

Identification of a lncRNA-miRNA-mRNA network based on competitive endogenous RNA theory reveals functional lncRNAs in hypertrophic cardiomyopathy

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Abstract. Hypertrophic cardiomyopathy (HCM) is an autosomal dominant disease that affects 1 in every 200 people in the general population, leading to cardiac ischemia, heart failure and increased risk of sudden death. Recently, accumulating evidence has suggested that long noncoding RNAs (lncRNAs) may serve specific roles in various biological processes and participate in the pathology of various diseases, including HCM. Although a large number of lncRNAs have been detected, the functions of lncRNAs in HCM are still unknown. In the present study, a global triple network based on competitive endogenous RNA (ceRNA) theory was constructed using data from the National Center for Biotechnology Information Gene Expression Omnibus. Furthermore, Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway analyses of mRNAs in the lncRNA-microRNA (miRNA)-mRNA network were performed using the Cytoscape plugins, BiNGO and Database. The lncRNA-miRNA-mRNA network was composed of 30 lncRNA nodes, 94 mRNA nodes and 8 miRNA nodes. Subsequently, hub nodes and the number of relationship pairs were analyzed and showed that 5 lncRNAs (ENST00000597346.1, ENST00000458178.1, ENST00000544461.1, ENST00000567093.1 and ENST00000571219.1) were closely related to HCM. Cluster module analysis and Random Walk with Restart of the ceRNA

network further confirmed the potential role of two lncRNAs (ENST00000458178.1 and ENST00000567093.1) in HCM. The present study provides a new strategy for identifying potential pathways associated with HCM or other diseases. Furthermore, lncRNA-miRNA pairs may be regarded as candidate diagnostic biomarkers or potential therapeutic targets for HCM.

Introduction

Hypertrophic cardiomyopathy (HCM) is one of the most common genetic heart disorders with a prevalence of 1 in 200 worldwide (0.6% of the general population) (1). HCM is characterized by increased thickness of the ventricular wall. Over 1,500 mutations in at least 11 genes, encoding components of the cardiac sarcomere-associated proteins, have been identified as potential cause for HCM (2-4). *MYH7* and *MYBPC3*, encoding β -myosin heavy chain and myosin-binding protein C, respectively, are the 2 most common genes involved, accounting for 50-60% of the HCM families. However, in about 40% of HCM patients, the causal genes remain to be identified, highlighting the need for precision medicine as genetic diversity of HCM-associated individuals (3,4). The clinical outcomes of HCM are diverse, ranging from no symptoms to cardiac ischemia, cardiac arrhythmia, congestive heart failure and other organ system dysfunctions (5). HCM is also associated with an increased risk of sudden death, heart failure and thromboembolic events, which results in an annual mortality rate of 1% worldwide (6).

A challenging characteristic of diagnosing HCM is the lack of association between genotype and phenotype, as family members carrying the same mutation develop different symptoms (7). Therefore, HCM is regarded as a highly complex disease due to its diverse clinical presentations, heterogeneous phenotypes, large number of mutations and broad spectrum of complications (4,7). The development of HCM likely results from a combination of endogenous genetic mutations with an exogenous decline in protein-protective mechanisms, and environmental factors such as lifestyle, blood pressure and physical exercise (8). In this regard, it is crucial to identify transcriptional profiles, epigenetic modifications and post-translational modifications which may initiate the onset of HCM.

In the last decade, accumulating evidence has indicated that rather than being transcriptional noise, diverse noncoding

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RNAs (ncRNAs) act as important regulators of HCM initiation and progression at the post-transcriptional level (9-15). To date, studies have primarily focused on small, noncoding endogenous RNAs (9,10). MicroRNAs (miRNAs) are 22-25 nucleotides in length and regulate gene expression by directly targeting mRNAs for degradation or translational repression (10). Several miRNAs, such as miR-21, miR-1, miR-29a, miR-133a and miR-130b have been described as important regulators of HCM in murine and human hearts (9,11-14). Furthermore, these miRNAs can be detected in the bloodstream, highlighting the possibility of their use as circulating biomarkers of HCM (11,14,15). However, the role of miRNAs in the progression of HCM remains to be elucidated.

In addition to miRNAs, long noncoding RNAs (lncRNAs) are known as important regulators of cardiac pathology (16,17). lncRNAs are transcripts >200 nucleotides in length with no protein-coding capacity. Based on their diverse biochemical roles, lncRNAs can perform their functions via RNA-DNA, RNA-RNA or RNA-protein interactions (18). Notably, lncRNAs have been reported to competitively interact with miRNAs and thus inhibit target mRNA degradation by a competitive endogenous RNA (ceRNA) regulatory mechanism (19,20). However, little is known regarding the function of lncRNAs in HCM, particularly in human hearts. Current studies of lncRNAs in HCM primarily describe expression profiling by RNA sequencing or microarray technology (21,22). However, the underlying mechanisms involving miRNAs or mRNAs are still unclear. Therefore, considering the large number lncRNAs expressed in the heart and limited knowledge of their function physiologically and pathophysiologically, it is hypothesized that functionally-related lncRNAs are functionally associated with mRNAs or miRNAs. Similar associations have been detected in several diseases such as ischemic cardiomyopathy (23), diabetic cardiomyopathy (24), and heart failure (25), but have not yet been revealed in HCM.

In the present study, a triple network was constructed using human data from the National Center for Biotechnology Information Gene Expression Omnibus (NCBI GEO). Based on the ceRNA mechanism in which lncRNA, miRNA and mRNA form a triplet, where the lncRNA and mRNA share the same miRNA. The present study constructed a lncRNA-miRNA-mRNA network with high reliability, providing a potentially novel understanding of the mechanisms of the development of HCM and potential therapeutic targets.

Materials and methods

Raw data. NCBI GEO (ncbi.nlm.nih.gov/geo/) is a publicly available genomics database containing data obtained from array- and sequence-based analysis. Users can query and download experimental data and curated gene expression profiles from NCBI-GEO. In the present study, human lncRNA and mRNA expression data were downloaded from dataset GSE68316 in myocardial tissues (21), and human miRNA expression data were downloaded from GSE36946 of surgical myectomy tissues. The age of patients corresponding to the heart samples in GSE68316 ranged from 31-60 years, whereas the age range of the patients in GSE36946 was 9-78 years; therefore, data for heart samples corresponding to patients aged from 31-60 years in GSE36946 were selected. Subsequently, the two datasets were

merged to construct a lncRNA-miRNA-mRNA network based on the ceRNA theory. The experimental design and flowchart of the steps performed in the present study are presented in Fig. 1.

Screening of differentially expressed lncRNAs, miRNAs and mRNAs. To detect differentially expressed lncRNAs, miRNAs and mRNAs between patients with HCM and healthy donors, two-class differential analyses was used. The threshold of dysregulated lncRNAs/mRNAs/miRNAs was a fold change of >2. Student's t-test was performed using SPSS 23.0 (IBM Corp.). $P < 0.05$ was considered to indicate a statistically significant difference.

