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Brief Communication

Investigating possible dilated cardiomyopathy targets via bioinformatic analysis

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Abstract: Dilated cardiomyopathy (DCM) is the most common cardiomyopathy associated with heart failure; however, the underlying mechanism remains unclear. Initially, gene expression data of patients with DCM from the GSE4172 and GSE21610 datasets were obtained from the Gene Expression Omnibus website. Differentially expressed genes (DEGs) were analyzed with a false discovery rate < 0.05 and log2 fold change > 1.2. Furthermore, both the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis and Gene Set Enrichment Analysis (GSEA) were used to investigate the functional annotations. STRING and Cytoscape tools were used to form the protein-protein interaction (PPI) network and authenticate hub genes. Thereafter, the signature of immune-related genes (IRGs) was selected from the DEGs and data via the IMMPORT website. Hub genes were selected from the differentially expressed IRGs that formed the PPI network. Finally, the receiver-operating characteristic curves of the key genes were measured as biomarkers of DCM. A total of 173 independent DEGs (103 upregulated and 70 downregulated genes) were found in the microarray datasets GSE4172 and GSE21610. KEGG analysis and GSEA indicated that the BMP signaling pathway and apoptosis-related signals have a key effect on DCM development. The 10 hub genes also indicated the key effect of the BMP signaling pathway on DCM. A total of 224 differentially expressed IRGs and 20 featured IRGs were identified. Finally, *BMP6*, *CD69*, *RUNX2*, and *SPP1* were identified as possible targets for DCM. Our data suggest a possible molecular regulatory mechanism for DCM therapy. Moreover, *BMP6*, *CD69*, *RUNX2*, and *SPP1* may have key effects on the development of DCM.

Keywords: Dilated cardiomyopathy, KEGG, gene set enrichment analysis, *BMP6*, *CD69*, *RUNX2*, *SPP1*

Introduction

Dilated cardiomyopathy (DCM) causes many adverse effects, such as fibrosis, left ventricular remodeling, and decreased left ventricle function [1]. The development of implantable cardioverter-defibrillators and other therapeutic strategies has substantially reduced the sudden death rate [2]. Nevertheless, exploring the underlying pathway and finding valuable biomarkers could provide new insights into DCM diagnosis and treatment.

The gene transcription profile provided new insights into DCM pathogenesis, revealing that signature genes, such as *BICD2* [3] and *KLF13* [4], are abnormally expressed in DCM and may contribute to the biological processes involved in DCM. Furthermore, *FBXO32* plays an important role in DCM via whole-exome sequencing [5]. Consequently, a systematic investigation of the transcriptional profile could provide new insights into the mechanisms underlying DCM [6].

Therefore, this study aimed to identify the possible targets and molecular mechanisms of DCM via bioinformatic analysis. First, gene profiles of patients with DCM and healthy donors were obtained from the Gene Expression Omnibus (GEO) to select differentially expressed genes (DEGs). Next, the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis and Gene Set Enrichment Analysis (GSEA) of the DEGs were accomplished to elucidate the potential molecular pathway of DCM. The protein-protein interaction (PPI) network created via the STRING and Cytoscape websites, along with the hub genes, was validated to identify potential targets in the progression of DCM. Additionally, we

classified and selected immune-related genes involved in DCM to reveal the underlying relationship between immunity and cardiomyopathy. Finally, the receiver-operating characteristic curves of the hub genes could provide new insights into the molecular mechanisms of DCM and its therapeutic guidelines.

Materials and methods

Data acquisition

Human DCM and transmural myocardial sample microarray gene expression profiles, including GSE4172 and GSE21610, were downloaded from the GEO website. The gene-level patterns of the cardiac samples were evaluated between the DCM patients and control group.

Detection of differentially expressed genes

The gene-level profile was obtained from the GSE4172 and GSE21610 datasets. The R package "limma" was used to evaluate the DEGs. A false discovery rate < 0.05 and log2 fold change > 1.2 were the thresholds to classify the DEGs.

