Identification of Core Gene Biomarkers in Patients With Hypertrophic Cardiomyopathy

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Research Article

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Abstract

Objective Hypertrophic cardiomyopathy (HCM) is a kind of common hereditary myocardial disease. However, there is still a lack of detailed research in the specific mechanism of HCM. In the present study, gene microarray data were used for bioinformatics analysis to explore differentially expressed genes (DEGs) and signaling pathways between HCM patients and normal control, which would provide suggestion for the prevention and treatment for the further study of HCM.

Methods: Gene microarray data of HCM patients and normal control were obtained from the Gene Expression Omnibus (GEO) database affiliated to National Center for Biotechnology Information (NCBI). Gene microarray data of 15 HCM patients and 10 normal people were respectively included. We compared the number of DEGs between the two groups, performed gene ontology (GO) enrichment analysis, kyoto encyclopedia of genes and genomes (KEGG) analysis, construct a protein-protein interaction (PPI) network based on the DEGs detected above.

Results: A total of 501 DEGs were selected through analysis, among which 275 were up-regulated and 226 were down-regulated. The DEGs are mainly concentrated in ribosomes, myocardial contractions and various signaling pathways. The down-regulated cellular components and molecular functions of HCM patients were mainly associated with ribosome-related components, and PPI network also found that the DEGs were mainly derived from the ribosome family. Upregulated pathways were mainly enriched in Hippo signaling pathway, PI3K-Akt signaling pathway, AMPK signaling pathway, and adrenergic signaling in cardiomyocytes.

Conclusion: By analyzing the DEGs between HCM and normal patients, differentially expressed genes, cellular components and biological processes were found. By exploring the specific mechanism of these DEGs and intervening HCM through various medical procedures, useful suggestions can be provided for targeted prevention, precise treatment and outcome improvement of HCM patients.

Introduction

Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiac disease and a common cause of sudden death among young people over the world, the incidence of HCM was about 1 in 500 persons[1, 2]. HCM is also a kind of autosomal dominant disease[3] more than 500 variants of HCM genotype changes have been identified. About 60% of adult patients with HCM have detectable genetic variations, while the rest genetic variations are unknown[4]. About 40-60% of HCM patients have multiple single disease-causing mutations. The most common mutations in HCM patients are myosin heavy chain (MYH7) and myosin binding protein C(MYBPC3) genes[5]. HCM can also cause by non-sarcomere gene mutations which associated with neuromuscular disease, metabolic disorders or genetic syndromes[6]. More and more studies suggested the diversity of genes involved in HCM recently[5]. More than 28,239 mutations in 27 genes associated with HCM have been detected[7]. However, the molecular mechanism of HCM is not completely understood, the diagnosis of HCM mainly relies on clinical manifestations and

ultrasound, due to the lack of timeliness and perceptiveness of diagnosis methods above, many patients be diagnosed as HCM when they underwent ventricular tachyarrhythmias and even sudden cardiac death[8], the life expectancy of many HCM patients has not significantly increased[9]. Therefore, it is necessary to search for new diagnosis methods and therapies against HCM on the basis of new genetic biomarkers.

Because of genetic diversity and genetic noise, gene detection and gene therapy of HCM patients still have limitations[10]. However, exploring the diverse genetic changes in the pathological process of HCM would enhance the understanding of molecular pathogenesis of HCM[11, 12]. Gene-based diagnostic tests can identify high-risk individuals and provide potential targets for the development of clinical drugs[6]. Microarray technology has been used to analyze the genetic spectrum in many cardiology diseases, considerable research data has been obtained[13, 14]. High-throughput sequencing and microarray probe have been used in patients with HCM[15-17]. Bioinformatics analysis can compare the gene expression levels of different patients and screen out DEGs between HCM and normal control, which has a great prospect in revealing the potential gene and pathway[18].

The data used in the present study was obtained from the National Center for Biotechnology Information (NCBI). We reanalyzed these data through bioinformatic methods such as DEGs screening, functional enrichment analysis and protein-protein interaction (PPI) network analysis, constructed DEGs of HCM patients, made biological annotation, identified important molecular and biological pathways in the pathological process of HCM. The detection of key gene and pathway provides ideas for the diagnosis and treatment of HCM patients, from molecular diagnosis to disease classification, from clinical treatment evaluation to prognosis prediction.

Methods

1. Source of Microarray data

Microarray data of HCM patients and normal control were obtained from the NCBI GEO database www.ncbi.nlm.nih.gov[19]. We searched "hypertrophic cardiomyopathy" and screened out myocardial samples of patients with HCM and normal control. In the present study, GSE68316 and GSE32453 data sets were obtained. Genetic data of 12 patients (7 patients with HCM /5 normal controls) and 13 patients (8 patients with HCM /5 normal controls) were included. Myocardium samples from patients with HCM were obtained from surgically resected myocardium, and control samples were obtained from normal myocardium.

2. Grouping and Identification of DEGs

Microarray data was divided into the HCM group and the control group the sample number of the HCM group was GSM1668287-GSM1668293 and GSM802932-GSM802939. The sample number of the control group was GSM1668294-GSM1668298 and GSM802940-GSM802944. Differential gene expression analysis was performed with the microarray data using online analysis tool GEO2R

(http://www.ncbi.nlm.nih.gov/geo/geo2r) [20]. The DEGs profile of HCM patients was constructed by correcting P < 0.01 and taking | LogFC | ≥2 as the selection criteria. After downloading the original TXT data, used online software ImageGP to display volcano plot and heat map.

(http://www.ehbio.com/ImageGP/index.php/Home/Index/index.html)

3. Enrichment Analysis of DEGs

David Database (https://david.ncifcrf.gov) was used for gene ontology (GO) function enrichment analysis[21, 22]. GO analysis includes biological process (BP), cellular component (CC) and molecular function (MF) analysis[23]. To better understand the interaction pathways of these DEGs, 132 up-regulated DEGs and 96 down-regulated DEGs were analyzed by Kyoto Encyclopedia of Genes and Genome (KEGG) pathways analysis[24, 25]. The BP, MF, CC and the KEGG pathways of the DEGs were constructed by ImageGP to visualize the above results. In GO and KEGG analysis, P < 0.01 was regard as statistically significant.

