Original Research

Bioinformatics analysis of the genes associated with co-occurrence of heart failure and lung cancer

Xiaoying Wang[1,2], Rui Song[3], Xin Li[4], Kai He[1,2], Linlin Ma[1] and Yanfei Li[1,2]

[1]Shanghai University of Medicine and Health Sciences Affiliated Zhoupu Hospital, Shanghai 201318, China; [2]Graduate School, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China; [3]Xuhui District Center for Disease Prevention and Control, Shanghai 200237, China; [4]Cardiovascular Medicine Department, East Hospital Affiliated to Tongji University, Shanghai 200120, China
Corresponding authors: Yanfei Li. Email: liyf@sumhs.edu.cn; Linlin Ma. Email: linlinma1986@gmail.com

Impact Statement

The co-existence of lung cancer (LC) in patients with heart failure (HF) has become increasingly common. This study first aimed to highlight the new understanding of this co-existence. The association with common pathophysiological risk factors should be emphasized in addition to cancer treatment-related cardiotoxicity leading to HF. Second, among the nine validated hub genes, the four shared validated genes including *SULF1*, *C1orf162*, *VSIG4*, and *LYVE1* have the potential to be biomarkers for the co-morbidity of them. Finally, functional analysis was performed with particular emphasis on extracellular matrix organization and regulation of leukocyte activation which suggests the basis of the co-pathogenesis of HF and LC is possibly attributed to the disorders of the immune system caused by these shared verified genes. And these genes provide opportunities to further define the underlying mechanisms and develop crossover strategies and therapeutic approaches to against the both pathologic states.

Abstract

Deaths of non-cardiac causes in patients with heart failure (HF) are on the rise, including lung cancer (LC). However, the common mechanisms behind the two diseases need to be further explored. This study aimed to improve understanding on the co-occurrence of LC and HF. In this study, gene expression profiles of HF (GSE57338) and LC (GSE151101) were comprehensively analyzed using the Gene Expression Omnibus database. Functional annotation, protein–protein interaction network, hub gene identification, and co-expression analysis were proceeded when the co-differentially expressed genes in HF and LC were identified. Among 44 common differentially expressed genes, 17 hub genes were identified to be associated with the co-occurrence of LC and HF; the hub genes were verified in 2 other data sets. Nine genes, including *ALOX5*, *FPR1*, *ADAMTS15*, *ALOX5AP*, *ANPEP*, *SULF1*, *C1orf162*, *VSIG4*, and *LYVE1* were selected after screening. Functional analysis was performed with particular emphasis on extracellular matrix organization and regulation of leukocyte activation. Our findings suggest that disorders of the immune system could cause the co-occurrence of HF and LC. They also suggest that abnormal activation of extracellular matrix organization, inflammatory response, and other immune signaling pathways are essential in disorders of the immune system. The validated genes provide new perspectives on the common underlying pathophysiology of HF and LC, and may aid further investigation in this field.

Keywords: Heart failure, lung cancer, co-occurrence, differentially expressed genes, hub genes, bioinformatics analysis

***Experimental Biology and Medicine* 2023; 248: 843–857. DOI: 10.1177/15353702231162081**

Introduction

Heart failure (HF) is a complex clinical syndrome,[1] which often occurs in combination with other diseases such as atherosclerosis, diabetes mellitus, and atrial fibrillation. However, a large proportion of the non-cardiovascular deaths in these patients are due to cancer;[2] nevertheless, this area has been relatively overlooked. As one of the most common diseases, lung cancer (LC) is also one of the leading causes of death among cancer patients worldwide.[3,4] The mortality associated with many types of cancer has reduced substantially due to recent improvements in cancer treatments; however, the co-morbidity burden of oncology patients has been increasing concomitantly. Cardiovascular disease is reported to be the most common cause of non-cancer death in patients with cancer, in whom there is an increased risk of HF events.[5] As this is mainly due to the cardiotoxicity of anticancer drugs and/or radiotherapy,[6] it is essential that the relationship between the two diseases is investigated in sufficient detail.

An accumulating body of evidence from experiments and clinical trials reveals numerous common features between the biological mechanisms underlying HF and cancer.[7] In one study, patients with HF were found to have a higher risk for developing cancer compared to the controls; among the site-specific cancers, LC demonstrated a consistently higher cumulative incidence in patients with HF.[8] In addition to the experimental and clinical evidence, studies have identified common risk factors linking cancer with cardiovascular disease; these include aging and an unhealthy lifestyle (e.g. smoking, unhealthy diets, physical inactivity, obesity, and alcohol misuse).[9] A well-known and modifiable risk factor for HF and LC is smoking.[10] These findings indicate a little-known but close association between HF and LC.

One research suggested that cancer is becoming an increasingly common threat in patients with HF.[5] The search for ways to address the co-occurrence of HF and LC should encourage and include a multidisciplinary approach, such as close communication between the fields of cardiology and oncology. However, the scientific evidence to support clinical decision-making is considerably limited. To overcome these challenges, two gene expression data sets were obtained from the Gene Expression Omnibus (GEO) database and analyzed to determine the common differentially expressed genes (DEGs) and their functions in HF and LC. Finally, four genes with the same expression trend were selected after validating hub genes in the other two data sets. The genes that were identified to link HF and LC may contribute to offer unknown evidence to reveal the biological mechanisms underlying the co-morbidity of the two diseases.

Materials and methods

Data source

As a publicly available database, the GEO (http://www.ncbi.nlm.nih.gov/geo/)[11] includes abundant genome sequencing data sets submitted by researchers. Search for relevant gene expression data sets from GEO using HF and LC as keywords. The included data sets should meet the following criteria: the two used expression profiles were not from the different sequencing platform and the included test samples are of human origin. Data sets containing the largest sample size were preferentially selected. Finally, two data sets (GSE57338 and GSE151101) were obtained (Affymetrix GPL11532 platform, Affymetrix Human Gene 1.1 ST Array). The GSE57338 data set contained 177 HF and 136 non-HF samples, respectively. The GSE151101 data set was generated from pulmonary tumor and matched unaffected (henceforth referred to as normal lung tissue) tissue and contained a total of 237 samples.

GEO2R web application for identifying DEGs

GEO2R (www.ncbi.nlm.nih.gov/geo/ge2r/), based on R language "LIMMA" package, is a function recently updated by the GEO.[12] It is used to implement sophisticated analysis of GEO data for calculating and visualizing the differential expression multiple. GEO2R could filter gene expression in a timely and more flexible manner for determining the DEGs between the samples of control and diseased groups. Only those genes that met the following selection criteria were as follows: adjusted P values < 0.05 and fold change (FC) $\geqslant 1.5$ were identified as DEGs. The common DEGs were filtered by the Sangerbox (http://vip.sangerbox.com/login.html) which is an online diagram tool.

Enrichment analyses of DEGs

Metascape (https://metascape.org/gp/index.html#/main/step1) is an effective and efficient tool for gene function annotation analysis, which combines gene annotation, pathway enrichment, membership analysis, and gene-related protein network analysis. Analysis reports with analytical resources and comprehensive gene list annotations are provided to experimental biologists through the fast one-click Express analysis interface. The gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichments of DEGs were conducted using the Metascape. Adjusted P values of <0.01 were considered significant. Terms with adjusted P values <0.01, an enrichment factor >1.5, and a minimum count of 3 were bridged and classified into clusters based on their high clustering similarities.

Protein–protein interaction network establishment

The STRING can be accessed online at https://string-db.org/. It can build a protein interaction network for researchers to integrate the connections between proteins, including the direct binding relationship and the relationship between indirect pathways.[13] The life of a cell partly depends on a complex web of functional interactions between biological molecules. Among these interactions, protein–protein interactions (PPIs) hold a particularly important position because of their versatility, specificity, and adaptability. A composite score greater than 0.4 was considered statistically significant when using STRING to search for interaction evidence.

Filtering and analysis of hub genes

Cytoscape (http://www.cytoscape.org) is a widely sourced and powerful online platform for network layout and query, for visualizing the integration of the network with other molecular states, and connecting the network to databases of functional annotations using straightforward plug-in architecture.[14] Five common algorithms (Stress, Betweenness, Degree, Maximum Neighborhood Component, and Maximal Clique Centrality) were applied to evaluate and screen hub genes using the cytoHubba plug-in of Cytoscape. The Molecular Complex Detection plug-in of Cytoscape was applied to identify densely connected network components.

Construction of a co-expression network

These co-expression networks of hub genes were constructed using GeneMANIA (http://www.genemania.org/). GeneMANIA is a reliable tool for speculating the gene function, analyzing gene lists, and performing functional assays and ranking of hub genes.[15]

Validation of hub gene expression

The GSE76701 and GSE118370 data sets were applied to confirm the expression of the selected hub genes. The GSE76701

data set consists of four HF and non-HF samples each. GSE118370 consists of six pulmonary tumor tissue and normal lung tissue samples each. The expression level of the obtained hub genes was initially tested for normality. If the gene expression met the normality test, the two sets of data were compared using the *t*-test; the non-parametric test was used in other cases. *P* values of <0.05 were considered significant.

Results

Identification and analysis of DEGs

The flowchart of this research is presented in Figure 1. DEGs (313 in GSE57338 and 1360 in GSE151101) were identified using the GEO2R web application (Figure 2(A) and (B)). On considering the intersection of the Venn diagram, 85 DEGs shared by HF and LC were obtained (Figure 2(C)). Genes with different expression trends in GSE57338 and GSE151101 were subsequently excluded (Figure 2(D) and (E) and Supplementary Table S1) to obtain 8 and 36 up- and down-regulated DEGs, respectively. A total of 44 genes were common between HF and LC and had the same expression trend; these were associated with the co-occurrence of LC and HF.

