

Comprehensive Analysis of a lncRNA-miRNA-mRNA Competing Endogenous RNA Network in Heart Failure

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Abstract

Background: Acute heart failure caused by progressive heart failure is a common disease in intensive care units (ICU). The growing incidence rate of heart failure and its high mortality rate result are very important sociosanitary problems. Therefore, it is important to identify the molecular mechanism by which heart failure occurs and to identify treatment for this mechanism. Recently, the mechanism of ceRNA has attracted increasing attention. The aim of the present study was to identify the candidate ceRNA network in the progression of heart failure.

Method: Microarray datasets GSE9128, GSE61741 and GSE77399 were downloaded from Gene Expression Omnibus (GEO) database. The differentially expressed genes (DEGs) were identified, and function enrichment analyses were performed. The protein-protein interaction network (PPI) was constructed and identification of hub-genes was performed using STRING and Cytoscape. Furthermore, according to the ceRNA theory, network of ceRNA was constructed.

Result: In the present study, based on the ceRNA theory and above series of analyses, network of ceRNA which include 7 mRNAs (BCL2A1, DUSP1, EGR1, MYC, NR4A2, PTGS2 and RAC2), 3 miRNAs (miR-20a, miR-129-59 and miR-185-5p) and 3 lncRNAs (GAS5, H19 and PCGEM1) were obtained.

Conclusion: In conclusion, these findings can be used to carrying on further study to identify the important roles of the ceRNA, biological function, appropriate treatment targets and biomarkers in the progression of heart failure.

Introduction

Acute decompensated heart failure is a growing major public health problem all through the world[1]. A variety of diseases including coronary artery disease, hypertension, cardiac valves disease and dilated cardiomyopathy contribute to the progression and occurrence of heart failure[2]. With the development of these diseases, cardiac remodeling and regeneration cause the development of heart failure[3]. Moreover, ventilator support, continuous renal replacement treatment and positive vasoactive agents are needed when the symptoms of acute heart failure occur in intensive care units. This is a huge economic burden on the health of the population. Some previous studies have showed that the mechanism of cardiac remodeling and regeneration[4]. However, some further investigations are needed to understand the molecular mechanism of heart failure.

LncRNA participated in the regulation of many physiological and pathological processes through a variety of mechanisms[5]. LncRNA can not only directly regulate the expressions of target genes, but also affect the expression of miRNA by binding miRNA, further affecting the expression level of target gene mRNA of miRNA[6]. Competing endogenous RNA (ceRNA) is widely involved in the regulation process of vital activities such as inflammation, apoptosis and differentiation. This regulatory mechanism also occurs in many cardiovascular diseases[7].

In this study, GSE9128, GSE61741 and GSE77399 were used from the Gene Expression Omnibus database to identify expressed mRNAs, miRNAs and lncRNAs to construct a ceRNA network. Therefore, the purpose of this study was to screen out differentially expressed lncRNA, miRNA and mRNA in the circulation of patients with heart failure, and to construct ceRNA network, so as to provide bases for finding molecular markers and therapeutic targets related to acute heart failure.

Materials And Methods

Data collection and identification of differentially expressed mRNAs, miRNAs and lncRNAs. The database of GEO (<http://www.ncbi.nlm.nih.gov/geo>) is a public functional genomics data repository of high throughput gene expression data, chips and microarrays[8]. In GEO, we found three datasets including GSE9128[9], GSE61741[10] and GSE77399[11] that met our retrieval requirement for study. The differentially expressed mRNAs, miRNAs and lncRNAs between healthy controls and heart failure were identified by an excellent web tool named GEO2R (<http://www.ncbi.nlm.nih.gov/geo/geo2r>). The adjusted P-values (adj. P) and Benjamini and Hochberg false discovery rate were applied to provide a balance between discovery of statistically significant genes. LogFC (fold change) >1 and adj. P-value <0.05 were considered statistically significant.

Functional and pathway enrichment analysis of DEGs. The biological function and signaling pathways analysis of differential genes obtained by the above methods were analyzed by the web database of DAVID. The web database of DAVID (<http://david.ncifcrf.gov>) is a common tool which is used to GO annotation and KEGG pathways enrichment of DEGs[12]. It can provide a comprehensive set of functional annotation information of genes and proteins. GO annotation is a main bioinformatics tool to annotate genes and analyze biological process of DEGs[13]. P<0.05 was considered statistically significant.

Identification of hub-genes and PPI network construction. In order to identify hub-genes in these DEGs, the database of STRING (Search Tool for the Retrieval of Interacting Genes <http://string-db.org>) was used to obtain the predicted interactions (version 10.0)[14]. The software of Cytoscape (version 3.6.1) is an open source bioinformatics software platform that can display visualization of PPI (protein-protein interaction) network[15]. There is an APP named MCODE (Molecular Complex Detection) in Cytoscape. MCODE can identify dense areas and most significant module from the network analyzed by STRING. The hub-genes were selected with degrees > 9.