DEG enrichment analyses

The gene ontology (GO)/KEGG enrichment method was used to explain the DEG molecular features using the DAVID tool [7]. Statistical significance was set at $P < 0.05$.

Identification of regulatory network for DCM by GSEA

GSEA was performed for functional annotation and to verify whether the DEGs exhibited significant differences between the DCM and control groups [8, 9].

Protein-protein interaction (PPI) network formation

The PPI network was used to identify the protein connections and selected hub genes. The PPI network of DEGs was formed using the STRING website [10]. Cytoscape (version 3.8.2) was used to visualize the relationship networks of the DEGs.

Hub gene selection and analyses

Hub genes were selected using CytoHubba and the Molecular Complex Detection tool (MCODE) of Cytoscape (version 3.8.2), which could screen the core genes in the PPI network. The principles used in MCODE were as follows: MCODE scores > 5, maximum depth = 100, cut-off = 2, k score = 2, and node score cut-off = 0.2. Additionally, cytoHubba was used to select hub genes.

Immune-related gene analyses

The IMMPORT website contains 1793 immune-related genes (IRGs) that can mediate the immune process [11]. The IRGs were selected from the DEGs and IRGs. The PPI network of the selected IRGs was constructed using the STRING website. The top 20 hub genes in the PPI network were then selected.

Receiver-operating characteristic (ROC) curve investigations

The selected signature IRGs were authenticated, gene-level values were exhibited, and ROC curves were constructed to evaluate the predictability of the signature IRGs.

Results

Detection of DEGs among DCM tissues and normal group

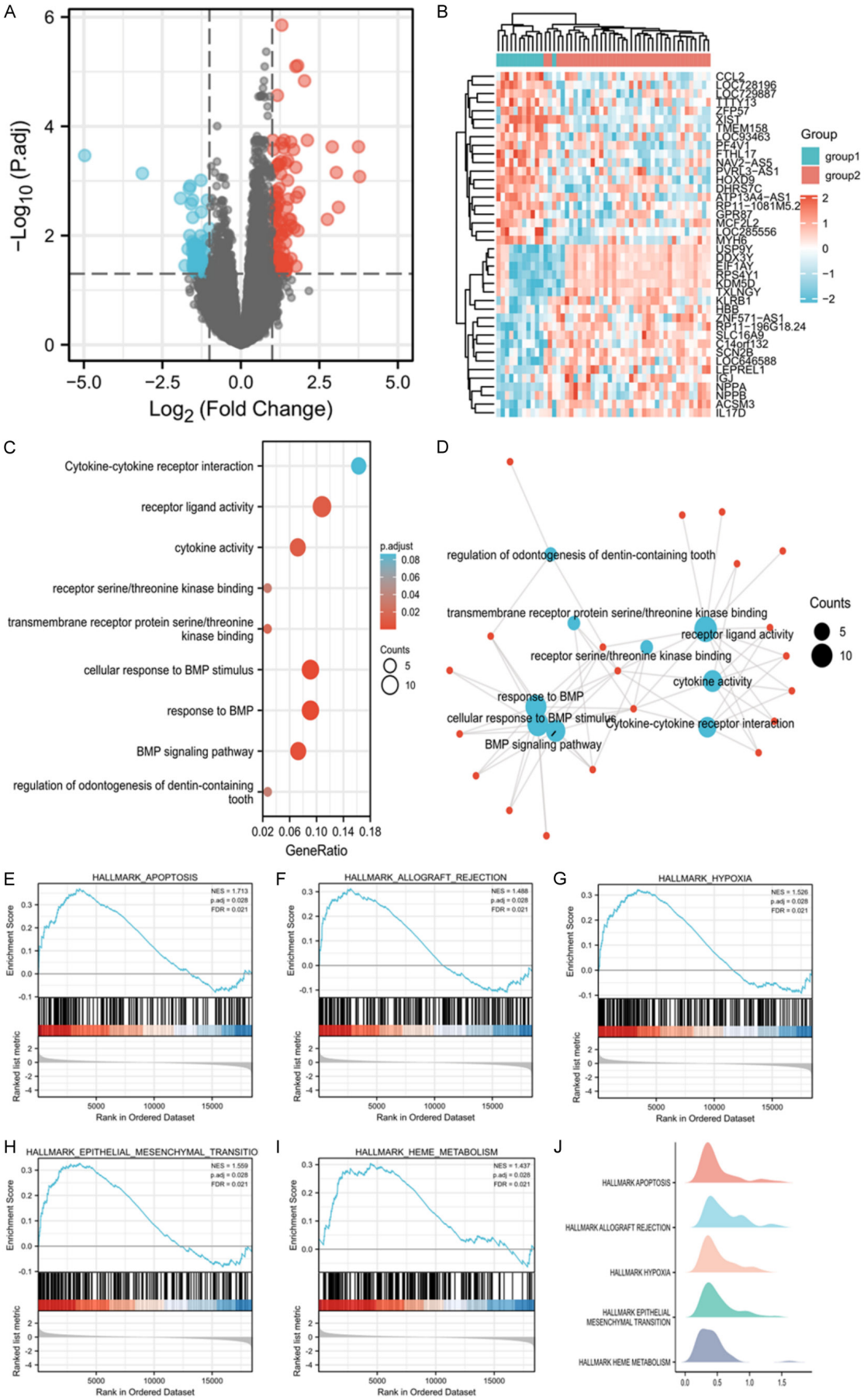
Based on the normalization of the gene expression profiles of GSE4172 and GSE21610, 173 DEGs were found, including 103 significantly upregulated and 70 notably downregulated (**Figure 1A**). The top 20 upregulated and downregulated genes were identified using a heatmap (**Figure 1B**).

