SERCA2a related miRNA miR-140-3p as a potential biomarker for chronic heart failure

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Abstract

Background

Multiple miRNAs were reported to be associated with sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a) in mediating the process of heart failure. However, whether these miRNAs are relevant to chronic heart failure (CHF) is rarely reported.

Methods

This study was designed to explore a new biomarker from the SERCA2a related candidate miRNAs for CHF. A total of 195 blood samples from 84 CHF patients, 86 non-heart failure patients and 25 healthy subjects were collected. The circulating expression of SERCA2a related candidate miRNAs (let-7a-5p, miR-24-3p, miR-25-3p, miR-133a-3p, miR-137a-3p, miR-140-3p, miR-141-3p, miR-142-3p, miR-148a-3p, and miR-153-3p) were detected with qRT-PCR. The area under curve value, correlations between miRNAs and NT-proBNP were applied to determine the relationship between CHF and the candidate miRNAs.

Results

The results showed among the 10 candidate miRNAs, only let-7a-5p, miR-25-3p, and miR-140-3p were significantly increased of CHF patients. However, only miR-140-3p showed the high area under the curve (AUC) value (ROC = 0.8873) and strong positive correlations (r = 0.5113) between miR-140-3p levels and NT-proBNP concentration. The level of miR-140-3p in LVEF < 45% of CHF patients was higher than in LVEF > 45%. However, there was no significant difference between hypertension and non-hypertension of the levels of miR-140-3p in CHF patients. Interestingly, the level of miR-140-3p was higher in NYHA IV CHF patients than in NYHA II or III. More important, the miR-140-3p level also declined when the heart function of NYHA IV patients was improved after treatment.

Conclusions

These results indicated circulation levels of miR-140-3p is related to the severity of chronic heart failure, and miR-140-3p may be an important biomarker for heart failure with reduced ejection fraction.

1. Introduction

Chronic heart failure (CHF) is the primary endpoint of various cardiovascular disorders, including atherosclerosis, ischemic cardiomyopathies, familiar cardiomyopathies, valvular-induced myocardial pathologies, as well as arterial hypertension [1, 2]. Appropriate use of biomarkers in clinical diagnosis is important for identifying heart failure (HF) in its early stage [3]. Experimental studies have demonstrated

that stabilization of Ca^{2+} cycling abnormalities in cardiomyocytes is very important in the pathophysiology of HF [4, 5]. SERCA2a is an important determinant of cardiac contractility and is responsible for Ca^{2+} re-uptake from cytosol during excitation-contraction coupling. Series of studies demonstrated the reduced expression or activity of SERCA2a is one of the hallmarks of heart failure, which suggest that SERCA2a is an important potential therapeutic target for heart failure [6–11]. In this regard, the SERCA2a and its regulators have shown to be important targets for intervention on heart failure [7, 9, 12, 13].

MicroRNAs (miRNAs) are 20–25 nucleotides and their major function is to mediate post-transcriptional gene silencing [14, 15]. Preclinical research into miRNAs has shown their important role in the control of calcium cycling and the development of ventricular hypertrophy and heart failure [16]. In the past ten years, many studies have demonstrated that different miRNAs regulated heart failure by targeting SERCA2a, such as let-7, miR25, miR-133, miR-142, and many others [17–26]. In the present study, ten candidate miRNAs (Let-7a-5p, miR-24-3p, miR-25-3p, miR-133a-3p, miR-137-3p, miR-140-3p, miR-141-3p, miR-142-3p, miR-148a-3p, and miR-153-3p), which are potential targeting SERCA2a (Table 2), are reported as important regulators in heart failure by mediating myocardial infarction, cardiac hypertrophy, cardiomyocytes apoptosis, autophagy, experimental autoimmune myocarditis, cardiac fibrosis, as well as other heart-related diseases [23, 27–34]. However, the level of these miRNAs in chronic heart failure and the relationship between these miRNAs and the severity of CHF have not yet been demonstrated. This study was designed to investigate the expression of these miRNAs in CHF patients and to explore the relationship between candidate miRNAs and the severity of CHF.

Table 2
MiRNAs information and target sites on ATP2A2 (SERCA2A)

ID	Accession	Target sites on ATP2A2	
hsa-let-7a-5p	MIMAT0000062	ATP2A2 target region Let-75-5p sequence	Position 490–497 of ATP2A2 3' UTR 5' ...CUGAGCAGAGUCUUGCUACCUCA... | | | | | | | | | 3' UUGAUAUGUUGGAUGAUGGAGU
hsa-miR-24-3p	MIMAT0000080	ATP2A2 target region miR-24-3p sequence	Position 307–313 of ATP2A2 3' UTR 5' ...UAUAAAAAAAAAUAGUUGAGCCAG... | | | | | | | | 3' GACAAGGACGACUUGACUCGGU
hsa-miR-25-3p	MIMAT0000081	ATP2A2 target region miR-25-3p sequence	Position 4450–4457 of ATP2A2 3' UTR 5' ...AGUAGACAGAUGUUGGUGCAAUA... | | | | | | | | 3' AGUCUGGCUCUGUUCACGUUAC
hsa-miR-133a-3p	MIMAT0000427	ATP2A2 target region miR-25-3p sequence	Position 1143–1149 of ATP2A2 3' UTR 5' ...CUGUAAAUAGCACAUGACCAAAU... | | | | | | | | 3' GUCGACCAACUUCCCCUGGUUU
hsa-miR-137-3p	MIMAT0000429	ATP2A2 target region miR-25-3p sequence	Position 3820–3826 of ATP2A2 3' UTR 5' ...CUCUCUUUCCUUUUCAGCAAUAC... | | | | | | | | 3' GAUGCGCAUAAGAAUUCGUUAUU
hsa-miR-140-3p	MIMAT0004597	ATP2A2 target region miR-25-3p sequence	Position 1278–1284 of ATP2A2 3' UTR 5' ...CAUUAACAGUCCUAACUGUGGUG... | | | | | | | | 3' GGCACCAAGAUGGGACACCAU
hsa-miR-141-3p	MIMAT0000432	ATP2A2 target region miR-25-3p sequence	Position 265–271 of ATP2A2 3' UTR 5' ...AUGAAAAAGCAUGUACAGUGUUC... | | | | | | | | 3' GGUAGAAAUGGUCUGUCACAAU

ID	Accession	Target sites on ATP2A2	
hsa-miR-142-3p	MIMAT0000434	ATP2A2 target region miR-25-3p sequence	Position 4315–4322 of ATP2A2 3' UTR 5' ...AUAAAUUCAUUAAAAACACUACA... | | | | | | | | 3' AGGUAUUUCAUCCUUUGUGAUGU
hsa-miR-148a-3p	MIMAT0000243	ATP2A2 target region miR-25-3p sequence	Position 471–478 of ATP2A2 3' UTR 5' ...AUAAGCCAAUUUUUCUGCACUGA... | | | | | | | | 3' UGUUUCAAGACAUCACGUGACU
hsa-miR-153-3p	MIMAT0000439	ATP2A2 target region miR-25-3p sequence	Position 1049–1055 of ATP2A2 3' UTR 5' ...GAACUAACACUAUUUUAUGCAAA... | | | | | | | | 3' CUAGUGAAAACACUGAUACGUU

2. Methods

2.1 Sample

This study was approved by the Ethics Committee of the Xiamen Cardiovascular Hospital of Xiamen University and received the patients' consent form. Eighty-four subjects who were diagnosed as CHF met the Framingham criteria had a circulating N-terminal pro-B-type natriuretic peptide (NT-proBNP) level over 1000 pg/ml. All the CHF patients on a scale of II to IV were categorized according to the New York Heart Association (NYHA) classification system. Correspondingly, eighty-six subjects and 25 healthy volunteers were enrolled as control groups. The non-heart failure patients were diagnosed with various cardiovascular diseases including coronary heart diseases, hypertension, valvular heart disease, arrhythmia, as well as other cardiovascular diseases, with a NT-proBNP value less than 100 pg/ml. The blood samples of non-heart failure and heart failure subjects, which were to determine the concentration of NT-proBNP of the inpatient, were obtained from the clinical laboratory of the hospital. Twenty-five healthy volunteers' blood samples were from the physical examination volunteers and were free from heart disease or other diseases.

