

Construction and analysis of the lncRNA-miRNA-mRNA network based on competitive endogenous RNA reveals functional genes in heart failure

GUOHONG WANG¹, XIANGHUI ZHENG^{2,3}, YANG ZHENG^{2,3}, RUI CAO⁴,
MAOMAO ZHANG^{2,3}, YONG SUN^{2,3} and JIAN WU^{2,3}

¹Department of Cardiovascular Center of Beijing Tongren Hospital, Affiliated to Capital Medical University, Beijing 100730; ²Department of Cardiology, The Second Affiliated Hospital of Harbin Medical University;

³The Key Laboratory of Myocardial Ischemia, Harbin Medical University, Ministry of Education;

⁴Cardiovascular Department, The First Hospital of Harbin, Harbin, Heilongjiang 150010, P.R. China

Received April 22, 2018; Accepted November 19, 2018

DOI: 10.3892/mmr.2018.9734

Abstract. Heart failure (HF) is a principal cause of morbidity and mortality worldwide, affecting an estimated 38 million people. Although significant progress has been made with respect to the underlying molecular mechanisms, the role of the competing endogenous RNA (ceRNA) network in the pathogenesis of HF remains largely unknown. In this study, an HF-associated ceRNA network was constructed based on the differentially expressed long noncoding RNAs (lncRNAs), microRNAs (miRNAs) and mRNAs obtained, respectively, from the GSE77399, GSE104150 and GSE84796 datasets. The ceRNA network consisted of 12 lncRNA nodes, 43 miRNA nodes, 343 mRNA nodes and 530 edges. Gene ontology and Kyoto Encyclopedia of Genes and Genomes pathway analysis demonstrated that the ceRNA network was primarily enriched in the immune response, inflammatory response and T cell and B cell receptor signaling pathways. In addition, three lncRNAs (growth arrest specific 5, taurine upregulated 1 and HOX transcript antisense RNA) and three miRNAs [hsa-miRNA (miR)-26b-5p, hsa-miR-8485 and hsa-miR-940] with higher node degrees compared with other genes were selected as hub nodes. The expression of hub nodes in patients with HF was verified by reverse transcription-quantitative polymerase chain

reaction analysis. The present study provided further insights into the important roles of the ceRNA network in HF development, and indicated the potential use of these hub nodes as diagnostic biomarkers and therapeutic targets.

Introduction

Heart failure (HF) is a global public health concern with an estimated prevalence of 38 million patients worldwide (1). HF is the end stage of various vascular diseases, including hypertension, valvular heart disease and myocardial infarction (2), and is commonly caused by ischemic heart disease (3). The heart responds to stress or cardiac insults via a compensatory increase in size and mass, termed cardiac hypertrophy. With the development of heart disease, the structure and metabolism of the heart alters to maintain cardiac function. However, due to the chronic accompanying stress, the ventricles dilate and the condition progresses to HF (4,5). Previous studies have provided novel insights into the pathobiology of HF and the underlying molecular mechanisms (6,7). However, the precise molecular etiology remains unclear, and thus requires investigation in order to identify novel therapeutic targets.

Studies have demonstrated that noncoding RNAs serve crucial roles in numerous diseases (8-10). They are classified into two classes: Small noncoding RNAs [microRNAs (miRNAs), small interfering RNAs and transfer RNAs] and long noncoding RNAs (lncRNAs). miRNAs and lncRNAs regulate each other in various signaling pathways and biological processes. Salmena *et al* (11) proposed the competing endogenous RNA (ceRNA) hypothesis, wherein lncRNAs harboring miRNA response elements compete with one another to bind to a common miRNA, and thereby act as molecular 'sponges' and de-repress the target genes of the miRNAs (11). A ceRNA network has been demonstrated in numerous diseases, particularly in cancer. Chen *et al* (12) identified an upregulation of the lncRNA long intergenic non-protein coding RNA 1234 (LINC01234) axis in gastric cancer tissues, wherein LINC01234 acted as the ceRNA for

Correspondence to: Professor Jian Wu, Department of Cardiology, The Second Affiliated Hospital of Harbin Medical University, 246 Xuefu Road, Nangang, Harbin, Heilongjiang 150010, P.R. China
E-mail: wujian780805@163.com

Abbreviations: HF, heart failure; lncRNAs, long noncoding RNAs; miRNAs, microRNAs; ceRNA, competing endogenous RNA; GEO, Gene Expression Omnibus; DELs, differentially expressed lncRNAs; DEMs, differentially expressed miRNAs; DEMs, differentially expressed mRNAs; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes

Key words: heart failure, miRNA, long noncoding RNA, competing endogenous RNA

miR-204-5p and modulated its endogenous target core-binding factor subunit β . However, no ceRNA networks have been associated with HF thus far.

In the present study, a ceRNA network was constructed using data from the GSE77399, GSE104150 and GSE84796 datasets downloaded from the Gene Expression Omnibus (GEO) database, to identify differentially expressed lncRNAs (DELs), miRNAs (DEMIs) and mRNAs (DEMs) using GEO2R. Subsequently, target lncRNAs and mRNAs of the miRNAs were predicted using the appropriate databases, and the ceRNA network was constructed and analyzed using Cytoscape. A total of six hub nodes were identified, including hsa-miR-26b-5p, hsa-miR-8485, hsa-miR-940, growth arrest specific 5 (GAS5), taurine upregulated 1 (TUG1) and HOX transcript antisense RNA (Hotair). The expression levels of hsa-miR-940, GAS5 and Hotair were increased and those of hsa-miR-26-5p, hsa-miR-8485 and TUG1 were decreased in patients with HF. The present study may not only provide a novel regulatory mechanism between noncoding and coding RNAs in HF, but also deepen the understanding of its development and progression.

Materials and methods

Data collection. The GEO (<https://www.ncbi.nlm.nih.gov/geo/>) is an international, public functional genomics data repository for high-throughput microarray and next-generation sequences (13). The recently published gene expression profiles related to HF (GSE77399, GSE104150 and GSE84796) were downloaded from GEO (<http://www.ncbi.nlm.nih.gov/geo/>). The GSE77399 lncRNA dataset was measured using GPL21384 Platforms (Human Disease-related lncRNA Profiler, System Biosciences, California, USA), and included data from 13 patients with HF and 12 healthy controls. The miRNA expression data in GSE104150 were based on GPL20712 Platforms (Agilent-070156 Human miRNA, miRNA version; Agilent Technologies, Inc., Santa Clara, CA, USA), and consisted of data from nine patients with HF and seven healthy controls. The mRNA expression data in GSE84796 were from 10 patients with HF and seven healthy controls, and were based on GPL14550 (Agilent-028004 SurePrint G3 Human GE 8x60 K Microarray, Probe Name Version; Agilent Technologies, Inc.).