4. Protein-Protein Interaction Network Analysis

The protein-protein interaction (PPI) network analysis of 132 up-regulated DEGs and 96 down-regulated DEGs was conducted in STRING database (https://string-db.org/) [26, 27]. In a PPI network, the node represents the protein, the edge between nodes represents the interaction between proteins. Cytoscape software was used to plot the result of protein interaction, visualize the PPI network, and calculate the number and connectivity of nodes. Protein interactions were screened using the interaction scoring criteria and module analysis was conducted using the Molecular Complex Detection (MCODE) plug-in. We set the degreed cut-off value as 2, node score cut-off value as 0.2, K score value as 3, Max. Depth value as 100, and MCODE score□10 as the standard. 12 different algorithms were used to screen the top 10 genes which had maximum connectedness by CytoHubba plug-in.

Results

1. Identification of DEGs

Microarray data used in this study were obtained from 25 samples in GPL20113 and GPL6104, including 15 samples of HCM patients and 10 samples of normal control. The basic information of the two platforms were shown in the table (Tab 1). The GEO2R online analysis tool was used to find out DEGs. A total of 501 DEGs were selected through analysis, among which 275 up-regulated DEGs and 226 down-regulated DEGs inside. These DEGs were shown in the volcano plot and the heat map (Fig 1). The top 8 up-regulated and top 8 down-regulated genes between HCM patients and normal controls were set out the table (Tab 2).

Among the 501 DEGs, the top 8 up-regulated genes were copper metabolism gene MURR1 domain containing 6□COMMD6□□coiled-coil domain containing 7□CCDC7□□ribosomal protein L26□RPL26□□ ribosomal protein S24□RPS24□□KIAA1841□ribosomal protein S7□RPS7□□prefoldin subunit 5□PFDN5□□

ribosomal protein L23（RPL23）. The top 8 down-regulated genes were selenium binding protein 1（SELENBP1）、trophinin associated protein（TROAP）、carbonic anhydrase 1（CA1）、erythrocyte membrane protein band 4.2（EPB42）、tensin 1（TNS1）、solute carrier family 4 member 1（SLC4A1）、solute carrier family 25 member（39SLC25A39）、BCL2-like 1（BCL2L1）(Tab 3).

2. GO function enrichment analysis

GO function analysis includes biological process (BP), cellular component (CC) and molecular function (MF). Top 10 up-regulated and top 10 down-regulated differently expressed biological processes between HCM patients and normal controls were shown in the table 3 (Tab 3). Top 10 up-regulated and top 10 down-regulated differently expressed cellular components between HCM patients and normal controls were shown in the table 4 (Tab 4). Top 10 up-regulated and top 7 down-regulated differently expressed molecular functions between HCM patients and normal controls were shown in the table 5 (Tab 5). ImageGP was used to visualize the above results, the above results were shown in GO enrichment plot (Fig 2).

The up-regulated biological processes are mainly enriched in myofibril assembly、regulation of smooth muscle contraction、smooth muscle cell-matrix adhesion、regulation of cell growth、potassium ion transport、positive regulation of epithelial cell apoptotic process、anatomical structure morphogenesis、muscle filament sliding、extrinsic apoptotic signaling pathway. The down-regulated biological processes are mainly enriched in SRP-dependent cotranslational protein targeting to membrane, viral transcription, nuclear-transcribed mRNA catabolic process, nonsense-mediated decay, translational initiation, translation, rRNA processing, hydrogen ion transmembrane transport, cytoplasmic translation, anterograde synaptic vesicle transport, mRNA splicing, via spliceosome (Fig 2).

In the cell component part, the upregulated cell component is mainly enriched in cytoskeleton, T-tubule, Z disc, cortical cytoskeleton, cytosol, plasma membrane, myofibril, axon, blood microparticle, microtubule organizing center. The downregulated cell components were mainly concentrated in cytosolic large ribosomal subunit, ribosome, intracellular ribonucleoprotein complex, nucleus, cytosol, focal adhesion, extracellular exosome, mitochondrial inner membrane, U4/U6 x U5 tri-snRNP complex, catalytic step 2 spliceosome (Fig 2).

In the cell molecular function, the upregulated molecular function is mainly enriched in potassium ion binding, actin filament binding, protein kinase binding protein binding, translation regulator activity, mRNA 3'-UTR binding, selenium binding, copper ion binding, mRNA 5'-UTR binding, heme binding. The downregulated molecular function was mainly concentrated in structural constituent of ribosome, RNA binding, poly(A) RNA binding, protein binding, mRNA binding, ubiquinol-cytochrome-c reductase activity, nucleotide binding (Fig 2).

3. KEGG pathway enrichment analysis

In order to have a more complete understanding of these selected DEGs, the KEGG pathway was analyzed by DAVID. We select the top 4 up-regulated and down-regulated DEGs, the details of these DEG2 were shown in Table 6 (Tab 6). We also used ImageGP to visualize the above results, the above results were shown in GO enrichment plot (Figure 2).

The up-regulated DEGs are mainly concentrated in the Hippo signaling pathway, PI3K-Akt signaling pathway, AMPK signaling pathway, Adrenergic signaling in cardiomyocytes pathway. The down-regulated DEGs are mainly concentrated in Ribosome signaling pathway, Oxidative phosphorylation signaling pathway, Cardiac muscle contraction signaling pathway, Spliceosome signaling pathway (Tab 6, Fig 2).

4. PPI network construction and key gene screening

132 up-regulated DEGs and 96 down-regulated DEGs were analyzed by PPI network in STRING, and Cytoscape software was used to reveal the results of protein interaction. Unconnected nodes in the network were hidden. The MCODE plug-in was used for module analysis, and it was found that the main DEGs came from the PRL (Phosphatase of Regenerating Liver) family, connecting 14 nodes and 89 edges. MCC algorithm was used to select genes with top 10 connectivity in PPI network, which were RPL26, RPL23, RPL21, RPS24, RPS3A, RPS7, MRPS7, RPL7, RPL5, and RPL22. These key genes were all up-regulated in HCM patients (Fig 3).

