Hub Targets Analysis of miRNA-mRNA-TF Network in Diabetic Cardiomyopathy

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Research Article

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ABSTRACT

Background: The pathogenic mechanism and development of the diabetic cardiomyopathy(DCM) has been generally explained, and it is clear that the microRNAs(miRNAs), mRNAs and transcription factors(TFs) participate in the process of the DCM disease. Yet, the hub targets of the disease progression are not clear. **Methods**: To figure out the problem, we downloaded data sets from the Gene Expression Omnibus(GEO) database (GSE44179 and GSE4745). The targeted mRNAs of miRNAs were downloaded from TargetScan, miRBD and microT-CDS database. Gene Ontology (GO) enrichment of miRNAs and mRNAs were analysed in DAVID.R studio software was used to visualize the results of screened targets and GO enrichment. Cytoscape software was used to visualize the miRNA-mRNA-TF interaction network and calculate the hub targets. **Results**: We filtered eight miRNAs, nine mRNAs and ten transcription factors(TFs) by bioinformatics analysis, and constructed a miRNA-mRNA-TF network. The top ten degrees of nodes in the

network are rno-miR-7a, Hnf4a, rno-miR-17, rno-miR-21, rno-miR-122, rno-miR-200c, Med1, Mlxipl, SP1 and rno-miR-34a, which were closely related to the process of DCM. **Conclusion**: This study revealed that rno-miR-7a, Hnf4a, rno-miR-17and rno-miR-21 may play vital role in the progress of diabetic cardiomyopathy.

Introduction

Diabetic cardiomyopathy(DCM) is a specific form of heart disease, induced by insulin resistance in heart tissue, hyperinsulinemia and hyperglycaemia, which are independent of other cardiac risk factors, including coronary artery disease and hypertension. These metabolic disturbances promote cardiac remodeling, fibrotic diastolic dysfunction and decreased ejection fraction in the DCM patients[1].The pathophysiology changes of diabetic cardiomyopathy were well explained, which contained cardiac hypertrophy, fibrosis and cardiac functional changes such as systolic and diastolic dysfunction[2].Recent evidences indicated that several microRNAs (miRNA) played critical role in the pathogenesis of DCM, and contributed to regulating genes related to cardiomyocyte hypertrophy, oxidative stress, cardiac fibrosis and apoptosis[3].Certain mRNAs and TFs were proved contribute to the pathology of DCM, as well[4].Whereas, the regulation relationship among miRNAs, mRNAs and TFs, and the hub targets of them are not clear in the DCM, yet. In order to figure out the problem, in this study, we focused on microRNAs and discussed the regulation relationship of miRNA-mRNA-TF network in the development of DCM and screened the hub nodes of the network, which might to be predictive and diagnostic biomarkers, and potential therapeutic targets.

Methods

Data source and screening

Data sets of microRNAs and mRNAs were downloaded from Gene Expression Omnibus(GEO)database(https://www.ncbi.nlm.nih.gov/geo). The miRNA expression profile data GSE44179 performed on Plat-form GPL14613 containing ventricle myocardium tissue from two healthy and four diabetic cardiomyopathy rattus

norvegicus models. The mRNA expression profile data were a part of GSE4745, which performed on Plat-form GPL85 and contained ventricle myocardium tissue from four healthy and four diabetic cardiomyopathy rattus norvegicus models after 42 days feeding. We got the differentially expressed miRNAs and mRNAs on GEO by analysis with GEO2R.We filtered the specific expression sequences by volcano plot and heat map of miRNAs and mRNAs in R studio (version 1.4.1717).The threshold value of log FC is >1.5 or <-1.5 and $p<0.05$.

GO enrichment analysis of miRNAs

We got Gene Ontology(GO)enrichment analysis result of miRNAs in DAVID(https://david.ncifcrf.gov) online, including molecular function (MF), Cellular component(CC) and Biological process(BP).We exhibited the GO enrichment analysis result by Pie Chart in R studio.

Prediction of the targeted mRNAs of miRNAs

The data sets of mRNA and miRNA which we obtained in GEO were from different samples, therefore, the realistic regulation relationship were unknown. Hence, we obtained the targeted mRNAs of miRNAs in three databases, including TargetScan(http://www.targetscan.org/vert_72), miRBD(http://mirdb.org)and microT-CDS(http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=microT_CDS/index).Then,we took the intersection of these three data and mRNAs we screened before to predict the targeted mRNAs of miRNAs. In the end, we got GO enrichment analysis result of targeted mRNAs in DAVID and exhibited the results by bubble chart and Chord chart of mRNAs of BP, CC and MF pathways in R studio.

Construction of miRNA-mRNA-TF network and GO enrichment analysis of mRNAs

We predicted the targeted TFs of miRNAs in TransmiR (v2.0) database(http://www.cuilab.cn/transmir). Then,we constructed the miRNA-mRNA-TF

network in Cytoscape (Version: 3.8.2),and we got the top 10 degrees notes by CytoHubba plug-in of Cytoscape. In the end, we got GO enrichment analysis result of mRNAs of network in DAVID and exhibited the results by bubble chart and Chord chart of mRNAs of BP, CC and MF pathways in R studio.

Statistical Analysis

The significant differences between the two groups were analyzed by Student's t-test. The value of $P<0.05$ or adjusted P value<0.05 was considered to be significant.