Screening of differentially expressed lncRNAs, miRNAs and mRNAs. The DELs, DEMIs and DEMs between the HF patients and healthy controls were analyzed by GEO2R (<https://www.ncbi.nlm.nih.gov/geo/geo2r/>). The false discovery rate (FDR) is a method of conceptualizing the rate of type I errors in null hypothesis testing when conducting multiple comparisons (14). GEO2R is able to calculate the FDR automatically. The screening thresholds were FDR corrected $P < 0.05$ and $|\log_2(\text{fold-change})| > 1$.

Prediction of target lncRNAs and mRNAs of miRNAs. The lncRNA targets of the miRNAs were predicted using DIANA-LncBase v2 (www.microrna.gr/LncBase), which identifies miRNA and lncRNA interactions derived from manually curated publications and the analysis of 153 AGO CLIP-Seq libraries (15). Data for miRNA and mRNA interactions were

downloaded from miRTarBase Release 7.0 (<http://mirtarbase.mbc.nctu.edu.tw/php/index.php>), which includes data collected via manual survey of the literature following systematic natural language processing of the text to filter research articles, and which are experimentally validated (16).

Construction of the lncRNA-miRNA-mRNA network. Based on the associations between lncRNAs, miRNAs and mRNAs, the ceRNA network was constructed via three steps: i) The upregulated and downregulated RNAs (lncRNAs, miRNAs and mRNAs) were assigned $|\log_2(\text{fold-change})| > 1$ with $P < 0.05$; ii) the lncRNAs and mRNAs that were regulated by a certain common miRNA were selected; and iii) the lncRNA-miRNA-mRNA network was constructed and visualized using Cytoscape version 3.5.1 (<https://cytoscape.org/>), and all node degrees of the ceRNA network were calculated.

Gene Ontology (GO) and pathway enrichment analysis. The GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses of the lncRNA-miRNA-mRNA network were performed using DAVID Bioinformatics Resources 6.8 (<https://david.ncifcrf.gov/tools.jsp>). DAVID provides a comprehensive set of functional annotation tools to determine the biological relevance of a large set of genes (17). $P < 0.05$ was considered statistically significant.

Participants. A total of 10 patients with HF and 10 age- and sex-matched healthy controls were recruited from The Second Affiliated Hospital of Harbin Medical University (Harbin, China) between December 2017 and January 2018. The diagnosis of HF was made according to the criteria listed in the 2017 ACC/AHA/HFSA Focused Update Guideline for the Management of Heart Failure (18). The inclusion criteria for the patients were: A primary diagnosis of chronic heart failure for at least 3 months; New York Heart Association (NYHA) classification I to III; and, > 18 years of age. The diagnosis of HF was made according to the criteria listed in the 2017 ACC/AHA/HFSA Focused Update Guideline for the Management of Heart Failure (12). The exclusion criteria for the patients included: Myocardial infarction within the previous month, unstable angina, uncontrolled hypertension, severe respiratory disease, decompensated non-cardiac disease, malignancy, physical disability and/or mental disease. The protocols of our study were in accordance with principles of the Helsinki Declaration and Good Clinical Practice and were approved by the Ethics Committee of the Second Affiliated Hospital of Harbin Medical University. The clinical characteristics of the study population are summarized in Table I.

Collection and analysis of human blood samples. Fasting blood (~ 5 ml) was collected from each participant and anticoagulated with ethylenediaminetetraacetic acid dipotassium salt. The samples were fractionated immediately via centrifugation at $2,000 \times g$ for 10 min at 4°C , and the plasma was stored at -80°C until use. The total cholesterol (TC), total triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and uric acid (UA) were measured using the Roche enzymatic method (Roche Diagnostics GmbH, Mannheim, Germany).

Table I. Characteristics of study subjects.

Characteristic	Control (n=10)	HF (n=10)	P-value
Age, years	69.20±1.806	67.80±2.480	0.6536
Sex, F/M	6/4	4/6	0.3711
Smoking, n (%)	3 (30%)	3 (30%)	1
Alcohol consumption, n (%)	3 (30%)	4 (40%)	0.6390
TC, mmol/l	4.210±0.5291	3.894±0.3850	0.6350
TG, mmol/l	2.536±0.7613	1.750±0.4679	0.3907
HDL-C, mmol/l	1.121±0.05417	1.154±0.1378	0.8261
LDL-C, mmol/l	2.097±0.2359	2.463±0.3403	0.3884
UA, μ mol/l	309.4±20.76	410.7±45.17	0.0566
NT-proBNP, pg/ml	668.0±369.3	10,139±4,224	0.0384
LVEF, %	61.10±0.7219	42.40±2.561	<0.0001
Hypertension, n (%)	8 (80%)	9 (90%)	0.5313
Diabetes, n (%)	3 (30%)	4 (40%)	0.6390

Values are presented as the mean \pm standard deviation unless stated otherwise. $P<0.05$ was considered indicate a statistically significant difference. TC, total cholesterol; TG, total triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; UA, uric acid; NT-proBNP, N-terminal B-type natriuretic peptide; LVEF, left ventricular ejection fraction; HF, heart failure; F, female; M, male.

N-terminal B-type natriuretic peptide (NT-proBNP) was measured by automated electrochemiluminescence immunoassay method using Elecsys and Cobas analyzers (Roche Diagnostics GmbH).

RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from the plasma samples using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA), as previously described (19). For miRNAs, RT-qPCR was performed using a Hairpin-it microRNA and U6 snRNA Normalization RT-PCR Quantitation kit (Shanghai GenePharma Co., Ltd., Shanghai, China). The conditions for the RT reaction were as follows: 30 min incubation at 25°C, 30 min at 42°C and 5 min at 85°C. The PCR conditions were 40 cycles of 12 sec at 95°C and 40 sec at 62°C. lncRNA-cDNA transcripts were amplified using a Transcriptor First Strand cDNA Synthesis kit (Roche Diagnostics, Basel, Switzerland), according to the manufacturer's protocol. The RT conditions were as follows: 60 min at 50°C and 5 min at 85°C. PCR was performed with Bestar SYBR Green qPCR Master Mix DBI Bioscience (Ludwigshafen, Germany) and the conditions were as follows: 40 cycles of 10 sec at 95°C, 30 sec at 60°C and 30 sec at 72°C. All reactions were performed in triplicate. The expression levels of miRNAs and lncRNAs relative to U6 and β -actin were respectively determined using the $2^{-\Delta\Delta C_q}$ method (19). The primers used are listed in Table II.

