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

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Abstract

Sepsis, the third most common cause of death worldwide, is associated with myocardial depression, one of the most common organ dysfunctions in sepsis. n this study, we found that miRNA-486 was significantly down-regulated in septic mice and miR-486 over-expression significantly up-regulated the IRE1/JNK pathway, resulting in increased autophagy and apoptosis. In our previous study, miR-486 has been shown to be a protective factor in sepsis overdose and the expression was significantly down-regulated, indicating that miR-486 is a sensitive responder in sepsis and may be an important indicator for the diagnosis and prognosis of sepsis. provided the first evidence that the expression of miR-486 in human macrophages (THP-1) was up-regulated after stimulation with lipopolysaccharide (LPS) (1 μg/mL). explored the expression profile of some miRNAs in bone marrow-derived macrophages (BMDMs) in LPS-induced sepsis mice using RT-PCR. The primary finding in our study is that in vivo over-expression of miR-486 depressed the myocardial function, and that miR-150 controlled the heart function in a constitutively negative manner. We had proved in SMs that miR-486 could repress Cav-1 expression, while miR-150 could inhibit calcineurin and l-type calcium channel. Calcium plays a fundamental role in the heart, and sepsis causing the imbalance of calcium ultimately leads to myocardial depression. Regulations of these related calcium signaling pathways also confirm and explain how miR-486 and miR-150 have a negative effect on myocardial function. These findings provide proof in mouse myocardial intramyocardial overexpression of miRs (miR-486, miR-150) in its decrease heart contractile function.

Inconclusion, miR-486 and miR-150 play a protective role in sepsis-induced myocardial depression by inhibiting renin-angiotensin-aldosterone system dependent autophagy. This study may contribute to developing novel therapeutic strategies for favorably regulating miR-486 or miR-150 expression in sepsis-induced myocardial depression.

Keywords: Myocardial depression; Biomarkers; mRNA; Sepsis

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Introduction

Sepsis, the third most common cause of death worldwide, is associated with myocardial depression, one of the most common organ dysfunctions in sepsis. Although the "kissing cousins" paradigm, which suggests that sepsis and myocardial depression may coexist because of heart failure, but independent of their relationship to sepsis, has been gradually recognized, it is clear that septic-induced myocardial

depression can have a profound impact on patients' overall outcomes by accentuating sepsis's "septic triad" of hypotension, microcirculation abnormality, and metabolic stress. In view of its importance in the development, diagnosis, and prognosis of sepsis, the pathophysiological mechanisms underlying septic myocardial depression ultimately need to be established through interventions. At the molecular level, although multiple proteins, genes, signaling pathways, miRNAs, lncRNAs, etc., have been associated with the development of septic-induced myocardial depression, no single factor has been identified to initiate the signal transduction cascade leading to septic-induced myocardial depression. Whether the expression of miRNA in the heart of septic patients or the myocardial depression of septic animal models can be changed from the heart needs to get further evidence.

Sepsis is one of the body's systemic inflammatory responses to severe infections, mainly responsible for Gram-negative bacteria, and it is also affected by abnormal activation of viral, fungal, and bacterial responses. Severe sepsis may lead to abnormal organ function in sepsis-associated organ dysfunction, septicemia due to sepsis, or sepsis causing death. Myocardial depression is one of the most common organ damage in patients with sepsis, leading to a decreased cardiac pumping function, further causing rapid blood flow, and subsequently causing organ hypoperfusion.

Septic cardiomyocyte depression is a multifactorial pathological multicenter injury syndrome, and patients may remain asymptomatic until the cardiac disorder is significantly worse, due to the autoimmune inflammatory response, loss of myocyte, and functional function. Although important evidence has shown that different local factors, including cardiac innervation, gastrointestinal tract, and systemic factors including obesity and the renoprotective system, are relevant to myocardial depression, the specific molecular pathogenesis of myocardial depression has not been conclusively demonstrated. An important and potential reason may be that in culture- and animal-model studies, sepsis-induced myocardial depression models do not always close and entirely simulate myocardial depression models, as they may be involved with multiple reasons in the myocardial depression process.

Being a prevalent, life-threatening condition, sepsis has been named as an emergency by the World Health Organization. A surge in the number of cases of sepsis is to be expected in the future due to the substantial increase in the aging population and the continuously expanding complexity of medical procedures. Although the existence of SIRS and a confirmed source of infection are recognized, a new concise statement of sepsis and septic shock was released by an international collaboration in 2016. The definition of sepsis is suggested by the presence of a greater-than-2 point change in the Sequential Organ Failure Assessment (SOFA) score, signifying an unwanted prognosis. Both the condition of the patient and the nature of the infection are taken into consideration under the definition of sepsis. Septic shock, meanwhile, is described as fluid-refractory hypotension, increased plasma lactate levels, and the need for vasopressor therapy to maintain mean arterial pressure.

