Original Article
Expression and function of miR-92a in ventricular remodeling after PCI treatment of acute myocardial infarction

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Abstract: Percutaneous coronary intervention is the major approach treating acute myocardial infarction (AMI). However, PCI easily causes myocardial ischemia reperfusion injury (MIRI) leading to ventricular remodeling. MiR-92a is correlated with AMI pathogenesis. Its expression and function during ventricular remodeling in AMI patients after PCI treatment remain unclear. AMI patients after PCI surgery received ultrasonic cardiography (UCG) for measuring ejection fraction (EF), left ventricular end systolic diameter (LVESD), left ventricular thickness, left ventricular end diastolic diameter (LVEDD). Real-time PCR was used to measure miR-92a expression and its correlation with ventricular remodeling related indexes. Wild type (WT) and miR-29a KD rats were established for MIRI model. M type ultrasound was used to evaluate cardiac function, whilst ELISA was used to test contents of type I collagen, interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α). Western Blot was used for detecting Bcl-2 protein expressions in myocardial cells. Patients after PCI had decreased EF, higher LVESD and LVEDD, lower left ventricular thickness, and enhanced miR-92a expression (P<0.05 compared to before surgery). MiR-92a was positively correlated with post-op EF and left ventricular thickness, and was negatively correlated with LVESD and LVEDD (P<0.05). MiR-92a KD MIRI rats had lower miR-92a expression, improved cardiac functions, lower type I collagen, higher Bcl-2, and decreased IL-6 and TNF-α expression (P<0.0 compared to WT).MiR-92a had elevated expression during ventricular remodeling. Inhibition of miR-92a could improve cardiac functions via suppressing type I collagen, decreasing apoptosis and inhibiting inflammation.

Keywords: MiR-92a, ventricular remodeling, acute myocardial infarction, percutaneous coronary intervention, cell apoptosis

Introduction

With transition of life styles, diet habitat, population aging and influences of social or mental factors, the incidence of acute myocardial infarction (AMI) is increasing by years [1]. Among all cardiovascular disease the most common and severe diseases are coronary heart disease and AMI [2]. As one common severe disorder of cardiovascular system, the timely treatment of AMI can help to improve patient's survival rate, life quality and prognosis [3]. Percutaneous coronary intervention (PCI) is the major approach for treating AMI currently [4]. With wide application of PCI, the mortality of AMI patients has been effectively decreased, with elongated survival time, but accompanied with rapid rise of heart failure

(HF) rate. PCI solves the issue of coronary artery flow, but inevitably leading to post-op HF in certain patients [5]. Previous study reported the occurrence of ventricular remodeling in some Ami patients after receiving PCI surgery, threatening prognosis [6]. After PCI, MIRI frequently occurs, leading to elevated secretion of inflammatory factors IL-6 and TNF-α, enhancing cell apoptosis, decreasing Bcl-2 expression, and remodeling ventricles [7, 8].

MicroRNA (miR) is widely distributed in both plant and animal cells, and is one type of regulatory small non-coding RNA [9]. MiR can negatively regulate gene expression via complete or incomplete binding with target mRNA molecules, or degrading mRNA/regulating protein translation via inhibiting expression of down-

stream target proteins [10]. With further study of miR, increasing knowledge has been obtained regarding the regulatory process of miR on biological functions. Current studies agreed that miR was one important regulatory factor for participating in cell growth, proliferation, differentiation, metabolism and apoptosis [11]. Abundant miR could participate in ventricular remodeling process [12]. Previous study found the correlation between miR-92a and AMI, during which the inhibition of miR-92a could exert protective effects on endothelial cells [13, 14]. The expression and functional role of miR-92a in ventricular remodeling of AMI patients after PCI treatment, however, has not been illustrated.

Materials and methods

General information

A total of 60 AMI patients who received PCI in the Xidian Group Hospital from January 2015 to January 2016. There were 32 males and 28 females, aging between 28 and 62 years old (average age = 36.3 ± 6.2 years). Inclusive criteria: All research objects fitted the diagnostic criteria of AMI including typical chest pain longer than 30 min but shorter than 12 h without relief by nitrate medication, novel signs of myocardial ischemia on ECG (ST segment change, left bundle branch block, pathology Q wave, dynamics of troponin (cTnI) and creatine kinase-MB isoenzyme (CK-MB), and receiving PCI for the first time within 24 h.