Statistical analysis. Categorical data are presented as counts and percentiles, and continuous variables as the mean \pm standard deviation. The data were analyzed using GraphPad Prism 7.0 (GraphPad Software, Inc., La Jolla, CA, USA). A two-tailed Student's t-test was used to evaluate the

differences between two groups. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Screening of DELs, DEMis and DEMs in patients with HF. A total of three gene expression profiles (GSE77399, GSE104150 and GSE84796) were downloaded from the GEO database, and uploaded to GEO2R to screen for the DELs, DEMis and DEMs between HF patients and healthy controls. A total of nine DELs were upregulated and 28 DELs were downregulated in the GSE77399 dataset (Table III). The GSE104150 dataset contained 96 upregulated and 75 downregulated DEMis (data not shown). There were 1,430 upregulated and 835 downregulated DEMs (total, 2,265) in the GSE84796 dataset (data not shown). The differentially expressed transcripts were selected for further analysis.

miRNA predicted target analysis. Target genes of the miRNAs were predicted using experimentally validated databases. The associations between the DELs and DEMis were first assessed using LncBase, which revealed 43 miRNA targets of 12 lncRNAs (Table IV). Subsequently, the associations between these 43 miRNAs and 2,265 DEMs were analyzed using miRTarBase to predict the miRNA targets, and 343 target mRNAs were identified (data not shown). Finally, the 12 co-expressed lncRNAs, 43 co-expressed miRNAs and 343 co-expressed mRNAs were selected to construct the ceRNA network.

lncRNA-miRNA-mRNA network construction. The lncRNA-miRNA-mRNA ceRNA network was constructed using Cytoscape 3.5.1, based on the above data. According to the ceRNA theory, miRNAs negatively regulate the expression

Table II. List of primers for reverse transcription-quantitative polymerase chain reaction analysis.

Name	Sense sequence (5'→3')	Antisense sequence (5'→3')
GAS5	CTTCTGGGCTCAAGTGATCCT	TTGTGCCATGAGACTCCATCAG
TUG1	TAGCAGTTCCCCAATCCTTG	CACAAATTCCTCATTCCTCC
Hotair	GGTAGAAAAAGCAACCACGAAGC	ACATAAACCTCTGTCTGTGAGTGCC
hsa-miR-26b-5p	CGCGGCTTCAAGTAATTCAGG	GTGCAGGGTCCGAGGTATTC
hsa-miR-8485	GCCTCACACACACACACACACGT	GTGCAGGGTCCGAGGT
hsa-miR-940	GCATCGTTCCTTCAAGCCGATCT	TGGGTGAGTCGTTCCGG
U6	GTCCTGGCAGATATACACTAAACAT	CTCACGCTTGAATTCATGCGGCTT
ACTB	CGTGGACATCCGCAAAGA	GAAGGTGGACAGCGAGGC

ACTB, β -actin; GAS5, growth arrest specific 5; TUG1, taurine upregulated 1; Hotair, HOX transcript antisense RNA; miR, microRNA.

Table III. DELs identified from GSE77399.

DELs	Gene names
Upregulated	PCAT-1, GAS5, LINC-ROR, VLDLR-AS1, LINC-RNA-SFMBT2, HOTAIR, EGOT, LOC285194, 7SK
Downregulated	H19, ANRIL, HOXA11AS, KRASP1, SOX2OT, BC017743, LIT, LUST, RMRP, BACE1AS, PCGEM1, SRA, BC043430, GAPDH2, PGK1, PCAT-14, Y4, ZEB2NAT, NCRMS, TUG1, H19-AS, PAR5, BC200, UCA1, Y5, IPW, MALAT1, HAR1B

DEL, differentially expressed long noncoding RNA.

Table IV. miRNAs that may target specific lncRNAs.

lncRNA	miRNAs
EGOT	hsa-miR-183-5p, hsa-miR-491-3p, hsa-miR-586
GAS5	hsa-let-7a-5p, hsa-let-7g-5p, hsa-let-7i-5p, hsa-miR-101-3p, hsa-miR-15a-3p, hsa-miR-23a-3p, hsa-miR-26b-5p, hsa-miR-382-3p, hsa-miR-382-3p, hsa-miR-542-3p, hsa-miR-576-5p, hsa-miR-590-3p, hsa-miR-7f-5p, hsa-miR-876-3p
Hotair	hsa-miR-491-3p, hsa-miR-548ap-3p, hsa-miR-548c-3p, hsa-miR-548f-3p, hsa-miR-548f-3p, hsa-miR-586, hsa-miR-597-5p, hsa-miR-8485
Linc-ROR	hsa-miR-1238-3p, hsa-miR-26b-5p, hsa-miR-302d-5p, hsa-miR-580-3p, hsa-miR-616-3p, hsa-miR-6883-3p, hsa-miR-8485
PCAT1	hsa-miR-548c-3p
VLDLR-AS1	hsa-miR-542-3p, hsa-miR-548f-3p
H19	hsa-miR-483-3p, hsa-miR-423-5p, hsa-miR-30d-5p, hsa-miR-940, hsa-miR-324-3p, hsa-miR-1825, hsa-miR-486-5p
HOXA11-AS	hsa-miR-940
SOX2-OT	hsa-miR-574-5p, hsa-miR-320a, hsa-miR-22-3p
BACE1-AS	hsa-miR-181a-5p
TUG1	hsa-miR-320c, hsa-miR-320a, hsa-miR-486-5p, hsa-miR-181a-5p, hsa-miR-320b, hsa-miR-1825, hsa-miR-1202, hsa-miR-30d-5p, hsa-miR-197-3p, hsa-miR-483-3p, hsa-miR-320d, hsa-miR-574-5p
BCYRN1	hsa-miR-22-3p, hsa-miR-320e

lncRNA, long noncoding RNA; miRNA, microRNA; GAS5, growth arrest specific 5; TUG1, taurine upregulated 1; Hotair, HOX transcript antisense RNA; EGOT, eosinophil granule ontogeny transcript; Linc-ROR, long intergenic non-protein coding RNA, regulator of reprogramming; PCAT1, prostate cancer associated transcript 1; VLDLR-AS1, VLDLR antisense RNA1; H19, H19, imprinted maternally expressed transcript; HOXA11-AS, HOXA11 antisense RNA; SOX2-OT, SOX2 overlapping transcript; BACE1-AS, BACE1 antisense RNA; BCYRN1, brain cytoplasmic RNA 1.