2.2 Serum preparation

The venous blood was collected after detecting the NT-proBNP and centrifuged at 3000 rpm for 5 min at room temperature. The resultant serum was aliquoted into tubes and stored at -80 °C for RNA isolation (as detailed below).

2.3 RNA Isolation, cDNA synthesis, and real-time PCR

All serum samples from the subjects in the three groups are extracted with the manufacturer's kit for total miRNA. MiRNA Reverse Transcription Kit was used for reverse transcription (RT) according to the manufacturer's recommendations. Briefly, 1 μg total mRNA was combined with dNTPs, MultiScribe™ reverse transcriptase, and the primer specific for the target miRNAs. The cDNA was diluted and used in PCR reactions. Realtime-PCR was performed according to the manufacturer's recommendation using the Bio-Rad CFX96.

2.4 Echocardiography

All patients with chronic heart failure underwent echocardiography for the detection of the heart function after admission. Echocardiographic examinations were performed by using an ACUSON SC2000 PRIME cardiac ultrasound system (SIEMENS Healthineers, Germany) equipped with a 3.5-MHz transducer. All the echocardiographic analysis was performed by an experienced clinical echocardiographer, who was blinded to the clinical information of the patients.

2.5 Statistical analysis

All the statistical calculations were performed using Graph Pad Prism 5.0 and data were presented as mean ± S.E.M or as median with interquartile range, as appropriate. The one-way analysis of variance (ANOVA) with a Fisher's least significant difference was used to determine whether the fold changes in miRNA levels were statistically significant among the 3 groups or CHF subgroups formed according to the disease severity. Spearman correlation coefficients were computed to assess the correlations between NT-proBNP and miRNA levels. We also assessed the discrimination of miRNA levels with a fitted logistic model, via the receiver operating characteristic (ROC) curve.

3. Results

3.1 Clinical characteristics of the patients.

The baseline and clinical characteristics and demographics of the three groups are shown in Table 1. The healthy control group enrolled 25 healthy subjects, the non-heart failure group consisted 86 patients with cardiovascular disease but not heart failure, including coronary artery disease (CAD), congenital heart disease (CHD), dissection of the aorta, angina pectoris, arrhythmia, hypertension, or/and diabetes, and the CHF group consisted of 84 subjects. None-heart failure and CHF groups were similar regarding age, gender, diabetes, hypertension, smoking habits but different NT-proBNP, echocardiographic and clinical diagnosis. In CHF cases, 48 (57%) subjects had an ejection fraction (EF) lower than 45%, whereas 36 (43%) subjects had an EF more than 45%.

Table 1
characteristics of patients.

	Healthy group	None-Heart failure group	Chronic heart failure group
patients(n)	25	86	84
Mean age, years	57.25 ± 14.36	53.89 ± 15.82	56.02 ± 12.99
Sex, male/female	17/8	47/39	46/38
NT-ProBNP, pg/ml	29.38 ± 4.28	50.38 ± 34.72	5182.61 ± 675.20**##
Diabetes, %(n)	NA	16(14)	14(12)
Smokers, %(n)	48(12)	43(37)	42(35)
Hypertension, %(n)	NA	55(47)	54(45)
NYHA grade, %			
class II	-	-	27
class III	-	-	28
class IV	-	-	29
LVEF	68.28 ± 1.32	67.91 ± 6.63	40.55 ± 7.69**##
>45%	-	-	36
<45%	-	-	48

3.2 Sequences and targeting sites of miRNA on SERCA2a.

As shown in Table 2, the sequences and binding sites of let-7a-5p, miR-24-3p, miR-25-3p, miR-133a-3p, miR-137-3p, miR-140-3p, miR-141-3p, miR-142-3p, miR-148a-3p and miR-153-3p on SERCA2a (ATP2a2) was shown. The potential targeting sites of corresponding miRNAs on SECA2a was explored with Target Scan (http://www.targetscan.org).

3.3 Circulating levels of SERCA2a related miRNAs in three groups.

The fold changes of miRNAs in three different groups are shown in Fig. 1. Compared to the non-HF group and healthy group, CHF patients had significantly higher serum levels of let-7a-5p (Fig. 1A), miR-25-3p (Fig. 1C), and miR-140-3p (Fig. 1F). In contrast, the serum levels of other miRNAs, miR-24-3p, miR-133-3p, miR-137-3p, miR-141-3p, miR-142-3p, miR-148a-3p, and miR-153-3p showed little increase in CHF patients than other two groups, and no significant difference was found.

3.4 Diagnostic accuracy of let-7a-5p, miR-25-3p, and miR-140-3p in chronic heart failure patients.

The ROC curve analysis was applied to further determine the diagnostic accuracy of the three up-regulated miRNAs (let-7a-5p, miR-25-3p and miR-140-3p) in chronic heart failure. As shown in the Fig. 2, the miR140-3p showed the highest area under curve value (AUC: 0.8873; 95% confidence interval: 0.8360 to 0.9387; P < 0.0001) (Fig. 2C) followed by miR-25-3p (AUC: 0.7556; 95% confidence interval: 0.6786 to 0.8345; P = 0.0008378) (Fig. 2B) and let-7a-5p (AUC: 0.5774; 95% confidence interval: 0.4898 to 0.6650; P = 0.07980) (Fig. 2A).

The Spearman correlation coefficient analysis was applied to further evaluate the relationship between candidate miRNAs and NT-proBNP levels. As shown in Fig. 3, the strongest positively correlation with NT-proBNP levels was observed for miR-140-3p (r = 0.4769; P < 0.0001) (Fig. 2F) followed by miR-25-3p (r = 0.4258; P < 0.0001) (Fig. 2D) and let-7a-5p (r = 0.1216; P = 0.1186) (Fig. 2E). These results indicated that miR-140-3p is more correlation with chronic heart failure than other SERCA2a related miRNAs.

3.5 Circulating miR-140-3p levels & disease severity

To investigate whether three up-expressed miRNAs are related to chronic heart failure severity or etiology, we categorized CHF patients according to their EF or NYHA class. Levels of circulating miR140-3p (Fig. 3C) were higher in subjects with EF of < 45%, compared to subjects with EF of > 45%, while the levels of circulating let-7a-5p (Fig. 3A) and miR-25-3p (Fig. 3B) were slightly higher in subjects with EF of < 45%, but this did not reach statistical significance. We also compared the levels of let-7a-5p (Fig. 3D), miR-25-3p (Fig. 3E), and miR-140-3p (Fig. 3F) in CHF patients with or without hypertension. The levels of let-7a-5p, miR-25-3p, and miR-140-3p showed no significant difference between none hypertension and hypertension CHF patients.

