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Research Article

Immune-Related Genes: Potential Regulators and Drug Therapeutic Targets in Hypertrophic Cardiomyopathy

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Background. Accumulating evidence shows that the innate immune system is a key player in cardiovascular repair and regeneration, but little is known about the role of immune-related genes (IRGs) in hypertrophic cardiomyopathy (HCM). *Methods*. The differential mRNA expression profiles of HCM samples were downloaded from the Gene Expression Omnibus (GEO) dataset (GSE89714), and the IRG expression profile was obtained from the ImmPort database. The regulatory pathways of IRGs in HCM were screened out through discrepantly expressive genes (DEGs) analysis, enrichment of gene function/pathway analysis, and protein-protein interaction (PPI) network. Besides, hub IRGs in the PPI network were selected for drug prediction. *Results*. A total of 854 genes were differentially expressed in HCM, of which 88 were IRGs. Functional enrichment analysis revealed that 88 IRGs were mainly involved in the biological processes (BP) of SMAD protein pathway, smooth muscle cell proliferation, protein serine/threonine kinase, and mitogen-activated protein kinase (MAPK) cascade. Cytokine-cytokine receptor interaction, TGFβ signaling pathway, PI3K-Akt signaling pathway, and MAPK signaling pathway were enriched in the pathway enrichment analysis of these 88 IRGs. Furthermore, the PPI regulatory network of IRGs was constructed, and 10 hub IRGs were screened out to construct a regulatory network for HCM. 4 transcription factors (TFs) were the major regulator of 10 hub IRGs. Finally, these 10 hub IRGs were entered into the pharmacogenomics database for prediction, and the relevant drugs were obtained. *Conclusions*. In this study, 10 hub IRGs were coexpressed with 4 TFs to construct a regulatory network for HCM. Drug prediction of these 10 hub IRGs proposed potential therapeutic agents that could be used in HCM. These results indicate that IRGs are potential regulators and drug therapeutic targets in HCM.

1. Introduction

Cardiomyopathy refers to a myocardial disease with cardiac dysfunction, among which hypertrophic cardiomyopathy (HCM) is the most common. HCM is typically characterized by abnormal left and/or right ventricular hypertrophy [1]. HCM is commonly known as hereditary cardiomyopathy [2, 3], which is estimated that more than 16 genes and 900 mutations are responsible for the occurrence of HCM [4–6]. Currently, HCM treatment mainly focuses on gene therapy [7], immune-modulatory therapy [8], and regenerative therapy [9–11].

An abundance of evidence shows that the immune system with the multiple therapeutic targets for HCM plays a pivotal role in cardiac tissue damage and repair [12–15] by blocking the proinflammatory pathway [16, 17] or regulating the circulation of immune cells and the regeneration of resident immune cells [18, 19]. Accumulating evidence indicates that the immune system is also involved in cardiac regeneration and has become a new hotspot in HCM regenerative

therapy [20]. To date, treating MYBPC3 mutations is the only feasible gene therapies of HCM [7]. Nevertheless, the effect of IRGs on the process of HCM remains unclear.

In our study, we performed a systematic investigation of the HCM-related IRGs to identify prognostic biomarkers of HCM. The differentially expressed genes were obtained based on HCM, and the prognostic IRGs were further identified. Besides, functional analysis and the PPI network further revealed that these genes were implicated in the regulation of many biological processes, including SMAD protein pathways, smooth muscle cell proliferation, protein serine/threonine kinase, and MAPK cascade. Mechanistically, these regulatory pathways are involved in cytokine-cytokine receptor interaction, TGFβ signaling pathway, PI3K-Akt signaling pathway, and MAPK signaling pathway. Finally, the abundance of 10 hub IRGs was screened, and these ten hub IRGs were used for drug prediction. Meanwhile, these 10 hub IRGs were coexpressed with 4 TFs to construct a regulatory network for HCM. Altogether, we provided new insights into the regulatory mechanism of the IRGs in HCM, which may be a new direction for the treatment of HCM.

2. Materials and Methods

2.1. Data Acquisition. Transcriptome RNA-sequencing data of HCM (GSE89714, analyzed on Illumina HiSeq 2000) was downloaded from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo), which contained data from 5 hypertrophic heart tissue samples and 4 normal samples. The IRGs were obtained from the ImmPort database (http://www.immport.org), including 17 immune categories according to their different molecular function, such as T-cell receptor signaling pathway, B-cell receptor signaling pathway, Natural Killer Cell Cytotoxicity, antimicrobials, cytokine, and TNF family receptors. The list of TFs was getting from the Cistrome database. The function of the IRGs was acquired from Genecards (http://www.genecards.org/).

2.2. Identification of Differentially Expressed Genes (DEGs). The R package "limma" and "voom" functions are used to normalize the data. DEGs between HCM and normal samples were screened using the limma package under the condition of Log fold change (log FC) > 1 and adj. P value < 0.05.

2.3. KEGG Pathway Enrichment Analysis. Kyoto Encyclopedia of Genes and Genomes (KEGG) is a database resource for understanding advanced functions and biological systems from large-scale molecular data generated by high-throughput experimental techniques. KEGG database was used to identify upregulation pathways by DAVID (version 6.8, https://david.ncifcrf.gov/) in the background of Homo sapiens. The pathway with a corrected P value < 0.05 was considered a significant difference.

2.4. GO Enrichment Analysis. Gene ontology (GO) is a major bioinformatics tool for annotating genes and analyzing the biological processes (BPs), cellular components (CCs), and molecular functions (MFs), respectively. GO analysis was performed by DAVID (version 6.8, https://david.ncifcrf.gov/), and P value < 0.05 was considered statistically significant.

2.5. PPI Network Analysis. The PPI regulatory network of IRGs was constructed using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; http://string-db.org) database (version 10.0), and interactions with a combined score > 0.4 were considered statistically significant. The PPI network result was displayed using the Cytoscape software version 3.4.0, and the most important modules were identified by Cytoscape's plugin molecular complex detection (MCODE) (version 1.4.2). The selection criteria of differentially expressed IRGs in HCM were as follows: MCODE score > 5, degree cut − off = 2, node score cut − off = 0.2, maximum depth = 100, and k score = 2. P value < 0.01 was considered statistically significant. The top 10 differentially expressed IRGs act as the hub genes by ranking the degree number of genes in the PPI network.

2.6. Drug Prediction. We used three pharmacogenomic databases for drug prediction of 10 hub genes: Drugbank online website (https://go.drugbank.com/), Drug-Gene interaction database (https://dgidb.genome.wustl.edu/), and Connectivity map (https://portals.broadinstitute.org/cmap/).

