European Review for Medical and Pharmacological Sciences 2019; 23: 6299-6306

MicroRNA-132 improves myocardial remodeling after myocardial infarction

L. CHEN[1], G.-Y. WANG[2], J.-H. DONG[2], X.-J. CHENG[1]

[1]Department of Cardiology, the People's Hospital of Rizhao, Rizhao, China
[2]Department of Cardiology, the Traditional Chinese Medicine Hospital of Rizhao City, Rizhao, China

Lin Chen and Guangyun Wang contributed equally to this work

Abstract. – OBJECTIVE: To elucidate the potential role of microRNA-132 in myocardial infarction (MI) and its underlying mechanism.

MATERIALS AND METHODS: The myocardial infarction model was established in WT and microRNA-132 KO mice using the LAD ligation method. WT mice were assigned into the control group (LAD ligation for MI) and sham group. After animal procedures, infarct size was calculated using hematoxylin and eosin (HE) staining and cardiac function was evaluated using echocardiography, respectively. By analyzing differentially expressed microRNAs relative to MI in a microarray, microRNA-132 was screened out and further verified by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). Hemodynamic parameters and cardiac function indexes in mice were accessed, including scar length/LV length, FS, dp/dtmax, dp/dtmin, ESV, EDV, EF and Tau_w.

RESULTS: QRT-PCR data showed a gradual decrease in microRNA-132 expression in the infarction zone, border zone and remote zone within 7 days after MI. Compared with mice in the control group, microRNA-132 KO mice showed a higher percentage of scar length/LV length at postoperative day 14 and day 28. MicroRNA-132 KO mice showed decreased FS, dp/dtmax and EF, but increased dp/dtmin, ESV and EDV. The injection of different concentrations of microRNA-132 mimics into mice (8 mg/kg, 16 mg/kg and 32 mg/kg) could reduce LVIDD, LVIDs, ESV, EDV, dp/dtmin and Tau_w. However, FS, EF and dp/dtmax increased by the injection of microRNA-132 mimics at postoperative day 28. The injection of 16 mg/kg microRNA-132 mimics significantly reduced the percentage of scar length/LV length in microRNA-132 KO mice than the control group and miR-CO group. After injection of 16 mg/kg microRNA-132 mimics, LVIDD and LVIDs markedly decreased at postoperative day 14 and day 28 compared with the control group and miR-CO group. However, FS was elevated by microRNA-132 mimics.

CONCLUSIONS: MicroRNA-132 is involved in the development of myocardial infarction. The microRNA-132 expression is upregulated after myocardial infarction, influencing infarct size and cardiac function.

Key Words:
MicroRNA-132, Myocardial infarction, Myocardial remodeling, Cardiac function.

Introduction

Myocardial infarction (MI) is an acute and severe cardiovascular disease that seriously threatens human health. With the widespread application of cardiac intervention and thrombolytic therapy, the mortality of MI has sharply dropped[1,2]. Since the survival of MI patients increases, the prevalence and incidence of heart failure (HF) after MI has risen greatly in recent years. National Health and Nutrition Examination Survey (NHANES) reported that the relative risk of HF incidence after MI is up to 8.1, which is the leading cause of HF[3]. HF is a serious event in the progression of cardiovascular disease, presenting high morbidity and mortality. It is predicted that the prevalence of HF will increase from 6 million to more than 8 million in 2030[4].

MicroRNAs are a class of non-coding RNAs with 20-25 nucleotides in length that negatively regulate gene expressions at the post-transcriptional level by binding to the 3' non-coding region (3'UTR) of the target gene mRNA[5]. Existing studies have shown that microRNAs are closely related to physiological and pathological processes of cardiovascular diseases. MicroRNAs are involved in the regulation of cardiomyocyte behaviors, including cardiac muscle contraction,

Corresponding Author: Xiaojing Cheng, MM; e-mail: chengxiaojing11@163.com

electrical conduction, cardiomyocyte growth, differentiation, senescence, and neovascularization[6-8]. Recent studies have shown that some non-coding RNAs including microRNAs could attenuate cardiovascular diseases by leading to acute MI, myocardial hypertrophy, myocardial fibrosis and neovascularization inhibition[9-11]. On the contrary, the protective effects of microRNAs are also identified by promoting cardiomyocyte regeneration, proliferation and differentiation, as well as inhibiting cardiomyocyte apoptosis, cardiac hypertrophy, fibroblast activation, and inflammatory response[12-17].

MicroRNA-132 exerts a regulatory role in cardiovascular diseases. Studies have found that chronic administration of angiotensin II (Ang II) in rats leads to hypertension and cardiac hypertrophy. MicroRNA-132 was found to be highly expressed in the heart, aortic wall and kidney of rats[18]. In Ang II-associated cardiovascular diseases, Ang II upregulates microRNA-132 level in vascular smooth muscle cells. Subsequently, activated MCP-1 regulates smooth muscle cell cycle and cell movement by acting on PTEN and CREB[19]. Here, the aim of this work was to investigate the potential role of microRNA-132 in MI and its underlying mechanism.

