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Over-expression of miR-486/miR-150 in sepsis: marker in myocardial depression function

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Abstract

Numerous biomarkers have been studied to identify the cause and severity of sepsis MicroRNAs (miRNAs) are non-coding RNA transcripts that regulate the expression of genes by repressing translation or degrading mRNA. Sepsis is a life-threatening condition and the major cause of mortality among patients in intensive care units, including acute phase proteins such as C-reactive protein (CRP). However, the lack of specify of these more frequently used biomarkers impedes the significant requirement to identify novel biomarkers for early sepsis detection. The role of miR-146a in the physiopathology of sepsis in myocardial depression continues to be poorly understood. miR-486/miR-150 may be used as a potential marker to difference sepsis induces myocardial function from non-sepsis causes.

Keywords: Myocardial depression; Biomarkers; mRNA; Sepsis

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Introduction

Sepsis is a medical condition characterized by a severe systemic inflammatory response due to an infection. At present, sepsis is the leading cause of death among patients admitted to the intensive care units (ICU) [1]. About 28.3% to 41% of all sepsis patients do not survive because of multiple organ failure. Indeed, sepsis starts with an initial systemic inflammatory response (SIRS), followed by severe sepsis with multiple organ dysfunction before progressing to septic shock with persistent hypotension. Moreover, sepsis might be misdiagnosed as the SIRS because the initial inflammatory response could also be caused by non-infectious substances such as burns, trauma and acute pancreatitis. There is thus a critical need to develop more accurate biomarkers for the diagnosis of sepsis [2].

A primary miRNA transcript (pri-miRNAs) is usually formed through transcription by RNA polymerase II in the nucleus as a long capped precursor miRNA or through maturation from introns [3]. The ribonuclease (RNase) III Dorsha enzyme and the DiGeorge Syndrome Critical Region 8 (DGCR8) protein form a microprocessor complex to process the 70–100 nt premature miRNA (pre-miRNA) from the pri-miRNA [4]. With the help of nuclear export transporter

Research Article doi: 10.18081/2333-5106/015-06/313-324

Exportin 5, the pre-miRNA is exported into the cytoplasm. At its terminal loop, the pre-miRNA interacts with RNase III endonuclease Dicer protein and the co-factor double-stranded transactivation-responsive RNA-binding protein (TRBP) to process the ~ 22 nt miRNA duplex [5].

The miRNA duplex is integrated into the RNA-induced silencing complex (RISC) which comprises of an argonaute (Ago) protein and a glycine-tryptophan repeat-containing protein of 182 kDa. (GW182). Previously, it was assumed that one strand at the 3′ end of the miRNA duplex (commonly called the "passenger strand") is released and degraded, while the other strand (called "guide strand") is remained in the RISC complex [6]. However, it has been demonstrated that both strands can be processed into mature miRNA and utilized for translational repression [7]. Mature miRNAs processed from the 3′ end are designated with a 3p suffix while miRNAs processed from the 5′end are identified with a 5p suffix. The RISC complex is directed to a 3′ or 5′ untranslated region (UTR) of an mRNA where there is base pairing with 2 to 8 nucleotides (called "seed sequence") of the mature miRNA [8].

Over the past decade, numerous studies have been conducted to identify biomarkers that are sufficient to define the stage and severity of sepsis and predict the best line of treatment. The major challenge is that these studies cannot identify the cause of the systemic inflammatory response between SIRS and sepsis [9].

Currently, biomarkers such as C-reactive protein (CRP), procalcitonin (PCT), Interlukin-6 (IL-6), sTREM-1, amongst others are being studied for their potential to detect SIRS caused by an infection. CRP, which is an acute phase protein found in the liver, is known to help in the removal of pathogens during infection [10]. PCT, a prohormone of calcitonin found in C-cells of the thyroid gland, are released in huge quantities into the blood stream during infection [11]. sTREM-1 is triggering receptors expressed on myeloid cells-1 which are released into plasma by phagocytes during bacterial or fungi infection [12]. These factors have potential to be an indicator of an infection-induced inflammatory response but experimental data have revealed they are also present during a non-infectious inflammatory response.