2.6.1. Drugbank Online Website. The proteins encoded by the 10 hub genes mentioned above were used in Drugbank online website for drug prediction (https://go.drugbank.com/), and the hub gene-protein-drug network was constructed by the Cytoscape software.

2.6.2. Drug-Gene Interaction Database. These 10 hub genes directly predicted drugs through the Drug-Gene interaction database (https://dgidb.genome.wustl.edu/) and visualized with the Cytoscape software.

2.6.3. Connectivity Map. These 10 hub genes were used for drug prediction with Connectivity map (https://portals.broadinstitute.org/cmap/); drugs with connectivity score below -0.879 (i.e., drugs most likely to reverse gene differential expression therapy for DCM) were selected. The connectivity score of each drug for each hub gene in the specific cell line was summarized; a heat map was made with the R software.

3. Results

3.1. Screening Results of Differential Genes and the Enrichment of Gene Function Analysis in HCM. GSE89714 was downloaded from the GEO database, which contained data from 5 hypertrophic heart tissue samples and 4 normal myocardial samples. 854 differentially expressed genes (DEGs) were screened, including 675 upregulated genes and 179 downregulated genes (Figure 1).

After functional enrichment analysis of 854 DEGs in HCM, we found that these 854 DEGs mainly participated in 10 biological processes (BP), including extracellular matrix organization, extracellular structure organization, skeletal system development, ossification, collagen fibril organization, urogenital system development, axon development, bone development, connective tissue development, and renal system development (Figure 2(a)). Additionally, these 854 DEGs were mainly expressed in the extracellular matrix,

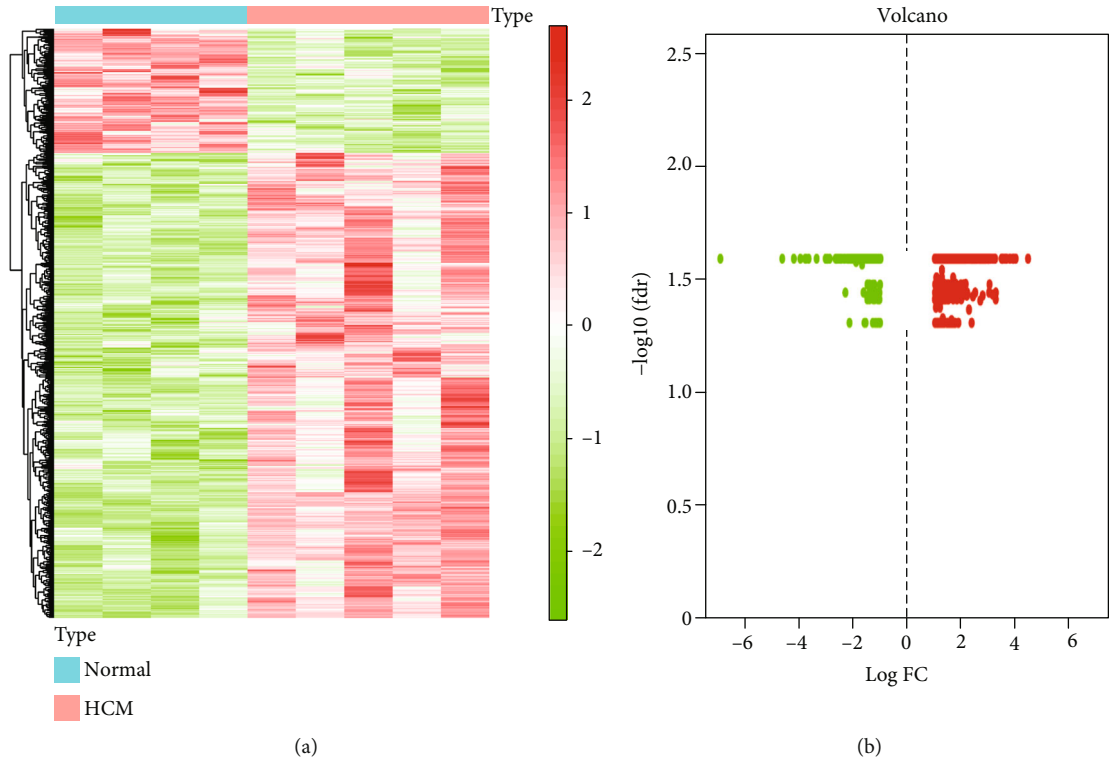


Figure 1: Differentially expressed genes in HCM. (a) The heat map of differentially expressed genes between HCM and normal tissues. Green dots represent lower expressed genes, and red dots represent highly expressed genes. (b) The volcano plot (b) of differentially expressed genes between HCM and normal tissues. Green dots represent lower expressed genes, and red dots represent highly expressed genes.

platelet alpha granule, basement membrane, endoplasmic reticulum lumen, collagen trimer, the complex of collagen trimers, and the cell-substrate junction (Figure 2(b)). Specifically, the molecular function (MF) of these 854 DEGs was mainly related to extracellular matrix structural constituent, collagen binding, glycosaminoglycan binding, extracellular matrix structural constituent conferring tensile strength, extracellular matrix structural constituent conferring compression resistance, heparin-binding, growth factor binding, growth factor activity, sulfur compound binding, and integrin binding (Figure 2(c)).

Subsequently, the results of the KEGG pathway show that there are 11 significantly upregulated pathways, including ECM-receptor interaction, focal adhesion, TGFβ signaling pathway, protein digestion and absorption, proteoglycans in cancer, PI3K-Akt signaling pathway, dilated cardiomyopathy (DCM), AGE-RAGE signaling pathway in diabetic complications, hypertrophic cardiomyopathy (HCM), amoebiasis, and cytokine-cytokine receptor interaction. The upregulated expression of the pathway is shown in Figure 2(d).

3.2. IRG Expression Profile of HCM and the Enrichment of Gene Function Analysis in HCM. The list of IRGs was downloaded through the ImmPort database. Meanwhile, the differentially expressed IRGs were extracted from GSE89714. 88 IRGs were differentially expressed in HCM. The expression levels of these 88 genes are listed in Supplementary Table S1, of which 16 are low expression and 72 are high expression (Figure 3(a)).

Through GO function enrichment analysis of 88 differentially expressed IRGs in HCM, we found that these 88 IRGs are mainly associated with the regulation of 10 biological processes (BP), including 4 processes of SMAD protein, 3 processes of smooth muscle cell proliferation, 2 processes of protein serine/threonine kinase, and MAPK cascade process (Figure 3(b)). Moreover, these 88 IRGs are mainly enriched in 10 Cell Components (CC), especially in platelet, extracellular matrix, vesicle lumen, granule lumen, mast cell, and so on (Figure 3(c)). Furthermore, the MF of these 88 IRGs is mainly correlated with receptor-ligand/regulator activity, growth factor activity/binding, cytokine activity/binding, hormone activity, G protein-coupled peptide receptor activity, and transmembrane receptor protein serine/threonine kinase binding (Figure 3(d)).

