Bioinformatics Analysis of a Regulatory lncRNA–miRNA–mRNA Network and Signaling Pathways in Patients With Cardiomyopathy

Tao Chen
Department of Cardiothoracic Surgery, The First Affliated Hospital of AnHui Medical University
Yu-Yao Liu
Department of Burns, The First Affliated Hospital of AnHui Medical University
Shu-Jun Li
Department of Pediatrics, Children's Hospital of AnHui Medical University
Hong Che
Department of Cardiothoracic Surgery, The First Affliated Hospital of AnHui Medical University
Sheng-Lin Ge (aydgsl@sina.com)
Department of Cardiothoracic Surgery, The First Affliated Hospital of AnHui Medical University

Research Article

Keywords: cardiomyopathy, bioinformatics analysis, microarray, lncRNA–miRNA–mRNA network, signaling pathway

Posted Date: October 12th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-936368/v1

Abstract

Background:Cardiomyopathy is a disease with a very low cure rate and a very complex pathogenesis.Although genes are known to play an important role in

various types of cardiomyopathy, the specific mechanism has not been well studied.

Methods: We screened the GSE29819 dataset from the Gene Expression Omnibus database (GEO) for long non-coding RNAs (lncRNAs) and messenger RNAs (mRNAs) with significant differences using R software. microRNAs (miRNAs) regulated by the lncRNAs were retrieved from the miRcode database. The downstream target genes of the miRNAs were predicted by miRDB, miRTarBase, and TargetScan, and genes consistent with the original dataset were selected to construct a lncRNA–miRNA–mRNA network. Finally, the biological effects of these genes were studied by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis.

Results: 223 differentially expressed genes and 13 differentially expressed lncRNAs has been screened in the cardiomyopathy tissues.After database screening and matching, the final lncRNA–miRNA–mRNA network included three lncRNAs, eight miRNAs, and nine mRNAs. These nine genes are mainly involved in the prolactin signaling pathway, EGFR tyrosine kinase inhibitor resistance, toxoplasmosis, the chemokine signaling pathway, and the PI3K–Akt signaling pathway. In the PI3K–Akt signaling pathway, the expression levels of these genes are significantly upregulated compared with the control group, and the main genes involved in regulation are *JAK2/ DDIT4/ FOXO3*. *JAK2* and *FOXO3* play an important regulatory role in the PI3K–Akt signaling pathway.

Conclusion: The *AC017002– miRNA-590-5p– FOXO3* network may be involved in the pathogenesis of cardiomyopathy, which will enable the determination of new markers to predict cardiomyopathy, and thus reduce the risk of developing the disease.

Introduction

Cardiomyopathy is a group of heterogeneous myocardial diseases that can result from various causes and leads to cardiac mechanical or electrical dysfunction, often manifested as ventricular hypertrophy or dilation. The disease can be limited to the heart itself, or can also occur as part of a spectrum of systemic disease. It can eventually lead to cardiac death or progressive heart failure. Cardiomyopathy can be classified into primary and acquired forms, which lead to different phenotypes, including dilated, hypertrophic, and restricted. Hypertrophic cardiomyopathy (HCM) is the most common primary cardiomyopathy. Dilated cardiomyopathy (DCM) can be hereditary or acquired. Other acquired cardiomyopathies include perinatal and stress ventricular cardiomyopathy, as well as rare forms, such as arrhythmogenic right ventricular cardiomyopathy (ARVC) and left ventricular noncompaction.

Long non-coding RNAs (lncRNAs) are endogenous RNA transcripts longer than 200 nucleotides that regulate gene expression from the epigenetic perspective but have no protein coding potential. lncRNAs

affect proteins through miRNAs and affect myocardial development. They are potential key regulators of various cardiovascular diseases.