Materials and Methods

Rat Model of Septic Shock

This study was approved by the ethical committees of American Cancer Society Surveillance and Health Services Research, and all experiments conformed to the relevant regulatory standards. Intraperitoneal injection of LPS was used to establish a rat septic shock model as described previously [13]. Briefly, male adolescent rats weighing 170 g to 190 g were anesthetized with 20% urethane (1 g/kg, 1 g urethane was added to 5 mL 0.9% saline). We separated the left femoral artery and connected it to the electrophysiological recorder (Biopac MP150 Biopac Systems, Goleta, CA, USA) to monitor the pressure and injected LPS intraperitoneally to establish the model (20 mg/kg, 10 mg LPS was added to 1 mL 0.9% saline) (Escherichia coli 055: B5, L-2880, Sigma-Aldrich, St. Louis, MO, USA). Control rats were

Research Article doi: 10.18081/2333-5106/015-06/313-324

treated with an equal volume of saline. Septic shock is established when the mean arterial pressure has decreased by 25% to 30%. Samples from the left ventricle were collected and divided into two parts. One part was fixed in 4% paraformaldehyde (PFA) for sectioning, and the other was stored at −80°C for qPCR and western blot.

Hematoxylin and Eosin (H&E) Staining

The heart samples were fixed in 4% paraformaldehyde (PFA) for 48 hours following dehydration and permeabilization, embedded with paraffin, and sectioned into 4 μm slices. These sections were deparaffinized, stained with hematoxylin for 10 minutes, and then stained with eosin for 1 minute. The sections were dehydrated through a graded alcohol series and observed under a light microscope at 40× magnification.

Terminal Deoxynucleotidyl Transferase dUTP Nick-End Labeling (TUNEL) Assay

H9C2 cardiomyocytes were fixed with 4% formaldehyde for 10 minutes after washing with PBS three times. Next, cells grown on coverslips or heart sections were subjected to the TUNEL assay according to the manufacturer's protocol (Wanlei Bio, Shenyang, China). The apoptotic cells were visualized by fluorescence microscopy.

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

Total RNA from the heart samples was extracted using RNAiso Plus (TaKaRa, Tokyo, Japan). cDNA was synthesized by reverse transcription with 1 μg total RNA using the PrimeScript™ RT Reagent Kit with gDNA Eraser (Perfect Real Time) (TaKaRa). RT-qPCR was performed using a 7500 Real-Time PCR System (Applied Biosystems, Waltham, MA, USA) according to the TB Green® Premix Ex Taq™ II (Tli RNase H Plus) kit instructions (TaKaRa). GAPDH was used as an endogenous control. The first strand of miR-150-5p was synthesized using the miRNA First Strand cDNA Synthesis Tailing Reaction Kit (Sangon Biotech, Shanghai, China) with 2 μg total RNA. RT-qPCR was performed as mentioned above.

The total protein from the tissue was extracted using a cell lysis solution (Beyotime Institute of Biotechnology) and phosphatase inhibitor (Meilun Bio, Dalian, USA) and quantified using the Bicinchoninic Acid (BCA) Protein Assay Kit (Beyotime). An equal amount of protein (40 mg/well) was separated by 10% and 12.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride membranes for 1.5 hours at 100 V. The membrane was then blocked with 5% BSA at room temperature for 1.5 hours. GAPDH, akt2, p-akt2, cleaved caspase3, bax, and bcl-2 primary antibodies were incubated with the membranes at 4°C overnight (Akt2; 1 : 200; Santa Cruz Biotechnology, Santa Cruz, CA, USA; p-Akt2; 1 : 500; Abcam, Cambridge, MA, USA; cleaved caspase3; 1 : 500; Cell Signaling Technology, Danvers, MA, USA; bax and bcl-2; 1 : 500; Proteintech; GAPDH; 1 : 10000; Abways). Subsequently, the membranes were incubated with the appropriate horseradish

Research Article doi: 10.18081/2333-5106/015-06/313-324

peroxidase-conjugated secondary antibody (Proteintech) at room temperature for 2 hours. The blotting was visualized using enhanced chemiluminescence (ECL detection kit, KeyGEN Biotech, Jiangsu, China) on the c300 Chemiluminescent Western Blot Imaging System (Azure Biosystems, Dublin, GA, USA).

Septic Shock-Induced Myocardial Apoptosis and Differentially Expressed MicroRNAs in Septic Shock Rats

As mentioned previously [14], our group has found that there is a positive relationship between the MAP and heart function. In this study, septic shock occurred at approximately 2 hours after LPS injection, and the MAP of the control groups remained unchanged (Figure 1(a)). H&E staining of the left ventricles showed that after 3, 6, and 12 hours of LPS treatment, infiltration of inflammatory cells occurred, there were changes in cell morphology and disordered cell arrangement with delivery time extension (Figure 1(b)), and the injury was most serious at the 12-hour time point. TUNEL-positive cells were increased in the LPS groups compared to the control groups (Figure 1(c)). Additionally, PCR also showed that the expression of caspase3 was increased in the heart of septic shock rats (Figure 1(d)), and similar results were observed when the protein level of cleaved caspase3 was measured (Figure 1(e)).

