

The Therapeutic Effect of miRNA-19a/19b on Heart Failure in Mice and the Mechanism of Myocardial Regeneration and Repair

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ABSTRACT

Heart failure is a common cardiovascular disease. The elderly have a high risk of illness. Many patients die of the disease every year. It has become a killer for all humans. Cardiovascular disease often triggers myocardial ischemia and myocardial cell apoptosis, resulting in myocardial damage and even heart failure in patients with cardiovascular disease. Related studies have shown that miRNA-19a/19b plays an important role in myocardial damage and heart failure character. The purpose of this article is to further explore the therapeutic effect of miRNA-19a/19b on heart failure and the regulation mechanism of myocardial regeneration and repair. In this paper, 40 male mice were used as experimental objects to perform ligation surgery on the coronary arteries of mice. To establish an animal model of myocardial infarction and heart failure, then inject miRNA-19a/19b reagent through the tail vein of the mouse, observe the changes of the myocardial cell and the area of the myocardial infarction by immunofluorescence PCR technology, and detect the myocardial tissue in the mouse. Specific changes in the expression of miRNA-19a/19b. The results of the study showed that miRNA-19a/19b can improve the cardiac function of mice. The cardiac function index of mice increased from the original (263.13 ± 5.26) to (385.48 ± 6.92), the area of myocardial infarction decreased by 27.5%, and the proliferation rate of myocardial cells increased by 18.6%, the rate of myocardial regeneration and repair increased by 20.4%. Therefore, it can be seen that miRNA-19a/19b has a significant effect on the treatment of heart failure in mice, can effectively improve heart function, and can promote the regeneration and repair of damaged myocardium, proving the feasibility of miRNA-19a/19b for cardiovascular disease.

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Introduction

Cardiovascular diseases, especially the damage and death of many cardiac functional myocardial organelles caused by myocardial ischemia, and other possible diseases of the heart system, such as heart failure, are gradually becoming the main killers of human health (1). For various types of multi-stage end-stage chronic cardiovascular disease, the current conventional medical methods can only effectively delay the continuous development of the patient's condition, but cannot be completely cured. In recent years, the continuous emergence of miRNA has provided new scientific prospects for modern people to re-understand the development process of this important human disease (1-3).

The miRNA-19a/19b is a member of the miR-17-92 gene cluster and participates in the regulation of various target genes in the human body. Malek analyzed and studied the mechanism of action of

miRNA-19a/19b in myocardial tissues, and found that miRNA-19a/19b can inhibit the apoptosis of cardiomyocytes by regulating the target gene SOCS1 (4). Khan research found that microRNA is a key regulator of cardiomyocyte movement. MiRNA-19a/19b plays an important role in many types of cardiovascular diseases. This discovery opens the door to the study of miRNA-19a/19b (5). Tian investigated whether miRNA-19a/19b is dysregulated during myocardial infarction and is directly related to the biological function of miRNA-19a/19b in the process of regulating cardiomyocytes, and found that miRNA-19a/19b expression is significantly reduced in myocardial damaged tissues (6). Li found through research that miRNA-19a/19b is closely linked to a variety of diseases, including neurodegenerative diseases, cardiovascular diseases, kidney diseases and tumors, and also pointed out that miRNA-19a/19b plays a role in the generation, development and

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function of the heart important regulatory role (7). Sen pointed out in the article that miRNA-19a/19b has a positive effect on the treatment of heart failure by regulating the expression of target genes CCND1 and CDK1, and also has a certain repair effect on myocardial damage, and introduced the process of the effect and pointed out the relevant. The principles and precautions are very helpful for later research (8). In summary, miRNA-19a/19b is very important for the treatment of cardiovascular diseases.

