Identification of potential core genes in atrial fibrillation based on bioinformatics analysis

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Abstract

OBJECTIVE: Atrial fibrillation (AF) is one of the most common arrhythmias, which is caused by a number of minor disorders caused by the heart owner's guide. It is noted in almost all organic heart disease and can also occur in non-organic heart disease, causing serious complications, such as heart failure and arterial embolism, seriously threaten people's health. Atrial fibrillation can cause serious complications, such as heart failure and arterial embolism, which seriously threaten people's health. Clinically, according to the characteristics of atrial fibrillation, atrial fibrillation is divided into paroxysmal atrial fibrillation, persistent atrial fibrillation, permanent atrial fibrillation (cannot be converted to sinus rhythm). Among them, persistent atrial fibrillation poses the greatest threat to people's health. So the main purpose of this study is to identify the differential genes in patients with permanent atrial fibrillation.

MATERIALS AND METHODS: To explore potential differential genes for permanent atrial fibrillation, we analyzed three microarray datasets GSE2240 derived from the Gene Expression Omnibus (GEO) database. We used the "limma" function package of R software to screen differentially expressed genes (DEGs), and used the "pheatmap" function package to construct heatmaps for the screened differential genes. Visualization and Integration Discovery (DAVID), Cytoscape, BMKcloud and String platforms were utilized for Genome Ontology (GO) analysis, Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, PPI (protein-protein

Interaction) network analysis, module analysis, and identification hub genes based on the selected differential genes.

RESULTS: 74 DEGs in total were identified, including 41 up- and 33 downregulated genes. These DEGs were mainly enriched in PI3K-Akt signaling pathway, cGMP-PKG signaling pathway, Focal adhesion, and so on. The module analysis filtered out 12 key genes, including PRL, CREB1, AGO2, NAMPT, IGFBP2, FGF7, ANGPT2, SYT13, NRXN1, AQP4, TCEAL2, and CPLX1. 2 Hub genes were identified, including IGFBP2, and FGF7.

CONCLUSIONS: IGFBP2 and FGF7 were identified as Hub genes of AF, which helped us to understand more deeply the pathophysiological mechanism of AF.

Introduction

Atrial fibrillation is the most prevalent arrhythmia in the population, which may lead to significant damage to quality of life and is associated with increased all-cause mortality. In the elderly, more than one third of strokes are associated with atrial fibrillation (1). Current census projections for 2050 suggest that the number of AF population in the United States, Europe and Japan will double to triple (2). However, epidemiological data may underestimate its actual prevalence as 40% of patients are asymptomatic. There is also evidence that the incidence of cardiovascular-related morbidity in patients with atrial fibrillation is significantly increased due to the association of atrial and ventricular mechanical or electrical failure, structural and hemodynamic changes, and thromboembolism events (3).

The patient's self symptom and ECG examination are the principal means of examination of atrial fibrillation at present. But most patients do not have obvious symptoms, and the diagnosis and treatment of these patients become particularly passive. Although many studies now show that multiple inflammatory factors are associated with atrial fibrillation(4,5), lack of specificity has not been clinically promoted. So finding more effective diagnosis and treatment can greatly reduce the burden of disease and improve the quality of life of patients. In recent years, with the development of gene chip technology, more and more gene expression profiles have served in cardiovascular clinical and research by gene chip technology. For example, through bioinformatics analysis, Da-Qiu Chen et al found that FADS2, LRRN3, GPR15, AK5, CXCL8 and IL1 β may be associated with the development of heart failure after myocardial infarction(6). G.-M. Li et al identified 8 differential genes through differential gene analysis of coronary heart disease and ischemic cardiomyopathy, in which PTGS2 and EGR1 are the most critical genes(7). Li et al compared the gene expression differences between the left atrium and right atrium, found that ACAT1, CRAD, GIN1, FTX, TCEAL2 and MCM3AP play a key role in the pathophysiological mechanism of AF, which may serve as a new therapeutic target for patients and contribute to a better understanding of the underlying mechanisms of AF(8). ZOU et al based on database analysis of atrial fibrillation and cardiogenic stroke, it was noted that the ZNF566, PDZK1IP1, ZFHX3, and PITX2 genes may be associated with atrial fibrillation-related stroke(9). However, integrated bioinformatics analysis method is rarely used to compare the atrial tissue gene expression of AF patients with SR people.

