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Analysis of Associated Genes and Biological Pathways Between Inflammatory Dilated Cardiomyopathy and Ischemic Cardiomyopathy by Bioinformatics

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Abstract

Objectives: To screen the associated genes and biological pathways of inflammatory dilated cardiomyopathy (DCMi) and ischemic cardiomyopathy (ICM) a transcriptome data method.

Materials and Methods: The differential genes (DEGs) were analyzed by the transcriptome data of DCMi and ICM in the comprehensive gene expression database, then the cluster analysis and Hub gene candidate genes were identified by Cytoscape, and the biological pathway of candidate genes was studied by GO and KEGG enrichment analysis.

Results: The common DEGs of DCMi and ICM were RPS4Y1 and MYH6. The biological processes in the GO analysis of DCMi are mainly related to the development and regulation of muscle and cardiomyocytes, while ICM is mainly related to biological processes such as extracellular matrix and collagen. Through KEGG analysis, we found that the DEGs in DCMi were mainly enriched in the PPAR signaling pathway (inhibition). In ICM, mainly enriched in ECM-receptor interaction (activation).

Conclusion: Our results reveal the related genes and biological pathways of DCMi and ICM, and we believe that the activation of the PPAR signaling pathway is expected to alleviate and improve myocardial inflammation. In ICM, it is possible to regulate the signal pathway of ECM- receptor interaction by increasing the transcriptional levels of COL3A1, COL1A1, and COL1A2, thus further promoting the progression of the disease.

Keywords: Bioinformatics, inflammatory dilated cardiomyopathy, ischemic cardiomyopathy, genes

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Introduction

Myocarditis is a long-term chronic myocardial inflammation with heterogeneous clinical manifestations, and its pathogenesis involves immune activation, including pro-inflammatory cytokines and autoantibodies triggered by the innate immune system[1]. Myocarditis can be caused by a variety of infectious pathogens, such as viruses, bacteria, chlamydia, rickettsia, fungi, and protozoa, as well as toxicity and hypersensitivity, among which the viral infection is deemed the most common cause of myocarditis[2], in the United States and Europe, coxsackievirus and parvovirus B19 are the main causes of myocarditis[3]. Patients with myocarditis often have systemic symptoms such as fever, myalgia, respiratory symptoms, gastroenteritis, chest pain, and palpitation. These non-specific symptoms are almost indistinguishable from those of acute coronary syndrome (ACS), non-ischemic cardiomyopathy, valvular disease, and pericarditis, so they bring greater challenges to the diagnosis and treatment of myocarditis[2]. Myocarditis will not only develop into inflammatory cardiomyopathy, and related studies have shown that about 1/3 of patients with myocarditis will develop inflammatory dilated cardiomyopathy. However, it is a serious disease associated with heart failure (HF)[4,5].

Ischemic heart disease (IHD) is one of the myocardial diseases with high morbidity and mortality[6]. Myocardial ischemia is called IHD, which can be divided into ACS and chronic coronary syndrome according to the cause of the disease[7]. ACS mainly results in a sudden limitation of coronary blood flow caused by acute lumen reduction or occlusions, such as thrombosis superimposed on atherosclerotic plaques and myocardial damage caused by sudden ischemia[8,9]. Chronic coronary syndrome, also known as chronic stable angina pectoris, refers to the chronic reduction of the coronary artery lumen due to atherosclerotic lesions, which limits coronary blood flow and causes ischemia when myocardial metabolic demand increases temporarily[6]. Long-term ischemia of the heart can lead to permanent myocardial dysfunction, HF, and even death[6].

Although inflammatory dilated cardiomyopathy and ischemic cardiomyopathy have different pathogenesis, both of them may eventually develop into HF. This may suggest that there may be a deeper link between DCMi and ICM. We hope to obtain the DEGs of DCMi and ICM through bioinformatics analysis, and through the further study of these candidate genes and biological pathways, to explore the pathogenesis and relationship between the two diseases, provide theoretical guidance for follow-up clinical research.