Materials and Methods

Establishment of MI Model in Mice

Male wild-type (WT) and microRNA-132 knockout (microRNA-132 KO) C57BL/6J mice with 23-27 g and 8-10 weeks old were selected. Mice were intraperitoneally anesthetized by pentobarbital sodium for tracheotomy. Left internal jugular vein catheterization was performed for connecting the small-animal ventilator. The skin was cut horizontally along the third to fourth intercostal spaces for heart exposure. The left anterior descending coronary artery (LAD) was ligated at 1 mm from the aortic root between the pulmonary conus and the auricula sinistra. Myocardium below the ligation turned pale and local myocardial movement weakened, suggesting the successful establishment of the MI model. Rats in the sham group received tracheotomy without performing LAD ligation. Penicillin was administrated for consecutive 3 days after surgery to prevent infection. 20 μL of microRNA control or microRNA-132 mimics (8 mg/kg, 16 mg/kg or 32 mg/kg) was proportionally mixed with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). The mixture was administrated into the left ventricular anterior wall myocardium three times per day, for consecutive 28 days. This study was approved by the Animal Ethics Committee of The People's Hospital of Rizhao Animal Center.

HE Staining

Paraffin specimens of heart tissues in the infarction zone, border zone and remote zone within 7 days after MI were fixed with 10% paraformaldehyde and stained with hematoxylin and eosin (n=5-7). Images were captured under a microscope (magnification 10×) and infarct size was calculated using the Image Pro Plus (version 6. 0, Media Cybernetics, USA).

RNA Extraction and Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Heart tissues in the infarction zone, border zone and remote zone within 7 days after MI were preserved in a -80°C refrigerator. 50-100 g heart tissue in each group was incubated with 1 mL of TRIzol (Invitrogen, Carlsbad, CA, USA) for extracting the total RNA and reversely transcribed into cDNA. After the complementary Deoxyribose Nucleic Acid (cDNA) was amplified, qRT-PCR was performed to detect the expressions of related genes. The amplification condition was 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 31 s. U6 was utilized as the reference control for microRNA-132. Primers used in this study were: microRNA-132, F: TGCGGGTGCTCGCTTCGGCAGC, R: CCAGTGCAGGGTCCGAGGT; U6, F: GCTTCGGCAGCACATATACTAAAAT, R: CGCTTCAGAATTTGCGTGTCAT.

Echocardiography

Postoperative echocardiography was performed in mice after inhalation anesthesia with Isoprene. The chest area was depilated, and the appropriate amount of ultrasonic glue was applied. Left-lateral position or dorsal decubitus of mice was applied for echocardiography. M-mode echocardiography was obtained using a small-animal ultrasound probe (model Veno2100) on the long axis of the parastolic left ventricle. LVIDs (left ventricular internal dimension systole) and LVIDD (left ventricular internal diastolic diameter) were recorded. Percentage of scar length/LV length, FS (fractional shortening), ESV (end-systolic volume), EDV (end-diastolic volume), EF (ejection fraction) and Tau_w (time constant of LV relaxation) were calculated.

Hemodynamic Monitoring

Postoperative hemodynamics in mice was monitored after inhalation anesthesia with Isoprene. A 20-G heparin-filled catheter (Spacelabs Medical, Inc., Redmond, WA, USA) was inserted from the right carotid artery to the left ventricle for connecting the biological signal collecting system. The maximum rate of increase in left ventricular pressure (dp/dtmax) and the maximum rate of decrease in left ventricular pressure (dp/dtmin) were recorded.

Statistical Analysis

Data were analyzed by using Statistical Product and Service Solutions (SPSS) 17.0 statistical software (SPSS Inc., Chicago, IL, USA). Quantitative data were represented as mean ± standard deviation ($\bar{x}\pm s$). Continuous variables were analyzed by the *t*-test or one-way ANOVA, followed by Post-Hoc Test (Least Significant Difference). $p<0.05$ was considered statistically significant.

Results

MicroRNA-132 Expression Decreased After MI

The results of HE staining showed that the infarction zone barely had survived cardiomyocytes, which were mainly collagen fibers and fibroblasts. The border zone consisted of fibroblasts and cardiomyocytes, with significant infiltration of inflammatory cells. The remote zone was mainly composed of cardiomyocytes. Pathological changes in the infarction zone, border zone and remote zone suggested the successful construction of the MI model (Figure 1A). Next, the heat map of microRNAs showed the differentially expressed microRNAs in the process of MI (Figure 1B). We found that microRNA-132 was markedly downregulated in MI, which was selected for further verification. QRT-PCR data showed a gradual decrease in microRNA-132 expression in the infarction zone, border zone and remote zone within 7 days after MI. Interestingly, microRNA-132 expression showed an increased trend with the distance away from the infarction zone (Figure 1C).

