Observational Study

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Correlation between elevated HCLS1 levels and heart failure

A diagnostic biomarker

Chunguang Li, MD[a], Li Zhang, MD[b,*], Long Zhang, MD[c], Guang Zhang, MD[a]

Abstract

The correlation between hematopoietic cell-specific lyn substrate 1 (HCLS1) expression levels and heart failure (HF) remains unclear. HF datasets GSE192886 and GSE196656 profiles were generated from GPL24676 and GPL20301 platforms in gene expression omnibus (GEO) database and differentially expressed genes (DEGs) were obtained, which was followed by weighted gene co-expression network analysis, protein-protein interaction (PPI) networks, functional enrichment analysis and comparative toxicogenomics database (CTD) analysis. Heatmaps of gene expression levels were plotted. TargetScan was used to screen miRNAs regulating central DEGs. A total of 500 DEGs were found and mainly concentrated in leukocyte activation, protein phosphorylation, and protein complexes involved in cell adhesion, PI3K Akt signaling pathway, Notch signaling pathway, and right ventricular cardiomyopathy. PPI network identified 15 core genes (HCLS1, FERMT3, CD53, CD34, ITGAL, EP300, LYN, VAV1, ITGAX, LEP, ITGB1, IGF1, MMP9, SMAD2, RAC2). Heatmap shows that 4 genes (EP300, CD53, HCLS1, LYN) are highly expressed in HF tissue samples. We found that 4 genes (EP300, CD53, HCLS1, LYN) were associated with heart diseases, cardiovascular diseases, edema, rheumatoid arthritis, necrosis, and inflammation. HCLS1 is highly expressed in HF and maybe its target.

Abbreviations: CTD = comparative toxicogenomics database, DEGs = differentially expressed genes, FC = fold change, FDR = false discovery rate, GEO = gene expression omnibus, GO = gene ontology, GSEA = gene set enrichment analysis, HCLS1 = Hematopoietic Cell-Specific Lyn Substrate 1, HF = heart failure, KEGG = Kyoto Encyclopedia of Genes and Genomes, PPI = protein-protein interaction, WGCNA = weighted gene co-expression network analysis

Keywords: diagnostic biomarker, differentially expressed genes, HCLS1, heart failure

1. Introduction

Heart failure (HF) refers to the inability of heart to effectively pump blood, resulting in body not receiving sufficient oxygen and nutrients. Its mortality rate is very high, causing millions of deaths globally every year. The incidence rate in young people is about 1% to 2%, while in elderly people it is over 10%.[1,2] Various diseases can also increase the incidence of HF. People with hypertension and diabetes have a risk of HF that is about 3 times higher than healthy individuals.[3,4] In young people, rate of HF occurrence is relatively equal between males and females. However, in elderly people, females are about 50% higher than males.[5] And there are also differences in different countries and regions. The incidence of HF varies greatly between different countries and regions. HF is generally more prevalent in high-income countries, while it is more commonly found at lower rates in low-income countries.[6] Smokers have a risk of HF that is about 1.5 times higher than nonsmokers, and people who drink excessively have a risk of HF that is about 2 times higher than those who do not drink or drink in moderation.[7] HF is caused by myocardial cell damage and ischemia that leads to impaired myocardial function. Due to the weakened pumping function of the heart, venous return is obstructed, causing fluid accumulation and retention. This can also affect the systemic circulation of the body, leading to hypoxia and malnutrition in other organs and tissues. The activation of the neuroendocrine system response in the body can further exacerbate HF. Respiratory symptoms may include shortness of breath, difficulty breathing, and coughing. Circulatory symptoms may include palpitations, chest tightness, and edema in the lower extremities. Digestive symptoms may include loss of appetite, nausea, and vomiting. Neurological symptoms may

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

The data in this article are from public databases and are exempt from ethical review.

[a] Clinical Lab Center, Beijing Luhe Hospital, Capital Medical University, Beijing, China, [b] Blood Transfusion Department, Beijing Luhe Hospital, Capital Medical University, Beijing, China, [c] Geriatric Medicine, Beijing Luhe Hospital, Capital Medical University, Beijing, China.

** Correspondence: Li Zhang, Blood Transfusion Department, Beijing Luhe Hospital, Capital Medical University, Beijing 101100, China (e-mail: lizhangli2580@163.com).*

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include insomnia, anxiety, and depression.[8] Heart gradually enlarges due to long-term stress, while the damage to myocardial cells intensifies fibrosis. Due to the decreased diastolic function of the ventricle and decreased cardiac pumping function, the systemic blood flow decreases, causing various tissue and organ ischemia and hypoxia, leading to impaired organ function throughout the body.[9] HF is a serious heart disease that, if left untreated, can lead to life-threatening conditions, severe symptoms, and a decrease in quality of life. Long-term treatment can also bring economic pressure. The causes of HF are not yet clear, in-depth research on molecular mechanisms of HF is important.

Bioinformatics is a powerful tool that can help scientists use computers to study biological data. By analyzing this data, they can understand how organisms work and even find ways to treat diseases. Bioinformatics is a broad study that is important for fields such as medicine, agriculture, and the environment. In conclusion, it not only provides new approaches for biologists, but also revolutionizes the fields of medicine and biotechnology.

Hematopoietic cell-specific lyn substrate 1 (HCLS1) encodes a protein that is involved in regulating proliferation, differentiation.[10] We used a public dataset to find the core genes between HF and healthy tissues through bioinformatics methods and then performed enrichment analysis to find important pathways.

2. Methods

2.1. HF data

HF datasets GSE192886 and GSE196656 profiles (GPl24676, GPl20301) were generated from GEO database (http://www.ncbi.nlm.nih.gov/geo/). GSE192886 included 5 HF, 5 healthy tissue samples and GSE196656 included 3 HF, 3 healthy tissue samples, to explore the differentially expressed genes (DEGs) of HF.