Materials and Methods

Search and Acquisition of Data

Use the "GEOquery" package of R software (version 4.0.2 r-project.org/) to download the microarray (microarray dataset) expression data sets GSE4172 and GSE5406 from GEO[10] (https://www.ncbi.nlm.nih.gov/geo/) database). The annotation platform of the gene expression profile GSE4172 is GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus2.0 Array. There were 12 samples, 4 healthy (control) and 8 DCMi. The annotation platform of the gene expression profile GSE5406 was GPL96 [HG-U133A] Affymetrix Human Genome U133A Array, 15 healthy samples, and 107 ICM samples.

Data Preprocessing and Differentially Genes Screening (DEGs)

We use "hgu133a" and "hgu133plus2" R packages in Bioconductor to obtain soft files on GPL96 and GPL570 platforms respectively and extract gene annotation information. In gene annotation, unannotated probes and probes mapped to multiple genes are screened. If multiple probes are mapped to the same gene, one of them is randomly retained in the data and represents the gene expression value. DEGs were screened by the limma package (version 4.0.3)[11]. The screening criteria for differential genes in GSE4172 were $p<0.05$ and $|\log2FC| \geq 1.5$. The DEGs in GSE5406 were screened by $p<0.05$ and $|\log2FC| \geq 1$. Pheatmap (version 4.0.3) and ggplot2 R

package (version 4.0.3) were used to process the screened differentially expressed genes and draw heat map and volcano map[12].

PPI Network Construction and Hub Gene Acquisition

All DEGs were uploaded to STRING (v.11.0) [STRING: functional protein association networks (string-db.rg)] to obtain a protein-protein interaction analysis of DEGs to predict the correlation of protein function. Then, we use Cytoscape (version 3.7.2) to analyze and visualize the biological networks and nodes of DEGs. MCC is the plug-in of cytoHubba and was calculated the top 10 Hub genes in DEGs. Finally, we used the MCODE plug-in (version 1.6.1) to cluster the DEGs. Degree cutoff, node score cutoff, and K-Core were set 2 and K-Core was set 100.

Correlation Analysis Between Gene Ontology and Function

To further explore the biological function of DEGs enrichment, we used "clusterProfiler" R package (3.18.1) to analyze the enrichment of gene ontology (GO) terms, including biological processes, molecular functions and cellular components. Then, to explore the signal pathways affected by differential genes, we used the "ggplot2" R package to analyze the KEGG pathways of up-and down-regulated genes. The standard for significant enrichment of differential genes in DCMi and ICM was adj ($p<0.05$)[13].

Results

Identification of Differential Genes

A total of 495 differential genes related to DCMi were identified in GSE4172, of which 258 were up-regulated and 237 down-regulated (Figure 1). A total of 37 differential genes related to ICM were identified in GSE5406, including 12 up-regulated and 25 down-regulated (Figure 1). Among them, there are two common genes. These common differential genes mainly include RPS4Y1 and MYH6.

