for exposure and development.

Luciferase Reporting Test

According to research reports, miR-101 has a binding site to lncRNA Mirt2. The Mirt2 Lenti-reporter-Luciferase wild-type vector and its mutant vector were constructed separately. The cells were divided into four groups: Mirt2 wt + miR-101 mimics, Mirt2 mut + miR-101 mimics, Mirt2 wt + NC, and Mirt2 mut + NC. The sample was loaded into a 24-well plate and left for 1 d. The wild-type vector (200 ng) and pRL-CMV vector (20 ng) were transfected into the Mirt2 wt, Mirt2 wt + miR-101 mimic group, and the mutant vector (200 ng) and Prl-CMV vector (20 ng) were transfected into Mirt2 mut, Mirt2 mut + miR-101 mimic group. At the same time, miR-101 mimic was transfected in the Mirt2 wt + miR-101 mimic and Mirt2 mut + miR-101mimic groups. After 48 h of transfection, Luciferase activity was detected using a Luciferase reporter detection system (Genechem, Shanghai, China).

Statistical Analysis

All data were measured 3 times, and the results are expressed as mean ± standard deviation (SD). The *t*-test was used for comparison between groups, and ANOVA followed by Post-Hoc Test (Least Significant Difference) was used for comparison between multiple groups. Data analysis was performed using Statistical Product and Service Solutions (SPSS) 22.0 software (Chicago, IL, USA), and graphs were produced using Graph Prism 8.0 software (San Diego, CA, USA). $p<0.05$ represents statistically significant differences.

Results

Sepsis Induced Cardiac Inflammation and Functional Changes In Rats

First, the cardiac function of the CLP group and the sham group was measured by echocardiography (Figure 1A-1D). Compared with those in the sham group, LVSP in the CLP group was significantly reduced, LVEDP was markedly increased, and EF and FS were also dramatically reduced. At the same time, the results of ELISA technology showed that the levels of cTn I and CK-MB in serum in CLP group were significantly increased, indicating that cardiac function