Prediction and construction network of mRNAs and miRNAs. The mRNA targets of miRNA were predicted using EVmiRNA (<http://bioinfo.life.hust.edu.cn/EVmiRNA>)[16]. Top 15 significantly differentially expressed miRNAs of GSE61741 were selected. The target genes of top 15 significantly differentially expressed miRNAs of GSE61741 and differentially expressed mRNAs of GSE9128 were intersected. Based on the negative regulation relationship between miRNAs and mRNAs, the miRNA-mRNA network was constructed and visualized using the software of Cytoscape (version 3.6.1).

Prediction and construction of the ceRNA network. The lncRNA targets of the miRNAs were predicted using DIANA-LncBase v2(<http://carolina.imis.athena-innovation.gr/>) [17]. Top 15 significantly differentially expressed miRNAs of GSE61741 were selected. The target lncRNAs of top 15 significantly differentially expressed miRNAs of GSE61741 and differentially expressed lncRNAs of GSE77399 were intersected. Based on the association of lncRNA, miRNA and mRNA, the alluvial of ceRNA was constructed and visualized using the web tool (<http://www.bioinformatics.com.cn>), an online platform for data analysis and visualization.

Results

Identification of differentially expressed mRNAs, miRNAs and lncRNAs in patients with heart failure. A total of three datasets including GSE9128, GSE61741 and GSE77399 were downloaded from the GEO database. GEO2R was used to screen for the differentially expressed mRNAs, miRNAs and lncRNAs between heart failure patients and healthy controls. There were 125 upregulated and 65 downregulated DEMs in the GSE9128 dataset. The plot of volcano and heat are shown in figure 1A and C. There were 38 upregulated and 39 downregulated DEMs in the GSE61741 dataset. The plot of volcano is shown in figure 1B. There were 18 upregulated and 9 downregulated DELs in the GSE77399 dataset. The plot of volcano and heat are shown in figure 2A and B.

Gene ontology enrichment analysis and KEGG pathway analysis. The DEGs were analyzed by GO term and KEGG pathway enrichment by DAVID. Gene Ontology functional enrichment analysis results showed that these DEGs were significantly enriched in negative regulation of phosphorylation, regulation of leukocyte activation, regulation of lymphocyte activation, negative regulation of immune system process, T cell activation, positive regulation of cytokine production leukocyte differentiation, negative regulation of protein phosphorylation and response to oxidative stress (Figure 3). There are several pathways associated with progression of heart failure. The analysis of KEGG pathway showed that these DEGs were significantly enriched in NOD-like receptor signaling pathway, Salmonella infection, NF-kappaB signaling pathway, C-type lectin receptor signaling pathway, Necroptosis, Apoptosis, TNF signaling pathway IL-17 signaling pathway and so on (Figure 4).

miRNA predicted target mRNA analysis and miRNA-mRNA network construction. Top 15 significantly differentially expressed miRNAs of GSE61741 were selected. The target genes of top 15 significantly differentially expressed miRNAs of GSE61741 and differentially expressed mRNAs of GSE9128 were intersected. Therefore, a total of 47 DEGs and 12 DEMs were obtained to construct miRNA-mRNA network. Based on the negative regulation relationship between miRNAs and mRNAs, the miRNA-mRNA network was constructed and visualized using the software of Cytoscape (version 3.6.1). The plot of miRNA-mRNA network is shown in figure 5.

miRNA predicted target lncRNA analysis. Based on the above data, target lncRNAs of above 12 DEMs and differentially expressed lncRNAs of GSE77399 were intersected. According to negative regulation

relationship between miRNAs and lncRNAs, only three pairs of lncRNA-miRNA including H19-has-miR-20a, PCGEM1-has-miR-129-5p and GAS5-has-miR-185-5p were obtained.

Hub-gene selection and ceRNA relationship construction. The most significant module of PPI network was obtained and visualized using Cytoscape. The hub genes were selected with degrees > 9. A total of 30 genes were identified as hub genes (Figure 6). According to above data, target mRNAs of -has-miR-20a, has-miR-129-5p, has-miR-185-5p and hub-genes were intersected. Based on the ceRNA theory, miRNAs negatively regulate the expression of mRNAs and lncRNAs, seven ceRNA relationships were obtained (Figure 7).

Discussion

Acute heart failure caused by progressive heart failure is a common disease in ICU. These patients often require mechanical ventilation and renal replacement therapy, which results in an increased economic burden for patients and their families[18]. Therefore, it is important to identify the molecular mechanism by which heart failure occurs and to identify treatment for this mechanism. Recently, the mechanism of ceRNA has attracted increasing attention. The relationship among lncRNA, miRNA and mRNA play an important role in the regulation of multiple processes. At present, numerous studies have been conducted on the ceRNA molecular mechanism of heart failure. Zhang et al have found that lncRNA-CHAR/miR-20b/PTEN play an important role in the progression of cardiac hypertrophy[19]. The research of Liang et al showed that lncRNA PFL contributes to cardiac fibrosis by acting miRNA-let-7d[20].