In order to explore the relationship between the severity of heart failure and the levels of three miRNAs, we compared the levels of three miRNAs in patients with different NYHA grade. The results showed that circulating levels of miR-140-3p (Fig. 4C) and miR-25-3p (Fig. 4B) increased with increasing NYHA class (P < 0.05), except let-7a-5p (Fig. 4A). We also explored the expression of miR-140-5p in CHF patients who have been treated and the heart function has improved. Interestingly, only the level of miR-140-5p was significantly decreased after the heart function was improved (Fig. 4F). These results indicated that the circulating levels of miR140-3p may be related to heart failure severity.

4. Discussion

Chronic heart failure (HF) is a global pandemic affecting millions of people worldwide [35]. Prediction of the of CHF patients is still a challenging task even using many clinical predictors [36]. Changed of Ca^{2+} homeostasis is an important feature of HF. SERCA2a is a transmembrane protein with a molecular mass of 110 kDa that can be divided into 3 distinct regions, which plays an important role in regulating Ca^{2+} levels in cardiomyocytes [37]. Reduced SERCA2a expression and/or activity are at least partly responsible for dysregulation of cellular Ca^{2+} homeostasis in heart failure [38].

Recently, several miRNAs have gained attention as potential biomarkers for personalized healthcare of patients with heart failure [39–42]. A series of studies have demonstrated that miR-24-3p, miR-133a-3p, miR-137-3p, miR-141-3p, miR-142-3p, miR-148a-3p and miR-153-3p are important regulators in several cardiovascular disease, which have an identical SERCA2a. For example, miR-24 regulates cardiac fibrosis [30], cardiac hypertrophy [43], myocardial infarction [44], cardiomyocytes apoptosis [45], and many other cardiovascular-related disease [46–48]. MiR-133 was reported to be associated with signs of heart failure in patients undergoing coronary bypass surgery [49], arrhythmogenesis in heart failure [21], modulating myocardial matrix remodeling [50], and regulating SERCA2a in congestive heart failure[17]. Alterations in the levels of several miRNAs (miR-1, miR-21, and miR-133) have been observed in human failing myocardium [51]. MiR-137 plays an important role in the pathophysiology of heart failure and reverse remodeling during mechanical support [28]. MiR-141-3p binds to STAT4 and suppresses its expression and regulates inflammation in experimental autoimmune myocarditis [29]. MiR-148-3p expression is altered in diabetic $Ins2^{+/-}$ Akita hearts [34]. MiR-153 was demonstrated as a regulator on cardiomyocytes autophagy [24] and on cardiac hypertrophy [33]. However, in the present study, the circulating levels of miR-24-3p, miR-133a-3p, miR-137-3p, miR-141-3p, miR-142-3p, miR-148a-3p, and miR-153-3p in chronic heart failure patients did not show significant alternation compared to non-HF patients or the healthy subjects. Interestingly, three candidate miRNAs (let-7a-5p, miR-25-3p, and miR-140-3p) showed a significant increase in CHF patients. Then, we explored the potential roles of these miRNAs as a novel biomarker for heart failure. Interestingly, the results found that the miR-140-3p levels, but not let-7a-5p and miR-25, in receiver operating characteristic curve (ROC) analysis shows a high AUC value, and the correlation between miR-140-3p and NT-proBNP present highest correlations compared to other studies. The value of ROC and the correlation value between NT-proBNP and miR-140-3p is higher than the previous studies of miR-423-5p [52] and miR-210 [53, 54] respectively in heart failure-related studies.

Let-7i is reported to be a biomarker for dilated cardiomyopathy [19]. Other previous studies have reported let-7 s regulates hypoxia-mediated cardiomyocytes apoptosis [55], cardiac dysfunction after myocardial infarction [27], or myocardial remodeling post-infarction in mice [56]. In the present study, we found that although the expression of let-7 is significantly increased in the serum of CHF patients, the ROC value is only 0.5774 and the r-value of the correlation between let-7 and NT-proBNP is only 0.1216, which is most less than these values of miR-140-3p and indicates that let-7 have not much correlation on CHF.

Multiple studies have demonstrated that inhibition of miR-25 improves cardiac contractility in the failing heart by targeting on SERCA2a [18, 20, 57, 58] or Six 1 [26]. Another study found a combination of miR-19b-5p, miR-221, miR-25-5p, and hypertension correlates with an increased heart failure risk in coronary heart disease patients [59]. However, there is not any study explored the expression of miR-25 in the serum of CHF patients. Our results showed that although the expression level of miR-25 is significantly increased in CHF patients compared to the non-heart failure patients or the healthy subjects, miR-25 may not be a predictor for CHF because there is little correlation value between the miR-25 expression and NT-proBNP.

Two previous studies have reported miR-140 plays important roles in cardiovascular diseases. One study found miR-140 is elevated in the right ventricles of pulmonary arterial hypertension rats and plays an important role in the pathogenesis of PAH-associated right ventricular dysfunction [22]. The other study explored the potential biomarker in heart failure patients, they found the failing heart released let-7b-5p, let-7c-5p, let-7e-5p, miR-122-5p, and miR-21-5p, and absorbed miR-16-5p, miR-17-5p, miR-27a-3p, miR-30a-5p, miR-30d-5p, miR-30e-5p, miR-130a-3p, miR-140-5p, miR-199a-5p, and miR-451a [60], but there was no in-depth study explored. Interestingly, in our present study, we found the circulating level of miR-140-3p significantly increased in CHF patients and shows more correlation between the expression level of miR-140-3p and NT-proBNP, and miR-140-3p shows more expression levels in LVEF < 45% and NYHA IV patients than others, which may explain the important role of miR-140 in regulating the progress of right ventricular dysfunction in the previous study [22]. More importantly, we also found the levels of miR-140-3p in NYHA IV patients can decrease along with the heart function improved, which means miR-140-3p may be an important heart failure indicator. These results indicated that miR-140-3p may be a novel biomarker for chronic heart failure patients.

Collectively, in the present study, we explored the expression of ten candidate miRNAs in CHF patients (let-7a-5p, miR-25, miR-24-3p, miR-133a-3p, miR-137-3p, miR-140-3p, miR-141-3p, miR-142-3p, miR-148a-3p, and miR-153-3p) which have the same target on the heart failure regulator SERCA2a. The results found that the expression of miR-24-3p, miR-133a-3p, miR-137-3p, miR-141-3p, miR-142-3p, miR-148a-3p, and miR-153-3p showed no alternation in CHF patients compared to other cardiovascular diseases patients and healthy subjects. The expression of let-7a-5p, miR-25-3p, and miR-140-3p showed an increase in CHF patients, and only miR-140-3p appear to be a biomarker for the diagnosis of CHF and the severity of chronic heart failure. However, the limitation of our studies is obviously that there are not enough numbers of subjects to prove our results and find. Larger numbers of clinical series are needed to verify for our conclusion.

