Original Article

MiR-24 expression in myocardial ischemia reperfusion induced by acute myocardial infarction after percutaneous coronary intervention treatment

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Abstract: Acute myocardial infarction (AMI) often leads to myocardial ischemia reperfusion (MIRI), thereby causing myocardial remodeling. Percutaneous coronary intervention (PCI) is the primary treatment of AMI, but easily results in myocardial ischemia-reperfusion injury, leading to myocardial remodeling. It was showed that miR-24 plays a role in cardiac remodeling. However, miR-24 expression and effect in AMI after PCI treatment have not been fully elucidated. A total of 80 cases of AMI patients after PCI surgery were enrolled. Information about echocardiography ejection fraction (EF), left ventricular end systolic diameter (LVESD), left ventricular thickness, and left ventricular end diastolic diameter (LVEDD) were recorded. MiR-24 expression before and after PCI treatment was detected by real-time PCR and analyzed with cardiac function. Rat AMI model was established and transfected by miR-24 lentivirus. Cardiac function in rats was assessed by M-mode ultrasound. Type I collagen content was determined by ELISA. Bax and Bcl-2 protein expressions in rat myocardial cells were tested by Western blot. EF reduced, LVESD and LVEDD increased, left ventricular thickness decreased, and miR-24 downregulated significantly in AMI patients after PCI compared with the preoperative group (P < 0.05). MiR-24 was positively correlated with left ventricular thickness and EF, and negatively correlated with LVESD and LVEDD (P < 0.05). Overexpression of MiR-24 in AMI rats obviously improved heart function index, reduced type I collagen content, downregulated Bax level, and enhanced Bcl-2 expression compared with AMI group (P < 0.05). MiR-24 downregulated in AMI and increased after PCI treatment. MiR-24 overexpression improved cardiac function through reducing type I collagen, regulating apoptosis balance, and alleviating MIRI.

Keywords: MiR-24, AMI, PCI, apoptosis

Introduction

Acute myocardial infarction (AMI) is a common clinical acute critical illness. With the accelerating pace of modern life, the changes of dietary habit, and the impact of aging, social and psychological factors, the incidence of AMI increased up 45–55/100,000 [1, 2]. AMI is often accompanied by myocardial ischemia and reperfusion (MIRI) and ventricular remodeling. Ventricular remodeling refers to the changes of geometry, form, and function of ventricular infarcted zone and non-infarcted zone. Ventricular remodeling is a slowly progressive process that can lead to progressive decline of ventricular dilation and contraction function, and potential ventricular arrhythmias, which eventually develops into chronic heart failure and induces sudden cardiac death because of low pump function [3, 4]. At first, ventricular remodeling maintains cardiac output as a compensatory mechanism. However, structure changes eventually lead to congestive heart failure because of the mismatching of preload and afterload and left ventricular dysfunction [5, 6]. Ventricular remodeling induced chronic heart failure is the severe or end stage of most primary cardiovascular disease and the leading cause of death of the patients with cardiovascular disease [7]. Myocar-
Cardiac extracellular matrix (ECM) change is important in the pathogenesis of ventricular remodeling. ECM remodeling makes arterial dilation to maintain normal vascular tone, resulting in monocytes/macrophages release of cytokines and growth factors that affect ECM production and degradation. Degradation of ECM components further recruits more inflammatory cells to the injured tissue, thus playing an important role in the change of myocardial collagen fiber \[8, 9\]. ECM contains various members, such as macromolecules and structural proteins, including collagen fibers, glycosaminoglycans, fibronectin, proteoglycans, elastin, and laminin proteoglycans. Myocardial collagen is the major component of ECM \[10, 11\]. Percutaneous coronary intervention (PCI) is the main treatment modality for AMI. With the widespread use of PCI, AMI mortality has been effectively reduced and the survival is prolonged \[12, 13\].

