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Identification of Potential Gene Interactions in Heart Failure Caused by Idiopathic Dilated Cardiomyopathy

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Background: Many heart failure (HF) cases are caused by idiopathic dilated cardiomyopathy (iDCM). This study explored the mechanisms of the development and progression of HF caused by iDCM.

Material/Methods: The gene expression profiles of 102 samples were downloaded from the GEO database (GSE5406). Differentially expressed genes (DEGs) were identified through GO analysis and a KEGG pathway analysis, respectively. A protein–protein interaction (PPI) network was constructed and analyzed to screen potential regulatory proteins. In addition, MCODE and a cytoHubba plugin were used to identify the module and hub genes of DEGs. Finally, transcription factors (TFs) were predicted using PASTAA. We did not perform whole-exome sequencing (WES) for detecting mitochondrial DNA (mtDNA).

Results: A total of 197 DEGs were screened, and 3 modules, and 4 upregulated and 11 downregulated hub genes were screened. The GO analysis focused on the terms and 12 KEGG pathways were enriched. The FOS, TIMP1, and SERPINE1 hub genes, as well as some key TFs, demonstrated important roles in the progression of HF caused by iDCM. CEBPD, CEBOB, CDC37L1, and SRGN may be new targets for HF in iDCM patients.

Conclusions: The identified DEGs and their enriched pathways provide references for exploring the mechanisms of the development and progression of HF patients with iDCM. Moreover, modules, hub genes, and TFs may be useful in the treatment and diagnosis of HF patients with iDCM. However, mtDNA was not investigated.

MeSH Keywords: Gene Expression Profiling • Heart Failure • Transcriptome

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Background

Despite the significant developments that have been made regarding the physiopathology of heart failure (HF), HF remains an increasing global epidemic, with an estimated >37.7 million individuals affected globally [1]. Among HF cases, idiopathic dilated cardiomyopathy (iDCM) has a high mortality rate and many patients are under the age of 10 years [2]. Additionally, iDCM accounts for approximately 50 000 hospitalizations and 10 000 deaths each year, and is responsible for about 25% of all cases of HF [3]. iDCM is characterized pathologically by left ventricle dilation, functionally by progressive contractile failure, and histologically by cardiomyocyte hypertrophy, the loss of myofibrils, and interstitial fibrosis [4]. However, its mechanism is not fully understood. The pathogenesis of HF arising from different etiologies varies [5]; thus, it is important to provide treatment targeted to the relevant etiology, especially in cases of iDCM with diversity pathogenesis.

Improving the outcome of patients with iDCM remains challenging for clinicians. Studies that focus on the treatment of iDCM-caused HF include urgent screening biomarkers that can make precise diagnoses. Syndecan-4 may be a useful biomarker for predicting adverse effects in iDCM patients, hepatocyte growth factor can be significantly increased in advanced HF patients with iDCM, and desmin (DES) is a good marker of HF development in patients with iDCM [6–8]. However, the cause of iDCM is unknown, more studies are needed that focus on its treatment in order to improve the outcome of HF patients with iDCM and to precisely diagnose the disease based on the screening of biomarkers. Therefore, to understand the mechanism, it is crucial to screen biomarkers with high specificity and sensitivity.

In this study, which was based on bioinformatics, samples of GSE5406 were used to analyze differential expression genes (DEGs), the enrichment of GO terms or pathways, and protein–protein interaction (PPI) in HF patients with iDCM in order to predict potential targets. We also analyzed the modules of each PPI network and sought to determine possible hub genes using cytoHubba and a network analyzer. We explored the functions of these modules. Finally, the common genes of the 3 methods were selected as the hub genes. Based on these hub genes, the key transcription factors (TFs) became evident.

Material and Methods

Data source

The gene expression profile of GSE5406 was downloaded from the GEO database (*http://www.ncbi.nlm.nih.gov/geo/)* using the platform GPL96 ([HG-U133A] Affymetrix Human Genome U133A Array; Affymetrix, Inc., Santa Clara, CA, USA). A total of 102 individuals were analyzed in this study. RNA samples for this dataset were extracted from explanted left ventricular (LV) myocardia of 86 patients with systolic HF due to iDCM (iDCM-HF), and 16 unused donor hearts with normal LV functions at the time of harvest were used as controls [9]. By applying whole-exome sequencing (WES), mitochondrial DNA (mtDNA) mutation in patients were identified, but we did not perform WES in this data source.

Data processing

The expression matrix retrieved from the GEO database was pre-processed using the robust multiarray analysis (RMA) method (*http://www.bioconductor.org/)*. The probe ID for each gene was then converted to a gene symbol using hgu133a.db, org.Hs.eg.db, and the annotate package in Bioconductor (*http://www.bioconductor.org/*). If a gene's symbols corresponded with multiple probes' IDs, the expression level of that gene was represented by the mean of the probes [10].

Identification of DEGs

A Linear Models for Microarray Data (LIMMA) package of *R* was used to identify the DEGs in the iDCM-HF samples compared with the normal LV samples [12]. The DEGs were selected with a threshold of |log2(fold change)|>0.589(fold change>1.5) and $p<0.05$. A heatmap and volcano plot were used to plot the samples and the DEGs using the pheatmap package in *R*.

Functional enrichment analysis

To further explore the biofunctions of the DEGs, a GO analysis and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were used. clusterProfiler (*http://www.bioconductor.org/packages/release/bioc/html/clusterProfiler.html*) in the *R* package was used to perform the KEGG (*http://www.genome.jp/kegg/*) enrichment analysis [13]. The cut-off was set at p<0.5.

Construction of the differential co-expression gene network

PPI are considered important for understanding the potential functions of a certain protein. In this study, the online database Search Tool for the Retrieval of Interacting Genes (STRING, stringdb.org) was utilized to evaluate the PPI among the DEGs [14]. To explore the regulatory mechanism of the DEGs in iDCM-HF, interactions with a medium confidence of a combined score >0.4 obtained from STRING were imported into the Cytoscape to construct the PPI network [15].

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Table 1. The KEGG pathway(p.adj<0.5).

ID	Description	Gene	P.adj
hsa04974	Protein digestion and absorption	SLC38A2, ATP1A1, COL1A2, COL15A1, COL21A1, COL1A1, COL3A1, ATP1A3	0.002989601
hsa04978	Mineral absorption	HMOX2, ATP1A1, MT1X, MT1M, MT2A, ATP1A3	0.002989601
hsa05205	Proteoglycans in cancer	LUM, MAP2K1, CDKN1A, FLNC, FZD7, ITGA5, PPP1R12B, COL21A1, THBS1, STAT3, MYC	0.002989601
hsa04510	Focal adhesion	MAP2K1, COL1A2, FLNC, ITGA5, PPP1R12B, PDGFD, COL1A1, THBS1, THBS4, SPP1	0.01037994
hsa04151	PI3K-Akt signaling pathway	ATF4, FGF1, MAP2K1, COL1A2, CDKN1A, ITGA5, AREG, PDGFD, COL1A1, THBS1, THBS4, MYC, SPP1	0.020270584
hsa04512	ECM-receptor interaction	COL1A2, ITGA5, COL1A1, THBS1, THBS4, SPP1	0.020270584
hsa04657	IL-17 signaling pathway	FOSL1, S100A9, CEBPB, CCL2, S100A8, FOS	0.031466966
hsa04010	MAPK signaling pathway	ATF4, FGF1, MAP2K1, FLNC, AREG, PDGFD, PLA2G4C, MYC, DUSP1, HSPA6, FOS	0.031466966
hsa04933	AGE-RAGE signaling pathway in diabetic complications	COL1A2, SERPINE1, COL1A1, STAT3, COL3A1, CCL2	0.033386478
hsa04066	HIF-1 signaling pathway	MAP2K1, SERPINE1, CDKN1A, NPPA, STAT3, TIMP1	0.033386478
hsa05219	Bladder cancer	MAP2K1, CDKN1A, THBS1, MYC	0.033386478
hsa05224	Breast cancer	FGF1, MAP2K1, CDKN1A, FZD7, MYC, HEY1, FOS	0.044949711