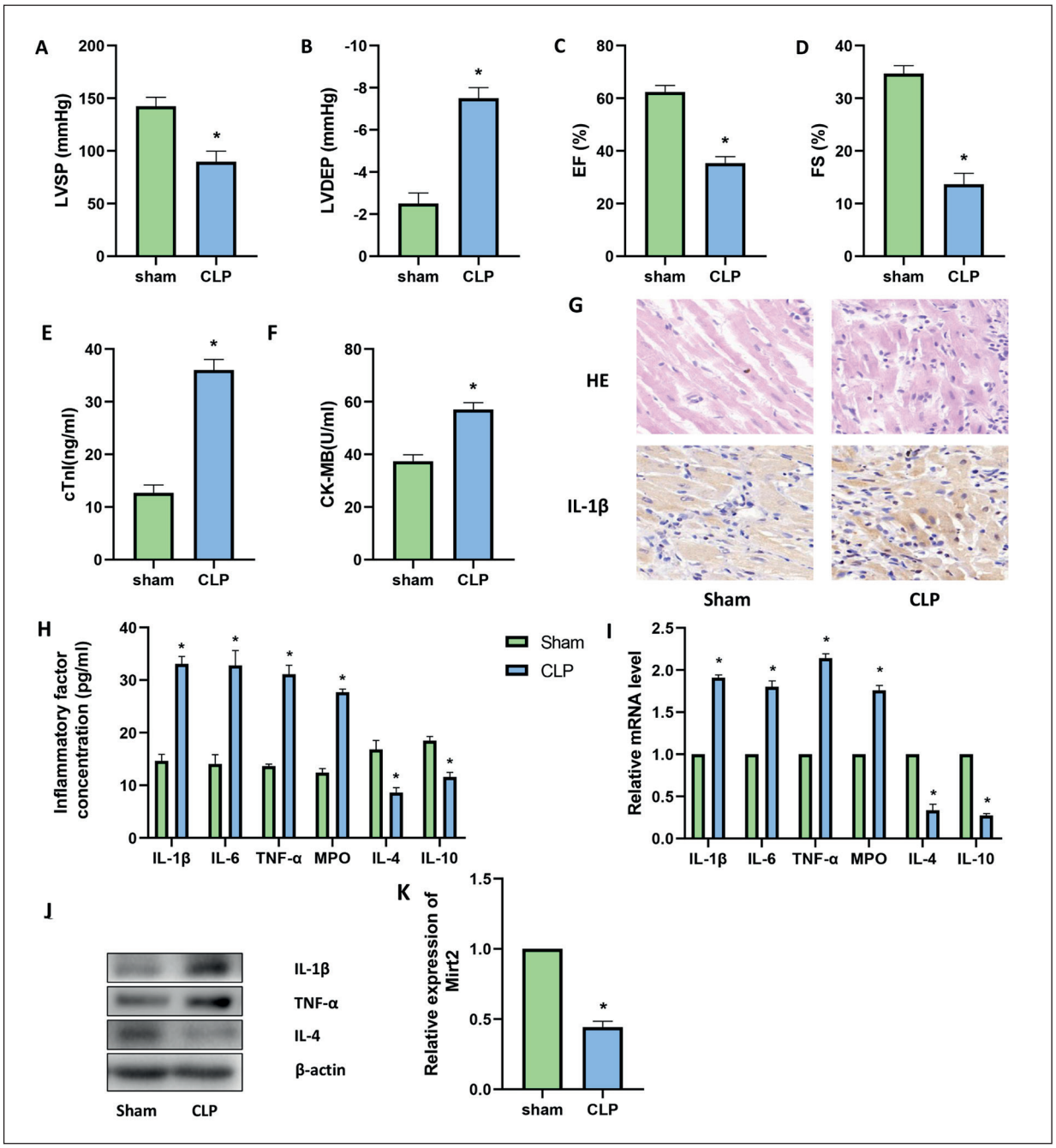

Figure 1. Sepsis induced cardiac inflammation and functional changes in rats. **A,** Echocardiographic detection of LVSP value. **B,** Echocardiographic detection of LVEDP value. **C,** Echocardiographic detection of EF value. **D,** Echocardiographic detection of FS value. **E,** ELISA detected serum cTn I content. **F,** ELISA detected serum CK-MB content. **G,** HE staining and immunohistochemical staining, (magnification: 400×). **H,** ELISA detected IL-1*β*, IL-6, TNF-*α*, MPO, IL-4 and IL-10 contents. **I,** RT-PCR detected IL-1*β*, IL-6, TNF-*α*, MPO, IL-4 and IL-10 expressions. **J,** WB detected IL-1β, TNF-α and IL-4 expressions. **K,** RT-PCR detected lncRNA Mirt2 expression. ("*" indicated statistical difference from the Sham group $p<0.05$).

was impaired in CLP group (Figure 1E and 1F). HE staining showed that myocardial fibers were arranged regularly in the sham group, and the cells had no degeneration and necrosis. However, the myocardial fiber structure of the CLP group was changed, with cell shrinkage, nuclear shrinkage, and widened intercellular spaces. Next, the expression of IL-1β in myocardial tissue was

detected by immunohistochemical staining. As a result, compared with that in the sham group, the expression of IL-1β in heart tissue of CLP group was significantly increased (Figure 1G). Next, ELISA was used to detect the expression of inflammatory factors in the serum. Similar to previous studies, the expressions of pro-inflammatory factors IL-1β, IL-6, TNF-α and MPO in the CLP group were significantly increased, while the expressions of anti-inflammatory factors IL-4 and IL-10 were significantly reduced (Figure 1H). In addition, RT-PCR was applied to detect the above-mentioned inflammatory factors, and obtained results were similar to those of ELISA (Figure 1I). At the same time, in order to detect changes in inflammatory factors at the translation level, WB was used to detect IL-1β, TNF-α, and IL-4. The results indicated that the expression of pro-inflammatory factors was significantly increased while the expression of anti-inflammatory factors was remarkably decreased in the CLP group (Figure 1J). Finally, RT-PCR found that the expression level of Mirt2 in the CLP group was decreased, compare with that in the sham group (Figure 1K).