Discussion

HCM is an autosomal dominant genetic disease caused by more than 1,400 mutations in 11 or more genes encoding cardiac myostatin, the character of which is asymmetric hypertrophy of the ventricular myocardium[9]. The clinical manifestations of HCM are multitudinous, some patients have mild symptoms, the other patients have fatigue, dyspnea, chest pain and other severe clinical manifestations, such as heart arrhythmia and sudden cardiac death[2]. Therefore, early disease identification and management of HCM would enhance patients' quality of life and prolong their survival. The treatment of HCM including lifestyle management, drug therapy and surgical therapy[1]. However, gene therapy of HCM is still in the exploratory stage. HCM is a highly complex and heterogeneous disease, about 40% gene mutations of HCM have not yet been identified, which set obstacle in the development of gene therapy[5].

Recently, the development of second-generation sequencing technologies, such as targeted gene sequencing, whole exon sequencing (WES) and whole genome sequencing appeared, these technologies can help to identify more HCM patients and discover more gene mutations. Integrated microarrays analysis from different data sets will obtain genome-wide expression profiling with more samples which will increase the statistical power than an individual microarray[28]. Screening DEGs and biological pathways that are abnormally expressed in the pathogenesis of HCM through bioinformatics analysis would shed light on the diagnosis and treatment of HCM[29].

In the present study, we obtained microarray data from two online data sets (Tab 1), a total of 501 DEGs were selected through analysis. We performed gene ontology (GO) enrichment analysis, kyoto encyclopedia of genes and genomes (KEGG) analysis, construct a protein-protein interaction (PPI) network based on the DEGs detected above. The results shown that the DEGs are mainly concentrated in ribosomes, myocardial contractions and various signaling pathways. The down-regulated cellular components and molecular functions of HCM patients were mainly associated with ribosome-related components, and PPI network also found that the DEGs were mainly derived from the ribosome family. Upregulated pathways were mainly enriched in Hippo signaling pathway, PI3K-Akt signaling pathway, AMPK signaling pathway, and adrenergic signaling in cardiomyocytes (Fig 4).

The results of the present study shown that by comparing the gene microarray data of HCM patients with normal control, the DEGs were mainly concentrated in myocardial contraction associated genes, cellular components, molecular functions and biological processes. The pathology of HCM include ventricular septum asymmetrical hypertrophy, hypertrophy and arrangement disorder of cardiomyocytes, and mitral valve antedisplacement. The pathophysiology of HCM is characterized by hypertrophy myocardial fibers and interlaced myocardial fibers' walking directions[30]. The results of present study shown that comparing with the normal control, upregulation cell component of HCM patients were cytoskeleton, T-tubule, Z disc, myogenic fiber and axon. The upregulation molecular functions were mainly concentrated in the actin filament binding. In the previous studies, the main genes that regulated in HCM were MYH7, MYBPC3, TNNT2 and TNNI3[31]. Abnormalities of these gene-encoded proteins can impair the function of the myocardium and lead to ventricular hypertrophy, cardiac dysfunction and fibrosis, thereby disrupting normal myocardial contraction[31]. In addition to the above marker genes, other HCM-related genes also encode sarcomere structural proteins and Z disc, troponin C, myosin and cardiac LIM proteins. Studies pointed out that HCM is not only a hereditary disease, but also a sarcomere related disease[32]. KEGG analysis in this study shown that HCM patients had decreasing myocardial systolic function when comparing with normal patients. An important abnormal pathological change in HCM is the reduction of contractile stress (the force per unit area) produced by the myocardial tissue, which is caused by cardiac muscle cell disorder and other abnormalities. The deterioration of contractile pressure leads to further abnormal in cardiac hypertrophy and histology change[33]. The structural disorder of cardiomyocytes is directly caused by the functional changes of myocardial cell-related mutations at the myocyte level[34]. Over the past decade, many studies focused on the important role of gene therapy in HCM, such as genome editing, exon jumping, allele-specific silencing, spliceose-mediated RNA trans-splicing and gene replacement techniques. These techniques have been tested for effectiveness in animal or human HCM pluripotent stem cell models[35]. However, with the development of gene sequencing technology and bioinformatics, researchers should pay more attention to gene groups rather than an individual gene, and recognize that HCM is not a disease caused by a single gene. In addition, early intervention against structural changes of HCM may become an important method for patients with HCM to slow down their disease progression and prevent long-term complications.

Present study shown that the downregulated cellular components of HCM patients were mainly concentrated in the cytosolic large ribosomal subunit and ribosome, and the downregulated molecular

functions were mainly concentrated in the structural constituent of ribosome and the RNA binding function of ribosome. PPI analysis also found that DEGs were mainly derived from PRL family. Ribosomal protein is the main component of ribosome and plays an important role in protein biosynthesis of cells[36]. Studies have shown that the knockdown of ribosome gene and its RNA splicing cofactors would reduce the proliferation and differentiation of cardiac myocytes. At the same time would increase the number of fibroblasts[37]. In drosophila, heart-specific ribosome knockdown during the embryonic stage leaded to a significant "no heart" phenotype[38]. Although cardiomyocyte hypertrophy compensates the increasing stress of ventricular wall, ribosomal regulatory signals have been shown to play an important role in the pathological remodeling of the heart. In chronic heart disease, the growth of such nonmitotic cardiomyocytes is often associated with interstitial fibrosis, increasing cell death and decreasing cardiac function[39]. Ribosome is involved in protein synthesis and plays an important role in the physiological and pathological process of myocardium. Therefore, the results of the present study suggest that the downregulation of ribosome family function may be one of the pathogenesis mechanisms of HCM. Recently, ribosome profiling (ribo-seq) and ribosome-tagging approach (ribo-tag) were used to monitor the change of gene expression in cardiac stress by purifying the labeled ribosomes of cardiac myocytes and deep sequencing of ribosome-protected mRNA fragments[40]. Therefore, ribosome may be one of the targets in the monitor and treatment of HCM, further studies need to focus on the role of ribosomes in HCM and interfering the pathogenesis of HCM through pharmacology, molecular biology and gene regulation technology.