Analysis of hub proteins

To obtain balance between the core genes and avoiding missing the key gene, we extracted the hub genes using cytoHubba. Through the cytoHubba plugin, 12 topological analysis methods were obtained [16]. The top 30 hub-forming genes/proteins (15%) were identified based on MCC, MNC, and DMNC, respectively. Then, the overlapping genes were selected as the hub genes. Finally, we found the common genes using Venn diagrams (*http://bioinformatics.psb.ugent.be/webtools/Venn/*).

Module

A Molecular Complex Detection (MCODE) analysis was performed to screen modules within the PPI network in the Cytoscape software [17]. MCODE was also used to identify the modules in the PPI network with a parameter degree cut-off ≥2 and a k-core ≥3.

Analysis of TFs

After selecting 15 hub genes, the TFs were predicted using PASTAA [18]. Four upregulated genes and 11 down-regulated genes were established, and the calculated *p* value and association score were used to evaluate the correlation between the disease and TFs through hypergeometric distribution.

Results

The DEGs

A total of 197 DEGs were finally screened out from the iDCM-caused HF samples to compare them with the normal left ventricle function samples, including 105 upregulated and 92 downregulated DEGs. The heat map for the DEGs is illustrated in Figure 1A, and the volcano plot is shown in Figure 1B. We did not perform whole-exome sequencing WES for detecting mtDNA.

Functional enrichment of DEGs

The GO enrichment analysis and KEGG pathway analysis revealed that the identified DEGs are significant. The top 10 most significant GO terms of the enriched biological processes (BPs) (Figure 2A), molecular functions (MFs) (Figure 2B), and cell components (CCs) (Figure 2C), respectively, are shown according to gene ratio. These include sulfur glycosaminoglycan binding, extracellular structure organization, and extracellular matrices. Moreover, the associated KEGG pathways most significantly enriched by DEGs are also displayed in Figure 3. The interaction with this pathway is represented by *R*, and Table 1 shows the detailed information of the KEGG pathway at a cutoff of p.adjust <0.5. The "PI3K-Akt signaling pathway",

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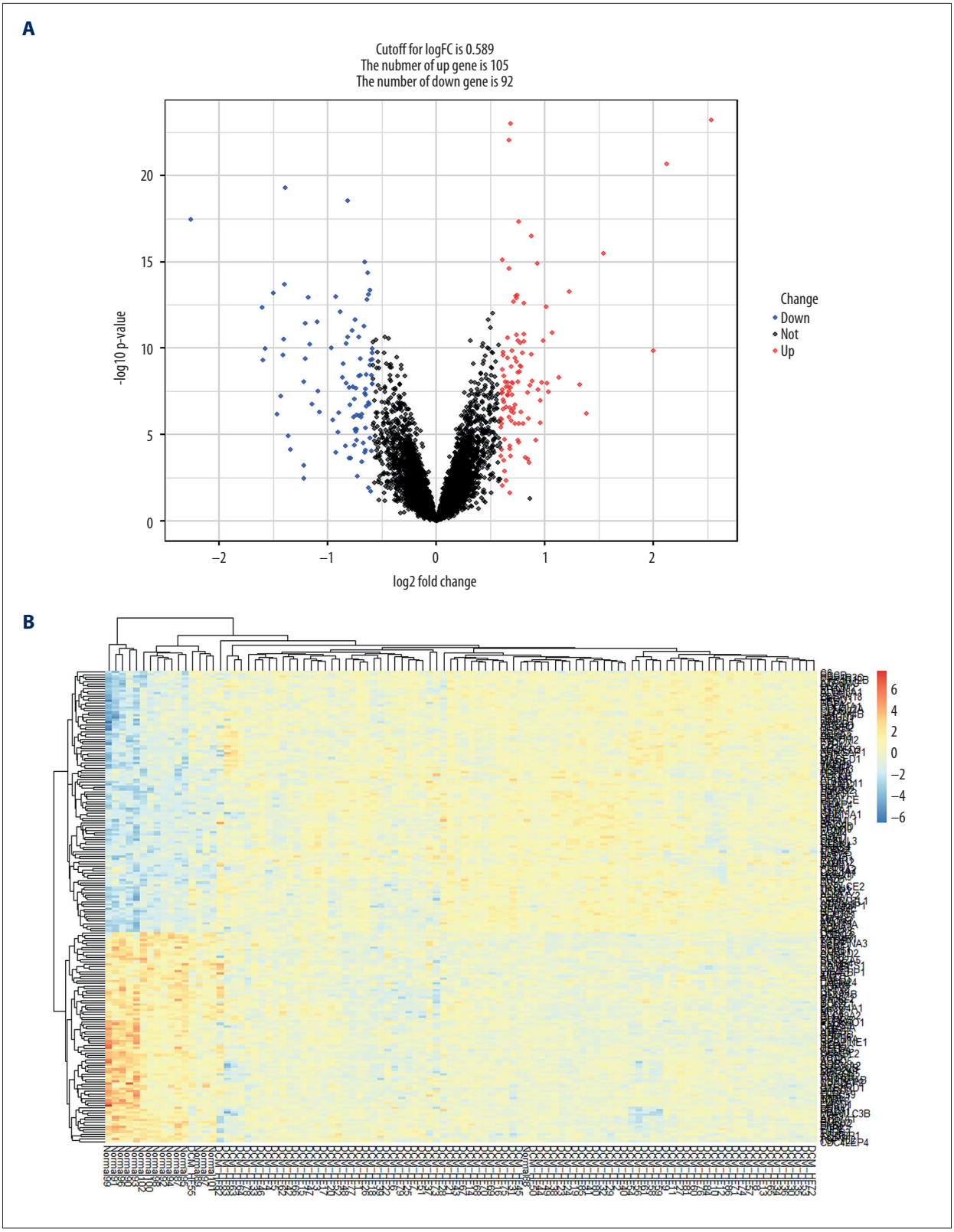

Figure 1. (**A**) Heatmap and (**B**) vocano plot for DEGs.