Exclusive criteria

Previous history of myocardial infarction; Firstl time of PCI treatment; Complicated with myocardial disease, pericardial disease, infectious pericarditis; Having acute heart failure (HF) when admission; With infectious disease, malignant tumor, severe diabetes, liver/kidney disease, pulmonary fibrosis, bone metabolic disorder, systemic immune disease and complication of malignant tumor; Or complicated with cardiac shock. This study has been approved by the ethical committee of the Xidian Group Hospital. All included subjects have informed consents and signed with this study.

Experimental animals

Healthy male Wistar rats (6-8 weeks old, body weight 230 ± 20 g, SPF grade) were purchased from laboratory animal center of the Xi'an Jiaotong University and were randomly divided into control and MIRI groups. Animals were kept in an SPF facility with fixed temperature (21 ± 1°C) and relative humidity (50-70%) with 12h light/dark cycle. MiR-92a KD rats were provide by Cygene (China).

Major reagents and equipment

Trizol reagent, RNA extraction kit, RT-PCR primer, reverse transcription (RT) kit, real-time PCR kit were purchased from Invitrogen (US). ELISA kit for Type I collagen, IL-6 and TNF-α were purchased from ebioscience (US). PVDF membrane was purchased from Pall Life Sciences (US). Western blotting reagents were purchased from Beyotime (China). ECL reagent was purchased from Amersham Biosciences (US). Rabbit anti-mouse Bcl-2 monoclonal antibody and goat anti-rabbit horseradish peroxidase (HRP)-labelled IgG secondary antibody were purchased from Cell Signaling (US). Lentivirus vector was purchased from Jikai Gene (China). Other common reagents were purchased from Sangon (China). ABI7900 HT Real-time PCR was purchased from ABI (US). Surgical microscope was purchased from Suzhou Instrument (China). Labsystem Version 1.3.1 microplate reader was purchased from Bio-rad (US).

PCI surgery

Percutaneous transluminal coronary angioplasty (PTCA) and stent endoprosthesis: following standard PCI approach, Judkins method was used for angiogram on bilateral coronary arteries to confirm IRA, followed by PTCA or stent implantation of coronary artery surgery. PTCA was directly applied into infarcted arteries, which were implanted for stent. Criteria of successful surgery: TIMI blood flow grade III, remaining stenosis <20%, no severe complication (death, re-infarction of myocardium and reestablishment of target vessels).

Ultrasonic cardiograph

Ultrasonic cardiograph was performed to evaluate left ventricular remodeling and cardiac function before surgery and 3 month after surgery, to quantify parameters including ejection fraction (EF), left ventricular end systolic diameter (LVESD), left ventricular end diabolic diameter (LVEDD) and ventricular wall thickness.

Recording of clinical information

All clinical information of included patients were recorded including sex, age, height and

Table 1. Primer synthesis sequences

Target gene	Forward primer 5'-3'	Reverse primer 5'-3'
GADPH	AGTGCCAGCCTCGTCTAG	CGTTTTGCCGTGGGTAG
Mir-92 (People)	TCTGAAGCGACGCAACTT	CGGGTCCGACATCA
Mir-92 (Rats)	GAACTACCAGTCAATCG	CAGGGACATCCTCTC

body weight, plus other disease, mediation and family history. Normal ultrasound cardiography and electrocardiogram (ECG) examination were conducted.

Rat AMI model preparation

Rat AMI model was prepared by balloon obstruction of left descending branch of coronary artery. After general anesthesia, wild type and MiR-92a KD rats were fixed in a supine position. After shaving and sterilization of anterior throat skin, tracheal intubation was performed under visual assistance. The ventilation machine was connected for assisted respiration (tidal volume 4 ml/kg, respiration frequency 80 per min). An incision was made via the fourth rib on left chest; the heart was then exposed by blunting separation of subcutaneous tissues and muscle. The left coronary artery was ligated using 7-0 nylon suture via 2/3 myocardial layer on the bottom edge (1~2 mm) of left ventricular ear. Electrocardiogram was monitored in real time during the surgery. The model was generated when heart tissues turned white accompanied with continuous elevation of ST segment (>1/2 R wave) in a single peak.