The pathogenesis of cardiomyopathy includes elevated oxidative stress, myocardial inflammation, apoptosis, and autophagy caused by metabolic disorders. Increasing numbers of lncRNAs have been found to be abnormally regulated in cardiomyopathy. The regulation of expression of specific lncRNAs such as *H19*, metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*), and myocardial infarction-associated transcript (*MIAT*) by genetic pathways can effectively regulate myocardial inflammatory apoptosis and autophagy during oxidative stress in experimental animals and affect the occurrence of cardiomyopathy. Currently, although lncRNAs are considered to be potential diagnostic and therapeutic targets for cardiomyopathy, little is known about their regulatory and functional roles in cardiomyopathy. *LNC-DACH1* (Dachshund homolog 1) affects cardiac function by regulating calcium ion changes in cardiomyocytes. As a precursor of miR-675, *H19* negatively regulates cardiomyocyte hypertrophy by inhibiting the expression level of CaMKIδ. *LNC-PLSCR4* affects the mitochondrial function of cardiomyocytes by regulating *miR-214-Mfn2*, thereby inhibiting the occurrence of cardiac hypertrophy. *LNC-MHRT* can also inhibit the expression of myocardial proteins by targeting *miR-145a-5p*, and ultimately inhibits KLF4 phosphorylation as a negative regulator of the pathogenesis of cardiac hypertrophy.

In this study, through the construction of a complete lncRNA–miRNA–mRNA network, the pathogenesis of cardiomyopathy was explored, and possible mechanisms of the occurrence of cardiomyopathy were found from a molecular perspective.

Materials And Methods

Raw data collection

In the GEO database (https://www.ncbi.nlm.nih.gov/geo/) we searched for “cardiomyopathy” and “long non-coding RNA” to obtain the required data. The inclusion criteria were: (1) expression profiling by array; (2) human data; (3) presence of a clear experimental group and control group; (4) a sample size greater than ten; and (5) presence of a gene symbol or gene sequence. Finally, we selected the GSE29819 dataset. This dataset analyzed left and right ventricular myocardial samples from six patients with ARVC, seven patients with idiopathic dilated cardiomyopathy, and six non-failing donor hearts (NF) that could not be transplanted for technical reasons.

Raw data pretreatment and screening

Raw GEO data are messy, repetitive, and incomplete. Therefore, we sorted the data and classified them as lncRNA and mRNA according to their gene attributes. We used the R language limma package (http://www.bioconductor.org/packages/release/bioc/html/limma.html) to identify and select significant differences in the lncRNAs and mRNAs, setting the filter conditions to log(fold change) > 1, adjustP < 0.05. Bayesian test and False Discovery Rate (FDR) correction were performed on the selected

experimental group and control group data to obtain the differential expression of the lncRNAs and mRNAs, and the results were visualized in a volcanic map and heat map.

Predicting potential miRNAs and genes

We searched the highly conserved miRNA families in the miRcode database (http://www.mircode.org/) and predicted miRNAs related to the lncRNAs we obtained. MiRDB (http://mirdb.org/), MiRTarBase (http://mirtarbase.cuhk.edu.cn/php/index.php) and TargetScan (http://www.targetscan.org/vert_72/) are three databases of miRNA–mRNA targeting relationships supported by experimental evidence. We used the predicted miRNAs to predict target genes from the three databases, and only target genes predicted in all three databases were included.

Construction of a lncRNA–miRNA–mRNA network

The predicted mRNAs in the database were numerous, and so we compared them with the mRNAs screened from the original data to preserve the genes present in the original data. This finalized the lncRNAs, miRNAs, and mRNAs that composed the network. We used Cytoscape software to construct the lncRNA–miRNA–mRNA network.

Functional enrichment and pathway analysis

To study the biological roles of these target genes and the biological signaling pathways involved, we used the “colorspace”, “stringi”, “DOSE”, “clusterProfiler”, and “Pathview” R data packages for GO and KEGG enrichment analysis, where the filtering criteria were P value < 0.05 and Q value < 0.05.

Results

Detection of differentially expressed genes and differentially expressed lncRNAs

We found 223 differentially expressed genes and 13 differentially expressed lncRNAs (*A2M-AS1*, *LPP-AS2*, *BDNF-AS*, *EP300-AS1*, *DAB1-AS1*, *LINC00208*, *LINC01428*, *SOCS2-AS1*, *AC017002*, *TRPM2-AS*, *LINC01300*, *AC002064*, and *FAM53B-AS1*) in the cardiomyopathy tissues (Table 1 and Figure 1).