Prediction of target lncRNAs and mRNAs of miRNAs. miRNA sequences were downloaded from miRBase (mirbase.org/) and lncRNA sequences were downloaded from the NCBI nucleotide database (ncbi.nlm.nih.gov/nucleotide/). The miRNA targets of lncRNAs were predicted and the minimum free energy of lncRNA-miRNA duplexes was calculated using RNAhybrid (bibiserv.cebitec.uni-bielefeld.de/rnahybrid) (26). miRNA-mRNA interactions were predicted using miRanda (<http://www.microrna.org/>) and TargetScan (targetscan.org/vert_72/).

Construction of a lncRNA-miRNA-mRNA network. Pearson's correlation coefficient was used to calculate the correlation between lncRNA and mRNA expression. The pairs of lncRNA-mRNA with r score >0.99 and P score <0.05 were regarded as target pairs. Among all the selected lncRNA-mRNA pairs, if the lncRNA and mRNA were both targeted and were sharing a common miRNA, the lncRNA-miRNA-mRNA ceRNA network was identified as a co-expression competing triplet. Cytoscape software (3.7.1) (27) was used to visualize the lncRNA-miRNA-mRNA network.

Functional enrichment analysis. To study functional enrichment, Gene Ontology (GO; geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG; genome.jp/kegg/) pathway analyses of mRNAs in the lncRNA-miRNA-mRNA network were performed using the Cytoscape plugins BiNGO and Database (<http://apps.cytoscape.org>). The GO database provides gene ontologies, annotations of genes and gene products based on terms. KEGG is a relational database comprising searchable molecular interaction pathways and reaction networks in metabolism, various cellular processes and multiple human diseases.

Reconstruction of a key lncRNA-miRNA-mRNA subnetwork. Cytoscape software was used to construct a new subnetwork extracted from all lncRNAs and their related miRNAs and mRNAs in the triple network. The target lncRNAs were identified by calculating the number and node of related lncRNA-miRNA-mRNA triplets,

Results

Screening for differentially expressed lncRNAs, miRNAs and mRNAs. Human lncRNA/mRNA expression data were obtained from GEO dataset GSE68316 and human miRNA expression data were downloaded from GEO dataset

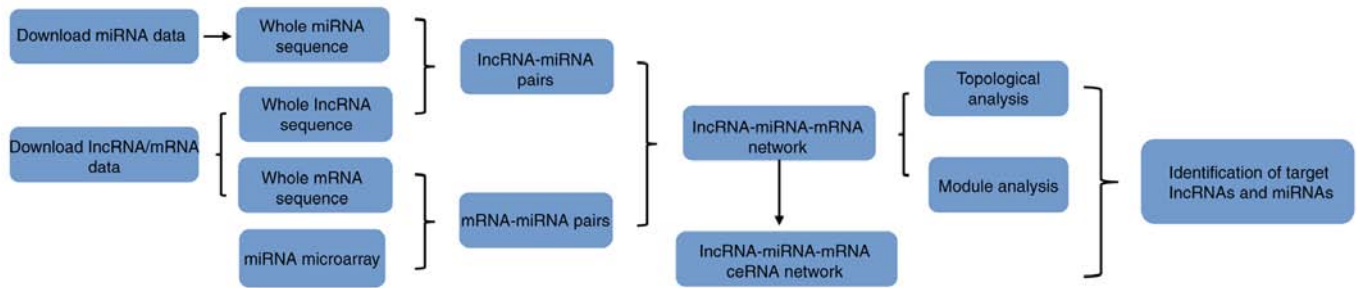


Figure 1. Schematic depiction of the experimental design and flowchart of the steps performed in the present study.

GSE36946. lncRNAs, mRNAs and miRNAs were considered as significantly differentially expressed when the fold change was >2 with $P < 0.05$. A total of 8 miRNAs (Table I), 431 lncRNAs and 609 mRNAs were selected for subsequent analyses. Considering the specific description of miRNAs in different microarrays, the names of selected miRNAs were redefined according to miRBase (Table I). Subsequently, the two datasets were merged to construct a ceRNA based lncRNA-miRNA-mRNA network.

ceRNA based lncRNA-miRNA-mRNA network. lncRNA-miRNA and miRNA-mRNA pairs were identified according to both base sequence and expression levels. A total of 181 miRNA-lncRNA pairs and 421 miRNA-mRNA pairs were predicted based on the intersecting elements (Fig. 2A and B). In addition, 442 lncRNA-mRNA pairs were further selected according to expression levels and ceRNA score (Fig. 2C). Finally, a network composed of 30 lncRNAs and 94 mRNAs (Fig. 2D), and a ceRNA network composed of 30 lncRNAs, 94 mRNAs and 8 miRNAs were constructed (Fig. 2E).

Topological analysis of the HCM-related lncRNA-miRNA-mRNA network. Hub nodes play important roles in biological networks. The topological properties of the HCM-related ceRNA network composed of lncRNA-miRNA-mRNA were first analyzed. The degree (the level of a gene related to its surroundings, where genes with a higher degree is more likely to be the core information exchange of the network), closeness (the length of information transferred from one node to the other) and betweenness (the function and influence of the corresponding gene in the whole network, where the larger the value is, the more capable of the gene is to participate in the communication between other genes) of the network were calculated and the topological features of all the nodes in the network were ranked. The top 20 genes in the network were listed in Table II. Interestingly, 5 lncRNAs appeared in the list. The number of primary lncRNA-miRNA pairs and secondary miRNA-mRNA pairs were calculated as presented in Table III. Among the top 10 lncRNA-miRNA pairs, the 5 lncRNAs identified in the ceRNA network were determined. Therefore, these 5 lncRNAs (ENST00000597346.1, ENST00000458178.1, ENST00000544461.1, ENST00000567093.1 and ENST00000571219.1) not only had higher betweenness and node degree but were also involved in a higher number of lncRNA-miRNA and miRNA-mRNA pairs, which suggested that these 5 lncRNAs may serve a role in the initiation and/or development of HCM.