In KEGG enrichment pathway analysis, it was found that upregulated pathways were mainly enriched in Hippo signaling pathway, PI3K-Akt signaling pathway, AMPK signaling pathway, and adrenergic signaling in cardiomyocytes.

Hippo signaling pathway is an enzymatic response signaling pathway, the activation of which mainly occurs in the proliferation and regeneration of adult cardiomyocytes and the process of heart failure[41]. Cardiomyocytes lack the ability of repair, so the myocardial injury such as myocardial infarction can lead to heart failure and sudden death[42]. This function is associated with the Hippo signaling pathway, which plays a key role in organ size control by inhibiting cell proliferation, apoptosis promotion, stem cell regulation and cell size limitation[43]. Activation of Hippo signaling results in quiescent state of cardiac fibroblasts, inactivation of Hippo signal leads the spontaneous transition of cardiac fibroblasts to myofibroblast status during disease-related stress, which is the foundation of fibrosis and ventricular remodeling[44]. The Hippo signal down-regulation has been manifested to inhibit cardiomyocyte proliferation and reduce the size of the heart[45]. In another study, Hippo signal deficiency has also been shown to inhibit cardiomyocyte proliferation, reverse systolic heart failure after myocardial infarction and reduce heart size[46]. Experimental studies also confirmed that Hippo/YAP signaling pathways and Hippo protein levels upregulated in HCM patient and rat models of aortic constriction surgery[47]. Therefore, activation of this type of pathway is a kind of “self-protective mechanism” of the heart, the suppression of this “over-protective mechanism” could be used as one intervention target in HCM patients.

Pi3k-akt3 signaling pathway plays an important role in cardiac development by promoting coronary angiogenesis, promoting the growth and survival of cardiomyocytes, maintaining cardiac systolic function, regulating signal transduction and inducing autophagy[48]. The results of the present study confirmed that the expression of PI3K-AKT3 signaling pathway was enhanced in HCM patients, which may be related to excessive proliferation of cardiomyocytes in HCM patients. It was found that P13KT had synergistic effect on cardiac hypertrophy controlled by P13Ka signal. P13K7 inhibits the activation of glycogen synthase kinase 3 in the downstream of insulin P13K/mAkt pathway, and the negative feedback enhances the downstream signal of P13Ka, thus inducing cardiac hypertrophy[49]. One of the function of Akt3 is to promote cell growth, and Akt3 transgenic mice shown obvious pathological hypertrophy in their 20 weeks[50]. Several studies confirmed that the PI3K/Akt pathway has a protective effect on diabetic cardiomyopathy and myocardial injury because of its restorative effects[51-53]. Activation of the PI3K/Akt pathway had a protective effect on dilated cardiomyopathy, but no study confirmed the protective effect of the PI3K/Akt pathway in HCM[54]. Therefore, the effect of the PI3K/Akt pathway in HCM can become a novel research direction in the future.

AMPK-activated protein kinase plays an important role in maintaining homeostasis of the heart. AMPK is a serine-threonine kinase that acts as a metabolic sensor in coordinating anabolic and catabolic activities in cells through phosphorylation of various proteins in metabolic pathways. In addition to playing a direct role of cardiomyocyte metabolism, AMPK can also directly or indirectly affect mitochondrial function regulation, post-translational acetylation, autophagy, mitochondrial autophagy, endoplasmic reticulum stress, apoptosis and other cellular processes[55]. A number of studies have confirmed the protective effect of AMPK in HCM through pharmacological means[56], gene therapy[57]. It has been demonstrated in animal experiments that HCM was associated with up-regulation of AMPK pathway[58]. The use of AMPK inhibitor complex C inhibited cardiac hypertrophy induced by epinephrine (PE)[59].In addition, mutations in the gene encoding AMPK activated protein kinase as well as mutations in mitochondrial DNA have been detected in HCM patients[60]. In HCM patients, the protective mechanism of AMPK may be related to its activation by cardiomyocyte hypertrophy. AMPK is a key regulator in hypertrophic inhibiting and cardiomyocyte integrity maintaining. Activation of AMPK can inhibit protein synthesis, glucose storage, improve cardiac fibrosis and hypertrophic process at the same time[61]. AMPK can serve as one of prevention and treatment target in HCM patients.

Activation of adrenergic receptor causes a variety of physiological responses, including cardiac contractions and secretion of renin by juxtamlomerular cells[62]. Adrenergic receptor activation is a main mechanism in improving cardiac performance under stress[63], and adrenaline is often used to induce cardiomyocyte hypertrophy and apoptosis in experiments[64]. Under adrenergic stimulation, the distribution of cAMP is highly restricted in different intracellular regions, thus promotes the selective phosphorylation of proteins in the contraction response of cardiomyocytes[65]. The enhancement of adrenergic signaling pathway in HCM patients indicated that the occurrence and development of HCM were closely related to neuroendocrine. Studies have shown increasing cardiac sympathetic activity in HCM, and increasing cardiac sympathetic activity is associated with impaired left ventricular diastolic and systolic functions[66]. The change of nerve afferent from local segmental hypertrophy is mainly

manifested as enhanced sympathetic activity, which is one of the inducers of cardiac hypertrophy[67]. Studies of pluripotent stem cell-derived cardiomyocytes have shown that arrhythmias in HCM patients depend on the concentration of epinephrine and the mutations of the gene[68]. It has been reported that the development of HCM in pheochromocytoma patients was caused by over activation of sympathetic nerve[69]. Studies have attempted to block cardiac adrenergic pathway in the prevention of ventricular remodeling and cardiac hypertrophy[70], beta-blockers would be chosen as the first choice in the treatment of HCM patients[71]. In the future, using pharmacology methods, neuroendocrine regulation and other means to inhibit adrenergic receptor signals in the prevention of the HCM is a research direction with broad theoretical basis and high feasibility.

