



Original Research

Comprehensive multi-factor analysis and exploration for the pathogenesis of non-ischemic cardiomyopathy and ischemic cardiomyopathy

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Abstract: Cardiomyopathy is a group of heterogeneous diseases that negatively affect cardiac function. Twenty-five years ago, clinical researchers began to realize that cardiomyopathy is an important and fairly common heart disease. Although many aspects of the pathogenesis of cardiomyopathy have been explored by biologists, the molecular mechanisms remain elusive. This study modularized the pathogenesis of non-ischemic cardiomyopathy and ischemic cardiomyopathy and finally explored their common core pathogenic driver genes. First, based on the normal expression profile data of patients with non-ischemic cardiomyopathy and ischemic cardiomyopathy, differential expression analysis was used to screen differentially expressed genes. Secondly, the co-expression analysis of differentially expressed genes was performed to obtain a co-expression module of genes. Thirdly, the enrichment analysis of GO functions and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway was conducted on the module genes. Finally, based on hypergeometric tests, non-coding RNA (ncRNA) and transcription factors with significant regulatory effects were predicted. In summary, we obtained 8 co-expression modules, of which HNF1B, PRDX3 genes had significant differences in expression in patients with cardiomyopathy, and had a positive regulatory role in the dysfunction module, so they were recognized as non-ischemic and key genes for non-ischemic diseases and ischemic cardiomyopathy. The enrichment results showed that the module genes were significant in the biological processes of neutrophil activation involved in immunoreaction, neutrophil-mediated immunity, neutrophil activation, and neutrophil degranulation, and significantly regulate the signal pathways such as vibrio cholerae infection. Finally, significant regulatory dysfunction modules of pivot ncRNAs (including MALAT1, miR-133a-3p, and miR-133b) and pivot TFs (including NFkB1, PML, and RELA, etc.) were identified. In summary, our work decodes a co-expression network involving the regulation of key genes in non-ischemic and ischemic cardiomyopathy. It helps to discover core dysfunction modules and potential regulatory factors, drive disease genes, and improve our understanding of its pathogenesis.

Key words: Cardiomyopathy; Co-expression module; Driver gene; Enrichment analysis; Regulatory factors.

Introduction

Cardiomyopathy (CMP) or myocardial infarction is a different type of myocardial disease in which the heart cannot provide sufficient blood flow to the body's organs, and the person has heart failure, which is usually related to arrhythmia. The three most common cardiomyopathy is dilated cardiomyopathy, the most common cardiomyopathy, hypertrophic cardiomyopathy or myocardial wall growth and thickening, and restrictive cardiomyopathy (reduced myocardial flexibility). Regardless of the cause of the disease, any heart problem can be called heart disease, and sometimes even cardiomyopathy, although it should be used in various heart diseases that cause acute heart failure (1-3).

Cardiomyopathy is a common debilitating disease with high mortality and low quality of life (1). Almost 50% of the patients died suddenly during childhood or adolescence or received heart transplantation, and these patients will be affected by cardiomyopathy (2). Among them, ischemic cardiomyopathy refers to the significant impairment of left ventricular function, which is caused by atherosclerotic coronary artery disease (3). The most common neurological diseases caused by cardiomyopa-

thy are ischemic stroke, followed by transient ischemic attack, syncope or vertigo, and the most common cardiomyopathy related diseases are muscular dystrophy, myofibril myopathy, congenital myopathy and metabolic myopathy (4). Cardiomyopathy is classified as an external factor, caused by external factors, such as hypertension, ischemia, inflammation, valve dysfunction, or internal factors, corresponding to myocardial disease without identifiable external causes (5). In terms of genetics, genetic variation of telomere-associated SNP and telomerase is related to ventricular arrhythmia in ischemic cardiomyopathy (6).

Long non-coding RNAs (lncRNAs) are a class of non-coding transcripts in the human genome that exceed 200 nucleotides in length. They perform their functions through a variety of mechanisms, including calling chromatin modification complexes to specific locations in the genome to form molecules Process bracket. They transcribe and regulate the expression of miRNA. Recent research highlights the increasingly important role of these lncRNAs in the pathogenesis of various diseases and challenges the fact that protein-coding genes are the only factor in the development of human diseases. According to the DNA Element Encyclopedia

project, protein-coding genes account for only 2 to 3% of the entire human genome, and more than 75% of the genome is copied into non-coding protein transcripts. In the 1950s, the discovery of non-coding RNA (ncRNA) changed the central phenomenon of molecular biology, but these transcripts have been known as spurious body “garbage” or scrambled transcriptional “noise” for decades (9-11).