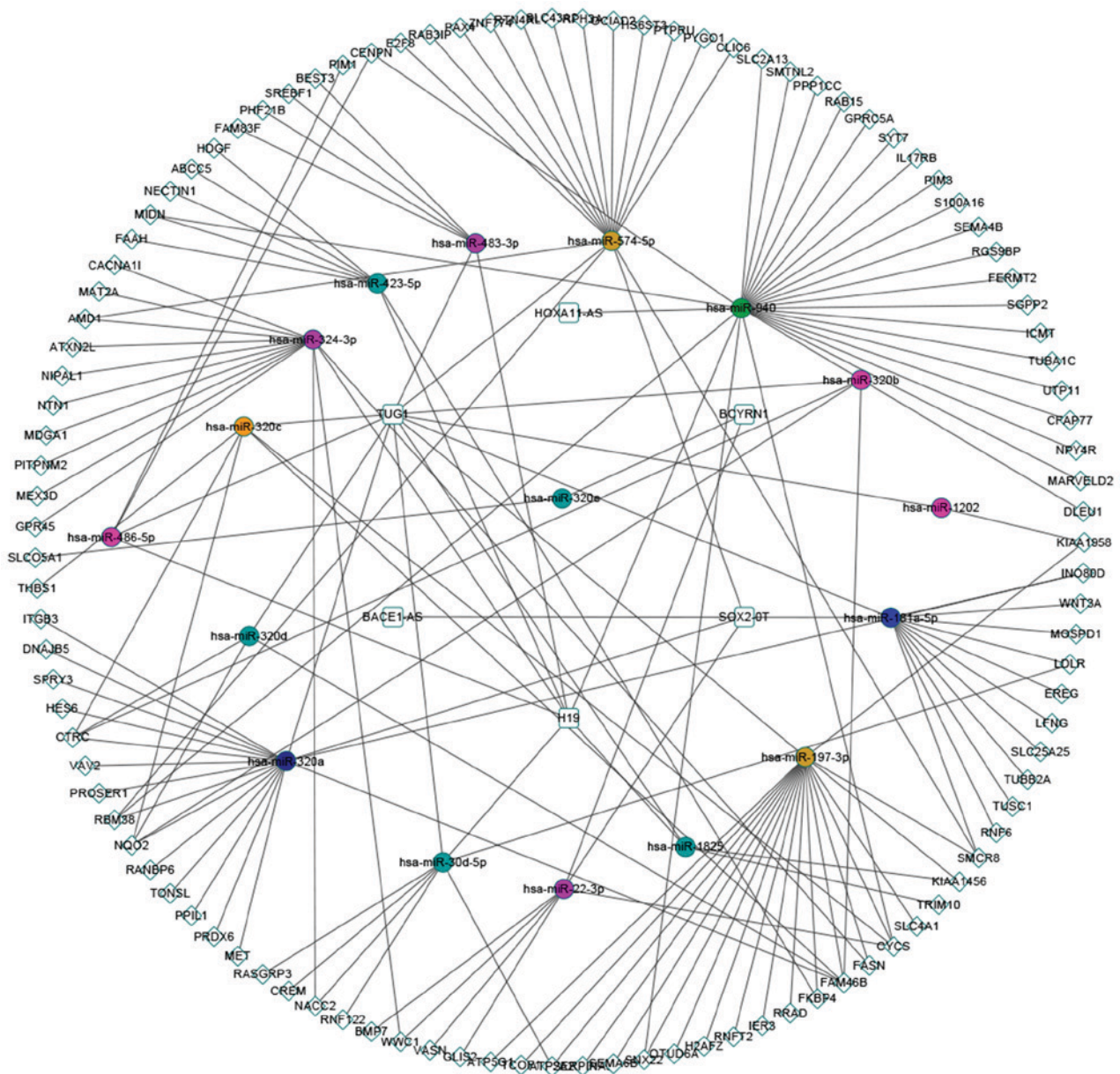


Figure 1. lncRNA-miRNA-mRNA competing endogenous RNA network of upregulated miRNAs. The squares represent lncRNAs, the circles represent miRNAs and the rhombuses represent mRNAs. There were six lncRNA nodes, 17 miRNA nodes and 107 mRNA nodes in the network. miRNA, microRNA; lncRNA, long noncoding RNA.

of lncRNAs and mRNAs. The ceRNA network of upregulated miRNAs is presented in Fig. 1, and consisted of six lncRNA nodes, 17 miRNA nodes, 107 mRNA nodes and 163 edges. The ceRNA network of the downregulated miRNAs (data not shown) included six lncRNA nodes, 26 miRNA nodes, 236 mRNA nodes and 367 edges. The top three ceRNA networks of downregulated miRNAs with high degrees are illustrated in Fig. 2.

Gene ontology enrichment analysis. The function of a ceRNA network depends on the functions of the constituent mRNAs. The biological functions of the DEMs were analyzed using DAVID. GO enrichment analysis of the downregulated miRNA ceRNA network is presented in Fig. 3A. HF is closely associated with immune dysfunction, and numerous GO terms associated with immune responses, including

‘adaptive immune response’, ‘inflammatory response’, ‘immune response’ and ‘neutrophil chemotaxis’ were obtained. The upregulated miRNA ceRNA network was enriched in terms such as ‘protein binding’, ‘negative regulation of axon extension involved in axon guidance’, ‘negative chemotaxis’ and ‘response to peptide hormone’ (Fig. 3B). These results indicated that the ceRNA network was primarily associated with immune responses in HF.

KEGG pathway analysis. The initiation and progression of HF are associated with alterations in a number of biological pathways. Therefore, a pathway enrichment analysis was performed on the ceRNA networks using KEGG. The down-regulated miRNA ceRNA network was primarily enriched in the pathways ‘natural killer cell mediated cytotoxicity’, ‘T cell receptor signaling pathway’ and ‘B cell receptor signaling