KEGG and GO enrichment analyses of DCM and normal tissues

Both the GO and KEGG results of the DEGs are shown in **Figure 1C** and **1D**. We demonstrated that both the BMP signaling pathway and cytokine-cytokine receptor interactions played a key role in the occurrence of DCM.

Detection of GSEA among DCM and normal tissues

GSEA was used to further characterize the signals enriched among the DEGs. As illustrated in **Figure 1E-J**, apoptosis, hypoxia, heme metabolism, and epithelial-mesenchymal transition pathways were closely correlated with DCM. These results implied that apoptosis and me-


A
−Log10 (P.adj)
Log2 (Fold Change)
B
Group
group1
group2
CCL2
LOC728196
LOC729887
TTTY13
ZFP57
XIST
TMEM158
LOC93463
PF4V1
FTHL17
NAV2-AS5
PVRL3-AS1
HOXD9
DHRS7C
ATP13A4-AS1
RP11-1081M5.2
GPR87
MCF2L2
LOC285556
MYH6
USP9Y
DDX3Y
EIF1AY
RPS4Y1
KDM5D
TXLNGY
KLRB1
HBB
ZNF571-AS1
RP11-196G18.24
SLC16A9
C14orf132
SCN2B
LOC646588
LEPREL1
IGJ
NPPA
NPPB
ACSM3
IL17D
C
Cytokine-cytokine receptor interaction
receptor ligand activity
cytokine activity
receptor serine/threonine kinase binding
transmembrane receptor protein serine/threonine kinase binding
cellular response to BMP stimulus
response to BMP
BMP signaling pathway
regulation of odontogenesis of dentin-containing tooth
p.adjust
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regulation of odontogenesis of dentin-containing tooth
transmembrane receptor protein serine/threonine kinase binding
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cytokine activity
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HALLMARK EPITHELIAL MESENCHYMAL TRANSITION
HALLMARK HEME METABOLISM

Figure 1. Volcanos plot and heatmap of the GSE4172 and GSE21610 datasets. A. Volcano plots of the distribution of differentially expressed genes (DEGs) in each dataset, fold change ≥ 1.2, P < 0.05. B. The expression heatmap of the top 20 upregulated and downregulated DEGs. C, D. The DEGs in GSE4172 and GSE21610 and the Gene Ontology annotation and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis. E-J. The GSEA analysis of the KEGG pathways significantly enriched in GSE4172 and GSE21610. Screening criteria of selected KEGG pathways: biological functions of pathways, FDR value < 0.25, and *p*-value < 0.05. ES, Enrichment Score; FDR, False Discovery Rate.