Thereafter, KEGG pathway enrichment analysis indicated that there are 30 significantly upregulated pathways in the microarray datasets, including cytokine-cytokine receptor interaction, TGFβ signaling pathway, viral protein interaction with cytokine and cytokine receptor, PI3K-Akt signaling pathway, pluripotency of stem cells regulation, MAPK signaling pathway, Hippo signaling pathway, proteoglycans in cancer, Ras signaling pathway, malaria, chemokine signaling pathway, calcium signaling pathway, hematopoietic cell lineage, amoebiasis, Rap1 signaling pathway, neuroactive ligand-receptor interaction, toxoplasmosis, cGMP-PKG signaling pathway, renal cell carcinoma, melanoma, axon guidance, Leishmaniasis, EGFR tyrosine kinase inhibitor resistance, bladder cancer, focal adhesion, gastric cancer,

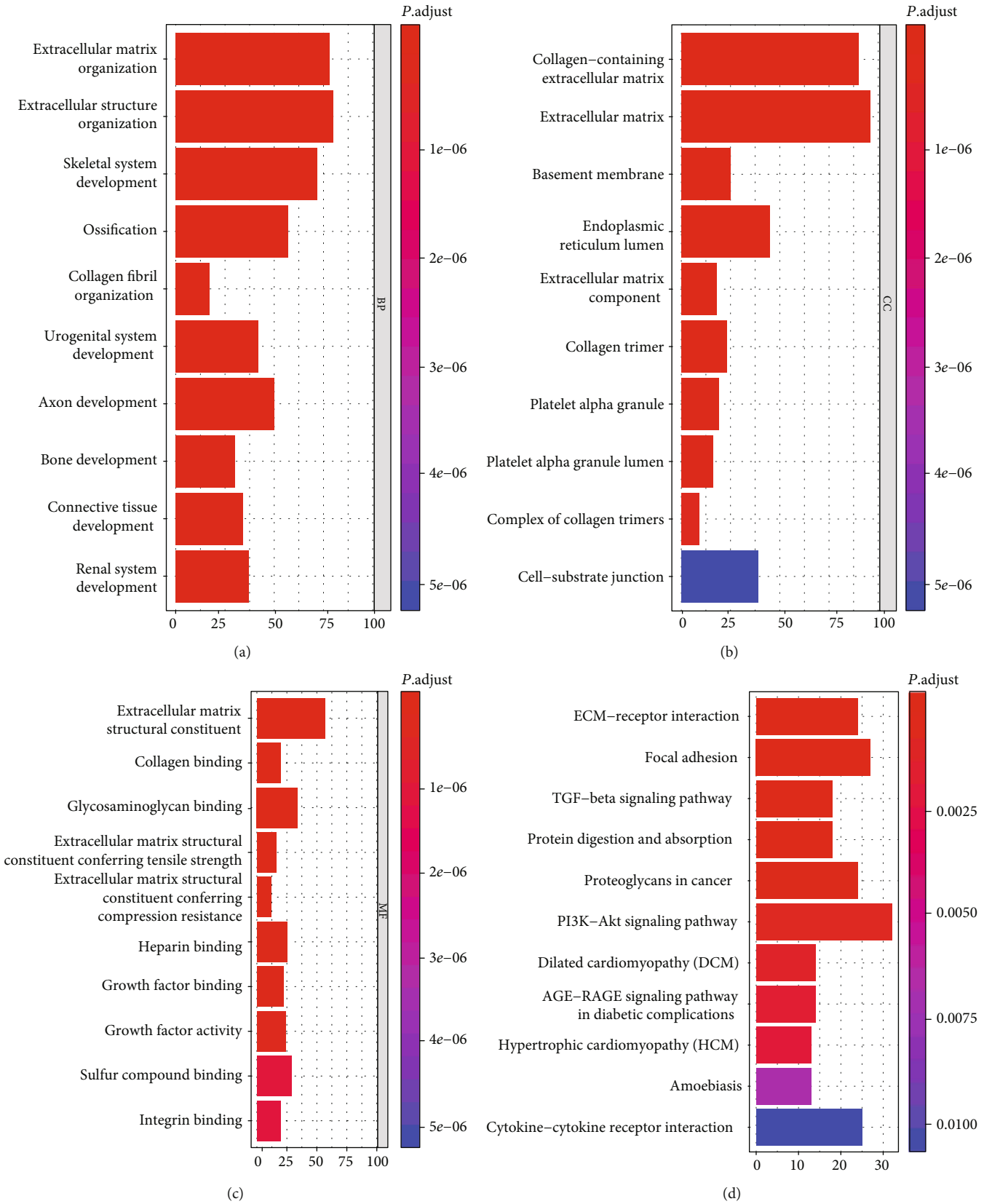


Figure 2: Gene functional enrichment of differentially expressed genes in HCM. (a) Gene ontology analysis: biological process of differentially expressed genes. (b) Gene ontology analysis: cellular component of differentially expressed genes. (c) Gene ontology analysis: molecular function of differentially expressed genes. (d) Encyclopedia of Genes and Genomes analysis: the top 10 significant pathways of differentially expressed genes.