In the present study, we analyzed the datasets of GSE9128, GSE61741 and GSE77399 to find more possible molecular mechanism of ceRNA in heart failure. Based on the ceRNA theory and above series of analyses, network of ceRNA which include 7 mRNAs (BCL2A1, DUSP1, EGR1, MYC, NR4A2, PTGS2 and RAC2), 3 miRNAs (miR-20a, miR-129-5p and miR-185-5p) and 3 lncRNAs (GAS5, H19 and PCGEM1) were obtained. MYC played critical roles in heart failure development progress[21]. NR4A2 is a member of the NR4A orphan nucleus receptor family. NR4A2 has protective function for cardiomyocytes against myocardial infarction[22]. DUSP1 can regulate cardiac metabolism. Overexpress DUSP1 can alleviate the fatal mitochondrial fission and provide a survival advantage to myocardial tissue[23]. The level of EGR1 is related to effectiveness of percutaneous coronary intervention. If the level of EGR1 is significant decreased in the early postoperative period, the patient may be suspected of having no-reflow[24]. PTGS2 (cyclooxygenase-2) represents a key enzyme in arachidonic acid metabolism in health and disease. It is expressed in several human tissues and induced in various cell types in response to inflammatory cytokine[25]. BCL2A1 and RAC2 have not been reported in the progression of heart failure. BCL2A1 is one of B-cell lymphoma2 (BCL2) proteins which are important cell death regulators. BCL2A1 is overexpressed in a variety of cancer cells, including hematological malignancies and solid tumors, and may contribute to tumor progression[26]. RAC2 is a GTPase that is exclusively expressed in hematopoietic cells. Mutations in RAC2 is associated with immunodeficiencies in some patients[27].

In previous study, the expression of miR-20a-5p is associated with the degree of left ventricular dilation[28]. Downregulation of miR-129-5p was observed in the serum of chronic heart failure patients. miR-129-5p mimic improved heart function and hemodynamic parameters[29]. At present, the study of miR-185-5p associated with heart failure is rare. There are some researches about miR-185-5p in the field of cancer. miR-185-5p was proved that it can inhibit cell metastasis of HCC by suppressing ROCK2[30]. Zhang et al found that expression of plasma H19 was high and it was independent predictors for coronary artery disease[31]. Li et al have found that the expression of GAS5 was low in peripheral blood of chronic heart failure[32]. These researches and findings are consistent with our results. At present, the study of PCGEM1 in heart failure was rare. There were some researches of PCGEM1 about cancers. In the research of Ho et al, they found that PCGEM1 is often upregulated in prostate cancer[33]. PCGEM1 also promotes cell proliferation, migration and invasion in cervical cancer[34]. Therefore, further study about the function and expression of PCGEM1 in heart failure is needed to identify. These findings indicate that these lncRNAs and miRNAs may have profound function in the progression of heart failure. Therefore, further studies about these mRNAs, lncRNAs and miRNAs are needed to identify their relationship and mechanisms.

There are several limitations in the present study. Firstly, we only have a result of bioinformatics analysis. Therefore, future in vitro and in vivo experiments are required to verify these results in heart failure pathology. Secondly, studies with larger cohorts of patients with heart failure are required to confirm the diagnostic and therapeutic value of the identified ceRNAs.

In conclusion, in the present study, we have performed a bioinformatics analysis to identify ceRNA network that may be involved in the progression of heart failure. In the present study, based on the ceRNA theory and above series of analyses, network of ceRNA which include 7 mRNAs (BCL2A1, DUSP1, EGR1, MYC, NR4A2, PTGS2 and RAC2), 3 miRNAs (miR-20a, miR-129-5p and miR-185-5p) and 3 lncRNAs (GAS5, H19 and PCGEM1) were obtained. These findings can be used to carrying on further study to identify the biological function, appropriate treatment targets and biomarkers in the progression of heart failure.

Declarations

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Conflict of interest

There was no conflict of interest between each author

Author contributions

Qinghui Fu contributed to the conception of the study;

Jun Hu and Enjiang Chen performed the bioinformatic analysis;

Rufang Jiang contributed significantly to analysis and manuscript preparation;

Xiaoqian Luo and Weina Lu performed the data analyses and wrote the manuscript;

Meiling Weng helped perform the analysis with constructive discussions.

Consent to participate

All the authors have consented to participate in this study.