Limitations

The present study has a few limitations: Firstly, this study used data from the Gene Expression Omnibus database affiliated to National Center for Biotechnology Information, using myocardial data from 25 samples. Because of the limitation of research data, the results of this study also have some limitations. Enlarging the samples can enhance the accuracy of the analysis. Secondly, present study only used bioinformatics analysis, further research needs to confirm these conclusions in animal experiments, such as comparing the protein expression and signaling pathway differences of the HCM patients and normal controls to verify the conclusion of present study. Finally, the present study searched for DEGs in HCM, which of them were expected to exert their effects in early diagnosis, disease prevention, treatment and prognosis improving of HCM. Further study should put forward potential intervention and be designed to validate the feasibility and the safety of these potential methods.

Declarations

Ethics approval and consent to participate

Not Applicable.

Consent for publication

Not Applicable.

Availability of data and materials

The data sets are obtained from public database Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo). The numbers of the datasets used in current study are GSE68316 and GSE32453.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' contributions

Xiaomeng Yang and Hong Jiang contributed to the conception of the study. Xiaomeng Yang performed the data analyses and written the manuscript. All authors read and approved the final manuscript.

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Tables

Table1 Detailed Data of GSE68316, GSE32453

	GSE68316	GSE32453
Platform	GPL20113	GPL6104
Organism	Homo Sapiens	Homo Sapiens
Sample Source	Myocardial Tissues	Myocardial Tissues
Sample number (HCM / Control)	7/5	8/5
Time of updating	Oct 10, 2019	Jan 18, 2013

Table 2 The top upregulated and downregulated differentially expressed genes in patients with HCM

DEGs	Gene	Gene Symbol	SPOT_ID	LogFC	adj.P.Val	P.Value
Upregulated	Homo sapiens COMM domain containing 6	COMMD6	NM_203497	4.062	9.37E-07	3.89E-09
	Homo sapiens coiled-coil domain containing 7	CCDC7	NM_145023	3.389	2.05E-06	1.33E-08
	Homo sapiens ribosomal protein L26	RPL26	NM_000987	3.364	2.50E-07	3.61E-10
	Homo sapiens ribosomal protein S24	RPS24	NM_001026	3.355	2.69E-07	4.34E-10
	Homo sapiens KIAA1841	KIAA1841	NM_032506	3.159	1.19E-06	5.26E-09
	Homo sapiens ribosomal protein S7	RPS7	NM_001011	3.039	1.64E-06	8.90E-09
	Homo sapiens prefoldin subunit 5	PFDN5	NM_002624	3.029	7.14E-07	2.46E-09
	Homo sapiens ribosomal protein L23	RPL23	NM_000978	2.934	5.01E-06	6.31E-08
Downregulated	Homo sapiens selenium binding protein 1	SELENBP1	NM_003944	-4.961	1.30E-08	2.73E-12
	Homo sapiens trophinin associated protein	TROAP	NM_001100620	-4.494	6.40E-07	1.97E-09
	Homo sapiens carbonic anhydrase 1	CA1	NM_001738	-4.467	1.08E-07	1.14E-10
	Homo sapiens erythrocyte membrane protein band 4.2	EPB42	NM_000119	-4.339	7.46E-08	4.19E-11
	Homo sapiens tensin 1	TNS1	NM_022648	-4.193	3.60E-07	6.47E-10
	Homo sapiens solute carrier	SLC4A1	NM_000342	-4.162	1.08E-07	8.55E-11

	family 4 member 1					
	Homo sapiens solute carrier family 25 member 39	SLC25A39	NM_016016	-3.995	4.48E-09	3.15E-13
	Homo sapiens BCL2 like 1	BCL2L1	NM_138578	-3.476	9.21E-08	5.83E-11

Table 3 Gene ontology analysis of differentially expressed genes in biological process（BP）

DEGs	Term	Count	Qvalue
Upregulated	myofibril assembly	3	3.39E-03
	regulation of smooth muscle contraction	3	5.15E-03
	smooth muscle cell-matrix adhesion	2	1.35E-02
	regulation of cell growth	4	1.72E-02
	iron ion homeostasis	3	1.76E-02
	potassium ion transport	4	1.83E-02
	positive regulation of epithelial cell apoptotic process	2	2.02E-02
	anatomical structure morphogenesis	4	2.47E-02
	muscle filament sliding	3	2.74E-02
	extrinsic apoptotic signaling pathway	3	3.30E-02
Downregulated	SRP-dependent cotranslational protein targeting to membrane	11	2.21E-11
	viral transcription	11	1.29E-10
	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	11	2.36E-10
	translational initiation	11	9.51E-10
	translation	13	2.56E-09
	rRNA processing	11	6.93E-08
	hydrogen ion transmembrane transport	5	2.03E-04
	cytoplasmic translation	4	2.22E-04
	anterograde synaptic vesicle transport	3	2.26E-03

	mRNA splicing, via spliceosome	6	4.16E-03

Table 4 Gene ontology analysis of differentially expressed genes in cellular component（CC）

DEGs	Term	Count	Qvalue
Upregulated	cytoskeleton	11	1.61E-04
	T-tubule	4	1.94E-03
	Z disc	5	7.49E-03
	cortical cytoskeleton	3	8.97E-03
	cytosol	33	1.20E-02
	plasma membrane	39	1.20E-02
	myofibril	3	1.43E-02
	axon	6	1.53E-02
	blood microparticle	5	1.76E-02
	microtubule organizing center	5	1.80E-02
Downregulated	cytosolic large ribosomal subunit	9	7.77E-10
	ribosome	10	6.41E-08
	intracellular ribonucleoprotein complex	9	1.93E-07
	nucleus	41	2.93E-04
	cytosol	29	5.04E-04
	focal adhesion	8	2.03E-03
	extracellular exosome	24	2.94E-03
	mitochondrial inner membrane	8	3.96E-03
	U4/U6 x U5 tri-snRNP complex	3	4.87E-03
	catalytic step 2 spliceosome	4	8.55E-03

Table 5 Gene ontology analysis of differentially expressed genes in molecular function（MF）