Functional enrichment analyses of common DEGs

Biological functions and pathways of 44 common DEGs were enriched for GO and KEGG pathway enrichment analysis using Metascape. This was useful in revealing the relationship between the two diseases. The GO analysis of Metascape results revealed that these genes were concentrated in extracellular matrix organization ($\log10(P) = -8.294$), inflammatory response ($\log10(P) = -7.266$), regulation of defense response ($\log10(P) = -6.403$), cell chemotaxis ($\log10(P) = -4.900$), and cellular transition metal ion homeostasis ($\log10(P) = -6.286$) (Figure 3(A)). KEGG pathway enrichment analysis demonstrated that these genes were mainly clustered in viral protein interaction with cytokine and cytokine receptor ($\log10(P) = -3.374$) and cytokine–cytokine receptor interaction ($\log10(P) = -3.050$) (Figure 3(B)).

PPI network creation and module analysis

The network of the common DEGs, which contained 43 nodes and 27 edges (Figure 4(A)), was built using the STRING database. The interactions in the network were visualized using Cytoscape. Genes that were related to each other were clearly identified (Figure 4(B)). This PPI network showed a correlation between the expression of most genes. One closely connected gene module was obtained using the Molecular Complex Detection plug-in of Cytoscape; it included three common DEGs (Figure 5(A)). As shown in Figure 5(B), the module analysis was retained as the functional description of the relevant parts. The functional description was mainly involved in metalloendopeptidase activity ($\log10(P) = -7.3$), metallopeptidase activity ($\log10(P) = -6.6$), and extracellular matrix organization ($\log10(P) = -6.1$) (Figure 5(B)).

Screening and analysis of hub genes

The top 20 hub genes (Table 1) were identified using the 5 algorithms of the cytoHubba plug-in. After considering the same part of the Venn diagrams, 17 shared hub genes were found and they included *CCL2*, *POSTN*, *ALOX5*, *PLA2G2A*, *C1orf162*, *FPR1*, *ADAMTS15*, *ADAMTS4*, *ADAMTS9*, *ALOX5AP*, *ANPEP*, *FAP*, *VSIG4*, *LYVE1*, *MT1A*, *STEAP4*, and *SULF1* (Figure 6(A)). The GeneMANIA database revealed the co-expression networks of the above genes and their related functions. The complex PPI networks showed 50.72% co-expression, 34.19% prediction, 7.24% physical interaction, 5.33% co-localization, 2.49% shared protein domains, and 0.03% genetic interaction (Figure 6(B)). These genes were mainly related to extracellular matrix organization, inflammatory responses, blood vessel morphogenesis, regulation of defense responses, cellular homeostasis, and regulation of leukocyte activation; these processes were evaluated by GO analysis (Figure 7).

Confirmation of hub gene expression

Among the 17 hub genes, the expression of the 9 genes including *ALOX5*, *FPR1*, *ADAMTS15*, *ALOX5AP*, *ANPEP*, *SULF1*, *C1orf162*, *VSIG4*, and *LYVE1* were found to be statistically significant in two other databases. The full names and related functions of the common hub genes are shown in Table 2.[16–24] The results showed that *SULF1* was significantly up-regulated and *C1orf162*, *VSIG4*, and *LYVE1* were significantly down-regulated in both GSE76701 and GSE118370 compared with normal tissue. These four genes can be regarded as important genes closely related to the co-morbidity of HF and LC. In addition to the results of enrichment analysis (Figure 7), the *SULF1* correlated with the extracellular matrix (ECM) and the *VSIG4* correlated with regulation of leukocyte activation; this may provide a basis for exploring the causes of co-morbidity between the two diseases.

As shown in Figure 8, *ALOX5*, *FPR1*, and *ADAMTS15* were significantly down-regulated in LC compared with normal tissue. *ALOX5AP* and *ANPEP* were significantly down-regulated in HF; these genes may be related to the eventual disease outcome in patients. The findings are summarized in Figure 9.

Discussion

HF and cancer, two of the most dangerous diseases to human health, cause a large number of deaths. The co-occurrence of cancer and HF has been described, and it significantly affects clinical outcomes.[7] However, the reason for this co-occurrence is unclear. The common risk factors between cancer and HF may partly explain their association.[10] Imaging and laboratory results or drugs may help detect unknown cancers in patients with HF during diagnosis and treatment.[25] However, the above explanation for co-occurrence is limited by the lack of comprehensive research. Fortunately, the link between cancer and HF is gaining attention. In this context, a study has shown a causal relationship between HF and colon cancer. The study concluded that the presence of HF led to a significantly increased intestinal tumor load in APCM in mice.[26] However, the pathways and underlying mechanisms were not fully elucidated and warrant further exploration. As the leading cause of cancer deaths worldwide,[4] the cumulative incidence of LC in HF patients remains at a high level

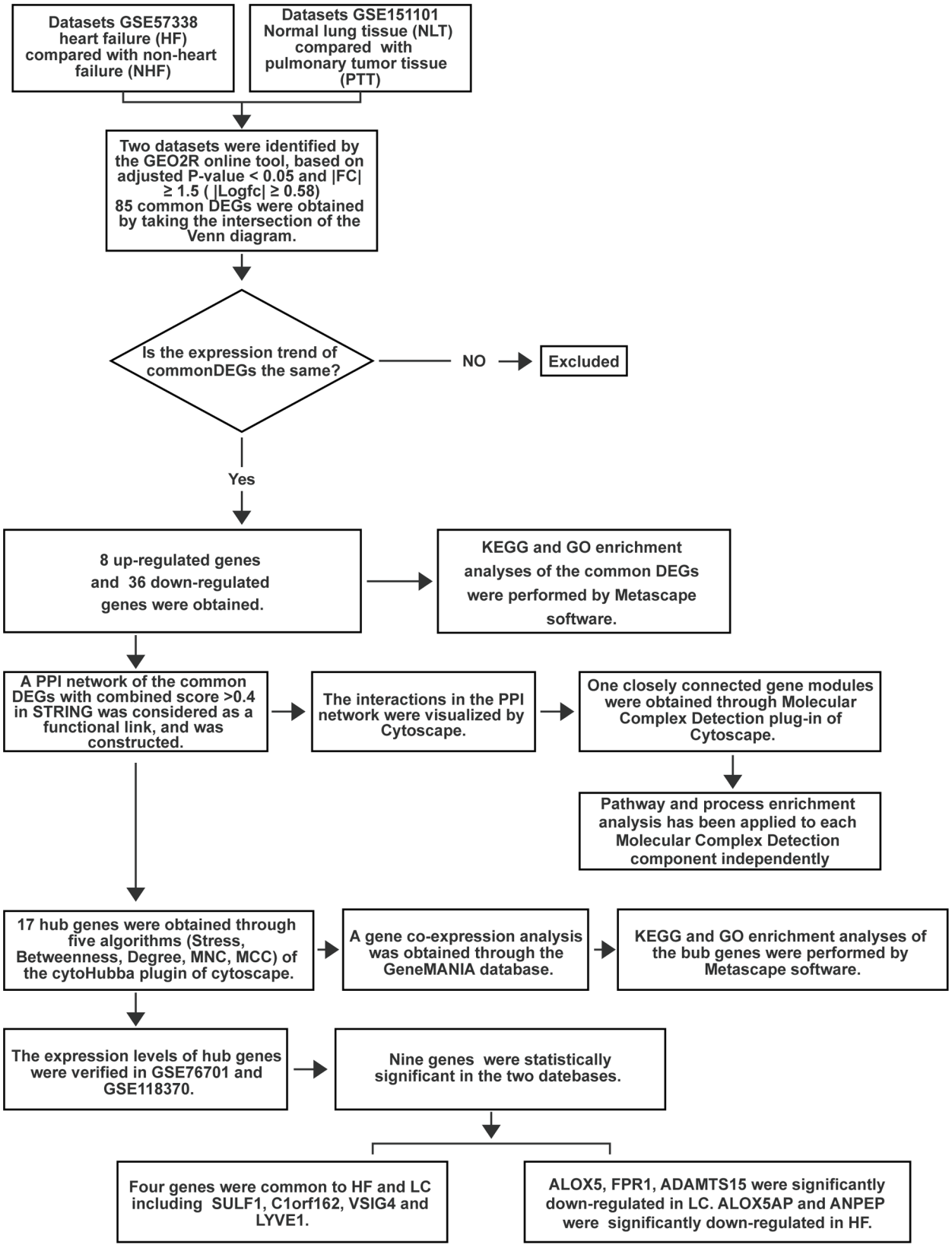

Figure 1. Research design flowchart.

compared with other cancers.[8] In order to reveal the underlying mechanisms and internal relationships responsible for the co-occurrence of HF and LC, this study attempted to explore their relationship using microarray data analysis.