Overexpression LncRNA Mirt2 Improved Cardiac Function and Reduces Inflammatory Response In CLP Rats

To test whether lncRNA Mirt2 has a protective effect on the heart, an AAV-lncRNA Mirt2 was first constructed, and then the virus was injected through the tail vein. Then, RT-PCR was used to detect the expression of lncRNA Mirt2 in the four groups of sham, CLP, CLP + AAV-lncRNA Mirt2, and CLP + AAV-NC. As a result, the expression of lncRNA Mirt2 was the highest in the CLP + AAV-lncRNA Mirt2 group (Figure 2A). Next, echocardiography was used to measure the cardiac function of the four groups of rats on the 5th day, and it was found that the CLP + AAV-lncRNA Mirt2 group had the most significant recovery of cardiac function on the 5th day (Figure 2B-2E). At the same time, the results of ELISA found that the serum levels of cTn I and CK-MB in the CLP group and CLP + AAV-NC group were the highest, while overexpression lncRNA Mirt2 could effectively inhibit the increase of cTn-I and CK-MB content (Figure 2F and 2G). On Day 5 of the tissue staining test, the heart tissue of the rat was found to have regular arrangement of myocardial fibers in the sham group, no degeneration and necrosis of the cells, and the lowest expression of IL-1β. The myocardial fiber structure of the CLP group and CLP + AAV-NC group changed, with cell shrinkage, nuclear shrinkage, widened intercellular space, and the highest expression of IL-1β, while the myocardial fibers in the CLP + AAV-lncRNA Mirt2 group were slightly disordered, cell degeneration and necrosis were reduced, and IL-1β expression was also reduced (Figure 2H). The results of WB also showed that the increased expression of pro-inflammatory factors IL-1β, IL-6, TNF-α and MPO and the decreased expression of anti-inflammatory factors IL- 4 and IL-10 caused by sepsis could be inhibited by overexpression lncRNA Mirt2 (Figure 2I). Next, the results of ELISA found that overexpression lncRNA Mirt2 significantly inhibited the increase of the expressions of pro-inflammatory factors, and promoted the anti-inflammatory factor expression (Figure 2J). At the same time, RT-PCR was used to detect the above-mentioned inflammatory factors, and the results obtained were similar to those of ELISA (Figure 2K).

MiR-101 Could Directly Target With LncRNA Mirt2

MiR-101 expression levels were first tested in the heart of the sham group and CLP group, and it was found that miR-101 expression was markedly increased in the CLP group (Figure 3A) and could directly bind to lncRNA Mirt2, thereby regulating downstream gene expression. Therefore, Dual-Luciferase reporter gene assay was used, and it was found that compared with that in the Mirt2 wt+miR-101 NC group, the luciferase activity in the Mirt2 wt + miR-101 mimic group was significantly reduced. There was no significant difference in Luciferase activity between the Mirt2 wt+miR-101 NC group and the Mirt2 mut + miR-101 mimic group. It was found that miR-101 could directly target Mirt2 (Figure 3B). Next, miR-101 expression was tested by RT-PCR after injecting AAV-lncRNA Mirt2 through the tail vein, and it as found that miR-101 expression was reduced in the AAV-lncRNA Mirt2 group (Figure 3C).

LncRNA Mirt2 Improved Cardiac Function and Inflammatory Response In CLP Rats by Regulating MiR-101

First, echocardiography was used to measure the cardiac function of CLP, CLP + AAV-lncRNA Mirt2, CLP + AAV-lncRNA Mirt2 + agomiR-101 four groups on the 5th day. As a