MicroRNA-132 KO Mice Showed Increased Myocardial Remodeling After MI

We further elucidated the regulatory effect of microRNA-132 on cardiac function after MI in mice of the sham group, control group and microRNA-132 KO group. Compared with mice in the control group, microRNA-132 KO mice showed higher percentage of scar length/LV length, indicating a larger infarct size (Figure 2A). FS in microRNA-132 KO mice showed a marked decrease at postoperative day 14 and day 28 than that at baseline and postoperative day 3, respectively (Figure 2B). At postoperative day 28, mice in the control group and microRNA-132 KO group showed lower dp/dtmax than those in the sham group, which was more pronounced in microRNA-132 KO group. On the contrary, dp/dtmin showed the opposite trend as the highest level was seen in microRNA-132 KO group (Figure 2C). Similarly, highest levels of ESV and EDV were observed in the microRNA-132 KO group compared with those in the sham group and control group at postoperative day 28, respectively (Figure 2D

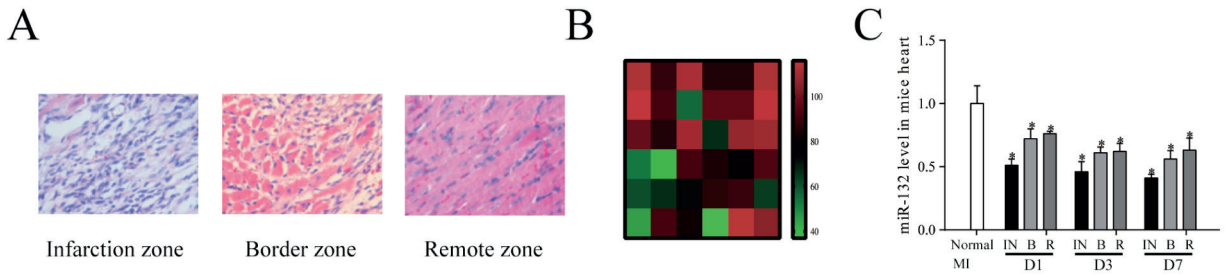

Figure 1. MicroRNA-132 expression decreased after MI. ***A,*** HE staining showed that the infarction zone almost had no survived cardiomyocytes, which were mainly collagen fibers and fibroblasts. The border zone consisted of fibroblasts and cardiomyocytes, with significant infiltration of inflammatory cells. The remote zone mainly composed of cardiomyocytes (magnification × 40). ***B,*** Heat map of microRNAs showed the differentially expressed microRNAs in the process of MI. Green represented lowly expressed microRNAs and red represented highly expressed ones. ***C,*** QRT-PCR data showed a gradual decrease in microRNA-132 expression in the infarction zone, border zone and remote zone within 7 days after MI. MicroRNA-132 KO, microRNA-132 knockout; IN, infarction zone; B, border zone; R, remote zone. **p*<0.05.