At present, researchers have made some progress in exploring the pathogenesis and treatment mechanism of ischemic cardiomyopathy and non-ischemic cardiomyopathy from the perspective of medicine and biology. Recent researches have demonstrated the important role of non-coding RNA in ischemic and non-ischemic cardiomyopathy (7). Among them, lncRNAs regulate the expression and function of ECM and myocardial fibrosis during the development of ischemic cardiomyopathy (ICM), which may represent new modifiers of cardiac function and heart disease, including ICM (8). Regulation of the Wnt signaling pathway and cell cycle plays a key role in the development of ischemic (ICM) and non-ischemic (NICM) cardiomyopathy (9). Meanwhile, most studies on the role of biomarkers in cardiomyopathy focus on myocardial stress (diuretic natriuretic peptide), injury (troponin), inflammation, and remodeling (fibrosis markers) (10). In addition, the expression of mir-21 in plasma of ICM patients was significantly increased, and the expression of trace RNA-21 in plasma was positively correlated with N-terminal B-type brain natriuretic peptide (NT-proBNP) and left ventricular end-diastolic volume (LVEDV) (11).

In the diagnosis and treatment of cardiomyopathy, after the demonstration, considering ischemic heart disease and patients with non-ischemic cardiomyopathy, the benefits of survival for subgroups for patients who have left ventricular dysfunction could be provided. Also, both appropriate and inappropriate ICD treatment for patients having ischemic and non-ischemic cardiomyopathy have connections with high-rate cut-off and delayed VT treatment of ICD programming. Besides, impaired congestive myocardial blood flow (MBF) is associated with increased mortality in ischemic and non-ischemic cardiomyopathy, which may be attributed to the instability of electricity induced VAs (14).

Instead, computed tomography angiography (CTA) seems to be a clinically appropriate and accurate diagnostic method that can exclude the cause of undetermined cardiomyopathy patients, which further supports the use of CTA to determine the cause of new cardiomyopathy of unknown etiology (15). These research results deepen our understanding of the pathogenesis of ischemic and non-ischemic cardiomyopathy and guide our further research (16-33). Although a series of studies on ischemic and non-ischemic cardiomyopathy have been reported, the overall effect of these achievements is still elusive. In order to thoroughly explore the common mechanisms of ischemic and non-ischemic cardiomyopathy, we conducted systematic module analysis and exploration to identify the dysfunction module and the core molecules between them, and further explore the key to the dysfunction module gene. In conclusion, our work details the potential mechanisms of multifactor-mediated functional modules in ischemic and non-ischemic cardiomyopathy and identifies com-

mon key genes as well as potential therapeutic targets and related biological processes. These processes may help to understand and treat ischemic and non-ischemic cardiomyopathy.

Materials and Methods

Data resources

The NCBI Gene Expression Omnibus database (GEO Dataset) (34) contains a broad classification of high-throughput experimental data, microarray-based single-channel, and double-channel assays on mRNA abundance and experimental data on genomic DNA and protein molecule. In addition, it includes data from non-array-based high-throughput functional genomics and proteomics techniques. We firstly collected a set of gene expression profiles of normal samples and samples associated with ischemic cardiomyopathy and non-ischemic cardiomyopathy from GEO, whose number was GSE9128 (35). The data set included 12 normal samples and 12 samples of ischemic cardiomyopathy and 12 samples of non-ischemic cardiomyopathy. Then, ncRNA-mRNA (protein) with score ≥ 0.5 were selected from RAID v2.0 database (36) for mutual antagonism, meanwhile, all human transcription factor target data were downloaded from and used in TRRUST v2 database (37) of the universal database for transcription research, to predict regulatory module factors.