Consent to publish

All the authors have consented to publish this study

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Figures

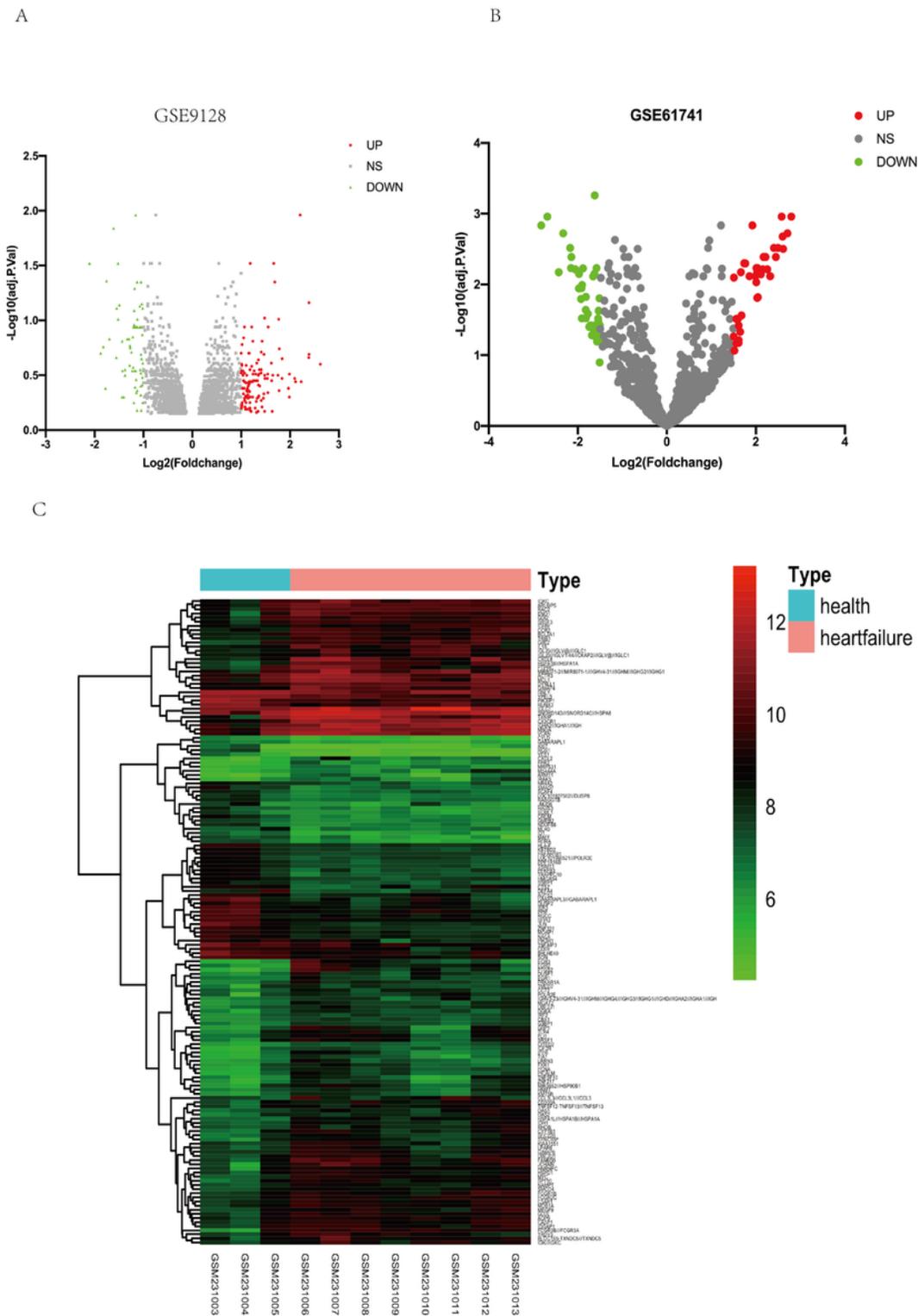
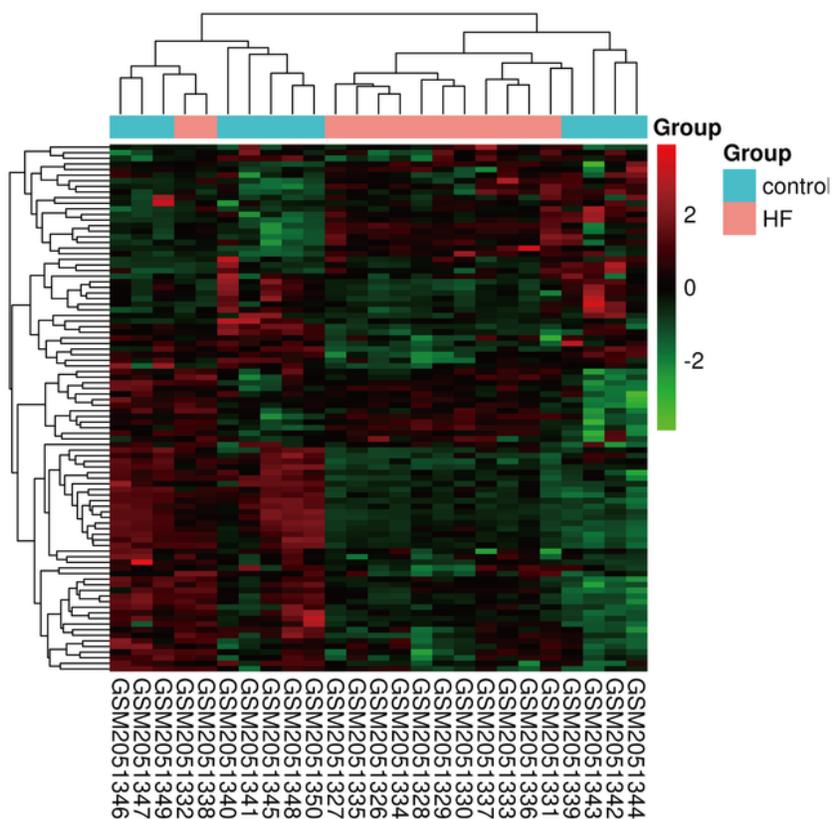


Figure 1

Volcano diagram of GSE9128 and GSE61741. Hot diagram of GSE9128. (A) volcano diagram of GSE9128 (B) volcano diagram of GSE61741 (C) Hot diagram of GSE9128. DEGs and DEMs were selected with a LogFC (fold change) >1 and adj. P-value <0.05 among above two expression profiling sets datasets.