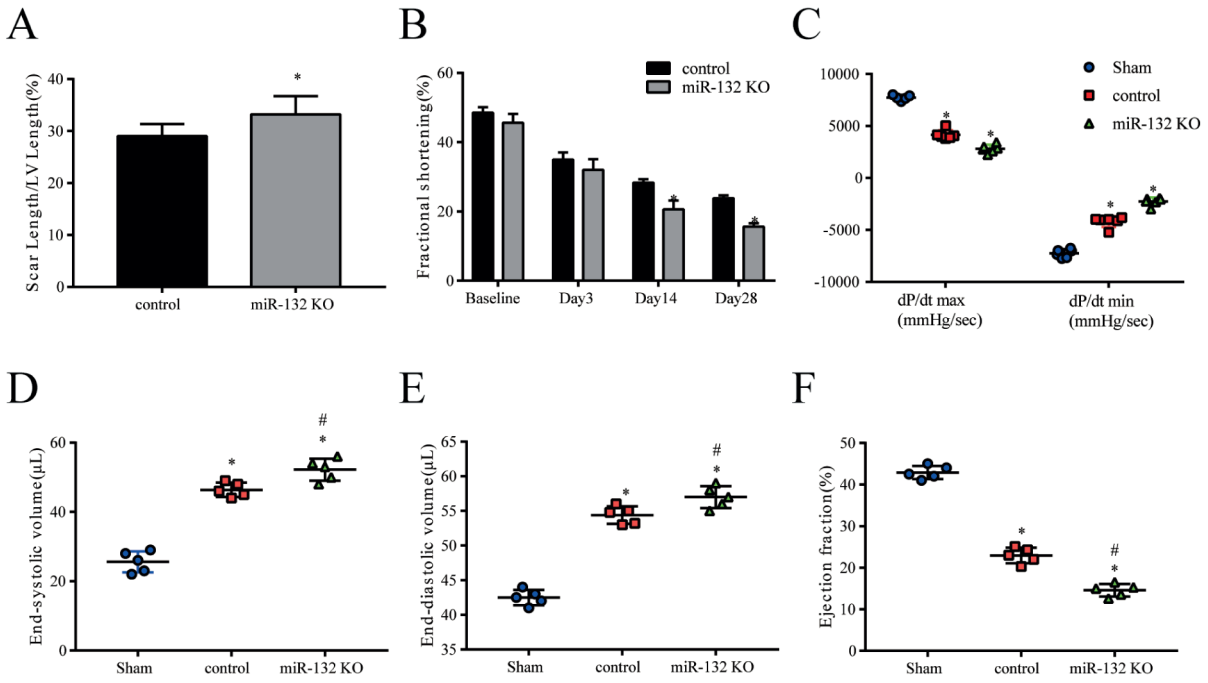

Figure 2. MicroRNA-132 KO mice showed increased myocardial remodeling after MI. ***A,*** Percentage of scar length/LV length in microRNA-132 KO mice and control mice. ***B,*** FS (%) in mice of control group and microRNA-132 KO group at baseline, postoperative day 3, day 14 and day 28. FS (%) =100 ×[(LVIDd− LVIDs)/LVIDd]. ***C,*** dp/dtmax and dp/dtmin (mmHg/sec) in mice of the sham group, control group and microRNA-132 KO group at postoperative day 28. ***D,*** ESV (μL) in mice of the sham group, control group and microRNA-132 KO group at postoperative day 28. ***E,*** EDV (μL) in mice of the sham group, control group and microRNA-132 KO group at postoperative day 28. ***F,*** EF (%) in mice of the sham group, control group and microRNA-132 KO group at postoperative day 28. *p<0.05 *vs.* sham group, #p<0.05 *vs.* control group.

and 2E). However, EF was lower in the control group and microRNA-132 KO group than that in the sham group. MicroRNA-132 KO mice exhibited the lowest level of EF (Figure 2F). These results suggested that microRNA-132 deficiency could aggravate HF phenotypes, impair ventricular dilatation and contractility.

MicroRNA-132 Decreased Myocardial Remodeling

Different concentrations of microRNA-132 mimics (8 mg/kg, 16 mg/kg and 32 mg/kg) were administrated into the left ventricular anterior wall myocardium at three days after MI. Three concentrations of microRNA-132 mimics all could reduce LVIDD (Figure 3A), LVIDs (Figure 3B), ESV (Figure 3D), EDV (Figure 3E), dp/dtmin (Figure 3H) and Tau_w (Figure 3I). However, FS (Figure 3C), EF (Figure 3F) and dp/dtmax (Figure 3G) increased by injection of microRNA-132 mimics. Further analysis on three concentrations of microRNA-132 mimics concluded that the high concentration of microRNA-132 mimics presented a better protective effect on cardiac function than low concentration. Those results suggested that microRNA-132 could reduce myocardial remodeling and cardiac function damage in a dose-dependent manner.

MicroRNA-132 Reduced Infarct Size and Myocardial Remodeling After MI

The injection of 16 mg/kg microRNA-132 mimics reduced percentage of scar length/LV length than that in the control group and miR-CO group, respectively. Reduced percentage of scar length/LV length indicated a decreased infarct size (Figure 4A). After injection of 16 mg/kg microRNA-132 mimics, LVIDD (Figure 4B) and LVIDs (Figure 4C) significantly decreased at postoperative day 14 and day 28 compared with those in the control group and miR-CO group, respectively. However, FS was elevated by microRNA-132 mimics (Figure 4D). We believed that microRNA-132 could reduce infarct size and myocardial remodeling after MI, which is time-dependent.

Discussion

Cardiomyocyte hypertrophy and apoptosis, as well as collagen deposition and fibrosis of the extracellular matrix can lead to the imbalance of myocardial cell and extracellular matrix. The compensated cardiac function gradually shifts to a decompensated state and ultimately leads to HF. MicroRNAs have been identified to influence myocardial remodeling by regulating cardiac hypertrophy, myocardial fibrosis and cardiomyocyte apoptosis.

Some certain microRNAs have been proved to be related to cardiac hypertrophy. Kumarswamy et al[19] established an *in vivo* HF model in MI rats, and found that overexpressed microRNA-1 after SERCA2a gene therapy reverses myocardial remodeling and improves cardiac function by