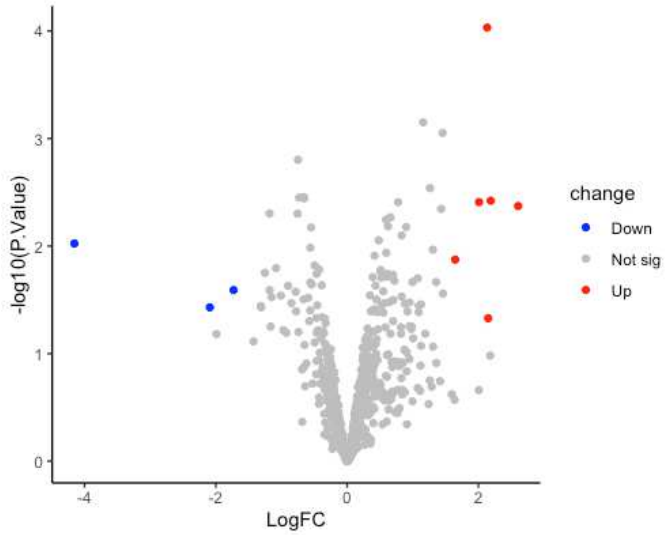
Results

The specific expressing miRNAs in DCM

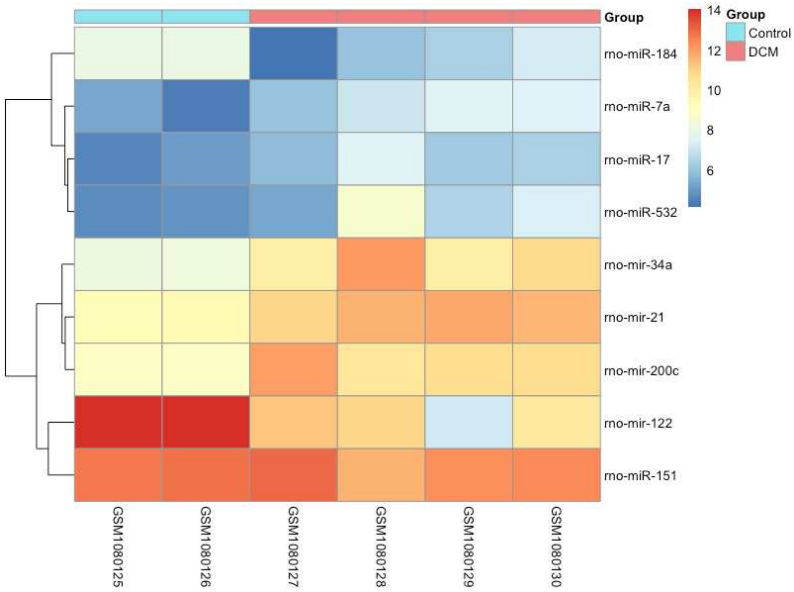
In volcano map, the cutoff value of log FC is ±1.5 and we screened nine specific miRNAs, including six upregulated miRNAs: rno-miR-21, rno-miR-7a, rno-miR-200c, rno-miR-34a, rno-miR-17 and rno-miR-532, and three downregulated miRNAs: rno-miR-122, rno-miR-151 and rno-miR-184(Figure1a). The heat map described the different expression between two groups(Figure1b). The specific expressing miRNAs are presented in table 1.

Table1: Special expressed miRNAs in DCM

Name	Log FC	P value	adj. P Value
rno-miR-21	2.133493	0.000093	0.0725
rno-miR-7a	2.189288	0.003785	0.2598
rno-miR-200c	2.009562	0.003896	0.2598
rno-miR-34a	2.60612	0.004241	0.2598
rno-miR-122	-4.154608	0.009463	0.3209
rno-miR-17	1.646546	0.013344	0.3582
rno-miR-151	-1.729582	0.025623	0.3764
rno-miR-184	-2.090834	0.037079	0.3764
rno-miR-532	2.148635	0.046863	0.4098



(a)



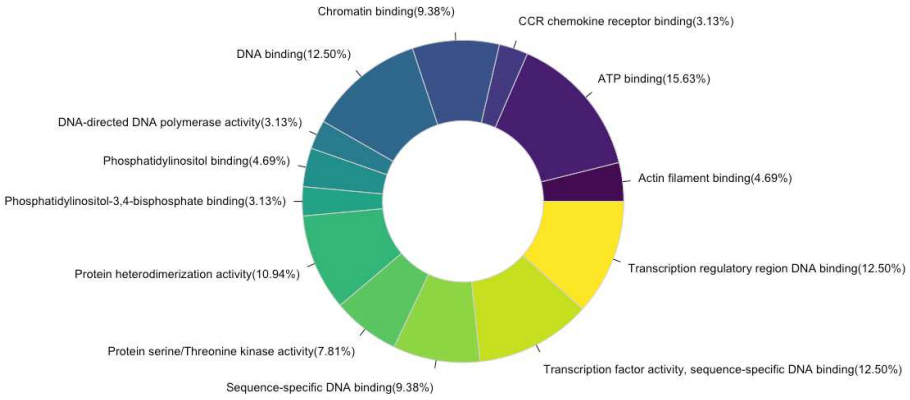
(b)

Figure1:(a) Volcano map of miRNAs. Red represent upregulated expression and blue represent downregulated expression of mRNAs. (b)Heat map of miRNAs. Red color: high expression, blue color: low expression of mRNAs.

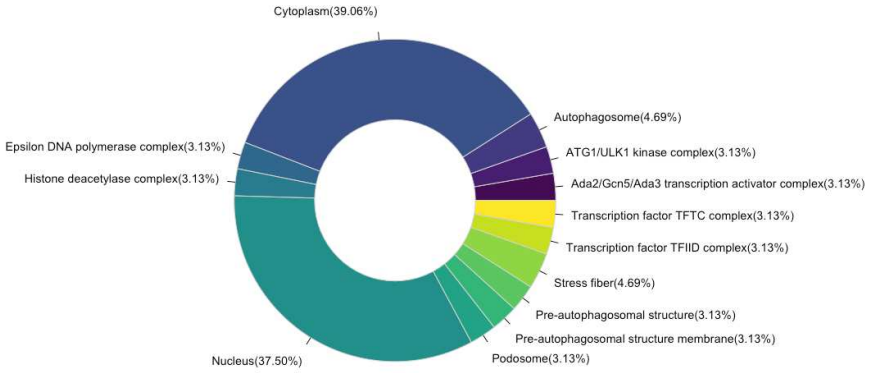
GO Enrichment Analysis of miRNAs

According to the molecular function (MF) terms by GO enrichment analysis, most of the miRNAs are related to ATP binding, DNA binding, transcription regulatory region DNA binding, transcription factor activity and sequence-specific DNA

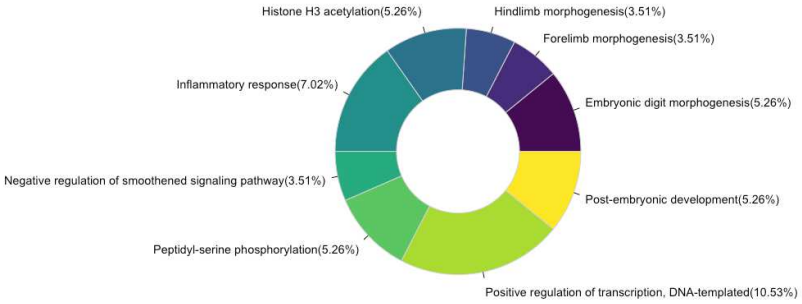
binding(Figure2a).In Cellular component(CC)terms, most of the miRNAs are related to cytoplasm and nucleus(Figure2b).In biological process(BP)terms, most of the miRNAs are related to positive regulation of transcription, DNA-templated and inflammatory response(Figure2c).