2.2. Remove patch effect

In this paper, we merged GSE192886 and GSE196656 datasets [DOD:10.1186/14721-105-13-335] by R software, then used limma package (version 3.42.2) to remove batch effect to obtain matrix.

2.3. DEGs

We adjusted raw *P* value using Benjamini Hochberg method. Folded change (FC) was calculated using false discovery rate (FDR). Threshold for DEG is $P < .05$, FC > 1.5, mapped volcanoes and obtained DEGs.

2.4. Weighted gene co-expression network analysis (WGCNA)

Scale-free co-expression networks were generated by R-package WGCNA. Set β value parameter to select for strong correlation between genes, classify genes with similar expression profiles into gene modules, perform average linkage hierarchical clustering, have a minimum size (gene set) of 30 gene treemaps. Set sensitivity to 3. A number of important modules were obtained, and a list of genes in modules was selected.

2.5. Protein-protein interaction (PPI) networks

PPI network formed by STRING database (confidence level > 0.4) was visualized and predicted core genes by Cytoscape software, calculated the best correlated 10 genes by 2 algorithms (MCC, MNC), respectively.

2.6. Functional enrichment analysis

DEGs screened by Venn diagram were entered into KEGG API (https://www.kegg.jp/kegg/rest/keggapi.html). Latest KEGG pathway gene annotations were acquired to map genes into background set for enrichment analysis using R-package cluster profile (version 3.14.3) to obtain results of gene set enrichment. GO annotations of genes from R-package org.hs.e.g..db (version 3.1.0). Map genes into background set as background, minimum gene set was 5, maximum gene set was 5000, *P* value < .05, FDR < 0.25. Metascape database (http://metascape.org/gp/index.html) was also used as an auxiliary analysis.

2.7. Gene set enrichment analysis (GSEA)

GSEA software (version 3.0) was downloaded from GSEA website (DOI: 10.1073/pnas.0506580102, http://software.broadinstitute.org/gsea/index.jsp). Samples were divided into

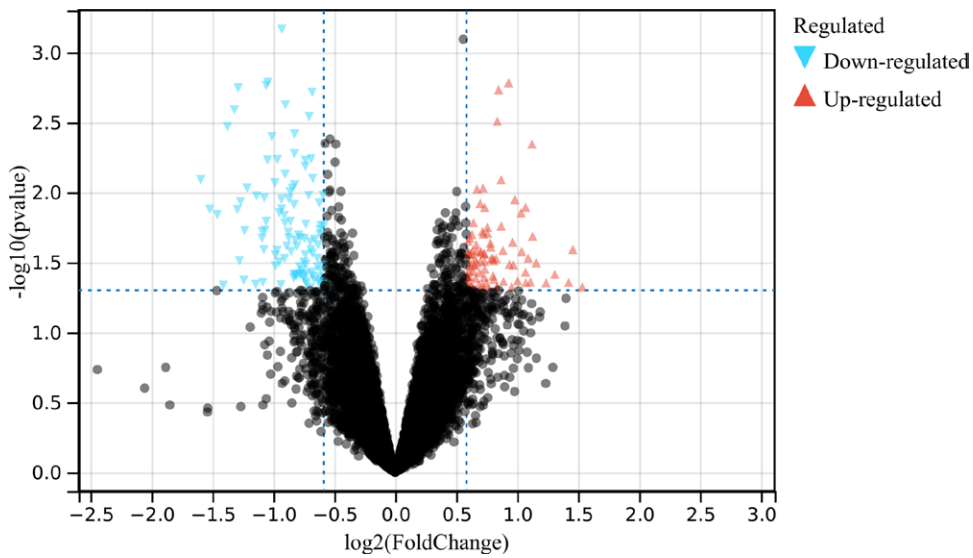

Figure 1. Analysis of differentially expressed genes (DEGs). 500 DEGs were identified.

HF and normal tissues, and molecular signature database was established (DOI: 10.1093/bioinformatics/btr260). We downloaded c2.cp.kegg.v7.4.symbols.gmt subset to evaluate relevant pathways and molecular mechanisms based on gene expression profiles and phenotypic grouping, setting minimum gene set of 5, maximum gene set of 5000, 1000 resampling, P value < .05, FDR < 0.25. GOKEGG analyses were performed genome-wide and formulated by GSEA.