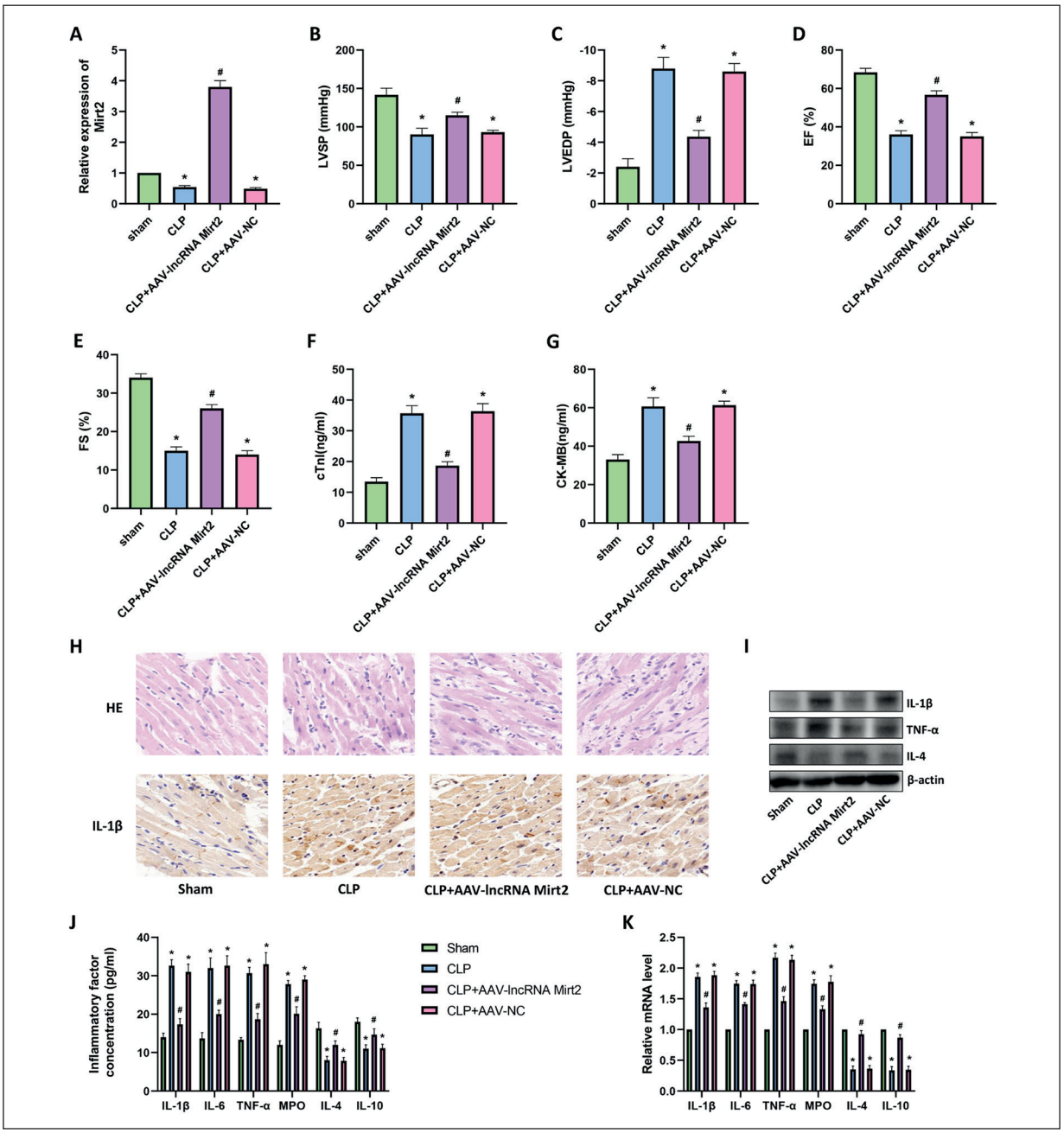

Figure 2. Overexpression lncRNA Mirt2 improved cardiac function and inflammatory response in CLP rats. **A,** RT-PCR detected lncRNA Mirt2 expression. **B,** Echocardiographic detection of LVSP value. **C,** Echocardiographic detection of LVEDP value. **D,** Echocardiographic detection of EF value. **E,** Echocardiographic detection of FS value. **F,** ELISA detected serum cTn I content. **G,** ELISA detected serum CK-MB content. **H,** HE staining and immunohistochemical staining, (magnification: 400×). **I,** WB detected IL-1β, TNF-α and IL-4 expressions. **J,** ELISA detected IL-1β, IL-6, TNF-α, MPO, IL-4 and IL-10 contents. **K,** RT-PCR detected IL-1β, IL-6, TNF-α, MPO, IL-4 and IL-10 expressions. ("*" indicated statistical difference from the Sham group $p<0.05$, "#" indicated statistical difference from the CLP group $p<0.05$).

result, it was found that miR-101 could inhibit AAV-lncRNA Mirt2 on the recovery of cardiac function in rats induced by sepsis, and had significant differences (Figure 4A-4D). At the same time, the serum levels of cTn I and CK-MB were tested, and it was found that the serum levels of cTn I and CK-MB were highest in the CLP group. The overexpression