Key lncRNA-miRNA-mRNA subnetworks. The 5 key lncRNAs in the ceRNA network were further investigated. The 5 lncRNAs were found to primarily target miR-10a-5p, miR-30c-3p, miR-1247-5p, miR-1268a and miR-144-5p (Table IV). The mRNAs and miRNAs associated with these 5 lncRNAs were identified in the global triple network and new subnetworks were constructed (Table IV). Among the 5 lncRNAs, sequence Basic Local Alignment Search Tool (BLAST) analysis showed that the sequence of lncRNA ENST00000597346.1 is part of ENST00000567093.1, and the sequence of ENST00000544461.1 is also part of ENST00000458178.1. ENST00000567093.1 and ENST00000458178.1 were conserved among human, mouse and rat genomes, whereas ENST00000571219.1 was only detected in the human genome. GO function and KEGG pathway analyses for the lncRNA-related mRNAs were then performed. For ENST00000458178.1, differentially expressed mRNAs were primarily enriched in 'Nucleobase-containing small molecule interconversion', 'Nucleobase-containing small molecule metabolic process' and 'Regulation of lipid metabolic process' (Fig. 3A). Enriched KEGG pathways included the 'AMPK signaling pathway', 'Pantothenate and CoA biosynthesis' and 'Arginine biosynthesis' (Fig. 3B), which shared a similar trend with ENST00000567093.1 (Fig. 3D and E). The subnetworks of ENST00000458178.1 and ENST00000567093.1 are presented in Fig. 3C and F, respectively. The biological processes, KEGG pathways and subnetwork of ENST00000571219.1-related mRNAs are presented in Fig. S1A-C. The HCM pathway was enriched in all 5 lncRNA-related KEGG pathways, indicating the potential roles of these 5 lncRNAs in HCM (Fig. 3B and E; Fig. S1B).

Module analysis of a HCM-related lncRNA-miRNA network. To further investigate the crosstalk between mRNAs and lncRNAs, a bidirectional hierarchical clustering analysis was performed using gplot in R software (version 3.5.1; <http://www.r-project.org/>). The heat map showed two modules screened by cluster analysis that were closely related to HCM (Fig. 4A-C). The two modules included two different gene populations. GO enrichment analysis and KEGG analysis of genes were performed in the modules (Fig. 4D-G). In module 1, 'Positive regulation of adenylate cyclase activity involved in G-protein-coupled receptor signaling pathways (GO: 0010579)' was significantly and closely related to HCM. KEGG pathway analysis further demonstrated that the 'AMPK signaling pathway' was the most significant signaling pathway in HCM. In module 2, 'Translation' had the most notable relationship with

Table I. Selected differently expressed miRNAs in samples of hypertrophic cardiomyopathy patients and healthy controls.

miRNA	Redefined name	P-value	Fold-change	Expression
hsa-miR-514	hsa-miR-514a-3p	1.72x10 ⁻⁶	3.069	Upregulated
hsa-miR-373	hsa-miR-373-3p	2.33x10 ⁻⁶	2.577	Upregulated
hsa-miR-30c-1 ^a	hsa-miR-30c-3p	2.49x10 ⁻³	2.451	Downregulated
hsa-miR-144 ^a	hsa-miR-144-5p	3.38x10 ⁻⁶	2.398	Downregulated
hsa-miR-1247	hsa-miR-1247-5p	1.80x10 ⁻³	2.290	Downregulated
hsa-miR-10a ^a	hsa-miR-10a-3p	6.63x10 ⁻⁶	2.286	Downregulated
hsa-miR-10a	hsa-miR-10a-5p	3.18x10 ⁻⁶	2.101	Downregulated
hsa-miR-1268	hsa-miR-1268a	2.30x10 ⁻⁶	2.096	Downregulated

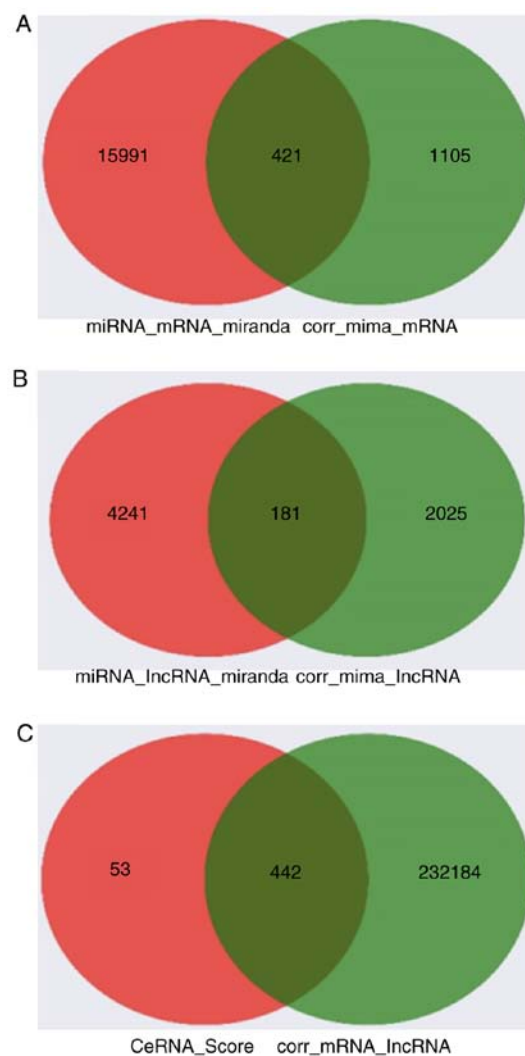
^aOriginal miRNA name.

Figure 2. View of the lncRNA-miRNA-mRNA network. (A) A total of 181 miRNA-lncRNA pairs and (B) 421 miRNA-mRNA pairs were identified according to both base sequence and expression level. (C) A total of 442 lncRNA-mRNA pairs were selected according to the ceRNA score and expression level. lncRNA, long non-coding RNA; miRNA, microRNA; ceRNA, competing endogenous RNA.

HCM, and KEGG pathway analysis showed that ‘Ribosome’ served a predominant role in HCM (Fig. 4D-G). Among all the lncRNAs in the two modules, 8 lncRNAs were included in the ceRNA network (ENST00000499521.2, ENST00000458178.1,

ENST00000567093.1, ENST00000591866.1, ENST00000367207.3, ENST00000425185.1, ENST00000596350.1 and ENST00000416301.1). According to the results of the current study, ENST00000458178.1 and

