

Original Article

Identification of microRNA expression in a rat model of post-infarction heart failure

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Abstract: Although the mechanisms for development and progression of heart failure (HF) have been extensively studied, the molecular mechanisms underlying the post transcriptional regulation of HF have not been fully elucidated. Functional miRNA studies reported that a variety of miRNAs play a role in pathogenic mechanisms leading to heart failure, such as remodelling, hypertrophy, apoptosis, and hypoxia. In the present study, six rats were assigned into two different treatment groups: rats with chronic heart failure (CHF group; n=3), and sham-operated rats (SO group; n=3). The miRCURY™ LNA Array (v.18.0, Exiqon) was utilized to detect the miRNA transcriptome in SO and CHF rats. And bioinformatics were utilized to identify putative miRNA target genes and their associated biological functions. Forty-one miRNAs were found to be differentially expressed between SO and CHF rats. The five most upregulated and downregulated miRNAs were selected to obtain more representative biological meanings for the putative genes. And the biological analysis further showed that the differentially expressed miRNAs are mainly involved in Wnt signaling pathway and TGF-beta signaling pathway. In conclusion, the data from the present study indicated changes in the miRNA expression levels in the rat myocardium under heart failure post myocardium infarction. When compared with that of the control group, heart failure exerted differential effects on miRNA expression. Bioinformatic analysis of the differentially expressed miRNAs showed that major affected pathways are known to be involved in cardiac remodeling and fibrosis.

Keywords: Heart failure, microRNA, rat, microarray, infarction

Introduction

Heart failure (HF) is a complex syndrome defined by a cardiac output inadequate to meet the metabolic demands of body tissues [1]. It results from a wide range of congenital and acquired cardiovascular or metabolic diseases leading to structural and functional impairment of the heart [2]. Although there have been dramatic innovations in medical and device treatments for heart failure in recent decades, the incidence of heart failure is increasing [3]. The heart failure syndrome affects an estimated more than 23 million people worldwide [4, 5]. Although the mechanisms for development and progression of HF have been extensively studied, the molecular mechanisms underlying the post transcriptional regulation of HF have not been fully elucidated [6-8].

MicroRNAs (miRNAs) are a kind of non-coding small RNAs that can post-transcriptionally regulate the expression of hundreds of their target genes. Functional miRNA studies reported that a variety of miRNAs play a role in pathogenic mechanisms leading to heart failure, such as remodelling, hypertrophy, apoptosis, and hypoxia [9-11]. Evidence from experimental platforms points to potential applications of miRNAs for diagnostic and therapeutic purposes [12, 13].

In the present study, the miRCURY™ LNA Array (v.18.0, Exiqon) was utilized to detect the miRNA transcriptome in SO and CHF rats. And bioinformatics were utilized to identify putative miRNA target genes and their associated biological functions. Forty-one miRNAs were found to be differentially expressed between SO and

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CHF rats. The five most upregulated and down-regulated miRNAs were selected to obtain more representative biological meanings for the putative genes. The biological analysis further showed that the differentially expressed miRNAs are mainly involved in adipocytokine signaling pathway, vascular smooth muscle contraction signaling pathway, Wnt signaling pathway and TGF-beta signaling pathway.

Materials and methods

Animals

Male Wistar rats (7-8 weeks old; weighing 250-300 g) were obtained from the Animal Center of the Chinese Academy of Medical Sciences (Beijing, China) for use as a model of CHF in the present study. The animals were housed in stainless steel wire-mesh cages (5 rats per cage) under standard laboratory conditions (25°C, relative humidity 60%, 12 hours dark/light periods) and were allowed free access to water and food. The investigations conformed to the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH Publication no. 85-23; revised 1985). The present study was approved by the Animal Research Ethics Committee of Shengjing hospital of China Medical University (Shenyang, China). The rats were assigned into two different treatment groups: Rats with chronic heart failure, (CHF group; n=3), and sham-operated rats, (SO group; n=3).

Rat model of CHF

Acute myocardial infarction was induced in the rats by left anterior descending artery ligation, as previously described [12]. Briefly, the rats were anesthetized via intraperitoneal injection of 10% chloral hydrate (3 ml/kg). The rats were then tracheotomied, intubated and mechanically ventilated, with arterial pH, PO₂ and PCO₂ maintained within the physiological range by supplying O₂ and altering the respiratory rate using an Ahx-300S Animal Respirator (Chengdu Tme Technology Co, Ltd., Chengdu, China). A thoracotomy was performed through the fourth intercostal space, the heart was exposed and an electrocardiogram was monitored on a BL-420S Data Acquisition & Analysis System (Chengdu Tme Technology Co, Ltd., China). A prolene suture (Ethicon, Inc., Somerville, NJ,

USA) was placed around the left coronary artery, close to its origin, and the ends were tied firmly in the CHF group and loosely in the SO group. Acute myocardial infarction was deemed successful on the basis of regional cyanosis of the myocardial surface distal to the suture, accompanied by elevation of the ST segment on the electrocardiogram. At 3 weeks-post myocardial infarction, echocardiography was performed on the surviving rats. The rats, which exhibited left ventricular wall infarction, with an infarcted area >40% of the left ventricular wall area were enrolled for the subsequent investigations. The infarcted rats were allocated into the CHF group (n=3). Heart rate, mean blood pressure, weight, and echocardiography were recorded, and blood samples were obtained in the rats of the two groups. The present study was approved by the ethics committee of Shengjing Hospital of China Medical University (Shenyang, China).

