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Bioinformatic analysis of microarray data reveals several key genes related to heart failure

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Abstract. – OBJECTIVES: Heart failure is a major public health problem worldwide. However, the molecular mechanism is still unclear. This study aims to discover differentially expressed genes (DEGs) between non-ischemic or ischemic heart failure samples and healthy control, which may be used for diagnosis and treatment of heart failure.

MATERIALS AND METHODS: Gene expression profile GSE9128 was downloaded from Gene Expression Omnibus, including 3 normal samples, 4 non-ischemic heart failure samples and 4 ischemic samples. Data processing and differential analysis were carried out with packages of R. Cluster analysis was also performed for all the samples to globally observe the difference among the three groups of samples. Interactors of the DEGs were retrieved with Osprey and then networks were constructed. The overlapping part of the network was selected out using Cytoscape, for which functional enrichment analysis was applied with DAVID tools.

RESULTS: A total of 293 and 133 DEGs were obtained for non-ischemic and ischemic heart failure, respectively. Two networks were established and then functional enrichment analysis revealed that "regulation of programmed cell death" was most significantly over-represented in common DEGs.

CONCLUSIONS: Genes differentially expressed in non-ischemic and ischemic heart failure can be biomarkers to distinguish the two types of heart failure. Besides, these genes can be targets to develop treatments.

Key Words:
Heart failure, Differentially expressed gene, Interaction network analysis, Functional enrichment analysis.

Introduction

Heart failure has become a major public health problem worldwide as its incidence is increasing year by year. It can result from a variety of cardiovascular diseases, including sustained pressure overload, myocardial ischemia and infarction, volume overload, as well as congenital and acquired cardiac disease[1].

From the perspective of molecular biology, heart failure is an overload cardiac disease caused by abnormalities of gene expression and regulation[2]. Considerable achievements have been made in disclosing the mechanisms of heart failure. Apoptosis plays a critical role in the development process[3,4]. Narula et al[5] verify the release of cytochrome c from mitochondria and activation of caspase-3 in human cardiomyopathy. Rössig et al[6] report that vitamin C can inhibit endothelial cell apoptosis in congestive heart failure. Oxidative stress contributes to apoptosis[7,8]. Inflammatory mediators (including cytokines, nitric oxide, and endothelin-1) are also involved in the progression[9,10].

With the in-depth study of the pathophysiology of heart failure, the treatment has switched from short-term corrective measures to long-term repair strategies with the aim of changing the biological properties of the heart. Reducing the death of myocardial cells and promoting the growth of new cells (including cell therapy) are two strategies to treat heart failure[11-13]. However, these strategies face big challenges in clinical applications, which verify the necessity of more fundamental researches.

In this study, gene expression data for non-ischemic and ischemic heart failure were compared with that of healthy control to screen out differentially expressed genes (DEGs). Further investigations with interaction network analysis and functional enrichment analysis were carried out to describe these genes in detail.

Materials and Methods

Microarray Data

Microarray data set GSE9128[14] was downloaded from Gene Expression Omnibus, including 3 healthy tissue samples, 4 ischemic tissue samples

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and 4 non- ischemic heart failure tissue samples. Data were collected with GPL96 [HG-U133A] Affymetrix Human Genome U133A Array. Both original data and probe annotation information were also acquired.

Data Pre-Processing and Differential Analysis

Raw data were converted into a recognizable format and then missing values were imputed[15], followed by standardization[16]. Two comparisons were performed: healthy control vs. non-ischemic heart failure and healthy control vs. ischemic heart failure. Differential analysis was conducted with package Linear Models for Microarray Data (limma[17]) of *R* language. p value < 0.05 and |logFC| > 1 were set as the cut-offs to screen out differentially expressed genes (DEGs).

Cluster Analysis

To globally present the difference in gene expression pattern between heart failure and healthy control, cluster analysis was conducted for all the samples[18].

Construction of Interaction Networks

Protein-protein interactions (PPIs) are the basis of biological functions[19] and thus they can provide insights into the underlying mechanisms[20]. In present study, interactors of the DEGs were retrieved with Osprey[21] and two corresponding networks were constructed. Osprey is designed to promote researches about PPIs and protein complexes, which integrates information from BIND[22] and GRID[23] and contains more than 50,000 interactions.