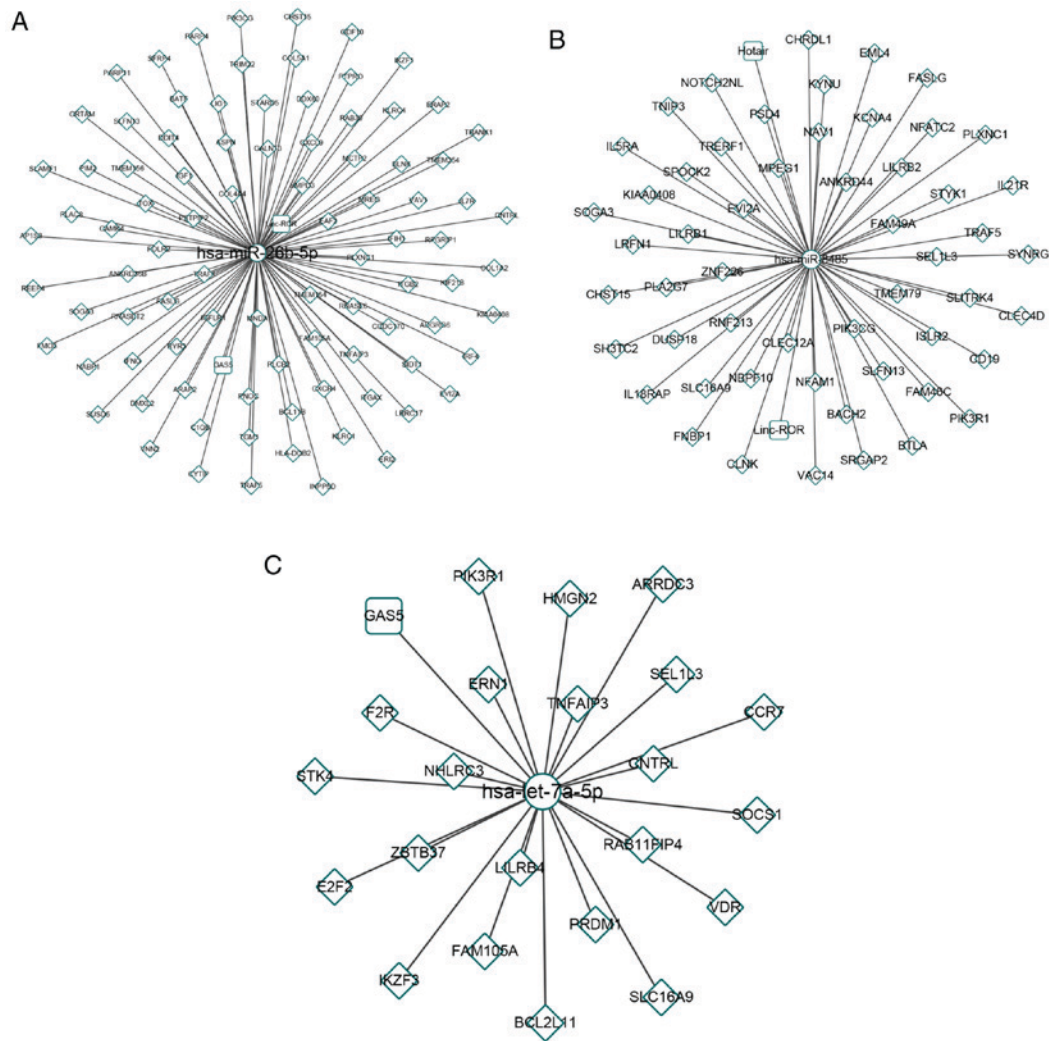


Figure 2. Top three miRNAs ranked by node degree. The sub-networks of (A) hsa-miR-26b-5p, (B) hsa-miR-8485 and (C) hsa-let-7a-5p are presented. The circles represent miRNAs, the squares represent long noncoding RNAs and the rhombuses represent mRNAs. miR/miRNA, microRNA.

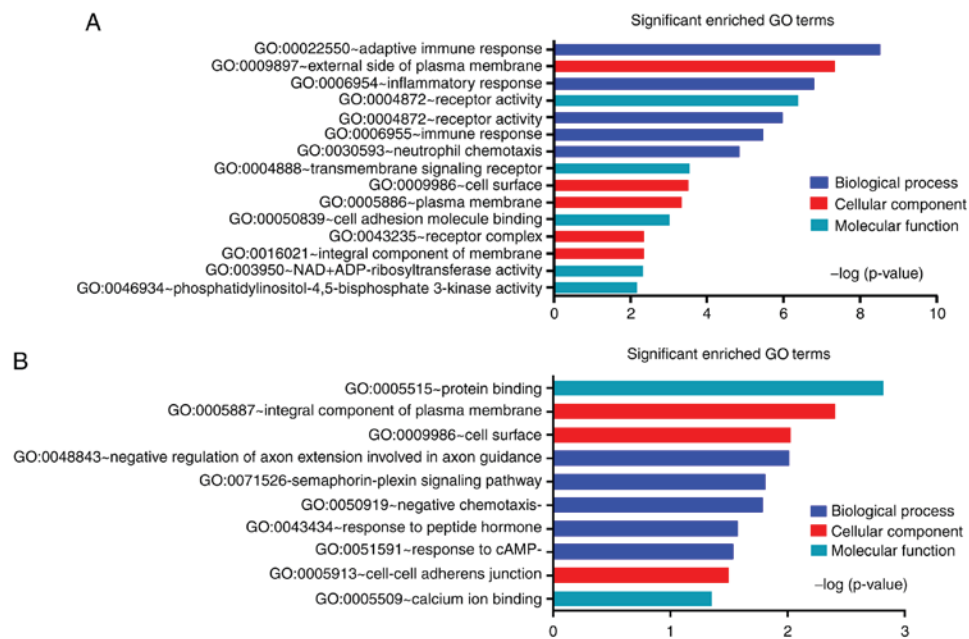


Figure 3. GO enrichment analysis. (A) Downregulated miRNA ceRNA network. (B) Upregulated miRNA ceRNA network. GO analysis classified the differentially expressed genes into three groups, including biological process, cellular component and molecular function. GO, gene ontology; miRNA, microRNA; ceRNA, competing endogenous RNA.

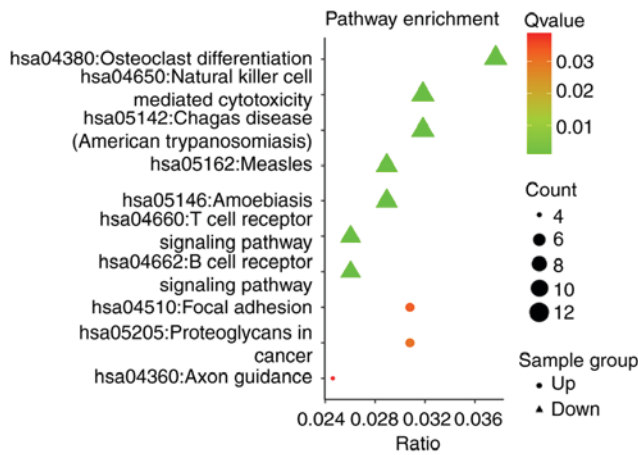


Figure 4. Kyoto Encyclopedia of Genes and Genomes pathway analysis. The triangles represent the downregulated miRNA ceRNA network and the circles represent the upregulated miRNA ceRNA network. miRNA, microRNA; ceRNA, competing endogenous RNA.

pathway' (Fig. 4). The upregulated miRNA ceRNA network was significantly enriched in the 'focal adhesion', 'axon guidance' and 'proteoglycans in cancer' pathways. KEGG analysis revealed that the ceRNA network in HF likely alters the pathways of immune regulation.

Topological analysis of the HF-associated ceRNA network. It is well known that hub nodes serve key roles in biological networks. Therefore, all the node degrees of the ceRNA network were calculated using Cytoscape. It has previously been reported that the degree of a hub node should exceed 5 (20). Based on this criterion, 38 nodes were identified as hub nodes, including five lncRNAs, 31 miRNAs, and two mRNAs (Table V). hsa-miR-26b-5p, hsa-miR-8485 and hsa-miR-940 were the top three hub miRNAs in the ceRNA network (Table V), and GAS5, TUG1 and Hotair had higher degrees compared with the other lncRNAs. These top hub nodes may serve a crucial role in the genesis and development of HF.

Validation of hub nodes in patients with HF. To further validate the importance of the hub nodes in HF, 10 patients with HF and 10 matched controls were enrolled to test for the serum levels of the different transcripts. The characteristics of the study population are presented in Table I. No significant differences were identified in terms of age, gender, smoking, alcohol consumption, TC, TG, HDL-C, LDL-C, UA, hypertension and diabetic status. Previous studies have demonstrated that HF-associated lncRNAs may be detected in heart tissue and plasma (21,22). The patients with HF had higher BNP ($10,139 \pm 4,224$ vs. 668.0 ± 369.3) and lower LVEF (42.40 ± 2.561 vs. 61.10 ± 0.7219) levels compared to the controls. In addition, the levels of hsa-miR-940, GAS5 and Hotair were higher in the patients with HF compared with the controls (Fig. 5). However, the circulating levels of hsa-miR-26-5p, hsa-miR-8485 and TUG1 were lower in patients with HF compared with the matched controls (Fig. 5). Taken together, the expression of the hub nodes (GAS5, TUG1, Hotair, hsa-miR-26b-5p, hsa-miR-8485 and hsa-miR-940) was significantly altered in patients with HF,

Table V. List of differentially expressed genes (node degree >5).