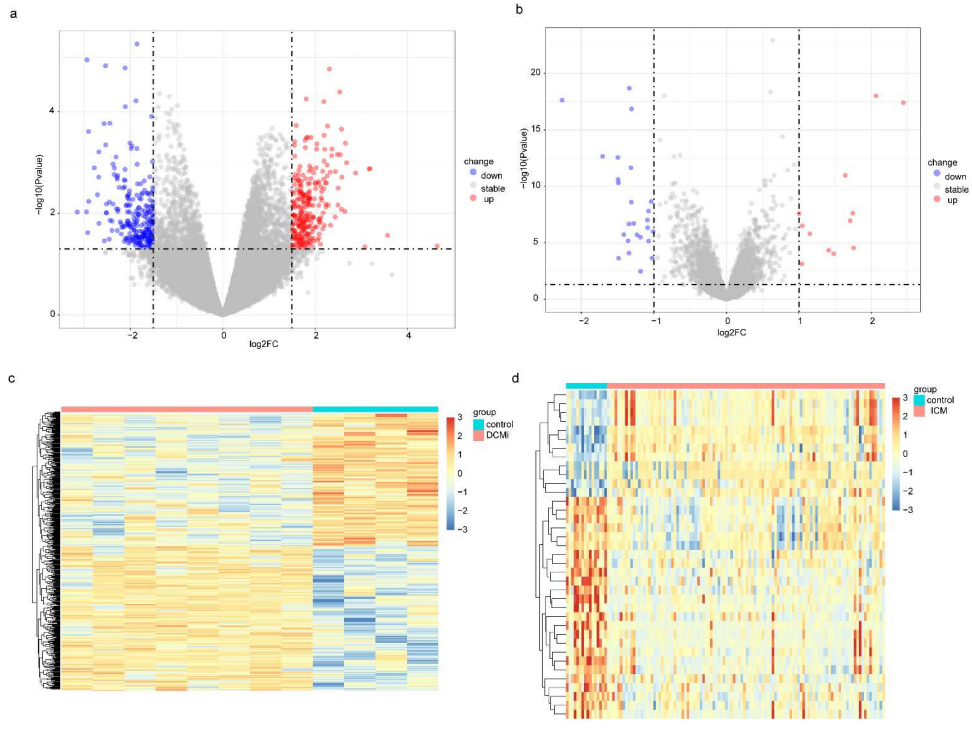

Figure 1. Volcano map and heat map of differential genes
(a) and (b) are the volcano maps of DCMi and ICM, respectively. (c) and (d) are heat maps of DCMi and ICM, respectively (DCMi: |log2FC|≥1.5, p<0.05. ICM: |log2FC|≥1.0, p<0.05)

Obtaining Hub Gene and Clustering Analysis

The top 10 genes ranked by DCMi and ICM were obtained according to the MCC algorithm (Table 1). The Hub genes of DCMi were mainly members of the fibroblast growth factor receptor family, including FGFR2 and FGFR4 (Figure 2). However, the Hub genes of ICM mainly include fibrosis-related genes such as COL3A1, COL1A1, and COL1A2 (Figure 2). Through cluster analysis, 9 clusters were obtained by DCMi (Figure 2 and Table 2), in which MYH6 is the common DEGs of DCMi and ICM. Two clusters were obtained by ICM (Figure 2 and Table 3).

Table 1. Calculates the Hub gene of the top ten of DCMi and ICM according to the MCC algorithm

Rank	DCMi		ICM	
	Name	Score	Name	Score
1	SHC1	111	COL1A1	181
2	SOS1	86	COL3A1	180
3	FGFR2	69	COL1A2	174
4	CTNNB1	64	LUM	144
4	JAK2	64	COL15A1	120
6	PDGFA	54	MXRA5	120
7	ADIPOQ	44	OGN	48
8	NTRK1	41	ASPN	24
9	FLT4	34	MYH6	20
10	FGFR4	33	MYOT	12

GO Analysis

To further study the effects of DEGs on disease-related signaling pathways, we enriched the DEGs of the two diseases by GO analysis (Figure 3). The results showed that DEGs of DCMi were mainly enriched in biological processes such as muscle organ development, regulation of muscle tissue development and regulation of striated muscle tissue development, and the common genes in the above biological processes included FGFR2, MYF5, SOX15, G6PD, RBP4 and BCL2, LUC7L, CTNNB1, RGS4, ARNTL, and CYP26B1 (Figure 3). The DEGs of ICM were mainly enriched in the extracellular matrix (ECM) organization, extracellular structure organization, and response to transforming growth factor-beta, in which the common genes in the above biological processes include COL3A1, COL1A1, and COL1A2 (Figure 3). In molecular function, ICM was enriched to contain the collagen ECM. Additionally, ICM is also enriched in a collagen trimer in cellular components. To sum up, the enrichment of DCMi is mainly related to muscle development and regulation, however, the accumulation of ICM in GO is mainly related to the ECM, collagen, and so on.

KEGG Enrichment Analysis

The DEGs of DCMi were enriched in 12 signal pathways by KEGG enrichment analysis (Figure 4a, $p \leq 0.05$). Among them, there were 2 up-regulated signal pathways and 10 down-regulated signal pathways. Up-regulated genes were mainly enriched in ABC transporters, while down- regulated genes were mainly enriched in