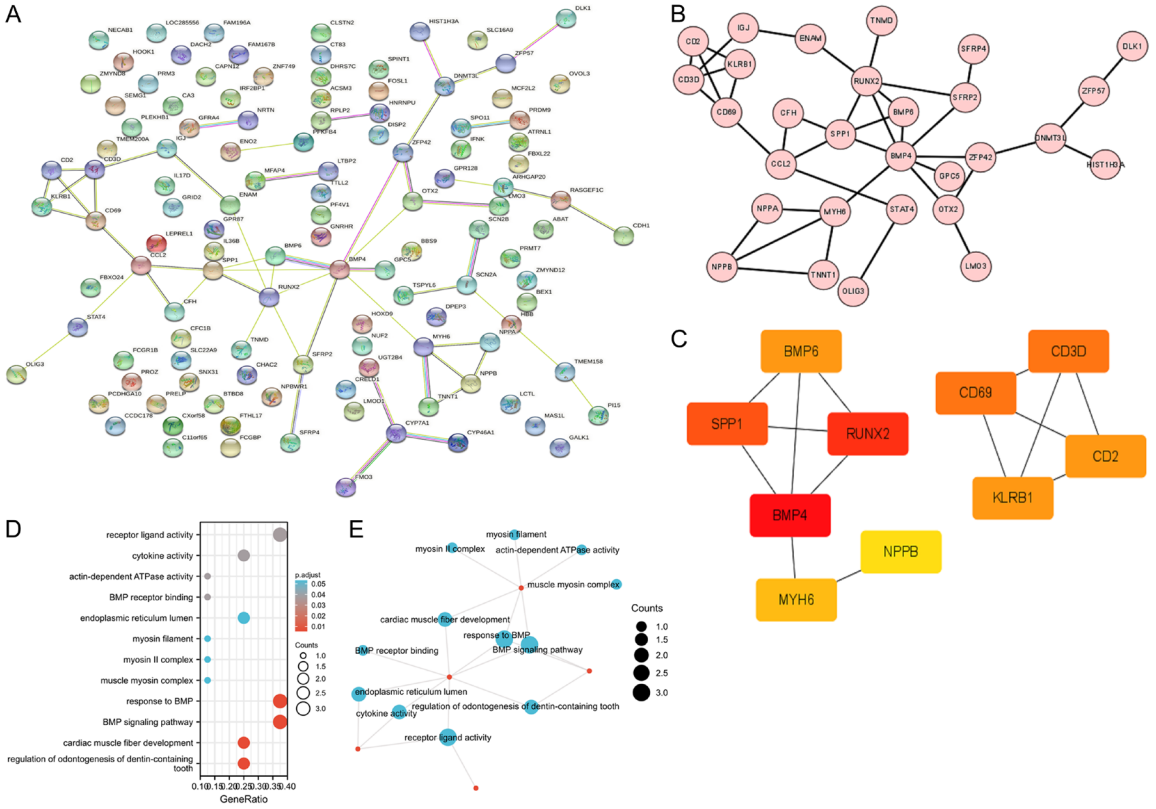

Figure 2. Protein-protein interaction (PPI) networks of the DEGs were constructed based on the STRING database and Cytoscape software. A. PPI network of the DEGs. B. The hub genes were screened by the MCDE from Cytoscape software. C. The hub genes were screened by the cytoHubba from Cytoscape software. D, E. The hub genes in GSE4172 and GSE21610 and the Gene Ontology annotation and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis.

tabolic disorders could be involved in DCM occurrence.