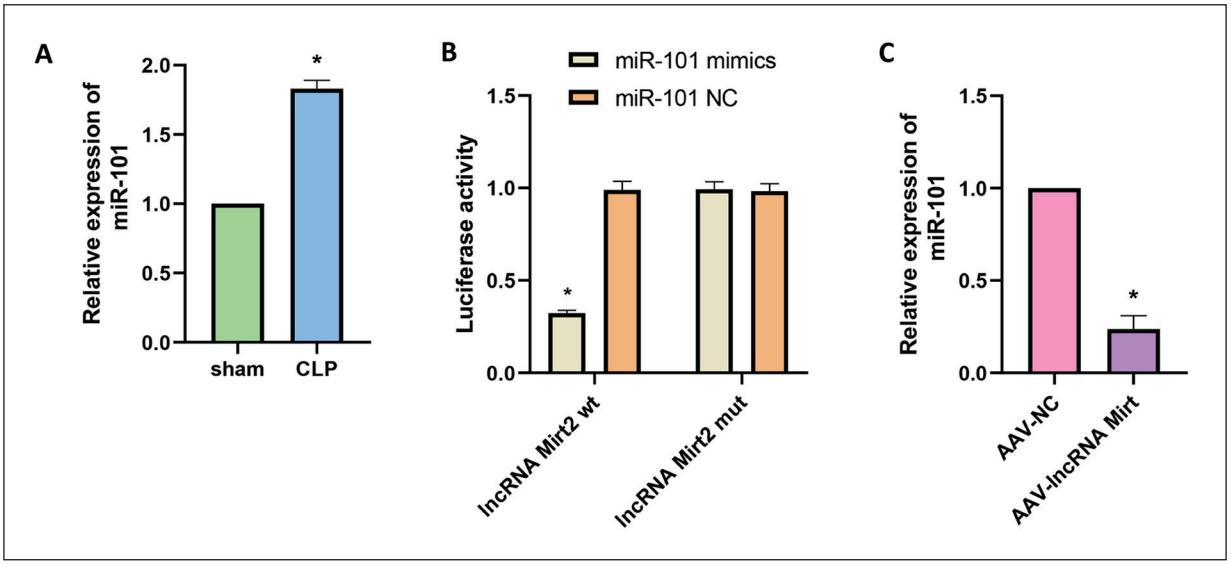

Figure 3. MiR-101 could be directly combined with lncRNA Mirt2. **A,** RT-PCR detected miR-101 expression. ("*" indicated statistical difference from the Sham group $p<0.05$). **B,** Luciferase reporting test. **C,** RT-PCR detected miR-101 expression. ("*" indicated statistical difference from the AAV-NC group $p<0.05$).

lncRNA Mirt2 could effectively inhibit the increase of serum cTn I and CK-MB levels, while agomiR-101 could effectively inhibit the protective effect of AAV-lncRNA Mirt2 on the heart (Figure 4E and 4F). On the 5th day of tissue staining test in rat heart tissue, it was found that AAV-lncRNA Mirt2 could significantly improve the structure of myocardial fibers, slow down cell shrinkage and widen intercellular space, and inhibit IL-1β expression, while agomiR-101 could effectively inhibit the protective effect of AAV-lncRNA Mirt2 on the heart, promote myocardial fiber arrangement disorder, cell degeneration and necrosis, and increase the expression of IL-1β (Figure 4G). For the detection of translation level, WB results also detected that both the increased expressions of pro-inflammatory factors IL-1β, IL-6, TNF-α and MPO and the decreased expressions of anti-inflammatory factors IL-4 and IL-10 caused by sepsis could be suppressed by overexpression lncRNA Mirt2, but this effect could be partially suppressed by agomiR-101 (Figure 4H). At the same time, the expression of inflammatory factors in serum was tested, and it was found that miR-101 could effectively inhibit the effect of AAV-lncRNA Mirt2 decreasing the pro-inflammatory factors expression and inhibiting anti-inflammatory factors expression increased (Figure 4I). Moreover, similar results were obtained by RT-PCR detection of the aforementioned inflammatory factors (Figure 4J).