DEGs	Term	Count	Qvalue
Upregulated	potassium ion binding	3	4.04E-03
	actin filament binding	5	1.30E-02
	protein kinase binding	8	1.51E-02
	protein binding	73	1.85E-02
	translation regulator activity	2	4.05E-02
	mRNA 3'-UTR binding	3	4.46E-02
	selenium binding	2	5.37E-02
	copper ion binding	3	5.67E-02
	mRNA 5'-UTR binding	2	6.02E-02
	heme binding	4	6.85E-02
Downregulated	structural constituent of ribosome	13	7.33E-10
	RNA binding	16	5.07E-08
	poly(A) RNA binding	22	6.42E-08
	protein binding	61	7.18E-05
	mRNA binding	4	2.30E-02
	ubiquinol-cytochrome-c reductase activity	2	4.75E-02
	nucleotide binding	5	8.92E-02

Table 6 KEGG pathway analysis of differentially expressed genes

Category	Term	Count	Qvalue	Genes
Upregulated	Hippo signaling pathway	5	2.84E-02	YWHAE, FZD3, PPP2R2B, CTNNA3, AJUBA
	PI3K-Akt signaling pathway	7	4.81E-02	CHRM2, YWHAE, VTN, PPP2R2B, FGF18, JAK2, BCL2L1
	AMPK signaling pathway	4	6.86E-02	SREBF1, PPP2R2B, STRADB, ADIPOR1
	Adrenergic signaling in cardiomyocytes	4	8.97E-02	PPP2R2B, TPM3, ATP1A2, MAPK12
Downregulated	Ribosome	13	1.80E-10	RPL5, RPL21, RPS7, RPL23, RPL22, RPS3A, MRPS7, RPL6, RPL7, RPL36AL, RPL26, RPL39, RPS24
	Oxidative phosphorylation	7	4.11E-04	NDUFA6, UQCRQ, PPA1, COX4I1, NDUFB2, COX7A2, UQCRH
	Cardiac muscle contraction	5	2.21E-03	UQCRQ, COX4I1, COX7A2, MYH6, UQCRH
	Spliceosome	5	1.66E-02	ISY1, SNRPB2, SNRPE, LSM5, LSM3

Figures

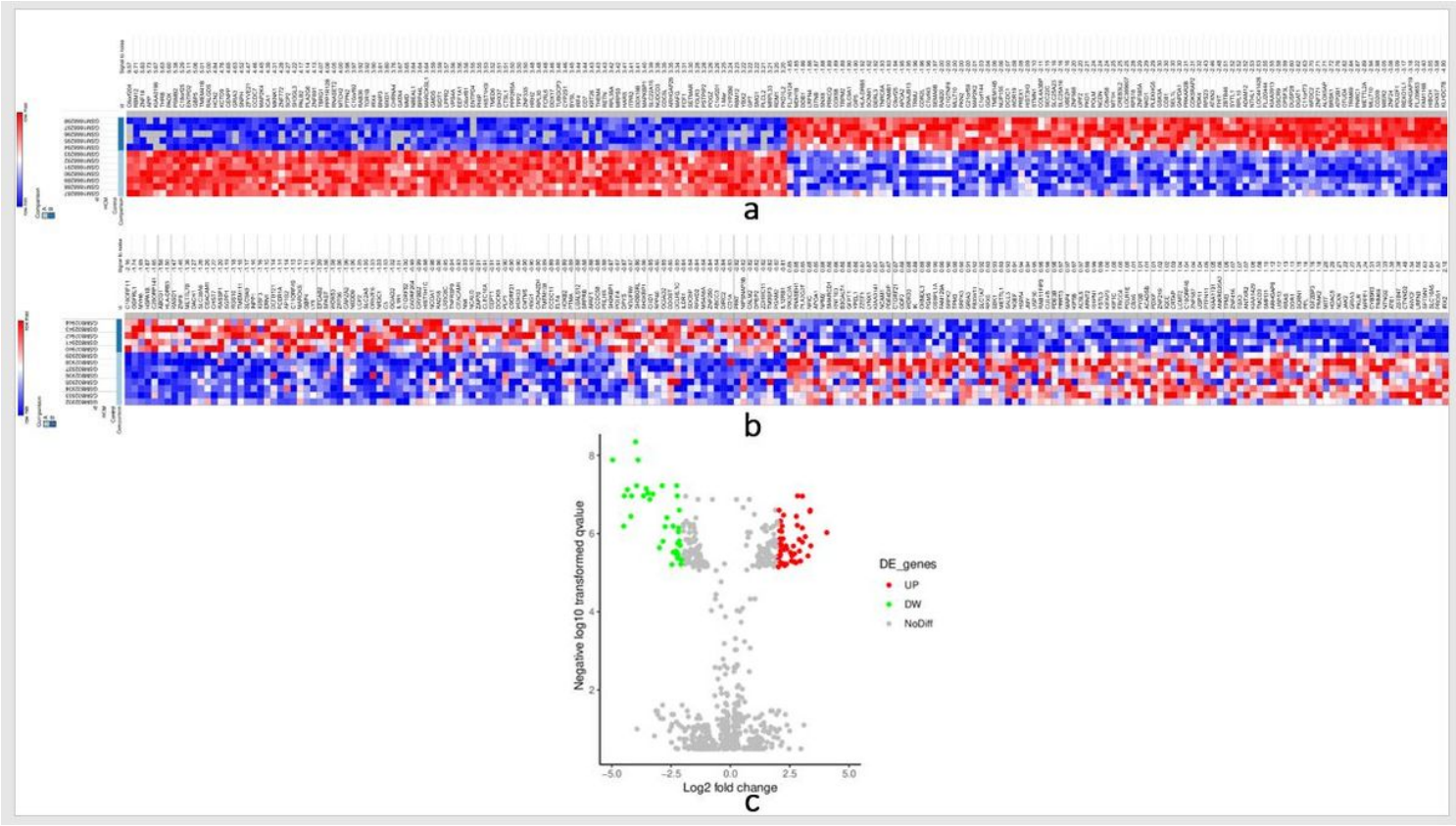

Figure 1

Volcano plot and heat map of the differentially expressed genes (DEGs) . (a) Volcano plot of DEGs from GPL20113. Green means downregulated DEGs; red means upregulated DEGs; gray means no difference. (b) Volcano plot of DEGs from GPL6104. (c) Heat map of upregulated DEGs and downregulated DEGs.