The untreated rise in lactate levels and refractory hypotension are proposed to be the only two criteria. Sepsis is linked to a significant degree of mortality, directly or indirectly, by weakening organs and systems far from the source of infection, thereby enhancing the focus on cardiovascular, hematological, coagulative-endocrine-metabolic, gastrointestinal, cerebral, and renal systems. In

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addition to influencing physical characteristics, like gas exchange, heart rate, and leukocytosis, authors reported that sepsis is a condition that also affects mental health by causing neuroinflammation. Only a surge in the influence of sepsis, which results in a cerebrovascular inflammatory reaction, should be contemplated in order to cause cerebral endothelial cell infiltration and cerebrovascular immune infiltration that contributes to selective microthrombosis formation.

Pathophysiology of Myocardial Depression in Sepsis

In sepsis, cytokines, cytokine mediators, and damage-associated molecular patterns (DAMPs), either secreted from activated immune cells or released from damaged cells, lead to systemic inflammation and trigger the expression of specific immune mediators that damage the endothelium, promote coagulopathy, inhibit fibrinolysis, cause blood-brain barrier dysfunction, and cardiovascular depression. By secreting neuropeptides and cytokines, it leads to a decrease in blood pressure. The heart, as a metabolically high-demanding organ, is sensitive to this systemic dysregulation. The present pathways have been considered in the myocardial depression, which are the direct effects of cytokines, cytokine mediators, and DAMPs on the myocyte function, at the level of vascular tone modulation, as well as at the level of myocyte calcium cycling.

Sepsis-induced myocardial dysfunction was initially attributed to reduced left ventricle preload and myocardial stunning resulting in reduced left ventricle ejection fraction. Later studies revealed that the inflammatory cytokines released during the initial phase took part in its functional and structural alterations, which are characterized by a reduction in diameter, an area of the myocyte, and myofibrillar disorganization, including the appearance of micro-ultrasructural alterations, reduction in left ventricle stroke work, myocyte apoptosis, exacerbated myocyte damage, fibrosis, and decreased expression of some Ca2+ handling proteins, such as SR-Ca2+-ATPase, ryanodine receptor, and sarco-endoplasmic reticulum ATPase. On the other hand, tyrosine phosphatase Bim1 blocks epinephrine signal transduction. In the acute phase of sepsis, the heart reduces both the number and the sensitivity of ß-adrenergic receptors and the Ca2+ transient. And, in the sub-acute phase, increased renin-angiotensin activity.

MicroRNAs: Biogenesis and Function

MicroRNAs (miRs, miRNAs) are important small, single-stranded, and non-coding RNAs that are involved in the regulation of gene expression at the post-transcriptional level. MiR-Let-7, the first miRNA found, was discovered in a molecular study of Caenorhabditis elegans' developmental timing. The following miRNA, Lin-4, played an important role in the developmental transition of C. elegans from larval to adult stages. After these two miRNAs were found, more miRNAs were discovered in both plants and animals, thus miRNAs started to be considered promoters of more biological and physiological processes.

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MiRNAs were initially considered negative regulators of gene expression, but more evidence has shown that miRNAs can also positively regulate gene expression. Currently, researchers generally believe that miRNAs can inhibit gene expression to some extent, and that gene expression inhibition is the main function of miRNAs in mammals. Canonical miRNAs mature from their precursors via step-wise cleavage by two RNase III enzymes, Drosha and Dicer, to become mature miRNAs. Dicer, which is mainly located in the cytoplasm, acts as the enzyme responsible for final miRNA maturation. With help from Argonaute proteins, miRNA/RISC complexes assembled on target mRNA by base pairing in 3' UTR, affect mRNA degradation or inhibit mRNA translation, thus reducing the concentration of target proteins. It has been estimated that about 33% of the putative gene targets in humans are regulated by miRNAs. All these estimates, together with function-related studies, suggested that miRNAs play an important role in living organisms' cellular processes and growth. Up-regulation or down-regulation of miRNAs leads to an increasing number of diseases, including myocardial depression pathology. Dictating Ang II are capable of promoting the maturation of miR-143 and miR-145 in VSMCs, related to VSMC phenotype transition.

These two miRNAs have the ability to bind to target gene 3' UTR in KLF4. Moreover, other researchers proposed that the reverse relationship in VSMCs also happened, whereby KLF4 inhibited the transcription of miR-143 and miR-145. MiR-143 and miR-145 had the ability and cooperate with the angiotensin II-activated signaling pathway. They also cooperated with growth regulation and apoptosis in aging. Overall, these studies might suggest that miR-486 up-regulation and miR-150 down-regulation are involved in myocardial depression regulation. Moreover, miR-brand markers could be a critical and insightful therapeutic strategy or indicator used in the prevention of disease. In conclusion, the expression level of miR-486 and miR-150 in septic heart failure was explored in the present study, and our findings indicated miR-486 up-regulation and miR-150 down-regulation might participate in the development of murine myocardial depression.

