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Investigating the expression of miRNA-133 in animal models of myocardial infarction and its effect on cardiac function

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Abstract. – OBJECTIVE: This study aimed to investigate the expression of miR-133 in animal models of myocardial infarction and its effect on cardiac function.

MATERIALS AND METHODS: Forty-five male-specific pathogen-free (SPF) C57/BL6 mice were selected, among which 35 were made for animal models of myocardial infarction and were enrolled into Model Group and another 10 were enrolled into Blank Control Group. Seven mice died in the model making. Ten mice randomly selected from the 28 mice successfully modeled were transfected with adenovirus carrying miRNA-133 and set as Virus Group, while the remaining 18 mice were randomly divided into Virus No-load Group and Model Group. Mice in Virus Group were transfected with adenovirus carrying miRNA-133, while those in Virus No-load Group were transfected with empty viral vectors without miRNA-133. The left ventricular ejection fraction (LVEF) and fractional shortening (FS) of the mice weeks after the infection were recorded and evaluated by echocardiography. The relative expression levels of miR-133 in the heart tissues of the four groups of mice were compared by Real Time-Polymerase Chain Reaction (RT-PCR).

RESULTS: The miR-133 expressions in Blank Control Group and Virus Group were higher than that of Model Group ($p<0.05$). Then, the myocardial infarction area of mice was compared. The LVEF and FS values of mice in Model Group, Virus No-load Group, and Virus Group were significantly lower than those in Blank Control Group, with the LVEF and FS values of Virus Group higher than that of Model Group and Virus No-load Group ($p<0.05$). The swimming time of Blank Control Group was significantly higher than that of Model Group and Virus Group ($p<0.05$), with Virus Group and Virus No-load Group having a greatly longer swimming time than Model Group ($p<0.05$). The myocardial infarction area of mice in Virus Group was significantly smaller than that in Model Group and Virus No-load Group, the difference was statistically significant ($p<0.05$). There was no significant difference in myocardial infarction area of mice between Model Group and Virus No-load Group ($p>0.05$).

CONCLUSIONS: MiR-133 was in a low expression state in the mice models of myocardial infarction and the overexpression of miR-133 could significantly improve the cardiac function index and motor function, as well as reduce the myocardial infarction area of mice with myocardial infarction. This could inspire new molecular therapy for the treatment of myocardial infarction.

Key Words:
MiRNA-133, Myocardial infarction, Mice models, Cardiac function.

Introduction

As people are pursuing ever-improving living standards, cardiovascular disease has posed a major threat to human life and health and is in a growing tendency to attack younger people[1,2]. Manifested as necrosis of the myocardium triggered by an irreversible ventricular remodeling and damage to cardiac function, myocardial infarction is a cardiovascular disease caused by the blockage of local blood vessels in the heart[3]. Long-term progressive heart failure, as a result of myocardial infarction, has a serious impact on the quality of life of patients[4]. At present, the main clinical treatment for heart failure after myocardial infarction is mainly instrument implantation and drug use, which gives no significant efficacy[5]. However, with the continuous development of molecular biology, Cao et al[6] in the study of animal models of myocardial infarction found that gene therapy could improve the cardiac function of myocardial infarction of animals to a certain extent.

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MicroRNA (miRNA), a single-stranded non-coding RNA of about 21-23 nucleotides in length, some of which have been found to be able to regulate the growth and development of the heart, is closely related to the occurrence of cardiovascular disease[7]. As a member of the microRNAs, miR-133 with high expression in the heart has been proved to play an important role in the psychological and pathological cardiovascular process[8]. Zhang et al[9] discovered the inhibitory effect of miR-133 on cardiomyocyte apoptosis when studying the influence of increased miR-133 expression by MAPK ERK1/2 activated by tanshinone. Other reports[10] discovered the low expression of miR-133 in both human and mice models of cardiac hypertrophy and found that high expression of miR-133 *in vitro* could inhibit cardiac hypertrophy. Previously, Liu et al[11] detected that miR-133 inhibited cardiac hypertrophy by negatively regulating the Ras homologous A and the cell division control gene 42.