(a)Molecular function terms for miRNAs



(b)Cellular component terms for miRNAs



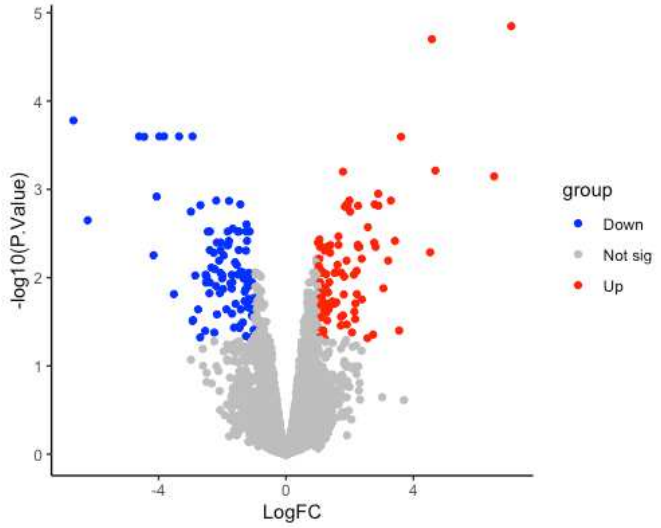
(c)Biological process terms for miRNAs

Figure2: GO enrichment analysis of miRNAs

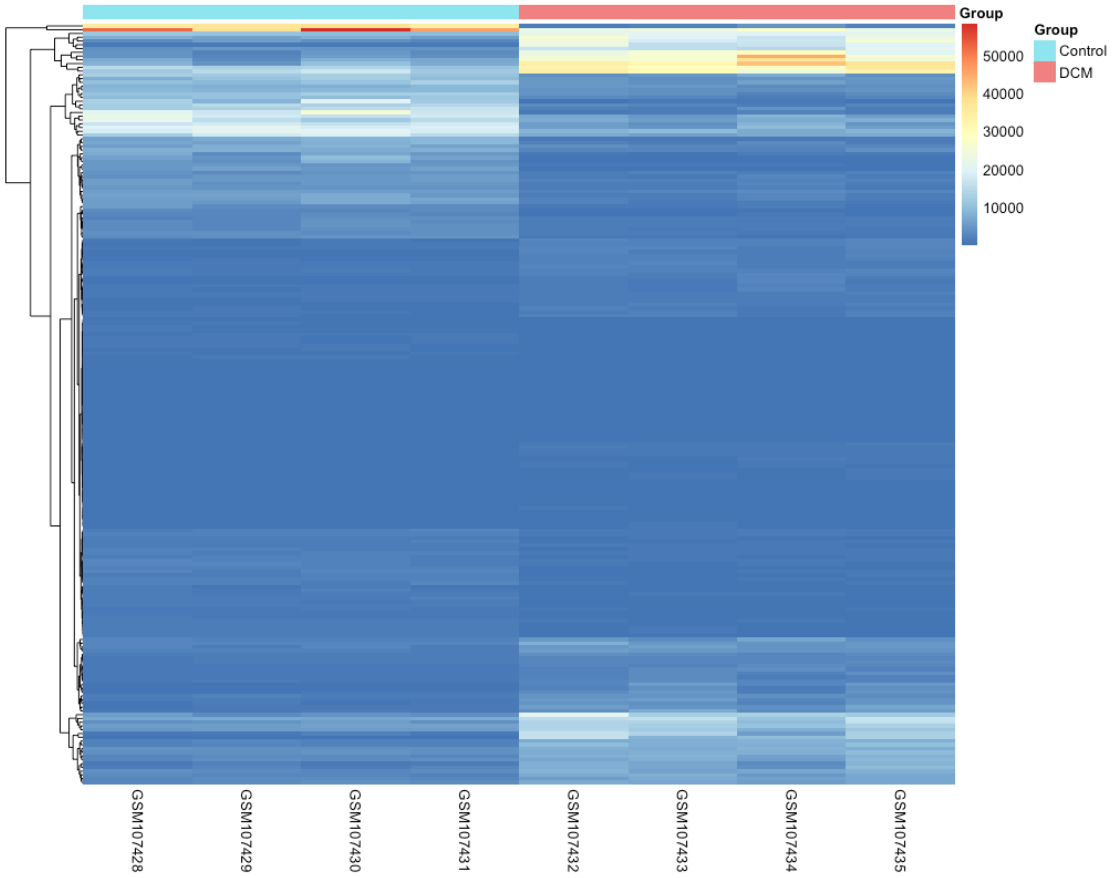
Different colors represent different term , the size of block represent the percentage.

The specific expressed mRNAs of DCM.

In volcano map, the cutoff value is ±1.5. We screened upregulated and downregulated expressing mRNAs(Figure3a). The heat map described the specific expressing mRNAs between two groups(Figure3b).



(a)



(b)

Figure3:(a) Volcano map of mRNAs. Red represents upregulated expressing mRNAs and blue represents downregulated expressing mRNAs. (b) Heat map of mRNAs. Red color: high expression, blue color: low expression.

Table2: Specific expressing mRNAs in DCM

mRNAs	Log FC	P value	adj. P Value
ACOT2	1.9339079	4.13E-06	0.0015127
SP1	1.1406845	1.33E-03	0.0396842
CYP26B1	3.29285	2.46E-06	0.0013413
TXNIP	1.6172858	6.44E-05	0.0071689
NR4A3	-2.5382219	1.36E-03	0.0401152
IGFBP3	1.9008582	5.48E-05	0.006414
COL1A1	-1.7432552	1.78E-04	0.0116197
DCLK1	-2.1640012	6.96E-04	0.0260704

miRNA-mRNA-TF Network in DCM

We obtained the targeted mRNAs of miRNAs in Targetscan, miRBD and microT-CDS databases on line. We took intersection of these three sets and miRNAs we screened previously(Table2). We searched targeted TFs of miRNAs in TransmiR(v2.0)and built network of miRNAs, mRNAs and TFs in Cytoscape(version 3.8.2)(Figure4).The network describes the relationship among miRNAs, mRNAs and TFs. The network links these factors and different expressing level sequences play different roles in the network. Top 10 degrees nodes of the network were screened by CytoHubba plug-in of Cytoscape, describing the specific expressing genes(Table3), including rno-miR-7a, Hnf4a, rno-miR-17, rno-miR-21, rno-miR-122, rno-miR-200c, Med1, Mlxipl, SP1 and rno-miR-34a. Top 3 miRNAs have the most degrees of nodes, including rno-miR-7a,rno-miR-17 and rno-miR-21,which are considered as critical roles in DCM.Hnf4a is the targeted TF of rno-miR-21,rno-miR-184,rno-miR-122, rno-miR-17,rno-miR-200c and rno-miR-7a.Med1 is the targeted TF of rno-miR-7a and rno-miR-200c.Mlxipl is the targeted TF of rno-miR-7a and rno-miR-200c.SP1 is the targeted mRNA of rno-miR-7a and rno-miR-34a.The pathways of these nodes played critical roles in DCM.