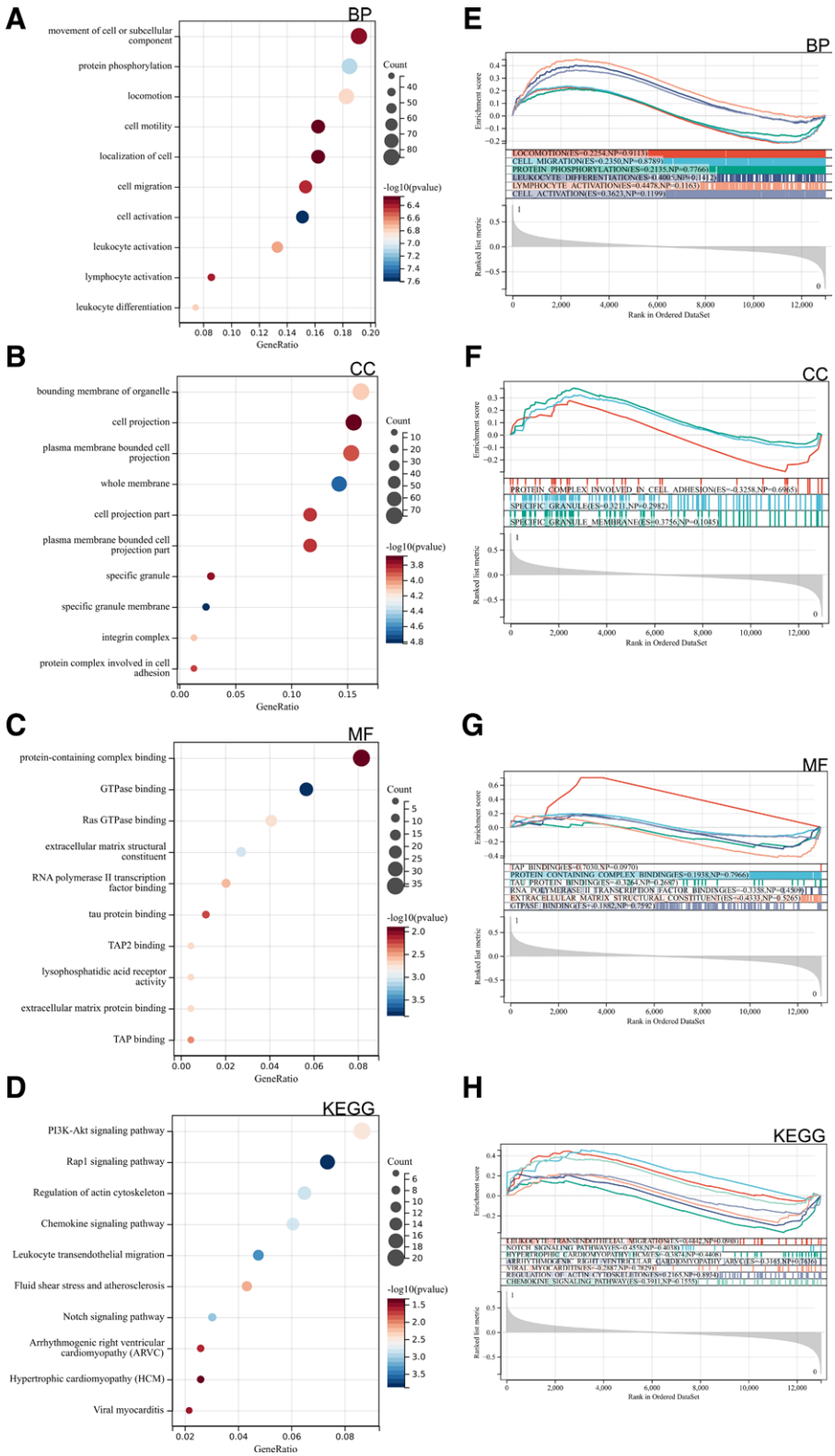

Figure 2. Functional enrichment analysis. (A, B, E, F) GO and KEGG. (C, D, G, H) GSEA.

2.8. Gene expression heatmap

R-package heatmaps were used to visualize differences of core genes between HF and healthy samples in GSE192886 and GSE196656.

2.9. Comparative toxicogenomics database (CTD) analysis

Bringing together information from fields of toxicology and genomics, CTD provides a wide variety of data that allows scientists to better understand how chemicals affect living organisms, as well as, health effects that may occur. We searched this site for core genes to discover disease information associated with them, and then used Excel to map the results.

2.10. miRNA

TargetScan can predict interaction between microRNAs (miRNAs) and mRNA targets. We used it to determine which miRNAs affect the expression levels of core genes.

3. Results

3.1. Analysis of DEGs

Following set threshold, a total of 500 DEGs were found in matrix of GSE192886 and GSE196656 (Fig. 1).