However, the current role of miR-133 expression in myocardial infarction has not been reported. Therefore, this study explored the expression of miR-133 in animal models of myocardial infarction and its effect on cardiac function to provide new solutions and ideas for the treatment of clinical myocardial infarction.

Materials and Methods

Animals

Animals: 45 male C57/BL6 SPF mice, aged 6-8 weeks, weighing 20-24 g, were all purchased from Shanghai SLAC Laboratory Animals Co., Ltd. [code number of production license: SCXK (Shanghai) 2012- 0002].

All mice were housed under normal 12-h circadian rhythm and kept at a constant temperature of 22°C, free to eat and drink. Experimental equipment: the medical small animal ventilator was purchased from Shanghai Yuyan Instruments Co., Ltd.; the surgical instrument was purchased from Shenzhen Ruiwode Life Science Co., Ltd., and Pelltobarbitalum Natricum at 1% concentration was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

Production of Mice Model of Myocardial Infarction

Mice were anesthetized by injecting Pelltobarbitalum Natricum at a concentration of 1% into the abdominal cavity at a dose of 0.1 ml/10g, 10 of which were enrolled into Sham-operated Group, receiving only thoracotomy without coronary artery ligation. The other 35 were enrolled into Myocardial Infarction Group. After the successful anesthesia, the mice were fixed on the mouse plate in a natural supine state and were operated with endotracheal intubation and connected to the ventilator. After the intubation, the ECG detector for the small animal was connected with the limbs of the mouse to record the changes of the electrocardiogram during the operation. The skin was then cut along the ribs in the 3rd to 4th intercostal space on the left side of the mouse to expose the heart with the help of a surgical spreader. Next, the left coronary artery was ligated about 2 mm below the left atrial appendage. If there were signs of whitened left ventricular anterior myocardium, weakened heartbeat, and excessive ST-T elevation shown on the ECG occurred, the models of myocardial infarction model were then successfully established and suturing was performed on the successful models. A total of 28 mice were successfully modeled in this experiment, 7 mice died during anesthesia and surgery, and the remaining mice were transfected with adenovirus.

Adenovirus Transfection and Test of Cardiac Function and Motor Function

Of the 28 mice that were successfully modeled, 10 were randomly selected to transfect with miRNA-133-bearing adenovirus and set as Virus Group, and the remaining 18 mice were randomly divided into Virus No-load Group and Model Group. Mice in Virus Group were transfected with adenovirus carrying miRNA-133, while those in Virus No-load Group were transfected with empty viral vectors without miRNA-133. Specifically, about 0.1 ml of adenovirus was injected at 5 points uniformly around the area of myocardial infarction of each mouse in Virus Group. Mice in Blank Control Group, Virus No-load Group, and Model Group were injected with the same dose of normal saline and the injection was conducted every other day for two weeks. On the 4th week after the adenovirus transfection, the cardiac function of mice was detected by echocardiography. Mice were connected to the echocardiographic instrument. Next, the left ventricular ejection fraction (LVEF) and the fraction shortening (FS) of the mice were recorded and evaluated. The motility of all mice 4 weeks after transfection was measured by recording the swimming time (from the time the mouse is put into the tank to the time

Table I. Related primer sequence table.

Factor	Forward primer	Reverse primer
miR-133	5'-TTTGGTCCCCTTCAAC-3'	5'-TAGCTATCCTTTGCT-3'
U6	5'-CTCGCTTCGGCAGCACA-3'	5'-ACGCTTCACGAATTTGCGT-3'

the mouse begins to sink) of each mouse in the swimming exhaustion experiments in which all the mice were placed in a 25°C water tank with a spilikin weighing about 1 g tied to the tail.

Detection of MiR-133 by RT-PCR

After killing the mice following the test and cutting up their heart tissue, the total RNA of the heart tissue was extracted by the TRIzol reagent, and the concentration and purity of the RNA were detected by ultraviolet spectrophotometry. 5 ul of RNA was taken from each group for reverse transcription according to the kit instructions, with the reaction conditions were 16°C for 15 min, 42°C for 42 min, and 85°C for 5 min, followed by qPCR. Reaction conditions: pre-denaturation at 95°C for 4 min. 40 cycles of 95°C for 15 s, 60°C for 40 s. 72°C terminal extension for 6 min. Using U6 as an internal reference, the relative expression of miR-133 in the heart tissues of each group of mice was statistically collected and compared by $2^{-\Delta\Delta CT}$ method. Specific primer sequences are shown in Table I.