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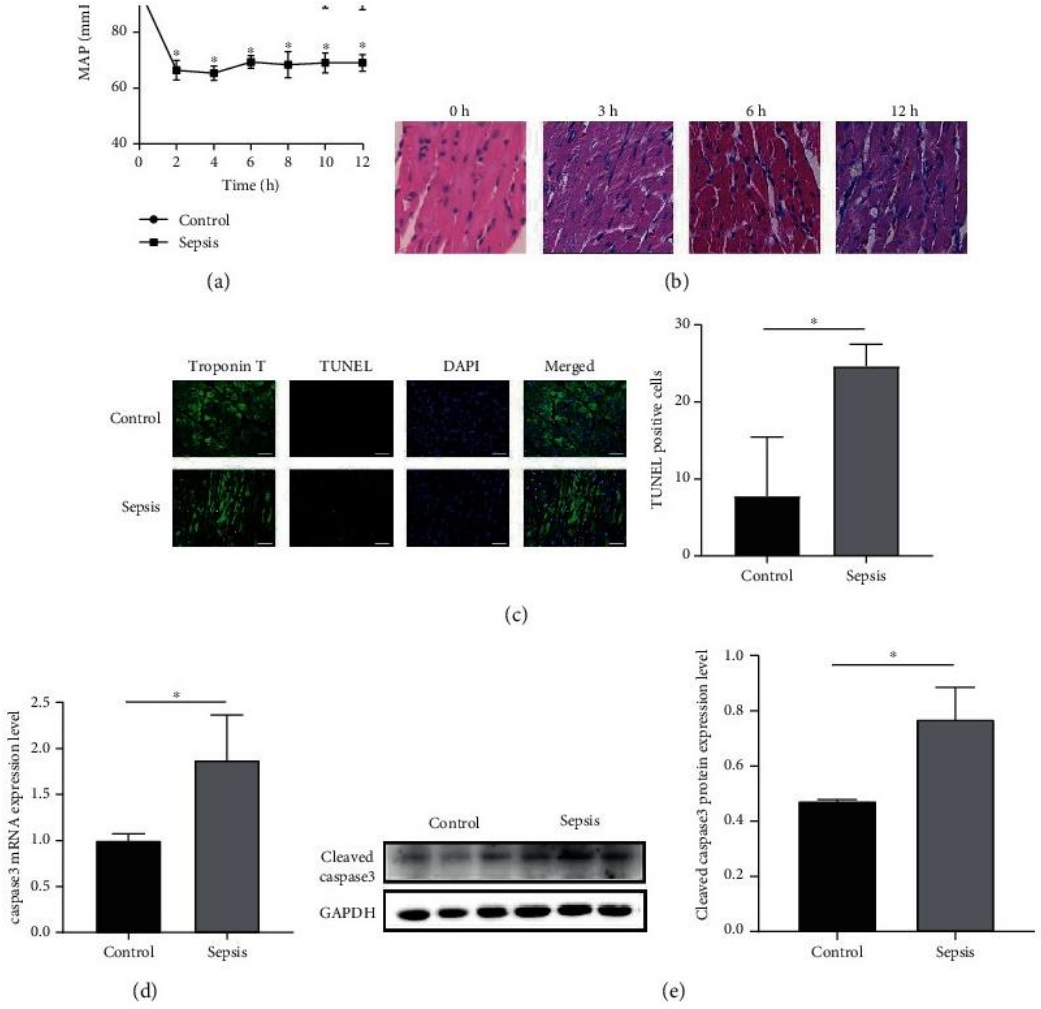

Figure 1.

Establishment of the rat septic shock model. (a) Mean arterial pressure of rats from the control and sepsis groups. (b) Pathological changes in rat heart tissues from the control and sepsis groups after LPS injection for 3, 6, or 12 hours by H&E staining. The following results were obtained from rat heart tissues 12 hours post-LPS treatment. (c) Apoptosis was detected using the TUNEL assay. Green represents the cardiomyocytes, red represents the TUNEL positive cells, and blue represents the cell nucleus. (d) The expression of caspase3 mRNA was analyzed by qPCR. (e) The expression of cleaved caspase3 protein was assayed by western blot. N = 10. Data are presented as mean ± standard deviation, repeated for three times. $^{*}P < 0.05$ compared to the control.