Analysis of co-expression

In order to explore the common drivers of ischemic and non-ischemic cardiomyopathy, we made a difference analysis between normal samples and samples of ischemic and non-ischemic cardiomyopathy and integrated the results of the two groups to obtain the differential gene expression profiles of ischemic and non-ischemic cardiomyopathy. At the same time, in order to explore the synergistic expression of differential genes in ischemic and non-ischemic cardiomyopathy, we used weighted gene co-expression network analysis (WGCNA) (38) to analyze the differentially expressed matrix of ischemic and non-ischemic cardiomyopathy and looked for the gene module of synergistic expression. First, the Coefficient of correlation index was weighted, that is, the Coefficient of gene correlation was raised to the power of N to calculate the Person Coefficient between any two genes. In the network, between genes, the associations are subject to scale-free network distribution, which makes the algorithm more biological significance. Then, between genes, hierarchical cluster dendrogram was constructed by the correlation coefficient. Different colors represented different modules while different branches of cluster dendrogram represented different gene modules. According to the regulation power of genes in each dysfunction module, we exploited the key genes that lead to the dysfunction module and consider them as the key genes that lead to the pathogenesis of ischemic and non-ischemic cardiomyopathy.

Functional and pathway enrichment

Exploring the functional and signaling pathways in which genes are involved often contributes to the study of the molecular mechanisms of disease, and enrichment analysis of the function and pathways of genes in

dysfunction modules is an effective means to explore the potential mechanisms of ischemic and non-ischemic cardiomyopathy. Therefore, we used the R language ClusterProfiler package (39) to respectively conduct the enrichment analysis on the Go function and KEGG pathway of the module genes of ischemic and non-ischemic cardiomyopathy. ClusterProfiler is a software package of Bioconductor, which can perform statistical analysis and visualization of functional clustering of gene sets or gene clusters. In addition, the BinGO (40) application of Cytoscape was also used to analyze the pathway of the integrated module network.

Transcription factors and ncRNA regulated the dysfunction modules

Transcription and post-transcriptional regulation of genes often take non-coding genes (ncRNA) and transcription factors (TF) as core drivers. Therefore, we scientifically predicted and tested its role in the dysfunction module of ischemic and non-ischemic cardiomyopathy. Pivot regulons, including ncRNA and TF, are defined as regulators that have a significant regulatory role in the pathogenesis of ischemic and non-ischemic cardiomyopathy. We require that there are more than two regulatory lines between each regulon and each module, and the significance p-value of the enriched target in each module based on the hypergeometric test calculation shall be < 0.01 .

Results

Determined the expression dysregulated molecules in non-Ischemic and ischemic cardiomyopathy

Biologists have conducted many experiments and studies on the pathogenesis of non-ischemic cardiomyopathy and ischemic cardiomyopathy and identified their potential pathogenic genes. However, the complex molecular linkages and overall effects of these genes are unclear. To observe the molecular changes in the pathogenesis of non-ischemic cardiomyopathy and ischemic cardiomyopathy, based on microarray data, we analyzed the gene differential expression between normal samples and non-ischemic cardiomyopathy and ischemic cardiomyopathy samples, respectively, and obtained the genes that may lead to the pathogenesis of non-ischemic cardiomyopathy and ischemic cardiomyopathy. By combining the results of the two groups, 2490 differentially expressed genes (DEGs) were obtained, and we believe that these differentially expressed genes contain dysregulated molecules of non-ischemic and ischemic cardiomyopathy.

Identified the functional modules associated with non-ischemic and ischemic cardiomyopathy

Modularity helps to perfect this is the representation of each system on its own, and modularity is the subsystem associated with complex systems around the world. In the global network, the interaction of the entire effect shows a global feature, which is a bridge between 2490 differentially expressed genes and their interacting cardiomyopathy genes, which play an important role in each elemental gene and construct an expression matrix. Each module not only interacts, but a module is a group of genes with a synergistic expression relationship, and