Declarations

Ethics approval and consent to participate: The experimental protocol was established, according to the ethical guidelines of the Helsinki Declaration and was approved by the Human Ethics Committee of Xiamen cardiovascular Hospital, Xiamen University. Written informed consent was obtained from individual or guardian participants.

Consent to Publish: All the authors agree to publish the article in the journal.

Availability of data and materials: The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Competing interests: None declared.

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Author's Contributions: G.L. conceived and designed the experiments; W.Y.W., T.Y., H.F.Q. performed the study; G.L., and X.X.Z., analyzed the data and wrote the paper; All the authors approved the submission.

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References

1. **Hulot JS, Senyei G, Hajjar RJ.** Sarcoplasmic reticulum and calcium cycling targeting by gene therapy. *Gene Ther*. 2012; 19: 596-9.
2. **Lompre AM, Hajjar RJ, Harding SE, Kranias EG, Lohse MJ, Marks AR.** Ca2+ cycling and new therapeutic approaches for heart failure. *Circulation*. 2010; 121: 822-30.
3. **Takeishi Y.** Biomarkers in heart failure. *Int Heart J*. 2014; 55: 474-81.
4. **Luo M, Anderson ME.** Mechanisms of altered Ca(2)(+) handling in heart failure. *Circ Res*. 2013; 113: 690-708.
5. **Marks AR.** Calcium cycling proteins and heart failure: mechanisms and therapeutics. *J Clin Invest*. 2013; 123: 46-52.
6. **Byrne MJ, Power JM, Preovolos A, Mariani JA, Hajjar RJ, Kaye DM.** Recirculating cardiac delivery of AAV2/1SERCA2a improves myocardial function in an experimental model of heart failure in large animals. *Gene Ther*. 2008; 15: 1550-7.
7. **del Monte F, Hajjar RJ, Harding SE.** Overwhelming evidence of the beneficial effects of SERCA gene transfer in heart failure. *Circ Res*. 2001; 88: E66-7.
8. **del Monte F, Williams E, Lebeche D, Schmidt U, Rosenzweig A, Gwathmey JK, Lewandowski ED, Hajjar RJ.** Improvement in survival and cardiac metabolism after gene transfer of sarcoplasmic reticulum Ca(2+)-ATPase in a rat model of heart failure. *Circulation*. 2001; 104: 1424-9.
9. **Kawase Y, Ly HQ, Prunier F, Lebeche D, Shi Y, Jin H, Hadri L, Yoneyama R, Hoshino K, Takewa Y, Sakata S, Peluso R, Zsebo K, Gwathmey JK, Tardif JC, Tanguay JF, Hajjar RJ.** Reversal of cardiac dysfunction after long-term expression of SERCA2a by gene transfer in a pre-clinical model of heart failure. *J Am Coll Cardiol*. 2008; 51: 1112-9.
10. **Lipskaia L, Chemaly ER, Hadri L, Lompre AM, Hajjar RJ.** Sarcoplasmic reticulum Ca(2+) ATPase as a therapeutic target for heart failure. *Expert Opin Biol Ther*. 2010; 10: 29-41.
11. **Lyon AR, Bannister ML, Collins T, Pearce E, Sepehripour AH, Dubb SS, Garcia E, O'Gara P, Liang L, Kohlbrenner E, Hajjar RJ, Peters NS, Poole-Wilson PA, Macleod KT, Harding SE.** SERCA2a gene transfer decreases sarcoplasmic reticulum calcium leak and reduces ventricular arrhythmias in a model of chronic heart failure. *Circ Arrhythm Electrophysiol*. 2011; 4: 362-72.

12. **Miyamoto MI, del Monte F, Schmidt U, DiSalvo TS, Kang ZB, Matsui T, Guerrero JL, Gwathmey JK, Rosenzweig A, Hajjar RJ.** Adenoviral gene transfer of SERCA2a improves left-ventricular function in aortic-banded rats in transition to heart failure. *Proc Natl Acad Sci U S A*. 2000; 97: 793-8.
13. **Sakata S, Lebeche D, Sakata Y, Sakata N, Chemaly ER, Liang L, Nakajima-Takenaka C, Tsuji T, Konishi N, del Monte F, Hajjar RJ, Takaki M.** Transcoronary gene transfer of SERCA2a increases coronary blood flow and decreases cardiomyocyte size in a type 2 diabetic rat model. *Am J Physiol Heart Circ Physiol*. 2007; 292: H1204-7.
14. **Gurha P.** MicroRNAs in cardiovascular disease. *Curr Opin Cardiol*. 2016; 31: 249-54.
15. **St Laurent G, Wahlestedt C, Kapranov P.** The Landscape of long noncoding RNA classification. *Trends Genet*. 2015; 31: 239-51.
16. **Kumarswamy R, Thum T.** Non-coding RNAs in cardiac remodeling and heart failure. *Circ Res*. 2013; 113: 676-89.
17. **Ning B, Qi X, Li Y, Liu H, Zhang F, Qin C.** Biventricular pacing cardiac contractility modulation improves cardiac contractile function via upregulating SERCA2 and miR-133 in a rabbit model of congestive heart failure. *Cell Physiol Biochem*. 2014; 33: 1389-99.
18. **Chen H, Liu S, Zhao C, Zong Z, Ma C, Qi G.** Cardiac contractility modulation improves left ventricular systolic function partially via miR-25 mediated SERCA2A expression in rabbit trans aortic constriction heart failure model. *J Thorac Dis*. 2018; 10: 3899-908.
19. **Satoh M, Minami Y, Takahashi Y, Tabuchi T, Nakamura M.** A cellular microRNA, let-7i, is a novel biomarker for clinical outcome in patients with dilated cardiomyopathy. *J Card Fail*. 2011; 17: 923-9.
20. **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: 531-5.
21. **Belevych AE, Sansom SE, Terentyeva R, Ho HT, Nishijima Y, Martin MM, Jindal HK, Rochira JA, Kunitomo Y, Abdellatif M, Carnes CA, Elton TS, Gyorke S, Terentyev D.** MicroRNA-1 and -133 increase arrhythmogenesis in heart failure by dissociating phosphatase activity from RyR2 complex. *PLoS One*. 2011; 6: e28324.
22. **Joshi SR, Dhagia V, Gairhe S, Edwards JG, McMurtry IF, Gupte SA.** MicroRNA-140 is elevated and mitofusin-1 is downregulated in the right ventricle of the Sugen5416/hypoxia/normoxia model of pulmonary arterial hypertension. *Am J Physiol Heart Circ Physiol*. 2016; 311: H689-98.
23. **Bush EW, van Rooij E.** miR-25 in heart failure. *Circ Res*. 2014; 115: 610-2.
24. **Zou Y, Liu W, Zhang J, Xiang D.** miR-153 regulates apoptosis and autophagy of cardiomyocytes by targeting Mcl-1. *Mol Med Rep*. 2016; 14: 1033-9.
25. **Tang QJ, Lei HP, Wu H, Chen JY, Deng CY, Sheng WS, Fu YH, Li XH, Lin YB, Han YL, Zhong SL.** Plasma miR-142 predicts major adverse cardiovascular events as an intermediate biomarker of dual antiplatelet therapy. *Acta Pharmacol Sin*. 2019; 40: 208-15.
26. **Oh JG, Jang SP, Yoo J, Lee MA, Lee SH, Lim T, Jeong E, Kho C, Kook H, Hajjar RJ, Park WJ, Jeong D.** Role of the PRC2-Six1-miR-25 signaling axis in heart failure. *J Mol Cell Cardiol*. 2019; 129: 58-68.