A



B

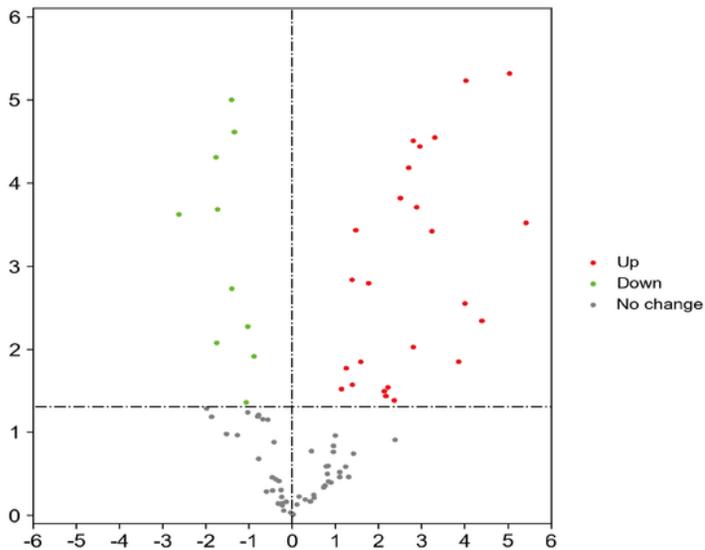


Figure 2

Volcano diagram and Hot diagram of GSE77399. (A) Hot diagram of GSE77399. (B) volcano diagram of GSE77399. DELs were selected with a LogFC (fold change) >1 and adj. P-value <0.05 in this expression profiling sets datasets.