Lnc Mirt2 Inhibited Inflammation Through the PI3K/AKT Signaling Pathway

The reason why PI3K/AKT signaling pathway is a hotspot in the regulation of the occurrence and development of cardiovascular diseases is that it plays an important role in the regulation of various cell functions (metabolism, growth, proliferation, survival, transcription and protein synthesis)[12,13]. The inflammatory response after myocardial injury can inhibit the PI3K/AKT signaling pathway. Thus, activating this pathway can effectively relieve myocardial inflammation and ventricular remodeling[14]. This may be related to the activation of this signaling pathway and the inhibition of the release of inflammatory factors such as tumor necrosis factor, thus reducing the inflammatory response of the damaged myocardium. Therefore, the expression of PI3K/AKT signaling pathway was detected. The results showed that p-PI3K and p-AKT protein expressions were significantly decreased in CLP group, and p-PI3K/PI3K ratio and p-AKT/AKT ratio were also significantly decreased. On the contrary, the expression of p-PI3K and p-AKT in CLP+ AAV-lncRNA Mirt2 group was significantly increased,

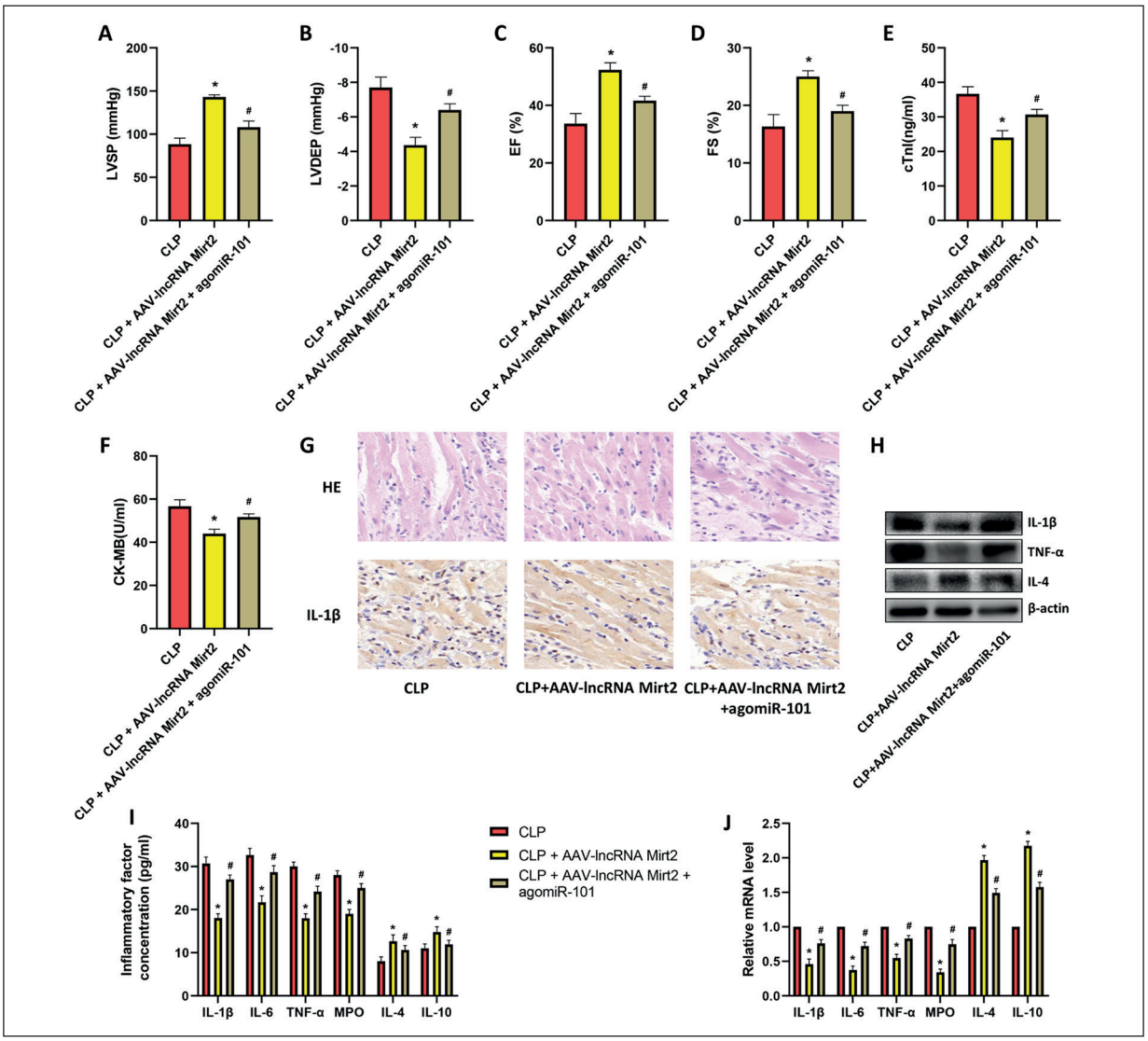

Figure 4. LncRNA Mirt2 improved cardiac function and inflammatory response in CLP rats by regulating miR-101. **A,** Echocardiographic detection of LVSP value. **B,** Echocardiographic detection of LVEDP value. **C,** Echocardiographic detection of EF value. **D,** Echocardiographic detection of FS value. **E,** ELISA detected serum cTn I