Table 1
13 lncRNAs with significant differences.

lncRNA	logFC	AveExpr	t	P.Value	adj.P.Val	β
A2M-AS1	1.20	6.77	6.28	0.0000002	0.00002	6.92
LPP-AS2	1.33	5.58	5.89	0.0000008	0.00005	5.72
BDNF-AS	-1.05	3.50	-4.38	0.0000906	0.00194	1.17
EP300-AS1	-1.05	6.20	-4.09	0.0002170	0.00372	0.34
DAB1-AS1	-1.58	2.88	-4.06	0.0002354	0.00395	0.26
LINC00208	-1.26	3.80	-3.45	0.0013713	0.01482	-1.40
LINC01428	1.05	2.91	3.09	0.0037173	0.03065	-2.33
SOCS2-AS1	1.12	3.97	3.04	0.0042134	0.03355	-2.45
AC017002	-1.07	3.88	-3.00	0.0047215	0.03654	-2.55
TRPM2-AS	1.09	3.14	2.98	0.0050516	0.03830	-2.61
LINC01300	-1.24	2.48	-2.96	0.0052182	0.03904	-2.64
AC002064	-1.14	2.68	-2.94	0.0055171	0.04073	-2.69
FAM53B-AS1	1.12	2.68	2.83	0.0073666	0.04954	-2.96

Construction of a lncRNA–miRNA–mRNA network

After database screening and matching, the final lncRNA–miRNA–mRNA network included three lncRNAs, eight miRNAs, and nine mRNAs. The lncRNAs all regulate multiple miRNAs simultaneously, for example *hsa-miR-22-3p* is regulated by *LINC00208* and *AC002064* simultaneously. Some miRNAs regulate multiple mRNAs, such as *hsa-miR-22-3p*, *hsa-miR-27a-3p*, and *hsa-miR-301b-3p*. Both *LDLR* and *SIK1* are regulated by multiple miRNAs (Figure 2).

GO and KEGG analysis

As can be seen from Figure 3, the nine genes identified are mainly involved in the biological processes of small molecule metabolic regulation, lipid biosynthetic regulation, reactive oxygen species metabolism, lipid biosynthetic regulation, and neuron death. These genes are also involved in the molecular functions of G protein-coupled receptor binding, 14-3-3 protein binding, and chromatin DNA binding. These genes play an upregulated role in reactive oxygen species metabolism and regulation of neuron death, and a downregulated role in lipid biosynthetic regulation.

These nine genes are mainly involved in the prolactin signaling pathway, EGFR tyrosine kinase inhibitor resistance, toxoplasmosis, the chemokine signaling pathway, and the PI3K–Akt signaling pathway (Figure 4). In the PI3K–Akt signaling pathway, the expression levels of these genes are significantly

upregulated compared with the control group, and the main genes involved in regulation are *JAK2/ DDIT4/ FOXO3*. It can be seen from Figure 4 that *JAK2* and *FOXO3* play an important regulatory role in the PI3K–Akt signaling pathway.

Discussion

Research has shown that, of the billions of protein-coding and non-coding RNAs (such as lncRNAs and miRNAs), only about 2% play an important role in the physiological and pathological development of cardiomyopathy. lncRNAs can regulate cardiac pathological processes through multiple molecular mechanisms, but they do not have the ability to directly encode proteins. At the same time, miRNAs are involved in various signaling pathways regulating pathological remodeling of the heart, such as cardiac hypertrophy, myocardial fibrosis, and myocardial infarction. A growing number of studies have found that lncRNAs may play an important role in regulating calcium processing in cardiomyocytes and the metabolic state of various cardiovascular diseases, such as HCM.

We constructed a lncRNA–miRNA–mRNA competing endogenous RNA (ceRNA) network related to cardiomyopathy to further explore the correlation between the different types of RNA and to reveal the mechanism behind the occurrence of cardiomyopathy. The construction of the lncRNA–miRNA–mRNA ceRNA network was based on the ceRNA hypothesis, in which lncRNAs regulate mRNA activity by isolating and binding miRNAs, thus playing the role of miRNA sponges.

We found that *AC017002– miRNA-590-5p– FOXO3* constituted the cardiomyopathy-related ceRNA network. *AC017002* is in a relatively prominent position in our ceRNA network. Studies have shown that *AC017002* may play a regulatory role in the occurrence of colon cancer. *miRNA-590-5p* may also be related to the occurrence of cancer. However, no studies have reported any associations between cardiomyopathy and *AC017002* or *miRNA-590-5p*.