In this research, we download GSE2240 data from the GEO database. The R software was first employed to screen the differential genes, and the volcano plot and heatmap were made. Secondly, DAVID online tools were applied for GO and KEGG pathway analysis to reveal the functional enrichment and related pathways of differential genes. Next builds the PPI network and does module analysis. Finally using cytoscape to find the Hub genes. Our result may provide a novel pathway for diagnosis and treatment of the AF in the future.

Materials And Methods

Data sources

Utilizing the keywords "atrial fibrillation," we screened the Gene Expression Omnibus database (GEO; www.ncbi.nlm.nih.gov/geo). Finally, the gene expression profile GSE2240 was downloaded. The expression profiling arrays was generated using GPL96 (HG-U133A) Affymetrix Human Genome U133A Array and using GPL97 (HG-U133B) Affymetrix Human Genome U133B Array. Each platform file includes 10 cases of samples from AF patients and 20 cases of samples from sinus rhythm (SR) people. Both of the two platforms were right atrial (RA) tissue.

Data preprocessing

Firstly, acquired RAW data were divided into atrial fibrillation group and control group, and then two sets of data were preprocessed using the affyPLM and affy package of R software. Finally, the preprocessed data were merged. After this, the probes were converted into gene symbols on the basis of the annotation

platform file .If there are multiple probes corresponding to a gene, take the average as the final value.If the probe without gene symbol was removed.

Identification of DEGs

The DEGs in atrial tissue samples from AF patients and SR people were identified using the Limma package in R Bioconductor. The adjusted p values were obtained by the Benjamini and Hochberg method. The screening criteria for DEGs were $|\log \text{ fold change (FC)}| > 1$ and adjusted $p < 0.05$. Then, heatmap plot were made using R software, to further analyze the DEGs.

GO and KEGG pathway enrichment analysis of DEGs

Database for Annotation, Visualization and Integrated Discovery (DAVID) (www.david.ncifcrf.gov/) were applied for Gene Ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, then use R software to visualize the results, to further understand the potential functions of the identified DEGs.

PPI network analysis, modules Analysis, and Selection of Hub Genes

PPI network analyses can show the functional link between proteins and proteins, using string software (www.string-db.org/cgi/input.pl) for PPI network analysis of differential genes. Subsequently, Cytoscape software was applied to visualize and analyze PPI network. Molecular Complex Detection (MCODE) can find the interacting dense region in the PPI network, and the dense regions of interest can also be extracted and visualized. So we use MCODE to discover modules across the network. The hub genes were identified by using the plug-in cytoHubba of the Cytoscape software, including Maximal Clique Centrality (MCC), Density of Maximum Neighborhood Component (DMNC) and Maximum Neighborhood Component (MNC).

Results

Identification of DEGs in atrial fibrillation

A total of 43 differential genes were selected based from GPL96, including 16 and 27 genes with up-regulated and down-regulated expressions, respectively. A total of 31 DEGs were obtained GPL97, including 25 up-regulated genes and 6 down-regulated genes, which were statistically significant (adjusted $p < 0.05$, $|\log \text{ fold change (FC)}| > 1$) (Table 1). The cluster heatmap plot of the DEGs are shown in Figure 1 and 2.

GO enrichment analysis of DEGs

The GO analysis consists of biological processes (BP), cellular component (CC) and molecular function (MF) terms. The different genes with adjusted p value < 0.05 were obtained from GO functional enrichment. The GO enrichment analysis results reveals : in the BP category, the DEGs were mainly

involved in cell adhesion, signal transduction, and negative regulation of cell growth; in the CC category, the DEGs were mainly involved in integral component of plasma membrane, and extracellular region; in the MF category, the DEGs were mainly enriched in hormone activity and calcium ion binding (Table 2 and Figure 3).

KEGG pathway analysis of DEGs

The KEGG pathways of the DEGs were analyzed using DAVID. As shown in Table 3 and Figure 4, the DEGs enriched in 8 pathways, especially PI3K-Akt signaling pathway, cGMP-PKG signaling pathway and so on.

Establishing the PPI Network, Conducting Modules Analysis, and Selection of Hub Genes

Use the string online tool to create a PPI network to gain a better understanding of the biological properties of differential genes. There were 66 nodes and 158 edges in this network, as showed in Figure 5. Subsequently, cytoscape is applied to confirm a vital module throughout the network, a total of 12 nodes and 28 edges in this module, as showed in Figure 6. 12 key genes were identified, including PRL, CREB1, AGO2, NAMPT, IGFBP2, FGF7, ANGPT2, SYT13, NRXN1, AQP4, TCEAL2, and CPLX1. These genes in the module were mainly enriched in the PI3K-Akt signaling pathway. Finally utilizing the cytoHubba plug-in to determine IGFBP2, and FGF7 as the Hub gene, as showed in Figure 7 and Table 4.