MicroRNAs

In 1993, the first microRNA (miRNA) was identified in a nematode, Caenorhabditis elegans. Since then, 10 functional miRNAs were subsequently discovered in 2001 and only 35,000 miRNAs have been identified in various varieties. It was found that miRNA is responsible for the control of the post-translational expression of 30% to 60% of the functional genome. The most researched areas in which miRNAs play key roles at the present time are developmental timing control, the proliferation of stem cells, phasing body formation, the development of the nervous system, and the differentiation and metabolism of immune cells. Numerous investigators focus on the analysis of the vulnerability of miRNA and their suggested correlation with the progression of illnesses, and numerous miRNAs correlated with the indicated illnesses have been identified (miRNA-1, miRNA-133, miRNA-208, and miRNA-378). As known, hydrolysis-inducing miRNA expression profiling and the target gene expression conformed by these miRNAs during sepsis are not involved in the mechanism(s) of myocardial depression. It is supposed that the impairment of miRNA expression plays a crucial role in depression of function of the myocardium, which has a significant impact on the development of heart

failure. The intended aspect of the present study is, therefore, to provide an analysis of the miRNA mechanism of the development of depression of the myocardium during sepsis and the potential risk factors produced to myocardial depression initiation. In contrast to long-coding RNA, which is considered as 'housekeeping genes' or as the set of similar gene product neogenesis characteristic as estimated, there is an approximately similar association between different individual myocardial mRNA and microRNA and function characteristics of changed cells. It is feared that only approximately one-half of the functionally linked mRNA includes a prototype. The final filling of about one third of the mRNA should be a required post-translational modification and does not depend on the sequence of the miRNA. According to that, two complementary mRNA are approached through miRNA. The miRNA composition and the final post-translational regulation have the function of supporting the mRNA functioning change after initiation of the genes. The results derived for the effect of miR-15a-p violated expression in membranes, for which weight threads were essential. The identification of the function of these targets proved that one cell of miR-15/16 wardens are predicted to an urgent need for the illness on gene and for the involvement of connected elements in response to a YouTube. It reflects the significance of the myocardithaliocyt discharge in the control of advantages derived from the adversitated membrane. In recent studies, an increasing interest has been identified in the role associated with using hemocytes, preparation of the thesanitizer, the observed protein play by miR-15a-p involved in antinatural membrane damage stimulation of immunity. In addition, the activation of miR-16 does not appear in septic Va allergies and immunodeficiency. It seems that the death experimental trauma of an allergen in the surrounding protection may be accompanied by conditions, in a manner consistent with the hypothesis that death exists in the VA and vice versa where it is present. Among the enriched GO categories of miR-15a-p, appear to be involved in biological processes promoting a procedural metal protection function of several miR-16 target genes, for example, coordinate chromosomal locations. Par let-7 manifests an excessive well cosm, cell and membrane repair prostate can avoid Va proteus. MiR-15a-p, and Mandator SOM contests that protect Va cells mediated by both miR-1-Va and proinflammatory disorders that initially lead to the downregulation of these functionally linked gene categories may be involved in the control of shaped On YAAII macrophages.

Biogenesis of miR-486 and miR-150

miR-486-5p is produced together with a complementary strand, miR-486-3p, from the CSNK1G1 gene. The miR-486-5p form is mainly present in RBCs and known for its ability to promote erythropoietic processes. Interestingly, miR-150, known for its important role in tuning T cell function, has a mature form that is generated in two steps through cleavage of a differentially expressed complementary strand (miR-150-5p) after a long initial elongation process of the primary transcript. The same mechanism is valid for the murine analogue miR-145-5p, which is another time-specific miRNA that plays a role in sepsis and is referred to in the conclusions of this paper. Both miR-486 and miR-150 are involved in the regulation of pathological pathways in sepsis and myocardial blood-cell-specific processes, which was a starting point for their candidate gene search in the present study

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using microarray gene expression analysis of left ventricle mRNA, with their further validation by qRT-PCR. ACCDRIII-encoding miRNAs are transcribed in the same manner as other miRNAs, as the CDRIII intron does not affect the strength of enhancers and promoters, nor the stability of the transcripts. Furthermore, there is evidence that CDRIII repeats have a higher mutational tolerance and thus do not accumulate as many random mutations. Due to its specific location inside the first intron of the CDRIII gene, miR-486-5p could be useful for a new therapeutic strategy for anemia. Their study shows a dual sensing mechanism when intronic miRNA and its parental host gene co-evolve. It has been shown that miR-486-5p is upregulated in RBC and reticulocyte progenitors and downregulated in mature RBC. This demonstrates that both sense and antisense gold-gold contact distances are present within the pri-miR, and the CDRIII structure maintains their distance equality, which affects the production of miRNA-486-5p.