Echocardiographic and hemodynamic measurements

Echocardiography was performed 3 weeks after surgery. A transthoracic 2D M-mode echocardiogram was obtained using a Philips iE33 (Philips Electronics, Amsterdam, The Netherlands), equipped with a transducer S12-4. The rats were anesthetized with 3 ml/kg 10% chloral hydrate, and echocardiography was performed. The chest each mouse was shaved and the rats were placed in a supine position. Images were captured by placing the transducer against the chest. Images were obtained by placing the transducer against the chest. Two-dimensional echocardiography measurements of left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD) and left ventricular wall thickness were recorded. Left ventricular fractional shortening (LVFS) was calculated according to the formula: $LVFS = (LVEDD - LVESD) / LVEDD \times 100\%$. Left ventricle ejection fraction (LVEF) was measured using the Biplane Modified Simpson's method.

Blood sampling

Blood samples (2 ml whole blood) were collected from each rat rapidly via the arterial catheter into a syringe (WeiGao group medical polymer Co., Ltd., Shundong, China). The rats were sacrificed by spinne dislocation at the end of

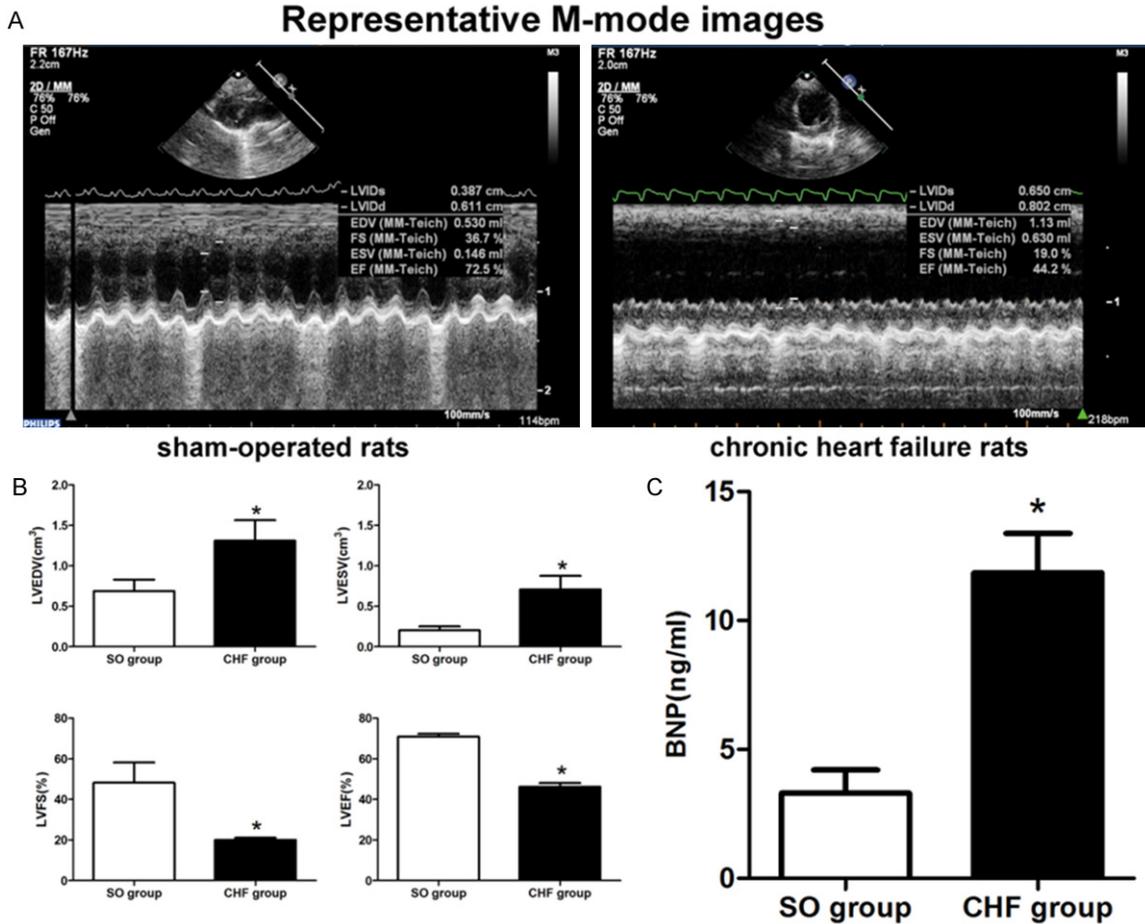


Figure 1. Echocardiographic, hemodynamic measurements and brain natriuretic peptide of CHF group and SO group. A: The representative M-mode image of CHF group and SO group. The rats of CHF group exhibited left ventricular wall infarction. B: Left ventricular end-diastolic volume (LVEDV); left ventricular end-systolic volume (LVESV); left ventricular fractional shortening (LVFS); left ventricular ejection fraction (LVEF) of CHF group and SO group. C: Brain natriuretic peptide (BNP) of CHF group and SO group. HF group, Heart failure after left anterior descending artery ligation, n = 3. SO group, sham operated rats, n = 3. Data are presented as mean ± standard deviation. *P<0.05 (refer to the CHF group vs. the SO group).

the experiments. For each rat, the entire heart was rapidly excised and washed with cold phosphate buffer (Experimental Center of Shengjing Hospital of China Medical University, Shenyang, China, containing 137 mmol/l NaCl, 2.7 mmol/l KCl, 10 mmol/l Na₂HPO₄, 2 mmol/l KH₂PO₄ (pH 7.4). The heart was then frozen at -80°C, prior to its use in the subsequent miRNA microarray analyses.

Detection of brain natriuretic peptide (BNP)

The blood samples were collected into serum separator tubes, and serum was obtained by centrifugation at 4,500 g for 15 min. The serum BNP was measured using a commercially avail-

able human/mouse/rat BNP enzyme immunoassay kit (RayBiotech, Norcross, GA, USA), according to the manufacturer's instructions. All samples and standards were measured in triplicate.

Methods of miRNA microarray