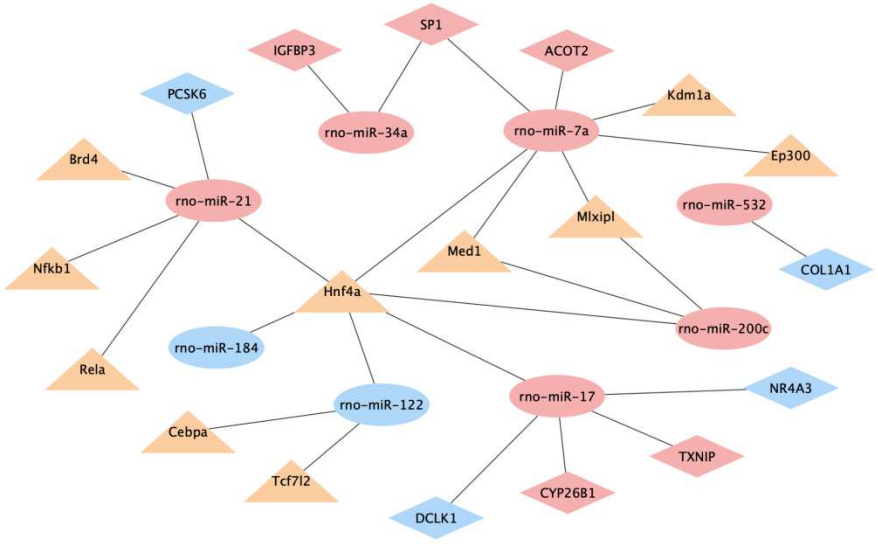


Figure4: miRNA-mRNA-TF Network in DCM

Ovals represent miRNAs, rhombus represent mRNAs and triangles represent TFs. Red

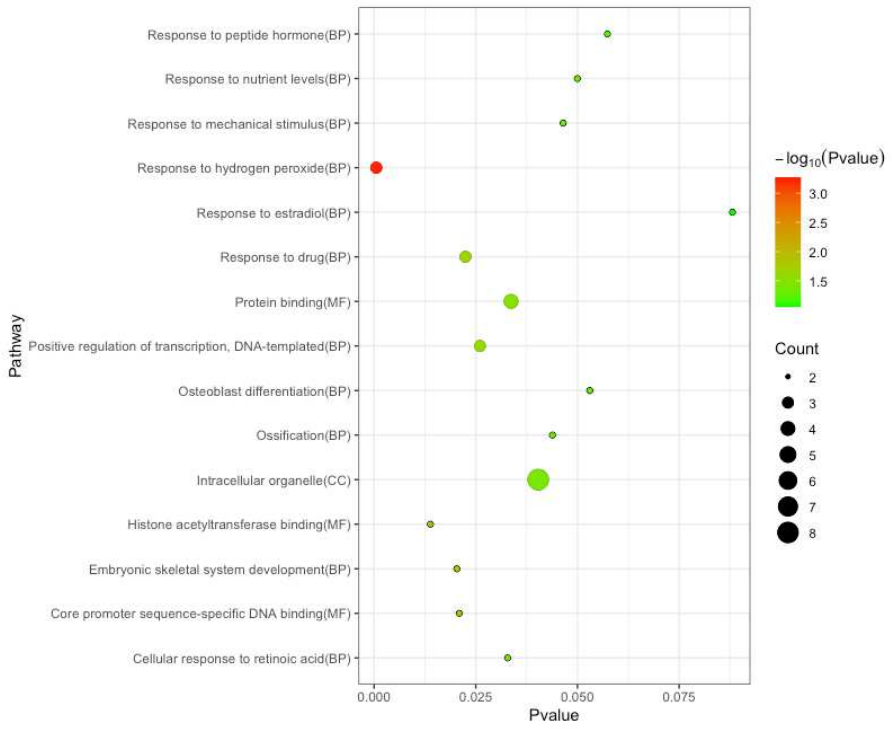
represent upregulated expression and blue represent downregulated.

Table3: Top 10 nodes in the TF-miRNA-mRNA network

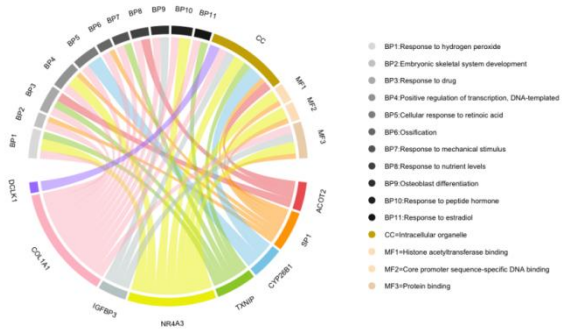
name	degree	closeness	betweenness
rno-miR-7a	7	13.66667	271
Hnf4a	6	14.58333	420.66667
rno-miR-17	5	11.95	172
rno-miR-21	5	11.95	172
rno-miR-122	3	10.61667	90
rno-miR-200c	3	10.61667	31
Med1	2	9.41667	4.66667
Mlxipl	2	9.41667	4.66667
SP1	2	9.66667	88
rno-miR-34a	2	7.75	46

GO Enrichment Analysis of mRNAs

We analysed GO enrichment of the eight mRNAs we identified previously in DAVID on line, except the mRNA PCSK6 on account of that we did not find the relevant pathways in DAVID. The bubble chart described the p value of BP,CC and MF pathways, and the size of the nodes represents the amounts of mRNAs involved in the pathways(Figure5a).The chord chart describes the relationship among miRNAs and pathways of BP,CC and MF(Figure5b).In BP pathways, most involved pathways were response to hydrogen peroxide, response to drug and positive regulation of transcription, DNA-templated. In addition, the mRNA COL1A1 was related to every BP pathway. The CC pathway, intracellular organelle, was related to all these eight mRNAs we screened. In MF pathways, the most involved pathway was protein binding. Furthermore, the mRNAs NR4A3 and SP1 were involved in every MF pathway we analysed.



(a) Bubble chart of GO Enrichment of mRNAs



(b) Chord chart of mRNAs in BP pathways, CC pathways and MF pathways

Figure5: GO Enrichment of mRNAs

(a)Bubble chart of GO Enrichment of mRNAs. The size of the circle represents amounts of the mRNAs. (b)Chord chart of mRNAs in BP pathways, CC pathways and MF pathways. The legend on the right illustrate the name of pathways and mRNAs.