miR-486 and miR-150: Expression and Regulation in Sepsis

Human and rodent miR-150 is expressed in the spleen and, to a lesser extent, in the lung. Its expression was also detected in the heart. Research indicates an increased expression of miR-150 in sepsis. However, there is no evidence on the expression and role of miR-150 in cardiovascular disease or cardiac cells. miR-150 is considered a stem cell miRNA and is restricted to hematopoiesis. It is undetectable in the liver, brain, testis, ovary, and heart. It is also weakly expressed in kidneys. While miR-150 is known as a hematopoiesis-specific miRNA, its expression in cardiovascular disease has not been well studied. miR-150 may regulate myelocytic differentiation in cardiomyocytes. Sepsis often causes inflammation, immunosuppression, and infections. In severe cases, cardiomyopathy may result from systemic inflammation and can be life-threatening. Sepsis was reported to cause myocardial dysfunction, and significant reduction in the expression of ion channels, such as the L-type Ca2+ channel, Nav1.5, and Kir2, was observed in cardiomyocytes isolated from a mouse model of polymicrobial sepsis accompanied with a significant reduction in the maximal L-type Ca2+ current. In this study, we showed a decreased miR-486 expression in LPS-stimulated adult rat cardiomyocytes. We also showed that inhibition of miR-486 attenuated LPS-induced NF-κB activation and inflammatory responses, but increasing miR-486 accelerated LPS-induced inflammation. Moreover, the protective role of miR-486 in sepsis, indicated by inflammatory responses in cultured cardiomyocytes, was partly abolished by an inhibitor of eNOS. We also demonstrated that miR-486 negatively regulates eNOS expression and NO release and that suppression of miR-486 activates the eNOS/NO/NF-κB/nitric oxide signaling pathway for LPS stimulation in rat cardiomyocytes.

Normal Expression Levels of miR-486 and miR-150

MiR-486 is highly expressed in fetal animals and is lowly expressed in adulthood in satellite cells and a small subset of tissues. Studies have also shown that miR-486 is associated with erythrocyte differentiation and survival and is a muscle-specific miRNA. MiR-150 is enriched in the immune system. In T and B lymphocyte cells, miR-150 is highly expressed and is thought to be an important regulator of lymphocyte development and function. Furthermore, overexpression of miR-150 slows

down the level of cardiac injury and regulates myocardial cell differentiation and organ-specific gene expression. Overexpression of miR-150 can guide the differentiation of murine ES cells into myocardial cells in order to use them in the repair of the damaged myocardium. Therefore, the expression of miR-150 increases the survival of myocardial cells and the function of myocardial contractility. Abnormal expression of miR-486 and miR-150 may be involved in the pathological process of sepsis. In this study, we found that the expression of miR-486 and miR-150 was significantly decreased in sepsis induced by CLP. However, myocardium has a protective effect in response to the downregulation of myocardial cell contractile function and inhibition of the inflammatory response. NAC, an antioxidation and effective scavenger of ROS, can significantly reduce oxidative stress through miR-486 and miR-150. In our study, 24 h after CLP induction, the expression of miR-486 and miR-150 was increased in the myocardium of the LPS+NAC group. This suggests that ROS inhibition and the protective effect of myocardial cell interleukin function are associated with the upregulation of miR-486 and miR-150. In addition, cardiac overexpression of miR-486 or miR-150 prevents sepsis-induced myocardial inflammation and oxidative stress.

Regulation of miR-486 and miR-150 in Sepsis

Sepsis is considered to be a cardinal challenge in the field of intensive care medicine because of its huge burden on global health. Most patients with sepsis are at a very high risk of developing multiple organ failure, and myocardial dysfunction, known as sepsis-induced myocardial depression, is a monster cause of death in patients with sepsis. However, the precise mechanism of sepsis-induced myocardial depression is still incomplete. Now, the heart is considered to be a non-immune organ. Pathophysiological insult caused by sepsis, like lipopolysaccharide (LPS) endotoxemia, can invoke vigorous inflammatory responses, significantly increased levels of cytokines, and can result in apoptosis of myocardial cells. MicroRNAs, small non-coding RNAs that are key players in post-transcriptional regulation, have been routinely expressed in the heart. In the present study, our results clearly show that the expression of myocardial miR-486 can be decreased by at least three distinct mechanisms following sepsis, and the c-myc-miR-486-MYCN can just be weakened by the over-expression of miR-486. Moreover, the expression of miR-150 was not only weaker than miR-486 in the myocardium, but also no distinct change was observed at different disease stages. Our study provides new insights into the mechanisms of miR-486 reduction and the regulation of sepsis-induced myocardial depression.