Table 2. Clustering results of differential genes in DCMi

Cluster	Gene	Nodes	Edges	Scores
1	*IKBKAP*, *NVL*, *TWISTNB*, *WDR3*, *NOM1*, *DDX52*	6	12	4.800
2	*WASL*, *DAAM1*, *MYH6*, *FMN1*, *TPM1*	5	9	4.500
3	*MAP3K1*, *FLT4*, *PDGFA*, *JAK2*, *SHC1*, *FGFR4*, and *NTRK1*	7	11	3.667
4	*RIMS1*, *UNC13C*, *PPFIA4*, *ERC2*	4	5	3.333
5	*CTNNB1*, *TCF7L2*, *ADIPOQ*, and *HIST1H2BN*	4	5	3.333
6	*CFD*, *RBP4*, *RARRES2*	3	3	3.000
7	*SREBF1*, *LPIN1*, *GPAT2*	3	3	3.000
8	*PMS1*, *XRCC6BP1*, *ZSWIM7*	3	3	3.000
9	*KIF17*, *TRAF3IP1*, *NPHP1*	3	3	3.000

the Peroxisome proliferator-activated receptor (PPAR) signaling pathway. The DEGs of ICM were predicted to have 18 signal pathways by KEGG enrichment analysis (Figure 4b). Among them, the up-regulated genes were enriched to 11 signal pathways, and the down-regulated genes were enriched to 7 signal pathways. Up-regulated genes were mainly enriched in the AGE-RAGE signaling pathway, diabetic complications, ECM-receptor interaction, and Diabetic cardiomyopathy. The down-regulated genes of ICM were mainly enriched in Mineral absorption and other pathways.