E

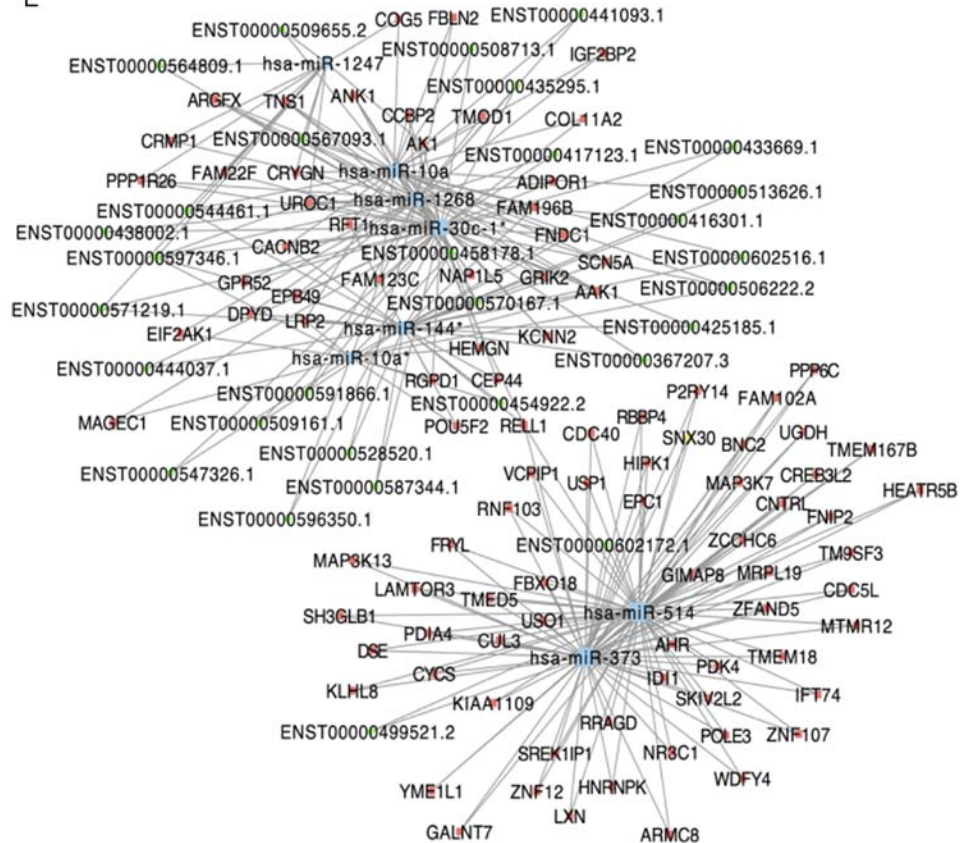


Figure 2. Continued. View of the lncRNA-miRNA-mRNA network. (D) View of the lncRNA-mRNA network. The squares represent mRNAs and the circles represent lncRNAs. (E) View of the lncRNA-mRNA-miRNA network. Red nodes represent mRNAs, green nodes represent lncRNAs and blue nodes represent miRNAs. lncRNA, long non-coding RNA; miRNA, microRNA; ceRNA, competing endogenous RNA.

Table II. Top 20 genes in degree, betweenness and closeness.

Betweenness	Closeness	Degree	Pagerank	Name	Gene type
2950	0.439597	60	0.107238	hsa-miR-514	miRNA
2950	0.439597	60	0.107238	hsa-miR-373	miRNA
1462.053	0.314904	46	0.06756	hsa-miR-30c-1 ^a	miRNA
2786.915	0.41195	34	0.049831	hsa-miR-1268	miRNA
568.3588	0.293722	31	0.04458	hsa-miR-10a	miRNA
2332.149	0.40184	30	0.0444	hsa-miR-144 ^a	miRNA
184.1859	0.274059	15	0.022549	hsa-miR-10a ^a	miRNA
147.3383	0.272917	14	0.020977	hsa-miR-1247	miRNA
132.3279	0.348404	6	0.008662	ENST00000597346.1	lncRNA
92.107	0.346561	5	0.007389	AAK1	mRNA
92.107	0.346561	5	0.007389	ENST00000458178.1	lncRNA
54.59979	0.337629	4	0.006136	ENST00000544461.1	lncRNA
4273.936	0.458042	4	0.006679	RELL1	mRNA
58.5175	0.344737	4	0.006111	SCN5A	mRNA
49.93945	0.344737	4	0.006131	FNDC1	mRNA
49.93945	0.344737	4	0.006131	FAM123C	mRNA
48.78154	0.341146	4	0.006126	ENST00000567093.1	lncRNA
48.78154	0.341146	4	0.006126	TNS1	mRNA
35.3917	0.332487	3	0.004916	ENST00000571219.1	lncRNA
28.90726	0.339378	3	0.004853	ARGFX	mRNA

^aOriginal miRNA name. miRNA, microRNA; lncRNA, long non-coding RNA; AAK-1, AP2-associated protein kinase 1; RELL1, RELT-like protein 1; SCN5A, sodium channel protein 5 subunit α ; FNDC1, fibronectin type III domain-containing protein 1; FAM123C, APC membrane recruitment protein 3; TNS1, tensin-1; ARGFX, arginine-fifty homeobox.

Table III. Number of lncRNA-miRNA and miRNA-mRNA pairs.

lncRNA	lncRNA-miRNA pairs	miRNA-mRNA pairs	Total number
ENST00000597346.1	6	58	64
ENST00000458178.1	5	58	63
ENST00000567093.1	4	53	57
ENST00000544461.1	4	44	48
ENST00000570167.1	3	51	54
ENST00000506222.2	3	50	53
ENST00000571219.1	3	44	47
ENST00000499521.2	2	238	240
ENST00000602172.1	2	238	240

miRNA, microRNA; lncRNA, long non-coding RNA.

ENST00000567093.1 not only had higher betweenness and node degree (Table II) but were also involved in a higher number of lncRNA-miRNA and miRNA-mRNA pairs (Table III), which were regarded as potential targets for HCM and were confirmed by module analysis.

Discussion

HCM is a commonly inherited cardiovascular disease that is the most frequent cause of sudden death in younger

individuals and functional disability resulting from heart failure and stroke (28). Although therapeutic advancements have been made in HCM, the treatment of HCM remains limited. Currently, major efforts have been made to detect the underlying mechanisms of HCM (29-31). ncRNAs including miRNAs and lncRNAs, have been identified as novel regulators underlying HCM (32-35). However, current studies of lncRNAs are focused on RNA sequencing or microarray technology (21,22), the functional studies are relative few and complicated compared to miRNAs. Therefore, it is necessary

Table IV. lncRNA-miRNA-mRNA networks of the 5 selected lncRNAs.

lncRNA	ceRNA score	miRNA	mRNA
ENST00000567093.1	1	hsa-miR-10a; hsa-miR-30c-1 ^a ; hsa-miR-1268; hsa-miR-1247	TNS1
ENST00000458178.1	0.687	hsa-miR-10a; hsa-miR-30c-1 ^a ; hsa-miR-1268	ANK1
ENST00000544461.1	0.666	hsa-miR-10a; hsa-miR-144 ^a ; hsa-miR-1268 hsa-miR-10a; hsa-miR-1268; hsa-miR-1247	KCNN2, SCN5A TNS1
ENST00000597346.1	0.666	hsa-miR-10a; hsa-miR-30c-1 ^a ; hsa-miR-1268	ANK1
ENST00000571219.1	0.666	hsa-miR-30c-1 ^a ; hsa-miR-1247 hsa-miR-144 ^a ; hsa-miR-30c-1 ^a	FAM22F, UROC1, AK1, CRYGN, PPP1R26, TNS1 RFT1, FAM196B, CACNB2, EPB49, RGPD1, GRIK2, SCN5A, AAK1

^aOriginal miRNA name. miRNA, microRNA; lncRNA, long non-coding RNA; ceRNA, competitive endogenous RNA; TNS1, tensin-1; ANK1, ankyrin-1; KCNN2, small conductance calcium-activated potassium channel protein 2; SCN5A, sodium channel protein 5 subunit α ; FAM22F protein; UROC1, urocanate hydratase; AK1, adenylate kinase isoenzyme 1; CRYGN, γ -crystallin N; PPP1R26, Protein phosphatase 1 regulatory subunit 26; RFT1, solute carrier family 52, riboflavin transporter, member 1; FAM196B, protein INSYN2B; CACNB2, voltage-dependent L-type calcium channel subunit β -2; EPB49, dematin; RGPD1, RANBP2-like and GRIP domain-containing protein 1; GRIK2, glutamate receptor ionotropic, kainite 2; AAK-1, AP2-associated protein kinase 1.