We identified 44 DEGs in both diseases, of which 17 were hub genes. The analysis showed that the 44 genes were clustered on ECM organization, inflammatory responses, blood vessel morphogenesis, regulation of defense responses,

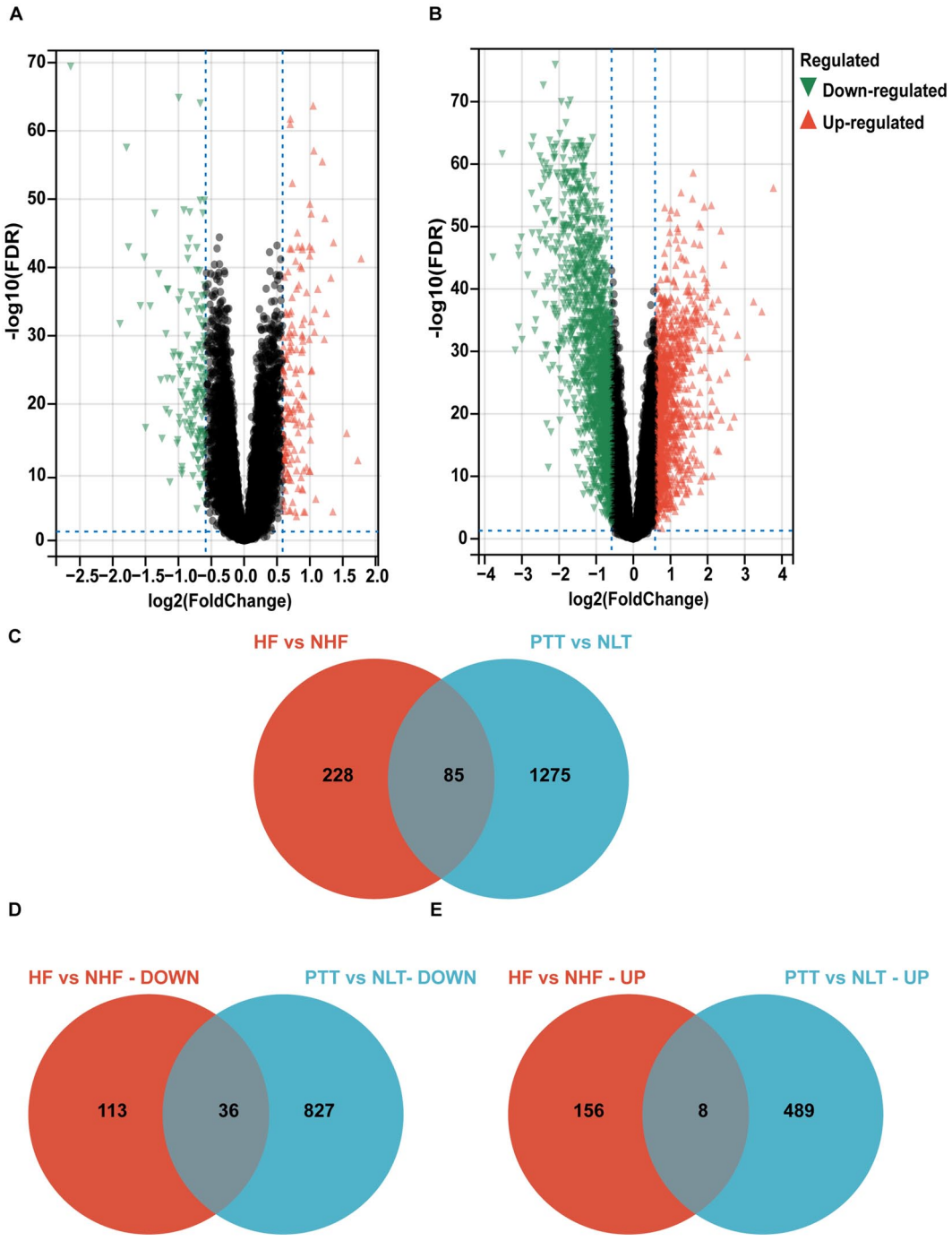

Figure 2. Volcano and Venn diagrams. (A) Volcano map of GSE57338. (B) The volcano map of GSE151101. Up-regulated genes in light red and down-regulated genes in light green. (C) Venn diagram of overlap of common DEGs. (D) Venn diagram of common down-regulated genes. (E) Venn diagram of common up-regulated genes.

cellular homeostasis, and regulation of leukocyte activation. Using hub gene enrichment analysis, this study demonstrated a common signaling pathway between HF and LC and provided new evidence for the association between the two diseases. Nine statistically significant genes were finally found via validation of hub genes in the other two data sets. The result in Figure 9 suggested that changes in the expression of *ALOX5*, *FPR1*, and *ADAMTS15* may contribute to disease progression in LC and *ALOX5AP* and *ANPEP* may contribute to disease progression in HF. Four genes including *SULF1*, *C1orf162*, *VSIG4*, and *LYVE1* were found to be common between HF and LC; this may offer a breakthrough in the search for mechanisms responsible for the concomitance of HF and LC.

SULF1 is a heparin-degrading endosulfatase.[27] Signaling processes modulated by SULF1 have been reported to be of close relevance for the development and expansion of cancer.[28] SULF1 also exerted tumor suppressive effects in various cancer models, with downregulation in hepatocellular carcinoma,[29] breast cancer,[30] gastric cancer,[30] and renal

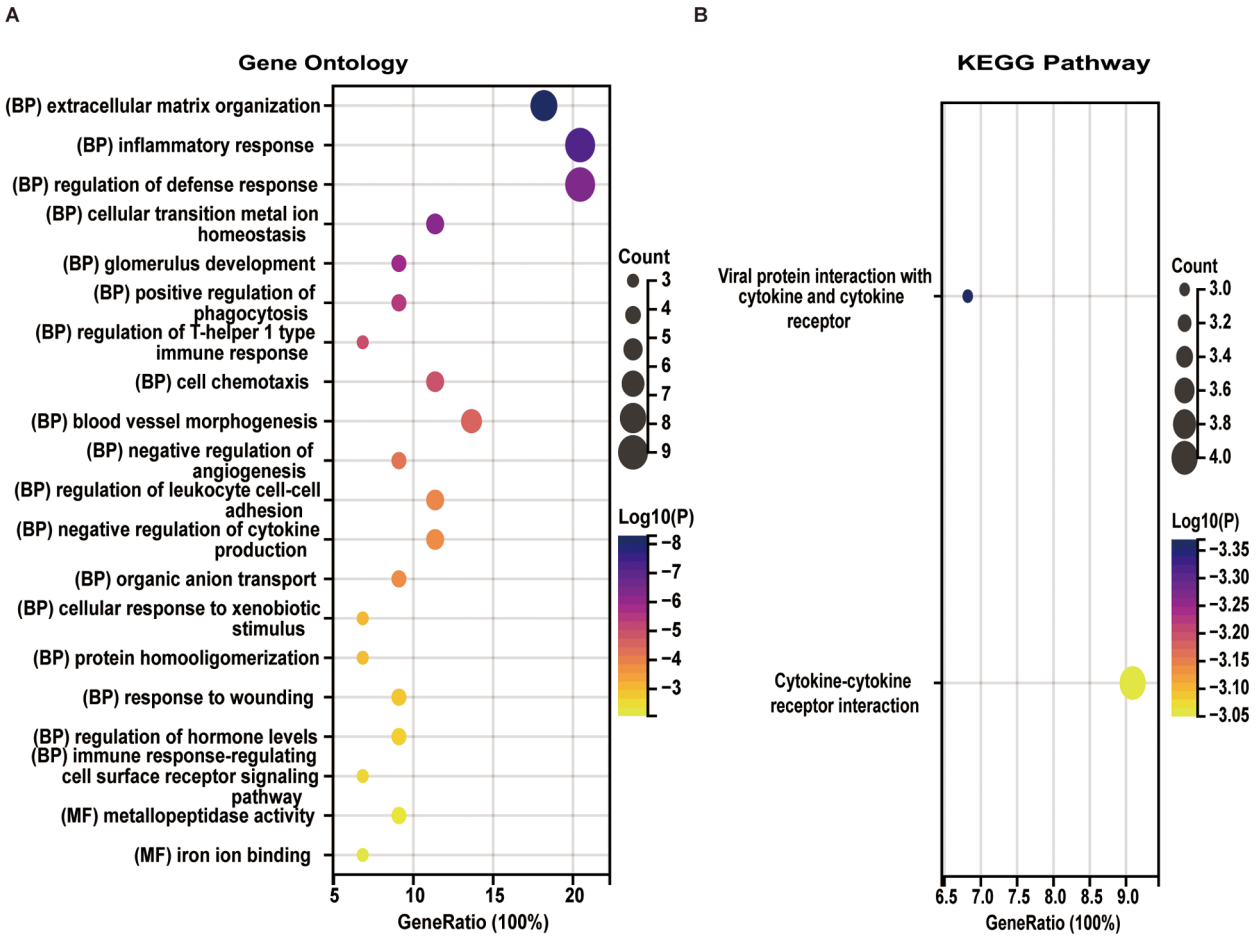

Figure 3. Common DEG enrichment analysis results. Enrichment analysis results of GO ontology (A) and KEGG pathways (B).

cancer.[30] However, one study offers conflicting result.[31] Rosen and Lemjabbar-Alaoui[31] analyzed the data from the ONCOMINE microarray database and found that SULF1 was clearly overexpressed in breast cancer, brain cancer, colorectal adenocarcinoma, and LC. More research is needed to explain this conflicting carcinogenic effect. This study also found higher *SULF1* expression in the tissue from HF and LC than in normal tissues; other studies have also confirmed its role in HF.[32] One study suggested that SULF1 is required for cardiac fibroblast activation and serves as a myofibroblast marker for myocardial fibrosis and HF.[33] Another study established heparan sulfate-editing Sulf as a key inducer of postinfarction angiogenesis and identified heparan sulfate sulfation as a direction of treatment for ischemic tissue repair;[34] this may serve as a foundation for research to identify molecular targets for the prevention of chronic HF. Based on the findings from these studies, we hypothesized that *SULF1* could be a key gene in HF and LC, and that its high expression in one of the conditions may be used as a biomarker for predicting the other disease.