Calculation of Myocardial Infarction Area

After the mice were sacrificed, hearts were removed. Their blood was washed away with phosphate-buffered saline (PBS) solution, blotted up with gauze and refrigerated at -20°C for 15 min. After serial section, hearts were put into 1% TTC solution and incubated in the dark for 15 min at 37°C. Myocardial infarction area was calculated, the percent of infarction (%) = infarction area/total area of cardiac section×100%.

Observation Indicators

On the 1st day and 4th week after the adenovirus transfection, the cardiac function of mice was detected by echocardiography, the left ventricular ejection fraction (LVEF) and the fraction shortening (FS) of mice were recorded and evaluated. The motility of all mice 4 weeks after the transfection was measured by recording the swimming time (from the time the mouse is put into the tank to the time the mouse begins to sink) of each mouse in the swimming exhaustion experiments in which all the mice were placed in a 25°C water tank with an object weighing about 1 g tied to the tail. The myocardial infarction area of mice in the four groups was recorded and compared.

Statistical Analysis

The collected data were statistically analyzed using SPSS 20.0 (Beijing, ND Times Technology Co., Ltd.). The measurement data were expressed as ($\overline{x}$±s). The comparison between the groups was analyzed by ANOVA. Pairwise comparison was detected by LSD/*t*-test. Comparison at different time points was analyzed by repeated ANOVA. Statistical difference was recognized if $p<0.05$.

Results

The Expression of MiR-133 in the Heart Tissue of Mice in the Four Groups 4 Weeks After the Transfection

The relative expressions of miR-133 in Blank Control Group (1.03±0.14) and Virus Group (1.13±0.17) were both statistically higher than that of Model Group (0.32±0.11) and Virus No-load Group (0.34±0.10) ($p<0.05$). The little difference between the relative expressions of miR-133 in Blank Control Group and Virus Group, and Virus No-load Group ($p>0.05$) suggested the successful transfection (Table II, Figure 1 for details)

Table II. Comparison of miR-133 expression in four groups of mice.

Factor	Blank Control Group n=10	Model Group n=9	Virus No-load Group n=9	Virus Group n=10	*F*	*p*
miR-133	1.03±0.14	0.32±0.11	0.34±0.10	1.13±0.17	99.43	<0.001

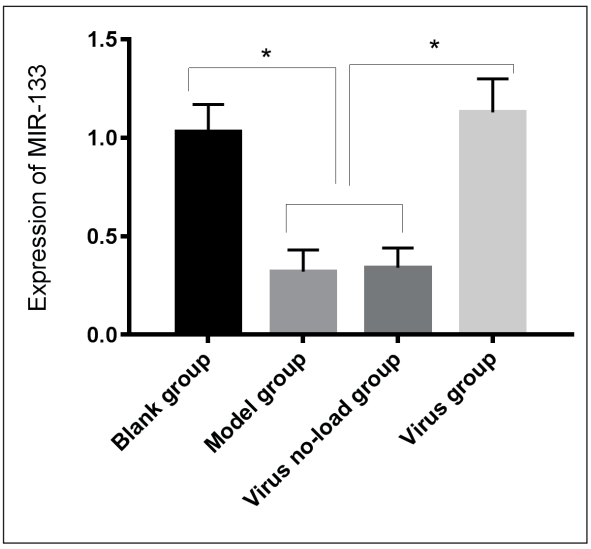

Figure 1. The expression of miR-133 in the heart tissues of the three groups of mice. The Blank Control Group and the Virus Group were not statistically different in the expression of miR-133 ($p>0.05$), but both statistically higher than that of the Model Group ($p<0.05$). Note: "*" means that $p<0.05$ when compared with the Model Group.