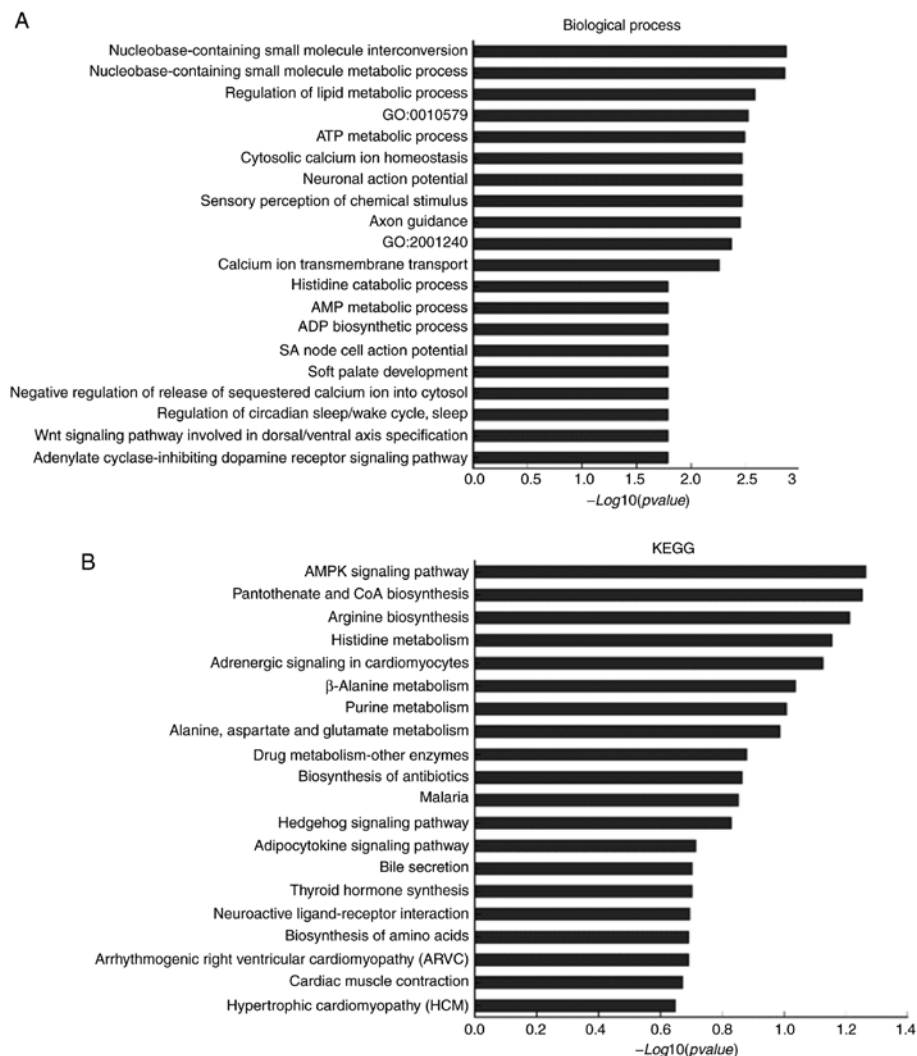


Figure 3. Functional analysis of differentially expressed mRNAs related to lncRNAs ENST00000597346.1 and ENST00000458178.1. (A) Biological processes and (B) KEGG analysis of differentially expressed mRNAs related to lncRNA ENST00000597346.1. lncRNA, long non-coding RNA; miRNA, microRNA; KEGG, Kyoto Encyclopedia of Genes and Genomes.