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Effect of miR-328 on inflammatory responses in sepsis rat model

The expression levels of TNF-α, IL-6, and IL-1β were significantly increased in the CLP group compared with the sham group, indicating that sepsis promoted inflammation. However, injection of miR-328 antagomir reduced the inflammatory response, resulting in decreased levels of TNF-α, IL-6, and IL-1β expression (P<0.01, Figure 2A–C). The results of this study demonstrated that miR-328 affects changes in the inflammatory response in sepsis.

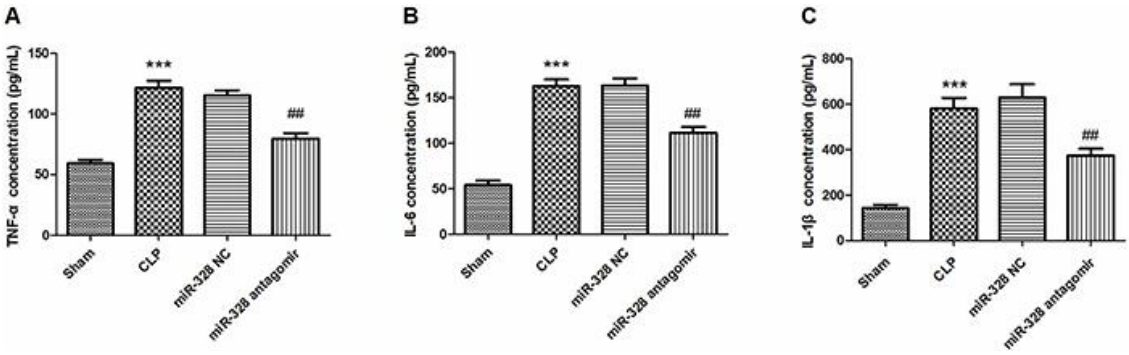

Figure 2.

Changes in inflammatory factors in the sepsis rat model by cecal ligation and perforation (CLP) and after injection of miR-328 antagomir. Data are reported as means±SD. ***P<0.001, compared with sham group; ##P<0.01, compared with CLP group (Student's *t*-test). NC: negative control. TNF-α: tumor necrosis factor-α; IL: interleukin.

Effect of miR-328 on cardiac dysfunction in sepsis rat model

The expression of miR-328 was significantly up-regulated in rat tissues and serum after CLP modeling, but the high expression was reversed when miR-328 antagomir was injected (P<0.01, Figure 3A and B). In addition, compared with the sham group, LVSP and $+dp/dt_{max}$ decreased significantly in the CLP group, while the levels of $-dp/dt_{max}$, LVEDP, cTnI, and CK-MB were significantly increased (P<0.01, Figure 3C–G). The experimental results showed that myocardial dysfunction occurred in the rat model of sepsis. However, when miR-328 antagomir was injected, myocardial dysfunction in sepsis rats was reversed, LVSP and $+dp/dt_{max}$ were significantly increased, levels of $-dp/dt_{max}$, LVEDP, cTnI, and CK-MB were significantly decreased (P<0.001, Figure 3C-G).