Discussion

Cardiovascular complications are the major cause of mortality and morbidity in diabetic patients. Ischaemic heart disease are primary in cardiovascular complications, however, the risk of heart failure also increases with or without myocardial ischaemia and hypertension. Cardiac hypertrophy is a salient feature of the diabetic

myocardium. The metabolic milieu associated with diabetes, such as hyperglycaemia, increased circulating fatty acids and triacylglycerols, hyperinsulinaemia, increased inflammatory cytokines, alter multiple molecular pathways within the cardiomyocyte, and then impair cardiac contractility and promote myocyte dysfunction, injury and cell death.

It proved that several critical miRNAs were associated with the pathogenesis of diabetic cardiomyopathy. The miRNAs regulated genes expression by repressing translation or promoting degradation of target mRNAs. Recent reports demonstrated that several miRNAs were involved in the pathogenesis of insulin resistance and type 2 diabetes, such as miR-143, miR-181, miR-103, miR-107 and miR-802.A change in myocardial miRNA content is relative to changes in cardiac function, such as miRNA-133[5].The miRNA-133 expression of myocardium was increased in the rabbit model of type 1 diabetes, and miRNA-133 regulated CTGF expression and modulated connective tissue content, which demonstrated that miRNA-133 is involved in fibrosis induction in DCM[6].

A research found that several specific mRNAs were differentially regulated in DCM, and most of the coding transcripts were associated with processes, such as inflammation, structural reorganization, metabolism, smooth muscle contraction, focal adhesion and repair contributing, which contribute to the development of DCM[7].

Several transcription factors(TFs) were involved in the changes of cardiac function, such as the mediator subunit 1(Med1). In mice models, Med1 deletion of cardiac resulted in changes of cardiac function, including left ventricular dilation, decreased ejection fraction, and pathological structural remodeling. Whereas, upregulation of Med1 occurs in both human and mice failing hearts, Kathryn M Spitler inferred that increased Med1 expression in failing hearts is a compensatory response[8].

The specific expression of sequences about mRNAs, miRNAs and TFs, which involved in the pathogenesis of DCM were explained broadly. However, the regulation relationship among these sequences and the hub targets are not clear, so, we started this study to figure out the problem.

In our study, we screened miRNAs and mRNAs, and searched targeted mRNAs and targeted TFs of miRNAs in DCM. We built a miRNA-mRNA-TF network with the sequences we filtered. The top 10 degrees of the network were considered as crucial roles in DCM, including rno-miR-7a, Hnf4a, rno-miR-17, rno-miR-21, rno-miR-122, rno-miR-200c, Med1, Mlxipl, SP1 and rno-miR-34a.

The top one expressed sequence was rno-miR-7a.MicroRNA miR-7,an evolutionarily ancient miRNA, played a crucial role in disease process of heart, brain, endocrine pancreas, skin and cancer, in human and mice[9].Several researches demonstrated that miR-7,the most sensitive regulator, was over expressed in both myocardial infarction and stage heart failure mouse models, which implies that it involved in pathology of myocardial infarction and stage heart failure[10].Evidence demonstrated that miR-7 was downregulated in end-stage dilated cardiomyopathy and they also showed that over expression of miR-7 reduced expression of ERBB2,which was critical for prevention of dilated cardiomyopathy[11].Moreover,miR-7 also showed highly conserved expression in insulin-producing cells of the animal kingdom. In mouse β-cells, miR-7 inhibited glucose-stimulated insulin secretion[12].

Over expressing miR-17 resulted in decreasing cell adhesion, migration and proliferation. Several evidence proved that the expression of miR-17 and fibronectin were negative correlation, so that miR-17 caused cellular defects for its repression of fibronectin expression. In the heart of upregulated miR-17 mice model, the expression of fibronectin were lower and the spaces between the papillary muscles were smaller, compared to wild-type mice models[13].Moreover,miR-17 promoted cardiomyocyte hypertrophy, proliferation, and survival. Evidence proved that miR-17 contributes to

exercise-induced cardiac growth and prevented ventricular remodeling, including attenuating cardiac apoptosis, decreasing fibrosis, and preserving cardiac function[14].Expression of miR-17 also suppressed mouse cardiac senescence[15].

In mammal organ systems, miR-21 is universally expressed, such as the heart. Cardiac hypertrophy is a common pathological response to cardiovascular diseases, including endocrine disorder, hypertension, ischemic heart disease and vascular disease. It proved that miR-21 was significantly upregulated in hypertrophic animal hearts. Cardiac fibrosis is a pathological feature of cardiac hypertrophy and heart failure,while,miR-21 was proved that significantly upregulated in cardiac fibroblasts[16].There was also strong evidence for the role of miR-21 in cardiac fibrosis[17].Transfection of primary cardiac fibroblasts with a synthetic miR-21 precursor gave rise to an increase in fibroblast growth factor 2 (FGF2) secretion. Concretely speaking, genes of encoding collagens and extracellular matrix molecules were highly upregulated in cardiac fibrosis, but they were reduced after specific inhibition of miR-21[18].

It proposed that miR-122 was a new biomarker to assess subclinical myocardial dysfunction, development of interstitial myocardial fibrosis, and the early stage of diabetic cardiomyopathy evolving toward heart failure. It proved that the progress of DCM was closely bound up with the development of interstitial myocardial fibrosis, which was triggered by the increase of miR-122.The miR-122 targeted the extracellular matrix and hence stiffening the myocardium[19].In the serum, the expression of miR-122 was relevant with whole body insulin insensitivity, body weight and triglyceride. The miR-122 was also considered as a key regulator of lipid metabolism. Inhibition of miR-122 by antagomir led to a significant decrease of triglyceride and fat accumulation in mice[20].But, downregulated expression of miR-122 also observed in HNF1A variant-induced diabetes, for the expression of miR-122 was regulated by transcription factors HNF1A[21].MicroRNA miR-122 was the most abundant liver miRNA with exquisite tissue specificity, and it was vital in the

maintenance of lipid and glucose homeostasis[22].Evidence proved that miR-122 knockdown promoted cell viability and inhibited apoptosis, on the contrary,miR-122 over expression suppressed viability and promoted apoptosis of cardiomyocyte in the myocardial cell of mice[23].The microRNA miR-122 we screened was downregulated myocardium of DCM rat model, however, most of the research studied the miR-122 in the serum. Based on the above evidences, we inferred that the downregulated miR-122 in myocardium of DCM mainly promoted cell viability and inhibited apoptosis, and decrease of triglyceride and fat accumulation.

Evidence proved that the expression of miR-200c increased in cardiomyocyte of DCM model rats. It suggested that miR-200c played a pro-hypertrophic role in high glucose induced cardiac hypertrophy through regulation of DUSP-1 and MAPK signaling pathway[24].