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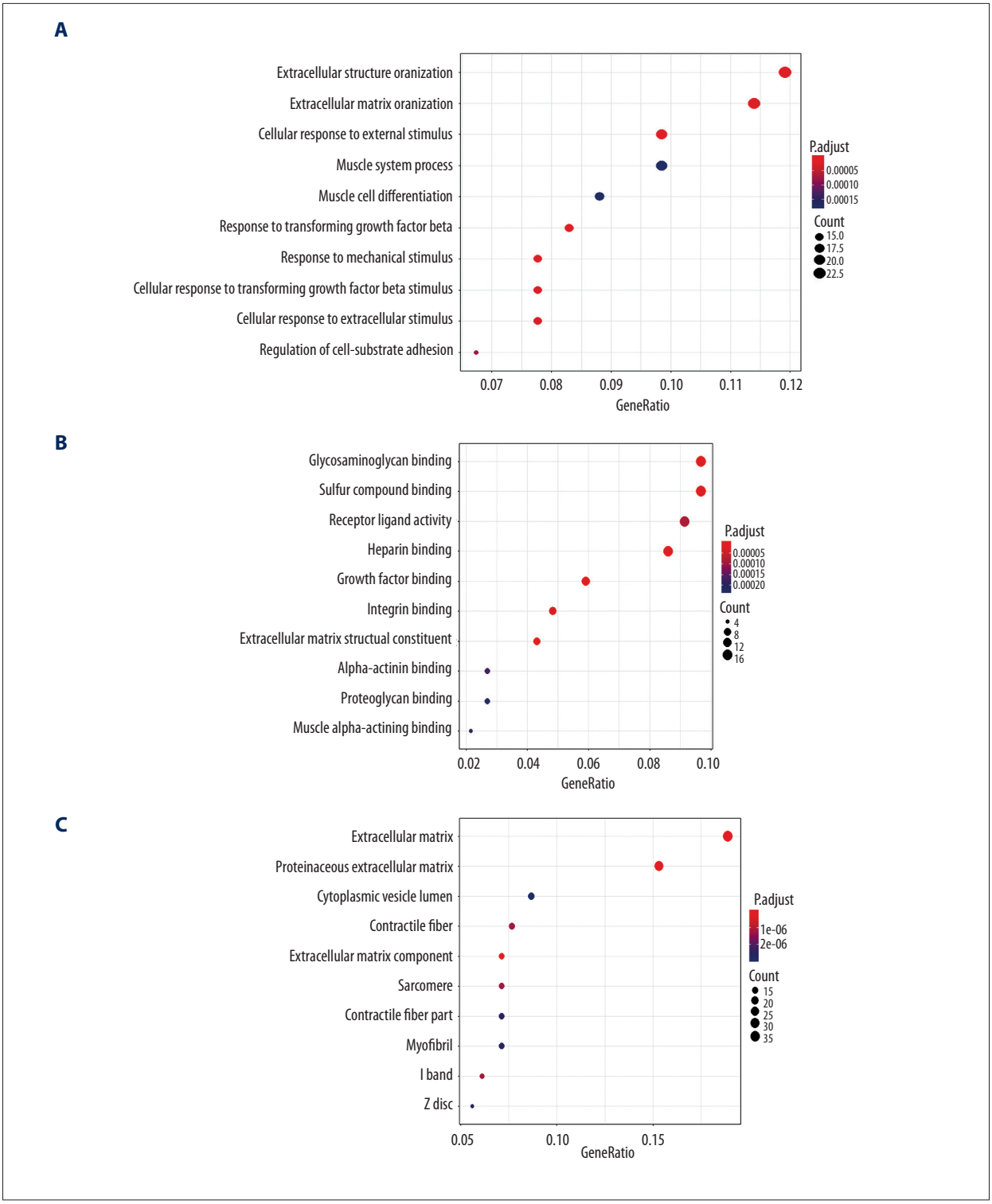

Figure 2. The top 10 significant GO terms of the enriched (**A**)biological processes (**B**) molecular function (**C**) Cell component.

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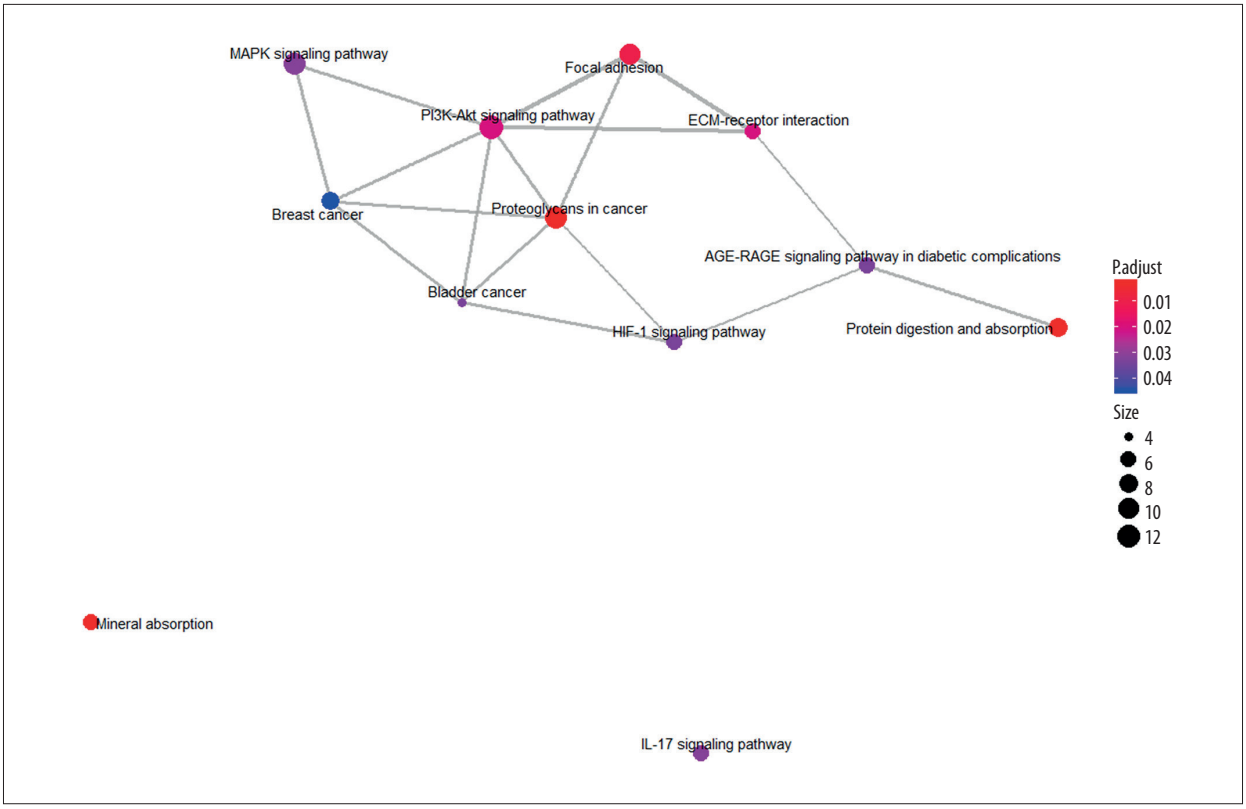

Figure 3. KEGG pathways enriched by DEGs and the relationship of passway (p.adjust <0.05).

"MAPK signaling pathway", and "proteoglycans in cancer" have the most links with the KEGG pathways.