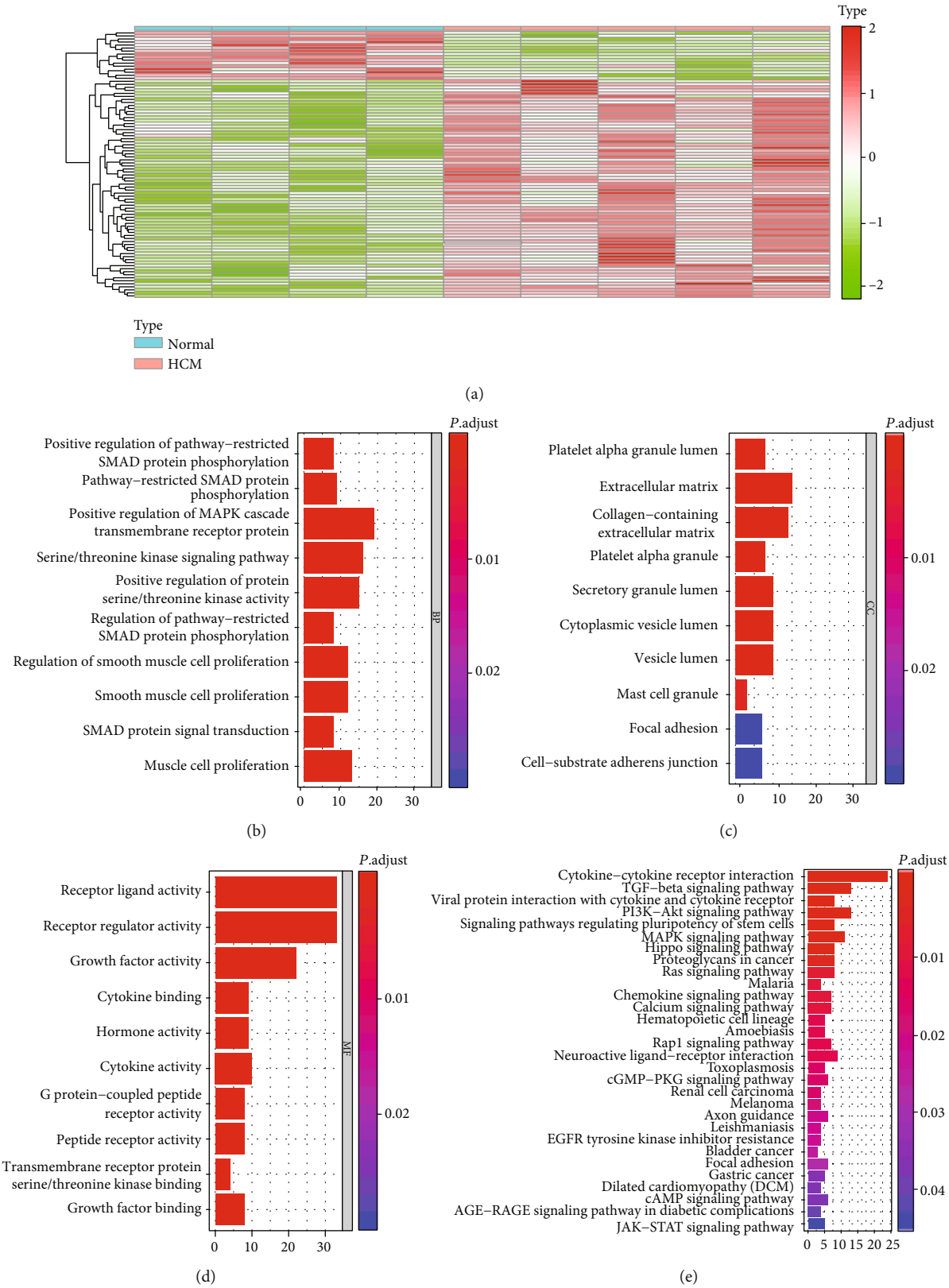


Figure 3: Differentially expressed IRGs in HCM and gene functional enrichment. (a) The differentially expressed IRGs between HCM and normal tissues. Green dots represent lower expressed genes, and red dots represent highly expressed genes. (b) Gene ontology analysis: biological process of differentially expressed IRGs. (c) Gene ontology analysis: cellular component of differentially expressed IRGs. (d) Gene ontology analysis: molecular function of differentially expressed IRGs. (e) Encyclopedia of Genes and Genomes analysis: the top 10 significant pathways of differentially expressed IRGs.