Cardiac Function of the Three Groups of Mice on 4th Week After the Adenovirus Transfection

The LVEF value of Model Group 4 weeks after the myocardial infarction was (43.26±0.83)%, and the FS value of Model Group 4 weeks after myocardial infarction was (23.56±1.23)%; LVEF value of Virus No-load Group 4 weeks after the myocardial infarction was (42.95±0.73)%, and the FS value of Virus No-load Group 4 weeks after the myocardial infarction was (23.31±1.22)%; the LVEF value of Blank Control Group 4 weeks after the myocardial infarction was (82.11±3.34)%, the FS value of the Blank Control Group 4 weeks after the myocardial infarction was (41.87±4.11)%; the LVEF value of Virus Group 4 weeks after the myocardial infarction was (67.17±4.15)%, the FS value of Virus Group 4 weeks after the myocardial infarction was (32.77±3.81)%. The LVEF and FS values of Model Group, Virus No-load Group, and Virus Group were significantly lower than those of Blank Control Group, with the LVEF and FS values of Virus Group higher than that of Model Group and Virus No-load Group, and the differences were statistically significant ($p<0.05$; table III for further information).

Motor Function Test of Mice in 4 Weeks After Transfection of Virus

The swimming time of Blank Control Group was significantly higher than that of Model Group, Virus No-load Group and Virus Group ($p<0.05$), with Virus Group having a greatly longer swimming time than Model Group and Virus No-load Group, the difference was statistically significant ($p<0.05$) (Table IV).

Comparison of Myocardial Infarction Area of Mice

Comparing the myocardial infarction area of mice, it was found that the myocardial infarction areas of mice in Model Group, Virus No-load Group, and Virus Group were (36.71±1.57)%, (35.11±1.21)% and (29.87±1.19)%, respectively. The myocardial infarction area of mice in Virus

Table III. Cardiac function of the three groups of mice on 4th week after the adenovirus transfection (%).

Factor	Blank Control Group n=10	Model Group n=9	Virus No-load Group n=9	Virus Group n=10	F	p
LVEF	82.11±3.34	43.26±0.83	42.95±0.73	67.17±4.15	412.5	<0.001
FS	41.87±4.11	23.56±1.23	23.31±1.22	32.77±3.81	456.8	<0.001

Table IV. Comparison of swimming time in four groups of mice.

Factor	Blank Control Group n=10	Model Group n=9	Virus No-load Group n=9	Virus Group n=10	F	p
Swimming time min*	10.34±1.43*	6.92±1.02*	6.81±1.06*	8.26±1.31	17.25	<0.001

Note: "*" means that $p<0.05$ when compared with Virus Group

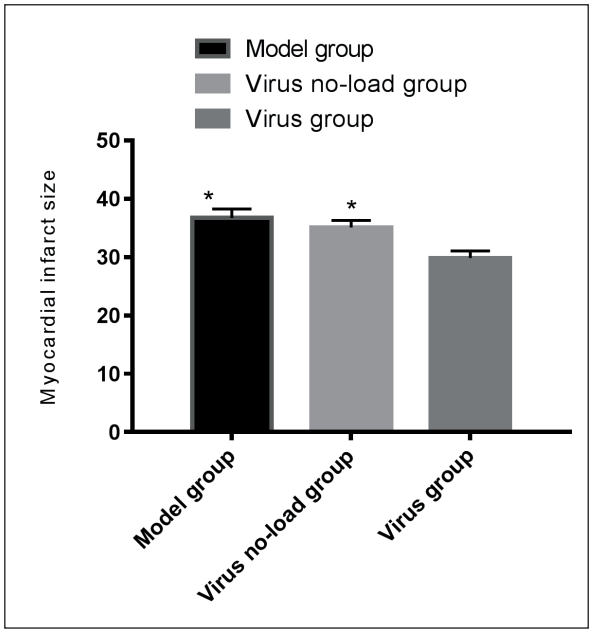

Figure 2. Comparison of LVEF values between the three groups of mice on the 1st day and 4th week after the adenovirus transfection. The LVEF values of the Blank Control Group were significantly higher than the Model Group and the Virus Group ($p<0.05$), while the LVEF values of the Virus Group were greatly higher than the Model Group, and the difference was statistically significant ($p<0.05$). Note: "*" means that $p<0.05$ when compared with the Model Group and the Blank Control Group.