miRNA microarray. The 7th generation of miRCURY™ LNA Array (v.18.0) (Exiqon) contains 3100 capture probes, covering all human, mouse and rat microRNAs annotated in miR-Base 18.0, as well as all viral microRNAs related to these species. In addition, this array contains capture probes for 25 miRPlus™ human microRNAs. RNA extraction. Total RNA was iso-

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lated using TRIzol (Invitrogen) and miRNeasy mini kit (QIAGEN) according to manufacturer's instructions, which efficiently recovered all RNA species, including miRNAs. RNA quality and quantity was measured by using nanodrop spectrophotometer (ND-1000, Nanodrop Technologies) and RNA Integrity was determined by gel electrophoresis. RNA labeling. After RNA isolation from the samples, the miRCURY™ Hy3™/Hy5™ Power labeling kit (Exiqon, Vedbaek, Denmark) was used according to the manufacturer's guideline for miRNA labeling. One microgram of each sample was 3'-end-labeled with Hy3™ fluorescent label, using T4 RNA ligase by the following procedure: RNA in 2.0 µL of water was combined with 1.0 µL of CIP buffer and CIP (Exiqon). The mixture was incubated for 30 min at 37°C, and was terminated by incubation for 5 min at 95°C. Then 3.0 µL of labeling buffer, 1.5 µL of fluorescent label (Hy3™), 2.0 µL of DMSO, 2.0 µL of labeling enzyme were added into the mixture. The labeling reaction was incubated for 1 h at 16°C, and terminated by incubation for 15 min at 65°C. Array hybridization. After stopping the labeling procedure, the Hy3™-labeled samples were hybridized on the miRCURY™ LNA Array (v.18.0) (Exiqon) according to array manual. The total 25 µL mixture from Hy3™-labeled samples with 25 µL hybridization buffer were first denatured for 2 min at 95°C, incubated on ice for 2 min and then hybridized to the microarray for 16-20 h at 56°C in a 12-Bay Hybridization Systems (Hybridization System-Nimblegen Systems, Inc., Madison, WI, USA), which provides an active mixing action and constant incubation temperature to improve hybridization uniformity and enhance signal. Following hybridization, the slides were achieved, washed several times using Wash buffer kit (Exiqon), and finally dried by centrifugation for 5 min at 400 rpm. Then the slides were scanned using the Agilent DNA Microarray Scanner (part number G2505C). Data analysis. Scanned images were then imported into GenePix Pro 6.0 software (Axon) for grid alignment and data extraction. Replicated miRNAs were averaged and miRNAs that intensities ≥ 30 in all samples were chosen for calculating normalization factor. Expressed data were normalized using the Median normalization. After normalization, differentially expressed miRNAs with statistical significance were identified through Volcano Plot filtering.