MicroRNA is a small molecule short-strand RNA made of 19-25 nucleotides with common molecular biological characteristics. MiRNA has a variety of function, such as regulating growth and development, and enhancing ability to adapt the environment. Its various presence makes it can be regulated by physiological and developmental signals \[14\]. MiR-24 is reported to play a role in myocardial infarction and cardiac remodeling \[15, 16\]. However, miR-24 expression in AMI, MIRI, and after PCI treatment has not been fully understood.

Materials and methods

General information

A total of 80 cases of AMI patients received PCI in Wuhan General Hospital of Guangzhou Military between Jan 2014 and Dec 2015 were enrolled, including 48 males and 32 females with mean age at 35.2±5.6 (26-58) years old. All the selected subjects accorded with the AMI diagnosis criteria published by AMI diagnosis and treatment guideline, including sustained typical chest pain for more than 30 min, typical ECG changes, CK/CK-MB or Troponin (Tn) dynamic changes. The patients received PCI for the first time with 24 h. Exclusion criteria contained patients received PCI treatment in the past. Patients combined with cardiomyopathy, pericardial disease, cardiac shock, and infective endocarditis, etc. Patients complicated acute heart failure on admission. Patients had other diseases, such as infectious disease, malignant tumor, diabetes, severe liver and kidney disease, pulmonary fibrosis, bone metabolic disease, systemic autoimmune disease, and malignant tumor complications, etc. This study was approved by the ethics committee in Wuhan General Hospital of Guangzhou Military and all enrolled patients or legal guardians had signed informed consent.

Main reagents and instruments

Trizol reagent, RNA extraction kit, RT-PCR primers, reverse transcription kit, and real-time PCR reagent were purchased from Invitrogen. Type I collagen ELISA kit was got from ebioscience. PVDF membrane was bought from Pall Life Sciences. Western blot related reagents were from Beyotime. ECL reagent was from Amersham Biosciences. Rabbit anti mouse Bcl-2 and Bax primary antibodies, and goat anti rabbit HRP labeled IgG secondary antibody were from Cell Signaling. Lentivirus was got from GenePharma. Other common reagents were from Sangon. ABI7900 HT real-time PCR was from ABI. Surgery microscopic instruments were from medical instrument factory in Suzhou. Labsystem Version 1.3.1 microplate reader was from Bio-rad.

Methods

PCI treatment: Percutaneous transluminal coronary angioplasty (PTCA) and carotid stenting method: Judkins method was applied to perform left and right coronary angiography to determine IRA. PICA or intracoronary stenting was performed using the standard method. In principle, PTCA and stent implantation were only directly carried out on infarction related artery. The success criteria of surgery were defined as TIMI flow at level 3, residual stenosis < 20%, no serious complications (death, myocardial re-infarction, and target vessel revascularization).

Ultrasonic cardiogram examination: Ultrasonic cardiogram examination was performed before or 3 months after surgery to evaluate left ventricular remodeling and cardiac function, including EF, LVESD, LVEDD, and RVWT.

Clinical record: The clinical information of enrolled patients was recorded, including gender, age, height, weight, drug history, or family
MiR-24 improves AMI after PCI

**Table 1. Primer sequence**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward 5’-3’</th>
<th>Reverse 5’-3’</th>
</tr>
</thead>
<tbody>
<tr>
<td>GADPH</td>
<td>AGTGCCAGCCTCGTCTCATAG</td>
<td>CGTTGAACCTTGCGTGGGTAG</td>
</tr>
<tr>
<td>Mir24 (human)</td>
<td>GATCTACGCAGCGAAGAATCCT</td>
<td>CTCTGGGACATCTCTCGTCA</td>
</tr>
<tr>
<td>Mir24 (rat)</td>
<td>CTACGGAAGATCTCAATAGCG</td>
<td>GGGACTCTCAATCCCTGTC</td>
</tr>
</tbody>
</table>