27. **Seeger T, Xu QF, Muhly-Reinholz M, Fischer A, Kremp EM, Zeiher AM, Dimmeler S.** Inhibition of let-7 augments the recruitment of epicardial cells and improves cardiac function after myocardial infarction. *J Mol Cell Cardiol*. 2016; 94: 145-52.
28. **Lok SI, van Mil A, Bovenschen N, van der Weide P, van Kuik J, van Wichen D, Peeters T, Siera E, Winkens B, Sluijter JP, Doevendans PA, da Costa Martins PA, de Jonge N, de Weger RA.** Post-transcriptional regulation of alpha-1-antichymotrypsin by microRNA-137 in chronic heart failure and mechanical support. *Circ Heart Fail*. 2013; 6: 853-61.
29. **Pan A, Tan Y, Wang Z, Xu G.** STAT4 silencing underlies a novel inhibitory role of microRNA-141-3p in inflammation response of mice with experimental autoimmune myocarditis. *Am J Physiol Heart Circ Physiol*. 2019; 317: H531-H40.
30. **Wang J, Huang W, Xu R, Nie Y, Cao X, Meng J, Xu X, Hu S, Zheng Z.** MicroRNA-24 regulates cardiac fibrosis after myocardial infarction. *J Cell Mol Med*. 2012; 16: 2150-60.
31. **Liu Y, Liang Y, Zhang JF, Fu WM.** MicroRNA-133 mediates cardiac diseases: Mechanisms and clinical implications. *Exp Cell Res*. 2017; 354: 65-70.
32. **Liu BL, Cheng M, Hu S, Wang S, Wang L, Tu X, Huang CX, Jiang H, Wu G.** Overexpression of miR-142-3p improves mitochondrial function in cardiac hypertrophy. *Biomed Pharmacother*. 2018; 108: 1347-56.
33. **Wang T, Zhai M, Xu S, Ponnusamy M, Huang Y, Liu CY, Wang M, Shan C, Shan PP, Gao XQ, Wang K, Chen XZ, Liu J, Xie JY, Zhang DY, Zhou LY, Wang K.** NFATc3-dependent expression of miR-153-3p promotes mitochondrial fragmentation in cardiac hypertrophy by impairing mitofusin-1 expression. *Theranostics*. 2020; 10: 553-66.
34. **Chavali V, Tyagi SC, Mishra PK.** Differential expression of dicer, miRNAs, and inflammatory markers in diabetic Ins2+/- Akita hearts. *Cell Biochem Biophys*. 2014; 68: 25-35.
35. **Savarese G, Lund LH.** Global Public Health Burden of Heart Failure. *Card Fail Rev*. 2017; 3: 7-11.
36. **Seronde MF, Vausort M, Gayat E, Goretti E, Ng LL, Squire IB, Vodovar N, Sadoune M, Samuel JL, Thum T, Solal AC, Laribi S, Plaisance P, Wagner DR, Mebazaa A, Devaux Y, network G.** Circulating microRNAs and Outcome in Patients with Acute Heart Failure. *PLoS One*. 2015; 10: e0142237.
37. **Periasamy M, Kalyanasundaram A.** SERCA pump isoforms: their role in calcium transport and disease. *Muscle Nerve*. 2007; 35: 430-42.
38. **Kawase Y, Ladage D, Hajjar RJ.** Rescuing the failing heart by targeted gene transfer. *J Am Coll Cardiol*. 2011; 57: 1169-80.
39. **Ovchinnikova ES, Schmitter D, Vegter EL, Ter Maaten JM, Valente MA, Liu LC, van der Harst P, Pinto YM, de Boer RA, Meyer S, Teerlink JR, O'Connor CM, Metra M, Davison BA, Bloomfield DM, Cotter G, Cleland JG, Mebazaa A, Laribi S, Givertz MM, Ponikowski P, van der Meer P, van Veldhuisen DJ, Voors AA, Berezikov E.** Signature of circulating microRNAs in patients with acute heart failure. *Eur J Heart Fail*. 2016; 18: 414-23.
40. **Pang PS, Cleland JG, Teerlink JR, Collins SP, Lindsell CJ, Sopko G, Peacock WF, Fonarow GC, Aldeen AZ, Kirk JD, Storrow AB, Tavares M, Mebazaa A, Roland E, Massie BM, Maisel AS, Komajda M,**

Filippatos G, Gheorghiade M, Acute Heart Failure Syndromes International Working G. A proposal to standardize dyspnoea measurement in clinical trials of acute heart failure syndromes: the need for a uniform approach. *Eur Heart J*. 2008; 29: 816-24.

41. **Vegter EL, van der Meer P, de Windt LJ, Pinto YM, Voors AA.** MicroRNAs in heart failure: from biomarker to target for therapy. *Eur J Heart Fail*. 2016; 18: 457-68.
42. **Weckbach LT, Grabmaier U, Clauss S, Wakili R.** MicroRNAs as a diagnostic tool for heart failure and atrial fibrillation. *Curr Opin Pharmacol*. 2016; 27: 24-30.
43. **Gao J, Zhu M, Liu RF, Zhang JS, Xu M.** Cardiac Hypertrophy is Positively Regulated by MicroRNA24 in Rats. *Chin Med J (Engl)*. 2018; 131: 1333-41.
44. **Fiedler J, Jazbutyte V, Kirchmaier BC, Gupta SK, Lorenzen J, Hartmann D, Galuppo P, Kneitz S, Pena JT, Sohn-Lee C, Loyer X, Soutschek J, Brand T, Tuschl T, Heineke J, Martin U, Schulte-Merker S, Ertl G, Engelhardt S, Bauersachs J, Thum T.** MicroRNA-24 regulates vascularity after myocardial infarction. *Circulation*. 2011; 124: 720-30.
45. **Wang L, Qian L.** miR-24 regulates intrinsic apoptosis pathway in mouse cardiomyocytes. *PLoS One*. 2014; 9: e85389.
46. **Li RC, Tao J, Guo YB, Wu HD, Liu RF, Bai Y, Lv ZZ, Luo GZ, Li LL, Wang M, Yang HQ, Gao W, Han QD, Zhang YY, Wang XJ, Xu M, Wang SQ.** In vivo suppression of microRNA-24 prevents the transition toward decompensated hypertrophy in aortic-constricted mice. *Circ Res*. 2013; 112: 601-5.
47. **Xu M, Wu HD, Li RC, Zhang HB, Wang M, Tao J, Feng XH, Guo YB, Li SF, Lai ST, Zhou P, Li LL, Yang HQ, Luo GZ, Bai Y, Xi JJ, Gao W, Han QD, Zhang YY, Wang XJ, Meng X, Wang SQ.** Mir-24 regulates junctophilin-2 expression in cardiomyocytes. *Circ Res*. 2012; 111: 837-41.
48. **Zhang X, Yoon JY, Morley M, McLendon JM, Mapuskar KA, Gutmann R, Mehdi H, Bloom HL, Dudley SC, Ellinor PT, Shalaby AA, Weiss R, Tang WHW, Moravec CS, Singh M, Taylor AL, Yancy CW, Feldman AM, McNamara DM, Irani K, Spitz DR, Breheny P, Margulies KB, London B, Boudreau RL.** A common variant alters SCN5A-miR-24 interaction and associates with heart failure mortality. *J Clin Invest*. 2018; 128: 1154-63.
49. **Danowski N, Manthey I, Jakob HG, Siffert W, Peters J, 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: 125-30.
50. **Duisters RF, Tijsen AJ, Schroen B, Leenders JJ, Lentink V, van der Made I, Herias V, van Leeuwen RE, Schellings MW, Barenbrug P, Maessen JG, Heymans S, Pinto YM, Creemers EE.** miR-133 and miR-30 regulate connective tissue growth factor: implications for a role of microRNAs in myocardial matrix remodeling. *Circ Res*. 2009; 104: 170-8, 6p following 8.
51. **Melman YF, Shah R, Das S.** MicroRNAs in heart failure: is the picture becoming less miRky? *Circ Heart Fail*. 2014; 7: 203-14.
52. **Kumarswamy R, Anker SD, Thum T.** MicroRNAs as circulating biomarkers for heart failure: questions about MiR-423-5p. *Circ Res*. 2010; 106: e8; author reply e9.