In addition to the findings from GO enrichment analysis (Figure 7), *SULF1* was found to be jointly involved in ECM organization (as shown in Figure 5(B)). The ECM is a sophisticated and dynamic construct, which provides signals to cells and regulates their behavior; it can maintain normal organs growth and tissue stability by triggering various biological activities, which are essential for living organisms.[35] ECM remodeling is essential for regulatory morphogenesis of intestines, lungs, and other organs; abnormal remodeling of the ECM may be one of the predisposing factors leading to various pathological states including fibrosis and cancer.[36] Under stress conditions, ECM macromolecules can lead to ventricular dysfunction and HF by acting as an indispensable role in driving cellular biological responses.[37] A better understanding on the mechanisms by which the ECM moderates organ structure and function and the ways in which ECM reshaping impacts disease progression will help advance the process of developing new therapeutic approaches. In addition to the direct targeting of tumor cells, which has received considerable attention in the field of cancer treatment, the interest in ECM targeting has also been increasing rapidly.[38,39] Research has shown that heparinase (which regulates the structure and function of heparan sulfate proteoglycan) and sulfatase (which removes 6-O-sulfate residues from heparan sulfate and modulates its binding to many cytokines and growth factors) can change the properties of ECM proteoglycans.[40] However, there is no firm evidence for the relationship between SULF1 and ECM; this requires further research. In this context, modulation of the ECM may be regarded as a possible strategy for controlling the properties of the tumor microenvironment.

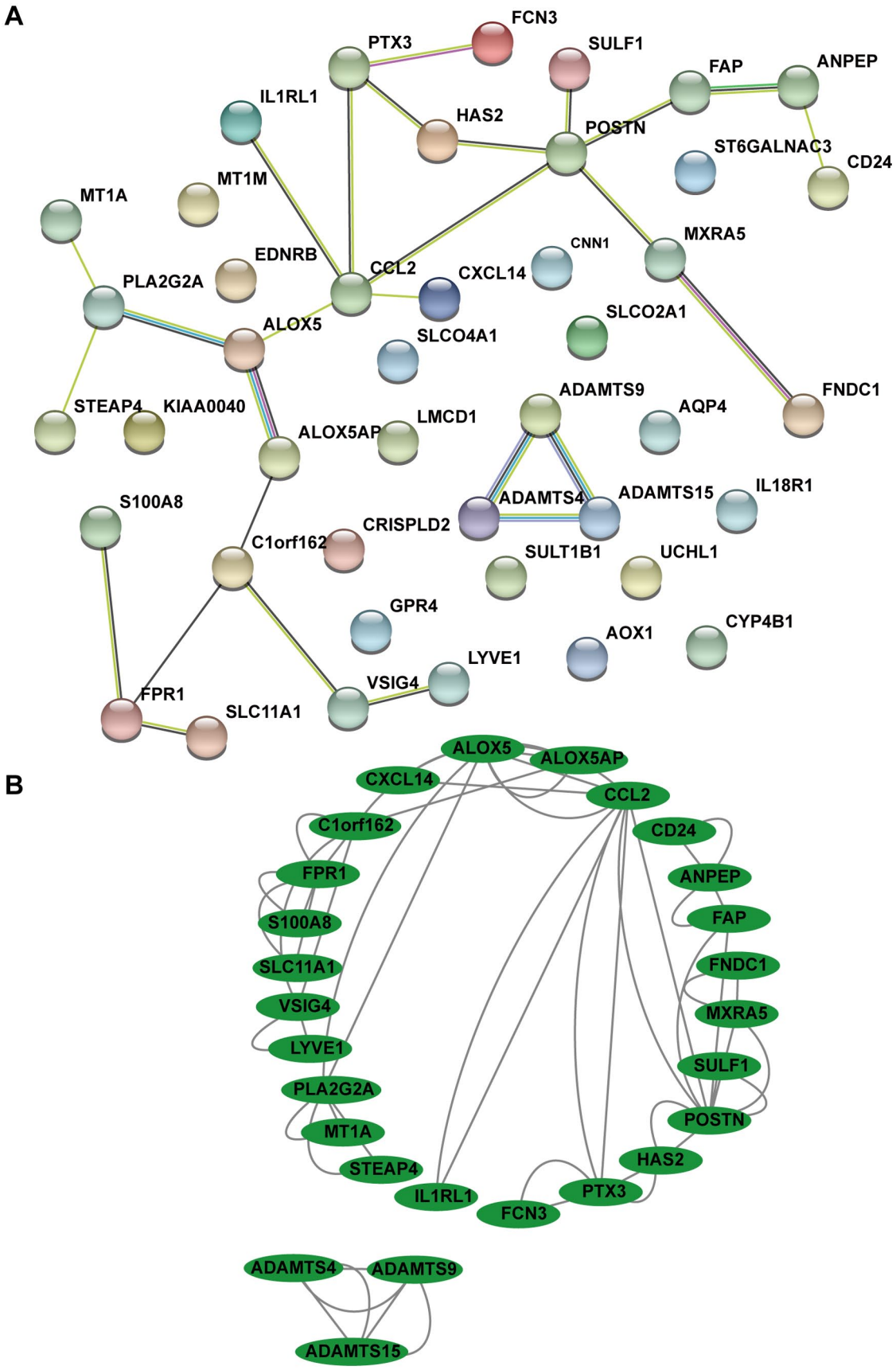

Figure 4. PPI network. (A) PPI network diagram. (B) Interactions in the PPI network.

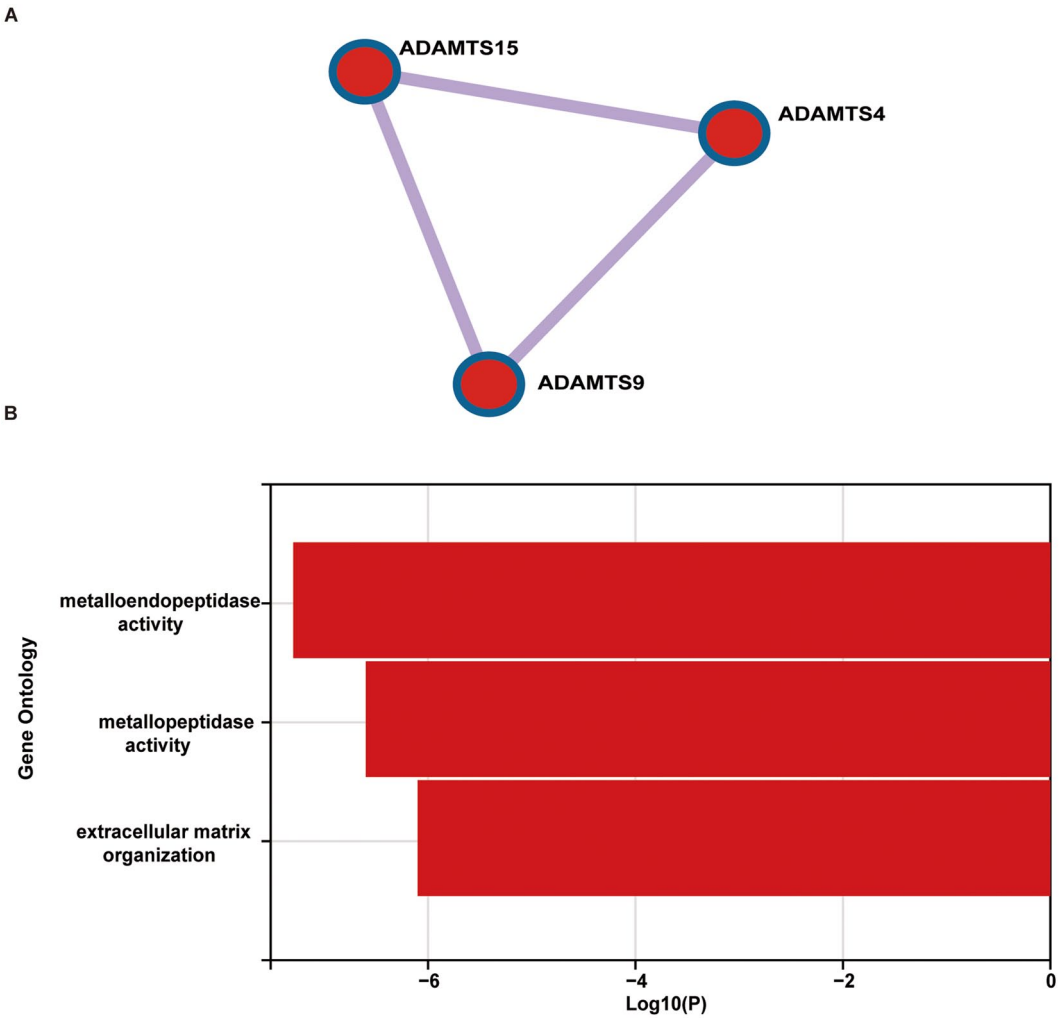

Figure 5. Remarkable gene module and enrichment analysis of the modular genes. (A) Remarkable gene clustering module. (B) Functional statement of the modular genes.

Table 1. Rank of the top 20 hub genes in cytoHubba.