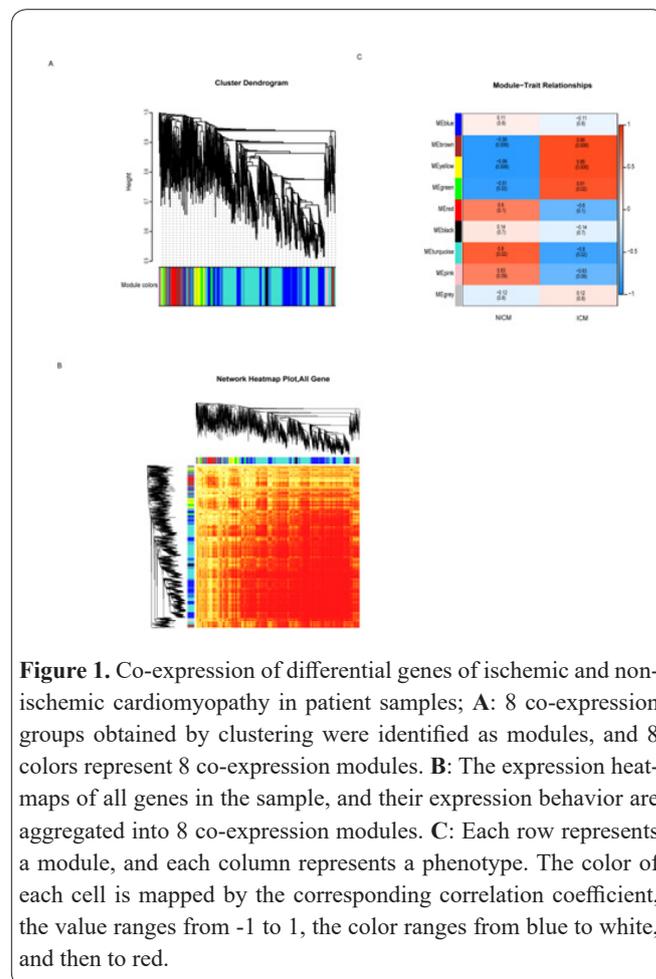
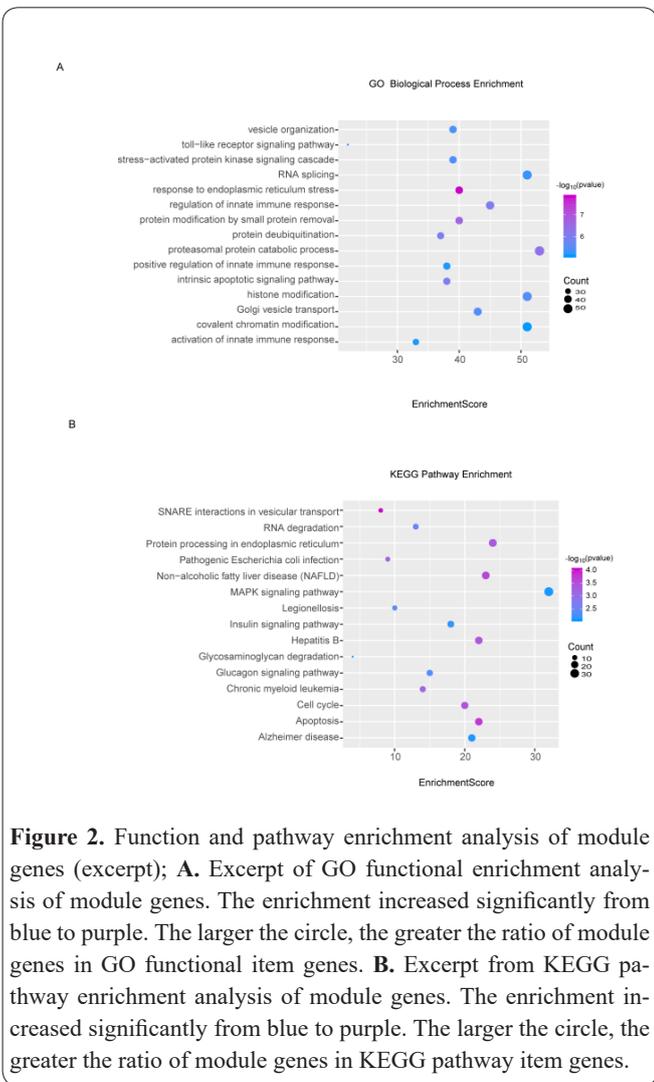


Figure 1. Co-expression of differential genes of ischemic and non-ischemic cardiomyopathy in patient samples; **A:** 8 co-expression groups obtained by clustering were identified as modules, and 8 colors represent 8 co-expression modules. **B:** The expression heatmaps of all genes in the sample, and their expression behavior are aggregated into 8 co-expression modules. **C:** Each row represents a module, and each column represents a phenotype. The color of each cell is mapped by the corresponding correlation coefficient, the value ranges from -1 to 1, the color ranges from blue to white, and then to red.

the genes of the same module have consistent expression behavior. Then, through weighted gene co-expression network analysis (WGCNA), genes are clustered into modules according to their expression behaviors in diseases. We obtained a total of 8 functional disorder modules (Figure 1A, B). Key genes of each module were identified based on functional disorder modules, and the core genes, based on HN1 and PRDX3, were obtained. According to the correlation between module and phenotype data, it shows that MEturquoise is correlated with non-ischemic cardiomyopathy, while MEbrown and MEyellow are correlated with ischemic cardiomyopathy (Figure 1C).