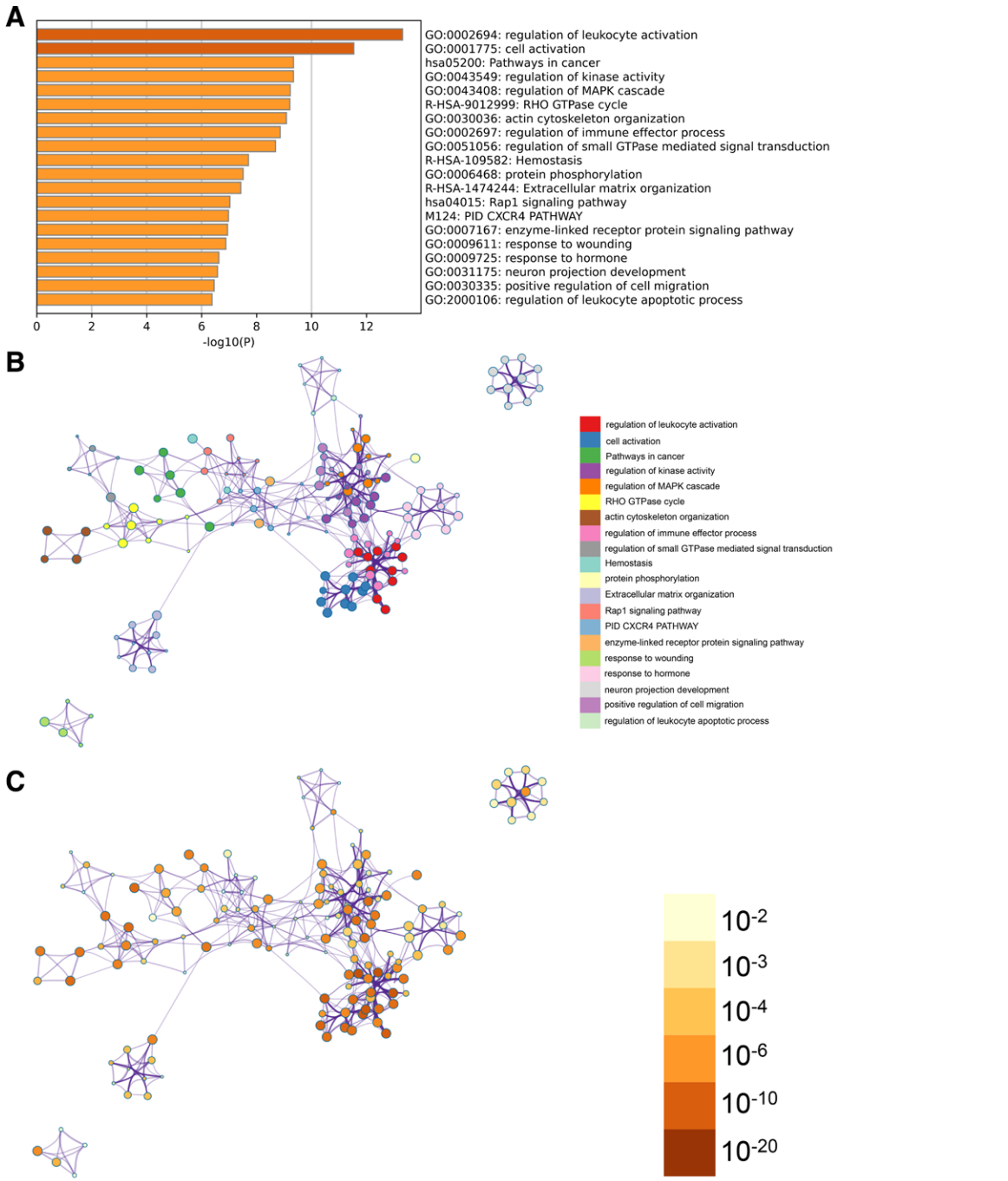

Figure 3. Metascape enrichment analysis. (A) Cancer pathways, regulation of immune effector processes, rapl signaling pathway, PID CXCR4 pathway were visible in go enrichment items in the enrichment items of metascape. (B)The enrichment network colored by enrichment term. (C) The enrichment network colored by *P* value.

3.2. Functional enrichment analysis

3.2.1. GO KEGG. In GO analysis, DESs mainly focused on leukocyte activation, protein phosphorylation, protein complexes involved in cell adhesion. In KEGG analysis, they mainly focused on PI3K Akt signaling pathway, rapl signaling pathway, Notch signaling pathway, right ventricular cardiomyopathy (Fig. 2A, B, E, F).

3.2.2. GSEA. Results of GSEA enrichment were similar to those of GOKEGG. DEGs focus on protein phosphorylation, leukocyte differentiation, notch signaling pathway, Notch signaling pathway, right ventricular cardiomyopathy, and protein complex involved in cell adhesion (Fig. 2C, D, G, H).

3.2.3. Metascape analysis. Cancer pathways, regulation of immune effector processes, rapl signaling pathway, PID CXCR4 pathway were visible in GO enrichment results of metascape (Fig. 3A). At the same time, network diagram and *P* value coloring map of enrichment results are also output to show correlation and confidence of enrichment items (Figs. 3B, C and 4).

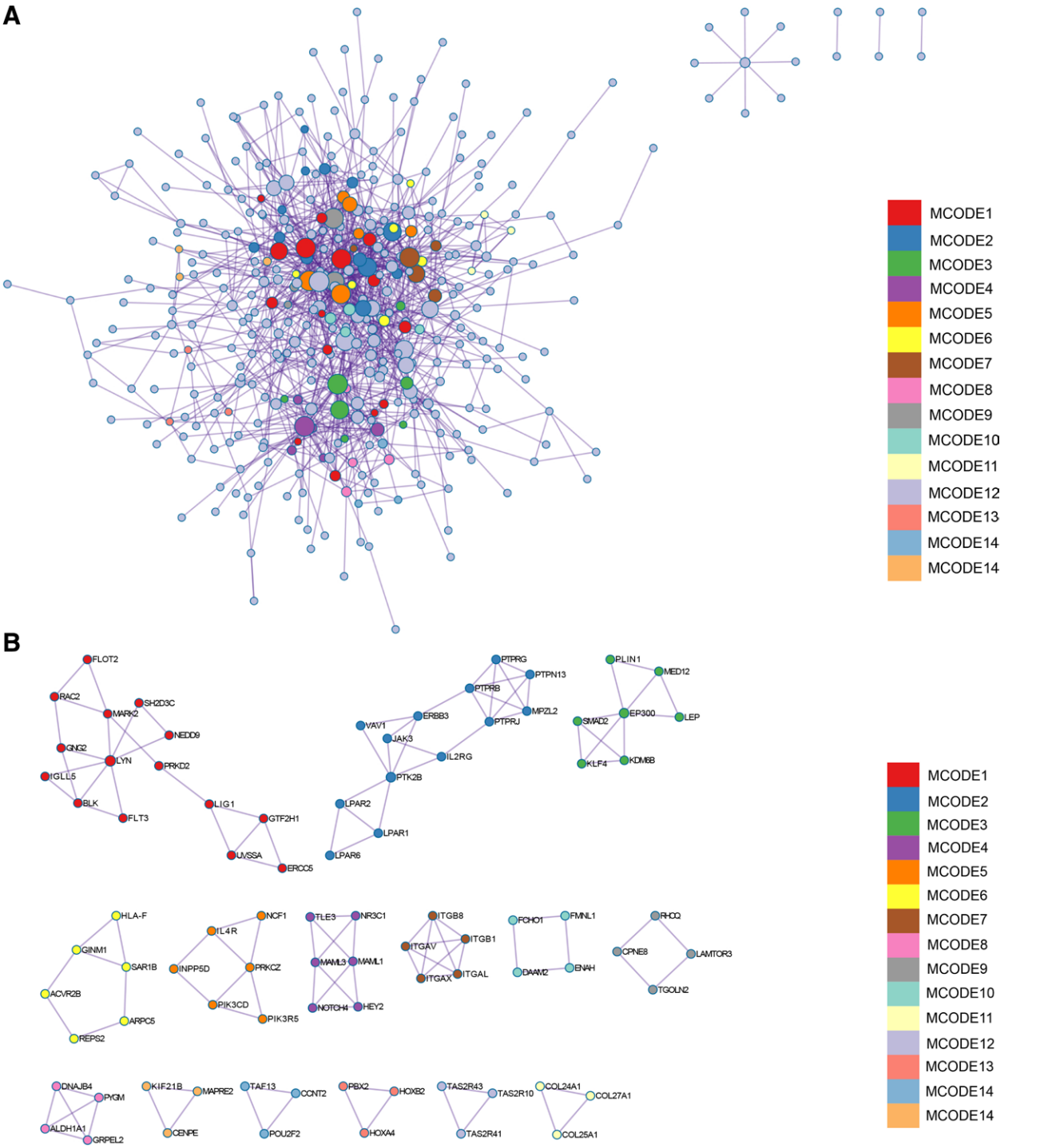

Figure 4. Metascape enrichment analysis.