Enriched GO Terms

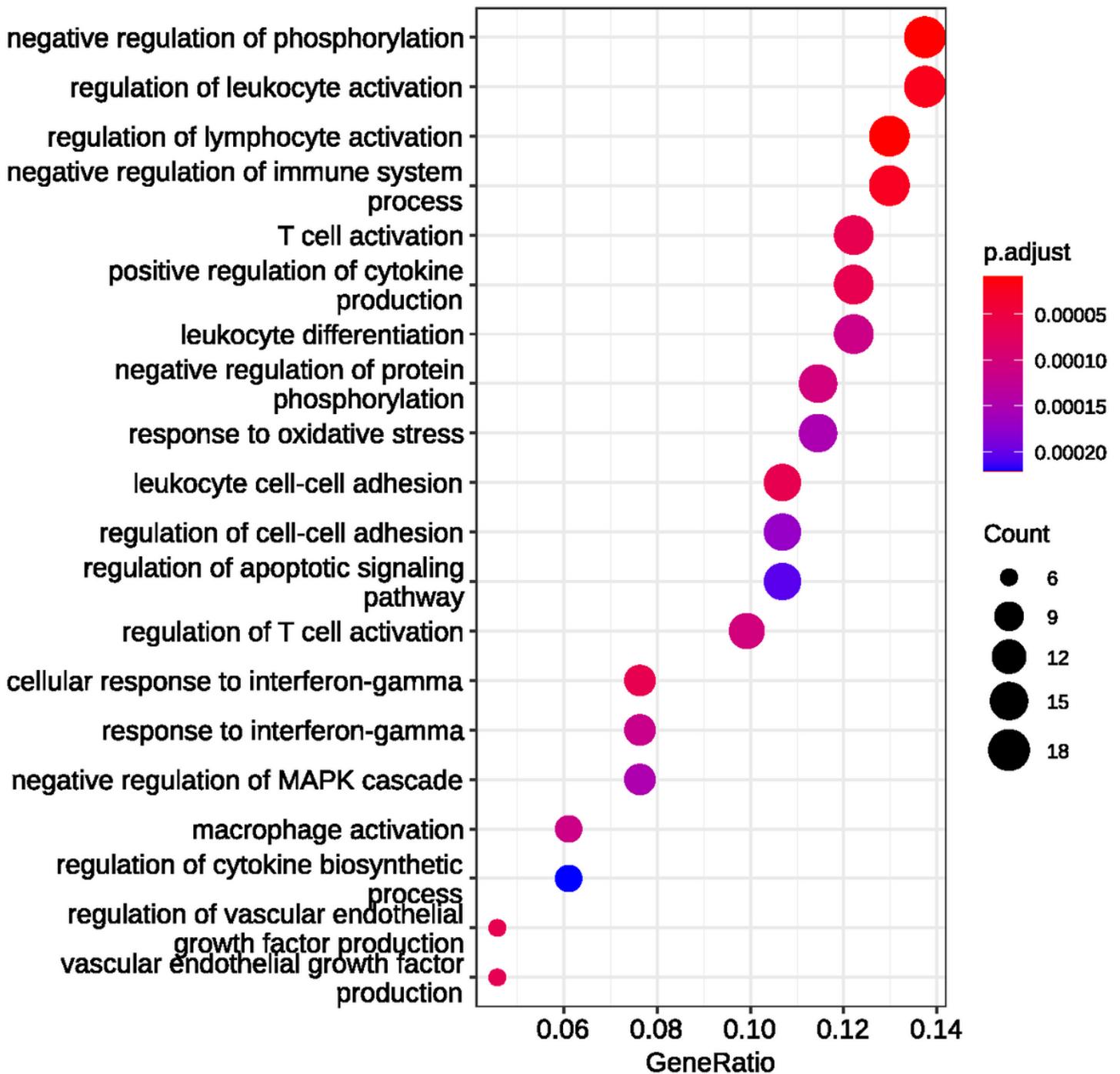


Figure 3

Top 20 significant GO terms that differentially expressed genes of GSE77399 were enriched in. GO, gene ontology; FDR, false discovery rate.

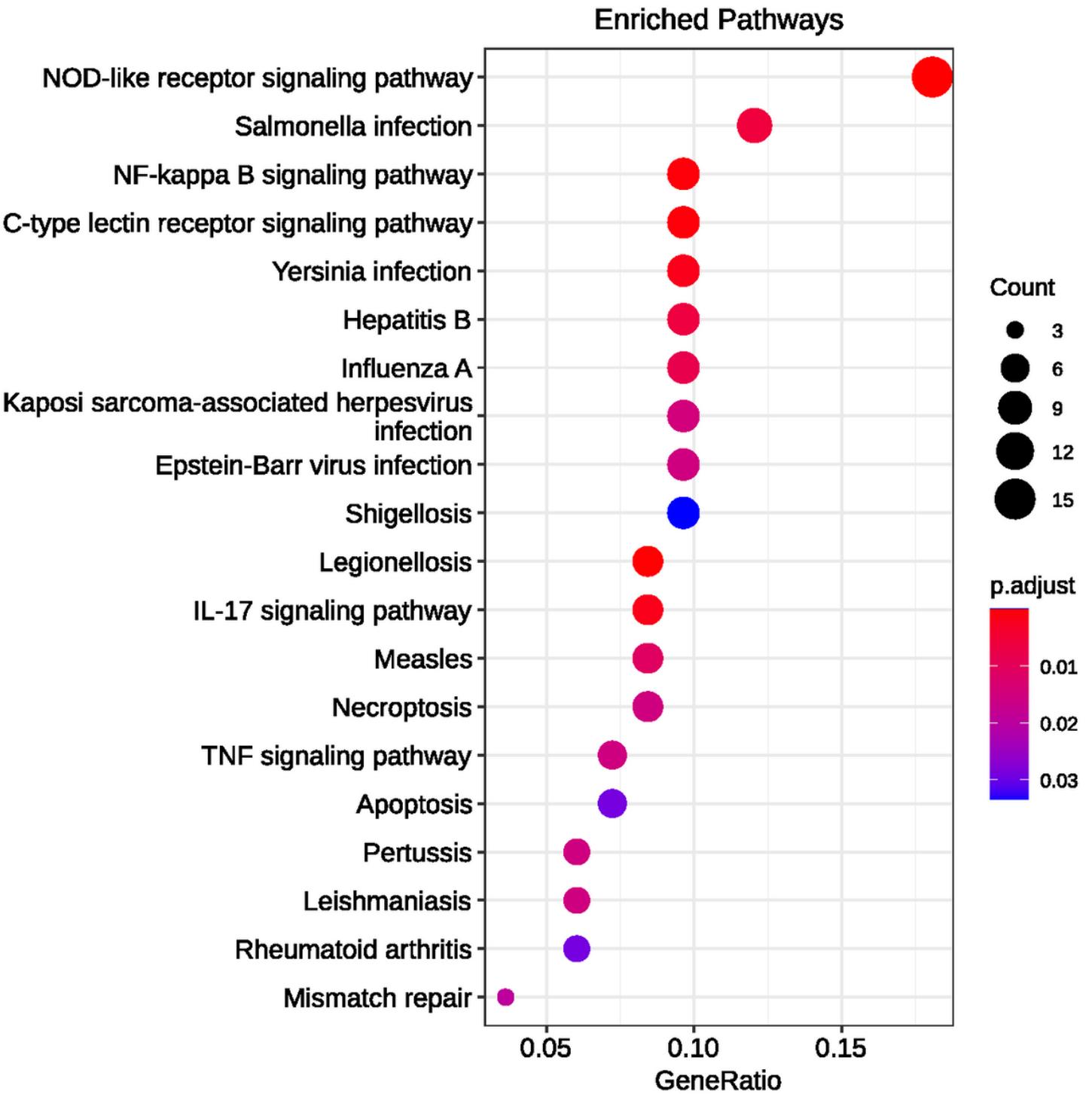


Figure 4

Top 20 significant KEGG pathway terms that differentially expressed genes of GSE77399 were enriched in. KEGG: Kyoto Encyclopedia of Genes and Genomes; FDR, false discovery rate.