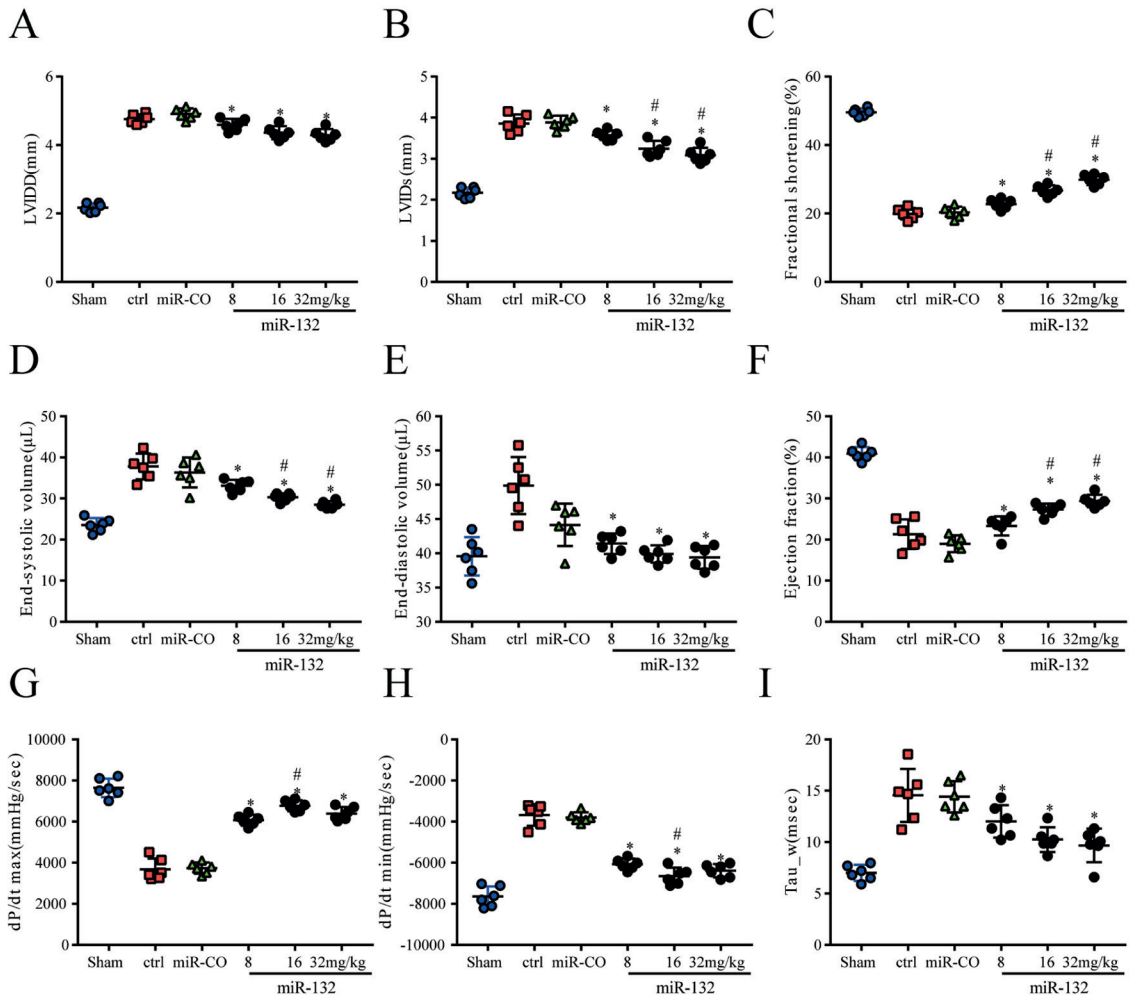

Figure 3. MicroRNA-132 decreased myocardial remodeling. Different concentrations of microRNA-132 mimics (8 mg/kg, 16 mg/kg and 32 mg/kg) were administrated into the left ventricular anterior wall myocardium at three days after MI. ***A,*** LVIDD (mm) in sham group, control group, miR-CO group, miR-132 8 mg/kg group, miR-132 16 mg/kg group and miR-132 32 mg/kg group. ***B,*** LVIDs (mm) in sham group, control group, miR-CO group, miR-132 8 mg/kg group, miR-132 16 mg/kg group and miR-132 32 mg/kg group. ***C,*** FS (%) in sham group, control group, miR-CO group, miR-132 8 mg/kg group, miR-132 16 mg/kg group and miR-132 32 mg/kg group. ***D,*** ESV (μL) in sham group, control group, miR-CO group, miR-132 8 mg/kg group, miR-132 16 mg/kg group and miR-132 32 mg/kg group. ***E,*** EDV (μL) in sham group, control group, miR-CO group, miR-132 8 mg/kg group, miR-132 16 mg/kg group and miR-132 32 mg/kg group. ***F,*** EF (%) in sham group, control group, miR-CO group, miR-132 8 mg/kg group, miR-132 16 mg/kg group and miR-132 32 mg/kg group. ***G,*** dp/dtmax (mmHg/sec) in sham group, control group, miR-CO group, miR-132 8 mg/kg group, miR-132 16 mg/kg group and miR-132 32 mg/kg group. ***H,*** dp/dtmin (mmHg/sec) in sham group, control group, miR-CO group, miR-132 8 mg/kg group, miR-132 16 mg/kg group and miR-132 32 mg/kg group. ***I,*** Tau_w (msec) in sham group, control group, miR-CO group, miR-132 8 mg/kg group, miR-132 16 mg/kg group and miR-132 32 mg/kg group. **p*<0.05 *vs.* control and miR-CO group, #*p*<0.05 *vs.* miR-132 8 mg/kg group. miR-CO, miR controls.