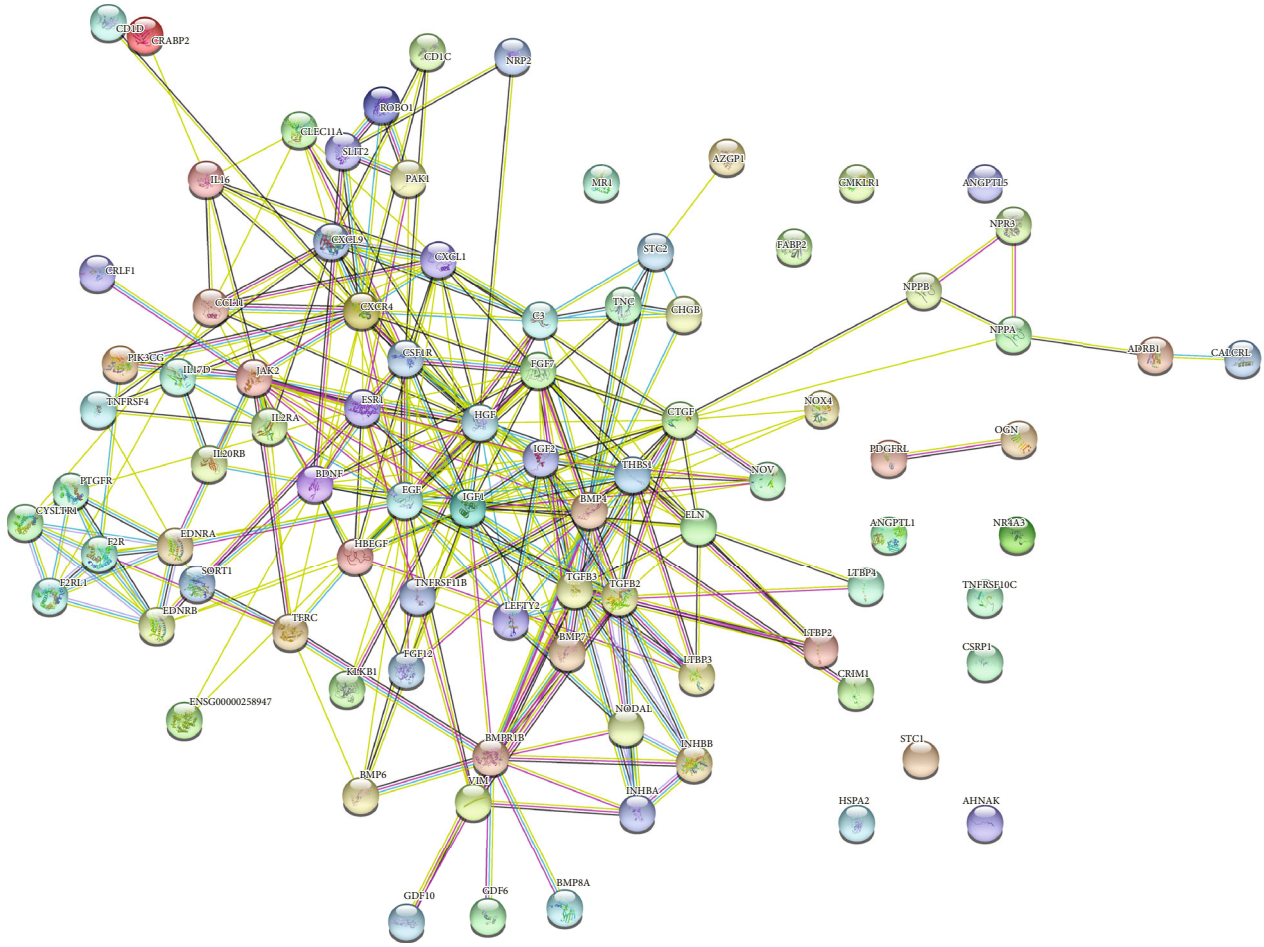


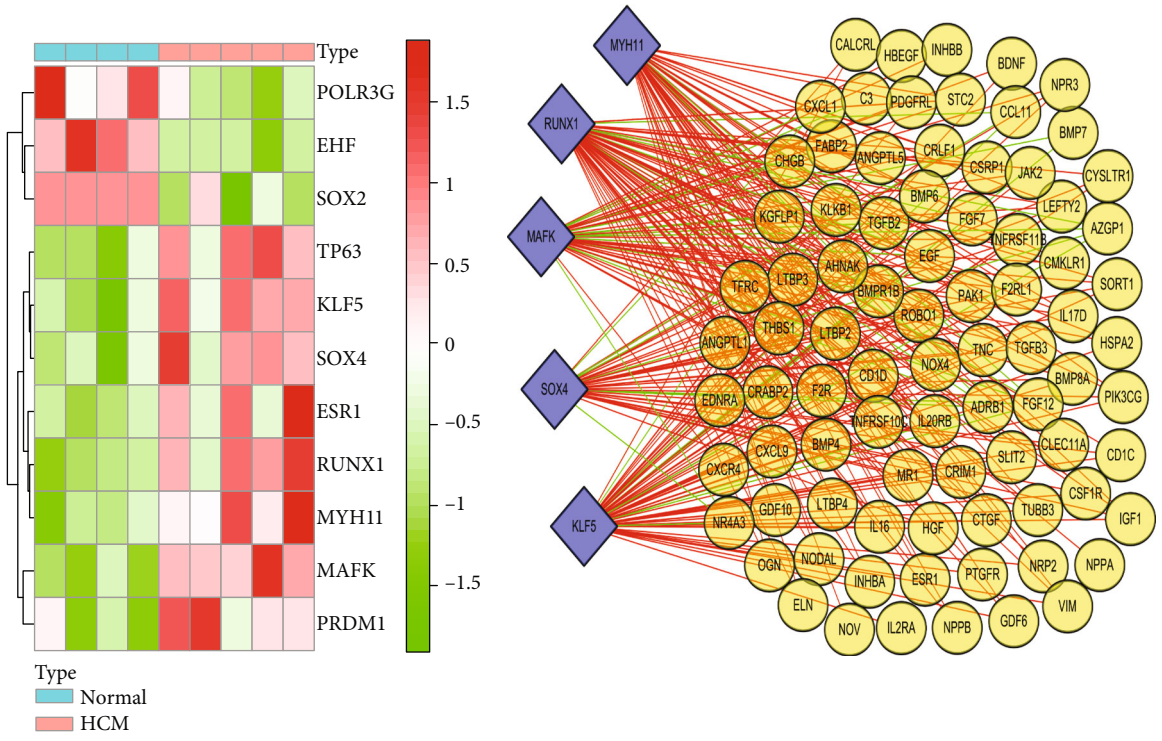
Figure 4: PPI network of IRGs in HCM.

dilated cardiomyopathy (DCM), cAMP signaling pathway, AGE-RAGE signaling pathway in diabetic complications, and JAK-STAT signaling pathway. The results are shown in Figure 3(e). Importantly, cytokine-cytokine receptor interaction, TGFβ signaling pathway, PI3K-Akt signaling pathway, and MAPK signaling pathway are the most obvious upregulation pathways (Figure 3(e)).

3.3. Screening of Differentially Expressed TFs and Coexpression Analysis of TFs and IRGs. The PPI regulatory network of IRGs was based on data collected from the STRING online database (https://string-db.org/). We found that IRGs interacted directly with each other through the analysis of the PPI network (Figure 4). The list of TFs was downloaded through the Cistrome database and screened out 11 differentially expressed TFs in HCM, including POLR3G, EHF, SOX2, TP63, SOX4, KLF5, ESR1, RUNX1, PRDM1, MAFK, and MYH11(Figure 5(a)). Next, coexpression analysis results of TFs and IRGs showed that there were 5 TFs coexpressed with 84 IRGs, including RUNX1, SOX4, KLF5, MAFK, and MYH11(Figure 5(b)). Notably, the 5 TFs are highly expressed in HCM. The expression levels are shown in Table 1. Finally, the regulatory network between 5 TFs and 84 IRGs was constructed by using the software of Cytoscape.

3.4. Identification of Hub IRGs and TF Regulatory Network of IRGs. The top 10 IRGs (EGF, IGF1, HGF, BMP4, CXCR4, CTGF, THBS1, TGFB2, JAK2, TGFB3) were selected as the hub genes. Obviously, they also interacted with each other (Figure 6(a)), especially for IGF1 and HGF, THBS1 and IGF1, THBS1 and HGF, THBS1 and BMP4, CTGF and IGF1, CTGF and HGF, TGFB3 and THBS1, and TGFB3 and CTGF (Figure 6(c)). Except for EGF, the other 9 genes were highly expressed in HCM (Table 1). The functional roles of 10 hub genes were downloaded from the online database (https://www.genecards.org/) and are shown in Table 2. Besides, coexpression analysis results showed that there were 4 TFs with a crucial regulatory role in 10 hub IRGs network, including SOX4, KLF5, MAFK, and MYH11 (Figure 6(b)).

3.5. Drug Prediction of Hub Genes. We used multiple pharmacogenomic databases for drug prediction of 10 hub genes and found that different prediction methods have different results. In Drugbank online website (https://go.drugbank.com/), there are proteins encoded by only 7 hub genes yielded related drugs; proteins encoded by other 3 hub genes have not related drugs (Figure 7(a)). In the Drug-Gene interaction database (https://dgidb.genome.wustl.edu/), all hub genes resulted in the corresponding drugs; only FRESOLIMUMAB and ABT-510 were mainly correspond to two



(a) (b)

Figure 5: TF regulatory network. (a) The differentially expressed TFs between HCM and normal tissues. Green dots represent lower expressed TFs, and red dots represent highly expressed TFs. (b) The TF regulatory network of IRGs.

Table 1: Expression levels of 5 TFs that regulate IRGs.

Gene	Normal myocardium	Hypertrophic cardiomyopathy	logFC	*P* value
MAFK	10.2	22.52	1.143	0.016
KLF5	1.35	3.36	1.316	0.019
RUNX1	1.075	4.5	2.067	0.016
SOX4	4.425	10.34	1.224	0.032
MYH11	37.075	88.28	1.252	0.016

genes, while other drugs are all only correspond to one gene (Figure 7(b)). In the Connectivity map (https://portals.broadinstitute.org/cmap/), 13 drugs with connectivity score below -0.879 were predicted, summarizing the connectivity scores of these 13 drugs with 10 hub genes and making a heat map with the R software (Figure 7(c)).