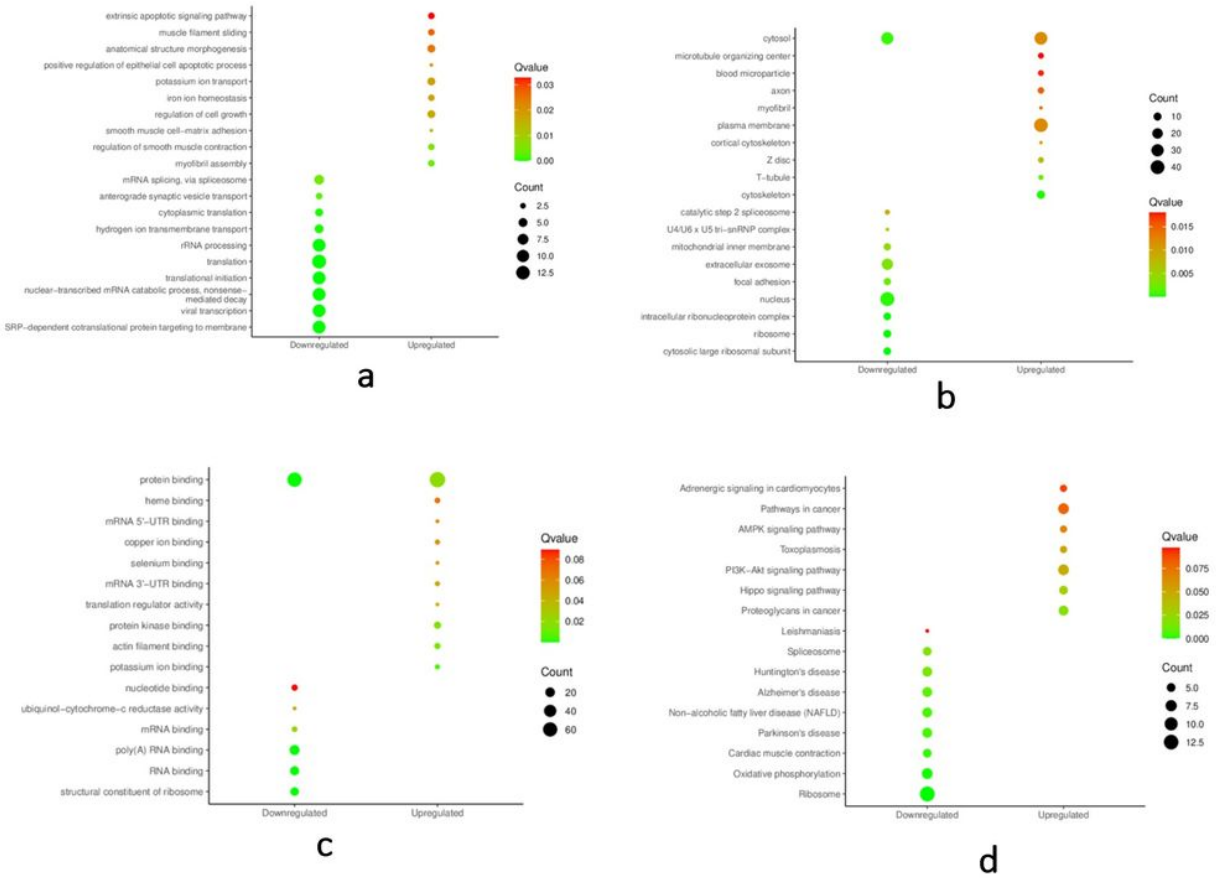

Figure 2

Gene ontology (GO) function analysis and kyoto encyclopedia of genes and genomes (KEGG) pathway analysis of HCM. (a) The enriched GO terms in the biological process (BP); (b) the enriched GO terms in the cellular component (CC); (c) the enriched GO terms in the molecular function (MF); (d) the enriched KEGG pathway in HCM.