Discussion

In this study, we performed an integrated analysis on gene expression profiles from atrial tissues without / with AF aiming to identify the DEGs, related key signaling pathways and Hub genes for the disease. A total of 74 DEGs, including 42 up and 32 downregulated genes, were identified from the GSE2240 database. The GO enrichment analysis showed that these differential genes associated with AF were mainly enriched in cell adhesion, signal transduction, negative regulation of cell growth, integral component of plasma membrane, extracellular region, hormone activity and calcium ion binding. From KEGG pathway enrichment analysis, we identified that these DEGs were mainly enriched in the pathway of the PI3K-Akt signaling pathway, cGMP-PKG signaling pathway. Through the construction and module analysis of the PPI network, we identified 12 key genes, including PRL, CREB1, AGO2, NAMPT, IGFBP2, FGF7, ANGPT2, SYT13, NRXN1, AQP4, TCEAL2, and CPLX1. Finally, IGFBP2 and FGF7 are regarded as Hub genes for the development of atrial fibrillation.

Insulin-like growth factor (IGF) binding protein 2 (IGFBP2) is a developmental regulatory gene highly expressed in embryonic and fetal tissues and significantly reduced after birth. It is also considered a systemic regulator of IGF and controls the activity, distribution, and function of IGFs in the pericellular space (10, 11, 12). IGFBP2 is found in serum and in various biological fluids, including amniotic membranes, follicles, cerebrospinal fluid and semen, and milk. IGFBP2 may play different physiological roles in growth and development according to their main structure and post-translational modifications (13). Most studies have shown that IGFBP2 plays a major role in the diagnosis, growth, invasion and metastasis of various tumors. For example: colorectal cancer (14), hepatocellular

carcinoma(15), bladder cancer(16), breast cancer(17), etc. In addition, a study suggests that increased expression of IGFBP2 in the hippocampus during neonatal development is associated with learning and memory, which provides insights into the cognitive needs of IGFBP2 in early life(18). In the field of cardiovascular disease, Han et al found that microRNA-873 alleviated myocardial damage in gestational diabetic rats by upregulated IGFBP2(19). However, there are limited studies on the relationship between IGFBP2 and atrial fibrillation. In the GO function enrichment analysis, we found that IBGF2 is mainly associated with extracellular signal transduction, and the occurrence of atrial fibrillation is also associated with multiple inflammatory signal transduction abnormalities, so we speculate that IBGF2 may be involved in the occurrence and maintenance of atrial fibrillation through this pathway. More experiments are required to verify the connection.

Fibroblast growth factors (FGFs) is a heparin-binding gene family involved in various cellular and pathophysiological processes such as embryonic development, cell growth, morphogenesis, tissue repair, tumor migration and invasion(20, 21, 22). FGFs perform different functions by binding and activating members of the FGF receptor (FGFR) family. FGF7, also known as keratinocyte growth factor, exerts its role in a paracrine manner and specifically activates the receptor tyrosine kinase acting on FGFR2 dimer, which in turn triggers a variety of signal transduction pathways, including the ras-mitogen-activated protein kinase (MAPK) and the phosphoinositide 3-kinase (PI3k) AKT signaling pathway (23). Downstream of the FGF receptor, PI3k can be activated, where it can signal through the classical PI3k/AKT pathway. This signaling pathway is found to dysbiosis in many inflammatory diseases, cancer, and fibrosis in which it contributes to the development of the disease (24, 25, 26, 27). Some researchers have found through animal experiments that resveratrol can reduce the incidence of AF through the PI3k/AKT signaling pathway (28). At the same time, a study also showed that activating the PI3k/AKT signaling pathway could reverse atrial remodeling and inhibit the occurrence of AF (29). In addition, several studies have revealed that activation of PI3k/AKT signaling pathway effectively inhibits atrial rhythm and reverses atrial remodeling (30, 31). In this study, we found that FGF7 was mainly enriched in the PI3k/AKT signaling pathway through KEGG analysis, so we hypothesized that FGF7 might be involved in the pathophysiological process of AF through the PI3k/AKT signaling pathway.