Interested in the functions and pathways involved in genes

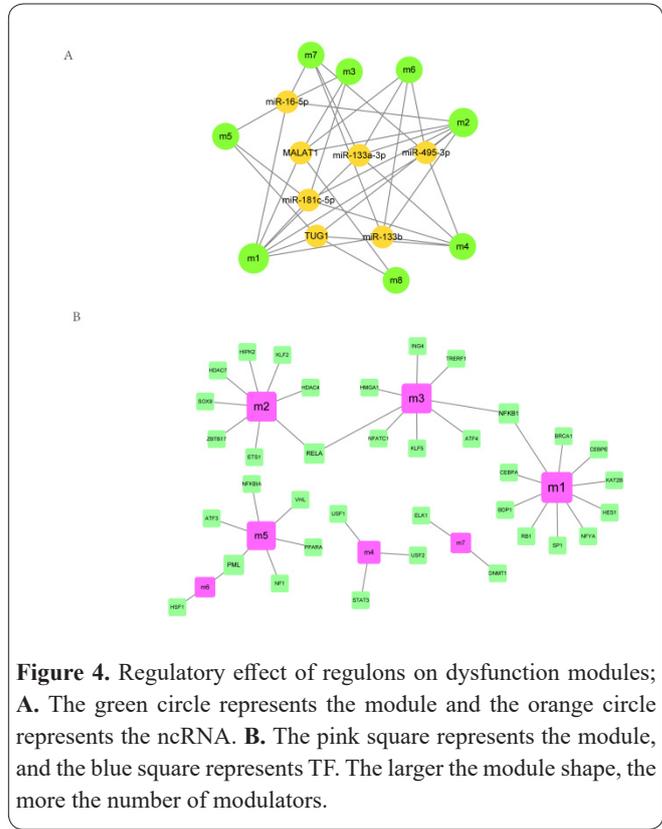
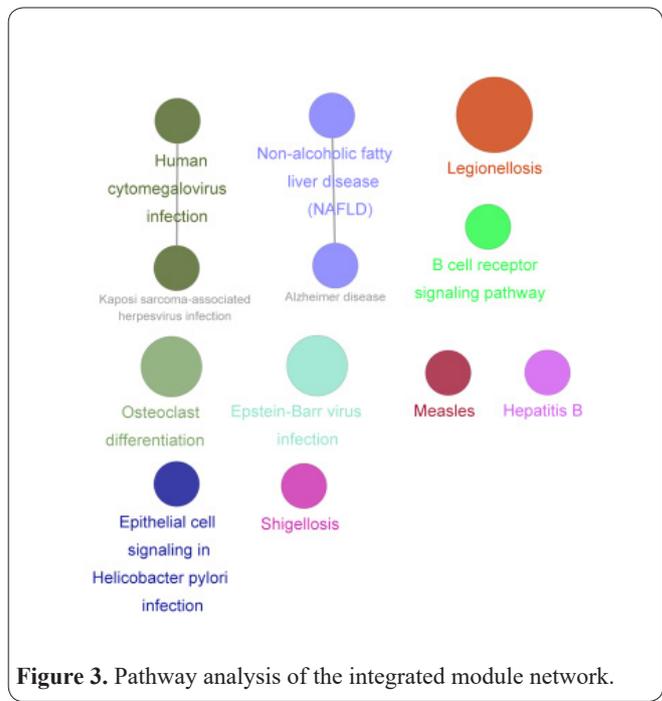
Functions and pathways are important mediators of disease physiological responses. Exploring the functions and pathways involved in dysfunctional module genes not only helps to determine the upstream and downstream relationships of genes in the same pathway within the module, but also helps to build a molecular bridge between the module and disease in systems biology, and Deepen the understanding of the underlying molecular mechanisms of diseases. We performed enrichment analysis on the GO function and KEGG pathway of 8 modules and obtained 1,482 biological processes (BP), 227 cell compositions (CC), 263 molecular functions (MF), and 55 KEGG pathways (Figure 2). It has been found that these functions are mainly concentrated in biological processes, such as neutrophil activation involved in immune responses, neutrophil-



mediated immunity, neutrophil activation, and neutrophil degranulation. On the other hand, the enrichment results of the KEGG pathway reflect that differentially expressed genes of non-ischemic and ischemic cardiomyopathy are mainly involved in signaling pathways such as *Vibrio cholerae* infection, and regulate the functions and pathways with the most genes in dysfunction modules. It may be considered to play the most critical role in the dysfunction module. In order to review the overall situation, we integrated a network of 8 modules and conducted path analysis through BinGO (Figure 3).