A, miRNA

Gene name	Node degree	Direction of regulation
hsa-miR-26b-5p	92	Down
hsa-miR-8485	56	Down
hsa-miR-940	27	Up
hsa-let-7a-5p	23	Down
hsa-miR-548c-3p	22	Down
hsa-miR-197-3p	20	Up
hsa-miR-320a	18	Up
hsa-miR-574-5p	17	Up
hsa-miR-183-5p	16	Down
hsa-miR-101-3p	14	Down
hsa-miR-324-3p	14	Up
hsa-miR-181a-5p	13	Up
hsa-miR-548f-3p	12	Down
hsa-miR-7f-5p	12	Down
hsa-miR-590-3p	12	Down
hsa-miR-23a-3p	12	Down
hsa-let-7g-5p	11	Down
hsa-let-7i-5p	10	Down
hsa-miR-616-3p	8	Down
hsa-miR-548ap-3p	8	Down
hsa-miR-30d-5p	8	Up
hsa-miR-580-3p	7	Down
hsa-miR-542-3p	7	Down
hsa-miR-491-3p	7	Down
hsa-miR-22-3p	7	Up
hsa-miR-423-5p	7	Up
hsa-miR-302d-5p	6	Down
hsa-miR-1238-3p	6	Down
hsa-miR-586	6	Down
hsa-miR-320c	6	Up
hsa-miR-483-3p	6	Up

B, lncRNA

Gene name	Node degree	Direction of regulation
GAS5	14	Up
TUG1	12	Down
Hotair	8	Up
Linc-ROR	7	Up
H19	7	Down

C, mRNA

Gene name	Node degree	Direction of regulation
HMGN2	6	Up
SLC16A9	6	Up

lncRNA, long non-coding RNA; GAS5, growth arrest specific 5; TUG1, taurine upregulated 1; Hotair, HOX transcript antisense RNA; Linc-ROR, long intergenic non-protein coding RNA, regulator of reprogramming; H19, H19, imprinted maternally expressed transcript; HMGN2, high mobility group nucleosomal binding domain 2; SLC16A9, solute carrier family 16 member 9.

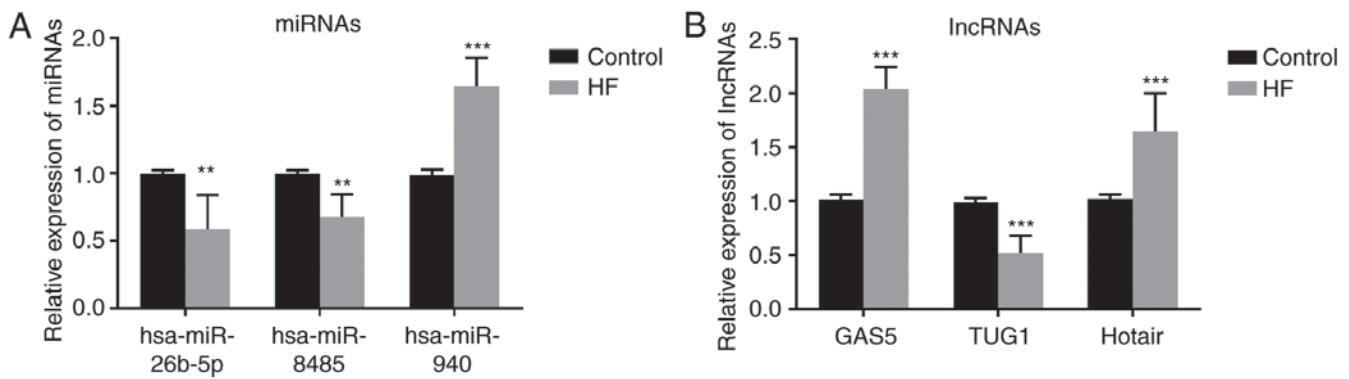


Figure 5. Hub nodes detection by reverse transcription-quantitative polymerase chain reaction. (A) The relative expression of hsa-miR-26b-5p and hsa-miR-8485 was decreased, and hsa-miR-940 was increased, in patients with HF. (B) The relative expression of GAS5 and Hotair was increased and TUG1 was decreased, in patients with HF. The data indicate the mean \pm standard deviation of triplicate samples, from three independent experiments. ** $P < 0.01$, *** $P < 0.001$ vs. respective control group. HF, heart failure; miRNA/miR, microRNA; lncRNA, long noncoding RNA; GAS5, growth arrest specific 5; TUG1, taurine upregulated 1; Hotair, HOX transcript antisense RNA.

and thus they may serve as biomarkers and therapeutic targets for HF. However, due the small size of the cohort, the clinical roles and biological functions of these hub genes require further validation with more experiments.

Discussion

HF is a chronic disease with high morbidity and mortality worldwide, which results in an increased economic burden for patients and their families (23). Although numerous studies have been conducted on the molecular mechanisms of HF, the pathogenesis of HF is not well understood and requires further research (23,24). Previous studies have largely focused on protein-coding genes (25). Recently however, with the development of high-throughput genomic platforms, noncoding RNAs, including miRNAs and lncRNAs, have gained more attention. They serve an important role in the regulation of multiple biological processes, including development, differentiation and metabolism (25,26). ceRNA is a common regulatory mechanism used by miRNAs and lncRNAs (11), and studies have demonstrated its involvement in various pathogenesises, including cancer (27-29). However, the ceRNA network in HF has not been systematically examined. In the present study, HF-associated gene expression datasets were downloaded from the GEO to construct the ceRNA network.

The present study identified the differentially expressed transcripts from GSE77399, GSE104150 and GSE84796, and predicted the target lncRNAs and mRNAs of miRNAs. Although the datasets were from different HF patients, as per previous studies, the differentially expressed genes could be analyzed for constructing an lncRNA-miRNA-mRNA ceRNA network (30,31). The ceRNA network of up-regulated miRNAs consisted of six lncRNA nodes, 17 miRNA nodes, 107 mRNA nodes and 163 edges. The ceRNA network of downregulated miRNAs consisted of six lncRNA nodes, 26 miRNA nodes, 236 mRNA nodes and 367 edges. GO analysis and KEGG pathway analysis demonstrated that the function of the ceRNA network was associated with immune regulation. The most significant biological process categories of GO terms were 'adaptive immune response', 'inflammatory response',