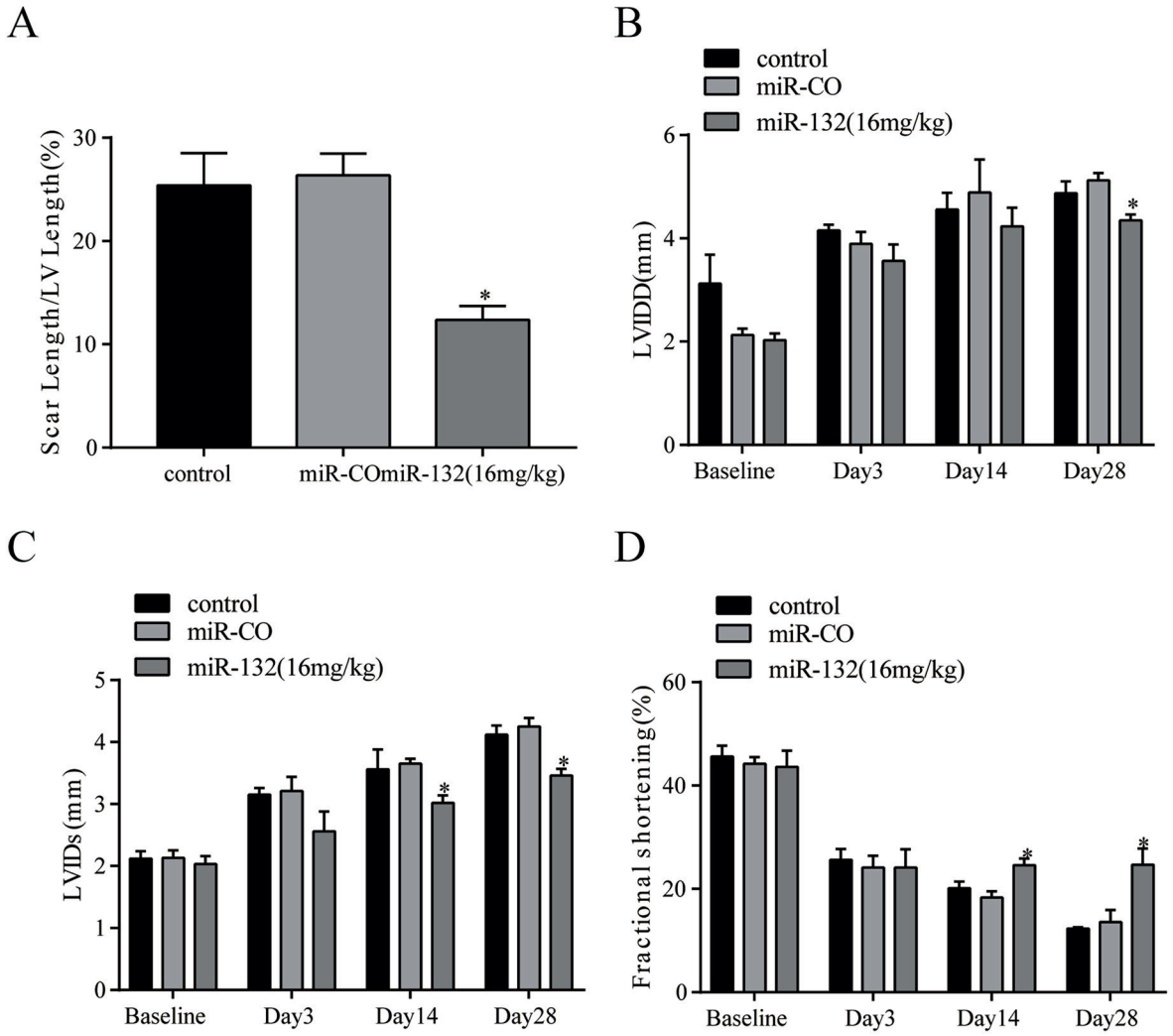

Figure 4. MicroRNA-132 reduced infarct size and myocardial remodeling after MI. ***A,*** Scar length/LV length in the control group, miR-CO group and miR-132 16 mg/kg group. ***B,*** LVIDD (mm) in control group, miR-CO group and miR-132 16 mg/kg group at baseline, postoperative day 3, day 14 and day 28. ***C,*** LVIDs (mm) in control group, miR-CO group and miR-132 16 mg/kg group at baseline, postoperative day 3, day 14 and day 28. ***D,*** FS (%) in control group, miR-CO group and miR-132 16 mg/kg group at baseline, postoperative day 3, day 14 and day 28. *$p<0.05$.