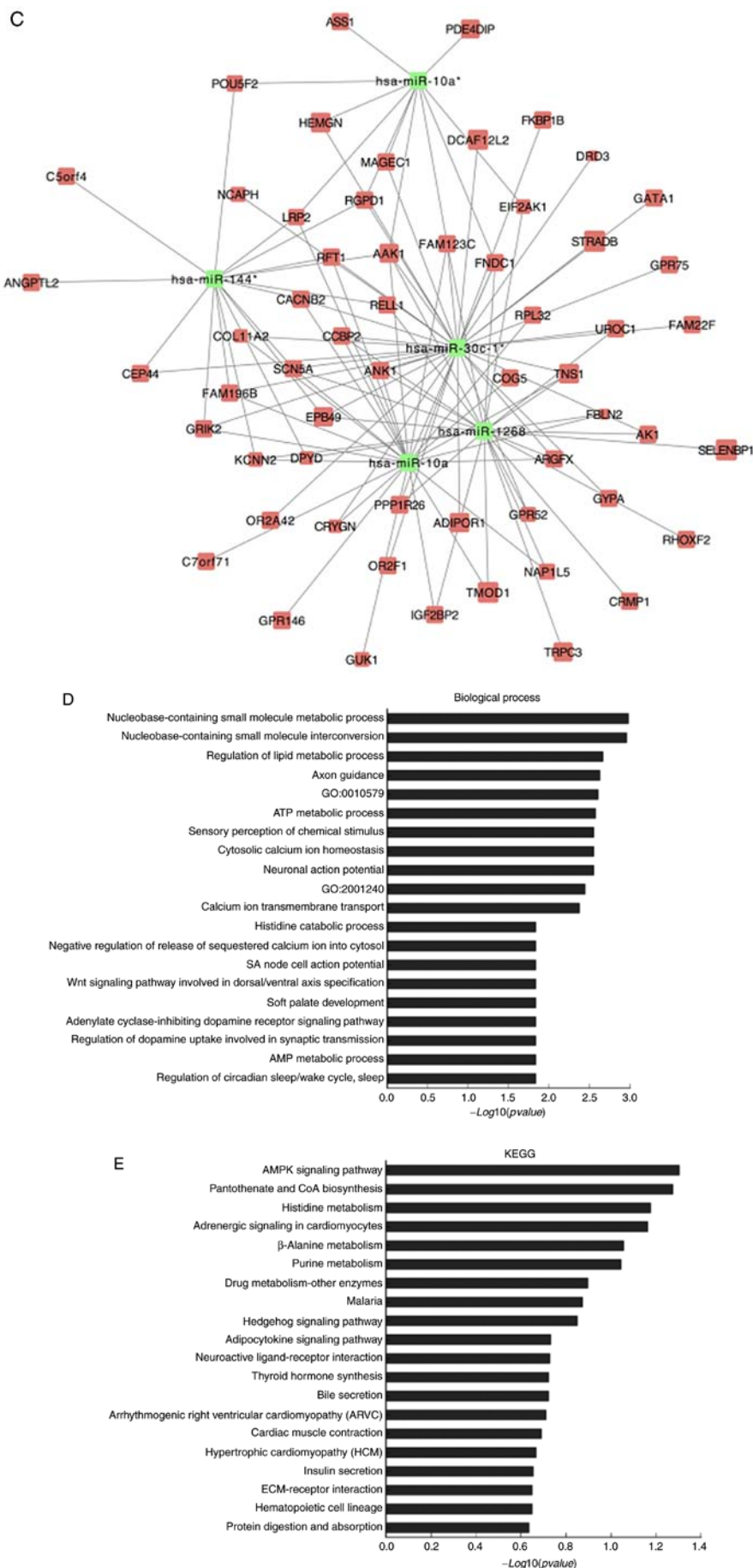


Figure 3. Continued. Functional analysis of differentially expressed mRNAs related to lncRNAs ENST00000597346.1 and ENST00000458178.1. (C) Subnetwork of lncRNA ENST00000597346.1 and ENST00000458178.1. (D) lncRNA ENST00000458178.1-related biological processes and (E) KEGG analysis of differentially expressed mRNAs. lncRNA, long non-coding RNA; miRNA, microRNA; KEGG, Kyoto Encyclopedia of Genes and Genomes.

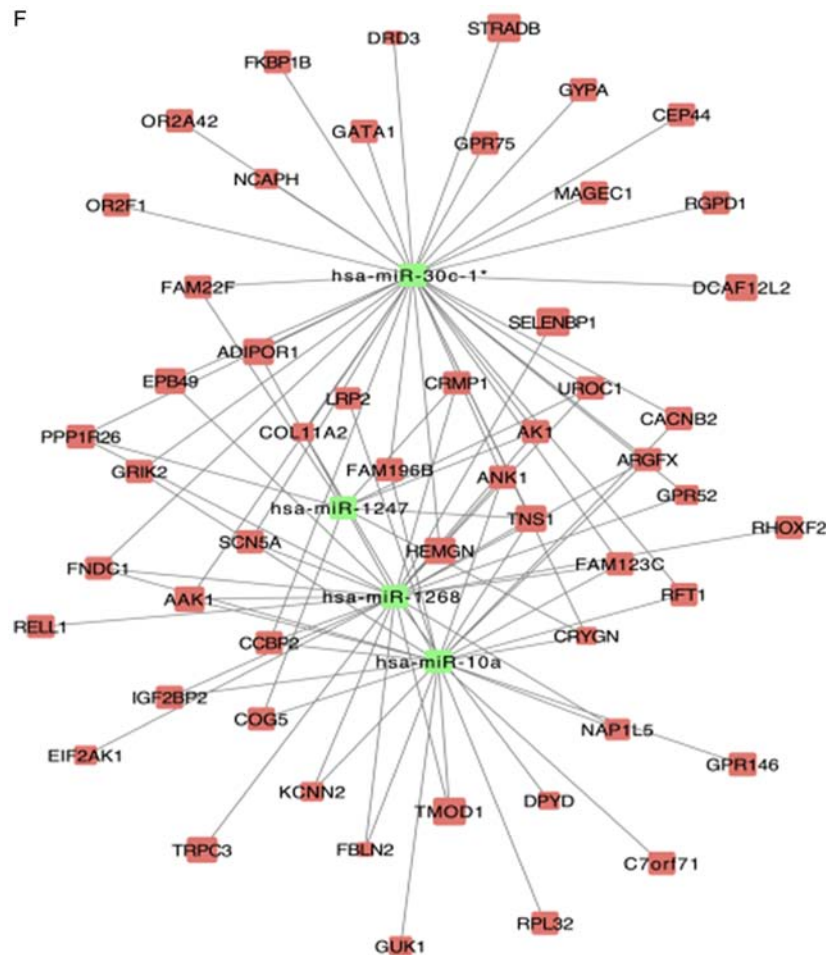
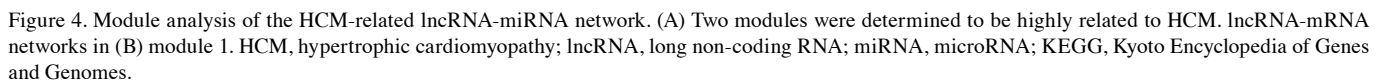


Figure 3. Continued. Functional analysis of differentially expressed mRNAs related to lncRNAs ENST00000597346.1 and ENST00000458178.1. (F) lncRNA ENST00000458178.1-related miRNA-mRNA subnetwork. Green nodes represent miRNAs and red nodes represent mRNAs. lncRNA, long non-coding RNA; miRNA, microRNA; KEGG, Kyoto Encyclopedia of Genes and Genomes.

to identify an efficient and accurate way to infer the potential function of lncRNAs with miRNAs and/or mRNAs whose functions have already been annotated. In the present study, interaction data from NCBI GEO (lncRNA-mRNA dataset: GSE68316, miRNA dataset: GSE36946) were used to generate a triple network based on ceRNA theory, which suggested that lncRNAs and mRNAs share the common miRNA in one triplet. The ceRNA network was composed of 30 lncRNA nodes, 94 mRNA nodes and 8 miRNA nodes. Subsequently, hub nodes and the number of relationship pairs were used to perform topological and subnetwork analysis. 2 lncRNAs (ENST00000458178.1 and ENST00000567093.1) were observed to be key topological nodes, whose node degrees and number of lncRNA-miRNA and miRNA-mRNA interaction pairs were higher compared with other lncRNAs, and these 2 lncRNAs were highly conserved in human, mouse and rat genomes. According to the ceRNA network, the 2 lncRNAs primarily targeted miR-10a-5p, miR-30c-3p, miR-1247-5p and miR-1268a.

A number of transcriptomic and proteomic profiling analyses of HCM have been previously performed and several dysregulated miRNAs have been identified. For example, Song *et al* (36) identified 13 dysregulated miRNAs involved in myocardial tissues in patients with HCM. The list of miRNAs in GSE36946 and previous reports on HCM or cardiac

hypertrophy were compared. Several miRNAs, including miR-10a, miR-30c and miR-373, were common between the present study and previous studies (37-41). Additionally, studies have shown that miR-10a was downregulated in a transverse abdominal aortic constriction-induced cardiac hypertrophy model and angiotensin II (Ang II)-stimulated cardiomyocytes. Overexpression of miR-10a in Ang II-treated cardiomyocytes ameliorated cell hypertrophy and decreased the expression of natriuretic peptides A and B by directly inhibiting T-box transcription factor TBX5 (37,38). Diabetic cardiomyopathy (DCM) is characterized by endothelial dysfunction, myocyte hypertrophy, necrosis, apoptosis and increased fibrosis deposition (39). A previous study demonstrated that cardiac miR-30c expression was decreased in rats and patients with DCM and in high HG-treated cardiomyocytes (40). Overexpression of miR-30c attenuated HG-induced cardiomyocyte hypertrophy by inhibiting cell division control protein 42 homolog and serine/threonine-protein kinase PAK 1 (40). Furthermore, a study reported that concurrent overexpression of miR-30c and miR-181a resulted in a greater decrease in cardiomyocyte hypertrophy and apoptosis via the p53-p21 pathway compared with the overexpression of miR-30c or miR-181a alone, suggesting a synergistic effect of these two miRNAs in DCM-induced cardiac hypertrophy (41). Contrary to our results, miR-373 levels were decreased in the



In the present study, GO and KEGG pathway analyses were used to assess biological functions that are enriched among differentially expressed coding genes. Owing to similar miRNA targets (miR-10a-5p, hsa-miR-30c-3p and miR-1268a), the significant GO terms of ENST00000567093.1 and ENST00000458178.1 shared common trends involving ‘Nucleobase-containing small molecule interconversion’.