Conclusions

In summary, our study offers an integrated bioinformatics analysis of DEGs of AF. In the present study, we identified some key genes and pathways, associated with AF. However, the key genes and signaling pathways related to AF derived from this study still need further experimental verification due to the defects of analytical methods and sample size.

Declarations

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Availability of data and materials

The datasets during the study are available from the corresponding author.

Authors' contributions

XP and ZZ designed the study. XP analyzed the data. XP and ZZ have approved the final manuscript.

Ethics approval and consent to participate

This study was based on a published research and no ethical approval and consent is required.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Kim NR, Choi CK, Kim HS, Oh SH, Yang JH, Lee KH, Kim JH, Park MS, Kim HY, Shin MH. Screening for Atrial Fibrillation Using a Smartphone-Based Electrocardiogram in Korean Elderly. *Chonnam Med J*. 2020;56:50–4.
2. Miura K, Ikemura N, Kimura T, Katsumata Y, Ueda I, Tanimoto K, Ohki T, Shinmura D, Negishi K, Fukuda K, et al. Treatment strategies and subsequent changes in the patient-reported quality-of-life among elderly patients with atrial fibrillation. *Am Heart J*. 2020;222:83–92.
3. Thijs V. Atrial fibrillation detection: fishing for an irregular heartbeat before and after stroke. *Stroke*. 2019;48:2671–7.
4. Qiu XY, Lu ST, Fan MK, Geng HH, Han ZY, Gao SP, Pan HY, Huang R, Pan M. Effects of Polymorphisms in Pre-miRNA on Inflammatory Markers in Atrial Fibrillation. *Chinese Clin Lab*. 2019;65:7–12.
5. Packer M. The epicardial adipose inflammatory triad: coronary atherosclerosis, atrial fibrillation, and heart failure with a preserved ejection fraction. *Eur J Heart Fail*. 2019;20:1567–9.
6. Li X, Li B. Identification of time-series differentially expressed genes and pathways associated with heart failure post-myocardial infarction using integrated bioinformatics analysis. *Mol Med Rep*. 2019;19:5281–90.
7. Li GM, Zhang CL, Rui RP, Sun B, Guo W. Bioinformatics analysis of common differential genes of coronary artery disease and ischemic cardiomyopathy. *Eur Rev Med Pharmacol Sci*. 2018;22:3553–

8. Li W, Wang L, Wu YYuan Z, Zhou J. Weighted gene co-expression network analysis to identify key modules and hub genes associated with atrial fibrillation. *Int J Mol Med*. 2019;45:401–16.
9. Zou R, Zhang D,Lv L, Shi W, Song Z, Yi B,Lai B, Chen Q, Yang S, Hua P. Bioinformatic gene analysis for potential biomarkers and therapeutic targets of atrial fibrillation-related stroke. *Journal of translational medicine*. 2019;17:45.
10. Zhou Z, Lu H, Zhu S, Gomaa A,Chen Z,Yan J, Washington K, El-Rifai W, Dang C, Peng D. Activation of EGFR-DNA-PKcs pathway by IGFBP2 protects esophageal adenocarcinoma cells from acidic bile salts-induced DNA damage.*J Exp. Clin Cancer Res*. 2019;38:13.
11. Zhu H, Zhang Y, Geng Y, Lu W,YinJ, Li Z,HuangL, Liu H, Xu N. IGFBP2 promotes the EMT of colorectal cancer cells by regulating E-cadherin expression. *Int J Clin Exp Pathol*. 2019;12:2559–65.
12. Yang D,QianH, Fang Z,Xu AZhao SLiu B, Li D. Silencing circular RNA VANGL1 inhibits progression of bladder cancer by regulating miR-1184/IGFBP2 axis. *Cancer medicine*. 2020;9:700–10.
13. Park SH, Kim KW, Kim JC. The Role of Insulin-Like Growth Factor Binding Protein 2 (IGFBP2) in the Regulation of Corneal Fibroblast Differentiation. *Invest Ophthalmol Vis Sci*. 2015;56:7293–302.
14. Zhu H, Zhang Y, Geng Y, Lu W,YinJ, Li Z,HuangL, Liu H, Xu N. IGFBP2 promotes the EMT of colorectal cancer cells by regulating E-cadherin expression. *Int J Clin Exp Pathol*. 2019;12:2559–65.
15. Lee CF, Ling ZQ, Zhao T, Lee KR. Distinct expression patternsin hepatitis B virus- and hepatitis C virus-infected hepatocellular carcinoma. *World J Gastroenterol*. 2008;14:6072–7.
16. Yang D,QianH, Fang Z,Xu AZhao SLiu B, Li D. Silencing circular RNA VANGL1 inhibits progression of bladder cancer by regulating miR-1184/IGFBP2 axis. *Cancer Med*. 2020;9:700–10.
17. Du Y, Wang P. Upregulation of MIIP regulates human breast cancer proliferation, invasion and migration by mediated by IGFBP2. *Pathol Res Pract*. 2019;215:152440.
18. Khan S, Lu X, Huang Q, Tang J, Weng J, Yang Z, Lv M, Xu X, Xia F, Zhang M,et al. IGFBP2 Plays an Essential Role in Cognitive Development during Early Life. *Adv Sci (Weinh)*. 2019;6:1901152.
19. Han N, Fang HY, Jiang JX, Xu Q. Down-regulation of microRNA-873 attenuates insulin resistance and myocardial injury in rats with gestational diabetes mellitus by up-regulating IGFBP2.*Am. J Physiol Endocrinol Metab*. 2020;3:2.
20. Shang A, Zhou C, Bian G, Chen W, Lu WWang W, Li D. miR-381-3p restrains cervical cancer progression by downregulating FGF7. *J Cell Biochem*. 2019;120:778–89.
21. Zhang X, Guo Y, Yang J,NiuJ,DuL, Li H, Li X. A functional variant alters binding of activating protein 1 regulating expression of FGF7 gene associated with chronic obstructive pulmonary disease. *BMC Med Genet*. 2019;20:33.
22. Correll KA, Edeen KE,Redente EF,Zemans RL,Edelman BL, Danhorn T, Curran-Everett D, Mikels-Vigdal A, Mason RJ. TGF beta inhibits HGF, FGF7, and FGF10 expression in normal and IPF lung fibroblasts. *Physiol Rep*. 2018;6:e13794.