Integration of Interaction Networks

The two networks for ischemic and non-ischemic heart failures were merged with Cytoscape[24] and the overlapping part was selected out.

Functional Enrichment Analysis for Overlapping Network

Functional enrichment analysis can be used to reveal altered biological functions based upon DEGs[25]. Therefore, it was performed for the DEGs in the overlapping network with DAVID[26]. FDR (False Discovery Rate) < 0.05 was set as the cut-off.

Results

Differentially Expressed Genes

The normalized gene expression data are shown in Figure 1. Differential analysis was performed for non-ischemic heart failure vs. healthy

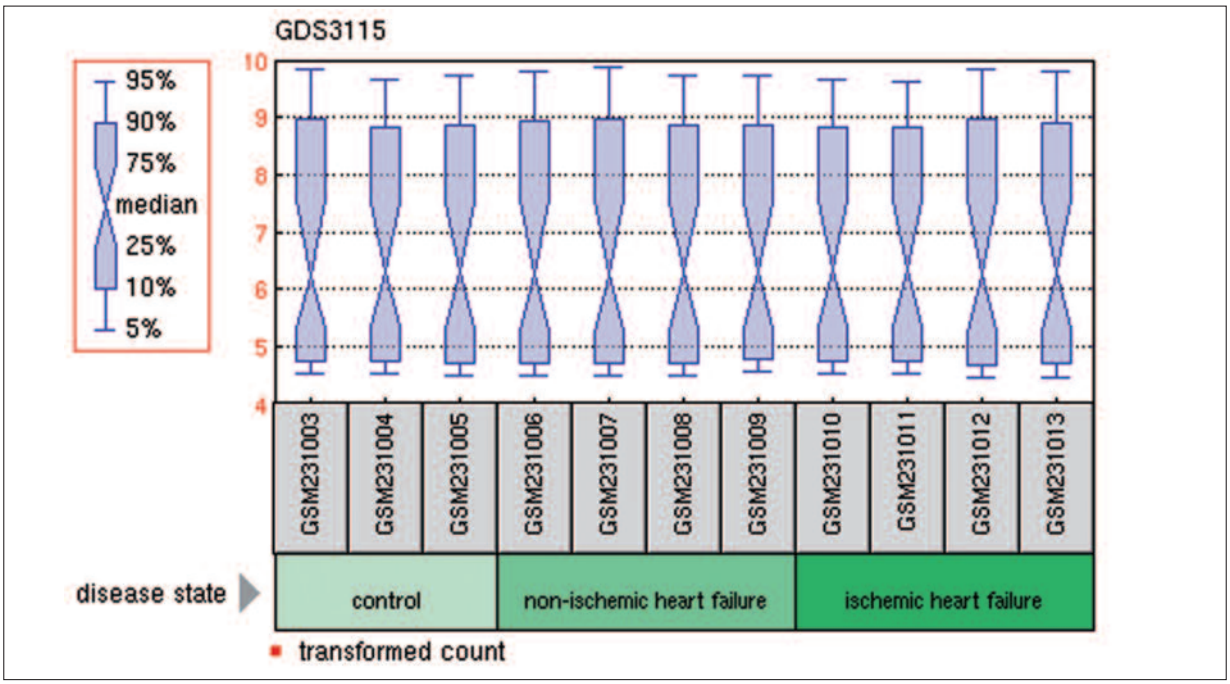

Figure 1. Box plot for normalized gene expression data. The medians are almost at the same level, indicating a good performance of standardization.

control and ischemic heart failure vs. healthy control. According to the criteria (p-value < 0.05 and |logFC| > 1), 293 and 133 DEGs were obtained, respectively.

Cluster Analysis Result

Cluster analysis was performed with gene expression data and the result is shown in Figure 2. There was evident difference among healthy control, non-ischemic heart failure and ischemic heart failure.

Interaction Networks

Interactors of the two groups of DEGs were searched with Osprey and then two corresponding networks were constructed for non-ischemic and ischemic heart failure (Figure 3 A and B).

Integrated Network

The above mentioned two networks were integrated with Cytoscape and the overlapping part was selected out (Figure 4). Genes included in this network was shared by both types of heart failure.