PPI of the DEGs

By integrating the DEG pairs with combined scores >0.4, PPI network analysis for genes in the green and red clusters with 144 nodes and 379 edges (Figure 4) accounted for 73.1% of DEGs. These include 12 upregulated genes at FC>2 (MXRA5, COL1A1, C6, LUM, FRZB, COL1A2, HBB, THBS4, COL15A1, ASPN, NPPA, and MATN2) and 24 downregulated genes at FC<0.5 (PDK4, LMCD1, SERPINE1, SLC19A2, RARRES1, MYOT, CNN1, SLC38A2, FCN3, PLIN2, PTX3, ANKRD2, FLNC, FKBP5, MYH6, CD163, NRAP, S100A8, S100A9, CYP4B1, PLA2G2A, MT1M, SERPINA3, and HOPX).

Module

In a pathogenetic network, some gene products have the same or similar functions. These are located in the same functional unit of the network, which is called a module, and they work together to carry out their biological functions [19]. Typical approaches to detecting functional units in PPI networks resort to functional modules [20]. Thus, we subsequently conducted a module analysis. When a "score ≥3" was defined as the cutoff criterion in MCODE, 3 clusters of modules (Module 1, Module 2, and Module 3) were identified from the PPI network visualized by STRING (Figure 5A–5C). The most significant cluster consisted of 10 nodes and 35 edges (Module 1). Furthermore, the MCODE analysis showed the 3 seed genes of each cluster; these were CEBPB, ISLR, and LDB3.

Hub gene selection

The hub genes were determined by overlapping the genes according to 3 ranked methods in cytoHubba. Fifteen hub genes were selected, and they included 4 upregulated genes and 11 downregulated genes. The overlapping of the DEGs in cytoHubba using the 3 methods is shown in Figure 5D. The details are shown in Table 2 and visualized in Figure 5E. Our results show the functions of 15 hub genes and their probable role in iDCM-caused HF; thus, they are likely to be novel therapeutic target genes.

Analysis of TFs

TFs that modulate gene expression in iDCM-HF patients, as predicted by PASTAA, are shown in Table 3. As indicated in Table 3A, DP-1 and E2f-1 TF families are at the top of the list in the downregulation of gene expression; however, DP-1 and E2f-1, as well as the TFs of AP-1 and Pax, occur frequently. In the upregulation of gene expression, the MiT/TFE family

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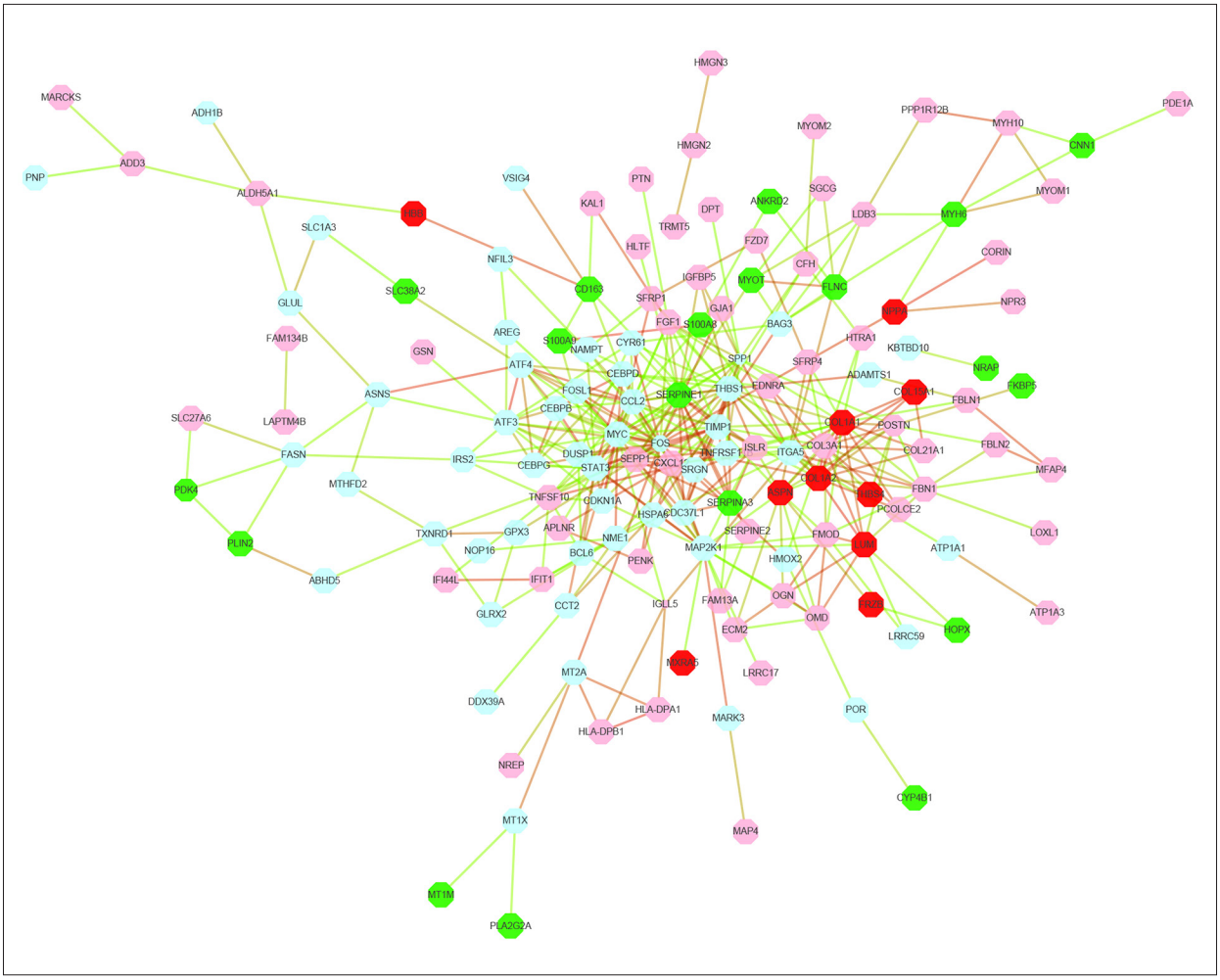

Figure 4. Outlines a PPI network analysis for DEGs. Key nodes in the giant network are highlighted in different colors: red is the up-regulated gene, and green corresponds with the down-regulated gene, respectively, and the dark red and green colors are the FC>2 and FC<0.5 genes, respectively. The smaller the *p* value, the larger node of the diameter. The closer the edge color is to pink, the greater the combined score.

attracted the most attention. Figure 6 shows the transcription factor-binding site as predicted by JASPAR.

Discussion

Many HF cases are caused by iDCM (30.7% of non-ischemic cardiomyopathy in HF patients) [21]. iDCM is a rare primary heart muscle disease with genetic, infective, and autoimmune etiologies that represents a peculiar model of HF with substantial differences from other etiologies [22]. Thus, revealing the initiation and development of HF caused by iDCM would benefit its diagnosis and treatment. Although significant progress has been made, and this study provides evidence that specific TFs identified in murine models (GATA and MEF2, NKX, and NFAT) are associated with human HF, the genes involved in iDCM largely remain unclear, and the corresponding regulatory TF levels also require attention [9]. However, to the best of our knowledge, there have been no studies aimed at determining potential genes associated with iDCM-caused HF. Therefore, we found novel targets for iDCM-HF patients by analyzing the microarray profiling data of gene expressions.