23. Huang T, Wang L, Liu D, Li P, Xiong H, Zhuang L, Sun L, Yuan X, Qiu H. FGF7/FGFR2 signal promotes invasion and migration in human gastric cancer through upregulation of thrombospondin-1. *Int J Oncol.* 2017;50:1501–12.
24. Carter E, Miron-Buchacra G, Goldoni S, Danahay H, Westwick J, Watson ML, Tosh D, Ward SG. (2014) Phosphoinositide 3-kinase alpha-dependent regulation of branching morphogenesis in murine embryonic lung: evidence for a role in determining morphogenic properties of FGF7. *PloS one* 9:e113555.
25. Li J, Zhang W, Zhu S, Shi F. Nitric Oxide Synthase Is Involved in Follicular Development via the PI3K/AKT/FoxO3a Pathway in Neonatal and Immature Rats. *Animals (Basel).* 2020;10:E248.
26. Liu D, Yang Y, Yan A, Yang Y. SPOCD1 accelerates ovarian cancer progression and inhibits cell apoptosis via the PI3K/AKT pathway. *Onco Targets Ther.* 2020;13:351–9.
27. Yin ZF, Wei YL, Wang X, Wang LN, Li X. Buyang Huanwu Tang inhibits cellular epithelial-to-mesenchymal transition by inhibiting TGF- β 1 activation of PI3K/Akt signaling pathway in pulmonary fibrosis model in vitro. *BMC Complement Med Ther.* 2020;20:13.
28. Chong E, Chang SL, Hsiao YW, Singhal R, Liu SH, Leha T, Lin WY, Hsu CP, Chen YC, Chen YJ, et al (2015) Resveratrol, a red wine antioxidant, reduces atrial fibrillation susceptibility in the failing heart by PI3K/AKT/eNOS signaling pathway activation. *Heart rhythm* 12:1046–1056.
29. Singhal R, Chang SL, Chong E, Hsiao YW, Liu SH, Tsai YN, Hsu CP, Lin YJ, Lo LW, Ha TL. Colchicine suppresses atrial fibrillation in failing heart. *Int J Cardiol.* 2014;176:651–60.
30. Liu SH, Hsiao YW, Chong E, Singhal R, Fong MC, Tsai YN, Hsu CP, Chen YC, Chen YJ, Chiou CW. Rhodiola Inhibits Atrial Arrhythmogenesis in a Heart Failure Model. *J Cardiovasc Electrophysiol.* 2016;27:1093–101.
31. Zhao J, Liu E, Li G, Qi L, Li J, Yang W. Effects of the angiotensin-(1–7)/Mas/PI3K/Akt/nitric oxide axis and the possible role of atrial natriuretic peptide in an acute atrial tachycardia canine model. *J Renin Angiotensin Aldosterone Syst.* 2015;16:1069–77.