Heart failure is the ultimate manifestation of the malignant development of cardiovascular diseases. At present, there are much researches on heart failure in my country. Through research, Xu found that the expression level of miRNA will be reduced after the mouse heart is damaged, which leads to the occurrence of heart failure, proving that miRNA plays an important role in the regulation of heart function (9). Nollet found through research that heart failure can continue to reduce the myocardial blood supply activity and increase the wall thickness of the local systolic chamber and thereby reduce the heart function, and pointed out that this is a danger signal that represents the risk of heart function disintegration, a high probability will cause the occurrence of death (10). In order to determine the correlation between heart rate failure and myocardial damage, Tavares conducted a series of investigations and studies and found that the degree of myocardial damage is a key factor in determining heart failure, and heart rate failure is often a complication of myocardial infarction (11). Chodkowska emphasized that although there is no clear pathophysiological way to study heart rate failure and myocardial damage, the researchers have proposed many theories, such as micro miRNA for the treatment of heart failure and myocardial infarction (12). Lesizza introduced the etiology and pathology of myocardial infarction in heart failure and confirmed that heart gene expression has changed significantly during heart failure, and some tiny genes have an abnormal expression in heart failure heart (13). It can be seen that heart rate failure is a very harmful disease, and the regeneration of new machines is the key to the treatment of heart failure.

This article is mainly to explore the therapeutic effect of miRNA-19a/19b on heart failure and the effect on the regulation mechanism of myocardial regeneration and repair. In this project, the animal

models of myocardial infarction and heart failure were established by ligating the coronary arteries of mice, and then miRNA-19a/19b reagent was injected through the tail vein of the mouse, and the mouse cardiomyocytes were observed by immunofluorescence PCR changes and specific circumstances of myocardial infarction area. The results show that miRNA-19a/19b can improve the cardiac function of mice. The cardiac function index of mice has increased from the original (263.13 ± 5.26) to (385.48 ± 6.92), the area of myocardial infarction has been reduced by 27.5%, and the proliferation rate of myocardial cells increased by 18.6%, the rate of myocardial regeneration and repair increased by 20.4%. The innovation of this research lies in the first in-depth detection of the gene expression level of the target sample receptor gene in the damaged tissues of the mouse heart and myocardium in various mouse hearts and myocardial tissues by quantitative PVM fluorescence irradiation technology and then in-depth analysis by applying statistical analysis methods. The expression was different among them, through in-depth analysis to detect the effect of miRNA-19a/19b on the proliferation and apoptosis of cardiomyocytes, and a new line of quantitative description of myocardial regeneration and repair through modeling. It can be seen that the innovation in this paper maximizes the research effect and the relevant data obtained are more accurate.

Materials and methods

Mechanism and Application of miRNA

MiRNA molecules are a class of highly conserved, non-linearly encoded macromolecules of 18 to 26 bases in length (14). At present, basic research has clarified that it has two mechanisms of photosynthesis regulation: one directly binds to the target receptor gene's incomplete complementation and prevents the translation of a miRNA gene; one principle is to directly bind to the target receptor gene's complete defect complementation, guidance a miRNA gene breaks at a specific target site, cleaves the target gene miRNA, and the degree of complete complementary binding of miRNA and its target receptor gene directly determines its photosynthesis regulation mode (15). A very complex network of gene regulation mechanisms is formed between miRNA and target cell genes. MiRNA directly affects the normal

expression of target cell genes, thereby directly regulating the normal proliferation, differentiation and apoptosis of target cells. Overexpression of miRNA-19b can lead to a decrease in the number of negative regulatory factors related to myocardial somatostatin and thyroid hormone-related protein 2 that promote cell proliferation and myocardial regeneration and lead to excessive myocardial cell apoptosis. This phenomenon is likely to cause myocardial Loss (16). The formula for calculating the number of negative regulatory factors in the myocardium is shown in 1.

$$N(s) = \frac{f^{(n+1)}(s, \mu m)}{(s+1)!} (s-s_0)^{n+1} \quad (1)$$

Where N represents the number of negative regulators and s represents the expression level of miRNA-19a.