Protein-protein interaction network and hub gene analyses

To select the key genes involved in DCM development, PPI network analysis was performed using the STRING website (**Figure 2A**). The top 30 hub genes were screened using the MCODE app from Cytoscape (**Figure 2B**). Meanwhile, the results from the Cytoscape CytoHubba app revealed the top 10 hub genes: *MYH6*, *SPP1*, *CD69*, *CD2*, *RUNX2*, *KLRB1*, *NPPB*, *BMP6*, *CD3D*, and *BMP4* (**Figure 2C**).

Detection of enrichment analyses of key genes

Enrichment analyses of the key genes are shown in **Figure 2D** and **2E**. We found that cardiac muscle fiber development and the BMP signaling pathway contributed to DCM occurrence, which prompted us to further investigate the related genes in this pathway.

Validation of the key genes and related genes expression

Their expression profiles were monitored to verify the effect of these key genes on the

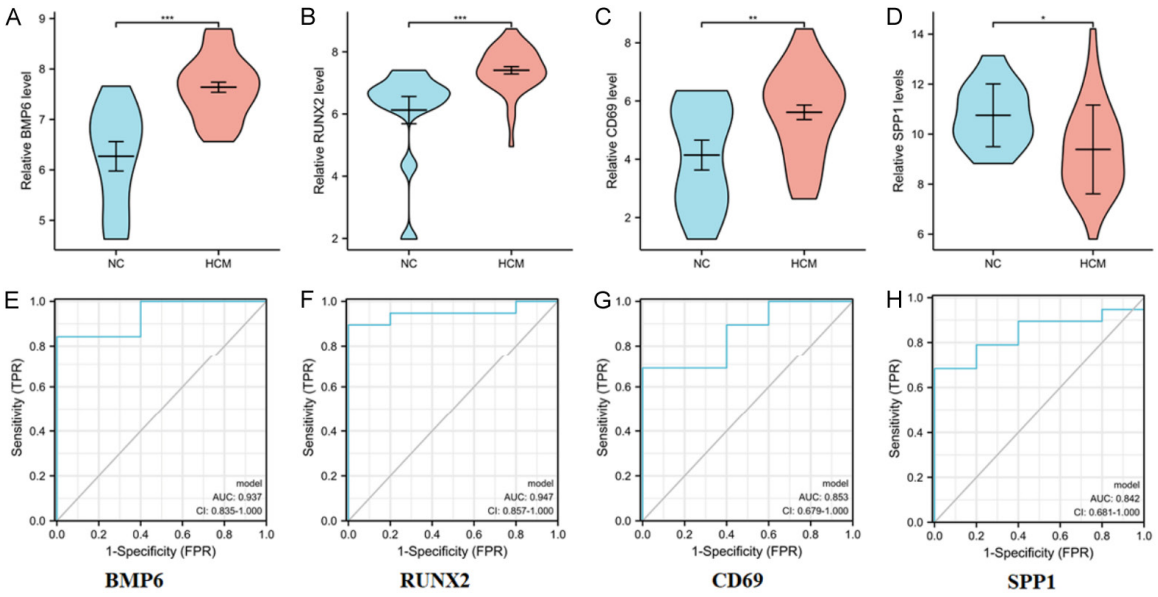

Figure 3. Verify the expression level and the prognostic value of key genes. A-D. The expression levels of hub genes (BMP6, CD69, SPP1and RUNX2) in GSE4172 and GSE21610. The *p*-value was from a Wilcoxon rank-sum test. *P < 0.05. **P < 0.01; ***P < 0.001. The y-axis represents the relative expression value log2 (TPM + 1). E-H. The receiver-operating characteristic curves of the four key genes in dilated cardiomyopathy (DCM).

occurrence of DCM. The key genes *BMP6*, *RUNX2*, and *CD69* were notably upregulated in the DCM group (**Figure 3A-D**), implying that *BMP6*, *RUNX2*, *SPP1,* and *CD69* participated in DCM occurrence.