Evaluation of post-op cardiac function index

M type ultrasound was used to describe the change of cardiac function within 24 h of disease onset and at 28 days post-op, including left ventricular quality index (LVMI), LVESD and LVEDD. In brief, rats were fixed in a supine position. Ultrasound probe (model 15L8) was placed near the chest for a horizontal section of left ventricular short axis mammillary muscle. After obtaining clear 2D image, M-type ultrasound cardiac graphic was applied to measure LVEDD and LVESD. The LVMI was calculated based on the formula.

Sample collection

Total of 5 ml blood samples were collected from the rat tail vein within 24 h of disease onset and at 28 d post-op. Blood samples were

centrifuged at 3000 rpm for 15 min. Serum was saved in new tubes and kept at -80°C for further experiments.

ELISA for type I, IL-6 and TNF-α contents

ELISA was employed to measure type I collagen, IL6 and TNF-α levels to analyze their correlations with ventricular remodeling and inflammatory factors following the manual instruction of ELISA kit. A linear regression function was generated based on concentrations and respective absorbance (A) values of standard samples. The concentration in serum sample was then deduced on the regression model from A value.

Real time PCR for miR-92a expression

Trizol reagent was used to extract total RNA form human peripheral blood and rat myocardial tissues. UV spectrometry was used to measure RNA purity and concentration. Reverse transcription was performed following manual instruction. PrimerPremier 6.0 was employed to design PCR primers, which were synthesized by Sangon (China) as shown in **Table 1**. Real-time PCR was performed to test target gene. Reaction conditions were: 52°C for 1 min, followed by 35 cycles each containing 90°C 30 s, 58°C 50 s and 72°C 35 s. Fluorescent quantitative PCR cycler was used to collect data. CT values of all standards were collected to plot the standard curve based on internal reference gene GAPDH. $2^{-\Delta Ct}$ method was used for semi-quantitative analysis.

Western blot for Bcl-2 protein expression

Total proteins were extracted from myocardial tissues. In brief, cells were lysed on ice for 15~30 min, with ultrasound treatment (5 s, 4 times). After centrifugation at 10000 g for 15 min (4°C), the supernatant was saved, quantified by Bradford method and stored at -20°C for Western blot assay. Proteins were separated in 10% SDS-PAGE, and were transferred to PVDF membrane by semi-dry method (150 mA, 1 h). Non-specific binding sites were removed by 5% defatted milk powder for 2 h. Anti-Bcl-2 monoclonal antibody (1:2000) was added for 4°C overnight incubation. After PBST washing, goat anti-rabbit secondary antibody (1:2000) was added for 30 min incubation at room tempera-

Table 2. Ultrasound cardiograph of AMI patients after PCI surgery

	Before surgery	3 month post-op
LVESD (mm)	30.19 ± 4.28	39.21 ± 5.77*
LVEDD (mm)	39.33 ± 5.21	49.17 ± 8.68*
EF (%)	51.32 ± 6.36	41.88 ± 7.13*
LVAWT (mm)	10.11 ± 0.75	8.82 ± 0.79*
LVPWT (mm)	9.65 ± 0.81	8.01 ± 0.52*

Note: *, P<0.05 compared to before surgery.
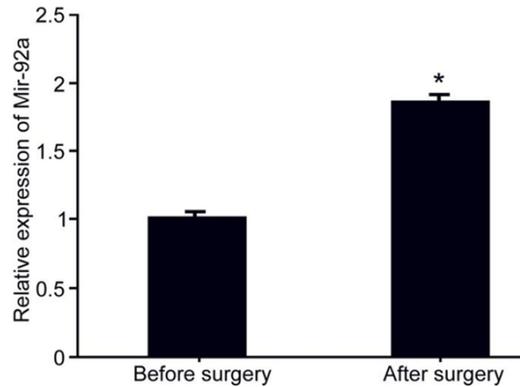
Figure 1. Expression of miR-92a on AMI patients before and after PCI surgery. *, P<0.05 compared to before surgery.