Bidirectional hierarchical clustering analysis was performed to investigate the crosstalk between mRNAs and lncRNAs. GO and KEGG pathway analyses of these 2 modules indicated that in module 1, the dysregulated genes

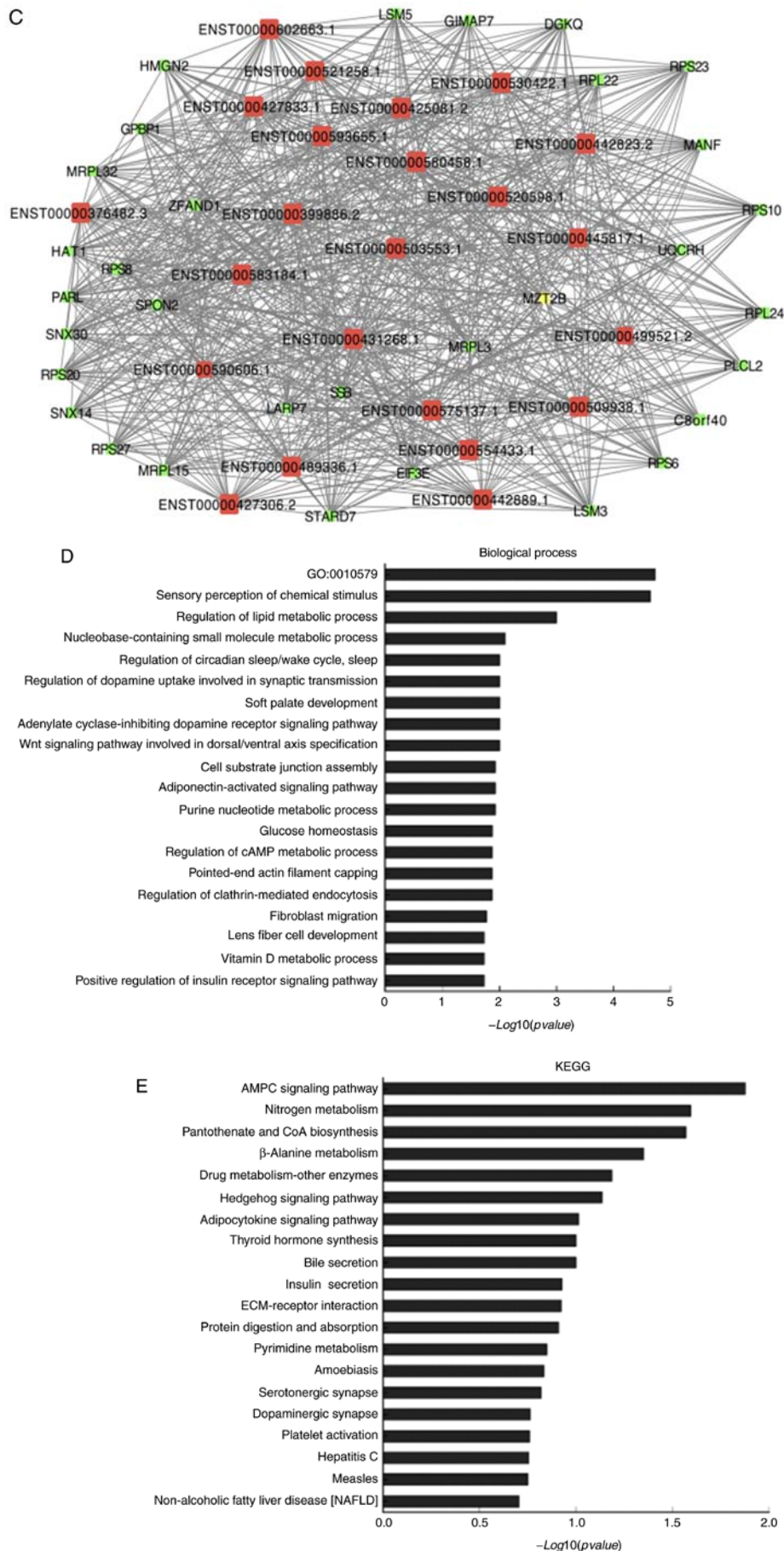


Figure 4. Continued. Module analysis of the HCM-related lncRNA-miRNA network. lncRNA-mRNA networks in (C) module 2. Red nodes represent lncRNAs and green nodes represent mRNAs. (D) Biological processes and (E) KEGG analysis of differentially expressed mRNAs related to module 1. HCM, hypertrophic cardiomyopathy; lncRNA, long non-coding RNA; miRNA, microRNA; KEGG, Kyoto Encyclopedia of Genes and Genomes.