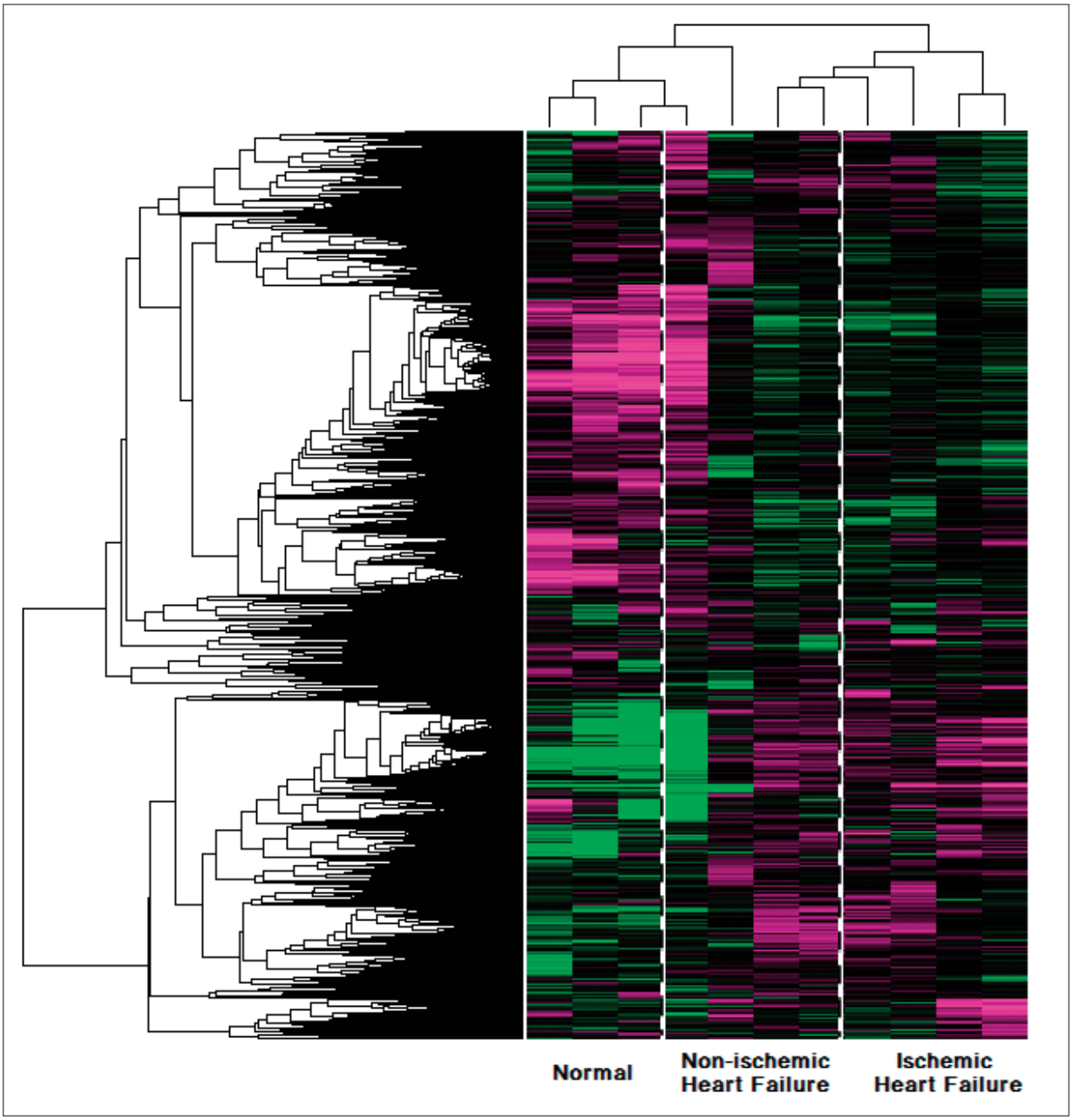

Figure 2. Cluster analysis result for gene expression data. Red indicates high expression while green for low expression level.