Table 3. Correlation analysis between miR-92a and cardiac function indexes

Post-op cardiac function index	Mir-24
LVESD (mm)	0.592
LVEDD (mm)	0.671
EF (%)	-0.725
LVAWT (mm)	-0.832
LVPWT (mm)	-0.716

ture. ECL reagent was then added for developing the membrane for 1 min, followed by X-ray exposure. The result was obtained by protein imaging system and Quantity One software (Gene, US) for measuring band density. Each experiment was replicated for four times (n = 4) for statistical analysis.

Statistical analysis

SPSS 19.0 software was used to process all data, of which measurement data were expressed as mean ± standard deviation (SD). The comparison of means among multiple groups was performed using one-way analysis of variance (ANOVA). A statistical significance was defined as P<0.05.

Results

Cardiac functions of AMI patients after PCI treatment

Analysis of cardiac function indexes was performed using ultrasound cardiograph in AMI patients before PCI surgery and at 3 month post-op follow-ups for further evaluation of left ventricular remodeling. Results showed significantly lower EF of left ventricle after surgery (P<0.05 compared to those before surgery). LVESD and LVEDD were all increased after the surgery (P<0.05 compared to before surgery). LVAWT and LVPWT found decreased ventricular wall thickness (P<0.05 compared to before surgery, **Table 2**). These results suggested significant change of cardiac functions in patients receiving PCI treatment, indicating ventricular remodeling, probably due to MIRI injury.

Expression of miR-92a in AMI patients after PCI surgery

Real-time PCR was used to test the expression change of miR-92a in AMI patients before and after PCI surgery. Results showed elevated miR-92a after PCI surgery (P<0.05 compared to before surgery, **Figure 1**).

Correlation between post-op miR-92a expression and cardiac function in AMI patients

We analyzed the correlation between miR-92a expression level in AMI patients 3 month after PCI surgery and cardiac function indexes from ultrasound cardiograph. Results showed negative correlation between miR-92a and left ventricular EF (P<0.05), and positive correlation between miR-92a and LVESD or LVEDD (P<0.05). MiR-92a was also negatively correlated with LVAWT and LVPWT (P<0.05). All these differences were of statistical significance (**Table 3**), indicating that miR-92a could work as one test index for myocardial ischemia-reperfusion and ventricular remodeling.

Expression of miR-92a in MIRI rats

Real-time PCR was used to test miR-92a expression in MIRI model on both wild type and miR-92a KD rats. Results showed elevated miR-92a expression in MIRI model rats of WT
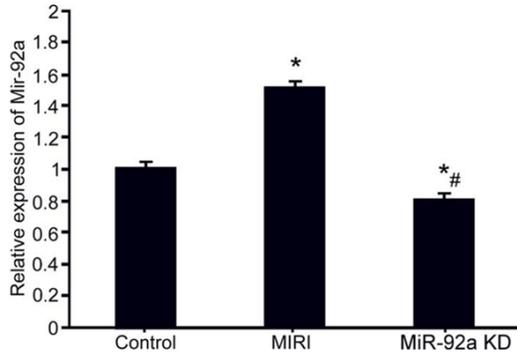
Figure 2. Expression change of MiR-92a in MIRI rats. *, P<0.05 compared to control group; #, P<0.05 compared to MIRI group.

Table 4. Effects of miR-92a inhibition of myocardial function of MIRI rats

Group	LVEDD (mm)	LVESD (mm)	LVMI
Control	0.47 ± 0.05	0.31 ± 0.03	2.51 ± 0.03
MIRI	0.62 ± 0.06*	0.44 ± 0.08*	3.85 ± 0.17*
miR-92a KD	0.56 ± 0.05#	0.36 ± 0.03#	2.87 ± 0.11#