Driven the co-pathological TF and ncRNA of non-ischemic cardiomyopathy and ischemic cardiomyopathy

From the perspective of system biology and system genetics, transcription and post-transcriptional regulation of genes have been considered as the key regulatory factors for the occurrence and development of diseases, among which transcription factors and ncRNAs are the common regulatory factors for expression and function. Although the regulation of single or several TF and ncRNA on the pathogenesis of non-ischemic and ischemic cardiomyopathy has been paid much attention by many biologists, few studies have focused on the bridging role of TF and ncRNA as a whole on the overall effect and development of dysfunction mechanism. Therefore, in this study, we conducted pivot analysis on co-expression modules based on the targeted regulatory relation-



ship of TF and ncRNA on module genes, in an attempt to explore the key regulons regulating the pathogenesis of non-ischemic and ischemic cardiomyopathy. The predicted results showed that a total of 957 ncRNAs involved 1348 ncRNA-module regulatory pairs, and 36 transcription factors involved 39 TF-module target pairs. The regulation conditions (Figure 4A, B) of regulatory factors in dysfunctional modules were observed by introducing the above results into the cytoscape separately. In addition, the number of regulatory modules of pivot regulators was statistically analyzed and found the dysfunctional modules with the most regulation by ncRNA (MALAT1, miR-133a-3p, and miR-133b) and TF (NFKB1, PML, and RELA). These transcription factors

and ncRNA may use mediating dysfunction modules to modulate the course of nonischemic and ischemic cardiomyopathy. Therefore, we identified these potential regulators as dysfunction molecules in the pathogenesis of nonischemic and ischemic cardiomyopathy.

Discussion

Ischemic cardiomyopathy is caused by atherosclerotic lesions in multiple coronary arteries, especially diffuse lesions, which can lead to severe myocardial dysfunction, enlarged heart, heart failure or arrhythmia, etc. (41, 42). Although the prevention, diagnosis, and treatment of cardiovascular diseases have been improved, the prevalence rate of ischemic dilated cardiomyopathy is still increasing in western countries (43). Although researchers have studied non-ischemic and ischemic cardiomyopathy in various ways, the pathogenesis remains unclear. In this study, we collected the gene expression profiles of ischemic and non-ischemic cardiomyopathy and normal samples from the NCBI Gene Expression Omnibus database (GEO Dataset), and based on the common difference of gene expression data on ischemic and non-ischemic cardiomyopathy, analyzed the gene dysfunction modules of ischemic and non-ischemic cardiomyopathy driven by transcription factors and ncRNA regulon, to deeply understand the molecular mechanisms of the pathogenesis of ischemic and non-ischemic cardiomyopathy. At the module level, the modules were significantly involved in biological processes such as neutrophil activation involved in immunoreaction, neutrophil-mediated immunity, neutrophil activation, and neutrophil degranulation. Meanwhile, it also significantly participates in the signaling pathway of vibrio cholerae infection. Among them, the matrix was released depending on the mediation of mechanism neutrophil and matrix metalloproteinase 9 activated myocardial ischemia/reperfusion (44). At the same time, in the inflammatory microvascular system, C15 rapidly regulates neutrophil physiology, induces adhesion cells to escape from the inflammatory endothelial cells, and reduces recruitment and cardiac injury in the model of myocardial infarction in neutrophil mice (45). And neutrophil degranulation is associated with ischemic reperfusion of the human heart during cardiopulmonary bypass (46). Besides, 6-gingerol inhibited cholera-induced pro-inflammatory cytokines in intestinal epithelial cells by regulating NF- κ B (47).