The forkhead box O3 (FOXO3) protein is one of the members of the FOXO protein family. The current study found that FOXO3 is involved in the regulation of many biological functions of the human body, including substance metabolism, protein conversion, cell survival, and cell death. There is growing interest in the role of FOXO3 in human health, and recent studies on FOXO3 have mainly focused on longevity, although the specific mechanism is still unclear. *FOXO3* is regulated by the PI3K–Akt signaling pathway. The PI3K–Akt signaling pathway plays an important role in heart development, participating in the development of the heart by promoting the growth and survival of cardiomyocytes, promoting coronary angiogenesis, maintaining cardiac systolic function, regulating signal transduction, and inducing autophagy. Our biological analysis showed that the expression of *FOXO3* was particularly prominent in patients with cardiomyopathy, indicating an association between *FOXO3* expression and the occurrence of cardiomyopathy.

FOXO3 may be related to cardiac senescence, myocarditis, myocardial ischemia/reperfusion injury, and myocardial fibrosis. Kong et al. found that dysregulation of autophagy could cause the occurrence of

cardiomyopathy, and FOXO3 could activate autophagy. Vahtola et al. found that the FOXO3 pathway was activated in diabetes-induced cardiac hypertrophy models in rats.

The *AC017002– miRNA-590-5p– FOXO3* network requires more cell and animal experiments to further confirm the binding relationship and regulatory mechanism. In conclusion, this study proposes the possibility of a lncRNA–miRNA–mRNA network for the pathogenesis of cardiomyopathy, which will enable the determination of new markers to predict cardiomyopathy, and thus reduce the risk of developing the disease.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable.

Authors' contributions

TC performed the data analysis and drafted the manuscript.YYL revised the fgures, and designed and supervised the study.SJL contributed to the manuscript revision. HC was trained in the use of the software. SLG determined the research direction and made the research plan.All authors read and approved the final manuscript.

Acknowledgements

We thank Catherine Perfect, MA (Cantab), from Liwen Bianji, Edanz Editing China (www.liwenbianji.cn/ac), for editing the English text of a draft of this manuscript.

References

1. Dadson, K., Hauck, L., & Billia, F. (2017). Molecular mechanisms in cardiomyopathy. Clinical science (London, England : 1979), 131(13), 1375–1392.
2. Maron BJ, Towbin JA, Thiene G, et al.; American Heart Association;Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; Council on Epidemiology and Prevention. Contemporary defnitions and classifcation of the cardiomyopathies: an American Heart Association Scientifc Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. Circulation. 2006;113(14):1807-1816.
3. Wang KC, Chang HY. Molecular mechanisms of long noncoding RNAs[J]. Mol Cell. 2011, 43 (6): 904-14.
4. Al-Hasani K, Mathiyalagan P, El-Osta A. Epigenetics, cardiovascular disease, and cellular reprogramming[J]. J Mol Cell Cardiol. 2019,128:129-33.
5. Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, et al. Natural RNA circles function as efficient microRNA sponges[J]. Nature. 2013,495(7441):384-8.
6. Li, M., Duan, L., Li, Y., & Liu, B. (2019). Long noncoding RNA/circular noncoding RNA-miRNA-mRNA axes in cardiovascular diseases. Life sciences, 233, 116440.
7. Pant, T., Dhanasekaran, A., Fang, J., Bai, X., Bosnjak, Z. J., Liang, M., & Ge, Z. D. (2018). Current status and strategies of long noncoding RNA research for diabetic cardiomyopathy. BMC cardiovascular disorders, 18(1), 197.
8. Liu, L., Zhang, D., & Li, Y. (2020). LncRNAs in cardiac hypertrophy: From basic science to clinical application. Journal of cellular and molecular medicine, 24(20), 11638–11645.
9. Cai B, Zhang Y, Zhao Y, et al. Long noncoding RNA-DACH1 (Dachshund Homolog 1) regulates cardiac function by inhibiting SERCA2a (sarcoplasmic reticulum calcium ATPase 2a). Hypertension. 2019;74:833-842.
10. Liu L, An X, Li Z, et al. The H19 long noncoding RNA is a novel negative regulator of cardiomyocyte hypertrophy. Cardiovasc Res.2016;111:56-65.
11. Lv L, Li T, Li X, et al. The lncRNA Plscr4 controls cardiac hypertrophy by regulating miR-214. Mol Ther Nucleic Acids. 2018;10:387-397.
12. Xu Y, Luo Y, Liang C, Zhang T. LncRNA-Mhrt regulates cardiac hypertrophy by modulating the miR-145a-5p/KLF4/myocardin axis. J Mol Cell Cardiol. 2020;139:47-61.
13. Luo Y, Xu Y, Liang C, Xing W, Zhang T. The mechanism of myocardial hypertrophy regulated by the interaction between MHRT and myocardin. Cell Signal. 2018;43:11-20.
14. Liu, L., Zhang, D., & Li, Y. (2020). LncRNAs in cardiac hypertrophy: From basic science to clinical application. Journal of cellular and molecular medicine, 24(20), 11638–11645.
15. Rinn JL, Chang HY. Genome regulation by long noncoding RNAs.Annu Rev Biochem. 2012;81:145-166.