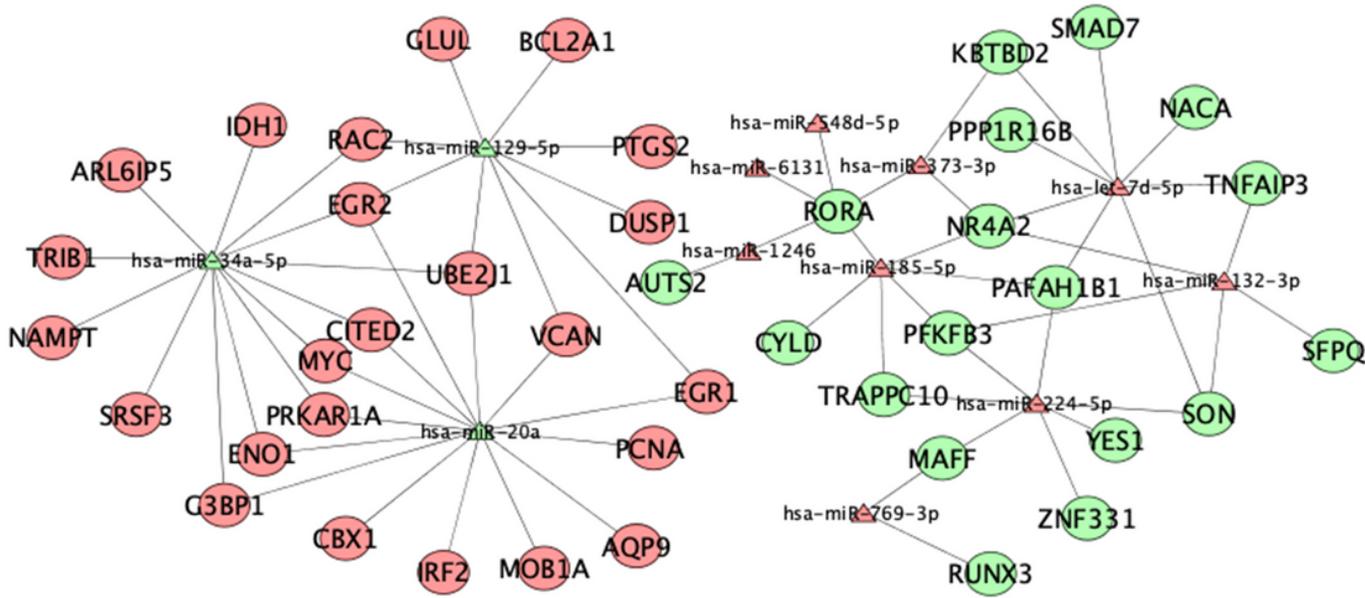


Figure 5

Diagram of the miRNA-mRNA network. Triangel representative of miRNA. Circle representative of mRNA. Red means up-regulated and green means down-regulated.