Statistical analysis

All statistical analyses were performed using SPSS 18.0 software (SPSS, Inc., Chicago, IL, USA). Data are expressed as the mean \pm standard deviation. Statistical analyses were performed using Student's t-test for comparison between two groups. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Characteristics of the rat models

The rats of CHF group exhibited left ventricular wall infarction. **Figure 1A** shows the representative M-mode image of CHF group and SO group. As shown in **Figure 1B**, 3 weeks post myocardial infarction, the LVEF and the LVFS value decreased significantly in the CHF group, compared with the SO group (LVEF, 45.97 ± 2.28 , vs. $71.30 \pm 1.93\%$, $P = 0.000$ and LVFS, 19.93 ± 1.27 , vs. $59.67 \pm 8.08\%$, $P = 0.001$). In addition, myocardial infarction increased the LVEDV and the LVESV in the CHF rats, compared with the SO group (LVEDV, 1.31 ± 0.26 , vs. 0.82 ± 0.09 cm³, $P = 0.038$ and LVESV, 0.71 ± 0.17 , vs. 0.24 ± 0.02 cm³, $P = 0.008$). **Figure 1C** shows serum levels of BNP increased significantly in the CHF rats compared with the SO rats, (11.85 ± 1.53 , vs. 3.30 ± 0.90 ng/ml, $P = 0.001$), 3 weeks post myocardial infarction.

miRNA expression is altered after heart failure

To analyze heart failure changes in miRNA expression in heart, miRNA microarray analysis was performed using arrays containing 3100 capture probes, covering all human, mouse and rat microRNAs annotated in miRBase 18.0, as well as all viral microRNAs related to these species. Total RNA was extracted from left ventricular of normal and CHF rats. miRNAs from CHF rats that were upregulated ($n = 29$) and downregulated ($n = 12$) at least 1.5-fold compared with those of normal rats were identified (**Figure 2**), and lists of the top five most differentially upregulated or downregulated miRNAs are presented in **Table 1**.

Prediction of target genes and functional annotation

Among the deregulated 41 miRNAs, the top 5 upregulated or downregulated miRNAs were

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Table 1. The top five most differentially upregulated or downregulated miRNAs

	Name	Fold change	P-value
Upregulated miRNAs	rno-miR-31a-5p	19.43721544	0.00014599
	rno-miR-205	5.120436317	0.021971604
	rno-miR-540-3p	4.735267606	0.035001886
	rno-miR-31a-3p	6.698152087	0.045896436
	rno-miR-495	4.693811775	0.000205327
Downregulated miRNAs	rno-miR-150-5p	0.423895779	0.001525308
	rno-miR-293-3p	0.314479374	0.04739143
	rno-miR-208a-3p	0.164477062	0.020222159
	rno-miR-183-3p	0.275752702	0.046690084
	rno-miR-133a-3p	0.414577468	0.000145687

miRNA, microRNA.

altered >2.0-fold (**Table 1**). So, the five most upregulated and downregulated miRNAs were selected to obtain more representative biological meanings for the putative genes. We used the Intersection of four miRNA target prediction database to identify putative target genes. TargetScan, miRanda, miRDB, miRWalk, four miRNA target prediction database, were used to identify 258 putative target genes for upregulated and downregulated miRNAs (**Table 2**). The analysis revealed 189 putative targets for the top 5 upregulated miRNAs and 69 putative targets for the top 5 downregulated miRNAs.

GO analysis was performed to determine the functions of the target genes regulated by these differentially expressed miRNAs. Each putative targets gene was subjected to GO analysis to reveal its cell function. GO was analyzed using three significant categories as follows: Molecular function, biological process and cellular component. The analysis revealed a wide distribution of cellular functions, which are presented in **Table 3**.

KEGG pathway annotations of the candidate target genes regulated by differentially expressed miRNAs were also performed. The result indicated that the significant pathways of the candidate target genes are mainly involved in Adipocytokine signaling pathway, vascular smooth muscle contraction signaling pathway, Wnt signaling pathway and TGF-beta signaling pathway, which are presented in **Table 4**.

Discussion

The present study demonstrated that heart failure post myocardium infarction induced

numerous changes in the miRNA expression levels of the rat myocardium. miRNAs from CHF rats that were upregulated (n=29) and downregulated (n=12) at least 1.5-fold compared with those of normal rats were identified. These findings indicate that the miRNA expression patterns with heart failure post myocardium infarction were distinct from those of the control group. In addition, the present study identified putative target genes of the up- and downregulated miRNAs

and categorized their reported biological functions.