Tables

Table 1
Screening upregulated and downregulated DEGs in atrial fibrillation

DEGs	Gene symbol
Upregulated(41)	SLC6A5 PCDHB3 AGPAT5 RPL3L PRL F2R RAPGEF6 DHRS9 IGFBP2 SPP1 THBS4 ANGPT2 LRRC2 COMP NPPB SHISA3 KIAA0368 REC114 DAPK1-IT1 USP34 LGR6 NRXN1 PRO2852 SNRPA1 TDRD6 LRRC16A DMXL1 CLSTN2 LINC00844 SYT13 IFNGR1 SLC20A1 AQP4 WDR11 NAMPT EGR1 LOC101930415 AGO2 INHBA NME7 PPP1CB
Downregulated(33)	PWAR5 ZC2HC1A DLEU2 MYBPC1 FGF7 MEIS3P1 SLC7A11 HPR GPR22 CYBRD1 ASTN2 CREB1 PCYOX1 ZBTB38 PPP1R1A PLXDC2 LYVE1 ALDH1A1 TNNI1 LRRC49 MCOLN3 MYLK ZNF148 MARCO DDR2 SLIT2 BMP10 TCEAL2 ATP1B4 DHRS9 LRRC2 CPLX1 LINC00702
Abbreviation: DEGs, differentially expressed genes	

Table 2
GO enrichment analysis of differential expressed genes

Term	Description	Count	p-value
GO:0005887	integral component of plasma membrane	14	5.80E-04
GO:0007155	cell adhesion	8	9.82E-04
GO:0005179	hormone activity	4	0.004134612
GO:0005576	extracellular region	13	0.005597677
GO:0007165	signal transduction	11	0.005974065
GO:0030308	negative regulation of cell growth	4	0.008442164
GO:0032870	cellular response to hormone stimulus	3	0.010553474
GO:0005509	calcium ion binding	8	0.011095583
GO:0005515	protein binding	40	0.013590848
GO:0030139	endocytic vesicle	3	0.013667389
GO:0071504	cellular response to heparin	2	0.017153266
GO:0008201	heparin binding	4	0.017625624
GO:0008083	growth factor activity	4	0.018211591
GO:0016525	negative regulation of angiogenesis	3	0.019418564
GO:0009611	response to wounding	3	0.020011285
GO:0005125	cytokine activity	4	0.02261623
GO:0005615	extracellular space	10	0.031210257
GO:0006810	transport	5	0.032042957
GO:0045893	positive regulation of transcription, DNA-templated	6	0.032211451
GO:0009986	cell surface	6	0.032683683
GO:0005102	receptor binding	5	0.03296048
GO:0004888	signaling receptor activity	4	0.037244056
GO:0007565	female pregnancy	3	0.037907457
GO:0046872	metal ion binding	13	0.04622821

Table 3
KEGG pathway analysis of DEGs.

Pathway	ID	Count	p-value	Genes
PI3K-Akt signaling pathway	hsa04151	8	6.94E-04	FGF7, CREB1, COMP, ANGPT2, PRL, SPP1, THBS4, F2R
cGMP-PKG signaling pathway	hsa04022	5	0.005199276	CREB1, ATP1B4, NPPB, PPP1CB, MYLK
Focal adhesion	hsa04510	5	0.013042299	COMP, PPP1CB, MYLK, SPP1, THBS4
Adrenergic signaling in cardiomyocytes	hsa04261	4	0.023568463	CREB1, PPP1R1A, ATP1B4, PPP1CB

Table 4
Hub genes based on cytoHubba

Projects	Methods in cytoHubba		
	MCC	DMNC	MNC
Gene symbol top 10	EGR1	TCEAL2	EGR1
	SPP1	SYT13	SPP1
	PRL	CPLX1	AQP4
	FGF7	AGO2	CREB1
	IGFBP2	NPPB	PRL
	CREB1	INHBA	MYLK
	ANGPT2	IGFBP2	ANGPT2
	MYLK	SLIT2	FGF7
	AQP4	FGF7	NRXN1
	NPPB	NAMPT	IGFBP2
Bold gene symbols were the overlap hub gene. Abbreviation: MCC, Maximal Clique Centrality; DMNC, Density of Maximum Neighborhood Component; MNC, Maximum Neighborhood Component.			

Figures

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Supplementary Files

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