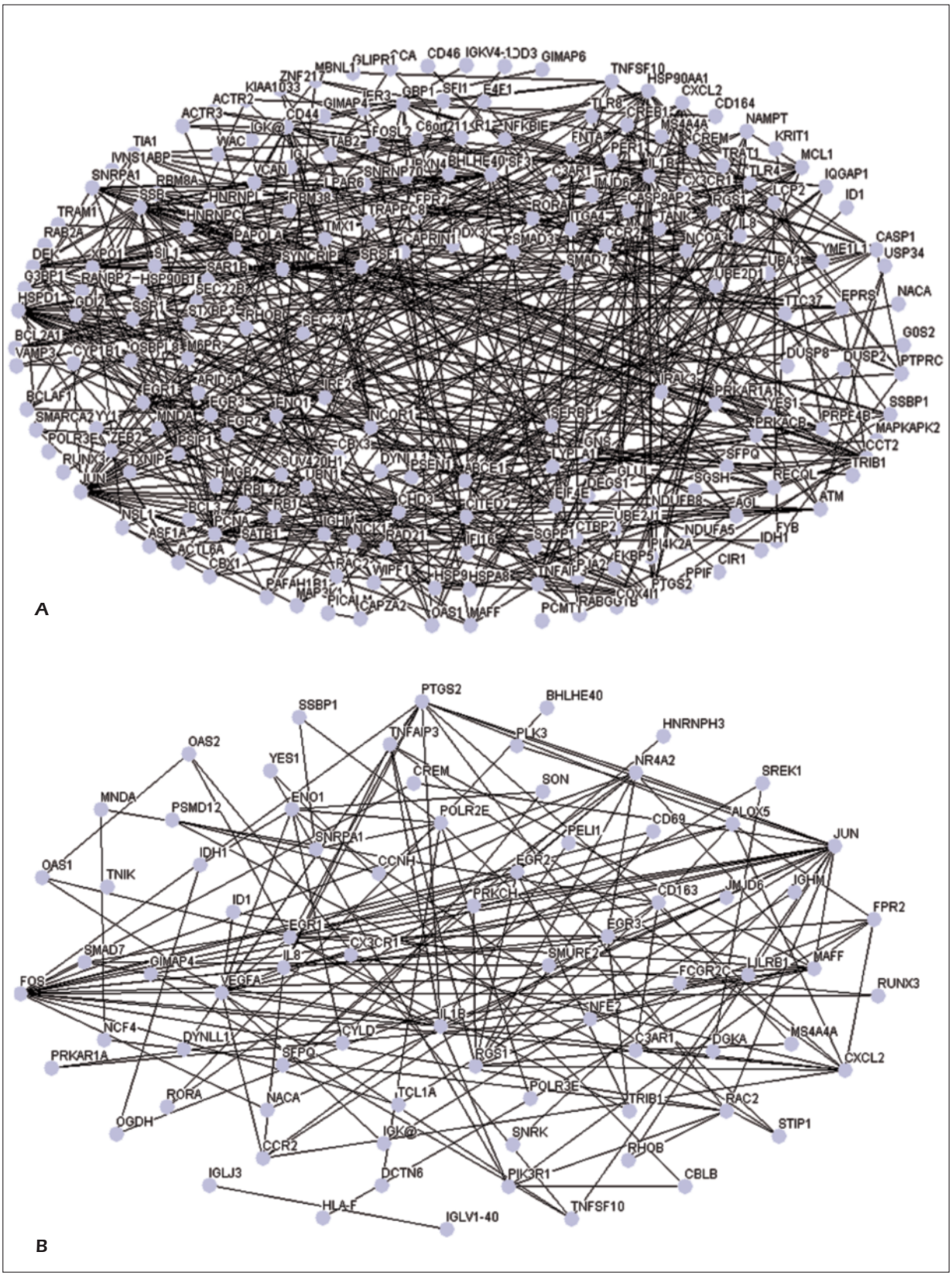

Figure 3. Interaction networks for differentially expressed genes from non-ischemic heart failure ***(A)*** and ischemic heart failure ***(B)*** with Osprey.