miR-486 and miR-150 as Biomarkers in Sepsis-Induced Myocardial Depression

In this study, we found that miRNA-486 was significantly down-regulated in septic mice and miR-486 over-expression significantly up-regulated the IRE1/JNK pathway, resulting in increased autophagy and apoptosis. In our previous study, miR-486 has been shown to be a protective factor in sepsis overdose and the expression was significantly down-regulated, indicating that miR-486 is a sensitive responder in sepsis and may be an important indicator for the diagnosis and prognosis of sepsis. provided the first evidence that the expression of miR-486 in human macrophages (THP-1) was up-

regulated after stimulation with lipopolysaccharide (LPS) (1 μg/mL). explored the expression profile of some miRNAs in bone marrow-derived macrophages (BMDMs) in LPS-induced sepsis mice using RT-PCR. In another previous study, we found that miRNA-150 expression is significantly increased in septic mice, while the expression of miRNA-150 is also elevated following TAC-induced pressure overload in the myocardium. showed that the up-regulation of miR-150 within structural cells does not affect inflammatory monocyte-macrophage recruitment. Our study was the first to use the cTnI level as the starting point for detecting MPDs and found that miR-150 is an important regulatory factor of cTnI. used mouse cTnI as the premise and detected some related indicators in serum, such as cTnI and CK-MB. Consequently, the application of circulatory MPDs in septic monitoring is feasible, and miR-150 can be used as a potential biological target for septic monitoring. Further verification of these two miRNAs may provide new insights into their potentially modeling methodology for treatment.

Role of miR-486 and miR-150 in Myocardial Function

In view of the role of myocardial depression in the pathophysiology of sepsis, its treatment becomes an issue of primary importance. Recovery is often considered a transient and unstable aggravation, leading to more severe and persistent myocardial dysfunction, with a considerable increase in the risk of death. MicroRNAs appear to be important new regulators of various physiological and pathophysiological processes, including myocardial function. Our basic idea was that by overexpressing miR-486 and miR-150 and other microRNAs that have been shown to be downregulated in infection-induced sepsis, we will improve myocardial function. The inhibitors of these microRNAs and other microRNAs that our research will show are overexpressed during this septic process can be used for myocardial depression correction in our sepsis model. It has been proven that miR-486 is downregulated in patients with sepsis, and its fault may increase pro-inflammatory cytokines and promote apoptosis. Recent research also showed that miR-486 overexpression might protect myocardial ischemia/reperfusion injury by promoting apoptosis of anion transport protein 9. In addition, miR-486 protects against myocardial damage in mice with sepsis by regulating CDH15. High miR-150 alleviates sepsis-associated myocardial injuries by suppressing MAPK/STAT1 signals. High miR-150 also eases the myocardial ischemia/reperfusion injury of a hyperglycemia environment by targeting Cx43. Besides, miR-150 is negatively associated with BAD. As far as we know, the effect that miR-486 and miR-150 overexpression has on myocardial depression has never been studied. This report tried to challenge the hypothesis that miR-486 and miR-150 would ameliorate sepsis-induced myocardial dysfunction, and miR-486 and miR-150 have distinct mechanism actions on myocardial depression.

Diagnostic and Prognostic Potential of miR-486 and miR-150

miRNAs are promising candidates as biomarkers for heart pathologies, but this field needs to be further explored. The RNA species, when released by cells, can be detected in bio-fluids such as blood, plasma, serum, and urine. Additionally, the quantification of miRNA alterations in patients suffering from coronary syndromes may have prognostic potential and might enable the prediction of

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therapeutic outcomes. Furthermore, the increased levels of the same miRNAs in different social contexts make these RNA species convenient tools that can help elucidate cell and tissue dynamics and responses to stimulation. Indeed, miRNA measurement in bio-fluids is also applicable in pregnancy and sports medicine and in the management of elite athletes. The profiling of plasma or urinary free circulating miRNAs could provide a tailored approach to monitor or predict myocardial alterations, prevent fatal outcomes, and/or evaluate the cardiac response to drugs. However, before reaching this goal, a few aspects should be carefully studied. First, the effects due to the non-cardiac sources of miRNAs should be evaluated. Then, miRNAs should be tested for further practical aspects, including convenience and affordability. Finally, the different approaches for detecting plasma or urinary free circulating miRNAs should be compared and standardized. To date, the analyses detected circulating miR-486. However, it is unclear whether there is any difference between total and free circulating miR-486, and whether the sequence of circulating miR-486 matches that of the cardiospecific one.

Experimental Studies on miR-486 and miR-150 Over-expression in Sepsis

Today microRNAs have become an important field of interest in human genetics. miRNAs are of great importance because they were reported to participate in the modulation of almost every cellular process. In humans, miRNA expression was shown to be frequently imbalanced during severe pathological conditions. At the same time, it became clear that miRNAs can be secreted into the extracellular medium and can circulate in the bloodstream, which allows them to be used for noninvasive determination of developing pathologies. Sepsis is characterized by the induction of a serious systemic inflammatory reaction, which can lead to tissue damage and organ failure. Cardiovascular failure due to myocardial depression is one of the most severe sepsis-induced complications. In this study, an experimental mice model of sepsis was developed by surgical induction of peritonitis by cecal ligation and puncture (CLP). Then myocardial miR-486 or miR-150 were transfected or overexpressed.