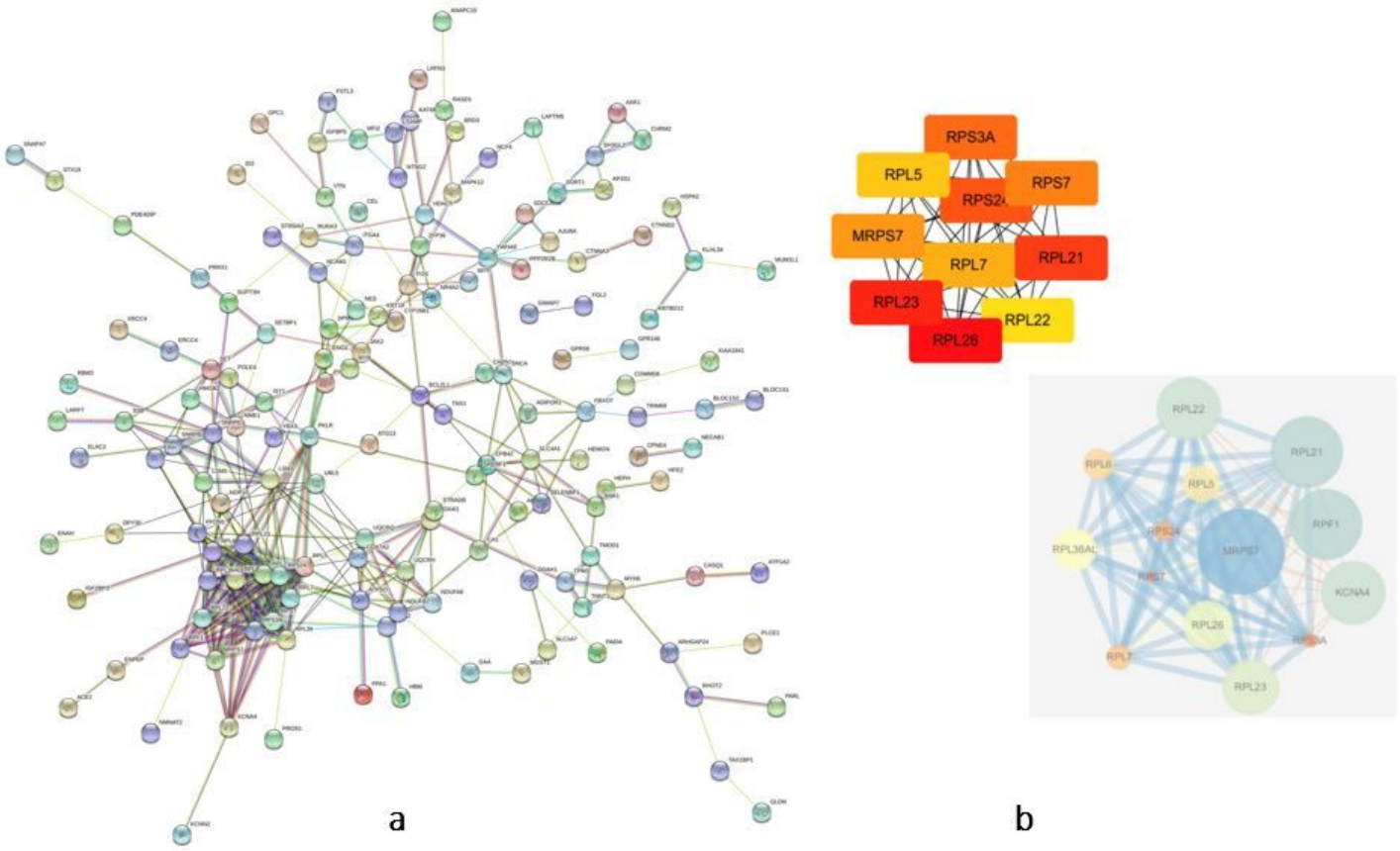

Figure 3

Protein–Protein Interaction (PPI) network and top module of hub genes. (a) The PPI network of all DEGs. (b) the PPI network of top 10 genes with high connectivity degree

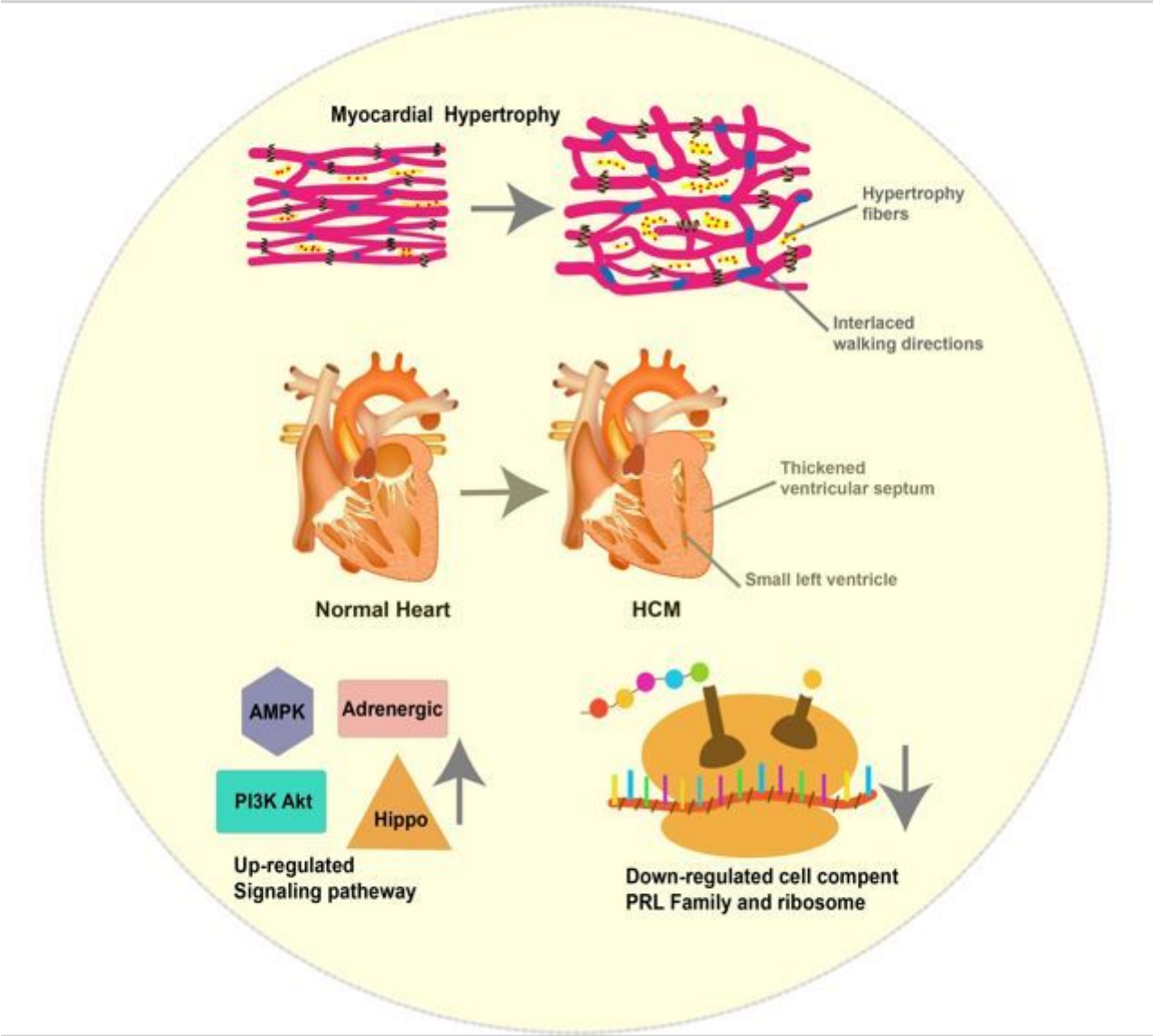

Figure 4

Potential therapeutic targets in HCM.