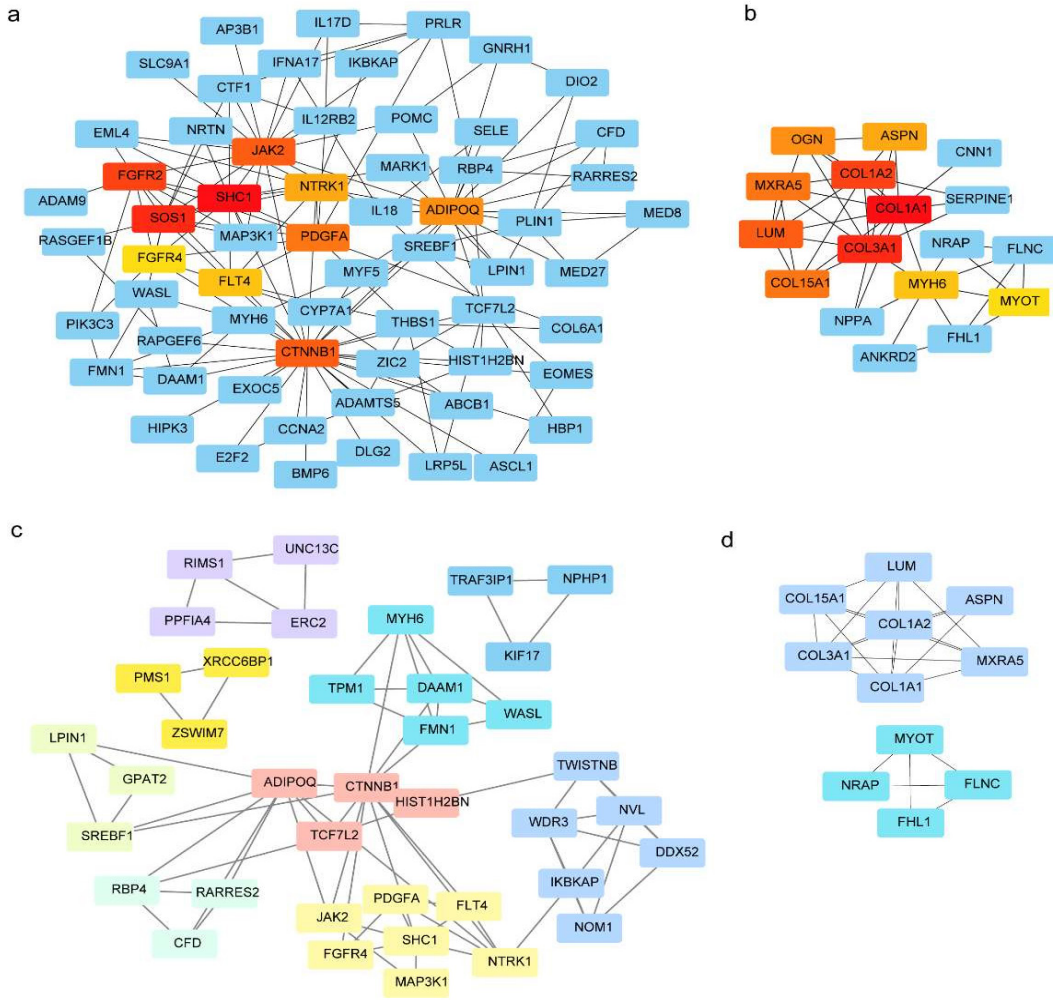

Figure 2. Hub genes and cluster analysis
(a) is the differential genes of DCMi calculated by the MCC algorithm and the top ten Hub genes, and (b) is the differential genes of ICM calculated by the MCC algorithm and the top ten Hub genes. (c) is the result of DCMi cluster analysis, and (d) is the result of ICM cluster analysis (different colors represent different clusters)

Table 3. Clustering results of differential genes in ICM

Cluster	Gene	Nodes	Edges	Scores
1	*COL3A1, LUM, COL1A2, COL15A1, COL1A1, MXRA5, ASPN*	7	18	6.000
2	*NRAP, FLNC, MYOT, FHL1*	4	5	3.333