53. **Zhao DS, Chen Y, Jiang H, Lu JP, Zhang G, Geng J, Zhang Q, Shen JH, Zhou X, Zhu W, Shan QJ.** Serum miR-210 and miR-30a expressions tend to revert to fetal levels in Chinese adult patients with chronic heart failure. *Cardiovasc Pathol*. 2013; 22: 444-50.
54. **Endo K, Naito Y, Ji X, Nakanishi M, Noguchi T, Goto Y, Nonogi H, Ma X, Weng H, Hirokawa G, Asada T, Kakinoki S, Yamaoka T, Fukushima Y, Iwai N.** MicroRNA 210 as a biomarker for congestive heart failure. *Biol Pharm Bull*. 2013; 36: 48-54.
55. **Joshi S, Wei J, Bishopric NH.** A cardiac myocyte-restricted Lin28/let-7 regulatory axis promotes hypoxia-mediated apoptosis by inducing the AKT signaling suppressor PIK3IP1. *Biochim Biophys Acta*. 2016; 1862: 240-51.
56. **Tolonen AM, Magga J, Szabo Z, Viitala P, Gao E, Moilanen AM, Ohukainen P, Vainio L, Koch WJ, Kerkela R, Ruskoaho H, Serpi R.** Inhibition of Let-7 microRNA attenuates myocardial remodeling and improves cardiac function postinfarction in mice. *Pharmacol Res Perspect*. 2014; 2: e00056.
57. **Dirkx E, Gladka MM, Philippen LE, Armand AS, Kinet V, Leptidis S, El Azzouzi H, Salic K, Bourajjaj M, da Silva GJ, Olieslagers S, van der Nagel R, de Weger R, Bitsch N, Kisters N, Seyen S, Morikawa Y, Chanoine C, Heymans S, Volders PG, Thum T, Dimmeler S, Cserjesi P, Eschenhagen T, da Costa Martins PA, De Windt LJ.** Nfat and miR-25 cooperate to reactivate the transcription factor Hand2 in heart failure. *Nat Cell Biol*. 2013; 15: 1282-93.
58. **Jeong D, Yoo J, Lee P, Kepreotis SV, Lee A, Wahlquist C, Brown BD, Kho C, Mercola M, Hajjar RJ.** miR-25 Tough Decoy Enhances Cardiac Function in Heart Failure. *Mol Ther*. 2018; 26: 718-29.
59. **Yao Y, Song T, Xiong G, Wu Z, Li Q, Xia H, Jiang X.** Combination of peripheral blood mononuclear cell miR-19b-5p, miR- 221, miR-25-5p, and hypertension correlates with an increased heart failure risk in coronary heart disease patients. *Anatol J Cardiol*. 2018; 20: 100-9.
60. **Marques FZ, Vizi D, Khammy O, Mariani JA, Kaye DM.** The transcardiac gradient of cardio-microRNAs in the failing heart. *Eur J Heart Fail*. 2016; 18: 1000-8.