inhibiting target genes of NCX-1. MicroRNA-499 expression is upregulated and promotes the development of cardiomyopathy in human decompensated cardiac hypertrophy. Wang et al[20] found that microRNA-499 upregulates expressions of CnAα and CnAβ, activates calcineurin activity and inhibits Drp1 dephosphorylation. Decreased mitochondrial aggregation of Drp1 reduces Drp1-mediated mitochondrial lysis, thereby exerting an anti-apoptosis effect. Some microRNAs are also closely related to myocardial fibrosis. Jiang et al[21] reported that microRNA-146b can inhibit Ang II-induced myocardial fibrosis in adult rats. MMP16, TRAF6 and IRAK1 are the potential target genes of microRNA-146b. It is reported that microRNA-146b regulates cardiac fibrosis by mediating matrix metalloproteinases and inflammatory cells. MicroRNA-133a is highly expressed in myocardium and skeletal muscle[22]. Northern blot analysis showed high expressions of microRNA-1 and microRNA-133a in mouse skeletal muscle and myocardium. MicroRNA-133a acts on the connective tissue growth factor (CTGF). The downregulation of microRNA-133 in heart diseases may result in an increased expression of CTGF in cardiomyocytes, leading to increased

extracellular matrix synthesis and accelerating fibrosis.

MicroRNAs also exert crucial functions in cardiomyocyte apoptosis. Heat shock protein (HSP) is closely related to cardiomyocyte apoptosis after MI. HSP60 can biodirectly regulate cell apoptosis, while HSP70 can inhibit apoptosis. Both *in vitro* and *in vivo* studies showed that the expressions of microRNA-1 and microRNA-206 were upregulated in cardiomyocytes after treatment with high concentration of glucose. Cardiomyocyte apoptosis is accelerated by regulating HSP60. MicroRNA-1 and microRNA-133 present antagonistic effects on cardiomyocyte apoptosis. Specifically, microRNA-1 inhibits HSP60 and HSP70, thus promoting cell apoptosis. However, microRNA-133 inhibits apoptosis by suppressing caspase-9[23].

In this work, we first constructed a MI model in mice. HE staining showed that the infarction zone had barely survived cardiomyocytes, which were mainly collagen fibers and fibroblasts. The border zone consisted of fibroblasts and cardiomyocytes, and the infiltration of inflammatory cells was significant. The remote zone was mainly composed of cardiomyocytes, altogether suggesting the successful construction of the MI model. Subsequently, qRT-PCR data showed a gradual decrease in microRNA-132 expression in the infarction zone, border zone and remote zone within 7 days after MI. However, microRNA-132 expression showed an increasing trend as away from the infarction zone. Echocardiography and hemodynamic data suggested that microRNA-132 deficiency aggravated HF phenotypes, impaired ventricular dilatation and contractility. On the contrary, supplementation with microRNA-132 alleviated MI-induced cardiac dysfunction in a dose-dependent manner. Supplementation with microRNA-132 also reduced myocardial infarct size and myocardial remodeling after MI in a time-dependent manner.

Conclusions

We found that the microRNA-132 expression was upregulated after myocardial infarction, leading to myocardial remodeling, which in turn affects infarct size and cardiac function.

Conflict of Interests

The Authors declare that they have no conflict of interests.

References

1) AlHabib KF, Sulaiman K, Al Suwaidi J, Almahmeed W, Alsheikh-Ali AA, Amin H, Al Jarallah M, Alfaleh HF, Panduranga P, Hersi A, Kashour T, Al Aseri Z, Ullah A, Altaradi HB, Nur Asfina K, Welsh RC, Yusuf S. Patient and system-related delays of emergency medical services use in acute ST-elevation myocardial infarction: results from the Third Gulf Registry of Acute Coronary Events (Gulf RACE-3Ps). PLoS One 2016; 11: e0147385.
2) Safi M, Khaheshi I, Memaryan M, Naderian M. Subcapsular liver hematoma after fibrino-lytic therapy for acute myocardial infarction: a rare case report. Acta Biomed 2017; 87: 318-320.
3) Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, Das SR, de Ferranti S, Despres JP, Fullerton HJ, Howard VJ, Huffman MD, Isasi CR, Jimenez MC, Judd SE, Kissela BM, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Magid DJ, McGuire DK, Mohler ER, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Rosamond W, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Woo D, Yeh RW, Turner MB. Heart disease and stroke statistics-2016 update: a report from the American Heart Association. Circulation 2016; 133: e38-e360.
4) Heidenreich PA, Albert NM, Allen LA, Bluemke DA, Butler J, Fonarow GC, Ikonomidis JS, Khavjou O, Konstam MA, Maddox TM, Nichol G, Pham M, Pina IL, Trogdon JG. Forecasting the impact of heart failure in the United States: a policy statement from the American Heart Association. Circ Heart Fail 2013; 6: 606-619.
5) He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. Nat Rev Genet 2004; 5: 522-531.
6) Verjans R, van Bilsen M, Schroen B. MiRNA deregulation in cardiac aging and associated disorders. Int Rev Cell Mol Biol 2017; 334: 207-263.
7) Boon RA, Iekushi K, Lechner S, Seeger T, Fischer A, Heydt S, Kaluza D, Treguer K, Carmona G, Bonauer A, Horrevoets AJ, Didier N, Girmatsion Z, Biliczki P, Ehrlich JR, Katus HA, Muller OJ, Potente M, Zeiher AM, Hermeking H, Dimmeler S. MicroRNA-34a regulates cardiac ageing and function. Nature 2013; 495: 107-110.
8) Wahlquist C, Jeong D, Rojas-Munoz A, Kho C, Lee A, Mitsuyama S, van Mil A, Park WJ, Sluijter JP, Doevendans PA, Hajjar RJ, Mercola M. Inhibition of miR-25 improves cardiac contractility in the failing heart. Nature 2014; 508: 531-535.
9) Zhang S, Cui R. The targeted regulation of miR-26a on PTEN-PI3K/AKT signaling path-way in myocardial fibrosis after myocardial infarction. Eur Rev Med Pharmacol Sci 2018; 22: 523-531.
10) Gao L, Liu Y, Guo S, Yao R, Wu L, Xiao L, Wang Z, Liu Y, Zhang Y. Circulating long noncoding RNA HOTAIR is an essential mediator of acute myocardial infarction. Cell Physiol Biochem 2017; 44: 1497-1508.