'immune response', 'negative chemotaxis' and 'response to peptide hormone', while the KEGG analysis demonstrated enrichment in 'natural killer cell mediated cytotoxicity', 'T cell receptor signaling pathway', 'B cell receptor signaling pathway' and 'focal adhesion'. These results are consistent with previous studies that have demonstrated that activation of the immune response contributes to the development of HF with reduced or preserved ejection function (32,33). In order to identify the key genes that may be used as potential diagnostic biomarkers and therapeutic targets of HF, the hub nodes were calculated. Hub nodes with high degree of connectivity to other nodes may be used as topological markers of the ceRNA network to evaluate the important genes (34). The top three miRNAs (hsa-miR-26b-5p, hsa-miR-8485 and hsa-miR-940) and three lncRNAs (GAS5, TUG1 and Hotair) were identified as the key hub nodes whose degrees were significantly higher compared with the other nodes. These hub genes were verified in patients with HF by RT-qPCR. Reduced levels of hsa-miR-26b-5p have been associated with increasing severity of HF (35), and a significant correlation was observed between hsa-miR-26b-5p and left ventricular end-systolic pressure and dp/dt_{max} values (36). hsa-miR-940 is enriched in human cardiac tissues (37), and is upregulated in patients with HF in response to acute exercise (38). Therefore, hsa-miR-940 may have beneficial effects in HF. hsa-miR-8485 has recently been demonstrated to bind to TDP-43, although limited knowledge is available as to its role in HF (38).

Cardiac fibrosis is an important characteristic of myocardial structural remodeling in HF (39). GAS5 is expressed at low levels in fibrotic cardiac tissue and serves a suppressive role in HF via negative regulation of miR-21 (40). Hotair is upregulated and may be a biomarker in patients with HF (41). Furthermore, a mechanistic study demonstrated that Hotair modulated phosphatase and tensin homolog expression in HF by competing with miR-19 (42). A previous study demonstrated the important role of TUG1 in coronary heart disease (43). These findings indicate that these miRNAs and lncRNAs may have profound implications for HF, and thus may be considered to be key regulatory genes.

In conclusion, based on the ceRNA hypothesis, a lncRNA-miRNA-mRNA ceRNA network was constructed for

HF. The present findings indicated that the ceRNA networks may serve important roles in the development of HF, and were enriched in immune response functions. hsa-miR-26b-5p, hsa-miR-8485, hsa-miR-940, GAS5, TUG1 and Hotair were identified as the key genes. The present study increased the understanding of the ceRNA-associated regulatory mechanism in HF, and helped to identify potential diagnostic biomarkers or therapeutic targets in HF.

Acknowledgements

The authors would like to thank Dr Yuanyuan Wei (China Pharmaceutical University, Jiangsu, China) for technical support and critical review of the manuscript.

Funding

This study was supported by the National Natural Science Foundation of China (grant nos. 81670373, 81670459 and 81771946).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

GW and XZ conceived and designed the study. YZ and RC performed the experiments. MZ, YS and JW analyzed the data. GW and XZ wrote the paper. YS and JW revised the manuscript and have given final approval of the version to be published.

Ethics approval and consent to participate

Human peripheral blood was isolated from patients with heart failure and healthy volunteers with their written informed consent. The project was approved by the Clinical Research Ethics Committee of The Second Affiliated Hospital of Harbin Medical University (Harbin, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Braunwald E: The war against heart failure: The Lancet lecture. *Lancet* 385: 812-824, 2015.
- Tham YK, Bernardo BC, Ooi JY, Weeks KL and McMullen JR: Pathophysiology of cardiac hypertrophy and heart failure: Signaling pathways and novel therapeutic targets. *Arch Toxicol* 89: 1401-1438, 2015.
- Moran AE, Forouzanfar MH, Roth GA, Mensah GA, Ezzati M, Flaxman A, Murray CJ and Naghavi M: The global burden of ischemic heart disease in 1990 and 2010: The Global burden of disease 2010 study. *Circulation* 129: 1493-1501, 2014.
- Tanai E and Frantz S: Pathophysiology of heart failure. *Compr Physiol* 6: 187-214, 2015.
- Kovács Á, Papp Z and Nagy L: Causes and pathophysiology of heart failure with preserved ejection fraction. *Heart Fail Clin* 10: 389-398, 2014.
- Zhang Y, Bauersachs J and Langer HF: Immune mechanisms in heart failure. *Eur J Heart Fail* 19: 1379-1389, 2017.
- Hulot JS, Ishikawa K and Hajjar RJ: Gene therapy for the treatment of heart failure: Promise postponed. *Eur Heart J* 37: 1651-1658, 2016.
- Xu S, Kamato D, Little PJ, Nakagawa S, Pelisek J and Jin ZG: Targeting epigenetics and non-coding RNAs in atherosclerosis: From mechanisms to therapeutics. *Pharmacol Ther*: Nov 12, 2018 (Epub ahead of print).
- Wong CM, Tsang FH and Ng IO: Non-coding RNAs in hepatocellular carcinoma: Molecular functions and pathological implications. *Nat Rev Gastroenterol Hepatol* 15: 137-151, 2018.
- Sallam T, Sandhu J and Tontonoz P: Long noncoding RNA discovery in cardiovascular disease: Decoding form to function. *Circ Res* 122: 155-166, 2018.
- Salmena L, Poliseno L, Tay Y, Kats L and Pandolfi PP: A ceRNA hypothesis: The rosetta stone of a hidden RNA language? *Cell* 146: 353-358, 2011.
- Chen X, Chen Z, Yu S, Nie F, Yan S, Ma P, Chen Q, Wei C, Fu H, Xu T, *et al*: Long noncoding RNA LINC01234 functions as a competing endogenous RNA to regulate CBFB expression by sponging miR-204-5p in gastric cancer. *Clin Cancer Res* 24: 2002-2014, 2018.
- Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, Marshall KA, Phillippy KH, Sherman PM, Holko M, *et al*: NCBI GEO: Archive for functional genomics data sets-update. *Nucleic Acids Res* 41(Database Issue): D991-D995, 2013.
- Zheng Y, Zheng X, Li S, Zhang H, Liu M, Yang Q, Zhang M, Sun Y, Wu J and Yu B: Identification of key genes and pathways in regulating immune-induced diseases of dendritic cells by bioinformatic analysis. *Mol Med Rep* 17: 7585-7594, 2018.
- Paraskevopoulou MD, Vlachos IS, Karagkouni D, Georgakilas G, Kanellos I, Vergoulis T, Zagganas K, Tsanakas P, Floros E, Dalamagas T and Hatzigeorgiou AG: DIANA-LncBase v2: Indexing microRNA targets on non-coding transcripts. *Nucleic Acids Res* 44 (D1): D231-D238, 2016.
- Chou CH, Shrestha S, Yang CD, Chang NW, Lin YL, Liao KW, Huang WC, Sun TH, Tu SJ, Lee WH, *et al*: miRTarBase update 2018: A resource for experimentally validated microRNA-target interactions. *Nucleic Acids Res* 46 (D1): D296-D302, 2018.
- Huang da W, Sherman BT and Lempicki RA: Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* 4: 44-57, 2009.
- Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE Jr, Colvin MM, Drazner MH, Filippatos GS, Fonarow GC, Givertz MM, *et al*: 2017 ACC/AHA/HFSA focused update of the 2013 ACCF/AHA guideline for the management of heart failure: A report of the American college of cardiology/American heart association task force on clinical practice guidelines and the heart failure society of America. *J Am Coll Cardiol* 70: 776-803, 2017.
- Zhang M, Liu F, Jia H, Zhang Q, Yin L, Liu W, Li H, Yu B and Wu J: Inhibition of microRNA let-7i depresses maturation and functional state of dendritic cells in response to lipopolysaccharide stimulation via targeting suppressor of cytokine signaling 1. *J Immunol* 187: 1674-1683, 2011.
- Han JD, Bertin N, Hao T, Goldberg DS, Berriz GF, Zhang LV, Dupuy D, Walhout AJ, Cusick ME, Roth FP and Vidal M: Evidence for dynamically organized modularity in the yeast protein-protein interaction network. *Nature* 430: 88-93, 2004.
- Gao L, Liu Y, Guo S, Yao R, Wu L, Xiao L, Wang Z, Liu Y and Zhang Y: Circulating long noncoding RNA HOTAIR is an essential mediator of acute myocardial infarction. *Cell Physiol Biochem* 44: 1497-1508, 2017.
- Greco S, Zaccagnini G, Fuschi P, Voellenkle C, Carrara M, Sadeghi I, Bearzi C, Maimone B, Castelvechio S, Stellos K, *et al*: Increased BACE1-AS long noncoding RNA and β -amyloid levels in heart failure. *Cardiovasc Res* 113: 453-463, 2017.
- O'Connor CM, Psotka MA, Fiuzat M, Lindenfeld J, Abraham WT, Bristow MR, Canos D, Harrington RA, Hillebrener M, Jessup M, *et al*: Improving heart failure therapeutics development in the United States: The heart failure collaboratory. *J Am Coll Cardiol* 71: 443-453, 2018.
- Pearson MJ, Mungovan SF and Smart NA: Effect of aerobic and resistance training on inflammatory markers in heart failure patients: Systematic review and meta-analysis. *Heart Fail Rev* 23: 209-223, 2018.