MiRNA-19a/b promotes myocardial hypertrophy by down-regulating anti-hypertrophic protein and inhibiting the function of miRNA-19a/b with antisense oligonucleotides slows the process of isoproterenol-induced cardiac hypertrophy (17). The pathological nature of myocardial hypertrophy is usually an important harbinger of heart failure. In clinical practice, we can effectively block the damage to the myocardial wall by effectively regulating the expression of certain cells located in miRNA. Early detection finds early treatment and makes it a long-term disease. Certain heart diseases in the compensated period can be maintained in their cardiovascular disease for long-term health, and miRNA-19a usually increases the expression level (18). As shown in Figure 1, the role of miRNA-19a/19b in myocardial blood vessels confirms that the expression of miRNA-19b in myocardial injury tissue is significantly increased, especially in myocardial vascular smooth muscle cells, after using miRNA-19a/19b inhibitors. Can not only reduce the degree of myocardial injury but also promote the proliferation of myocardial vascular smooth muscle cells, miRNA-19a/19b target genes PTEN and Bcl2 mediate this process.

Myocardial dysfunction and chronic heart failure are mainly attributed to two types of myocardial exercise remodeling and contraction processes, and diseases that recur during cardiac exercise due to large external pressure loads (19). The pathogenesis of

myocardial infarction and myocardial hypertrophy is one of the main causes of heart failure (20). In each peripheral clinical area of acute myocardial infarction, the expression of miRNA-19b type in this family is significantly down-regulated. The down-regulated heart rate miRNA-19a may also release many related response factors around human fibrosis and myocardial infarction. This area is heavily aggregated to form fibrous tissue, which greatly reduces the blood compliance and vasodilation function of the human heart (21). The results of these clinical studies indicate that up-regulation of miRNA-19a/19b expression can effectively prevent false myocardial wall damage, maintain cardiac function and promote myocardial regeneration and repair (22).

Object selection

In this experiment, 40 two-month-old mice were selected as the research object, with a weight of (130±5.0) g, purchased from the Shanghai keno animal experiment company. Select the laboratory that has been disinfected and pollution-free as the experimental site. The temperature of the laboratory is 26-30 degrees centigrade, the moisture in the air is about 16.5-19.8%, the exposure time of the sun is 8:00-17:00, and the laboratory keeps. Ventilation was performed to ensure that the oxygen content in the air is sufficient, rearing in the standard clean rearing environment required by the state. Before the experiment, mice were screened for health, excluding unhealthy mice, and miRNA-19a/19b reagent was injected through the tail vein of the mice.

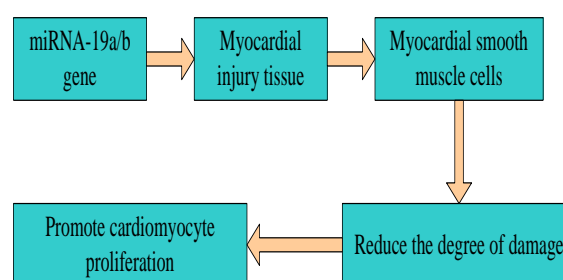


Figure 1. The role of miRNA-19a/b in vascular diseases

Related equipment

The main equipment used in this experimental institute: anesthesia machine, LUM heart ultrasound system, SYS-100 fluorescence microscope. TR paraffin slicer, transmission electron microscopy, ultra-clean workbench, frozen slicer, pure water

instrument, BL electrophoresis tank (USA BL Company), fluorescence measuring instrument PPL, cryogenic centrifuge, S-8000 ultracentrifuge, chemical reaction luminescence instrument, PCR detector, fluorescence quantitative detector. XCN pipette, biochemical incubator, electrocardiogram examination instrument, cardiac pacing, magnetic stirrer. The reagents used in this experimental study are shown in Table 1.