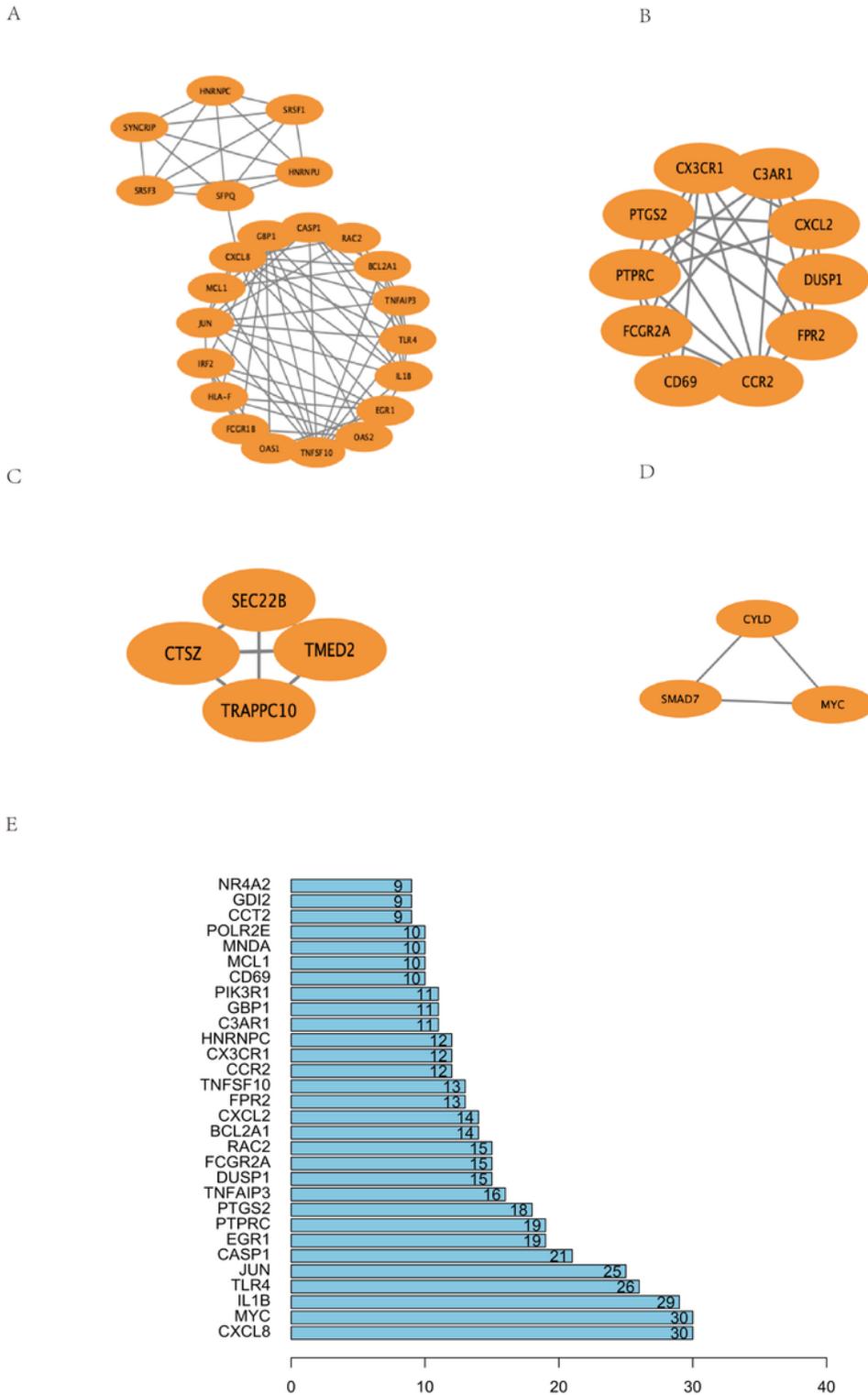


Figure 6

Protein-protein interaction networks of the DEGs in GSE77399. (A)-(D) The most significant module was obtained from PPI network using Cytoscape. (E) A total of 30 genes were identified as hub genes with degrees > 9.

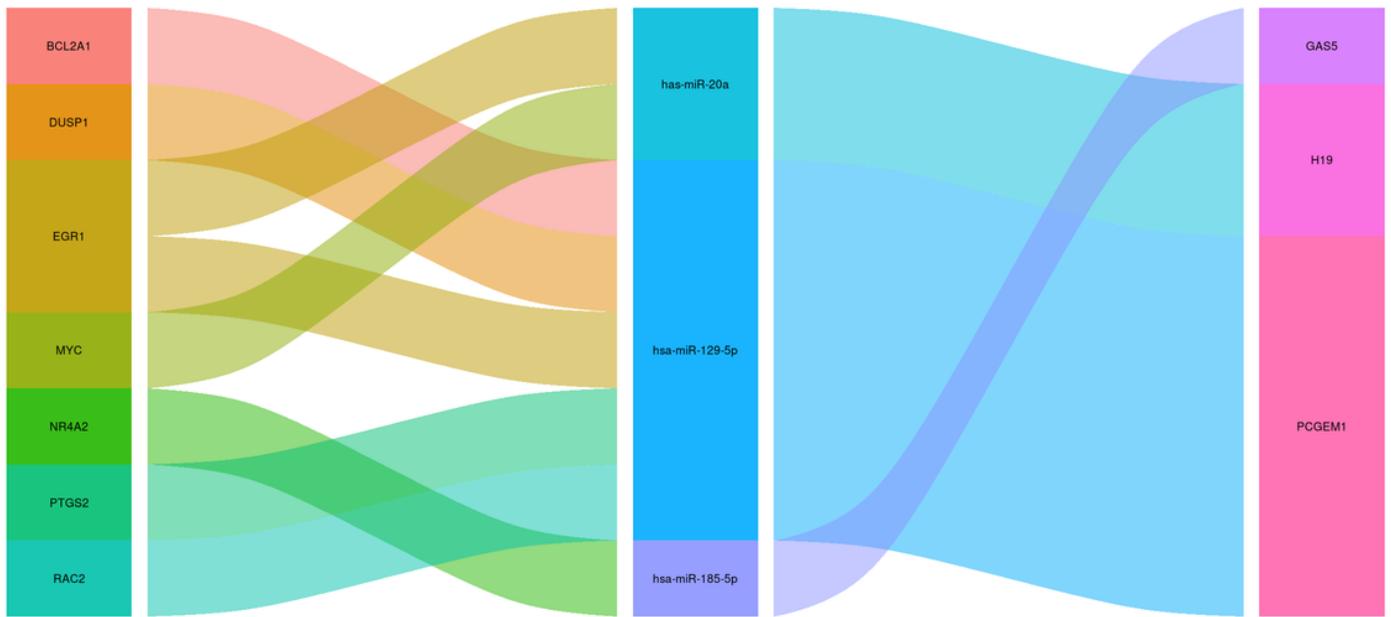


Figure 7

Sankey diagram for the ceRNA network in heart failure. Each rectangle represents a gene, and the connection degree of each gene is visualized based on the size of the rectangle.