4. Discussion

HCM is an inherited myocardial disease with cardiac dysfunction and genetic disorder [21–23]. Accumulating evidence revealed that the immune system plays a key role in heart injury responses [24–26]. It has been reported that myocardial aging is largely associated with changes in gene expression patterns of immune response [27]. Therefore, it is plausible that the development of HCM is attributed to abnormal IRG expression. In our results, a total of 854 mRNAs were differentially expressed in HCM patients, of which 88 were IRGs, including 16 low-expressed IRGs and 72 high-expressed IRGs. 10 hub IRGs (IGF1, CTGF, TGFB2, TGFB3, HGF, BMP4, CXCR4, THBS1, EGF, JAK2) were selected to construct a regulatory network in HCM, suggesting that these 10 hub IRGs might be the new therapeutic targets for the intervention of HCM.

Insulin-like growth factors 1 (IGF1) is a hormone with pleiotropic effects, which consists of 70 amino acids and is regulated by IGF-binding proteins (IGFBPs) [28–31]. It is generally accepted that IGF1 exhibits a protective effect on myocardium by regulating autophagy, precursor cell differentiation, extracellular matrix activity, and myocardial fibrosis [32, 33]. In our results, insulin-like growth factor 1 (IGF1) and IGF-binding protein (CTGF) are two hub IRGs in HCM. Moreover, CTGF and IGF1 were highly expressed in HCM and significantly interacted with each other($R = 0.92$). Accordingly, IGF1 may provide a novel therapeutic direction for HCM. A plethora of evidence showed that IGF1 could regulate the metabolism of cardiomyocytes through MAPK [34–36] and PI3K-Akt pathway [37]. In the present study, GO function enrichment analysis showed that IRGs participate in the process of MAPK cascade, and the KEGG pathways are enriched in the PI3K-Akt signaling pathway and MAPK signaling pathway. Therefore, PI3K-Akt and MAPK signaling pathway may be the regulatory mechanism of IGF1 on the pathogenesis of HCM.

TGFβs are multifunctional cytokines with three isoforms (TGFβ1, 2, and 3) encoded by three distinct genes, which are essential mediators of cardiac repair [38–42]. Extensive

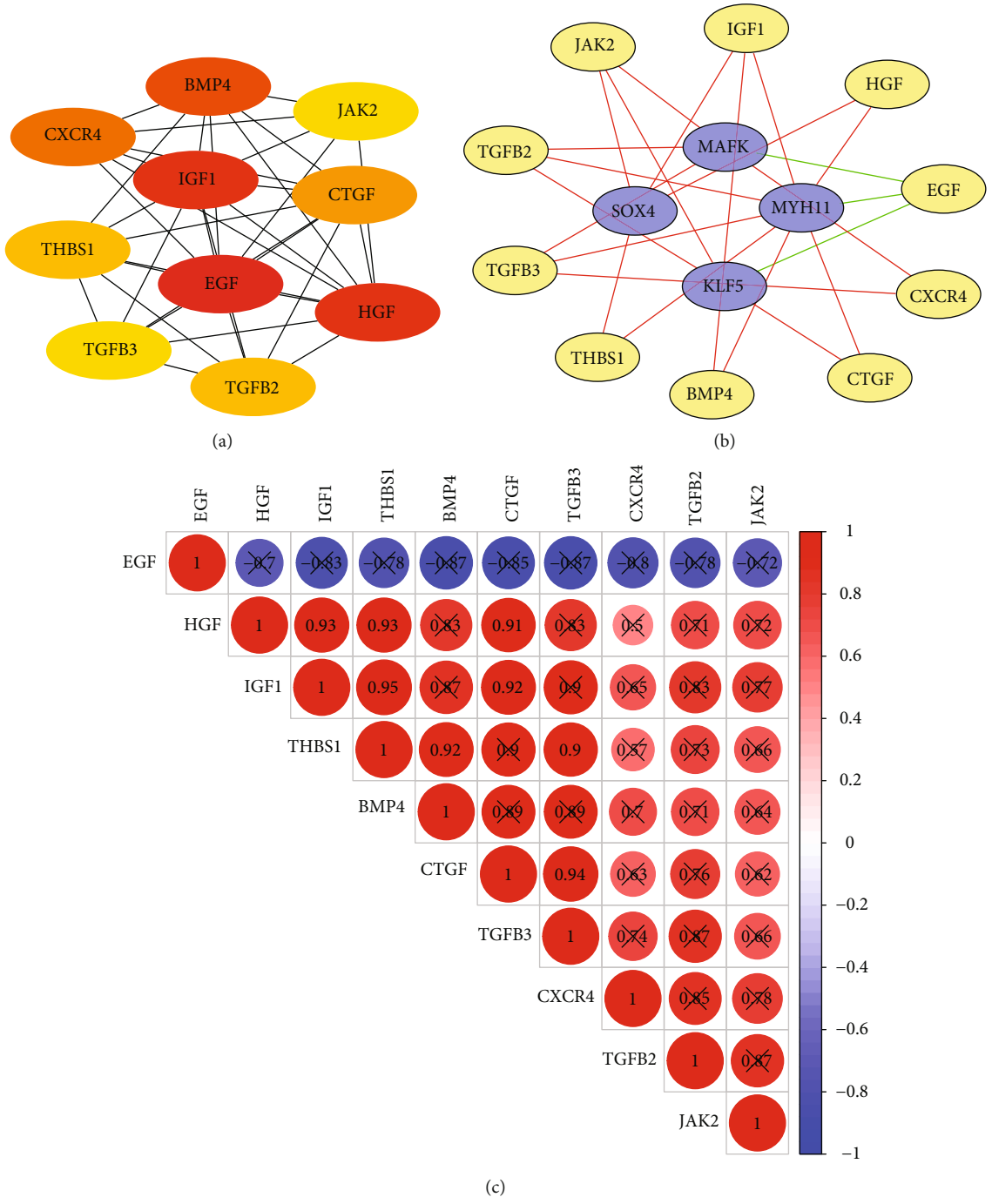


Figure 6: Identification of hub IRGs and the TF regulatory network: (a) the interaction network of hub IRGs; (b) the TF regulatory network of hub IRGs; (c) the correlation coefficient of hub IRGs.

evidence suggests that TGFβ is produced by various cells in response to tissue injury, especially in injured myocardial regions. In general, TGFβ isoforms show different expression patterns in the injured myocardium: TGFβ1 and TGFβ2 are induced in the early stage, whereas TGFβ3 shows a delayed and prolonged upregulation. Importantly, our results reveal that transforming growth factor β2 proprotein (TGFB2) and transforming growth factor β3 (TGFB3) are two hub IRGs in HCM. Moreover, TGFB2 and TGFB3 were highly expressed in HCM, indicating a promising therapeutic target of TGFβ for HCM. TGFβ can be activated by SMAD [43, 44]. Meanwhile, TGFβ-SMAD3 signaling pathway is an important mediator of cardiac fibroblasts and is expected to be an attractive therapeutic target for HCM [45–48]. In our results, these 88 IRGs are involved in the regulation of 4 biological processes of SMAD protein, including positive regulation of pathway-restricted SMAD protein phosphorylation, pathway-restricted SMAD protein phosphorylation, regulation of pathway–restricted SMAD protein phosphorylation, and SMAD protein signal transduction. Furthermore, the

Table 2: Functional roles of the 10 hub genes.