A number of miRNAs determined to be differentially expressed in the present study are known to be involved in function in the rat myocardium. Firstly, some of microRNAs we found in the present study have been reported to have close relationship with heart failure. For example, recently miR-150 has been described as a regulator in cardiac hypertrophy. In a mouse model, a study identified the crucial role of miR-150 in protecting against cardiac hypertrophy and fibrosis in response to pressure overload through the downregulation of serum response factor [13]. Prior study have showed a change in several circulating miRNA levels after left ventricular assist device implantation, with decreased levels of the myomirs miR-208a after 3 months [14]. Another study validated miR-208 as a potent therapeutic target for the modulation of cardiac function and remodeling during heart disease progression [15]. Rooij et al found mutant mice overexpressing miR-208 to not develop cardiomyocyte fibrosis despite being exposed to an increased after load [16, 17]. miR-133a, a muscle specific miRNA, is functionally cooperative in promoting mesoderm differentiation in embryonic stem (ES) cells whilst repressing ectodermal and endodermal differentiation [1, 18, 19]. Loss-of-function miR-133a mutants exhibit increased proliferation of cardiomyocytes and up-regulation of smooth muscle cell-specific genes. miR-133 regulates cardiomyocyte proliferation by repressing cyclin D2 and serum response factor [20]. In both animal and human models, miR-133 was identified as regulator of cardiac

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Table 2. Predicted target of the top five most differentially upregulated or downregulated miRNAs

	Target genes
<i>Upregulated miRNAs</i>	Serpib2, Trim39, Tmem50b, Dhrs8, Mgrn1, Siah1a, Kihl12, Vil2, RGD708449, MGC124555, Podxl, Rnd3, Acs1, RGD1359713, Nars, Fkbp9, Trak2, RAMP4, Sel1h, MGC109455, Ssbp3, Esrrg, SPP24_RAT, Pja2, RGD1305453, RGD1305440, Mmd, Errfi1, St6galnac3, Bet1l, RGD1305246, Cep55, RGD1305314, Prdx3, Ap3m1, Paqr4, Pcdha2_predicted, Pcdha2, Pcdhac2, Pir, Drb1, Dctn2, Tmbim1, Cnbp1, Slc1a4, Shank1, C1qtnf6, Mfng, Arf3, Errfi1, Clp1, Slc25a19, Cep55, Abca2, Slc5a7, Xrcc4, Lypla1, Fbxo11, Dncli2, Vnn1, Flt1, Sgk, Tmed9, LOC305166, Nuak2, Bat1a, Cpox, Gmfb, Siah1a, SEPT7, RGD1309892, Sdc1, Rg9mttd1, Btbd15, Pcdha2_predicted, Pcdha2, Pcdhac2, Khdrbs2, Tcfcp2l2, Ssr1, Zfp297b, Yt521, Hspa5, Akr1b4, Acs14, Dusp1, Tgfb1, Ptn, Smad1, Tagln3, Dnajb9, Ppp6c, Cbfb, Ccng1, Strn3, Stx6, Slc12a2, LOC303059, Akap6, Ube4a, Serpine1, Pafah1b2, Nupl1, Cirh1a, Nip7, Pscdbp, TSEN34, Tmpo, Mgat2, Hnrph1, 7-Mar, Tnfsf4, Grm6, Asah1, RGD1306658, LOC683711, Dusp6, Rab18, RGD1359509, Prei3, Gpr64, Mtr, Smad7, Ccna2, Psip1, Gad1, Fcgr3a, RGD1306635_predicted, Afap, Cand1, Pigm, Kihl24, MGC124653, Klf5, Ddit4, Arl5b, Kcnk10, Ppp1cc, Ankr46, Ube2v2, B3galt3, Ssbp3, Cnbp1, Cugbp1, Gucy1b3, MGC108974, Cmpk, Cd164, Irs1, Dclk2_predicted, Ube1c, Tsc22d3, Git2, Cd44, Lrrn1, Chfr, Ptk2, Gatad2b, Actc1, Them4, Snx27, Zfp422, Tgif, F101B_RAT, Pvalb, Cacna1c, Tnfaip1, Myod1, Sycp1, Tob2, Slc16a1, Slc17a6, Apba2, Prom2, Slc38a2, Bcat1, Ube2z, Crkr, MGC94113, Lphn2, Stat3, Psme3, Adm, Kpna2, Clcf1, Cep55, Scd2, Nolc1
<i>Downregulated miRNAs</i>	Dhrs8, Sgk, Syt2, Rab8a, Timm17a, Ptbp1, Clta, Gpm6a, Glra2, Ppp2ca, Ppp2cb, Zfp672, Agt, Whsc2, Sec61a1, Cmpk, Vps54, Ndrgr1, Slc6a1, Sv2a, MGC94600, RGD1305778, Cybasc3, Ncoa4_predicted, Drd1a, Freq, Plp2, RGD1305117, RGD708449, Hspa5, Acs14, Tmem24, Rad23b, Ppp2r1a, St3gal2, Cd276, Gdi2, Egr2, Mtfmt, Abcb9, Pvr12, Mcf2l1, Rnf34, Hspb8, Gpi, Ptk9, Bcat1, Commd7, Ap3m1, RGD1305356, Gpr85, Vil2, Commd10, Ppp2r1a, Tmpo, Ppp2ca, Bnip3l, Prkch, Ppp2cb, Snx1, Ssrp1, Amd1, Irs1, Vtcn1, Wbp11, Scsep1, Plcb4, Sfrs2

miRNA, microRNA.