**Table 2. Cardiac ultrasonic testing in AMI patients before and after PCI (X ± S)**

<table>
<thead>
<tr>
<th></th>
<th>Pre-operation</th>
<th>3 months after surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVESD (mm)</td>
<td>31.37±3.26</td>
<td>38.72±5.81*</td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>40.21±6.47</td>
<td>47.36±8.55*</td>
</tr>
<tr>
<td>EF (%)</td>
<td>50.72±7.31</td>
<td>43.12±6.68*</td>
</tr>
<tr>
<td>LVAWT (mm)</td>
<td>9.13±0.89</td>
<td>8.67±0.81*</td>
</tr>
<tr>
<td>LVPM (mm)</td>
<td>9.08±0.62</td>
<td>8.14±0.43*</td>
</tr>
</tbody>
</table>

*P < 0.05, compared with pre-operation.

history. Conventional ultrasonic cardiogram and ECG were applied before PCI treatment.

**Experimental animals grouping and treatment:**
A total of 60 healthy male Wistar rats were randomly divided into three groups as control, AMI group, and miR-24 group. Left anterior descending coronary artery balloon block method was adopted to establish rat AMI model, while miR-24 lentivirus was transfected to AMI rats to construct miR-24 rat.

**Rat AMI modeling:** The rat was fixed on supine position and received tracheal intubation to connect with breathing machine (tidal volume at 4 ml/kg and respiratory rate at 80 bpm). The incision was made at the fourth rib on the left of sternum to expose the heart. A 7-0 nylon thread was passed through the two-thirds of myocardium at 1-2 cm inferior margin of left auricle to ligate the left coronary artery. ECG was real-time monitored in the process of experiment. Myocardium whitening and continuous arched ST segment elevation (> 1/2 R wave) in unimodal curve were considered as successful modeling.

**MiR-24 lentivirus construction and transfection:** Lentivirus vector and miR-24 plasmid were co-transfected to 293T cell line. After packaging virus particles and concentration, miR-24 plasmid lentivirus were injected to local myocardial tissue at 4×10⁷ TU.

**Postoperative cardiac function evaluation:** Cardiac function after I/R in each group was assessed by M type ultrasound at 24 h after onset and 28 days after surgery. Left ventricular mass index (LVMI), and ventricular systolic and diastolic diameter were calculated. The 15L8 ultrasound probe was put on the rats near the sternum to obtain clear 2D image through the level of short axis papillary muscle in left ventricular besides the sternum. It was further converted into M type echocardiography to measure LVEDD and LVESD. And calculate LVMI.

**Sampling:** A total of 5 ml caudal vein was extracted at 24 h after onset and 28 days after surgery. After centrifuged at 3000 rpm for 15 min, the serum was set in Eppendorf tube and stored at -80°C.

**ELISA**
Type I collagen content was detected by ELISA to analyze the correlation with ventricular remodeling. Standard substance was used to draw the linear regression equation, whereas the sample OD value was calculated based on the equation.

**Real-time PCR**
Total RNA was extracted from human peripheral blood and rat myocardial tissue using Trizol and quantified by ultraviolet spectrophotometer. Total RNA was further reverse transcribed to cDNA using the specific primers designed by PrimerPremier 6.0 (Table 1). Real-time PCR was performed at 54°C for 1 min, followed by 35 cycles of 90°C for 30 s, 58°C for 50 s, and 72°C for 35 s. The relative expression was calculated by 2⁻ΔCt method using GAPDH as control.

**Western blot**
Myocardial tissue protein was treated by lysis on ice for 15–30 min and ultrasonicated for 5 s×4 times. After centrifuged at 10,000 g at 4°C for 15 min, the supernatant was moved to a new Ep tube. The protein was separated by 10% SDS-PAGE and transferred to PVDF membrane at 160 mA for 1.5 h. After blocked by 5% skim milk for 2 h, the membrane was incubated in primary antibody (Bax for 1:1000, Bcl-2 for 1:2000) at 4°C overnight. Next, the membrane
MiR-24 improves AMI after PCI

Real-time PCR was applied to test miR-24 expression changes in AMI patients before and after PCI. It was found that miR-24 obviously reduced after PCI compared with pre-operative group (P < 0.05) (Figure 1).