Maximal clique centrality	Maximum neighborhood component	Degree	Stress	Betweenness
CCL2	*ADAMTS15*	*CCL2*	*ALOX5*	*CCL2*
POSTN	*ADAMTS4*	*POSTN*	*CCL2*	*ALOX5*
ALOX5	*ADAMTS9*	*ALOX5*	*ALOX5AP*	*POSTN*
PLA2G2A	*LYVE1*	*PLA2G2A*	*C1orf162*	*ALOX5AP*
C1orf162	*MT1A*	*C1orf162*	*POSTN*	*C1orf162*
FPR1	*STEAP4*	*FPR1*	*FPR1*	*PLA2G2A*
PTX3	*SULF1*	*PTX3*	*FAP*	*FPR1*
ADAMTS15	*ALOX5*	*ADAMTS15*	*ANPEP*	*FAP*
ADAMTS4	*CCL2*	*ADAMTS4*	*VSIG4*	*PTX3*
ADAMTS9	*PLA2G2A*	*ADAMTS9*	*PTX3*	*ANPEP*
ALOX5AP	*ALOX5AP*	*ALOX5AP*	*PLA2G2A*	*VSIG4*
ANPEP	*C1orf162*	*ANPEP*	*MXRA5*	*MXRA5*
FAP	*ANPEP*	*FAP*	*HAS2*	*HAS2*
VSIG4	*FAP*	*VSIG4*	*LYVE1*	*LYVE1*
MXRA5	*CD24*	*MXRA5*	*MT1A*	*MT1A*
HAS2	*VSIG4*	*HAS2*	*STEAP4*	*STEAP4*
LYVE1	*FPR1*	*LYVE1*	*ADAMTS15*	*ADAMTS15*
MT1A	*IL1RL1*	*MT1A*	*ADAMTS4*	*ADAMTS4*
STEAP4	*POSTN*	*STEAP4*	*ADAMTS9*	*ADAMTS9*
SULF1	*CXCL14*	*SULF1*	*SULF1*	*SULF1*

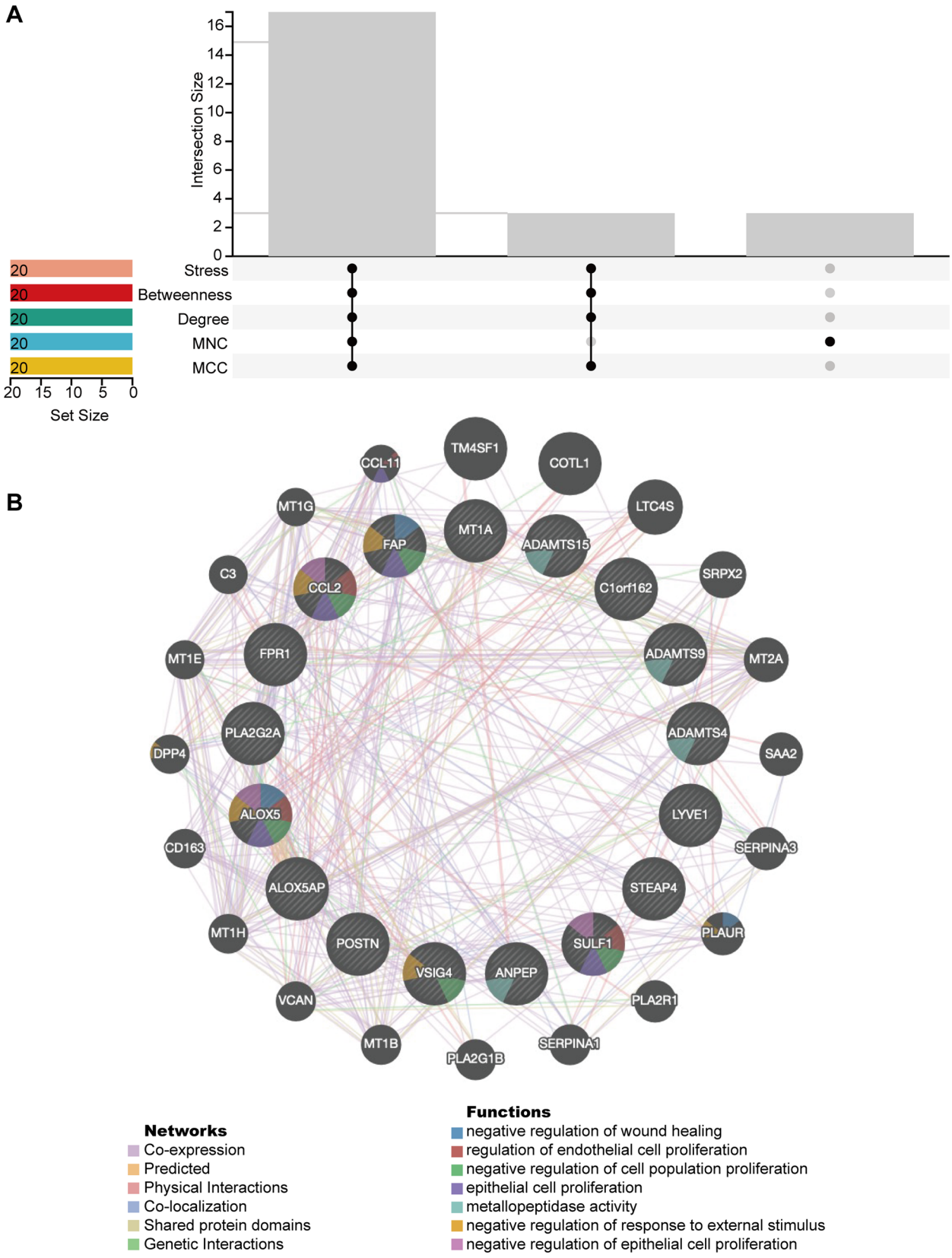

Figure 6. Venn diagram and co-expression network of hub genes. (A) Venn diagram of 17 hub genes. (B) The co-expression network and related functions of 17 hub genes.

V-set immunoglobulin-domain-containing 4 (VSIG4), is a membrane protein of the immunoglobulin superfamily[21] and a complement receptor. VSIG4 may mediate clearance of C3b-opsonized pathogens by binding the complement component, C3b.[41] VSIG4 can also bind to unidentified T-cell ligands or receptors to inhibit interleukin-2 production and

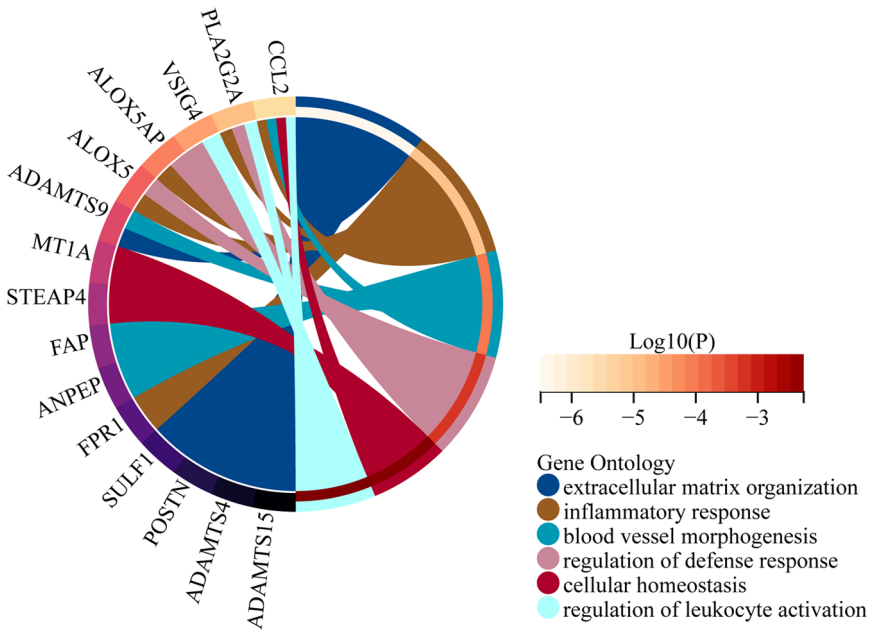

Figure 7. GO enrichment analysis of the hub genes.

Table 2. Details of the validated genes.

1	*ALOX5*	Arachidonate 5-lipoxygenase	Catalyzes the first step in leukotriene biosynthesis, and thereby plays a role in inflammatory processes; belongs to the lipoxygenase family[16]
2	*FPR1*	Formyl peptide receptor 1	fMet-Leu-Phe receptor; high affinity receptor for *N*-formyl-methionyl peptides (fMLPs), which are powerful neutrophil chemotactic factors. Binding of fMLP to the receptor stimulates intracellular calcium mobilization and superoxide anion release. This response is mediated via a G-protein that activates a phosphatidylinositol-calcium second messenger system[17]
3	*ADAMTS15*	A disintegrin and metalloproteinase with thrombospondin motifs 15; ADAM metallopeptidase with thrombospondin type 1 motif 15	
4	*ALOX5AP*	Arachidonate 5-lipoxygenase-activating protein; required for leukotriene biosynthesis by ALOX5 (5-lipoxygenase)	Anchors ALOX5 to the membrane. Binds arachidonic acid, and could play an essential role in the transfer of arachidonic acid to ALOX5. Binds to MK-886, a compound that blocks the biosynthesis of leukotrienes;[18] belongs to the MAPEG family (a.k.a. FLAP, Q5VV04, AY619687)
5	*ANPEP*	Alanyl aminopeptidase, membrane; aminopeptidase N	Broad specificity aminopeptidase[19] which plays a role in the final digestion of peptides generated from hydrolysis of proteins by gastric and pancreatic proteases. Also involved in the processing of various peptides including peptide hormones, such as angiotensin III and IV, neuropeptides, and chemokines. May also be involved the cleavage of peptides bound to major histocompatibility complex class II molecules of antigen presenting cells.[20] May have a role in angiogenesis and promote cholesterol crystallization; aminopeptidases
6	*C1orf162*	Transmembrane protein C1orf162, chromosome 1 open reading frame 162	
7	*VSIG4*	V-set and immunoglobulin domain-containing protein 4	Phagocytic receptor, strong negative regulator of T-cell proliferation and IL2 production. Potent inhibitor of the alternative complement pathway convertases, immunoglobulin-like domain containing[21]
8	*LYVE1*	Lymphatic vessel endothelial hyaluronic acid receptor 1	Ligand-specific transporter trafficking between intracellular organelles (TGN) and the plasma membrane. Plays a role in autocrine regulation of cell growth mediated by growth regulators containing cell surface retention sequence binding (CRS).[22] May act as a hyaluronan (HA) transporter,[23] either mediating its uptake for catabolism within lymphatic endothelial cells themselves or its transport into the lumen of afferent lymphatic vessels for subsequent re-uptake and degradation in lymph nodes
9	*SULF1*	Extracellular sulfatase Sulf-1	Exhibits arylsulfatase activity and highly specific endoglucosamine-6-sulfatase activity.[24] It can remove sulfate from the C-6 position of glucosamine within specific subregions of intact heparin. Diminishes HSPG (heparan sulfate proteoglycans) sulfation, inhibits signaling by heparin-dependent growth factors, diminishes proliferation, and facilitates apoptosis in response to exogenous stimulation

MAPEG: Membrane-Associated Proteins in Eicosanoid and Glutathione metabolism.