Validation of featured genes and formation of diagnosis model

Our findings showed that the average AUC scores of *BMP6*, *RUNX2*, *CD69*, and *SPP1* were 0.937, 0.947, 0.853, and 0.842, respectively. The AUCs of these genes were significant, indicating good discriminatory ability for DCM diagnosis (**Figure 3E-H**).

Immune-related gene selection

According to the data provided by the IMMPORT website and DEGs, 224 differentially expressed IRGs were selected (**Figure 4A**). To determine connectivity, 224 differentially expressed IRGs were constructed using the STRING website (**Figure 4B**). The top 20 hub genes were selected and are shown in **Figure 4C**. Interestingly, *SPP1* was found in both the hub and immune-related gene groups. *SPP1* expression significantly decreased in the DCM group (P < 0.05, **Figure 3D**).

Discussion

DCM is a common cardiovascular disease that causes fatal arrhythmia, heart failure, and stroke. Nonetheless, the mechanism underlying DCM occurrence remains unclear. Consequently, it is vital to identify the mechanisms and select new effective targets for treating DCM using bioinformatic analysis. Due to difficulty in procuring the heart tissue from both the patients and healthy people, most previous studies used peripheral blood samples. To maximize the different gene expression profiling between the DCM patients and healthy controls, we selected human DCM and transmural myocardial microarray gene expression profiles, including GSE4172 and GSE21610. We selected 173 DEGs from the GSE4172 and GSE21610 databases; 103 were significantly upregulated and 70 were significantly downregulated between the DCM and control groups. The KEGG analysis showed that these DEGs mostly participated in cytokine-cytokine receptor interactions and the BMP signaling pathway.

Moreover, the GSEA data indicated that cell apoptosis and metabolic disorders might facilitate the occurrence of DCM. Hub genes were selected from the PPI network and then