In a DCM rat model, the expression of miR-34a was upregulated.While,miR-34a mimic induced H9c2 cell apoptosis in HG condition[25]. Evidence also demonstrated that miR-34a reduced type I collagen production, cell viability, and migration and increased apoptosis of CFs by targeting Pin-1 signaling, so that it could attenuate myocardial fibrosis[26].

Ferroptosis is a form of regulated cell death, which is characterized by an excessive degree of iron accumulation and lipid peroxidation. Liver-enriched transcription factor Hnf4a suppresses ferroptosis via modulation of the expression of potential anti-ferroptosis regulators or pro-ferroptosis regulators[27].Moreover,Hnf4a is the main regulator of glucose stimulated insulin secretion in pancreas[28].

Mediator (Med), an evolutionarily conserved protein complex, mediates distinct protein-protein interactions. It demonstrated that Med1 involved in modifying glucose and lipid metabolism, and adipocyte differentiation[29].Med1 is also dynamically expressed in cardiac development and disease, with prominent upregulation of Med1

in both human and murine failing hearts. In cardiac-specific Med1 knockout mouse models, it observed changes in cardiac function, including pathological structural remodeling, left ventricular dilation and decreased ejection fraction[8].

It demonstrated that cells coordinate lipid storage with metabolic gene regulation by lipid droplet binding of the MLX family of glucose-sensing transcription factors, such as MLXIPL. In this process, it proposed that the bond of MLXIPL and accumulating LDs restrict glucose-stimulated gene transcription[30].

It reported that SP1 directly regulated a number of cardiac genes, including ANF, connexin40, sarcoplasmic reticulum Ca^{2+}ATPase (SERCA), cardiac α-actin and cardiac troponin T (cTnT), and so on. Upregulation of SP1 was observed in cardiac hypertrophy rat model, while, it proved that SP1 participated in process of cardiac hypertrophy[31].

Generally speaking, the top degrees of the network are rno-miR-7a, HNF4A, rn o-miR-17 and rno-miR-21, while,these three miRNAs are all upregulated in DCM rat model. MicroRNA rno-miR-7a mainly take part in the pathology of myocardial infarction and heart failure, and inhibiting glucose-stimulated insulin secretion. Upregulated microRNA miR-17 suppresses the repression of fibronect in and promoting cardiomyocyte hypertrophy, proliferation, and survival. Micro RNA miR-21 promoted the myocardial fibrosis and myocardial hypertrophy. Li ver-enriched transcription factor HNF4A was also the most specific transcriptio n factor of the TFs we screened. HNF4A mainly involved in ferroptosis and r egulator of glucose stimulated insulin secretion in pancreas. We screened the vi tal genes and worked the network and function of them in DCM by reference from researches before, however, there are some imperfections in our study. A ctually, the relationship among the genes, expression quantity and the function in DCM of rat model or human are not clear. So, we need more experiments to verify the results we worked in this study.

Conclusion

In our study, we built a miRNA-mRNA-TF network of DCM. In this network, top 10 degrees genes are rno-miR-7a, Hnf4a, rno-miR-17, rno-miR-21, rno-miR-122, rno-miR-200c, Med1, Mlxipl, SP1 and rno-miR-34a.To be more specific, rno-miR-7a, HNF4A, rno-miR-17 and rno-miR-21 play even more important roles among them. In brief, these specific genes play vital role in DCM, and they may provide innovations for the diagnosis and therapy of DCM.

Author contributions statement

Conception: D.G. and B.R.; Methodology: D.G., B.R. and K.H.; Analysis: B.R., K.H., and Y.T.; Writing: B.C. and K.H.; Supervision: D.G., Y.W. and M.Y.

Competing interests

The authors declare no competing interests.

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Figures

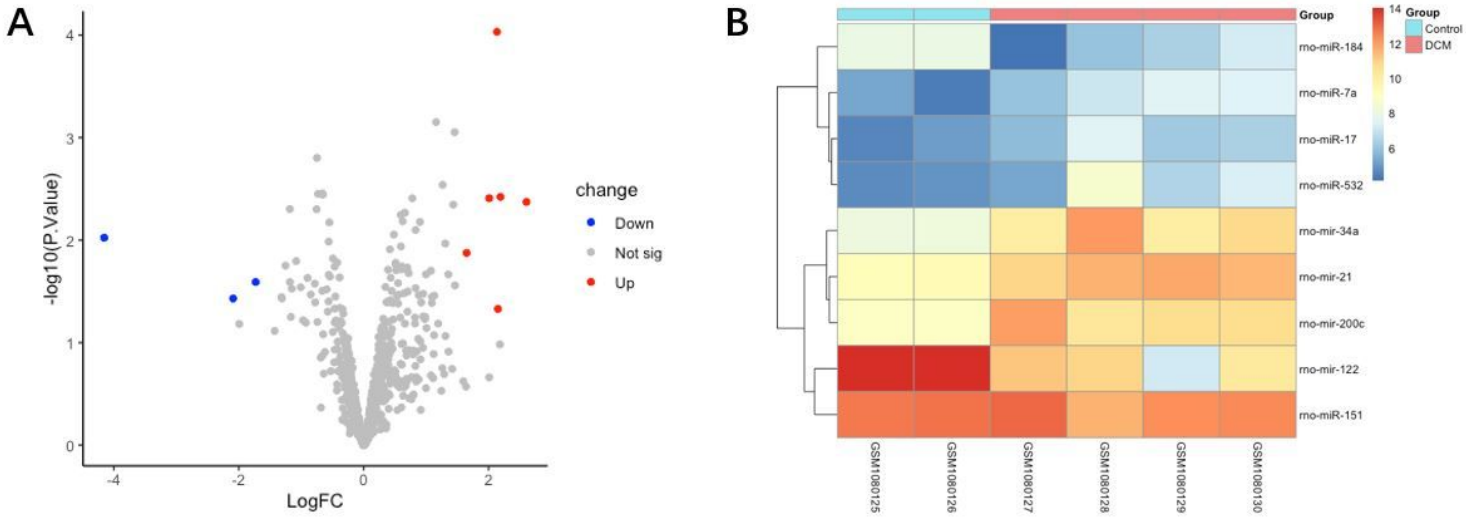

Figure 1

(a) Volcano map of miRNAs. Red represent upregulated expression and blue represent downregulated expression of mRNAs. (b)Heat map of miRNAs. Red color: high expression, blue color: low expression of mRNAs.