content. **F,** ELISA detected serum CK-MB content. **G,** HE staining and immunohistochemical staining. (magnification: 400×) **H,** WB detected IL-1β, TNF-α and IL-4 expressions. **I,** ELISA detected IL-1*β*, IL-6, TNF-*α*, MPO, IL-4 and IL-10 contents. **J,** RT-PCR detected IL-1β, IL-6, TNF-α, MPO, IL-4 and IL-10 expressions. ("*" indicated statistical difference from the CLP group $p<0.05$, "#" indicated statistical difference from the CLP+AAV-lncRNA Mirt2 group $p<0.05$).

p-PI3K /PI3K ratio and p-AKT /AKT ratio were also significantly increased (Figure 5).

Discussion

One of the major fatal consequences of sepsis is the development of cardiac dysfunction, and the key predictor of survival in patients with sepsis is the degree of myocardial damage[15]. In recent years, the mortality rate of sepsis and related complications in intensive care units (ICUs) at home and abroad has increased year by year. Statistics show that there are more than 600,000 sepsis patients in North America each year, with a mortality rate of 30-50%. In China, ICUs have a high incidence of sepsis, occupying a large amount of medical resources, and sepsis often evolves into adverse clinical outcomes[16,17].

The immune response to sepsis is extremely complex. In the early stages of sepsis, the innate

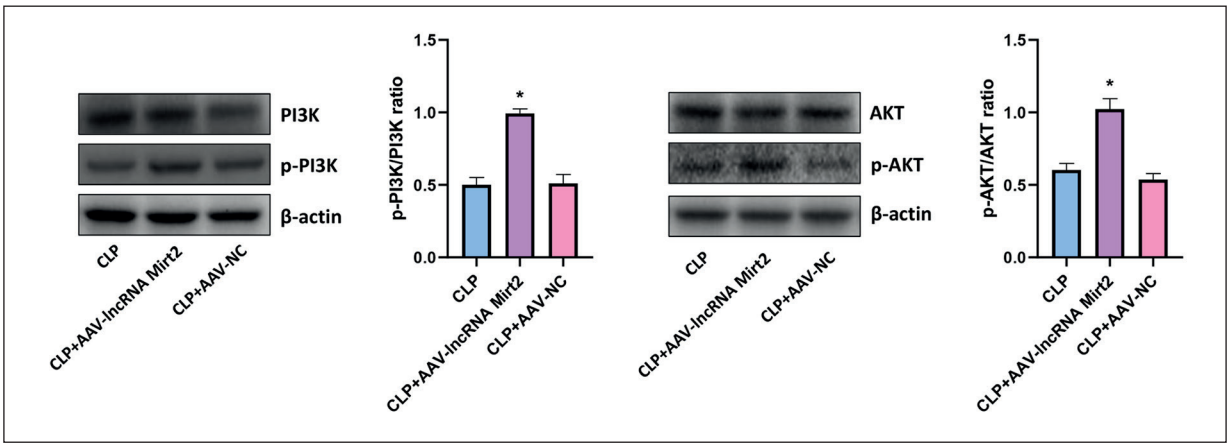

Figure 5. Lnc Mirt2 inhibited inflammation through the PI3K/AKT signaling pathway. WB detected PI3K, p-PI3K, AKT and p-AKT expressions, ("*" indicated statistical difference from the CLP group $p<0.05$).