16. Goff LA, Rinn JL. Linking RNA biology to lncRNAs. Genome Res. 2015;25:1456-1465.
17. Wehbe N, Nasser SA, Pintus G, Badran A, Eid AH, Baydoun E.MicroRNAs in cardiac hypertrophy. Int J Mol Sci. 2019;20(19):4714.
18. Bar C, Chatterjee S, Thum T. Long noncoding RNAs in cardiovascular pathology, diagnosis, and therapy. Circulation.2016;134:1484-1499.
19. Viereck J, Thum T. Long noncoding RNAs in pathological cardiac remodeling. Circ Res. 2017;120:262-264.
20. Lyu, L., Xiang, W., Zhu, J. Y., Huang, T., Yuan, J. D., & Zhang, C. H. (2019). Integrative analysis of the lncRNA-associated ceRNA network reveals lncRNAs as potential prognostic biomarkers in human muscle-invasive bladder cancer. Cancer management and research, 11, 6061–6077.
21. Wang DZ, Chen GY, Li YF, Zhang NW. Comprehensive analysis of long non-coding RNA and mRNA expression profile in rectal cancer. Chin Med J (Engl). 2020;133(11):1312-1321.
22. Chen, G. Q., Liao, Z. M., Liu, J., Li, F., Huang, D., & Zhou, Y. D. (2021). LncRNA FTX Promotes Colorectal Cancer Cells Migration and Invasion by miRNA-590-5p/RBPJ Axis. Biochemical genetics, 59(2), 560–573.
23. Xu Y, Han T, Zhu Y, Chen Q. miR-590-5P inhibits the progression of tongue squamous cell carcinoma by targeting FasL. Int J Clin Exp Pathol. 2017;10(12):11880-11887. Published 2017 Dec 1.
24. Shen, B., Yu, S., Zhang, Y., Yuan, Y., Li, X., Zhong, J., & Feng, J. (2016). miR-590-5p regulates gastric cancer cell growth and chemosensitivity through RECK and the AKT/ERK pathway. Onco Targets and therapy, 9, 6009–6019.
25. Jiang X, Xiang G, Wang Y, et al. MicroRNA-590-5p regulates proliferation and invasion in human hepatocellular carcinoma cells by targeting TGF-β RII. Mol Cells. 2012;33(6):545-551.
26. Stefanetti, R. J., Voisin, S., Russell, A., & Lamon, S. (2018). Recent advances in understanding the role of FOXO3. F1000Research, 7, F1000 Faculty Rev-1372.
27. Morris, B. J., Willcox, D. C., Donlon, T. A., & Willcox, B. J. (2015). FOXO3: A Major Gene for Human Longevity–A Mini-Review. Gerontology, 61(6), 515–525.
28. Du WW, Yang W, Chen Y, et al. Foxo3 circular RNA promotes cardiac senescence by modulating multiple factors associated with stress and senescence responses. Eur Heart J. 2017;38(18):1402-1412.
29. Lin, J., Lin, H., Ma, C., Dong, F., Hu, Y., & Li, H. (2019). MiR-149 Aggravates Pyroptosis in Myocardial Ischemia-Reperfusion Damage via Silencing FoxO3. Medical science monitor : international medical journal of experimental and clinical research, 25, 8733–8743.
30. Loebel, M., Holzhauser, L., Hartwig, J. A., Shukla, P. C., Savvatis, K., Jenke, A., Gast, M., Escher, F., Becker, S. C., Bauer, S., Stroux, A., Beling, A., Kespohl, M., Pinkert, S., Fechner, H., Kuehl, U., Lassner, D., Poller, W., Schultheiss, H. P., Zeller, T., … Skurk, C. (2018). The forkhead transcription factor Foxo3 negatively regulates natural killer cell function and viral clearance in myocarditis. European heart journal, 39(10), 876–887.