On the other hand, at the molecular level, we have exploited a total of 8 key genes, including HN1 and PRDX3, through co-expression analysis. These core genes are not only significant in differential expression but also play an important regulatory role in dysfunction modules. Among them, by enhancing MYC activity, HN1 promotes the migration, invasion, and tumorigenesis of breast cancer (48). In patients with ischemic cardiomyopathy, the expression of elongation factor Tu (a molecule involved in protein synthesis) and PRDX3 in the isolated mitochondria was significantly increased and was involved in the stress response (49). Meanwhile, human ARHGDIG as a GDP dissociation inhibitor (GDI) for Rho protein, which plays a major role in regulating GTP enzymatic activity (50). In addition, mutations in transport protein particle complex

C2 (TRAPPC2) are associated with spinal retardation dysplasia (SED) in skeletal diseases (51). On the other hand, the transforming growth factor β (TGF β)/Smad signaling pathway is activated in SMC4-transduced glioma cells and inhibited in SMC4-silenced glioma cells, facilitating the invasiveness of SMC4-mediated glioma cells (52). Among the 8 key genes mentioned above, except that PRDX3 is involved in the pathogenesis of cardiomyopathy, studies of other genes have not found any influence on cardiomyopathy. However, our analysis shows that they are involved in the dysfunction modules of non-ischemic and ischemic cardiomyopathy, and play an important role in the common pathogenesis, which is the key direction of future research.

Besides, 957 ncRNA were predicted to participate in the pathogenesis of non-ischemic and ischemic cardiomyopathy through the mediating module, and their varying abnormal expressions in non-ischemic and ischemic cardiomyopathy were verified based on the difference analysis. According to the statistical analysis, we determined that MALAT1, miR-133a-3p, miR-133b, miR-16-5p, miR-181c-5p, miR-495-3p, and TUG1 have significant effects on the 5 dysfunctional modules and are the genes that regulate the most modules. Among them, by negatively regulating mir-145 / Bnip3, lncRNA-MALAT1 can eliminate the cardioprotective effect of fentanyl while lncRNA-MALAT1 is sensitive to H/R injury, and the down-regulation of MALAT1 is helpful to reduce the apoptosis of cardiac myocytes (53). By targeting COL1A1, miR-133a-3p inhibits cell proliferation, invasion and migration and promotes apoptosis of ESCC cells (54). Meanwhile, miR-133b inhibits the proliferation of cardiac myocytes and the release of cytokines TNF- α and IL-6 by targeting Rab27B, and alleviates the myocardial injury caused by CVB3 infection (55). In addition, the decreased expression of miR-16-5p is helpful to the proliferation, survival, and resistance to cytotoxic therapy of glioma cells (56). On the other hand, by inducing cell cycle arrest and apoptosis of breast cancer, in-vitro experiments have shown that TUG1 overexpression significantly inhibits cell proliferation (57). Studies on miR-133a-3p, miR-16-5p, miR-181c-5p, miR-495-3p and TUG1 have not found their effects on the pathogenesis of cardiomyopathy, but our analysis shows that they are the genes that regulate the most modules and play an important role in the pathogenesis of ischemic and non-ischemic cardiomyopathy. This needs further study. Other ncRNAs that significantly regulate the dysfunction modules of ischemic and non-ischemic cardiomyopathy may also be involved in the basic pathogenesis of ischemic and non-ischemic cardiomyopathy, which may serve as candidate molecules for further molecular experimental verification.

Finally, we identified differential expressions in varying degrees of 36 transcription factors and significantly modulated the common dysfunction modules of ischemic and nonischemic cardiomyopathy. According to regulatory analysis, NFKB1, PML, and RELA significantly regulate the two modules, and these regulatory factors may play an important role in the pathogenesis of ischemic and non-ischemic cardiomyopathy. Among them, studies on human heart failure have shown that nuclear factor B (NF- κ B) is activated in cardiac myo-

cytes for the long term, indicating important participation of NF- κ B in cardiac remodeling (58). Meanwhile, promoter polymorphisms associated with smaller NF κ B1 gene activation were also associated with dilated cardiomyopathy (59). PML ubiquitination and degradation pathways regulate cellular and non-cellular components of the tumor microenvironment to enhance immune evasion and metastasis (60). Moreover, by increasing the activity of the NF- κ B pathway and p65-mediated transcription, it is suggested that catalase improves diabetic autophagy in part, while both activation of apoptosis and high glucose-induced autophagy fluxes were hugely attenuated by p65 siRNA (61-63). At the same time, other transcription factors that significantly regulate the dysfunction modules of ischemic and non-ischemic cardiomyopathy may also participate in the basic process of ischemic and non-ischemic cardiomyopathy, which needs to be verified by experiments.

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