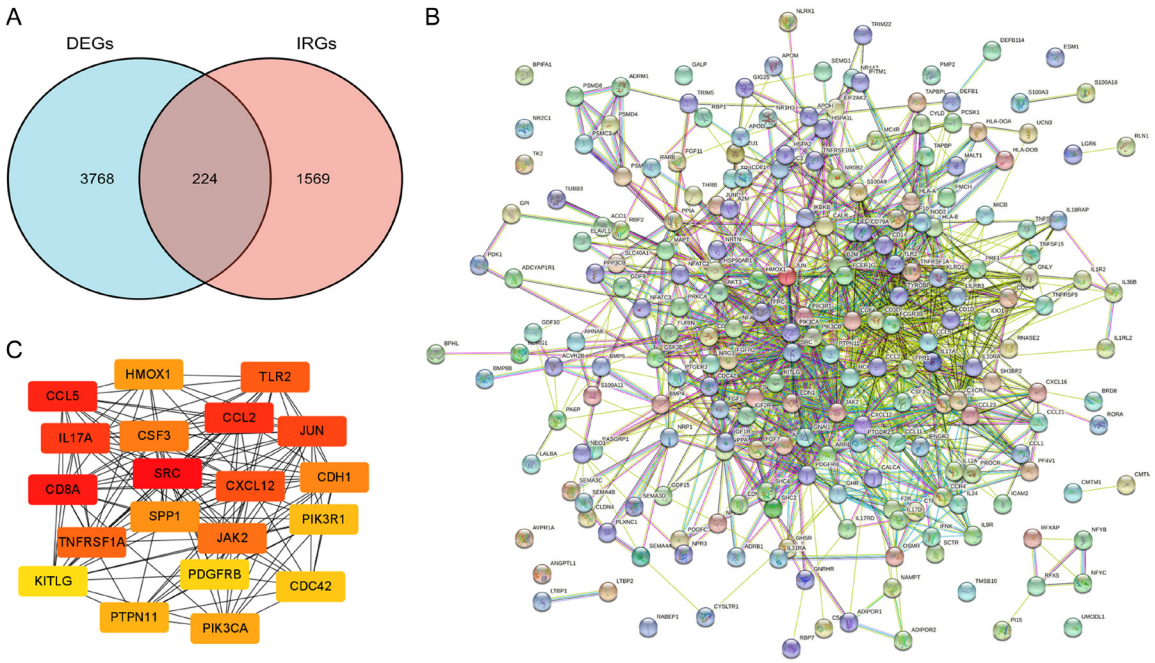

Figure 4. The differentially expressed immune-related genes and protein-protein interaction PPI network. A. The intersection data of DEGs and immune-related genes (IRGs) were presented. B. The PPI network of the differentially expressed IRGs. C. The top 20 genes with most adjacent nodes among the PPI network were screened by the cytoHubba from Cytoscape software.

enriched and analyzed. The molecular roles of these key genes indicated that the BMP signaling pathway could contribute to DCM pathogenesis. The IRGs were then obtained via the IMMPORT website and the featured IRGs were selected between the DEGs and IRGs. In addition, 224 IRGs were screened and the featured IRGs were verified. Finally, four key genes (*BMP6*, *RUNX2*, *CD69*, and *SPP1*) were regarded as potential biomarkers based on their significant AUC scores.

BMPs participate in vascular development, such as endothelial cell (ECs) differentiation and venous specification. Many BMP proteins, such as BMP4 and BMP6, are abnormally expressed in hypoxic ECs. Moreover, BMP6 has an anti-angiogenic effect on ECs via regulating VEGFR2 levels [12]. Furthermore, BMP6 induces the expression of the Hippo pathway, which regulates many cell functions, including cellular proliferation and apoptosis that have been linked to DCM development [13].

Runx2, an important transcription factor in bone development, is a critical mediator of adverse cellular effects on the cardiovascular system. However, whether Runx2 causes DCM development is still unclear. Both macrophages and cardiomyocytes undergo the remodeling processes, which finally causes DCM [14]. Moreover, Runx2 induces inflammatory molecules and macrophage infiltration, implying that Runx2 could play a crucial role in cardiovascular diseases, including DCM by regulating macrophage infiltration and inflammation [15]. As mentioned above, Runx2 may be a potential target for treating DCM.

CD69 participates in alterations in the immune response. CD69 deficiency increases the incidence of an acute stroke brain injury by stimulating a pro-thrombotic endothelial cell phenotype via *VWF* upregulation. Thus, CD69 deficiency is closely associated with worsened ischemic stroke outcomes. Moreover, neutrophil infiltration of the ischemic tissue is upregulated in *CD69*-knockdown mice [16]. As abnormal inflammation is a basic mechanism involved in DCM, CD69 may be a biomarker for cardiovascular-related diseases, including DCM.

Our study also showed that the featured IRGs might act as biomarkers via bioinformatic analysis of the transcriptome gene expression pro-

files of patients with DCM and normal controls. Interestingly, SPP1 is involved in myocardial infarction [17]. However, whether SPP1 could cause DCM is still unclear. Interstitial fibrosis and abnormal extracellular matrix protein production are well-known characteristics of DCM [18, 19]. SPP1 expression can regulate cell function by incorporating microenvironmental signals [20]. Together, this reveals that SPP1 is a possible biomarker for cardiovascular diseases, including DCM.

This study had some limitations. First, this study aimed to study the potential clinical value of the single hub genes in the DCM diagnosis; however, it needs further research to assess diagnostic efficacy with the identified genes in combination rather than individually. Second, although the four potential biomarkers were authenticated using GSE4172 and GSE21610 gene expression profiles, future studies need to generate more data to validate our findings. Thus, the biological roles of the four hub genes (*BMP6*, *CD69*, *RUNX2*, and *SPP1*) must be determined using *in vitro* and *in vivo* models.

Conclusions

In this study, we highlighted four hub genes (*BMP6*, *CD69*, *RUNX2*, and *SPP1*) as possible biomarkers and therapeutic targets for DCM treatment. This systematic investigation of key gene expression patterns could provide new insights into the molecular mechanisms of DCM.

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Disclosure of conflict of interest

None.

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