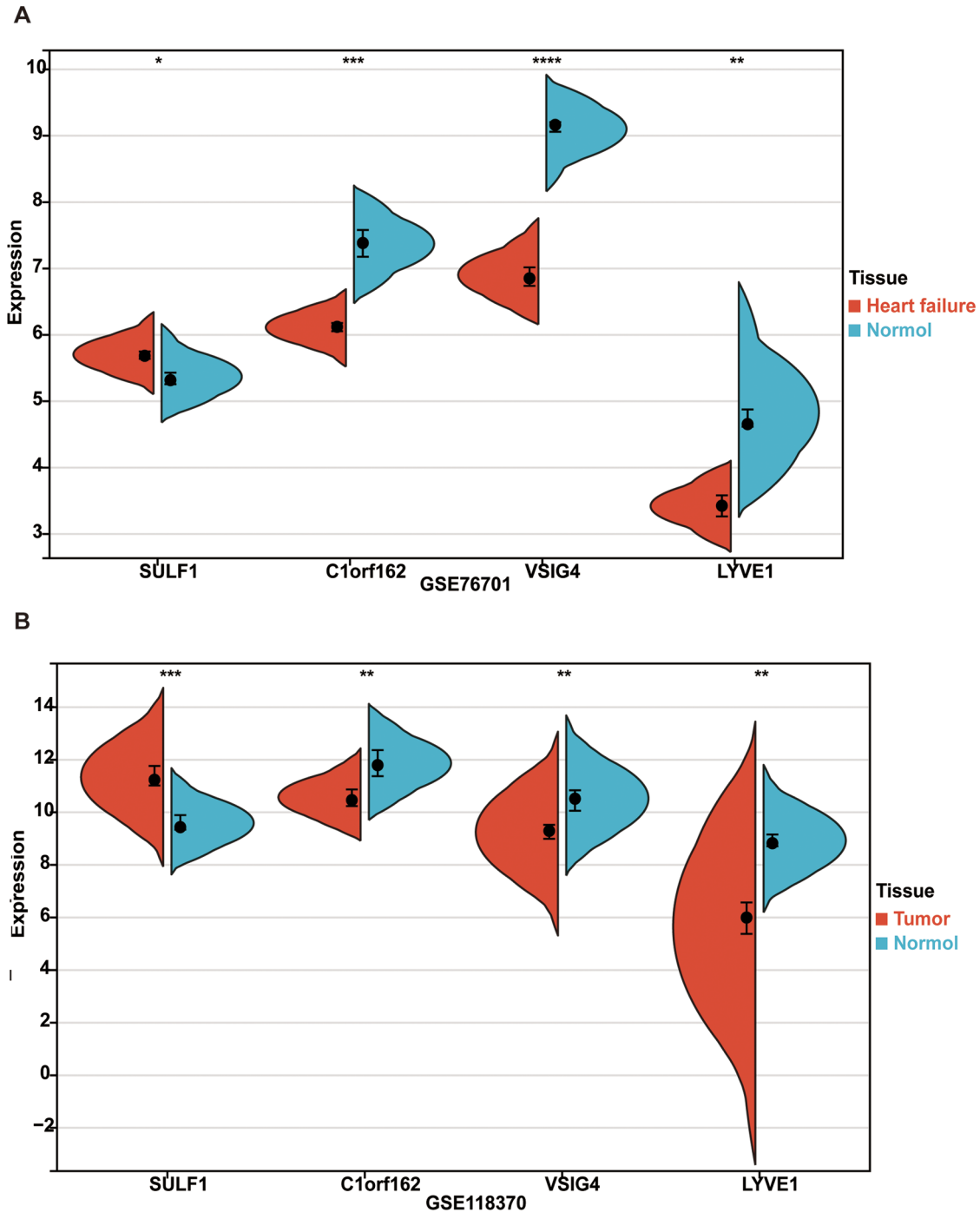

Figure 8. Genes with the same expression trends in both GSE76701 and GSE118370. (A) Expression level of four genes in GSE76701. (B) Expression level of four genes in GSE118370. *P* values < 0.05 were considered statistically significant.
$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$.

T-cell proliferation.[21] It can negatively regulate innate inflammatory responses.[42] The data from a study suggested that deficiency of VSIG4 in high-fat diet-induced obesity and hepatitis mouse model accelerated the severity of the inflammatory response.[43] Inflammation has been well documented as an influential trigger for the development and progression of HF[44] and LC.[45] VSIG4 maybe a prospective target for treating HF and LC caused by dysregulation of inflammatory responses.

As shown in Figure 7, VSIG4 was found to be involved in the regulation of leukocyte activation; this may be related to its role in HF and LC. Leukocytes, as immune cells derived from hematopoietic stem cells of the bone marrow, play certain functions in inflammation and immune responses.[46] All immune cells recruited to the site of action play critical roles in the microenvironment and can participate in tumor development and progression.[47] The interaction between tumor cells and immune cells can lead to the aggregation of

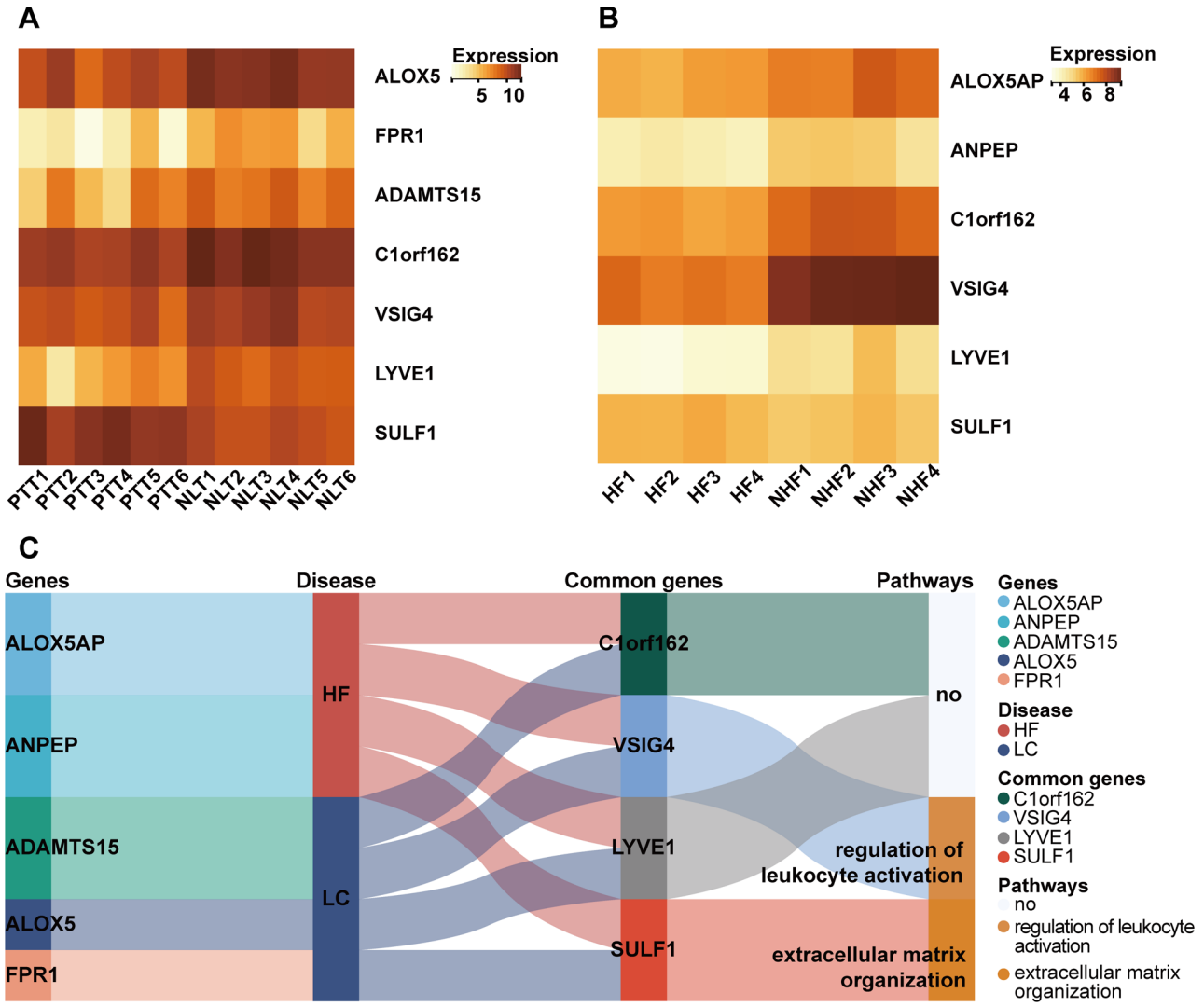

Figure 9. Expression of these validated genes and the involved pathways. (A) Heatmap of the expression of these validated genes in GSE118370. (B) Heatmap of the expression of these validated genes in GSE76701. (C) Validated genes and pathways involved in HF (heart failure) and LC (lung cancer).

tumor cells; this also explains the differences in host protection and tumor-shaping functions of the immune system in cancer.[48] The existing literature suggests that leukocytes reduce damage and protect the host through multiple and elaborate mechanisms during inflammation.[49] Several links between leukocyte driven inflammation and cardiac fibrosis ultimately lead to adverse outcomes such as HF, with an increase in mortality.[50] We hypothesized that this may be attributed to the low expression of VSIG4, which partially affects leukocyte activation and leads to immune disorders; the antitumor and heart-defending effects are not balanced in these patients. The exact underlying mechanisms and effects of VSIG4–leukocyte interaction in the development and progression of HF and LC remain unclear. Further studies are necessary to bridge the knowledge gap regarding VSIG4 with regulation of leukocyte activation.