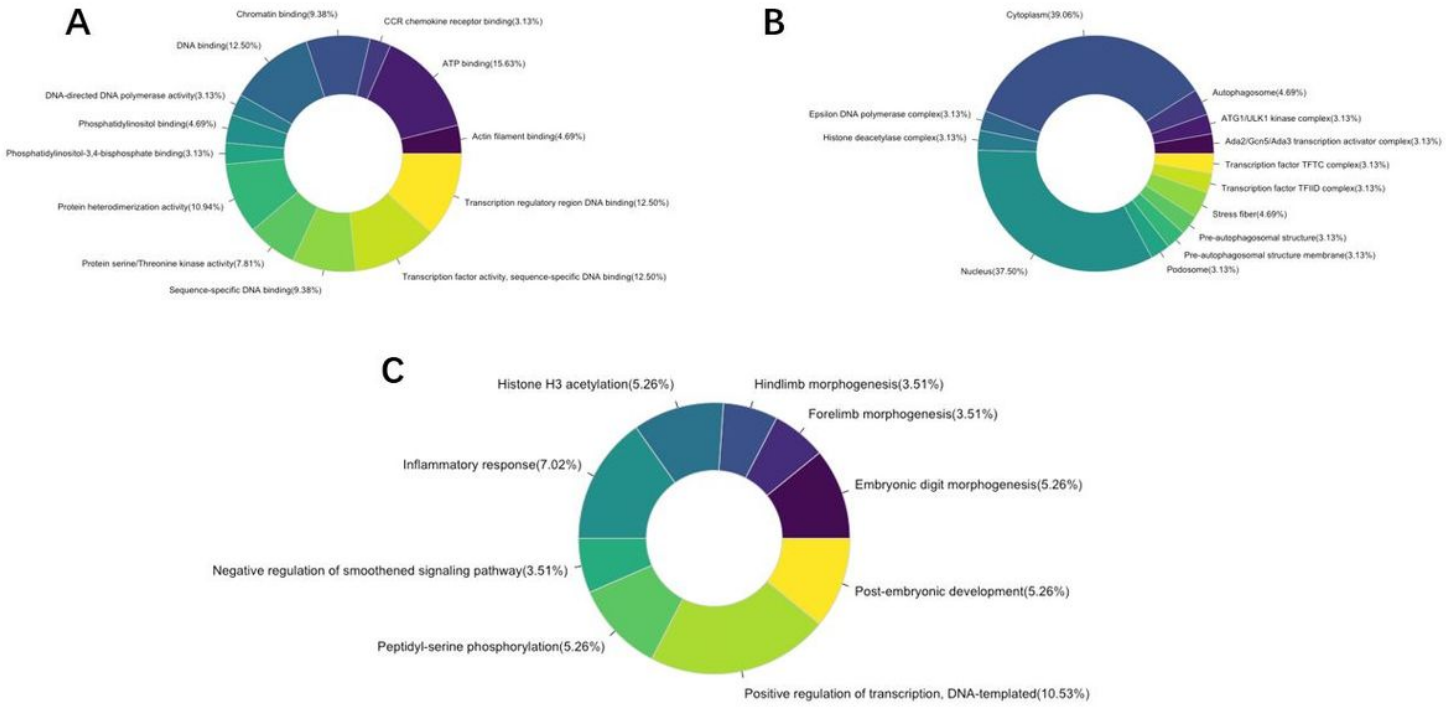

Figure 2

GO enrichment analysis of miRNAs Different colors represent different term , the size of block represent the percentage.

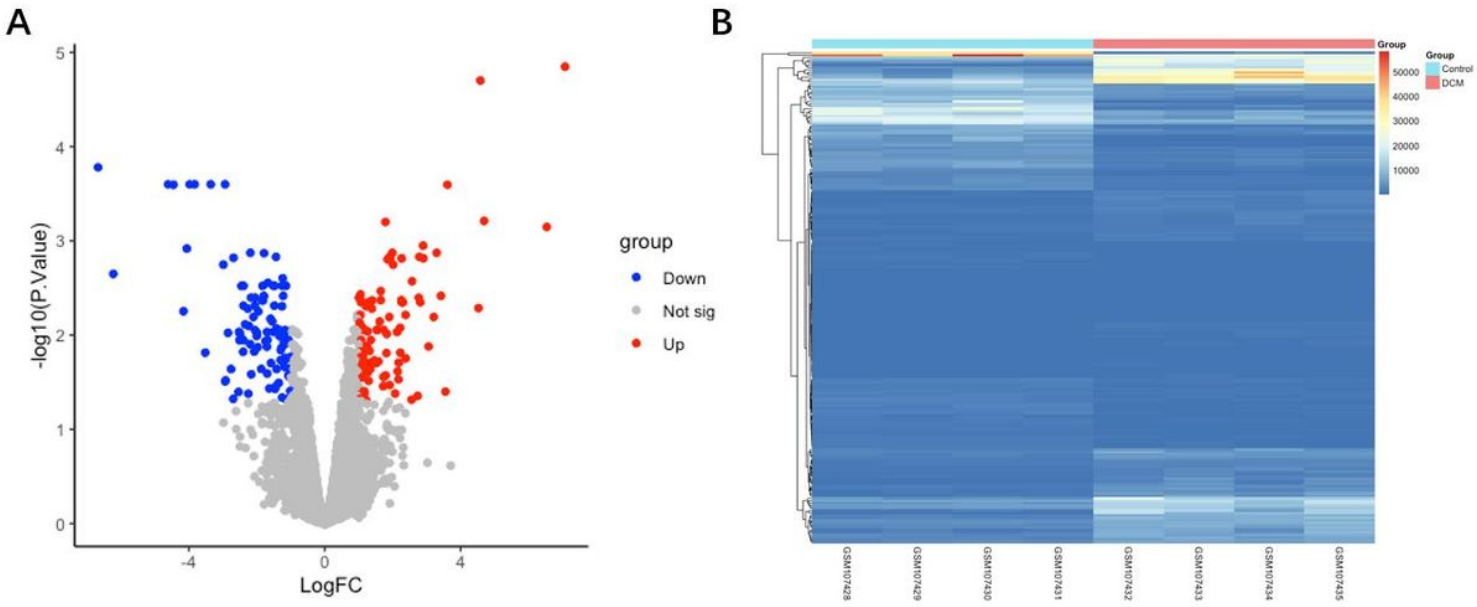

Figure 3

(a) Volcano map of mRNAs. Red represents upregulated expressing mRNAs and blue represents downregulated expressing mRNAs. (b) Heat map of mRNAs. Red color: high expression, blue color: low expression.