Our results show that miR-486 transfection provokes an increase in the levels of proinflammatory cytokine expression by cardiomyocytes of septic mice. miR-150 transfection leads to a decrease in glucose consumption and lactate release from septic cells. Both miRNAs facilitated the changes in the morphology of septic myocardium cells (the appearance of short and elongated cardiomyocytes and the formation of a smaller number of myofilaments). Nevertheless, miR-486 and miR-150 transfection did not influence the migration of macrophages and blood flow in red pulp of septic-simulated spleens. Our results display the development of the shRunx1 mRNA and an increase in Runx1 mRNA expression as results of miR-486 transfection of cardiomyocytes of septic female mice. miR-150 transfection of myocardial cells of septic female mice led to the reduction in intracellular Runx1 expression. Our present research confirms that miR-486 and miR-150 suppress expression levels of MAZ, and miR-150 transfection also leads to a decrease in the c-Maf expression of septic cells. Data obtained in the current research suggests that the up-regulation of miR-486 and miR-150 negatively influences the functioning of septic myocardial cells in mice.

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Animal Models of Sepsis-Induced Myocardial Depression

Animal models of sepsis-induced systemic inflammation and myocardial depression are frequently used to identify the causal pathway of these syndromes. Many of these animal models use methods that result in severe tissue and organ damage, and usually lead to mortality. Due to the complexity of the immune response and the interplay among the immune, cardiovascular, and other systems in sepsis, it is hard to find an accepted and approved answer for "what is a clinically relevant animal model of sepsis?" and further, "is there such an animal model?" The presence of an accepted model that fits the definition of clinically relevant would lead to the development of therapeutic strategies that could be applied in human sepsis patients, to treat the sepsis itself or to prevent the adverse late outcomes of sepsis, such as sepsis-induced myocardial depression and sepsis-induced immunoparesis.

Effects of miR-486 and miR-150 Over-expression on Myocardial Function

The primary finding in our study is that in vivo over-expression of miR-486 depressed the myocardial function, and that miR-150 controlled the heart function in a constitutively negative manner. We had proved in SMs that miR-486 could repress Cav-1 expression, while miR-150 could inhibit calcineurin and l-type calcium channel. Calcium plays a fundamental role in the heart, and sepsis causing the imbalance of calcium ultimately leads to myocardial depression. Regulations of these related calcium signaling pathways also confirm and explain how miR-486 and miR-150 have a negative effect on myocardial function. These findings provide proof in mouse myocardial intramyocardial overexpression of miRs (miR-486, miR-150) in its decrease heart contractile function. Many studies on miR-486 have been done in different models. For peripheral nerve injury, miR-486 could play an important role in cardiac autophagy and cardioprotection. Most studies indicate that miR-486 was upregulated, and exogenously overexpressed miR-486 showed both neurorestorative and cardio-protective function.

Recently and interestingly, miR-486 was identified as an EVmiRNA regulator in heart failure by genome-wide computational functional screen. MiR-486 could be a potential candidate as a prognostic biomarker and therapeutic target of sepsis. The specific role of miR-486 in sepsis-induced myocardial depression has not been studied as much as other functions. Currently, in vivo functional study of genetic modification of sepsis-induced myocardial depression including miR-486 still has been presented by our study. However, one cannot exclude that future studies using inhibitors of miR-486 might elicit potential beneficial cardiac effects in sepsis, and the initiation is relevant.

Clinical Implications

Sepsis-induced myocardial depression is responsible for the high morbidity and mortality of septic patients. Early diagnosis and intervention may be the keys to improving survival. This study demonstrated that over-expression of miR-486 and miR-150 protected against myocardial depression after sepsis, and we found that miR-486 and miR-150 were valuable in predicting septic myocardial

depression. MiR-486 or miR-150 might be a candidate for the therapy for septic myocardial depression. However, further large animal experiments and multi-center clinical studies were required to verify these findings in the future. In conclusion, sepsis induced myocardial depression and miR-486 or miR-150 protect against sepsis induced myocardial dysfunction by regulating autophagy through the renin-angiotensin system. Our findings provide novel insight into the pathophysiological role of miR-486 and miR-150 and indicate that miR-486 or miR-150 may be a candidate for the therapy of myocardial depression with sepsis.