Note: *, P<0.05 compared to control group; #, P<0.05 compared to MIRI group.
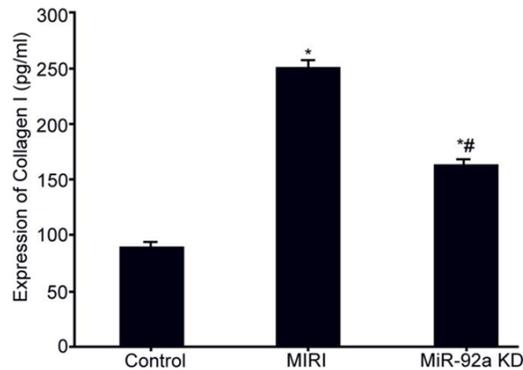
Figure 3. Effects of miR-92a inhibition on type I collagen expression in MIRI rats. *, P<0.05 compared to control group; #, P<0.05 compared to MIRI group.

animals (P<0.05 compared to control group. MiR-92a expression level was significantly decreased in miR-92a KD MIRI rats (P<0.05, **Figure 2**).

Effects of miR-92a expression inhibition on myocardial function of rats

M type ultrasound was used to compare the effect of miR-92a expression inhibition on change of cardiac function in MIRI rats. Results
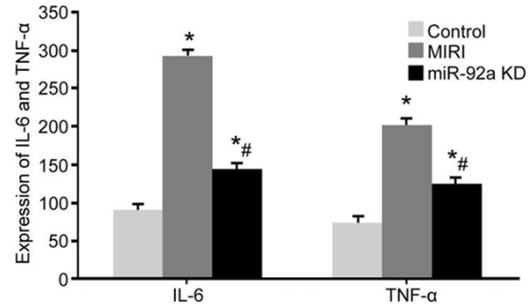
Figure 4. Effects of miR-92a inhibition on serum inflammatory factor in MIRI rats. *, P<0.05 compared to control group; #, P<0.05 compared to MIRI group.

showed elevated LVESD and LVEDD in wild type MIRI rats (P<0.05 compared to control group). In miR92a KD MIRI rat model, miR-92a expression was inhibited, accompanied with lower LVESD and LVEDD (P<0.05 compared to MIRI group). Meantime, LVMI showed similar change of LVESD and LVEDD, indicating that miR-92a inhibition significantly improved cardiac function of MIRI rats, further improving ventricular remodeling (**Table 4**).

Effects of miR-92a inhibition on type I collagen expression in MIRI rats

ELISA was used to analyze the effect of miR-92a inhibition on type I collagen in myocardium of MIRI rats. Results showed elevated type I collagen contents in wild type MIRI rats (P<0.05 compared to control group). In miR-92a KD MIRI rat model, miR-92a expression was inhibited, accompanied with decreased type I collagen content (P<0.05 compared to MIRI group, **Figure 3**). Therefore, inhibition of miR-92a overexpression significantly inhibits type I collagen expression in MIRI.

Effects of miR-92a inhibition of serum inflammatory factors of MIRI rats

ELISA was used to analyze the effect of miR-92a inhibition on serum levels of inflammatory factors IL-6 and TNF-α in serum of MIRI rats. Results showed elevated expression of IL-6 and TNF-α expression in wild type MIRI rats (P<0.05 compared to control group). In miR-92a KD MIRI rat model, miR-92a expression was inhibited, showing elevated expression of IL-6 and TNF-α (P<0.05 compared to MIRI group, **Figure 4**). Therefore, miR-92a inhibition could remarkably inhibit secretion of inflammatory factors in MIRI rats, further regulating MIRI.
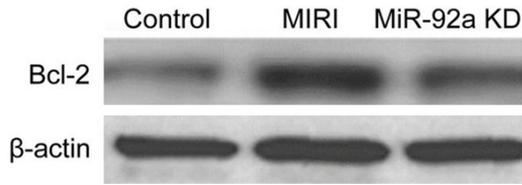
Figure 5. Effects of miR-92a inhibition on Bcl-2 expression in myocardium of MIRI rats.
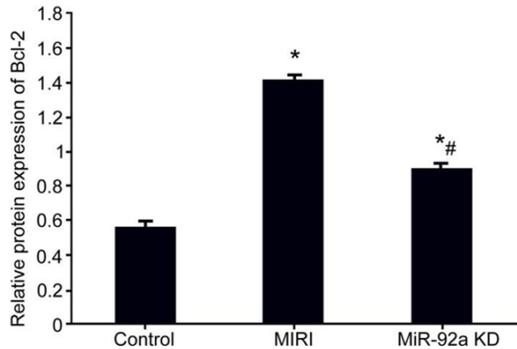
Figure 6. Analysis of miR-92a inhibition's effects on Bcl-2 expression of MIRI rat myocardium. *, P<0.05 compared to control group; #, P<0.05 compared to MIRI group.