11) Hinkel R, Penzkofer D, Zuhlke S, Fischer A, Husada W, Xu QF, Baloch E, van Rooij E, Zeiher AM, Kupatt C, Dimmeler S. Inhibition of microRNA-92a protects against ische-mia/reperfusion injury in a large-animal model. Circulation 2013; 128: 1066-1075.

12) Ong SG, Lee WH, Huang M, Dey D, Kodo K, Sanchez-Freire V, Gold JD, Wu JC. Response to letter regarding article, "cross talk of combined gene and cell therapy in ischemic heart disease: role of exosomal microRNA transfer". Circulation 2015; 131: e385.

13) Stanczyk J, Ospelt C, Karouzakis E, Filer A, Raza K, Kolling C, Gay R, Buckley CD, Tak PP, Gay S, Kyburz D. Altered expression of microRNA-203 in rheumatoid arthritis syno-vial fibroblasts and its role in fibroblast activation. Arthritis Rheum 2011; 63: 373-381.

14) Hu S, Huang M, Li Z, Jia F, Ghosh Z, Lijkwan MA, Fasanaro P, Sun N, Wang X, Martelli F, Robbins RC, Wu JC. MicroRNA-210 as a novel therapy for treatment of ischemic heart disease. Circulation 2010; 122: S124-S131.

15) Dong S, Cheng Y, Yang J, Li J, Liu X, Wang X, Wang D, Krall TJ, Delphin ES, Zhang C. MicroRNA expression signature and the role of microRNA-21 in the early phase of acute myocardial infarction. J Biol Chem 2009; 284: 29514-29525.

16) Fabbri M, Paone A, Calore F, Galli R, Gaudio E, Santhanam R, Lovat F, Fadda P, Mao C, Nuovo GJ, Zanesi N, Crawford M, Ozer GH, Wernicke D, Alder H, Caligiuri MA, Nana-Sinkam P, Perrotti D, Croce CM. MicroRNAs bind to Toll-like receptors to induce promet-astatic inflammatory response. Proc Natl Acad Sci USA 2012; 109: E2110-E2116.

17) Jiang X, Ning Q, Wang J. Angiotensin II induced differentially expressed microRNAs in adult rat cardiac fibroblasts. J Physiol Sci 2013; 63: 31-38.

18) Jin W, Reddy MA, Chen Z, Putta S, Lanting L, Kato M, Park JT, Chandra M, Wang C, Tangirala RK, Natarajan R. Small RNA sequencing reveals microRNAs that modulate an-giotensin II effects in vascular smooth muscle cells. J Biol Chem 2012; 287: 15672-15683.

19) Kumarswamy R, Lyon AR, Volkmann I, Mills AM, Bretthauer J, Pahuja A, Geers-Knorr C, Kraft T, Hajjar RJ, Macleod KT, Harding SE, Thum T. SERCA2a gene therapy restores microRNA-1 expression in heart failure via an Akt/FoxO3A-dependent pathway. Eur Heart J 2012; 33: 1067-1075.

20) Wang JX, Jiao JQ, Li Q, Long B, Wang K, Liu JP, Li YR, Li PF. miR-499 regulates mito-chondrial dynamics by targeting calcineurin and dynamin-related protein-1. Nat Med 2011; 17: 71-78.

21) Jiang X, Ning Q, Wang J. Angiotensin II induced differentially expressed microRNAs in adult rat cardiac fibroblasts. J Physiol Sci 2013; 63: 31-38.

22) Yu H, Lu Y, Li Z, Wang Q. microRNA-133: expression, function and therapeutic potential in muscle diseases and cancer. Curr Drug Targets 2014; 15: 817-828.

23) Xu C, Lu Y, Pan Z, Chu W, Luo X, Lin H, Xiao J, Shan H, Wang Z, Yang B. The muscle-specific microRNAs miR-1 and miR-133 produce opposing effects on apoptosis by target-ing HSP60, HSP70 and caspase-9 in cardiomyocytes. J Cell Sci 2007; 120: 3045-3052.