Table 3. GO analysis of target genes regulated by the top five most differentially upregulated or downregulated miRNAs

	<i>Upregulated miRNAs</i>	<i>Downregulated miRNAs</i>
<i>BP</i>	Modification-dependent protein catabolic process, modification-dependent macromolecule catabolic process, proteolysis involved in cellular protein catabolic process, cellular protein catabolic process, protein catabolic process, regulation of transcription cell cycle, cellular macromolecule catabolic process, macromolecule catabolic process, response to organic substance, intracellular signaling cascade, positive regulation of transcription, positive regulation of gene expression, positive regulation of macromolecule biosynthetic process, response to endogenous stimulus, positive regulation of macromolecule metabolic process, positive regulation of cellular biosynthetic process, regulation of cell proliferation, positive regulation of biosynthetic process, regulation of apoptosis, Transcription, regulation of programmed cell death, positive regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process, positive regulation of nitrogen compound metabolic process	Protein transport, establishment of protein localization, protein localization
<i>CC</i>	Plasma membrane, nuclear lumen, Nucleoplasm, membrane-enclosed lumen, intracellular organelle lumen, organelle lumen, Cytosol, endomembrane system, cell projection, organelle envelope	Plasma membrane, plasma membrane part, organelle membrane, cytosol
<i>MF</i>	Enzyme binding	None

miRNA, microRNA; MF, Molecular function; BP, biological process; CC, cellular component.

Table 4. Significant pathway of target genes regulated by the top five most differentially upregulated or downregulated miRNAs

	<i>Upregulated miRNAs</i>	<i>Downregulated miRNAs</i>
<i>KEGG_PATHWAY</i>	Adipocytokine signaling pathway, Vascular smooth muscle contraction, Lysosome	Long-term depression, Tight junction, Wnt signaling pathway, TGF-beta signaling pathway Oocyte meiosis Lysosome

miRNA, microRNA.

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hypertrophy, with lower levels in heart failure and cardiac hypertrophy compared with controls [9, 21-23] miR-133a also plays an important role in β -adrenergic signaling pathway and Akt pathway [9].

Some of microRNAs we found in this study is new to heart failure. miRNA-31 can manifest antifibrotic effect via downregulating Islet-1, a key factor during the TGF- β -induced epithelial-mesenchymal transition [24]. These findings identify atrial-specific up-regulation of miR-31 in human AF as a key mechanism causing atrial dystrophin and nNOS depletion, which in turn contributes to the atrial phenotype begetting this arrhythmia. A study described miR-495 mediated the expression of endothelial or angiogenic genes by directly targeting vascular endothelial zinc finger 1. After transplantation in immunodeficient myocardial infarction mice, the derived endothelial cells significantly increased neovascularization in the infarcted heart, prevented functional worsening, and attenuated expansion of infarct size. The functional integration of the implanted ECs into coronary networks was also enhanced by inhibiting miR-495 [25].

In addition, the present study identified putative target genes of the up- and downregulated miRNAs and categorized their reported biological functions by GO into molecular function (MF), biological process (BP), cellular component (CC). Among the Significant pathway of target genes regulated by the top five most differentially upregulated or downregulated miRNAs, Wnt signaling pathway and TGF-beta signaling pathway were assigned to the highest number of target genes. Wingless related integration site (Wnt) signaling has proven to be a fundamental mechanism in cardiovascular development as well as disease [26]. There is increasing evidence that Wnt signaling is involved in adverse cardiac remodeling and the progression to heart failure [27]. TGF- β is a powerful profibrotic cytokine with diverse and often contradictory functions [28]. TGF- β has both profibrotic and hypertrophic actions and has been linked to fibrosis in heart disease [28, 29]. In animal models of ischemic HF, positive benefits from inhibition of TGF- β signaling is controversial as inhibition of TGF- β before or immediately following MI has been shown to increase mortality and exacerbate dysfunction, yet inhibition

started 24 hours post-MI attenuated remodeling and improved function [28, 30-33].

In conclusion, the data from the present study indicated changes in the miRNA expression levels in the rat myocardium under heart failure post myocardium infarction. When compared with that of the control group, heart failure exerted differential effects on miRNA expression. Bioinformatic analysis of the differentially expressed miRNAs showed that major affected pathways are known to be involved in cardiac remodeling and fibrosis.