Table 1. The reagents used in this experimental study are shown in Table 1

Item name	Quantity used	Item source
M199 medium	300ml	Jiangsu chemical reagent company
Ammonium nitrate phosphate	280ml	American mute
Collagenase D	200ml	German borsch
Cell purifier	550mg	Nanjing chemical company

Establishment of mouse heart failure model

In order to conduct experiments, we established a heart failure model of coronary artery ligation in mice. Anesthetize mice by intraperitoneal injection of hydrated chloral hydrate (0.5 ml / 100 g). If corneal reflex does not occur after injection and there is no retraction reaction by pinching the toes, it is suitable for anesthesia (23). Cut the tracheal cannula of the mouse and connect it to the ventilator with a tidal volume of 1.3 to 1.8 ml/100 g and a frequency of 40 times/min. Rapid thoracotomy at the third and fourth intercostal spaces on the left side to prevent exposure of the right heart, and during rapid thoracotomy 3/0 rapid thoracotomy, about 5 above and below the opening of the left anterior descending branch of the right coronary artery (lad). At a millimeter of the myocardial space, the tube is sutured and ligated (24). Gently return the heart to the thoracic cavity and carefully sew the skin of the ribcage. Except for the animals not ligating the experimental control group, the operation of the animals in the simulated surgery is the same as described above. After LAD ligation, localized lesions were found on the surface of the LAD blood supply area, including a sharp drop in the SY segment of the electrocardiogram, blood pressure, and the anterior wall and apex of the left ventricle. All of the above phenomena indicate that the model has been successfully established (25).

Mouse myocardial infarction model

Place the anesthetized mouse supine and fix it on the iodophor test stand. After the iodophor disinfected the precordial area of the mouse by normal blood disinfection, the fifth end of the mouse sternum was excised with two eye swabs. The posterior intercostal space between the six chests was quickly cut into the needle tissue from the mouse skin and the lower layer of the needle, and the incision was 1 cm. Next, use two sutures with purse thread sutures to cut the upper and lower layers of the skin into needles. Pierce the suture of the two-pin wallet thread and bind it to the tissue for initial use (26). Quickly cut the trachea through the circulation of one or two branch trachea under the thyroid of the mouse and quickly connect it to the abdominal ventilator of the mouse and the animal (mouse breathing frequency 50 times/min, tidal volume 10 ml/100 g. The respiration frequency ratio is 3:5). After suturing the intercostal wound of the mouse with a needle, use your hands or fingers to quickly squeeze the chest cavity of the mouse to quickly expel the excess air in the mouse chest cavity, and use the assistant at the same time, and then tighten the chest. Suture the needle and close its inner layer and lung skin tissue layer (27).

Pathological examination of the myocardium

The pathological and morphological changes of left ventricular myocardial tissue were evaluated by PCR fluorescence detection. The specific steps are as follows: Immediately remove the left ventricular and left ventricular myocardial valve tissues of the removed mice in a 5% paraformaldehyde center and fix them for about 50 hours. After conventional gradient dehydration, ethanol and ethanol dehydration, secondary trimethyl benzene transparency, wax immersion and dye embedding, staining and re-dressing, remove the untrimmed myocardial tissue mass with an automatic microtome and cut it into 5 Slices 25 mm wide (5).

Heart function test in mice

Cardiac ultrasound examination: After pacing was stopped, 7% pentobarbital sodium 49mg/kg was intraperitoneally anesthetized, the heart rate was recorded, and the electrocardiographic instrument was used to determine the myocardial ejection fraction of the mouse and the short-axis shortening rate of the

heartbeat. Hemodynamic detection uses a multi-channel physiological signal acquisition and processing system:

Results and discussion

Mouse Cardiac Function Test Results

The relevant data is shown in Table 2, the results of the study showed that compared with before miRNA-19a/19b injection, the cardiac ejection fraction, short-axis shortening rate of beating heart, and cardiac output blood pressure of the mice were significantly reduced; the differences were statistically significant ($P < 0.05$). After the establishment of myocardial infarction and heart failure animal models, the heart rate and left ventricular diastolic pressure of mice increased significantly.