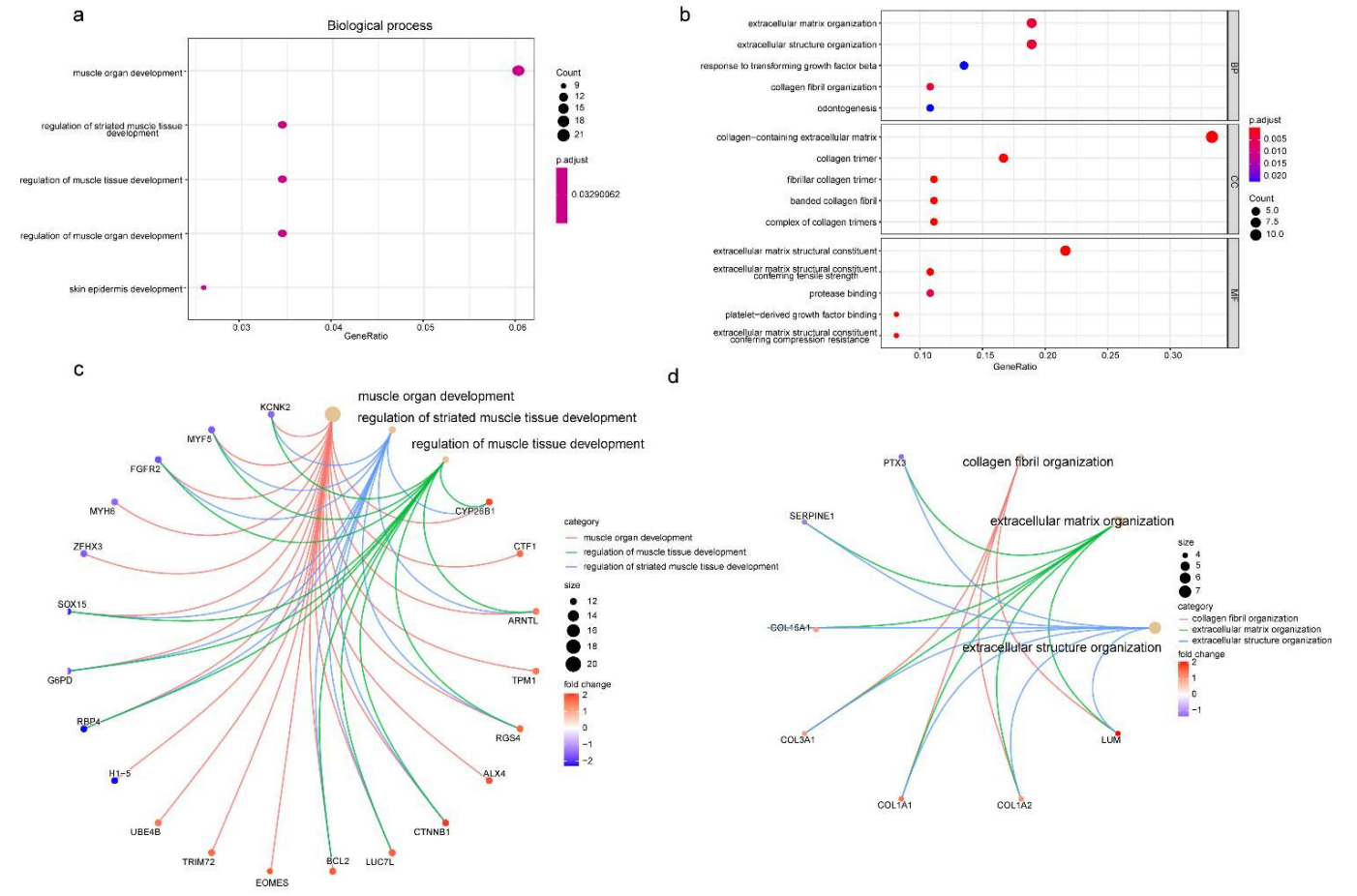

Figure 3. GO analysis results of DEGs
(a) is the result of GO analysis of differential genes of DCMi, and (b) is the result of GO enrichment of differential genes of ICM. (c) is the display of common genes in the first three biological processes of DCMi enrichment, and (d) is the display of common genes in the first three biological processes of ICM enrichment

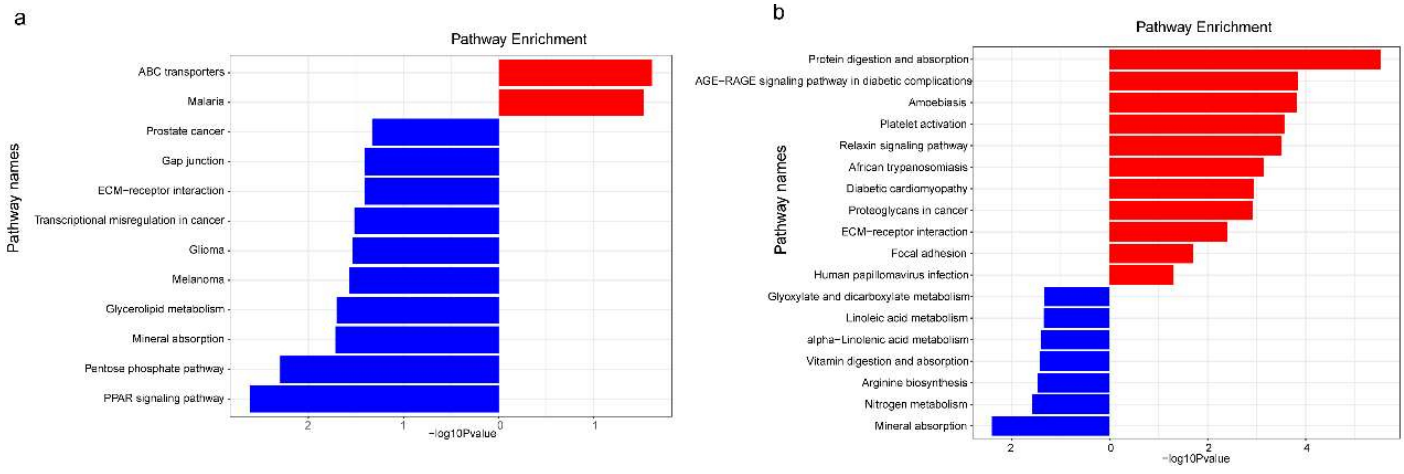

Figure 4. Enrichment of the KEGG signal pathway of DEGs. (a) and (b) are the result of KEGG enrichment in DCMi and ICM

Discussion

Through the analysis of the microarray expression data sets of DCMi and ICM, we found that the common genes of the two diseases include RPS4Y1 and MYH6. A comprehensive analysis of Hub genes and GO enrichment, we found that the DEGs of DCMi enriched in the biological process mainly include FGFR2 and MYF5. In ICM, the DEGs involved in the biological process are mainly fibrosis-related genes such as COL3A1, COL1A1, and COL1A2.