Group was significantly smaller than that in Model Group and Virus No-load Group, the difference was statistically significant ($p<0.05$). There was no significant difference in myocardial infarction area between Model Group and Virus No-load Group ($p>0.05$), as shown in Figure 2.

Discussion

With high mortality and disability rate in cardiovascular disease, myocardial infarction is a disease which has a serious impact on patients' quality of life[12]. After myocardial infarction, local myocardial tissue loss and necrosis will occur, followed by lost contraction and electrocoupling function. Then, the scar tissue will be formed by myocardial remodeling and fibrous tissue hyperplasia as a substitution of necrotic myocardial tissue. However, scar tissue without electro-biological properties and contractility to support the contractile function of the heart after myocardial infarction will cause a further decrease in the cardiac function[13,14]. Considering the fact that no satisfactory efficacy is achieved at present in treating myocardial infarction, it is in an urgent need to find a new effective treatment method[15]. Molecular biology being in the spotlight of current research, miR-133, as one of the miRNAs with specific expression of striated muscle tissue, has been discovered by studies to have a significant impact on the physiological and pathological processes of the heart, such as cardiac development and arrhythmia[16,17]. Feng et al[18] found that the increased miR-133 expression by tanshinone IIA could inhibit the incidence of myocardial apoptosis in mice with heart failure. Also, Myers et al[19] found that miR-133 was found to be an anti-apoptotic factor, suppressed the cell apoptosis by inhibiting the expression of Caspase-9. Since no research has been reported on the expression of miR-133 in myocardial infarction and its influence on cardiac function, this work explored the expression of miR-133 in animal models of myocardial infarction and its effect on cardiac function.

In this report, to study the effect of overexpression on miR-133 on myocardial function in mice, we used the adenovirus carrying mir-133 to transfect some mice of the successful models of myocardial infarction. According to the comparison of miR-133 expressions in the heart tissues of the four groups of mice four weeks after the transfection, little difference existed between the expressions of miR-133 in Blank Control Group and Virus Group ($p>0.05$) which were both statistically significantly higher than that of Model Group and Virus No-load Group ($p<0.05$), suggesting the successful transfection and the low miR-133 expression in the mice models of myocardial infarction. The low miR-133 expression in the myocardium after myocardial infarction was also discovered by Wang et al[20] in their study of the effect of miR-133 on myocardial remodeling, but its specific mechanism of action was not detailed. We evaluated cardiac function and motor function in the four groups of mice 4th week after the transfection, and found that the LVEF and FS values of mice in Model Group, Virus No-load Group and Virus Group were significantly lower than those of Blank Control Group, with the LVEF and FS values of Virus Group higher than that of Model Group and Virus No-load Group ($p<0.05$). The swimming time of Blank Control Group was significantly higher than that of Model Group, Virus No-load Group and Virus Group ($p<0.05$), with Virus Group having a greatly longer swimming time than Model Group and Virus No-load Group ($p<0.05$). Thus, a conclusion was drawn that miR-133 was in a low expression state in the mice models of myocardi-

al infarction, and the overexpression of miR-133 could significantly improve the cardiac function index and motor function of mice with myocardial infarction. Discovered in Chen et al[21] team's study of miR-133a, mesenchymal stem cells with overexpression of miR-133 could effectively improve cardiac function in mice with myocardial infarction, the mechanism of which was that the mesenchymal stem cells with overexpression of miR-133 could significantly inhibit the expression of SNAR1, verifying the conclusion of this work. Finally, we compared the myocardial infarction area of mice and the results showed that the myocardial infarction area of mice in Virus Group was significantly smaller than that in Model Group and Virus No-load Group ($p<0.05$). There was no significant difference in myocardial infarction area between Model Group and Virus No-load Group ($p>0.05$). Results suggest that the overexpression of miR-133 in myocardial infarction mice helps reduce the size of myocardial infarction.

Conclusions

We showed that miR-133 was in a low expression state in the mice models of myocardial infarction and the overexpression of miR-133 could significantly improve the cardiac function index and motor function of mice with myocardial infarction, which could inspire a new molecular therapy for the treatment of myocardial infarction.

Conflict of Interests

The Authors declared that they have no conflict of interests.

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