Figures

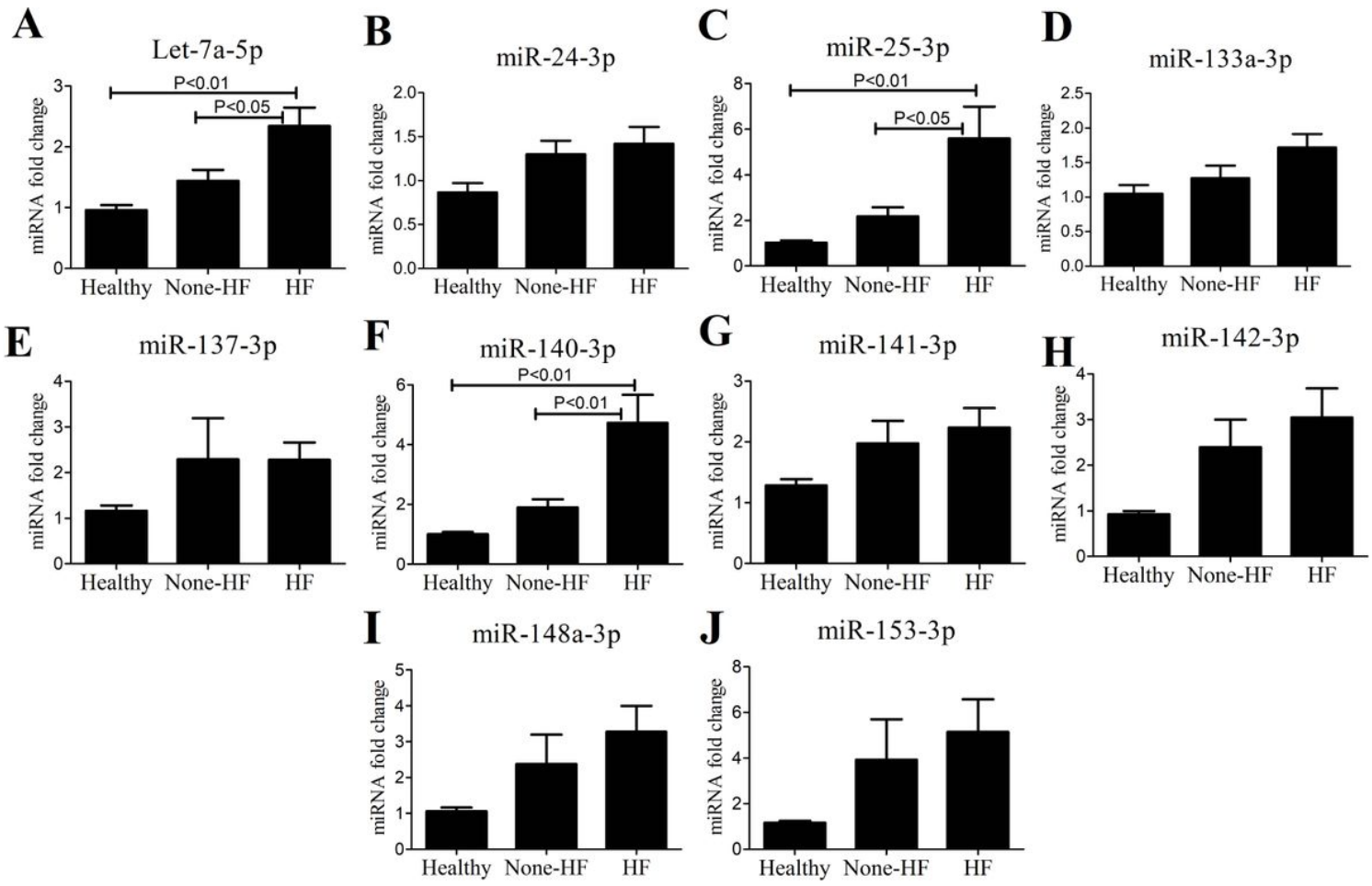

Figure 1

Expression profiling of the candidate miRNAs. Circulating levels of let-7a-5p (A), miR-24-3p (B), miR-25-3p (C), miR-133a-3p (D), miR-137-3p (E), miR-140-3p (F), miR141-3p (G), miR-142-3p (H), miR-148-3p (I) and miR-153-3p (J) in healthy group (Healthy), none-chronic heart failure patients group (none-HF) and chronic heart failure group (HF) of subjects. Data are presented as means ± SEM.

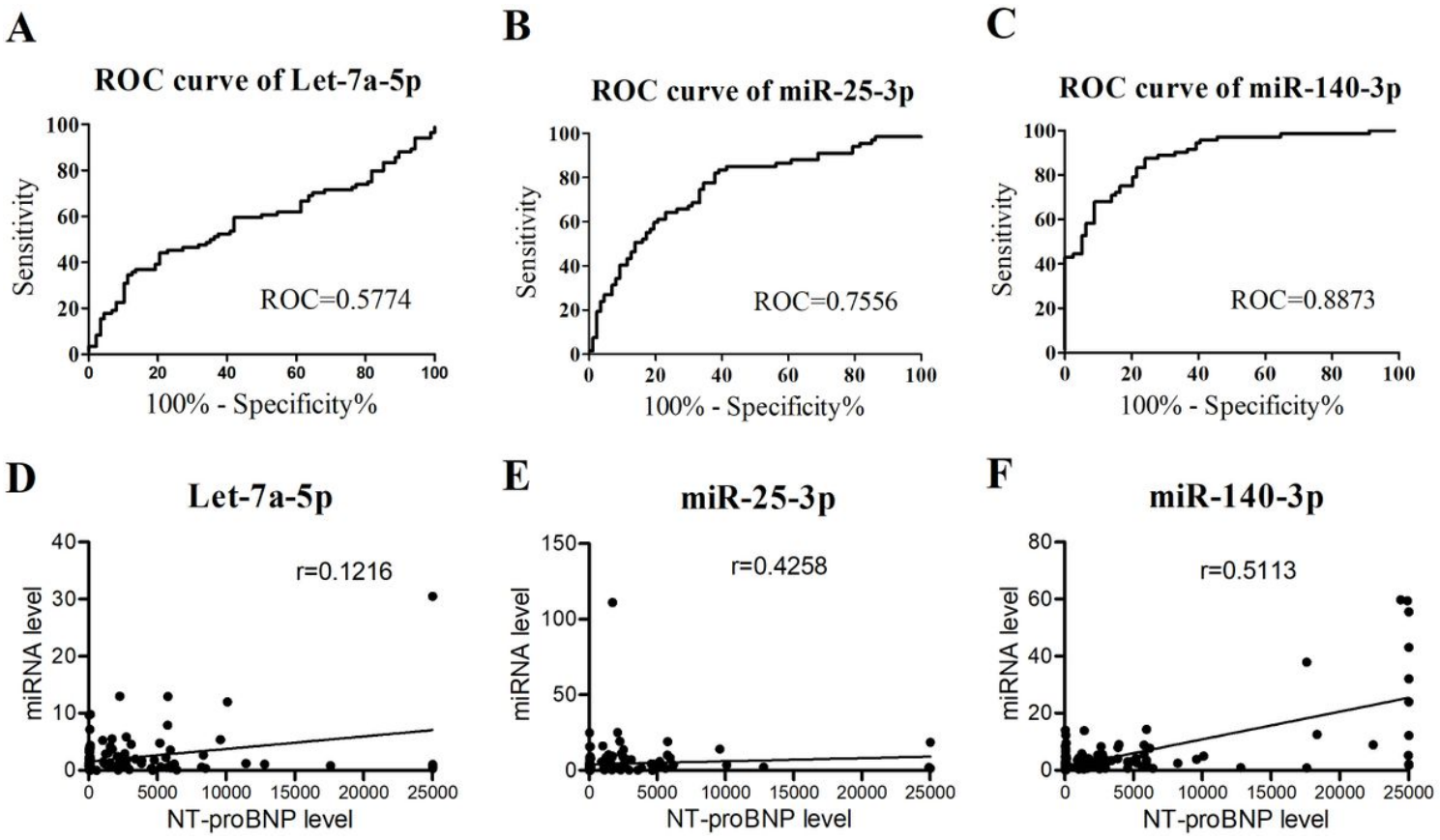

Figure 2

The receiver operating characteristic (ROC) curve analysis and correlations between NT-proBNP and miRNAs levels. The ROC analysis showed among the let-7a-5p (A), miR-25-3p (B) and miR-140-3p (C). The correlation between NT-proBNP and miRNAs levels was observed for let-7a-5p (D), miR-25-3p (E) and miR-140-3p (F).