Consequently, 197 significantly altered genes were identified for iDCM in HF patients; among them, the obviously regulated gene was marked in a PPI network (Figure 4). The enriched DEGs are found beyond the cell, markedly in an extracellular structure organization and extracellular matrix (ECM), as indicated by GO analysis. The ECM continues to be widely studied in the heart. It has been reported recently that ECM proteoglycan fibromodulin is upregulated in clinical and experimental HF, and affects cardiac remodeling [23]. Increased ECM deposition is a hallmark of dilated cardiomyopathy (DCM), hypertrophy, and HF in humans [24]. Of course, other issues,

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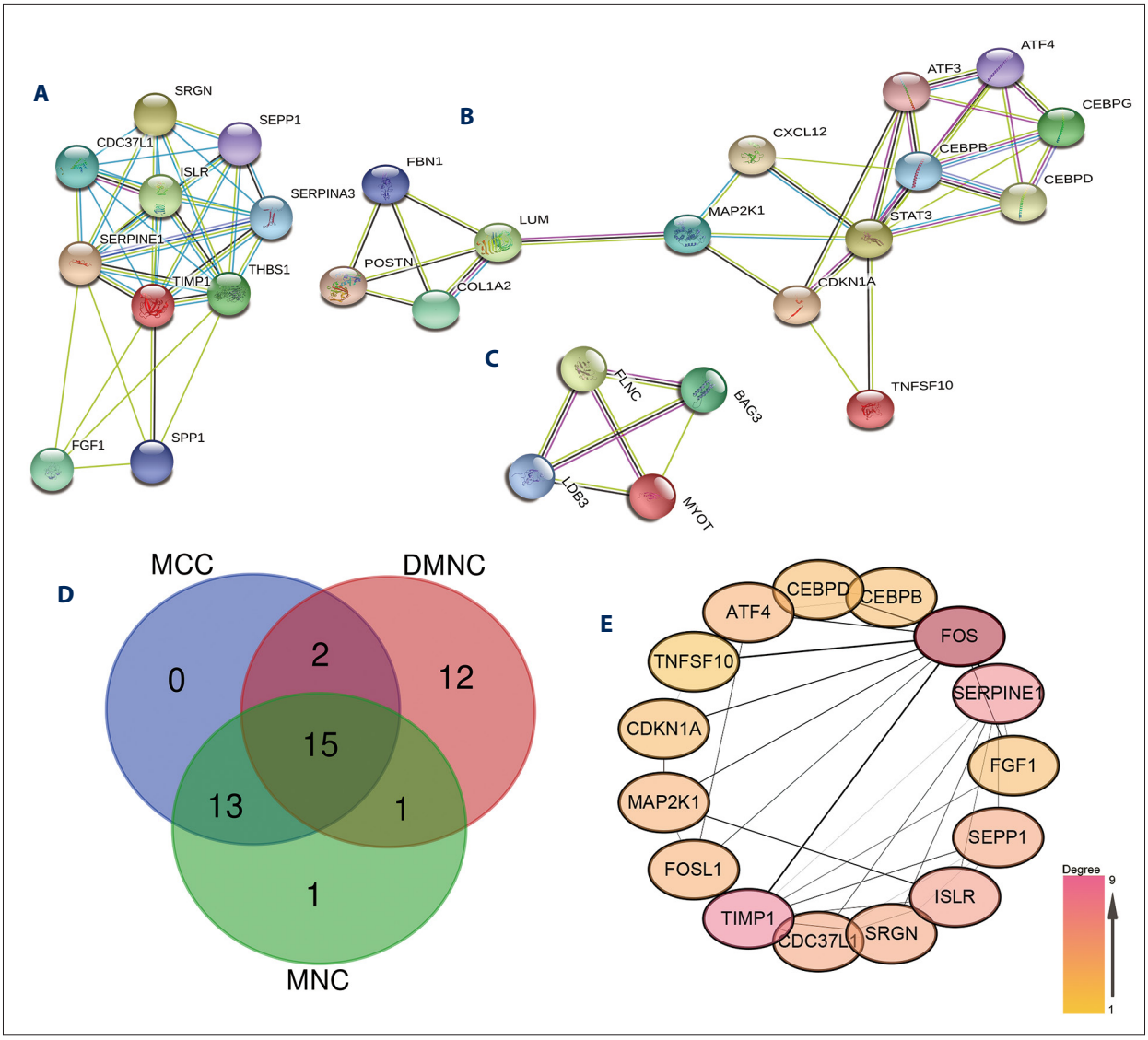

Figure 5. Three modules by STRING: Module 1 (**A**), Module 2 (**B**), and Module 3 (**C**). Overlapping DEGs among cytoHubba of the three methods (**D**). PPI network of the 15 hub genes (**E**). The wider the edge, the greater the edge betweenness

Table 2. The information of 15 hub gene.

Gene	FC	*P*	Degree
FOS	0.646836359	0.011938948	10
MAP2K1	0.640915732	1.48E-13	4
FGF1	1.674161905	8.91E-14	3
CEBPB	0.636011871	0.00000402	3
TNFSF10	1.687192949	0.00000229	2
CDC37L1	0.607610736	0.000000779	5
ATF4	0.646899144	7.88E-14	4

Gene	FC	*P*	Degree
SERPINE1	0.377215132	3.06E-11	7
SEPP1	1.653991537	0.00000233	5
CDKN1A	0.561612771	5.28E-11	3
ISLR	1.828979092	3.34E-17	6
SRGN	0.577572122	0.000235932	5
FOSL1	0.56105762	1.11E-08	4
TIMP1	0.590064052	0.000000976	8
CEBPD	0.559030961	0.0000465	3

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such as glycosaminoglycan binding and integrin binding, are also becoming areas of concern.

It can be seen that the PI3K-Akt signaling pathway has the most interactions with other pathways (Figure 3). HF is a major symptom in the progression of cardiac hypertrophy [25], and a spectrum of hypertrophy occurs in DCM [26]. Accumulating evidence strongly suggests that the PI3K-Akt signaling pathway plays an important role in controlling hypertrophic change and preserving cardiac function during the progression of HF in cardiac hypertrophy [25]. After manipulation of cardiac PI3K-Akt signaling by an apoptosis regulator, cardiomyocyte death occurs in doxorubicin-induced cardiomyopathy mice [27]. Notably, multiple significant pathways for genes have been identified, including some pathways associated with cancer, which may bring about new options for IDMC-caused HF patients. The KEGG pathways are clearly tied to each other.

Among the 15 selected hub genes, FOS, TIMP1, and SERPINE1 are the top 3-degree genes. Because it is miR-146a-targeted, the Fos-AP-1 pathway has the capacity to inhibit the activity of MMP-9, which is an AP-1 target gene involved in cardiac remodeling, myocardial dysfunction, and the progression of HF [28]. Collagen turnover biomarkers are correlated with failing heart diastolic function [29]. SERPINE1 was found in other microdata analyses as a significantly regulated gene in dilated ischemic cardiomyopathy, and TIMP1, SERPINE1, and SPP1 were evident from the early pre-phenotype stage of HF in hypertrophic cardiomyopathy [30]. FGF1 was unchanged in its expression in HF dilated, ischemic, and inflammatory cardiomyopathy. Additionally, idiopathic cardiomyopathy needs more research [31]. eIF2α-ATF4-CHOP signaling is one of the major endoplasmic reticulum stress signaling pathways causing cardiac hypertrophy [32]. CDKN1A (p21) knockout mice (p21KO) were found to develop age-dependent cardiac hypertrophy and HF by 10 months of age [33]. The activation

Table 3. Transcription factor that hub gene expression predicted.