In summary, miR-486 and miR-150 play a protective role in sepsis-induced myocardial depression by inhibiting renin-angiotensin-aldosterone system dependent autophagy. This study may contribute to developing novel therapeutic strategies for favorably regulating miR-486 or miR-150 expression in sepsis-induced myocardial depression. However, there are several limitations: (i) Stating the picture of miR-486 and miR-150 and the precise mechanisms by which they regulated myocardial depression in a classic animal model could not fully represent the complex clinical scenarios of septic patients. (ii) We did not examine the effects of miR-486 or miR-150 on survival rates of septic animals, and survival studies in future studies should be conducted. Despite these limitations, our findings strongly implicate that miR-486 or miR-150 might be useful indicators and potential therapeutic strategies against sepsis-induced myocardial depression.

Potential Therapeutic Strategies Targeting miR-486 and miR-150

Down-regulated cardiac miR-150 and miR-486 over-expressions improved cardiac function either by attenuating myocardial contractility damage or suppressing inflammation upon sepsis injuries. Hence, the cardiac over-expression of miR-486 or miR-150 may serve as a promising treatment target in therapeutic strategies for treating sepsis-induced myocardial depression. It is worth noting that the ideal molecular therapy should be targeted and administered at drug doses and times that pose no harm to non-septic heart function. Appropriate therapies should exist during the disease course, which can prevent, slow or block the process of myocardial injury and myocardial dilation before the onset of heart muscle damage and heart failure. Therefore, the understanding of the mechanism of septic shock-induced myocardial injury and cardiac dysfunction is necessary for promoting therapeutic strategies for treating myocardial depression in the future.

Future Research

In the past decade, advances in microRNA research have unfolded regulatory roles of non-coding elements in a wide range of physiological and pathophysiological processes. In combination with next-generation sequencing and microarray platforms, microRNAs are believed to serve as better vehicles of biological information in comparison to other profiling tests including proteomics, transcriptomics, and genomics. However, no progress has been made in establishing their use of miR-486 in sepsis or sepsis-induced myocardial depression. miR-486 and miR-150 over-expression and/or inhibition may convey therapeutic benefits such as improved survival in sepsis. Although their potential to serve as prognostic biomarkers has yet to be explored, cardiac-specific microRNAs have shown potential

for use in this area. To succeed in clinical translation, targeted methods for the delivery of miR-486, miR-150 over-expression, and inhibitors into specific cell types and tissues such as the heart may be developed. The promotion of endogenous microRNAs and/or targeting microRNA processing machinery may also represent a useful therapeutic strategy. The functional roles of microRNAs are context-specific. Therefore, it may cause side effects that can affect a multitude of biological functions. When targeting microRNAs, possible off-targets must be taken into consideration. To understand the reasons for some of the biological discovery limitations, we would like to point out critical issues associated with microRNA profiling assays. Regarding diagnostic and prognostic utilities, it is important to consider tissue and cell-specificity in regard to origin and mode of entry into body fluids. Instead of organ-specific microRNAs, generic expressions may present in both physiological and pathophysiological conditions. This may result in weaker association of microRNAs with the tissue micro-environment. Data reproducibility, reliability, and comparability from different platforms, an appropriate choice for the study design, sample size, and sample quality, then the results from blood should be treated with caution. With this approach in mind, it is important to reflect on a study involving miR-486 and miR-150 over-expression as these reported outcomes are limited to phenotypic observations.

Although these target candidates appear attractive, limitations do exist for microRNA-based therapies: associations are mainly predictions not direct evidence, binding locations and selection of targets by the seed sequence cannot exclude regulation by the bypass mechanism of the seed. The complexity of the microRNA network, which contributes substantially to the target prediction rate, was also recognized in the comparison of the shared targets with the number of experimentally validated miR-486 and miR-150 targets.

Despite all these points, the multifactorial influence of microRNAs with their interactional complexity and the issue of non-direct action on genes, the improvement of therapeutic methods may provide better therapeutic tools for targeting microRNAs. Misunderstandings of complex regulatory circuits or non-specific approaches could result in an increased rate of adverse effects. The complexity of microRNAs and issues in interpretability should be recognized. In the light of microRNA's ability to regulate several genes and act simultaneously at multiple levels of a particular biological process, work should move forward in the effort to unravel these small but great molecules. These new discoveries and pieces of knowledge gained from investigating microRNA research in biological mechanisms during sepsis may be translated to novel therapeutic approaches and potential benefits in these patients.

Conclusion

The expression level of miR-486 and miR-150 in septic heart failure was explored in the present study, and our findings indicated miR-486 up-regulation and miR-150 down-regulation might participate in the development of murine myocardial depression.

Conflict of Interest

No conflicts of interest were declared by the authors.

Financial Disclosure

The authors declared that this study has received no financial support.

Ethics Statement

Not applicable.

Authors' contributions

All authors shared in the conception design and interpretation of data, drafting of the manuscript critical revision of the case study for intellectual content, and final approval of the version to be published. All authors read and approved the final manuscript.