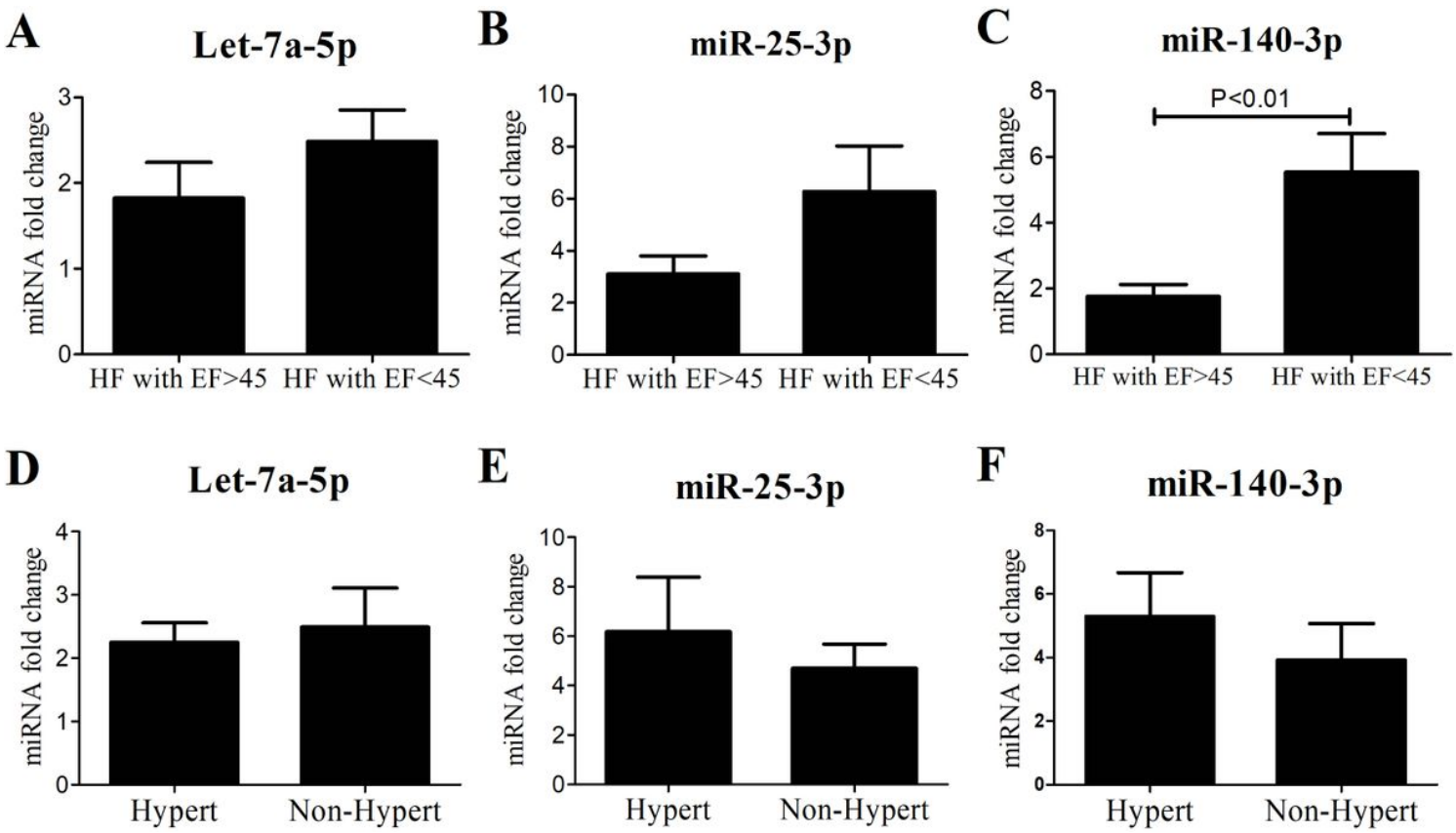

Figure 3

Comparison of miRNAs levels of CHF patients according to their LVEF values and with or without hypertension. The circulation expression of let-7a-5p (D), miR-25-3p (E) and miR-140-3p (F) were respectively shown between patients with a LVEF ≤ 45% (n=53) and those who had a LVEF > 45% (n=31), and the levels of let-7a-5p (D), miR-25-3p (E) and miR-140-3p (F) in CHF patients with or without hypertension. Data are presented as means ± SEM.

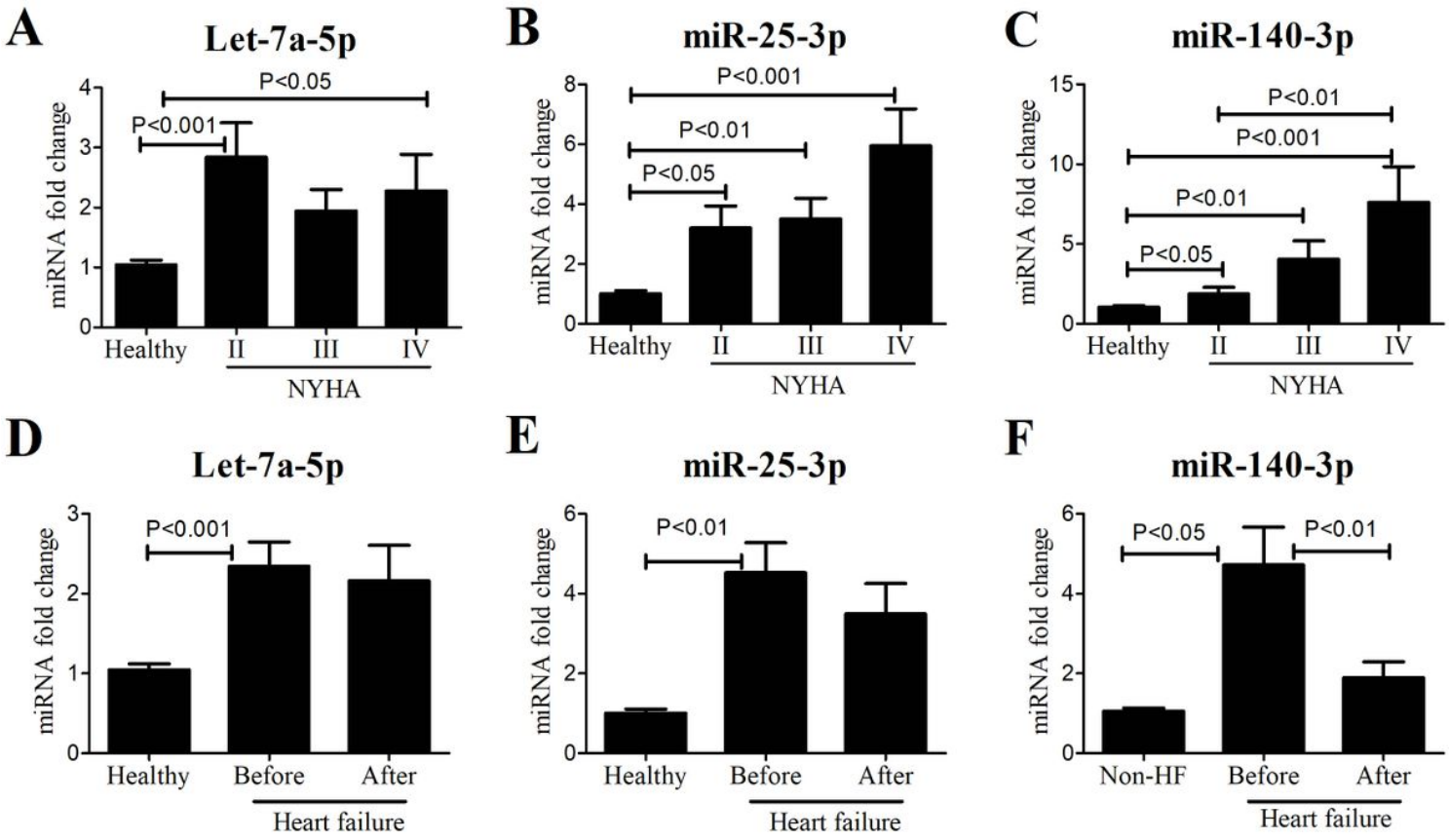

Figure 4

Comparison of miRNAs levels of CHF patients with different NYHA grades, and before or after treatment of NYHA IV CHF patients. CHF patients with NYHA class II (n=27), III (n=28) and IV (n=28) were compared to healthy subjects among let-7a-5p (A), miR-25-3p (B) and miR-140-3p (C). The levels of let-7a-5p (D), miR-25-3p (E), and miR-140-3p (F) of 20 NYHA IV patients, who were treated and with improved heart functions, were compared to healthy subjects in NYHA IV CHF patients before or after treatment. Data are presented as means ± SEM.