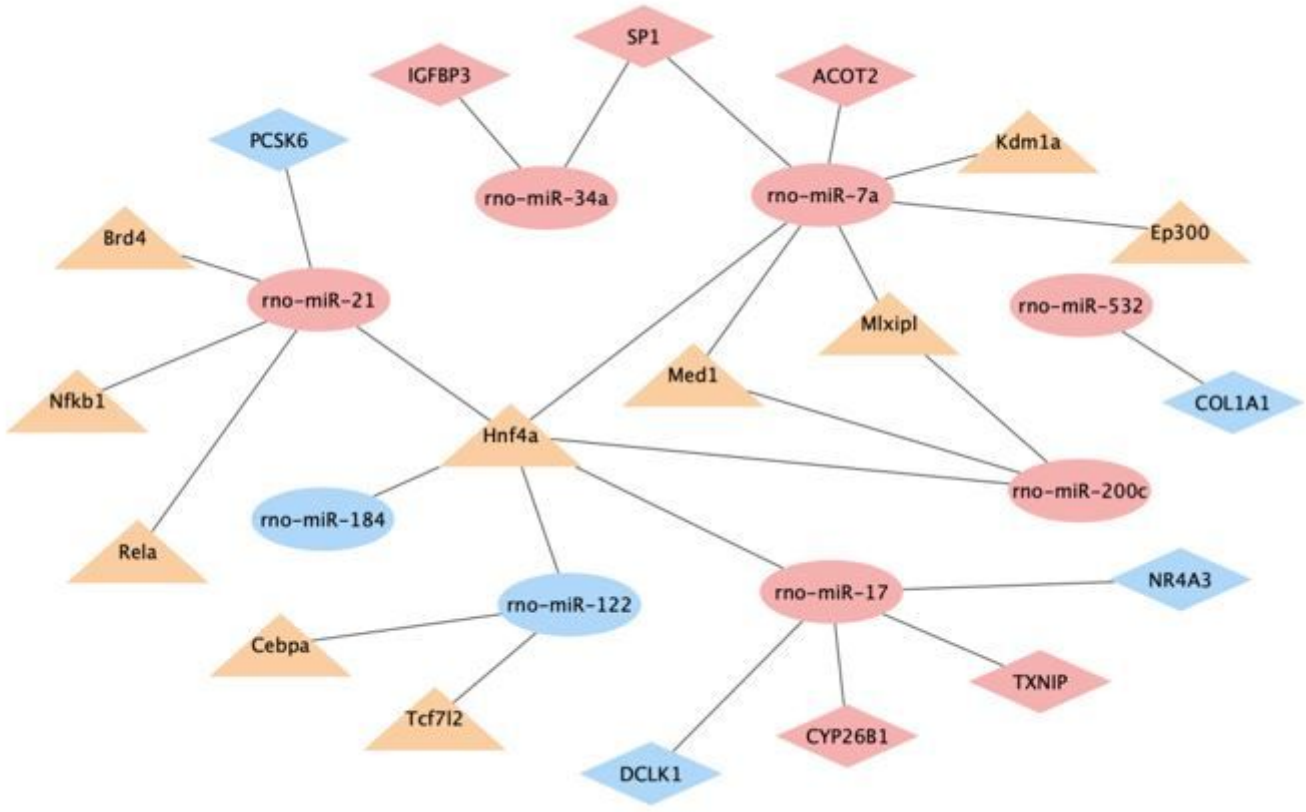

Figure 4

miRNA-mRNA-TF Network in DCM Ovals represent miRNAs, rhombus represent mRNAs and triangles represent TFs. Red represent upregulated expression and blue represent downregulated.

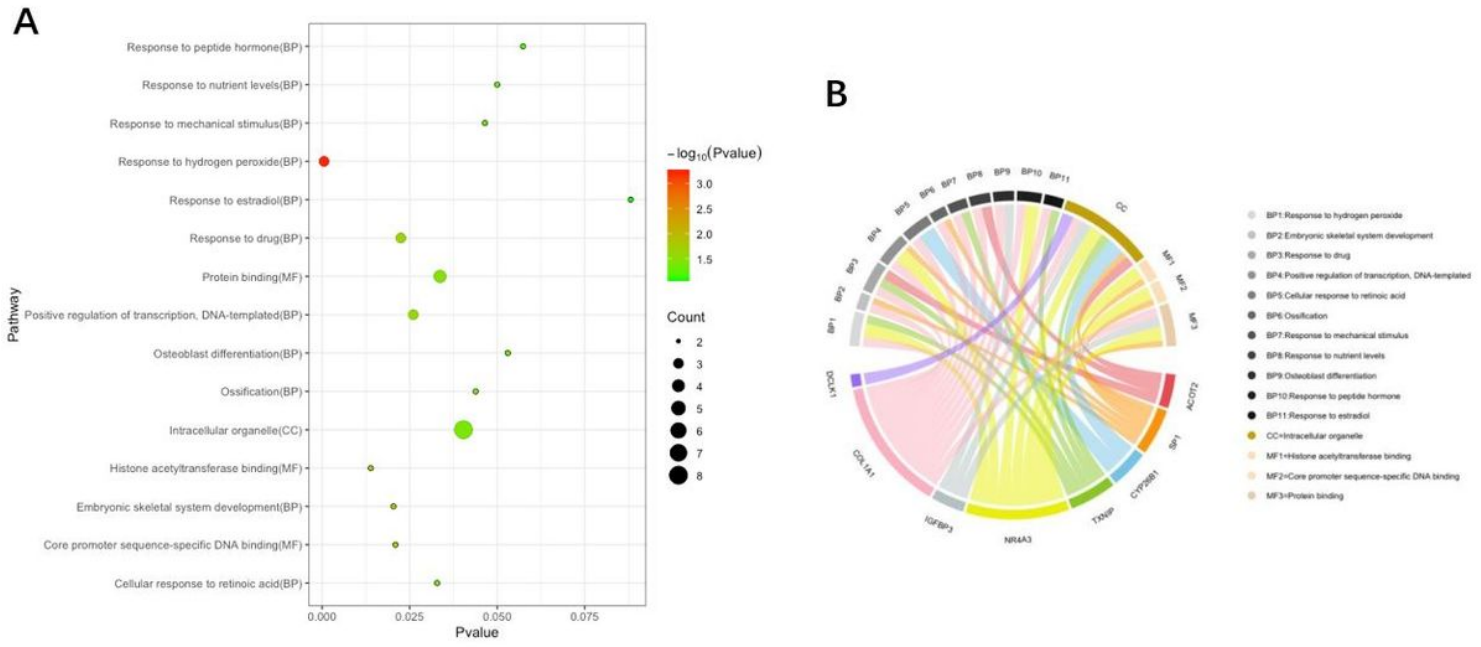

Figure 5

GO Enrichment of mRNAs (a)Bubble chart of GO Enrichment of mRNAs. The size of the circle represents amounts of the mRNAs. (b)Chord chart of mRNAs in BP pathways, CC pathways and MF pathways. The legend on the right illustrate the name of pathways and mRNAs.