As one of the most abundant glycosaminoglycans in ECM, lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1) is a type I integral membrane protein.[51] The lack of LYVE1 in mice prevented docking of leukocyte docking, which can worsen chronic inflammation and long-term deterioration of cardiac function.[52] A study has shown that LYVE1 functions to maintain vascular homeostasis[53] by binding to hyaluronic acid expressed in smooth muscle cells; this work underscores the tremendous complexity and plasticity of recruiting and resident cardiac macrophages in this state of stability, especially within infarcted tissue. Therefore, a loss of LYVE1 + macrophages may disrupt adaptive healing mechanisms, impairing the opportunity for effective cardioprotection; this results in the development of HF. All these studies demonstrate that LYVE1 exists as an essential constituent of cardiac function. Findings from previous studies also suggest that LYVE1 may be involved in lymphatic hyaluronan transport and participate in tumor metastasis.[54] The results from this study suggest that *LYVE1* expression was down-regulated in LC tissue. It is worth noting that a previous study reported an association between LYVE1 and clinicopathological factors and survival.[55] The role of LYVE1 in HF and LC therefore needs further investigation.

At present, data pertaining to *C1orf162* are extremely limited; these need to be supplemented in future studies. Although four validated genes related to HF and LC (besides *C1orf162*) have been explored separately in previous studies, the common molecular mechanisms between them have been less studied by bioinformatics approaches. With the high co-morbidity rate of HF and LC, we studied and characterized for the first time the common DEGs and pivotal genes for both pathological states. Four validated genes were finally obtained; they helped to further clarify the common underlying mechanisms between HF and LC.

Certain limitations exist in this study. First, this was a retrospective analysis; as the scope of retrospective analyses is limited, external verification will be needed to verify our findings. Second, sufficient sample size is necessary; more HF and LC samples will be needed to validate our findings. Third, preclinical models mimicking the human scenario of co-existence of both pathologies will be needed for future studies. Finally, the function of the four validated genes needs further verification *in vitro* model; this will be a priority for our future work.

In conclusion, we found numerous close but obscure connections between HF and LC, that may be mediated by the validated genes. Based on the results of GO enrichment analysis, HF and LC co-occurrence is possibly related to the disorders of the immune system. In this context, the abnormal activation of ECM organization, inflammatory response, and other immune signaling pathways needs particular attention. The findings suggest that crosstalk within the immune system may be responsible for cell cycle changes in different organs. The common validated genes have potential for use as biomarkers for the co-occurrence of the two diseases. This means that by monitoring the abnormal expression of these genes, we can predict whether a disease is likely to develop into a combination of two diseases. Patients with high expression of *SULF1* gene and low expression of *C1orf162*, *VSIG4*, and *LYVE1* genes may be predicted to have a higher chance of co-morbidity HF and LC. Similarly, the *ALOX5*, *FPR1*, and *ADAMTS15* genes may be predicted to partially beneficial in the disease progression of LC, and the *ALOX5AP* and *ANPEP* genes may partially contribute to the disease progression of HF. In addition to clinical data, scientific experiments based on preclinical models (mimicking the human scenario of co-existence of both pathologies) are urgently needed to verify the potential biomarkers we screened. Once these genes can be validated, they can be used as indicators to predict disease diagnosis and prognosis. In the future, based on the common validated genes between LC and HF, it is possible to provide specific drug or therapeutic target sites to treat the co-morbidities of the two diseases, or to guide clinical drug use, thus providing occasions for developing the crossover strategies and treatments to fight both diseases.

AUTHORS' CONTRIBUTIONS

XW, YL, and LM designed the study. XW wrote the initial version of the manuscript and revised the final version. RS, XL, and KH performed data collection and analyzed the data. YL and LM supervised the work. All authors contributed to the manuscript and approved the submitted version.

ACKNOWLEDGEMENTS

The authors acknowledge the Innovative Team of Intelligent Inspection and Active Health (ITIH) for supporting this study.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was financially supported by the Natural Science Foundation of Shanghai (21ZR1428400) and the Technical Standard Special Project of the Shanghai Science and Technology Commission (19DZ2201000).

DATA AVAILABILITY

The data sets for this study can be found in the GEO (http://www.ncbi.nlm.nih.gov/geo/).

ORCID ID

Xiaoying Wang https://orcid.org/0000-0001-9850-7800

SUPPLEMENTAL MATERIAL

Supplemental material for this article is available online.

REFERENCES

1. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE Jr, Drazner MH, Fonarow GC, Geraci SA, Horwich T, Januzzi JL, Johnson MR, Kasper EK, Levy WC, Masoudi FA, McBride PE, McMurray JJ, Mitchell JE, Peterson PN, Riegel B, Sam F, Stevenson LW, Tang WH, Tsai EJ, Wilkoff BL. 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association task force on practice guidelines. *J Am Coll Cardiol* 2013;**62**:e147–239
2. Shi C, van der Wal HH, Silljé HHW, Dokter MM, van den Berg F, Huizinga L, Vriesema M, Post J, Anker SD, Cleland JG, Ng LL, Samani NJ, Dickstein K, Zannad F, Lang CC, van Haelst PL, Gietema JA, Metra M, Ameri P, Canepa M, van Veldhuisen DJ, Voors AA, de Boer RA. Tumour biomarkers: association with heart failure outcomes. *J Intern Med* 2020;**288**:207–18
3. Thakur SK, Singh DP, Choudhary J. Lung cancer identification: a review on detection and classification. *Cancer Metastasis Rev* 2020;**39**:989–98
4. Nasim F, Sabath BF, Eapen GA. Lung cancer. *Med Clin North Am* 2019;**103**:463–73
5. Anker MS, Sanz AP, Zamorano JL, Mehra MR, Butler J, Riess H, Coats AJS, Anker SD. Advanced cancer is also a heart failure syndrome: a hypothesis. *J Cachexia Sarcopenia Muscle* 2021;**12**:533–7
6. Jerusalem G, Lancellotti P, Kim SB. HER2+ breast cancer treatment and cardiotoxicity: monitoring and management. *Breast Cancer Res Treat* 2019;**177**:237–50
7. Cuomo A, Pirozzi F, Attanasio U, Franco R, Elia F, De Rosa E, Russo M, Ghigo A, Ameri P, Tocchetti CG, Mercurio V. Cancer risk in the heart failure population: epidemiology, mechanisms, and clinical implications. *Curr Oncol Rep* 2020;**23**:7
8. Kwak S, Kwon S, Lee SY, Yang S, Lee HJ, Lee H, Park JB, Han K, Kim YJ, Kim HK. Differential risk of incident cancer in patients with heart failure: a nationwide population-based cohort study. *J Cardiol* 2021;**77**:231–8
9. Tu H, Wen CP, Tsai SP, Chow WH, Wen C, Ye Y, Zhao H, Tsai MK, Huang M, Dinney CP, Tsao CK, Wu X. Cancer risk associated with chronic diseases and disease markers: prospective cohort study. *BMJ* 2018;**360**:k134