31. Vivar, R., Humeres, C., Anfossi, R., Bolivar, S., Catalán, M., Hill, J., Lavandero, S., & Diaz-Araya, G. (2020). Role of FoxO3a as a negative regulator of the cardiac myofibroblast conversion induced by TGF-β1. Biochimica et biophysica acta. Molecular cell research, 1867(7), 118695.
32. Kong, D. K., Georgescu, S. P., Kyriakis, J. M., & Goruppi, S. (2010). Deficiency of transcriptional regulator p8 induces autophagy and causes impaired cardiac function. Autophagy, 6(5), 652–654.
33. Vahtola E, Louhelainen M, Merasto S, et al. Forkhead class O transcription factor 3a activation and Sirtuin1 overexpression in the hypertrophied myocardium of the diabetic Goto-Kakizaki rat. J Hypertens. 2008;26(2):334-344.

Figures

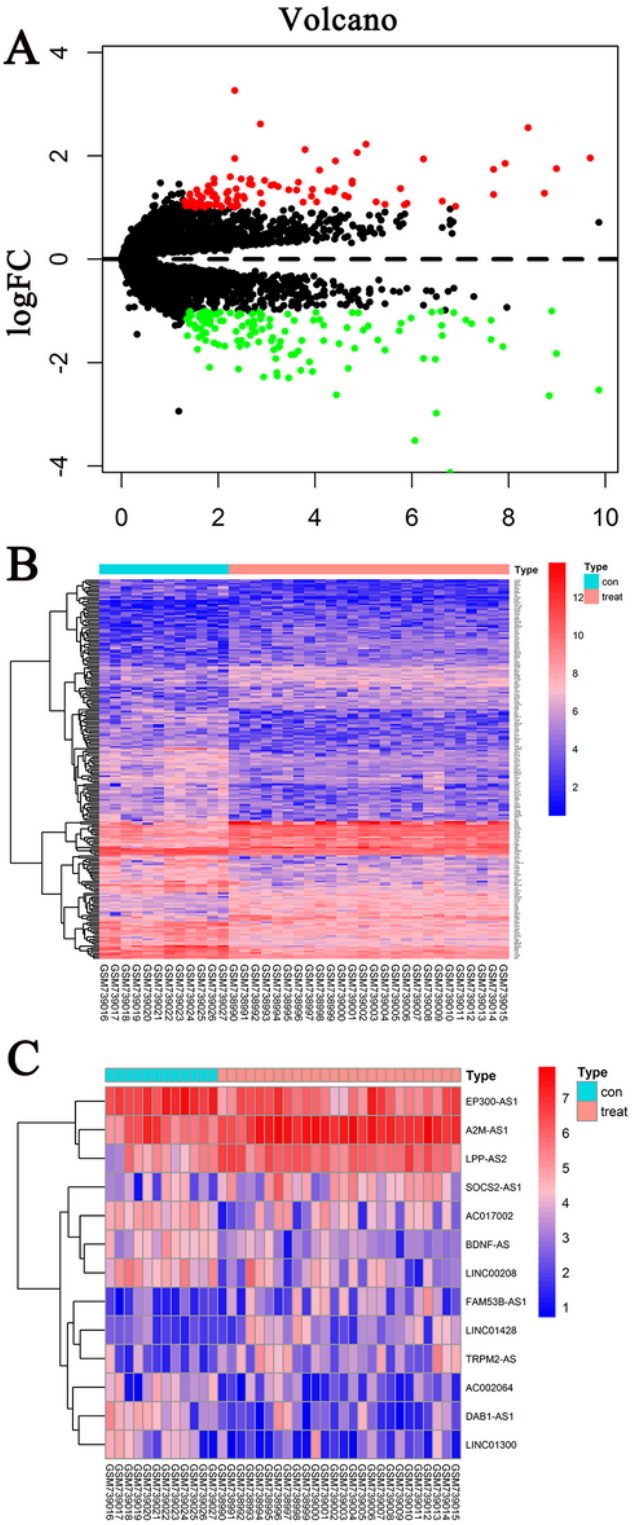


Figure 1

Differentially expressed genes.(A)Volcano plots of DElncs and DEmRs. LncRNAs and mRNAs with significant differences were screened according to logfold change > 1 and adjust P < 