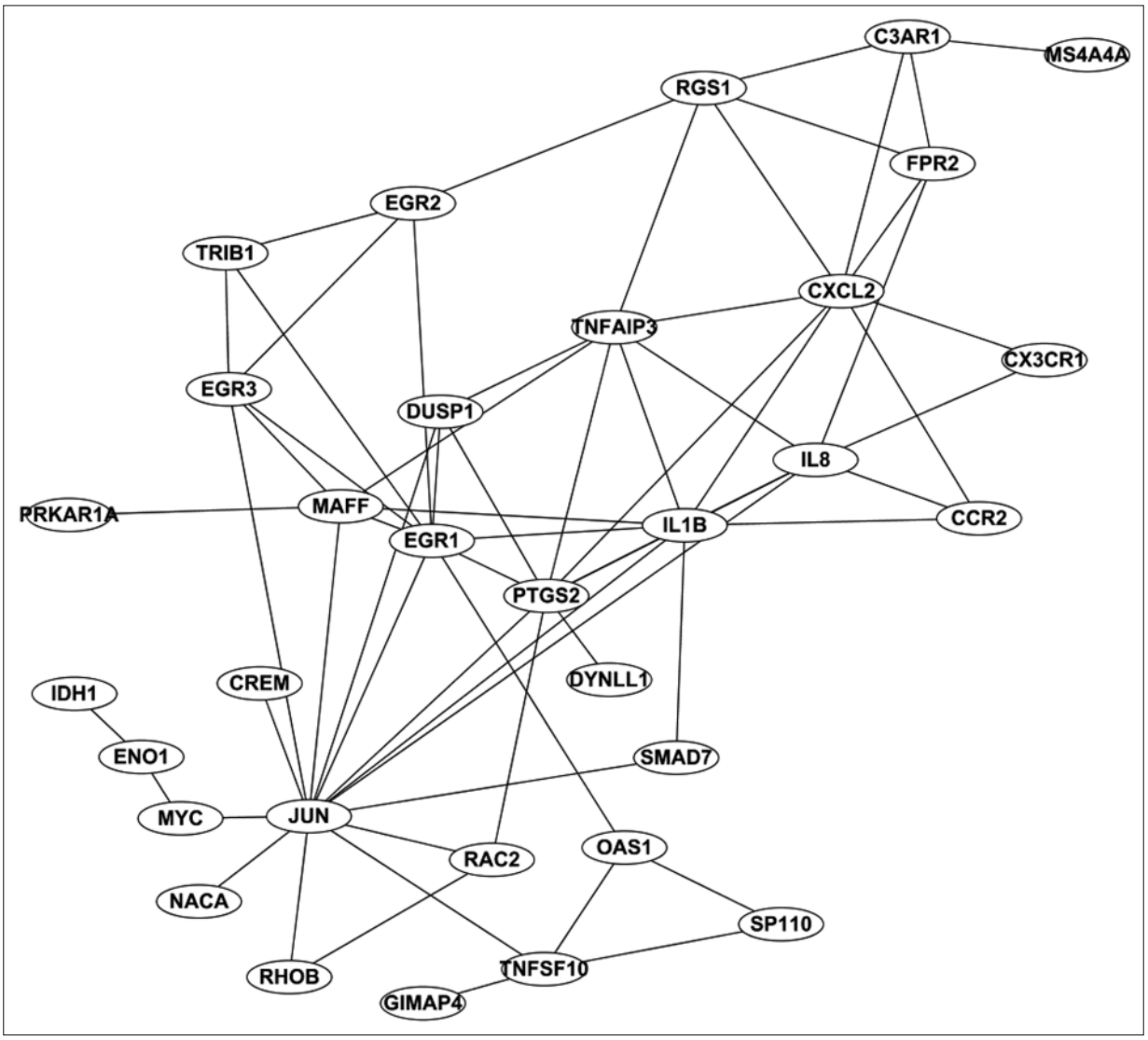

Figure 4. The overlapping part after network integration.

Functional Enrichment Analysis Result

Functional enrichment analysis was applied for the DEGs in the integrated network with DAVID and the result is shown in Table I. A total of 7 terms were significantly over-represented while "regulation of programmed cell death" was the most significant one, including 12 DEGs (IER3, TNFSF10, PTGS2, DUSP1 DYNLL1, JUN, IL1B HSPA1A, TNFAIP3, MYC, RUNX3, CITED2). In additional, the intracellular signaling cascade was

Table I. Functional enrichment analysis result for DEGs in the overlapping network.