No.	Gene symbol	Full name	Function
1	EGF	Epidermal growth factor	EGF stimulates the growth of various epidermal and epithelial tissues in vivo and in vitro and of some fibroblasts in cell culture.
2	IGF1	Insulin like growth factor 1	The insulin-like growth factors, isolated from plasma, are structurally and functionally related to insulin but have a much higher growth-promoting activity.
3	HGF	Hepatocyte growth factor	Activating ligand for the receptor tyrosine kinase MET by binding to it and promoting its dimerization.
4	BMP4	Bone morphogenetic protein 4	Induces cartilage and bone formation. Also acts in mesoderm induction, tooth development, limb formation, and fracture repair.
5	CXCR4	C-X-C motif chemokine receptor 4	Receptor for the C-X-C chemokine CXCL12/SDF-1 that transduces a signal by increasing intracellular calcium ion levels and enhancing MAPK1/MAPK3 activation.
6	CTGF	IGF-binding protein 8	Major connective tissue mitoattractant secreted by vascular endothelial cells.
7	THBS1	Thrombospondin-1	Adhesive glycoprotein that mediates cell-to-cell and cell-to-matrix interactions.
8	TGFB2	Transforming growth factor beta-2 proprotein	Associates noncovalently with TGF-beta-2 and regulates its activation via interaction with "milieu molecules."
9	JAK2	Janus kinase 2	Nonreceptor tyrosine kinase involved in various processes such as cell growth, development, differentiation, or histone modifications.
10	TGFB3	Transforming growth factor beta 3	Precursor of the latency-associated peptide (LAP) and transforming growth factor beta-3 (TGF-beta-3) chains, which constitute the regulatory and active subunit of TGF-beta-3, respectively.

results of KEGG pathway enrichment analysis showed that the TGFβ signaling pathway is upregulated. Collectively, the above results suggest that two hub IRGs (TGFB2 and TGFB3) regulated the TGFβ-SMAD signaling pathway and participate in the pathological process of HCM.

In the regulatory networks of the 10 hub IRGs, 4 TFs (SOX4, KLF5, MAFK, and MYH11) were coexpressed with these 10 hub IRGs, which suggests that SOX4, KLF5, MAFK, and MYH11 are the major regulators of IRGs during HCM. In detail, SOX4 is a member of the Sox (for Sry-box) family, which is essential for the regulation of embryonic development and cell fate. Also, SOX4 is required for the development of cardiac outflow tract. SOX4 deficiency even causes congenital heart defects in embryonic mice [49–51]. As a member of the Krüppel-like factors (KLFs) family, KLF5 is intimately correlated with cardiovascular remodeling, cardiac energetic, and cardiac fibroblasts [52–55]. Notably, KLF5 has recently been proved as a novel disease gene of familial dilated cardiomyopathy (DCM) and negatively regulates HCM [56, 57]. MAFK, a member of the small Maf family, is an important contributor to hematopoiesis [58]. MAFK is well known for its role in modulating NF-κB activity and controlling immune-inflammatory responses [59, 60]. MYH11 is a smooth muscle myosin belonging to the myosin heavy chain family, which plays a vital role in human disease familial hypertrophic cardiomyopathy (FHC) [61, 62]. In our results, these 4 TFs were not only coexpressed with 10 hub genes but also correlated with each other to form a regulatory network. The network may be a potential therapeutic target for HCM.

Recent studies revealed that the immune system plays a pivotal but complex role in myocardial injury and repair [63, 64]. During the cardiac injury, the immune mechanism is activated, which induces an inflammatory response, and subsequently triggers reparative pathways to promote wound healing [65]. Surprisingly, the overall mobilization of the immune system follows a consistent pattern in response to tissue injury: when an injury occurs, the dying cardiomyocytes trigger an acute inflammatory response and are recognized by resident immune cells, such as macrophages, innate lymphoid cells (ILC), and mast cells (MC) [66]. Subsequently, these resident immune cells also participate in the removal of dying cardiomyocytes, the reconstruction of tissue matrix, the restriction of the spread of inflammatory molecules, and the maintenance of local homeostasis [67, 68]. Besides, macrophages/monocytes secrete various chemokines and cytokines that recruit immune cells to promote wound healing [69]. These processes are highly consistent with the cell constituents (CC) of 88 IRGs, which are differentially expressed in our results. In our results, 88 IRGs are primarily enriched in the collagen-containing extracellular matrix, secretory granule lumen, cytoplasmic vesicle lumen, mast cell granule, and cell-substrate adherens junction.

In this study, we used three pharmacogenomic databases for drug prediction of 10 hub genes and found that different prediction methods have different results. In Drugbank online website (Figure 7(a)), proteins encoded by only 7 hub genes yielded related drugs. In the hub gene-drug network (Figure 7(b)), there are two drugs FRESOLIMUMAB and ABT-510, corresponding to two hub genes at the same time, indicating that these two drugs may be vital therapeutic molecules. Connectivity map prediction results (Figure 7(c)) clearly showed that all 13 drugs could upregulate the differentially underexpressed hub genes, that is, EGF. All these 13 drugs could downregulate most of the differentially highly expressed 9 hub genes. These drugs may be potentially effective therapeutic molecules in the future and may inspire the design of similarly structured active drug molecules.