RPS4Y1 encodes the ribosomal protein S4, which is central to the correct development of individuals. RPS4Y1 can accelerate the loss of HUVEC activity induced by high glucose, so RPS4Y1 may inhibit cell viability by inducing mitochondrial- dependent apoptosis[14]. On the other hand, RPS4Y1 may lead to cell death by mediating pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α, and IL-8. Previous studies have shown that the expression of inflammatory factors such as IL-1β, IL-6, and TNF-α are significantly up- regulated in DCMi and ICM, so the up-regulated expression of RPS4Y1 in DCMi and ICM is likely to promote the development of the disease through inflammatory factors.

The *MYH6* gene encodes the α heavy -chain subunit of cardiac myosin and is central to myocardial development. The down-regulation of *MYH6* gene expression may cause the atrial septal defect, and the mutation of the *MYH6* allele can inhibit hypertrophic cardiomyopathy[15]. In our study, the mRNA levels of MYH6 in both DCMi and ICM were significantly down-regulated, so we think it may be related to the development of the disease[16].

Through KEGG analysis, we found that the down-regulated genes in DCMi were mainly enriched in the PPAR signaling pathway. PPARs, a transcription factor belonging to the nuclear receptor superfamily, contains the following three subtypes: PPARα, PPARγ, and PPARβ/δ. It heterodimerizes with the retinoid X receptor and binds to a specific response element called the PPAR response element in the target gene promoter[17]. PPARα and PPARγ have been demonstrated to be expressed in many cell types[18,19]. PPARγ is expressed in cardiomyocytes and is central to cardiovascular diseases such as atherosclerosis, cardiac hypertrophy, and myocardial infarction, and the lack of PPARγ signal transduction may be a reason for developing diabetic cardiomyopathy[20,21]. IL-17 is secreted by T-helper 17 (Th17) cells, a subgroup of CD4+T cells. IL-17 is involved in the pathogenesis of autoimmune myocarditis. IL-17 neutralization can reduce the severity of myocarditis[22]. PPARα may provide a new idea for treating autoimmune myocarditis. Our results also found that the PPAR signal pathway was inhibited in DCMi, so we think it may promote the progression of the disease.

In ICM, the up-regulated genes were mainly enriched in ECM-receptor interaction. Myocardial ECM is central to maintaining normal cardiac structure and function. Under normal circumstances, the synthesis and degradation of myocardial collagen fibers are in dynamic balance. In many cardiovascular diseases, the quantity, proportion, structure, and morphology of myocardial interstitial collagen change, accompanied by the imbalance of collagen production and degradation (collagen production increase and degradation decrease). Finally, myocardial interstitial fibrosis leads to an increase in myocardial stiffness, and even HF[23].

Our study found that the Hub gene of ICM mainly includes COL3A1, COL1A1, and COL1A2, in which COL3A1 and COL1A2 encode type I and III collagen (ColI and ColIII), respectively. ColI and ColIII are the main fibrous collagen produced by fibroblasts, including cardiac fibroblasts[24]. ColI and ColIII are the main components of ECM proteins, accounting for 80% and 12%, respectively[25]. Related studies have shown that COL3A1 and COL1A2 are mainly highly expressed in ICM and dilated cardiomyopathy in cardiovascular diseases[26,27]. Our results show that the differential genes in ICM are significantly enriched in the ECM-receptor interaction signal pathway. Therefore, we believe that ICM may regulate the ECM-receptor interaction signal pathway by increasing the transcriptional levels of COL3A1, COL1A1, and COL1A2, to further promote the progress of the disease.

Conclusion

We found that RPS4Y1 and MYH6 are common genes for DCMi and ICM. In DCMi, the PPAR signaling pathway is inhibited in DCMi, which may lead to uninhibited differentiation of Th17 cells and promote IL-17 secreted by Th17 to further mediate the pathogenesis of myocarditis. In ICM, it is possible to regulate the signal pathway of ECM- receptor interaction by increasing the transcriptional levels of COL3A1, COL1A1, and COL1A2, thus further promoting the progression of the disease.

Ethics

Ethics Committee Approval: This study does not require.

Informed Consent: This study does not require.

Peer-review: Externally peer-reviewed.

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