Term	Count	*p*-value
GO:0043067~regulation of programmed cell death	12	4.21E-04
GO:0010941~regulation of cell death	12	4.35E-04
GO:0007242~intracellular signaling cascade	12	0.012739
GO:0006355~regulation of transcription, DNA-dependent	15	0.011555
GO:0051252~regulation of RNA metabolic process	16	0.005698
GO:0006350~transcription	20	5.12E-04
GO:0045449~regulation of transcription	22	0.001077

also enriched, including DGKA, RGS1, DUSP1, RAC2, IL8, SMAD7, CREM, PRKAR1A, CCR2, IL1B, RHOB, and TRIB1 genes.

Discussion

In this study, gene expression data for ischemic and non-ischemic heart failure were compared with that of healthy control and a range of DEGs were revealed. These genes could be beneficial in gain understandings about the pathogenesis of heart failure. Interaction networks were constructed for each type of heart failure. To further describe the roles of core genes in the process, the overlapping DEGs were selected out. According to functional enrichment analysis, they were closely associated with regulation of apoptosis.

Our finding was in accordance with previous reports as apoptosis is involved in the development of heart failure[27,28]. Since cardiomyocyte apoptosis is one of the factors constributing to decreased cardiac contractility, researches on the regulatory mechanisms of apoptosis can help to prevent and treat heart failure[29,30]. A total of 12 DEGs were found to be related to apoptosis and some of them might be potential biomarkers. Immediate early response 3 (IER3) regulates Fas- or tumor necrosis factor type alpha-induced apoptosis and increases expression rapidly in response to cellular stresses[31,32]. It's also associated with the physiology of the cardiovascular system. Elevated blood pressure and cardiac hypertrophy after ablation of the IER3 gene are observed[33]. Wittchen et al[34] report the up-regulation of IER3 in human inflammatory cardiomyopathy. Dual specificity phosphatase 1 (DUSP1) plays an important role in the human cellular response to environmental stress[35] as well as in the negative regulation of cellular proliferation[36,37]. Bueno et al[38] confirm that it limits the cardiac hypertrophic response via reducing signaling from all three major MAPK branches. Besides, Sakai et al[39] find that it is up-regulated in mouse heart after single chlorpromazine administration. Runt-related transcription factor 3 (RUNX3) is a member of the runt domain-containing family of transcription factors. Torquati et al[40] find that it inhibits cell proliferation and induces apoptosis by reinstating transforming growth factor beta responsiveness in esophageal adenocarcinoma cells. Cbp/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 2 (CITED2) inhibits transactivation of HIF1A-induced genes by competing with binding of hypoxia-inducible factor 1-alpha to p300-CH1. It is necessary for heart development through a Nodal-Pitx2c pathway[41]. Van Oort et al[42] find that CITED2 is differentially expressed in calcineurin transgenic mice upon myocyte-enhancer factor-2 inhibition, suggesting that it may contribute to contractile dysfunction.

In addition, several genes associated with intracellular signaling cascade were also identified. Regulator of G-protein signaling 1 (RGS1) is a member of the regulator of G-protein signaling family and takes a part in the regulation of G-protein-coupled receptor (GPCR) signaling. Riddle et al[43] indicate that RGS proteins are regulators of cardiovascular physiology and potentially novel drug targets as well. Mittmann et al[44] report that the upregulation of RGS4 in heart failure diminishes Gq/11-mediated signaling and can be involved in the desensitization of Gq/11-mediated positive inotropic effects. Ras-related C3 botulinum toxin substrate 2 (RAC2) regulates a variety of cellular events, such as cell growth and cytoskeletal reorganization. Stockwell[45] finds that patients who have variations in the MRP2, RAC2, and HFE genes have up to a three-fold higher risk of developing heart failure after transplant.

Conclusions

Our study discovered a range of DEGs which could be good directions of future researches aiming to disclose the mechanisms of heart failure. More importantly, several interesting DEGs associated with apoptosis and signal transduction were revealed, which might be targeted to modulate the development process and thus treat the disease ultimately.

Acknowledgements

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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