The present study has some limitations. It has a small number of samples. Secondly, further study of using quantitative real time-PCR technique to verify microarray results is needed. Furthermore, the target genes and pathways of CHF associated miRNAs remain elusive and deserve further investigations.

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Disclosure of conflict of interest

None.

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References

- [1] Wong LL, Wang J, Liew OW, Richards AM and Chen YT. MicroRNA and heart failure. *Int J Mol Sci* 2016; 17: 502.
- [2] Writing Committee Members, Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE Jr, Drazner MH, Fonarow GC, Geraci SA, Horwich T, Januzzi JL, Johnson MR, Kasper EK, Levy WC, Masoudi FA, McBride PE, McMurray JJ, Mitchell JE, Peterson PN, Riegel B, Sam F, Stevenson LW, Tang WH, Tsai EJ, Wilkoff BL; American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American

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- Heart Association Task Force on practice guidelines. *Circulation* 2013; 128: e240-327.
- [3] Chaudhry SP and Stewart GC. Advanced heart failure: prevalence, natural history, and prognosis. *Heart Fail Clin* 2016; 12: 323-333.
- [4] Bui AL, Horwich TB and Fonarow GC. Epidemiology and risk profile of heart failure. *Nat Rev Cardiol* 2011; 8: 30-41.
- [5] Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Franco S, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Huffman MD, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Magid D, Marcus GM, Marelli A, Matchar DB, McGuire DK, Mohler ER, Moy CS, Mussolino ME, Nichol G, Paynter NP, Schreiner PJ, Sorlie PD, Stein J, Turan TN, Virani SS, Wong ND, Woo D and Turner MB. Heart disease and stroke statistics—2013 update: a report from the American Heart Association. *Circulation* 2013; 127: e6-e245.
- [6] Liu X, Meng H, Jiang C, Yang S, Cui F and Yang P. Differential microRNA expression and regulation in the rat model of post-infarction heart failure. *PLoS One* 2016; 11: e0160920.
- [7] Zhu X, Wang H, Liu F, Chen L, Luo W, Su P, Li W, Yu L, Yang X and Cai J. Identification of microRNA networks in end-stage heart failure because of dilated cardiomyopathy. *J Cell Mol Med* 2013; 17: 1173-1187.
- [8] Naga Prasad SV, Duan ZH, Gupta MK, Surampudi VS, Volinia S, Calin GA, Liu CG, Kotwal A, Moravec CS, Starling RC, Perez DM, Sen S, Wu Q, Plow EF, Croce CM and Karnik S. Unique microRNA profile in end-stage heart failure indicates alterations in specific cardiovascular signaling networks. *J Biol Chem* 2009; 284: 27487-27499.
- [9] Vegter EL, van der Meer P, de Windt LJ, Pinto YM and Voors AA. MicroRNAs in heart failure: from biomarker to target for therapy. *Eur J Heart Fail* 2016; 18: 457-468.
- [10] Melman YF, Shah R and Das S. MicroRNAs in heart failure: is the picture becoming less miRky? *Circ Heart Fail* 2014; 7: 203-214.
- [11] Tijssen AJ, Pinto YM and Creemers EE. Non-cardiomyocyte microRNAs in heart failure. *Cardiovasc Res* 2012; 93: 573-582.
- [12] Li Y, Xuan YH, Liu SS, Dong J, Luo JY and Sun ZJ. Short-term vagal nerve stimulation improves left ventricular function following chronic heart failure in rats. *Mol Med Rep* 2015; 12: 1709-1716.
- [13] Liu W, Liu Y, Zhang Y, Zhu X, Zhang R, Guan L, Tang Q, Jiang H, Huang C and Huang H. MicroRNA-150 protects against pressure overload-induced cardiac hypertrophy. *J Cell Biochem* 2015; 116: 2166-2176.
- [14] Akat KM, Moore-McGriff D, Morozov P, Brown M, Gogakos T, Correa Da Rosa J, Mihailovic A, Sauer M, Ji R, Ramarathnam A, Totary-Jain H, Williams Z, Tuschl T and Schulze PC. Comparative RNA-sequencing analysis of myocardial and circulating small RNAs in human heart failure and their utility as biomarkers. *Proc Natl Acad Sci U S A* 2014; 111: 11151-11156.
- [15] Montgomery RL, Hullinger TG, Semus HM, Dickinson BA, Seto AG, Lynch JM, Stack C, Latimer PA, Olson EN and van Rooij E. Therapeutic inhibition of miR-208a improves cardiac function and survival during heart failure. *Circulation* 2011; 124: 1537-1547.
- [16] Schulte C, Westermann D, Blankenberg S and Zeller T. Diagnostic and prognostic value of circulating microRNAs in heart failure with preserved and reduced ejection fraction. *World J Cardiol* 2015; 7: 843-860.
- [17] van Rooij E, Sutherland LB, Qi X, Richardson JA, Hill J and Olson EN. Control of stress-dependent cardiac growth and gene expression by a microRNA. *Science* 2007; 316: 575-579.
- [18] Wystub K, Besser J, Bachmann A, Boettger T and Braun T. miR-1/133a clusters cooperatively specify the cardiomyogenic lineage by adjustment of myocardin levels during embryonic heart development. *PLoS Genet* 2013; 9: e1003793.
- [19] Chen JF, Mandel EM, Thomson JM, Wu Q, Callis TE, Hammond SM, Conlon FL and Wang DZ. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. *Nat Genet* 2006; 38: 228-233.
- [20] Liu N, Bezprozvannaya S, Williams AH, Qi X, Richardson JA, Bassel-Duby R and Olson EN. microRNA-133a regulates cardiomyocyte proliferation and suppresses smooth muscle gene expression in the heart. *Genes Dev* 2008; 22: 3242-3254.
- [21] Care A, Catalucci D, Felicetti F, Bonci D, Adario A, Gallo P, Bang ML, Segnalini P, Gu Y, Dalton ND, Elia L, Latronico MV, Hoydal M, Auctor C, Russo MA, Dorn GW 2nd, Ellingsen O, Ruiz-Lozano P, Peterson KL, Croce CM, Peschle C and Condorelli G. MicroRNA-133 controls cardiac hypertrophy. *Nat Med* 2007; 13: 613-618.
- [22] Danowski N, Manthey I, Jakob HG, Siffert W, Peters J and Frey UH. Decreased expression of miR-133a but not of miR-1 is associated with signs of heart failure in patients undergoing coronary bypass surgery. *Cardiology* 2013; 125: 125-130.
- [23] Dong DL, Chen C, Huo R, Wang N, Li Z, Tu YJ, Hu JT, Chu X, Huang W and Yang BF. Reciprocal repression between microRNA-133 and calcineurin regulates cardiac hypertrophy: a novel