Effects of miR-92a inhibition on Bcl-2 expression in MIRI rat myocardium

Western blot was used to test the effect of miR-92a inhibition on Bcl-2 expression in myocardial tissues of MIRI rats. Results showed decreased Bcl-2 expression in myocardium of MIRI rats after miR-92a inhibition (P<0.05 compared to control group). In miR-92a KD MIRI rat model, miR-92a expression was decreased, accompanied with higher Bcl-2 expression (P<0.05 compared to MIRI group, **Figures 5**, **6**).

Discussion

PCI treatment alleviates mortality rate and extends patient's survival period at certain extents in AMI patients. However, it frequently induces MIRI for ventricular remodeling and heart failure [15]. Ventricular modeling refers to morphological, structural and functional change at both infarction and non-infarction areas, leading to progressive dilation of ventricles, and decrease of constriction function [16]. During the progression of chronic ventricular remodeling, impaired function of blood

ejection, leading to increased incidence of arrhythmia. Under sever conditions, chronic heart failure may occur, event causing cardiac death [17]. Therefore chronic heart failure, caused by ventricular remodeling may be terminal stage of cardiovascular disease [18]. miRNA plays important regulatory roles in diseases including hypertension, coronary heart disease and ventricular remodeling [19]. The role and mechanism of AMI on ventricular remodeling and MIRI were investigated after PCI treatment.

miR-92a is one recently discovered miR in cardiovascular disease with research interests. Previous study found positive correlation between elevated circulation of miR-92a and systolic pressure, thus becoming one marker for atherosclerosis [20]. Elevated expression of miR-92a occurs in HF patients, making it one HF marker and to explain related pathogenesis mechanism [21]. In myocardial infarction and coronary heart disease, miR-92a expression was found to be up-regulated [22]. The inhibition of miR-92a during AMI pathogenesis exerts protective effects on endothelial cells [13, 14]. Therefore, this study hypothesized that miR-92a exerted functions during both ventricular remodeling and MIRI after PCI treatment. Results showed elevated miR-92a in AMI patients after PCI treatment, accompanied with myocardial remodeling. MiR-92a was negatively correlated with EF and left ventricular thickness, and was positively correlated with LVEDS and LVEDD, indicating that miR-92a could work as one index reflecting cardiac function. Further study utilized WT and miR-92a KD rats to establishment MIRI rat mode. Results showed that MIRI model on miR-92a KD rats had improved cardiac function index. More than 90% of collagen fibers in myocardium belongs to type I collagen. Due to it rigidity, extensibility and lower elasticity, type I collagen plays an important role in forming and maintain ventricular wall tensions, as increased type I collagen fibers led to higher rigidity [23]. Bcl-2 is one important member of apoptosis protein family with an anti-apoptotic nature to directly regulate programmed cell death [24]. On the other hand, elevated secretion of inflammatory factors IL-6 and TNF-α could lead to inflammation, which aggravates MIRI injury, leading to ventricular remodeling or even heart failure [25]. This study demonstrated that miR-92a KD rats inhibited miR-92a expression, further decreas-

ing type I collagen contents in MIRI rat model, which also had higher Bcl-2 expression, inhibition of inflammatory factor secretion, thus benefiting fibrosis after myocardial infarction, regulating apoptosis/anti-apoptosis homeostasis, decreasing MIRI and improving heartbeat.

Conclusion

MiR-92a had elevated expression in ventricular remodeling. Inhibition of miR-92a could improve cardiac functions during ventricular remodeling via decreasing type I collagen, suppressing apoptosis and inhibiting inflammation. Therefore, miR-92a could work as one index reflecting post-op cardiac functions after PCI, and provide novel targets of treating MIRI and ventricular remodeling.

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Disclosure of conflict of interest

None.

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