immune response of the innate body is hyperactive, manifested as a cascade of cascades of inflammatory factors. The inflammatory cells involved are mainly neutrophils and monocytes/macrophages, which are also the body's first line of defense[18]. In fact, when Gram-negative bacilli invade the body, endotoxin LPS interacts with Toll like receptors (TLRs), many signaling pathways such as mitogen-activated protein kinase (MAPK) are activated, eventually inducing an inflammatory response[19]. In addition, adaptive immunity is also involved in the pathogenesis of sepsis[20]. The inflammatory cells involved are B lymphocytes and T lymphocytes. The hypothesis of compensatory anti-inflammatory response syndrome is that the imbalance between pro-inflammatory and anti-inflammatory responses in the body is involved in the occurrence and development of sepsis. When TLRs signaling pathway is activated, T helper cell 17 (Th17) can produce a large number of high mobility group protein 1 (HMGB-1), IL-1β, IL-6, TNF-α, which in turn causes the body's pro-inflammatory response. T Helper cells 2 (Th2) and Regulatory T cells (Treg) release anti-inflammatory mediators such as IL-4 and IL-10 to prevent pro-inflammatory reactions in the body. The imbalance of anti-inflammatory response and pro-inflammatory response leads to aggravation of systemic inflammatory response[21]. In the late stage of sepsis, due to the apoptosis of a large number of immune cells, the body is in an "immune paralysis" state, and its ability to resist external pathogens has decreased. Bacteria and bacterial pathogens can infect the body and repeatedly increase body damage.

Non-coding RNAs (ncRNAs) participate in the immune response during sepsis by regulating gene expression at multiple levels, including transcription and translation[22]. In the LPS-induced sepsis model, the activation of RLR4 undergoes signal transduction, activating protein kinases IRAK-1 and TRAF-6, and then forming a waterfall-like cascade of inflammatory factors by activating (nuclear factor-k-gene binding) NF-kB[23]. Taganov et al[24] found that the expression of miRNA-146a in LPS-activated monocytes was up-regulated and negatively regulated the expressions of IRAK-1 and TRAF-6, suggesting that miRNA-146a may be a NF-kB dependent gene, which negatively feedback regulates immune signal transduction and inhibits the release of inflammatory factors. During the body's immune response, lncRNAs can regulate the secretion of inflammatory factors, whose damage in LPS-induced sepsis mice is characterized by increased intestinal microvascular permeability, interstitial edema, leukocyte infiltration, and plasma TNF-α, IL-1β, myeloperoxidase (MPO), intercellular adhesion molecule-1 (ICAM-1) abnormal expressions. In addition, the inducible nitric oxide synthase (iNOS) mRNA expression and NF-kB protein expression showed also apparent elevated tendency[25]. LncRNA Mirt2 is a member of ncRNAs involved in the pathophysiology of various diseases in the body. As a negative regulator of inflammation, lncRNA Mirt2 is concerned by researchers, and lncRNA Mirt2 participates in the regulation of inflammatory response through interaction with miRNA-101. In this research, it was found that overexpression lncRNA Mirt2

could reduce the secretion of pro-inflammatory factors and increase the anti-inflammatory factors by inhibiting the expression of miRNA-101, thereby alleviating CLP-induced changes in cardiac structure and function.

MiR-101 inhibits tumor cell proliferation by blocking the PI3K/AKT signaling pathway by targeting MALAT-1[26]. The current research also detected an association between miR-101 and the PI3K/AKT signaling pathway, and the cross talk between Lnc Mirt2 and miR-101 was mainly explored. It was found that miR-101 inhibited the inflammatory response by activating the PI3K/AKT signaling pathway. This study will provide a new target for the treatment of sepsis.

Conclusions

This study investigated the effects of lncRNA Mirt2 expression on sepsis inflammation and cardiac function from animal experiments. By comparing the expression levels of lncRNA Mirt2 in the hearts of sepsis rats and sham rats, it was found that the expression of lncRNA Mirt2 was reduced in sepsis rats. Adenoviral vector transfection technology up-regulated the expression of lncRNA Mirt2 in a sepsis rat model, which could reverse the excessive secretion of pro-inflammatory factors and improve cardiac contractility through the PI3K/AKT signaling pathway. It is suggested that lncRNA Mirt2 is involved in the regulation of sepsis inflammation and cardiac function, and may provide a heart target for the diagnosis and treatment of sepsis.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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