(**A**) Top 20 TFs of up-regulted hub genes.

Rank	Matrix	Transcription factor	Association score	P-Value
1	E2F1_Q4_01	Dp-1, E2f-1	3.956	7.15E-04
2	MTATA_B	N/A	3.872	9.01E-04
3	AHRARNT_01	Ahr, Arnt	3.829	9.59E-04
4	ATF6_01	Atf6	3.829	9.59E-04
5	E2F_Q3_01	Dp-1, E2f-1	3.646	1.45E-03
6	E2F_Q3	N/A	3.237	3.07E-03
7	AP4_Q6	Ap-4	3.129	3.76E-03
8	TAXCREB_02	Creb, Deltacreb	2.979	6.02E-03
9	CBF_01	N/A	2.472	1.59E-02
10	PAX3_01	Pax-3	2.425	1.84E-02
11	USF_Q6_01	Usf-1, Usf1	2.425	1.84E-02
12	PAX3_B	Pax-3	2.425	1.92E-02
13	AP4_Q5	Ap-4	2.345	2.11E-02
14	AP2ALPHA_01	Ap-2alpha, Ap-2alphaa	2.327	2.29E-02
15	AP2GAMMA_01	Ap-2gamma	2.327	2.29E-02
16	SRF_01	Srf	2.225	2.83E-02
17	TCF11MAFG_01	Lcr-f1, Mafg	2.158	3.09E-02
18	E2F1_Q3	E2f-1	2.123	3.53E-02
19	USF_01	Usf1	2.116	3.56E-02
20	E4F1_Q6	N/A	2.071	3.77E-02

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(**B**) Top 20 TFs of down-regulted hub genes.

Rank	Matrix	Transcription factor	Association score	P-Value
1	TFE_Q6	Mitf, Tfe3-l	3.595	6.30E-04
2	MYCMAX_02	Max1, C-myc	3.173	1.37E-03
3	CDPCR3_01	Cutl1	2.967	3.15E-03
4	IRF7_01	Irf-7a	2.876	3.51E-03
5	DR1_Q3	Coup-tf1, Coup-tf2	2.372	1.31E-02
6	LMO2COM_02	Lmo2	1.921	2.91E-02
7	SMAD_Q6_01	Smad1, Smad1.1	1.917	2.91E-02
8	GATA1_02	Gata-1	1.917	2.91E-02
9	E2F1_Q3_01	E2f-1	1.868	3.16E-02
10	GATA1_04	Gata-1	1.825	3.33E-02
11	HSF_Q6	Hsf	1.822	3.58E-02
12	NRSF_Q4	Nrsf, Rest	1.745	3.84E-02
13	TFIIA_Q6	Tfiia-alpha/beta, Tfiia-gamma	1.743	3.84E-02
14	COREBINDINGFACTOR_Q6	N/A	1.677	4.34E-02
15	FAC1_01	Fac1	1.676	4.34E-02
16	MYOGENIN_Q6	Myogenin	1.65	4.51E-02
17	CDPCR3HD_01	Cutl1	1.619	5.01E-02
18	CIZ_01	Ciz6-1, Ciz8	1.618	5.01E-02
19	FOXP1_01	Foxp1a	1.618	5.01E-02
20	ISRE_01	N/A	1.618	5.01E-02

of CEBP, along with the upregulation of its lipogenesis targets, accounts for lipid storage and acts as a hallmark of arrhythmogenic right ventricular cardiomyopathy (another type of cardiomyopathy) [34]. However, CEBPD and CEBOB require further study, as do CDC37L1 and SRGN. These may be new targets for HF in iDCM patients.

Three modules were extracted from the PPI network through the MCODE analysis. In the present study, MAP2K1 drew strong interest. Protein kinase regulation and function are usually studied in proliferating cells in relation to cancer, as these are attractive therapeutic targets. Other microarray data for human HF has also found them to be downregulated [35]. MAP2K1 is a genetic cause of pediatric hypertrophic cardiomyopathy [36]. The pathogenic sequence variants in the genes of the RAS-MAPK pathway suggest that genetic lesions that promote signaling dysregulation contribute to disease pathogenesis and progression in children with iDCM [37]. Due to its importance, it is expected that MAPK will be further explored. Strangely, Immunoglobulin Superfamily Containing Leucine-rich Repeat (ISLR) is expressed in the heart tissue; however, there have been no further details reported in the heart. Thus, more studies are necessary to validate these findings.

TFs may play an important role in iDCM-HF patients. TFE3, together with the microphthalmia-associated transcription factor (MITF), belong to the MiT/TFE family. Of the 4 members of the MiT/TFE family, TFE3 is the least studied, and its function has not been widely investigated [38]. However, our findings suggest that further research as it relates to the cardiovascular system is necessary. Efficient DNA-binding requires dimerization with itself or with another MiT/TFE family member, such as TFE3 [39]. The role of MITF in the development and proliferation of mast cells is well-established [40]. Mast cells are important in remodeling the ventricular function in HF and dilated cardiomyopathy [41–43], and MITF may be involved in guinea pig cardiac hypertrophy and cardiomyocyte survival [44]. E2F1 was recently detected to be a novel regulator of metabolism [45], as it promotes apoptosis in all cells, including those in the heart [46]. E2F1-regulated miR-30b and

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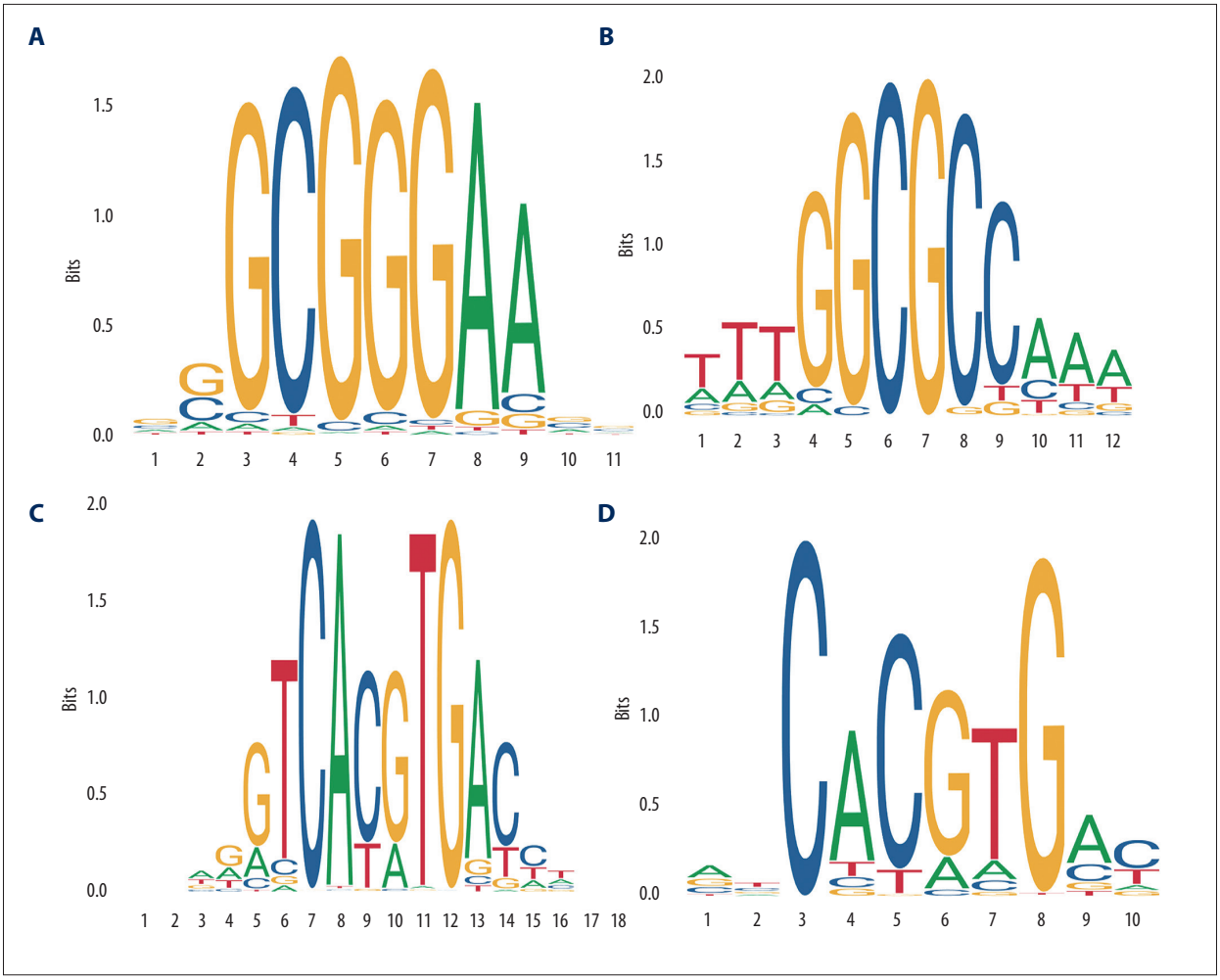