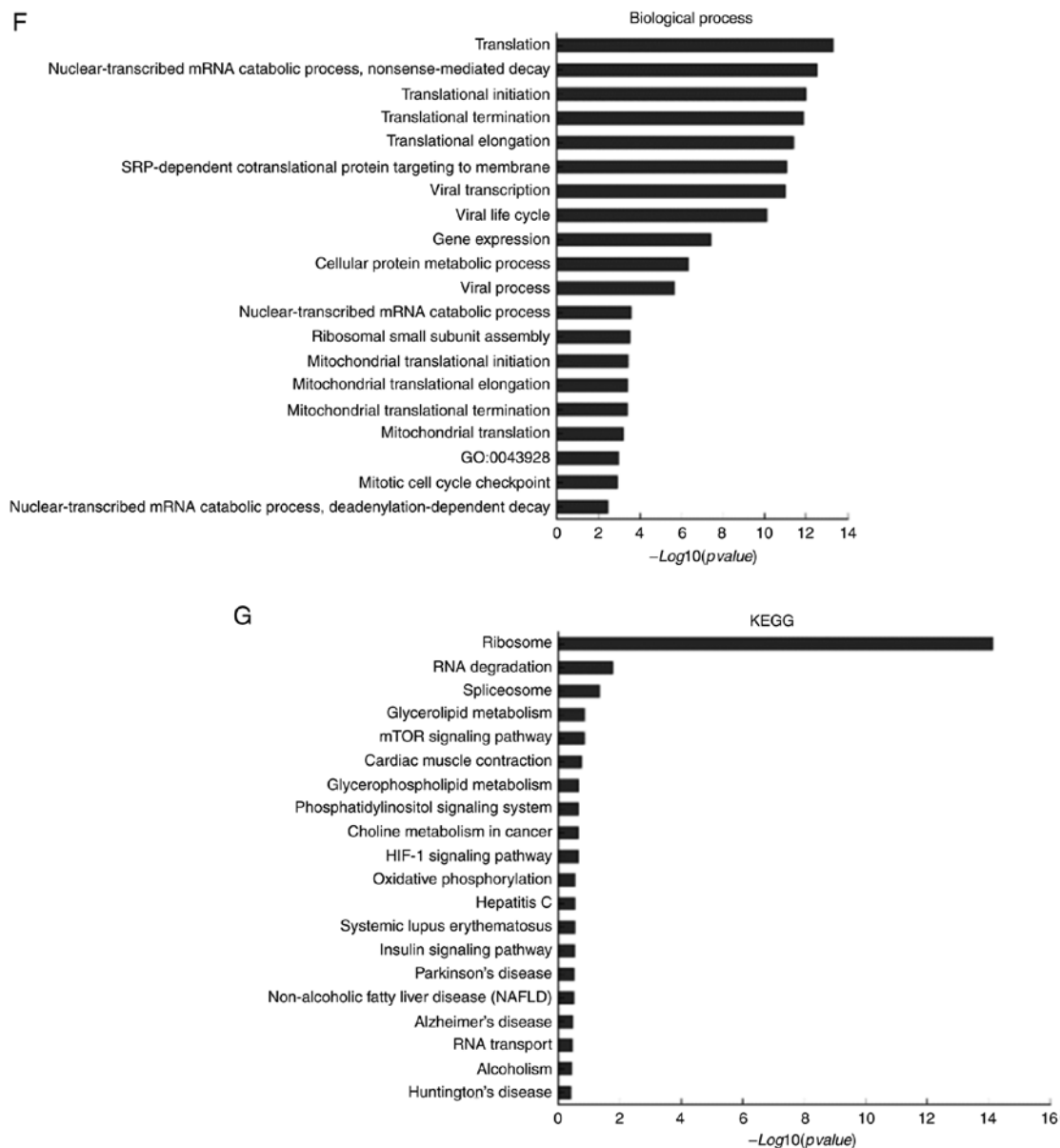


Figure 4. Continued. Module analysis of the HCM-related lncRNA-miRNA network. (F) Biological processes and (G) KEGG analysis of differentially expressed mRNAs related to module 2. HCM, hypertrophic cardiomyopathy; lncRNA, long non-coding RNA; miRNA, microRNA; KEGG, Kyoto Encyclopedia of Genes and Genomes.

were primarily involved in metabolic regulation, including lipid regulation, nucleobase-containing small molecule metabolic process and CoA biosynthesis, which has been discussed earlier. In module 2, the mRNAs were primarily enriched in translational regulation, including translation initiation, termination and elongation, ribosome and RNA degradation. Once the transcriptional process is impaired, the expression levels of numerous proteins are dysregulated, and this may alter normal physiological processes. To the best of our knowledge, there are no previous studies which have addressed translational regulation in HCM, and thus, it would be meaningful to investigate transcriptional dysfunction in HCM. Nevertheless, additional studies are required to fully understand the molecular mechanisms underlying HCM.

Currently, identifying ncRNA-disease associations is playing an increasingly vital role in diagnostic and therapeutic tools for diseases including HCM. However, since the fact that

it is expensive and time-consuming via experimental studies to uncover associations between ncRNAs and disease, novel and effective computational models for the identification of ncRNAs associated with HCM or other diseases are being developed. Several novel computational methods have been used to calculate potential ncRNAs-disease association scores (48,49). lncRNAs have emerged as one of the largest and significantly diverse type of RNA family (50,51). The biological role and functions of lncRNAs are diverse and still mostly uncharacterized. Their target-mimetic and sponge/decoy function on miRNAs have been identified. Previous studies demonstrated that lncRNAs can act as miRNA sponges, reducing their regulatory effect on mRNAs (52,53). This function introduces an extra layer of complexity in the miRNA-mRNA interaction network. In addition, while several studies examining lncRNAs or miRNAs in cardiac hypertrophy have been performed (54-56), to the best of our knowledge, there

are relatively fewer studies focusing on a ceRNA network between lncRNAs and miRNAs of HCM (57,58), particularly in human models. In the present study, a ceRNA based lncRNA-miRNA-mRNA network in human HCM models was constructed. A total of 2 lncRNAs (ENST00000458178.1 and ENST00000567093.1) were determined to be key topological nodes and highly conserved in human, mouse and rat genomes. According to the ceRNA network, the 2 lncRNAs primarily targeted miR-10a-5p, miR-30c-3p, miR-1247-5p and miR-1268a, which were highly related to the development of HCM. Therefore, the study provides a framework for constructing powerful computational methods in human HCM models to predict potential lncRNA-miRNA-disease associations and select the most promising lncRNAs/miRNAs related to HCM or other diseases for experimental validation.

However, there are several disadvantages to using bioinformatics analysis. Firstly, the changes and potential functions of lncRNAs/miRNAs/mRNAs are hypothetical, and need to be further verified in animal or human models of hypertrophic cardiomyopathy. Secondly, a lncRNA-miRNA-mRNA ceRNA network was constructed by combining two different GSE datasets (GSE68313 and GSE36946); there may be missing and overlapping data between different databases. HCM myocardial samples will be collected and a complete lncRNA/miRNA/mRNA profile will be performed in future studies. The functional role of the 2 lncRNAs on HCM and the relationship between lncRNAs and miRNAs detected in the present study will be further explored. The underlying mechanisms can be investigated by western blotting and proteomics studies. A luciferase reporter assay will also be performed to confirm the target genes of miRNAs in future work.

The present study has some limitation. Due to the lack of samples, the changes in lncRNAs, miRNAs and mRNAs were not validated in myocardial tissues from patients with HCM, and thus, there may have been false positives. Additionally, during the process of converting gene IDs from different databases, a number of genes may have been lost, which may have decreased the accuracy of the present results. Finally, the present study was primarily focused on alterations in lncRNAs/miRNAs in HCM samples, whereas the specific functions of ceRNAs in HCM remain unknown, therefore the underlying biological functions and mechanisms warrants further exploration.

In conclusion, a ceRNA based lncRNA-miRNA-mRNA network was constructed in the present study, providing a new strategy for studying HCM and other diseases. Furthermore, lncRNA-miRNA pairs may be regarded as candidate diagnostic biomarkers or potential therapeutic targets for treatment of HCM.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the GEO repository. GSE36949: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE36949> GSE68316: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE68316>

Authors' contributions

FH and JS analyzed, interpreted and ensured the quality of the data. LT conceived and designed the study. LY and XH developed the methodology. LT wrote and reviewed the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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