10. Meijers WC, de Boer RA. Common risk factors for heart failure and cancer. *Cardiovasc Res* 2019;**115**:844–53
11. Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, Marshall KA, Phillippy KH, Sherman PM, Holko M, Yefanov A, Lee H, Zhang N, Robertson CL, Serova N, Davis S, Soboleva A. NCBI GEO: archive for functional genomics data sets – update. *Nucleic Acids Res* 2013;**41**:D991–195
12. Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, Ellis B, Gautier L, Ge Y, Gentry J, Hornik K, Hothorn T, Huber W, Iacus S, Irizarry R, Leisch F, Li C, Maechler M, Rossini AJ, Sawitzki G, Smith C, Smyth G, Tierney L, Yang JY, Zhang J. Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol* 2004;**5**:R80
13. Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, Doncheva NT, Legeay M, Fang T, Bork P, Jensen LJ, von Mering C. The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res* 2021;**49**:D605–112
14. Doncheva NT, Morris JH, Gorodkin J, Jensen LJ. Cytoscape StringApp: network analysis and visualization of proteomics data. *J Proteome Res* 2019;**18**:623–32
15. Franz M, Rodriguez H, Lopes C, Zuberi K, Montojo J, Bader GD, Morris Q. GeneMANIA update 2018. *Nucleic Acids Res* 2018;**46**:W60–4
16. Tantisira KG, Drazen JM. Genetics and pharmacogenetics of the leukotriene pathway. *J Allergy Clin Immunol* 2009;**124**:422–7
17. Forsman H, Kalderén C, Nordin A, Nordling E, Jensen AJ, Dahlgren C. Stable formyl peptide receptor agonists that activate the neutrophil NADPH-oxidase identified through screening of a compound library. *Biochem Pharmacol* 2011;**81**:402–11
18. Nunns GR, Stringham JR, Gamboni F, Moore EE, Fragoso M, Stettler GR, Silliman CC, Banerjee A. Trauma and hemorrhagic shock activate molecular association of 5-lipoxygenase and 5-lipoxygenase-activating protein in lung tissue. *J Surg Res* 2018;**229**:262–70
19. Sørensen KD, Abildgaard MO, Haldrup C, Ulhøi BP, Kristensen H, Strand S, Parker C, Høyer S, Borre M, Ørntoft TF. Prognostic significance of aberrantly silenced ANPEP expression in prostate cancer. *Br J Cancer* 2013;**108**:420–8
20. Norris R, O'Keeffe MB, Poyarkov A, FitzGerald RJ. Peptide identification and angiotensin converting enzyme (ACE) inhibitory activity in prolyl endoproteinase digests of bovine α(s)-casein. *Food Chem* 2015;**188**:210–7
21. Widyagarini A, Nishii N, Kawano Y, Zhang C, Azuma M. VSIG4/CRIg directly regulates early CD8(+) T cell activation through its counter-receptor in a narrow window. *Biochem Biophys Res Commun* 2022;**614**:100–6
22. Liu NF, Yu Z, Luo Y, Sun D. A LYVE-1/CRSBP-1 mutation in inherited primary lymphedema. *Lymphology* 2017;**50**:9–15
23. Jackson DG. Hyaluronan in the lymphatics: the key role of the hyaluronan receptor LYVE-1 in leucocyte trafficking. *Matrix Biol* 2019;**78–79**:219–35
24. Joy MT, Vrbova G, Dhoot GK, Anderson PN. Sulf1 and Sulf2 expression in the nervous system and its role in limiting neurite outgrowth in vitro. *Exp Neurol* 2015;**263**:150–60
25. Eikelboom JW, Connolly SJ, Bosch J, Shestakovska O, Aboyans V, Alings M, Anand SS, Avezum A, Berkowitz SD, Bhatt DL, Cook-Bruns N, Felix C, Fox KAA, Hart RG, Maggioni AP, Moayyedi P, O'Donnell M, Rydén L, Verhamme P, Widimsky P, Zhu J, Yusuf S. Bleeding and new cancer diagnosis in patients with atherosclerosis. *Circulation* 2019;**140**:1451–9
26. Meijers WC, Maglione M, Bakker SJL, Oberhuber R, Kieneker LM, de Jong S, Haubner BJ, Nagengast WB, Lyon AR, van der Vegt B, van Veldhuisen DJ, Westenbrink BD, van der Meer P, Silljé HHW, de Boer RA. Heart failure stimulates tumor growth by circulating factors. *Circulation* 2018;**138**:678–91
27. Yi X, Liu Y, Zhou B, Xiang W, Deng A, Fu Y, Zhao Y, Ouyang Q, Liu Y, Sun Z, Zhang K, Li X, Zeng F, Zhou H, Chen BT. Incorporating SULF1 polymorphisms in a pretreatment CT-based radiomic model for predicting platinum resistance in ovarian cancer treatment. *Biomed Pharmacother* 2021;**133**:111013
28. Bret C, Moreaux J, Schved JF, Hose D, Klein B. SULFs in human neoplasia: implication as progression and prognosis factors. *J Transl Med* 2011;**9**:72
29. Lai JP, Thompson JR, Sandhu DS, Roberts LR. Heparin-degrading sulfatases in hepatocellular carcinoma: roles in pathogenesis and therapy targets. *Future Oncol* 2008;**4**:803–14
30. Chen Z, Fan JQ, Li J, Li QS, Yan Z, Jia XK, Liu WD, Wei LJ, Zhang FZ, Gao H, Xu JP, Dong XM, Dai J, Zhou HM. Promoter hypermethylation correlates with the Hsulf-1 silencing in human breast and gastric cancer. *Int J Cancer* 2009;**124**:739–44
31. Rosen SD, Lemjabbar-Alaoui H. Sulf-2: an extracellular modulator of cell signaling and a cancer target candidate. *Expert Opin Ther Targets* 2010;**14**:935–49
32. Wu J, Subbaiah KCV, Xie LH, Jiang F, Khor ES, Mickelsen D, Myers JR, Tang WHW, Yao P. Glutamyl-prolyl-tRNA synthetase regulates proline-rich pro-fibrotic protein synthesis during cardiac fibrosis. *Circ Res* 2020;**127**:827–46
33. Korf-Klingebiel M, Reboll MR, Grote K, Schleiner H, Wang Y, Wu X, Klede S, Mikhed Y, Bauersachs J, Klintschar M, Rudat C, Kispert A, Niessen HW, Lübke T, Dierks T, Wollert KC. Heparan sulfate-editing extracellular sulfatases enhance VEGF bioavailability for ischemic heart repair. *Circ Res* 2019;**125**:787–801
34. Theocharis AD, Skandalis SS, Gialeli C, Karamanos NK. Extracellular matrix structure. *Adv Drug Deliv Rev* 2016;**97**:4–27
35. Bonnans C, Chou J, Werb Z. Remodelling the extracellular matrix in development and disease. *Nat Rev Mol Cell Biol* 2014;**15**:786–801
36. Frangogiannis NG. The extracellular matrix in ischemic and nonischemic heart failure. *Circ Res* 2019;**125**:117–46
37. Seo BR, DelNero P, Fischbach C. In vitro models of tumor vessels and matrix: engineering approaches to investigate transport limitations and drug delivery in cancer. *Adv Drug Deliv Rev* 2014;**69–70**:205–16
38. Berezin V, Walmod PS, Filippov M, Dityatev A. Targeting of ECM molecules and their metabolizing enzymes and receptors for the treatment of CNS diseases. *Prog Brain Res* 2014;**214**:353–88
39. Skandalis SS, Aletras AJ, Gialeli C, Theocharis AD, Afratis N, Tzanakakis GN, Karamanos NK. Targeting the tumor proteasome as a mechanism to control the synthesis and bioactivity of matrix macromolecules. *Curr Mol Med* 2012;**12**:1068–82
40. Lu P, Takai K, Weaver VM, Werb Z. Extracellular matrix degradation and remodeling in development and disease. *Cold Spring Harb Perspect Biol* 2011;**3**:a005058.
41. Helmy KY, Katschke KJ Jr, Gorgani NN, Kljavin NM, Elliott JM, Diehl L, Scales SJ, Ghilardi N, van Lookeren Campagne M. Crig: a macrophage complement receptor required for phagocytosis of circulating pathogens. *Cell* 2006;**124**:915–27
42. Small AG, Al-Baghdadi M, Quach A, Hii C, Ferrante A. Complement receptor immunoglobulin: a control point in infection and immunity, inflammation and cancer. *Swiss Med Wkly* 2016;**146**:w14301
43. Li Y, Sun JP, Wang J, Lu WH, Xie LY, Lv J, Li HX, Yang SF. Expression of Vsig4 attenuates macrophage-mediated hepatic inflammation and fibrosis in high fat diet (HFD)-induced mice. *Biochem Biophys Res Commun* 2019;**516**:858–65
44. Wu CK, Lee JK, Chiang FT, Yang CH, Huang SW, Hwang JJ, Lin JL, Tseng CD, Chen JJ, Tsai CT. Plasma levels of tumor necrosis factor-α and interleukin-6 are associated with diastolic heart failure through downregulation of sarcoplasmic reticulum Ca2+ ATPase. *Crit Care Med* 2011;**39**:984–92
45. Albrengues J, Shields MA, Ng D, Park CG, Ambrico A, Poindexter ME, Upadhyay P, Uyeminami DL, Pommier A, Küttner V, Bružas E, Maiorino L, Bautista C, Carmona EM, Gimotty PA, Fearon DT, Chang K, Lyons SK, Pinkerton KE, Trotman LC, Goldberg MS, Yeh JT, Egeblad M. Neutrophil extracellular traps produced during inflammation awaken dormant cancer cells in mice. *Science* 2018;**361**:eaao4227
46. Suess PM. Effects of polyphosphate on leukocyte function. *Prog Mol Subcell Biol* 2022;**61**:131–43
47. Stoiber D, Assinger A. Platelet-leukocyte interplay in cancer development and progression. *Cells* 2020;**9**:855

48. Mittal D, Gubin MM, Schreiber RD, Smyth MJ. New insights into cancer immunoediting and its three component phases – elimination, equilibrium and escape. *Curr Opin Immunol* 2014;**27**:16–25
49. Kondo M. Lymphoid and myeloid lineage commitment in multipotent hematopoietic progenitors. *Immunol Rev* 2010;**238**:37–46
50. Okyere AD, Tilley DG. Leukocyte-dependent regulation of cardiac fibrosis. *Front Physiol* 2020;**11**:301
51. Banerji S, Ni J, Wang SX, Clasper S, Su J, Tammi R, Jones M, Jackson DG. LYVE-1, a new homologue of the CD44 glycoprotein, is a lymph-specific receptor for hyaluronan. *J Cell Biol* 1999;**144**: 789–801
52. Vieira JM, Norman S, Villa Del Campo C, Cahill TJ, Barnette DN, Gunadasa-Rohling M, Johnson LA, Greaves DR, Carr CA, Jackson DG, Riley PR. The cardiac lymphatic system stimulates resolution of inflammation following myocardial infarction. *J Clin Invest* 2018;**128**:3402–12
53. Lim HY, Lim SY, Tan CK, Thiam CH, Goh CC, Carbajo D, Chew SHS, See P, Chakarov S, Wang XN, Lim LH, Johnson LA, Lum J, Fong CY, Bongso A, Biswas A, Goh C, Evrard M, Yeo KP, Basu R, Wang JK, Tan Y, Jain R, Tikoo S, Choong C, Weninger W, Poidinger M, Stanley RE, Collin M, Tan NS, Ng LG, Jackson DG, Ginhoux F, Angeli V. Hyaluronan receptor LYVE-1-expressing macrophages maintain arterial tone through hyaluronan-mediated regulation of smooth muscle cell collagen. *Immunity* 2018;**49**:326–417
54. Jackson DG. The lymphatics revisited: new perspectives from the hyaluronan receptor LYVE-1. *Trends Cardiovasc Med* 2003;**13**:1–7
55. Hwang J, Kang MH, Yoo YA, Quan YH, Kim HK, Oh SC, Choi YH. The effects of sonic hedgehog signaling pathway components on non-small-cell lung cancer progression and clinical outcome. *World J Surg Oncol* 2014;**12**:268

(Received October 31, 2022, Accepted January 3, 2023)