Correlation analysis of miR-24 expression with cardiac function index in AMI patients

Correlation analysis demonstrated that miR-24 expression was positively correlated with LVEF, LVAWT, and LVPWT (P < 0.05), whereas negatively correlated with LVESD and LVEDD at pre-operation and 3 months after operation (P < 0.05) (Table 3), indicating that miR-24 could be treated as one of the indicators of MIRI.

MiR-24 expression in AMI rat

Real-time PCR was used to test miR-24 expression in AMI rat after miR-24 overexpression. It was revealed that miR-24 expression significantly downregulated in AMI rat compared with control (P < 0.05). MiR-24 overexpression obviously promoted miR-24 expression compared with AMI group (P < 0.05) (Figure 2).

The impact of miR-24 overexpression on AMI rat myocardial function changes

M mode echocardiography was adopted to determine the effect of miR-24 overexpression on AMI rat myocardial function. The results demonstrated that LVESD and LVEDD significantly elevated in AMI group compared with control (P < 0.05). MiR-24 overexpression obviously reduced LVESD and LVEDD compared

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Table 3. Correlation analysis of miR-24 expression with cardiac function index in AMI patients

<table>
<thead>
<tr>
<th>Mir-24</th>
<th>Pre-operation</th>
<th>3 months after surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVESD (mm)</td>
<td>-0.54</td>
<td>-0.681</td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>-0.423</td>
<td>-0.561</td>
</tr>
<tr>
<td>EF (%)</td>
<td>0.458</td>
<td>0.615</td>
</tr>
<tr>
<td>LVAWT (mm)</td>
<td>0.627</td>
<td>0.721</td>
</tr>
<tr>
<td>LVPWT (mm)</td>
<td>0.716</td>
<td>0.784</td>
</tr>
</tbody>
</table>

Statistical analysis

The statistical analysis was performed on SPSS 19.0 software. Measurement data was depicted as mean ± standard deviation and analyzed by one-way ANOVA. P < 0.05 was considered as statistical significance.

Results

Cardiac function examination in AMI patients before and after PCI

Ultrasonic cardiogram examination was performed before or 3 months after surgery to evaluate left ventricular remodeling and cardiac function. It was showed that EF significantly reduced after surgery compared with 24 h with-

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Real-time PCR was applied to test miR-24 expression changes in AMI patients before and after PCI. It was found that miR-24 obviously reduced after PCI compared with pre-operative group (P < 0.05) (Figure 1).

Correlation analysis of miR-24 expression with cardiac function index in AMI patients

Correlation analysis demonstrated that miR-24 expression was positively correlated with LVEF, LVAWT, and LVPWT (P < 0.05), whereas negatively correlated with LVESD and LVEDD at pre-operation and 3 months after operation (P < 0.05) (Table 3), indicating that miR-24 could be treated as one of the indicators of MIRI.

MiR-24 expression in AMI rat

Real-time PCR was used to test miR-24 expression in AMI rat after miR-24 overexpression. It was revealed that miR-24 expression significantly downregulated in AMI rat compared with control (P < 0.05). MiR-24 overexpression obviously promoted miR-24 expression compared with AMI group (P < 0.05) (Figure 2).

The impact of miR-24 overexpression on AMI rat myocardial function changes

M mode echocardiography was adopted to determine the effect of miR-24 overexpression on AMI rat myocardial function. The results demonstrated that LVESD and LVEDD significantly elevated in AMI group compared with control (P < 0.05). MiR-24 overexpression obviously reduced LVESD and LVEDD compared
MiR-24 improves AMI after PCI

ELISA was used to assess the impact of miR-24 overexpression on type I collagen expression in AMI rat. It was presented that type I collagen content markedly increased in AMI group compared with control (P < 0.05). MiR-24 overexpression apparently reduced type I collagen level compared with AMI group (P < 0.05), revealing that miR-24 overexpression can suppress type I collagen expression (Figure 3).