Figure 6. The predicted transcription factors binding site. DP-1 (**A**) E2F-1 (**B**) MITf (**C**) TFE3 (**D**)

miR-421 protect the heart from ischemia and infraction injuries [47,48]. E2F-dependent transcription can be stimulated by TFDP1, which is found in the promoter region of a number of genes [49]. DP1 also binds DNA cooperatively with E2F family members [50]. The E2F1: DP complex appears to mediate both cell proliferation and apoptosis. Previously studied TFs, such as GATA and FOX, were also included in the TF list (Table 2) [9].

The present study could provide new understanding of the mechanism of HF in iDCM patients. However, there are several limitations that should not be ignored. First, the control sample size of the non-failing myocardium was too small. Further studies with larger sample sizes are needed to verify our findings. Second, as only 1 microarray profile was analyzed, it may be impossible to avoid bias. Third, it would be more optimal if the study used advanced *in vivo* and *in vitro* experiments to confirm the effects of aberrant genes and TFs in the patients and controls. In addition, idiopathic dilated cardiomyopathy may be a consequence of a wide variety of causes, including virus-mediated diseases, immune dysregulation, and toxic, metabolic, inherited, and tachycardia-induced conditions [51,52]. A different etiology of iDCM may make a difference in HF progression. Therefore, a more detailed etiological division of iDCM in HF patients should be encouraged to further verify the results of our investigation. Last but not least, the heart has high energy requirements, in addition to nuclear genes, mutation of 37 mtDNA may also cause iDCM. Since the mutational load varies considerably from individual to individual and from mutation to mutation, it is difficult to predict the influence of mtDNA mutations on the risk of heart failure. No mtDNA data were assessed in this study, so further research on mtDNA mutation by WES is needed to explore the development of HF.

Conclusions

Several potentially relevant genes and TFs were identified in HF patients with iDCM, including 15 hub genes, including FOS, TIMP1, and SERPINE1, as well as some key TFs, such as DP-1,

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E2f-1, TFE3, and MITF. CEBPD, CEBOB, CDC37L1, and SRGN may be new targets for HF in iDCM patients. Our study provides greater understanding of the gene expression profile in iDCM-caused HF, but we did not investigate mtDNA.

References:

1. Bui AL, Horwich TB, Fonarow GC: Epidemiology and risk profile of heart failure. Nat Rev Cardiol, 2011; 8(1): 30–41
2. Luciani M, Del MF: Insights from second-line treatments for idiopathic dilated cardiomyopathy. J Cardiovasc Dev Dis, 2017; 4(3): pii: E12
3. Codd MB, Sugrue DD, Gersh BJ, Melton LJ: Epidemiology of idiopathic dilated and hypertrophic cardiomyopathy. A population-based study in Olmsted County, Minnesota, 1975–1984. Circulation, 1989; 80(3): 564–72
4. Cahill TJ, Ashrafian H, Watkins H: Genetic cardiomyopathies causing heart failure. Circ Res, 2013; 113(6): 660–75
5. Yang G, Chen S, Ma A et al: Identification of the difference in the pathogenesis in heart failure arising from different etiologies using a microarray dataset. Clinics (Sao Paulo), 2017; 72(10): 600–8
6. Bielecka-Dabrowa A, von HS, Aronow WS et al: Heart failure biomarkers in patients with dilated cardiomyopathy. Int J Cardiol, 2013; 168(3): 2404–10
7. Wang Y, Moreira MC, Khan A et al: Prognostic significance of circulating levels of hepatocyte growth factor in patients with Chagas' disease and idiopathic dilated cardiomyopathy. Cardiology, 2012; 121(4): 240–46
8. Pawlak A, Gil RJ, Kulawik T et al: Type of desmin expression in cardiomyocytes – a good marker of heart failure development in idiopathic dilated cardiomyopathy. J Intern Med, 2012; 272(3): 287–97
9. Hannenhalli S, Putt ME, Gilmore JM et al: Transcriptional genomics associates FOX transcription factors with human heart failure. Circulation, 2006; 114(12): 1269–76
10. Irizarry RA, Hobbs B, Collin F et al: Exploration, normalization, and summaries of high-density oligonucleotide array probe level data. Biostatistics, 2003; 4(2): 249–64
11. Ritchie ME, Phipson B, Wu D et al: limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res, 2015; 43(7): e47
12. Yu G, Wang LG, Han Y, He QY: clusterProfiler: An R package for comparing biological themes among gene clusters. OMICS, 2012; 16(5): 284–87
13. Szklarczyk D, Franceschini A, Wyder S et al: STRING v10: Protein-protein interaction networks, integrated over the tree of life. Nucleic Acids Res, 2015; 43(Database issue): D447–52
14. Szklarczyk D, Morris JH, Cook H et al: The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res, 2017; 45(D1): D362–68
15. Chin CH, Chen SH, Ho CW et al: A hub-attachment based method to detect functional modules from confidence-scored protein interactions and expression profiles. BMC Bioinformatics, 2010; 11(Suppl. 1): S25
16. Bader GD, Hogue CW: An automated method for finding molecular complexes in large protein interaction networks. BMC Bioinformatics, 2003; 4: 2
17. Roider HG, Manke T, O'Keeffe S et al: PASTAA: Identifying transcription factors associated with sets of co-regulated genes. Bioinformatics, 2009; 25(4): 435–42
18. Min F, Gao F, Liu Z: Screening and further analyzing differentially expressed genes in acute idiopathic pulmonary fibrosis with DNA microarray. Eur Rev Med Pharmacol Sci, 2013; 17(20): 2784–90
19. Zhang XF, Dai DQ, Ou-Yang L, Wu MY: Exploring overlapping functional units with various structure in protein interaction networks. PLoS One, 2012; 7(8): e43092
20. Shore S, Grau-Sepulveda MV, Bhatt DL et al: Characteristics, treatments, and outcomes of hospitalized heart failure patients stratified by etiologies of cardiomyopathy. JACC Heart Fail, 2015; 3(11): 906–16
21. Merlo M, Stolfo D, Anzini M et al: Persistent recovery of normal left ventricular function and dimension in idiopathic dilated cardiomyopathy during long-term follow-up: Does real healing exist. J Am Heart Assoc, 2015; 4(1): e001504
22. Andenæs K, Lunde IG, Mohammadzadeh N et al: The extracellular matrix proteoglycan fibromodulin is upregulated in clinical and experimental heart failure and affects cardiac remodeling. PLoS One, 2018; 13(7): e0201422
23. Hughes CJR, Jacobs JR: Dissecting the role of the extracellular matrix in heart disease: Lessons from the drosophila genetic model. Vet Sci, 2017; 4(2): pii: E24
24. Aoyagi T, Matsui T: Phosphoinositide-3 kinase signaling in cardiac hypertrophy and heart failure. Curr Pharm Des, 2011; 17(18): 1818–24
25. Benjamin IJ, Schuster EH, Bulkley BH: Cardiac hypertrophy in idiopathic dilated congestive cardiomyopathy: A clinicopathologic study. Circulation, 1981; 64(3): 442–47
26. Kitamura Y, Koide M, Akakabe Y et al: Manipulation of cardiac phosphatidylinositol 3-kinase (PI3K)/Akt signaling by apoptosis regulator through modulating IAP expression (ARIA) regulates cardiomyocyte death during doxorubicin-induced cardiomyopathy. J Biol Chem, 2014; 289(5): 2788–800
27. Palomer X, Capdevila-Busquets E, Botteri G et al: miR-146a targets Fos expression in human cardiac cells. Dis Model Mech, 2015; 8(9): 1081–91
28. Sanchis L, Andrea R, Falces C et al: Prognosis of new-onset heart failure outpatients and collagen biomarkers. Eur J Clin Invest, 2015; 45(8): 842–49
29. Tsoutsman T, Wang X, Garchow K et al: CCN2 plays a key role in extracellular matrix gene expression in severe hypertrophic cardiomyopathy and heart failure. J Mol Cell Cardiol, 2013; 62: 164–78
30. Polyakova V, Loeffler I, Hein S et al: Fibrosis in end-stage human heart failure: Severe changes in collagen metabolism and MMP/TIMP profiles. Int J Cardiol, 2011; 151(1): 18–33
31. Yao Y, Lu Q, Hu Z et al: A non-canonical pathway regulates ER stress signaling and blocks ER stress-induced apoptosis and heart failure. Nat Commun, 2017; 8(1): 133
32. Hauck L, Grothe D, Billia F: p21(CIP1/WAF1)-dependent inhibition of cardiac hypertrophy in response to Angiotensin II involves Akt/Myc and pRb signaling. Peptides, 2016; 83: 38–48
33. Chen L, Yang F, Chen X et al: Comprehensive myocardial proteogenomics profiling reveals C/EBPα as the Key Factor in the lipid storage of ARVC. J Proteome Res, 2017; 16(8): 2863–76
34. Fuller SJ, Osborne SA, Leonard SJ et al: Cardiac protein kinases: The cardiomyocyte kinome and differential kinase expression in human failing hearts. Cardiovasc Res, 2015; 108(1): 87–98
35. Ware SM: Evaluation of genetic causes of cardiomyopathy in childhood. Cardiol Young, 2015; 25(Suppl. 2): 43–50
36. Kaski JP, Syrris P, Shaw A et al: Prevalence of sequence variants in the RAS-mitogen activated protein kinase signaling pathway in pre-adolescent children with hypertrophic cardiomyopathy. Circ Cardiovasc Genet, 2012; 5(3): 317–26
37. Ploper D, De Robertis EM: The MITF family of transcription factors: Role in endolysosomal biogenesis, Wnt signaling, and oncogenesis. Pharmacol Res, 2015; 99: 36–43
38. Miller AJ, Levy C, Davis IJ et al: Sumoylation of MITF and its related family members TFE3 and TFEB. J Biol Chem, 2005; 280(1): 146–55
39. Martina JA, Diab HI, Li H, Puertollano R: Novel roles for the MiTF/TFE family of transcription factors in organelle biogenesis, nutrient sensing, and energy homeostasis. Cell Mol Life Sci, 2014; 71(13): 2483–97
40. Batlle M, Pérez-Villa F, Lázaro A et al: Correlation between mast cell density and myocardial fibrosis in congestive heart failure patients. Transplant Proc, 2007; 39(7): 2347–49
41. Janicki JS, Brower GL, Levick SP: The emerging prominence of the cardiac mast cell as a potent mediator of adverse myocardial remodeling. Methods Mol Biol, 2015; 1220: 121–39
42. Patella V, de Crescenzo G, Lamparter-Schummert B et al: Increased cardiac mast cell density and mediator release in patients with dilated cardiomyopathy. Inflamm Res, 1997; 46(Suppl. 1): 31–32
43. Gómez LA, Gunturiz ML: [Identificación y expresión diferencial del factor de transcripción asociado a microftalmia en corazón y cardiomiocitos aislados de cobayo, posible papel en hipertrofia y en viabilidad.] Biomédica 2014; 34: 387–402 [in Spanish]

Conflict of interest

None.

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44. Denechaud PD, Fajas L, Giralt A: E2F1, a novel regulator of metabolism. Front Endocrinol (Lausanne), 2017; 8: 311
45. Mayor J, Westendorp B, Salih M, Tauna BS: The role of the E2F6 transcription repressor in dilated cardiomyopathy. American Heart Association Scientific Sessions, At Orlando, Florida, 2009; Volume, 120
46. Wang K, Zhou LY, Wang JX et al: E2F1-dependent miR-421 regulates mitochondrial fragmentation and myocardial infarction by targeting Pink1. Nat Commun, 2015; 6: 7619
47. Wang K, An T, Zhou LY et al: E2F1-regulated miR-30b suppresses Cyclophilin D and protects heart from ischemia/reperfusion injury and necrotic cell death. Cell Death Differ, 2015; 22(5): 743–54
48. Wu CL, Zukerberg LR, Ngwu C et al: *In vivo* association of E2F and DP family proteins. Mol Cell Biol, 1995; 15(5): 2536–46
49. Helin K, Wu CL, Fattaey AR et al: Heterodimerization of the transcription factors E2F-1 and DP-1 leads to cooperative trans-activation. Genes Dev, 1993; 7(10): 1850–61
50. Felker GM, Thompson RE, Hare JM et al: Underlying causes and long-term survival in patients with initially unexplained cardiomyopathy. N Engl J Med, 2000; 342(15): 1077–84
51. Hofstra PDRMW, Veldhuisen PDDJV, Tintelen JPV: Inherited cardiomyopathies. Br Med J, 2011; 343(7833): 1106–10

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