3.3. WGCNA

Soft threshold power in WGCNA was set as 9 (Fig. 5A and B). We generated gene-level clustering tree to obtain the core modules (Fig. 5C). We calculated expression correlation of module eigenvectors with genes, according to cutoff criteria (|MM| > 0.8). Modules with a distance of <0.25 were merged, obtaining 30 co-expression modules. Interactions between these modules were then analyzed (Fig. 5D). We generated correlation heatmaps of modules and phenotypes (Fig. 5E) and GS-MM correlation scatter plots (Fig. 5F and G).

3.4. PPI networks

We imported DEGs into Cytoscape for visualization (Fig. 6A), using 2 algorithms to calculate hub genes (Fig. 6B and C), 15 genes (hcls1, fermt3, CD53, CD34, Itgal, ep300, lyn, Vav1, itgax, LEP, itgb1, IGF1, MMP9, Smad2, Rac2) were obtained. In PPI network previously generated in metascape, core genes (hcls1, fermt3, CD53, CD34, Itgal, ep300, lyn, Vav1, itgax, LEP, itgb1, IGF1, MMP9, Smad2, Rac2) were obtained. There are similarities between the 2 results, which further increase credibility of the results.

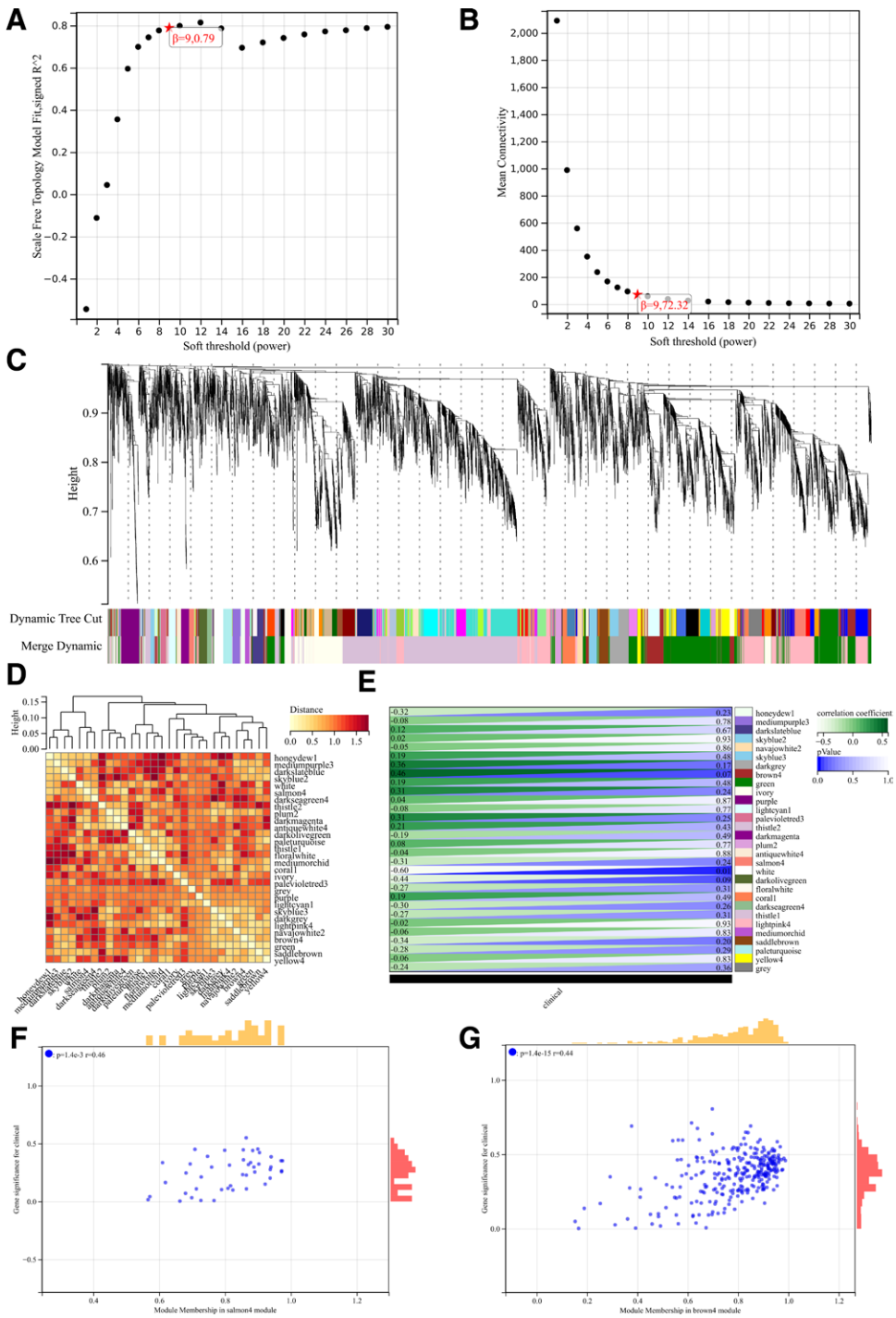

Figure 5. WGCNA. (A, B) The soft threshold power in the WGCNA was set to 9. (C) A hierarchical clustering tree of all genes was constructed, and significant modules were generated. (D) Interactions between these modules were analyzed. (E) Generated module to phenotype correlation heatmaps. (F, G) GS to MM correlation scatter plots for the associated hub genes. WGCNA = weighted gene co-expression network analysis.