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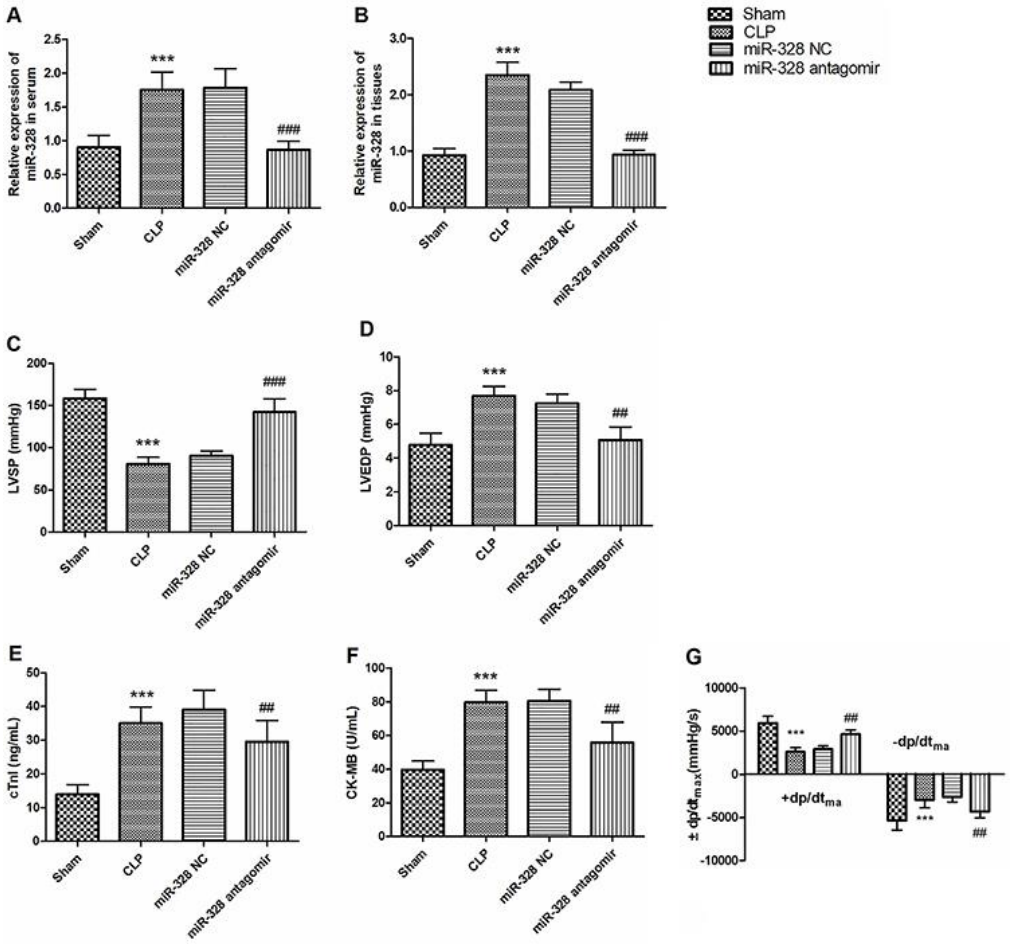

Figure 3.
Effect of miR-328 on cardiac dysfunction in a rat model of sepsis. A and B, Changes in expression levels of miR-328 in serum and myocardial tissue after establishment of a rat model of sepsis and after injection of miR-328 antagomir. C-G, Modeling of sepsis in rats and changes in cardiac hemodynamics and serum myocardial injury after miR-328 antagomir injection. Data are reported as means±SD. ***P<0.001, compared with sham group; ##P<0.01, ###P<0.001, compared with CLP group (Student's *t*-test). CLP: cecal ligation and perforation; NC: negative control; LVSP: left ventricular systolic pressure; LVDEP: left ventricular end-diastolic pressure; cTnI: serum cardiac troponin I; CK-MB: myocardial kinase isoenzyme; $\pm dp/dt_{max}$: maximum rate of increase/decrease in left ventricular blood pressure.

Discussion

Sepsis-induced myocardial depression has received much attention recently due to its high risk of mortality. Because of the high mortality, it is necessary to elucidate the mechanisms underlying its pathogenesis. Numerous studies, including basic research and clinical trials, have confirmed that many regulatory factors play a crucial role in the pathogenesis of sepsis-induced myocardial depression [14]. Apoptosis has been demonstrated to be one of the main causes of decreased heart function [15-17]. We have observed apoptosis in the cardiomyocytes of septic shock rats and H9C2 cardiomyocytes stimulated with LPS, and we have also confirmed that apoptosis plays a more significant role in sepsis-induced myocardial depression

in children [18]. In the present study, we also observed similar results; the apoptotic rate and the expression of cleaved caspase3 protein were increased in LPS-treated rats compared to the control group. These results indicate that cell apoptosis occurs during sepsis-induced myocardial depression, but the specific mechanism by which this occurs is unclear [19-22]. Previous studies have demonstrated that miRNAs participate in sepsis-induced myocardial depression in various ways. One is miRNAs regulate target genes expression negatively at posttranscription level. It has been reported that in the rats septic model injected by LPS for 24 h, the expression of miR-146a increased, which inhibited the activation of toll-like receptor 4/nuclear factor kappa-B (TLR-4/NF-κB) signaling pathways and alleviated the cardiomyocytes apoptosis and injury caused by sepsis. The other is that miRNAs involve in the pathogenesis of diseases as a downstream molecule of long noncoding RNAs (lncRNAs) [23].

Conclusion

miR-328 was highly expressed in the serum of patients with sepsis, and down-regulation of miR-328 can alleviate cardiac dysfunction and inflammatory response in sepsis. miR-328 can be used as a diagnostic marker for sepsis for early diagnosis and treatment.

Competing interests

The authors declare that they have no competing interests.

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