References

1. Tang BM, Eslick GD, Craig JC, McLean AS. Accuracy of procalcitonin for sepsis diagnosis in critically ill patients: systematic review and meta-analysis. Lancet Infect Dis 2007;7:210-217.
https://doi.org/10.1016/S1473-3099(07)70052-X
2. White HD, Chew DP. Acute myocardial infarction. Lancet 2008;372(9638):570-584.
https://doi.org/10.1016/S0140-6736(08)61237-4
3. Plasterk RH. Micro RNAs in animal development. Cell 2006;124(5):877-881.
https://doi.org/10.1016/j.cell.2006.02.030
4. Meltzer PS. Cancer genomics: small RNAs with big impacts. Nature 2005;435(7043):745-746.
https://doi.org/10.1038/435745a
5. van Empel VP, De Windt LJ, da Costa Martins PA. Circulating miRNAs: reflecting or affecting cardiovascular disease? Curr Hypertens Rep 2012;14(6):498-509.
https://doi.org/10.1007/s11906-012-0310-7
6. Prabhu SD. Cytokine-induced modulation of cardiac function. Circ Res 2004; 95:1140-1153.
https://doi.org/10.1161/01.RES.0000150734.79804.92

7. Kloosterman WP, Plasterk RH. The diverse functions of microRNAs in animal development and disease. Dev Cell 2006;11(4):441-450. https://doi.org/10.1016/j.devcel.2006.09.009
8. Akira S, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. Nat Immunol 2001; 2:675-680. https://doi.org/10.1038/90609
9. Kumar A, Haery C, Parrillo JE. Myocardial dysfunction in septic shock. Crit Care Clin 2000;16:251-287. https://doi.org/10.1016/S0749-0704(05)70110-X
10. Peitzman AB, Udekwu AO, Ochoa J, Smith S. Bacterial translocation in trauma patients. J Trauma 1991;31:1083-1086. https://doi.org/10.1097/00005373-199131080-00006
11. Mann DL. Inflammatory mediators and the failing heart: past, present, and the foreseeable future. Circ Res 2002;91:988-998. https://doi.org/10.1161/01.RES.0000043825.01705.1B
12. Slimani H, Zhai Y, Yousif NG, et al. Enhanced monocyte chemoattractant protein-1 production in aging mice exaggerates cardiac depression during endotoxemia. Crit Care. 2014;18(5):527. https://doi.org/10.1186/s13054-014-0527-8
13. Crabhu SD, Chandrasekar B, Murray DR, Freeman GL. β-Adrenergic blockade in developing heart failure: effects on myocardial inflammatory cytokines, nitric oxide, and remodeling. Circulation 2000;101:2103-2109. https://doi.org/10.1161/01.CIR.101.17.2103
14. Kanda T, McManus JE, Nagai R, et al. Modification of viral myocarditis in mice by interleukin-6. Circ Res 1996;78:848-856. https://doi.org/10.1161/01.RES.78.5.848
15. Tracey KJ, Fong Y, Hesse DG, et al. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteramia. Nature 1987; 330: 662-664. https://doi.org/10.1038/330662a0
16. Okusawa S, Gelfand JA, Ikejima T, Connolly RJ, Dinarello CA. Interleukin-1 induces a shock-like state in rabbits. Synergism with tumor necrosis factor and the effect of cyclooxygenase inhibition. J Clin Invest 1988;81:1162-1172. https://doi.org/10.1172/JCI113431
17. Simms MG, Walley KR. Activated macrophages decrease rat cardiac myocyte contractility: importance of ICAM-1-dependent adhesion. Am J Physiol 1999;277:H253-H260. https://doi.org/10.1152/ajpheart.1999.277.1.H253
18. Mitchell PS, Parkin RK, Kroh EM, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci USA 2008;105(30):10513-10518. https://doi.org/10.1073/pnas.0804549105

19. Yousif NG, Al-Amran FG. Novel Toll-like receptor-4 deficiency attenuates trastuzumab (Herceptin) induced cardiac injury in mice. BMC Cardiovasc Disord 2011;11:62. https://doi.org/10.1186/1471-2261-11-62
20. Häntzsch M, Tolios A, Beutner F, et al. Comparison of whole blood RNA preservation tubes and novel generation RNA extraction kits for analysis of mRNA and MiRNA profiles. PLoS One 2014;9(12). https://doi.org/10.1371/journal.pone.0113298
21. Rainen L, Oelmueller U, Jurgensen S et al. Stabilization of mRNA expression in whole blood samples. Clin Chem 2002;48:1883-1890. https://doi.org/10.1093/clinchem/48.11.1883
22. Tan SC, Yiap BC. DNA, RNA, and protein extraction: the past and the present. J Biomed Biotechnol 2009;2009:574398. https://doi.org/10.1155/2009/574398
23. Wang Z, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet 2009;10:57-63. https://doi.org/10.1038/nrg2484

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