Table 2. Heart rate and left ventricular diastolic pressure in mice; HR (A), LVEF (B), LXESP (C), LVEDP (D)

Group	Ejection fraction	Short axis shortening rate	Left ventricular pressure	P
A	36.81 ± 2.57	78.44 ± 6.59	124.22 ± 11.5	0.0247
B	44.36 ± 3.18	81.08 ± 5.35	136.80 ± 15.2	0.0178
C	49.25 ± 4.61	72.64 ± 5.93	155.14 ± 10.7	0.0367
D	58.72 ± 5.36	90.27 ± 6.71	169.75 ± 19.6	0.0219

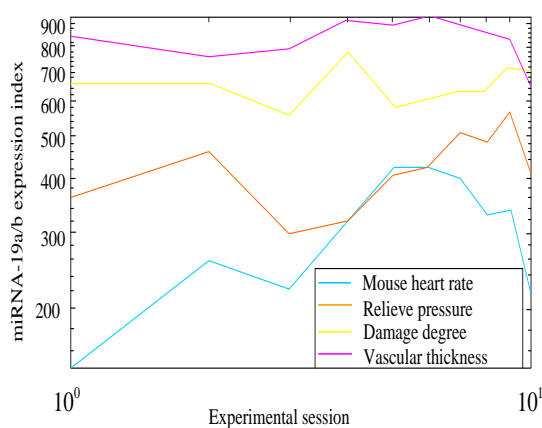


Figure 2. Expression index of miRNA-19a/19b in mouse myocardium

Expression of miRNA-19a/19b in Animal Models of Mouse Heart Failure and Myocardial Infarction

The results showed that the expression of miRNA-19a/19b in the mouse myocardium gradually

decreased two days after the establishment of the mouse heart failure animal model. As shown in Figure 2, the expression index of miRNA-19a/19b in the mouse myocardium was reduced from the original (305.28 ± 8.86) decreased to (171.55 ± 5.32), the difference was statistically significant ($P < 0.05$), indicating that heart rate failure and myocardial damage can inhibit the expression of miRNA-19a/19b to a certain extent.

Protective Effect of miRNA-19a/19b on Mouse Heart After Myocardial Infarction

After the modeling was completed, the heart tissue of the mice was taken and the area of myocardial infarction was measured. The study found that the area of myocardial infarction in mice became much smaller. As shown in Figure 3, miRNA-19a/19b can make mouse myocardium. The infarct area was reduced by 27.5%, indicating that miRNA-19a/19b has a certain protective effect on the infarcted myocardium.

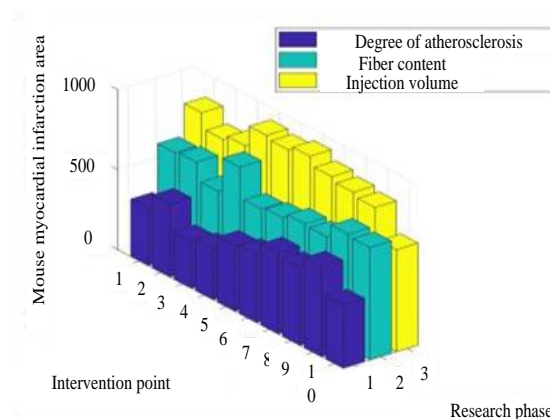


Figure 3. The protective effect of miRNA-19a/19 On Mouse Myocardium

Result Analysis of Mirna-19a/19b on the Treatment of Heart Failure

After the establishment of an animal model of heart failure, the cardiac function of mice was detected by electrocardiogram and ultrasound detector. As shown in Figure 4, after injection of miRNA-19a/19b, the mice's cardiac function indexes FS and EF were relatively increased. The functional index rose from the original (263.13 ± 5.26) to (385.48 ± 6.92), proving that miRNA-19a/19b has a good therapeutic effect on heart failure in mice.