0.05.Red dots representing significantly upregulated ones, green dots representing significantly downregulated ones, and black dots representing no differences ones. (B)Heat map of DEmRs. Red box plot represents upregulated DEmRs, while blue represents downregulated DEmRs.(C) Heat map of DElncs. Red box plot

represents upregulated DElncs, while blue represents downregulated DElncs. DElncs: differentially expressed lncRNAs; DEmRs: differentially expressed mRNAs.

lncRNA	miRNA	mRNA
LINC00208	hsa-miR-22-3p	LRRC1/DDIT4
	hsa-miR-125b-5p	STAT3
AC017002	hsa-miR-590-5p	FOXO3
	hsa-miR-27a-3p	LDLR/LPCAT1
	hsa-miR-135a-5p	JAK2
	hsa-miR-142-3p	SIK1
AC002064	hsa-miR-301b-3p	LDLR/SIK1
	hsa-miR-22-3p	LRRC1/DDIT4
	hsa-miR-1297	ADM

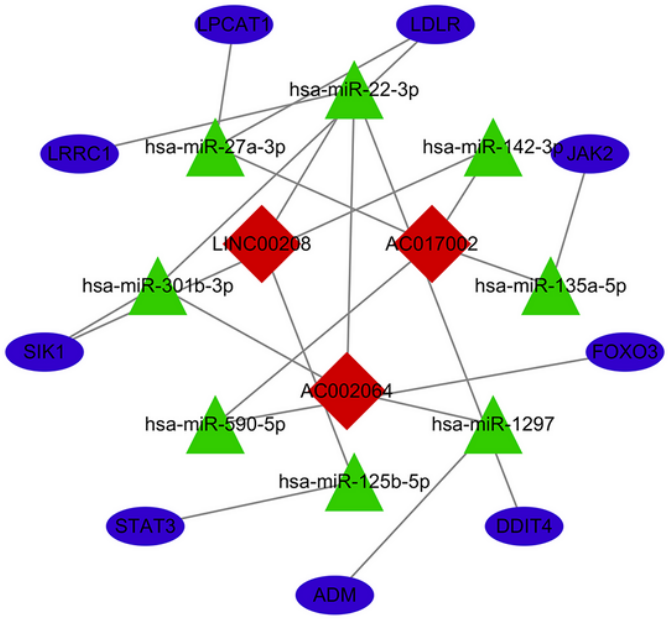


Figure 2

LncRNA miRNA mRNA networks. Red rhombus represent lncRNAs, green triangles represent miRNAs, and blue ovals represent mRNAs. The lines represent the relationship of regulation.