The influence of miR-24 overexpression in Bcl-2 and Bax expression in myocardium of AMI rat

Western blot was applied to detect the influence of miR-24 overexpression in Bcl-2 and Bax expression in myocardium of AMI rat. The results demonstrated that Bcl-2 decreased, while Bax elevated in myocardium from AMI group compared with control, leading to the ratio of Bcl-2 and Bax reduce (P < 0.05). MiR-24 overexpression obviously enhanced Bcl-2 expression and declined Bax level compared with AMI group, resulting in the ratio of Bcl-2 and Bax elevation (P < 0.05) (Figure 4).

Discussion

The incidence of cardiovascular disease keeps high in recent years, accounting for the leading cause of death worldwide that seriously damages to human health. AMI showed the highest incidence, the severe damage, and extremely high mortality [17]. The wide application of PCI effectively reduces the mortality of AMI patients and prolongs the survival time. Though PCI solves the problem of the coronary artery recanalization, some patients inevitably occur heart failure after operation. It was reported that about 30% of STEMI patients underwent PCI surgery still occur ventricular remodeling, leading to poor prognosis [18, 19]. Therefore, further in-depth clarifying the mechanism of ventricular remodeling after myocardial infarction, predicting the occurrence and prognosis of heart failure after myocardial infarction in early stage, and accurate assessment of the severity of heart failure can not only provide the basis for selecting the appropriate treatment, but also supply new drug treatment target to delay the development of heart failure after myocardial infarction and forecast for high-risk patients [20].

MiR-24 was found to participate in the regulation of cardiovascular disease and can promote the survival of cardiac cells [21, 22]. This study proved that miR-24 expression decreased during the onset of AMI. Myocardial remodeling after PCI leaded to miR-24 further downregulation. MiR-24 was positively correlated with EF and LVWT, whereas negatively correlated with LVESD and LVEDD, indicating that miR-24 could be treated as one of the cardiac function indexes for MIRI. Moreover, we transfected miR-24

Table 4. The impact of miR-24 overexpression on AMI rat myocardial function changes (X ± S)

<table>
<thead>
<tr>
<th>Group</th>
<th>LVEDD (mm)</th>
<th>LVESD (mm)</th>
<th>LVMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.47±0.05</td>
<td>0.31±0.03</td>
<td>2.51±0.03</td>
</tr>
<tr>
<td>AMI group</td>
<td>0.62±0.06*</td>
<td>0.44±0.07*</td>
<td>3.85±0.17*</td>
</tr>
<tr>
<td>MiR-24 group</td>
<td>0.56±0.05*</td>
<td>0.39±0.03*</td>
<td>3.19±0.03*</td>
</tr>
</tbody>
</table>

*P < 0.05, compared with control. *P < 0.05, compared with AMI group.
lentivirus to the myocardium of AMI rat and found that miR-24 overexpression can improve the cardiac function of AMI rat. Type I collagen accounted for more than 90% in myocardium. It plays an important role in forming and maintaining ventricular wall tension because of its high rigidity and low extensibility and elasticity. Type I collagenous fiber elevation will increase the stiffness [23]. Bax and Bcl-2 are important members of the apoptosis protein family. Bcl-2 is an anti-apoptotic protein, while Bax is a pro-apoptotic protein. Their imbalance directly affects cell apoptosis [24]. This study confirmed that miR-24 reduced the content of type I collagen, contributing to suppress the impact of fibrosis after myocardial infarction. It further alleviated Bax expression and enhanced Bcl-2 level, thus to regulate apoptosis balance to decrease MIRI and improve cardiac function.

**Conclusion**

MiR-24 downregulated in AMI and increased after PCI treatment. MiR-24 overexpression improved cardiac function through decreasing type I collagen, regulating apoptosis imbalance, and alleviating MIRI. MiR-24 could be treated as an cardiac function indicator for AMI and PCI, and provide new potential target for the treatment of MIRI.

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**Disclosure of conflict of interest**

None.

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**References**

MiR-24 improves AMI after PCI


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