3.5. Gene expression heatmap

We derived visual expression difference Heat map between HF and healthy tissue, 4 core genes (ep300, CD53, hcls1, lyn) were highly expressed in HF tissue samples, lowly expressed in healthy tissue samples. It may have a modulating effect on HF (Fig. 7).

3.6. CTD analysis

In CTD results, 4 genes (ep300, CD53, hcls1, lyn) were found to be associated with heart disease, CVD, arthritis rheumatoid, necrosis, and inflammation (Fig. 8).

3.7. miRNAs analysis

We found that related miRNA of ep300 gene is hsa-mir-150-5p; Related miRNAs of CD53 gene are hsa-mir-325-3p; Related miRNAs of lyn gene are hsa-mir-30c-5p, hsa-mir-30b-5p, hsa-mir-30a-5p (Table 1).

4. Discussion

HF is a serious chronic disease that can lead to the heart inability to effectively pump blood to various parts of the body, thereby affecting normal body function. It can cause significant harm to a patient life safety, quality of life, and economic burden. The molecular mechanisms of HF are very complex and involve many changes at the cellular and molecular levels. The balance of calcium ions in cardiac cells is compromised, resulting in mechanisms of calcium ion disorder such as calcium ion leakage, impaired calcium ion clearance, reverse transport of calcium ions, and changes in calcium ion sensitivity. These can cause abnormalities in the contraction and relaxation functions of cardiac cells, leading to the occurrence and development of HF.[11,12] Inflammation causes increase in concentrations of inflammatory mediators in blood of HF patients. These mediators mainly involved in HF through mechanisms such as promoting cardiac fibrosis, causing damage to vascular endothelial function, and activating the neuroendocrine system.[13,14] The release of many harmful substances and intracellular contents from apoptotic cardiomyocytes can lead to exacerbation of the inflammatory response and further acceleration of cardiomyocyte death, thereby worsening the condition of HF.[15] In HF patients, autophagic process of myocardial cells is often inhibited, leading to the accumulation of aging proteins and damage to cell function. Myocardial cell autophagy also affects severity and prognosis of HF.[16] Heart needs to consume large amounts of ATP Energy metabolism abnormalities, such as mitochondrial dysfunction, accumulation of metabolic waste, increased oxidative stress,