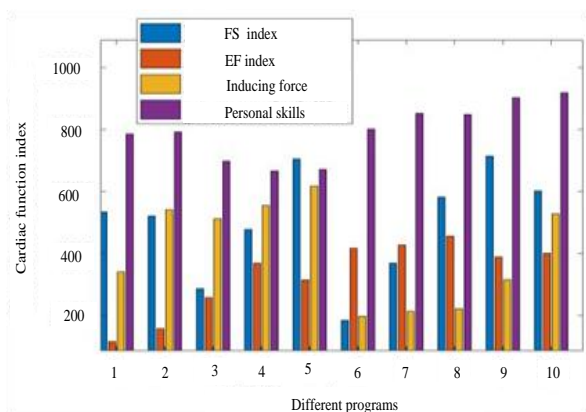


Figure 4. Result analysis of miRNA-19a/19b on the treatment of heart failure

Effect of miRNA-19a/19b on myocardial regeneration and repair

The study found that miRNA-19a/19b promotes the proliferation of myocardial cells in mice with myocardial damage and heart failure by regulating the expression of target genes CCND1 and CDK1. As shown in Figure 5, it increased by 18.6% and the rate of myocardial regeneration and repair increased by 20.4%, proving that miRNA-19a/19b is very helpful for the repair and repair of myocardium in mice after heart failure or myocardial infarction.

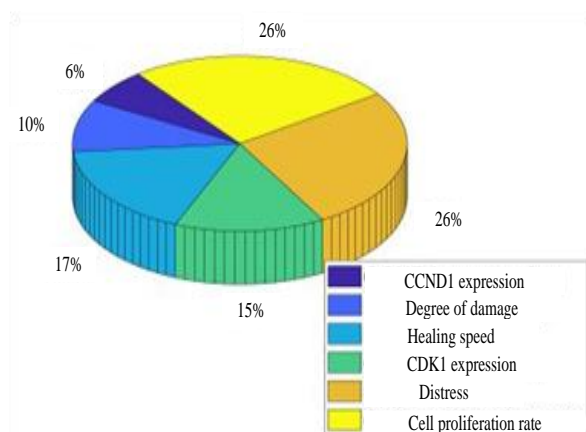


Figure 5. Effect of miRNA-19a/19b on myocardial regeneration and repair

The development and development of cardiovascular diseases (such as myocardial infarction and heart failure) is a complex process, each stage involves different miRNAs, and a gene may be regulated by multiple miRNAs (28). Long-term research on the role of miRNA in the pathological regulation of chronic heart failure has made great

progress. In just a few years, the application research of miRNA in the development of cardiovascular disease and prevention of other diseases has developed into a hotspot of medical research in the field of cardiovascular disease in China, but miRNA and related cardiovascular disease-related research are still in the early stages. In this article, we review the role of miR-19a and miR-19b in protecting the heart after MI. Adult myocardial infarction and perfusion of miR-19a/19b mimics into the myocardium can effectively prevent cardiac dysfunction caused by myocardial infarction. The study found that miR-19a/19b protects and treats the heart after myocardial infarction.

This study found that miRNA-19a/19b protects the heart after myocardial infarction by targeting two distinct biological processes, thus exhibiting two stages of heart protection. This article confirmed that miRNA-19a/19b can promote the proliferation of cardiomyocytes after myocardial infarction. Immediate injection of miRNA-19a/19b into the heart of myocardial infarction can reduce myocardial cell death and dysfunction caused by myocardial infarction, and improve the survival rate of mice after myocardial infarction. At the same time, miRNA-19a/19b protects the heart by down-regulating the immune inflammation of the damaged heart in the early stages of heart damage. Combined with recent research, cell movement and immune response play a major protective role in regulating heart damage and remodeling.

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None.

Interest conflict

The authors declare no conflict of interest.

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