25. Wang KC and Chang HY: Molecular mechanisms of long noncoding RNAs. *Mol Cell* 43: 904-914, 2011.
26. Ulitsky I and Bartel DP: lincRNAs: Genomics, evolution, and mechanisms. *Cell* 154: 26-46, 2013.
27. Shuwen H, Qing Z, Yan Z and Xi Y: Competitive endogenous RNA in colorectal cancer: A systematic review. *Gene* 645: 157-162, 2018.
28. Yang C, Wu D, Gao L, Liu X, Jin Y, Wang D, Wang T and Li X: Competing endogenous RNA networks in human cancer: Hypothesis, validation, and perspectives. *Oncotarget* 7: 13479-13490, 2016.
29. Qi X, Zhang DH, Wu N, Xiao JH, Wang X and Ma W: ceRNA in cancer: Possible functions and clinical implications. *J Med Genet* 52: 710-718, 2015.
30. Jiang H, Ma R, Zou S, Wang Y, Li Z and Li W: Reconstruction and analysis of the lncRNA-miRNA-mRNA network based on competitive endogenous RNA reveal functional lncRNAs in rheumatoid arthritis. *Mol Biosyst* 13: 1182-1192, 2017.
31. Pang L, Hu J, Zhang G, Li X, Zhang X, Yu F, Lan Y, Xu J, Pang B, Han D, *et al*: Dysregulated long intergenic non-coding RNA modules contribute to heart failure. *Oncotarget* 7: 59676-59690, 2016.
32. Dick SA and Epelman S: Chronic heart failure and inflammation: What do we really know? *Circ Res* 119: 159-176, 2016.
33. Fildes JE, Shaw SM, Yonan N and Williams SG: The immune system and chronic heart failure: Is the heart in control? *J Am Coll Cardiol* 53: 1013-1020, 2009.
34. Browne AL, Charmsaz S, Varešlija D, Fagan A, Cosgrove N, Cocchiglia S, Purcell S, Ward E, Bane F, Hudson L, *et al*: Network analysis of SRC-1 reveals a novel transcription factor hub which regulates endocrine resistant breast cancer. *Oncogene* 37: 2008-2021, 2018.
35. Ovchinnikova ES, Schmitter D, Vegter EL, Ter Maaten JM, Valente MA, Liu LC, van der Harst P, Pinto YM, de Boer RA, Meyer S, *et al*: Signature of circulating microRNAs in patients with acute heart failure. *Eur J Heart Fail* 18: 414-423, 2016.
36. Vegter EL, Ovchinnikova ES, Silljé HHW, Meems LMG, van der Pol A, van der Velde AR, Berezikov E, Voors AA, de Boer RA and van der Meer P: Rodent heart failure models do not reflect the human circulating microRNA signature in heart failure. *PLoS One* 12: e0177242, 2017.
37. Liang D, Xu X, Deng F, Feng J, Zhang H, Liu Y, Zhang Y, Pan L, Liu Y, Zhang D, *et al*: miRNA-940 reduction contributes to human tetralogy of fallot development. *J Cell Mol Med* 18: 1830-1839, 2014.
38. Xu T, Zhou Q, Che L, Das S, Wang L, Jiang J, Li G, Xu J, Yao J, Wang H, *et al*: Circulating miR-21, miR-378, and miR-940 increase in response to an acute exhaustive exercise in chronic heart failure patients. *Oncotarget* 7: 12414-12425, 2016.
39. Nevers T, Salvador AM, Velazquez F, Ngwenyama N, Carrillo-Salinas FJ, Aronovitz M, Blanton RM and Alcaide P: Th1 effector T cells selectively orchestrate cardiac fibrosis in nonischemic heart failure. *J Exp Med* 214: 3311-3329, 2017.
40. Tao H, Zhang JG, Qin RH, Dai C, Shi P, Yang JJ, Deng ZY and Shi KH: LncRNA GAS5 controls cardiac fibroblast activation and fibrosis by targeting miR-21 via PTEN/MMP-2 signaling pathway. *Toxicology* 386: 11-18, 2017.
41. Greco S, Zaccagnini G, Perfetti A, Fuschi P, Valaperta R, Voellenkle C, Castelveccchio S, Gaetano C, Finato N, Beltrami AP, *et al*: Long noncoding RNA dysregulation in ischemic heart failure. *J Transl Med* 14: 183, 2016.
42. Lai Y, He S, Ma L, Lin H, Ren B, Ma J, Zhu X and Zhuang S: HOTAIR functions as a competing endogenous RNA to regulate PTEN expression by inhibiting miR-19 in cardiac hypertrophy. *Mol Cell Biochem* 432: 179-187, 2017.
43. Wu Z, Zhao S, Li C and Liu C: LncRNA TUG1 serves an important role in hypoxia-induced myocardial cell injury by regulating the miR-145-5p-Binp3 axis. *Mol Med Rep* 17: 2422-2430, 2018.



This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.