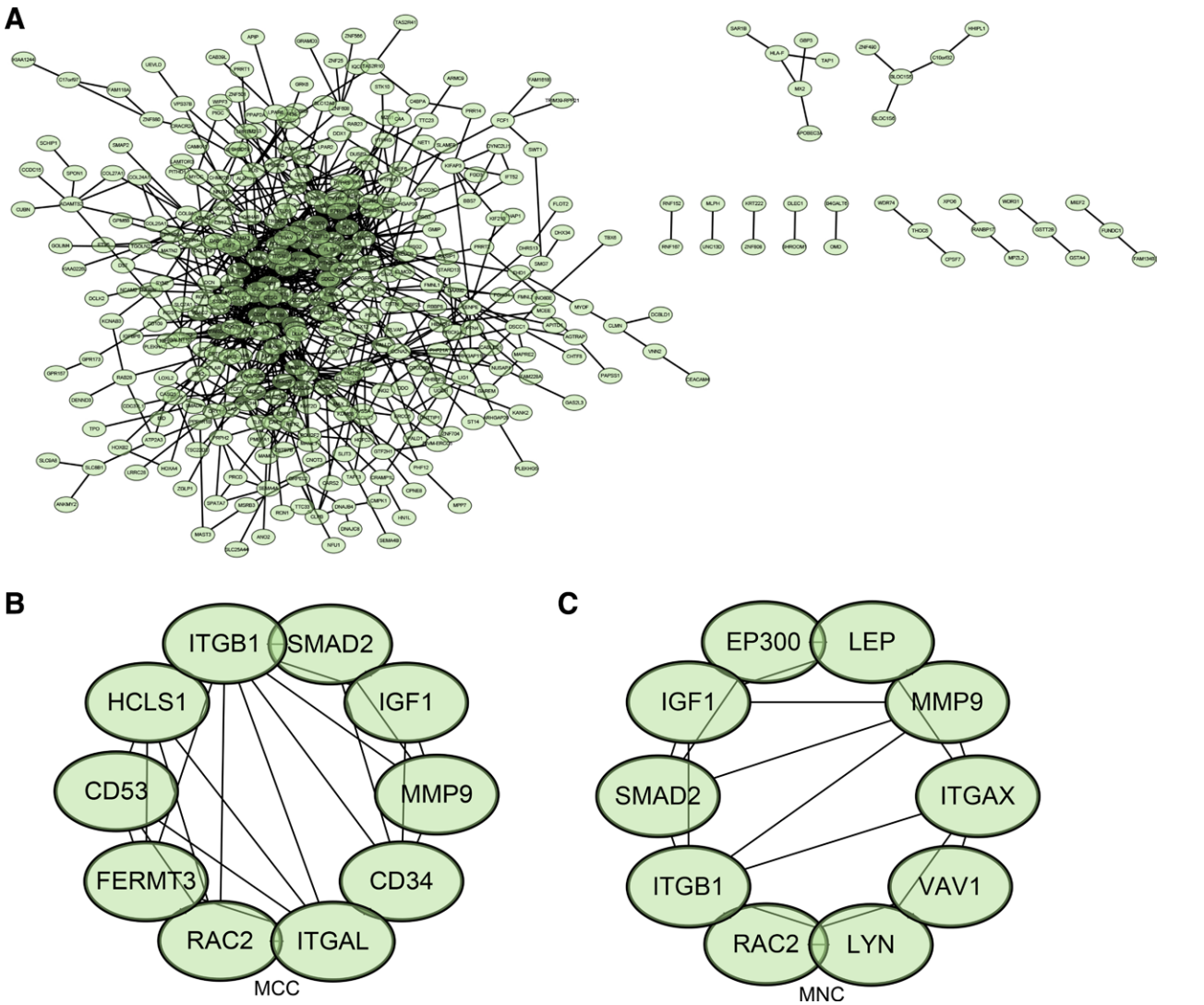

Figure 6. Construction and analysis of protein-protein interaction (PPI) networks. (A) The PPI network. (B, C) Two different algorithms to identify hub genes.

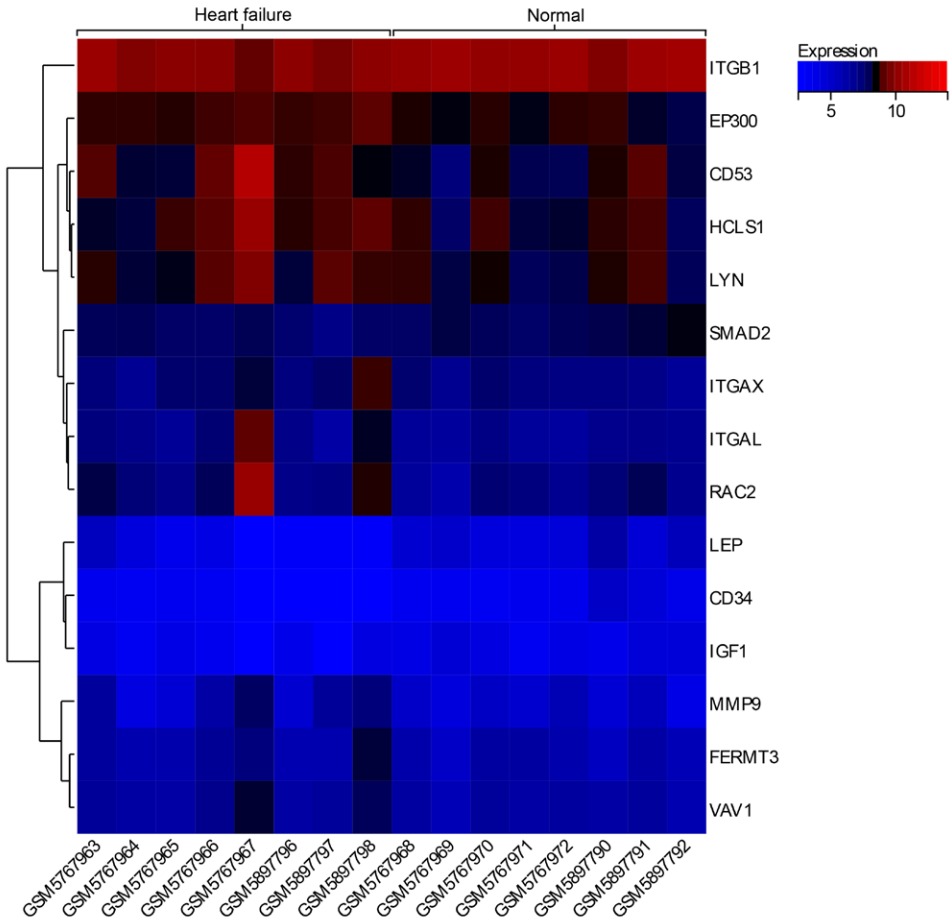

Figure 7. Gene expression heatmap. 4 core genes (ep300, CD53, hcls1, lyn) were highly expressed in the HF tissue samples and lowly expressed in the normal tissue samples.