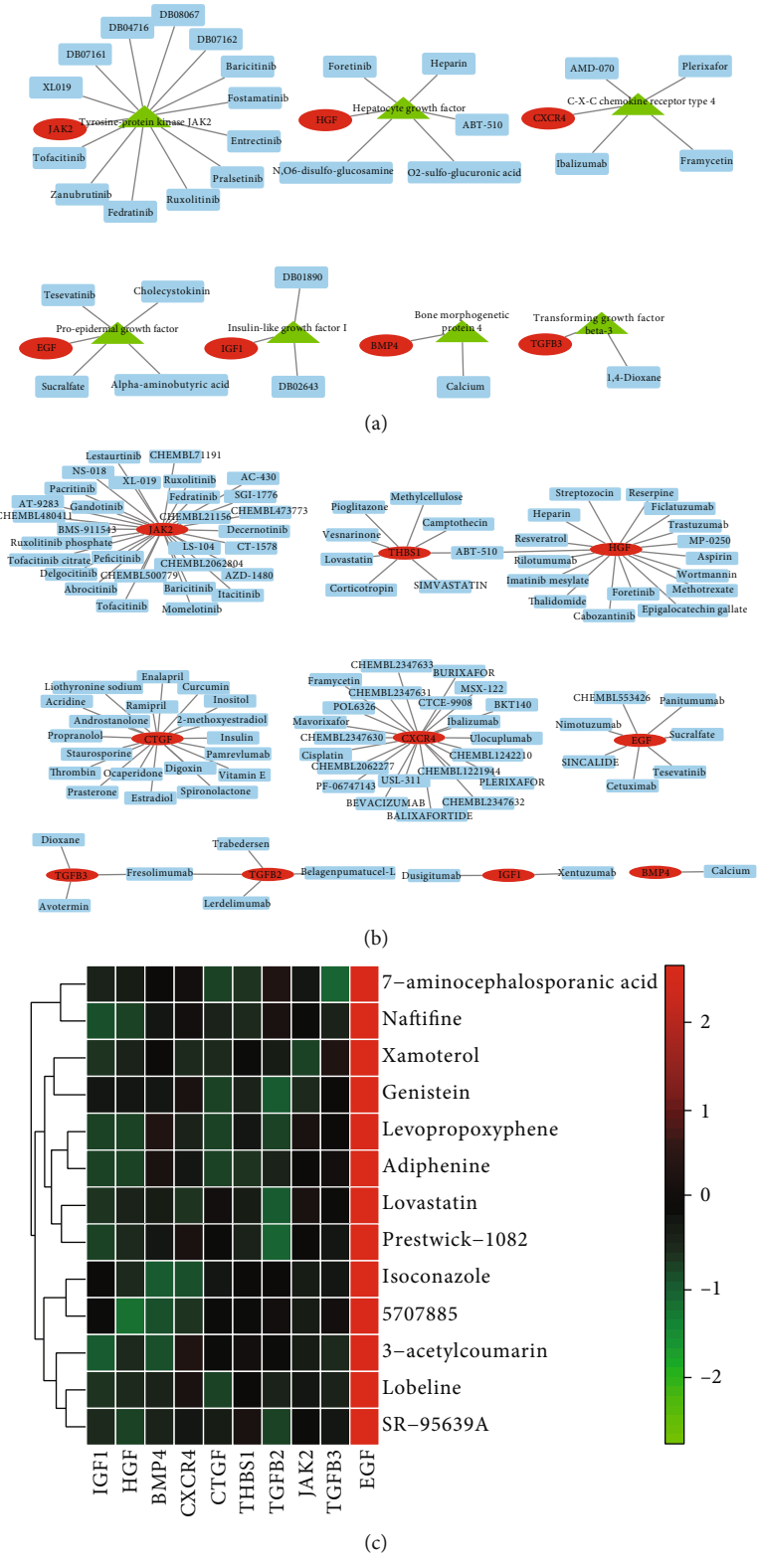


Figure 7: Drug prediction of 10 hub IRGs. (a) Hub gene-protein-drug network. Red ovals represent hub genes, green triangles represent proteins encoded by hub genes, and blue rectangles are drugs. (b) Hub gene-drug network. Red indicates hub genes, and blue indicates drug. Among them, the 78 drugs corresponding to JAK2 showed only the top 30, and all 78 drug names can be obtained in Supplementary Table S2. (c) Heat map of the effect of drugs on hub genes expression. Green indicates that the drug can downregulate the hub gene expression, red marks that the drug can upregulate the hub gene expression, and black means that the drug and the hub gene expression have little correlation.

Generally, cardiac injury activates immune mechanisms, which depend on the close interaction of cells, the regeneration of cardiac resident immune cells, and immune cells [70, 71]. In the process of myocardial injury and repair, immune cells, like $CD4^+$ T cells, $CD8^+$ T cells, B cells, natural killer cells (NK), and macrophages, are the main regulator in wound healing, which includes resident immune cells and circulating immune cells from the blood [72–74]. As mentioned previously, the resident immune cells coordinate the removal of dying cardiomyocytes, the reconstruction of tissue matrix, the restriction of the spread of inflammatory molecules, and the maintenance of local homeostasis. In addition, excessive inflammatory signals can also trigger extramedullary hematopoiesis of the spleen and produce new immune cells that are recruited to the injured site to alleviate tissue injury [75]. Therefore, the balance between self-maintenance of the cardiac resident immune cells and the recruitment of circulating immune cells is crucial for the potential of cardiac regeneration [76]. At present, the replacement of lost cardiomyocytes is the most important strategy for the treatment of cardiomyopathy. In the past, the regenerative therapy of HCM is mainly focused on the "stem cell," especially for pluripotent stem cell-derived cardiomyocytes [77, 78]. However, it seems that "stromal cell types" have got more attention, particularly including resident immune cells and innate immune system. Therefore, the cardiac resident immune cells become a hotspot in cardiac regeneration of HCM [20]. Moreover, macrophages are also implicated in heart regeneration [79, 80]. In our results, the KEGG pathway of 88 differentially expressed IRGs is also mostly enriched in signaling pathways that regulate pluripotency of stem cells and hematopoietic cell lineage, suggesting that IRGs might be another hotspot in cardiac regeneration of HCM by regulating pluripotency of stem cells and hematopoietic cell lineage.

5. Conclusions

IRGs are a key player in the progression of hypertrophic cardiomyopathy, which is mainly involved in immune regulation, cardiac regeneration, and gene therapy. Meanwhile, 10 hub IRGs were coexpressed with 4 TFs to construct a regulatory network for HCM. Mechanistically, PI3K-Akt and MAPK signaling pathway are the main regulatory mechanism of IGF1, and TGFβ-SMAD signaling pathway is another regulatory mechanism in the pathological process of HCM. Drug prediction of these 10 hub IRGs proposed potential therapeutic agents that could be used in HCM. All these data suggest that IRGs may be a new direction for the treatment of HCM.

Data Availability

The data used to support the findings of this study are available in the Gene Expression Omnibus (GEO) repository (https://www.ncbi.nlm.nih.gov/gds).

Conflicts of Interest

The authors declare that they have no competing interests.

Acknowledgments

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Supplementary Materials

Supplementary 1. Table S1: the expression levels of 88 IRGs.

Supplementary 2. Table S2: 78 drug names corresponding to JAK2.

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