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- mechanism for progressive cardiac hypertrophy. *Hypertension* 2010; 55: 946-952.
- [24] Bronnum H, Andersen DC, Schneider M, Nosent AY, Nielsen SB and Sheikh SP. Islet-1 is a dual regulator of fibrogenic epithelial-to-mesenchymal transition in epicardial mesothelial cells. *Exp Cell Res* 2013; 319: 424-435.
- [25] Liang J, Huang W, Cai W, Wang L, Guo L, Paul C, Yu XY and Wang Y. Inhibition of microRNA-495 enhances therapeutic angiogenesis of human induced pluripotent stem cells. *Stem Cells* 2017; 35: 337-350.
- [26] Pahnke A, Conant G, Huyer LD, Zhao Y, Feric N and Radisic M. The role of Wnt regulation in heart development, cardiac repair and disease: a tissue engineering perspective. *Biochem Biophys Res Commun* 2016; 473: 698-703.
- [27] Hermans KC and Blankesteyn WM. Wnt signaling in cardiac disease. *Compr Physiol* 2015; 5: 1183-1209.
- [28] Edgley AJ, Krum H and Kelly DJ. Targeting fibrosis for the treatment of heart failure: a role for transforming growth factor-beta. *Cardiovasc Ther* 2012; 30: e30-40.
- [29] Lim H and Zhu YZ. Role of transforming growth factor-beta in the progression of heart failure. *Cell Mol Life Sci* 2006; 63: 2584-2596.
- [30] Frantz S, Hu K, Adamek A, Wolf J, Sallam A, Maier SK, Lonning S, Ling H, Ertl G and Bauersachs J. Transforming growth factor beta inhibition increases mortality and left ventricular dilatation after myocardial infarction. *Basic Res Cardiol* 2008; 103: 485-492.
- [31] Ikeuchi M, Tsutsui H, Shiomi T, Matsusaka H, Matsushima S, Wen J, Kubota T and Takeshita A. Inhibition of TGF-beta signaling exacerbates early cardiac dysfunction but prevents late remodeling after infarction. *Cardiovasc Res* 2004; 64: 526-535.
- [32] Ellmers LJ, Scott NJ, Medicherla S, Pilbrow AP, Bridgman PG, Yandle TG, Richards AM, Protter AA and Cameron VA. Transforming growth factor-beta blockade down-regulates the renin-angiotensin system and modifies cardiac remodeling after myocardial infarction. *Endocrinology* 2008; 149: 5828-5834.
- [33] Okada H, Takemura G, Kosai K, Li Y, Takahashi T, Esaki M, Yuge K, Miyata S, Maruyama R, Mikami A, Minatoguchi S, Fujiwara T and Fujiwara H. Postinfarction gene therapy against transforming growth factor-beta signal modulates infarct tissue dynamics and attenuates left ventricular remodeling and heart failure. *Circulation* 2005; 111: 2430-2437.