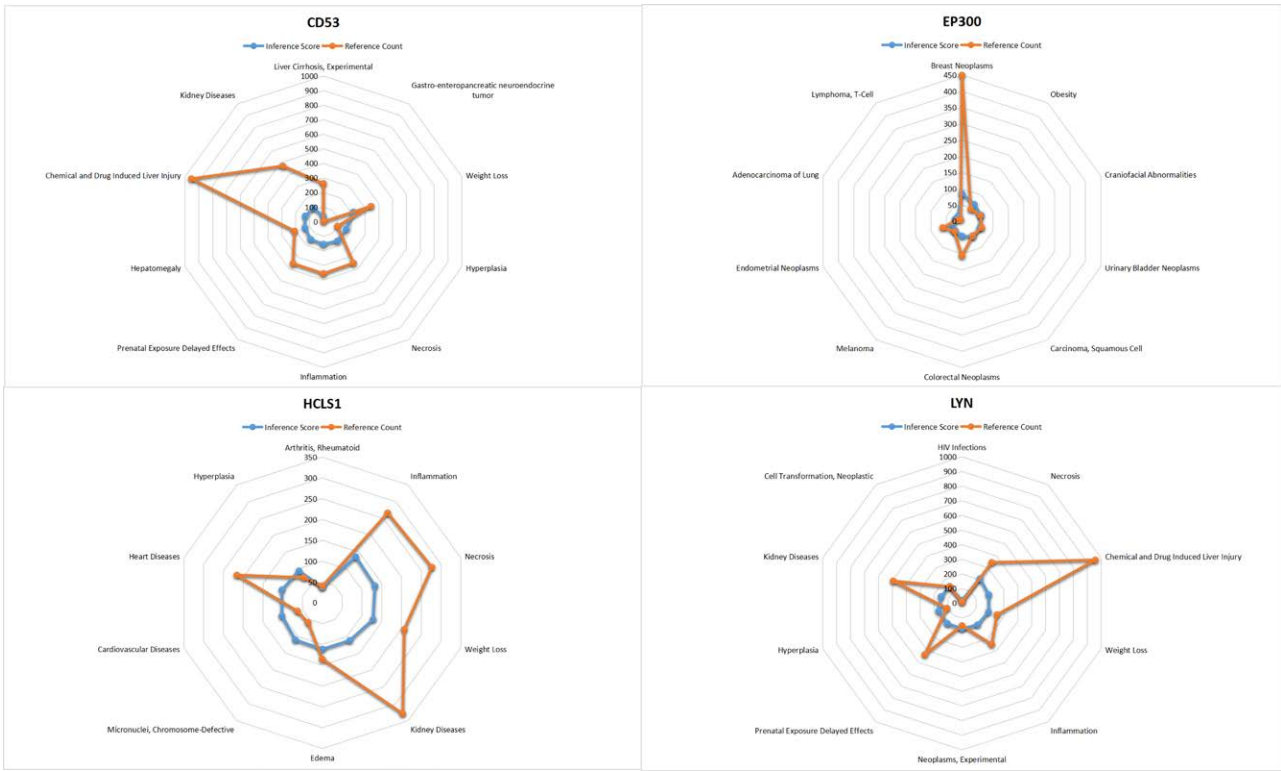

Figure 8. CTD analysis. Four genes (ep300, CD53, hcls1, lyn) were found to be associated with heart disease, CVD, edema, arthritis rheumatoid, necrosis, inflammation.

Table 1

A summary of miRNAs that regulate hub genes.

	Gene	MIRNA		
1	EP300	hsa-miR-150-5p		
2	CD53	hsa-miR-325-3p		
3	LYN	hsa-miR-30c-5p	hsa-miR-30b-5p	hsa-miR-30a-5p
4	HCLS1	none		

and decreased activity of glyceraldehyde-3-phosphate dehydrogenase, can lead to HF.[17,18] Myocardial cell hypertrophy can lead to changes in membrane potential and increased electrophysiological instability, promoting the occurrence of arrhythmias, and can also cause mitochondrial dysfunction, resulting in reduced intracellular ATP synthesis.[19] Enlargement and injury of myocardial cells promote extracellular matrix synthesis and secretion in the cardiac extracellular matrix, leading to the accumulation and fibrosis of the cardiac extracellular matrix, which results in a decrease in cardiac tissue contractile function. The increase in end-diastolic volume of the ventricle also changes the interaction between myocardial cells and the extracellular matrix, affecting signal transduction and regulation in myocardial cells.[20,21]

Autoimmune abnormalities in HF have been a mechanism of increasing interest in recent years. The production of autoantibodies is an important manifestation of the autoimmune abnormalities in HF. Various autoantibodies against myocardial cells, mitochondria, and ribosomal proteins can be detected in the serum of HF patients. These autoantibodies can affect the function of myocardial cells in a variety of pathways The autoimmune abnormalities in HF may be related to various factors such as infection, tumors, and inflammation. Due to abnormal changes in the immune system, HF patients are prone to complications such as myocarditis and myocardial fibrosis, which can lead to worsening of HF. HF patients experience a series of abnormal changes in their immune system, including the production of autoantibodies, release of cytokines.[22,23] T cells also participate in occurrence of autoimmune abnormalities in HF. T cells in HF patients can be activated and release various cytokines. These cytokines can directly damage myocardial cells, or indirectly affect the function of myocardial cells by inducing the production of autoantibodies. Targeted drugs have emerged for the molecular mechanisms of HF, such as inhibitors of myocardial cell calcium ion channels, inhibitors of inflammatory factors, inhibitors of myocardial cell apoptosis, etc. The development and application of these targeted drugs can make the treatment more precise and individualized, thus improving the therapeutic effect.[24] HCLS1 is overexpressed in HF, higher expression of HCLS1, worse the results.

HCLS1 can function in various cell types. HCLS1 protein is a non-tyrosine kinase substrate highly expressed in hematopoietic cells. It is a substrate protein in the Lck/Yes signaling pathway that can activate lyn tyrosine kinase by binding to the SH2 domain. HCLS1 is implicated in many biological processes, such as signal transduction, cell polarity, movement, adhesion in immune cells, and macrophages.[25,26] Evidence of involvement of HCLS1-associated protein X-1 in pathogenesis of heart disease has also been found.[27] It has also been shown that the central gene HCLS1, in conjunction with other genes, maybe a targeted therapeutic approach for chronic chagasic cardiomyopathy.[28] Therefore, HCLS1 may affect calcium signaling, intracellular signaling pathway regulation, cardiac contraction and relaxation function, etc. The absence of HCLS1 may lead to abnormalities in these processes, ultimately leading to HF.[29,30]

Above studies also support our view that HCLS1 may affect cardiomyocyte apoptosis, myocardial fibrosis, myocardial cell energy metabolism disorder. Higher expression of HCLS1 in HF, worse the results.

However, the study has some flaws, we did not overexpress the gene or knock down the gene in animals, so the specific function of the gene has not been verified. We should also make an in-depth and comprehensive exploration of this issue in the future.

5. Conclusion

Based on the medical research reviewed, it can be concluded that HCLS1 is highly expressed in HF, higher levels of HCLS1 mean worse outcomes.

Author contributions

Conceptualization: Chunguang Li.
Data curation: Li Zhang, Long Zhang.
Formal analysis: Li Zhang, Guang Zhang.
Methodology: Chunguang Li, Long Zhang.
Software: Li Zhang.
Validation: Chunguang Li, Guang Zhang.
Visualization: Chunguang Li, Li Zhang, Long Zhang, Guang Zhang.
Writing – review & editing: Chunguang Li.
Writing – original draft: Li Zhang.

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