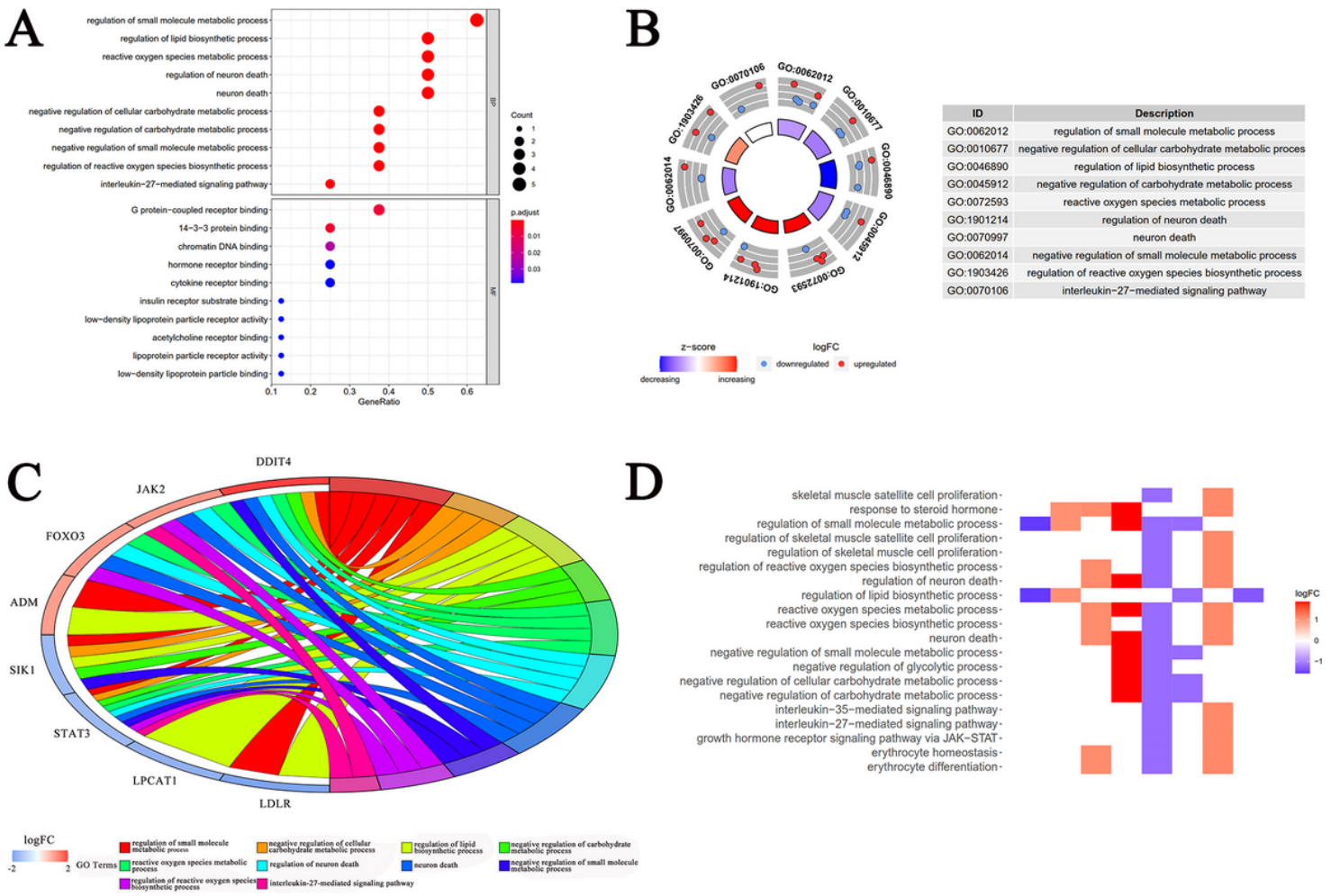
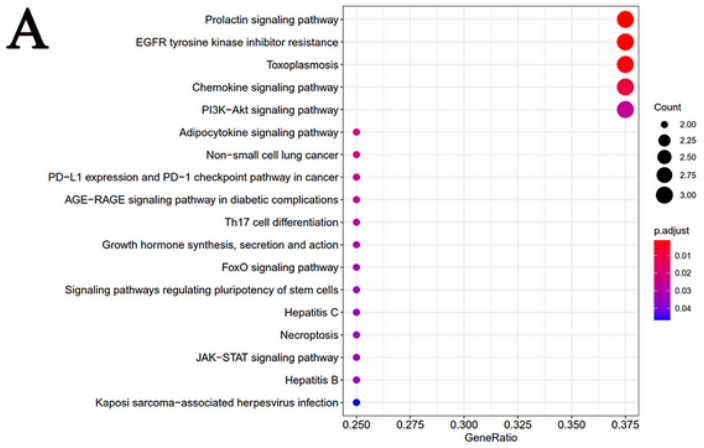
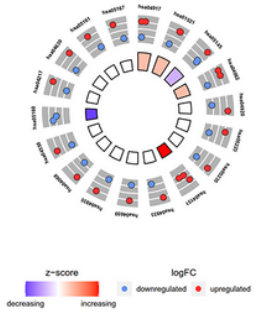


Figure 3

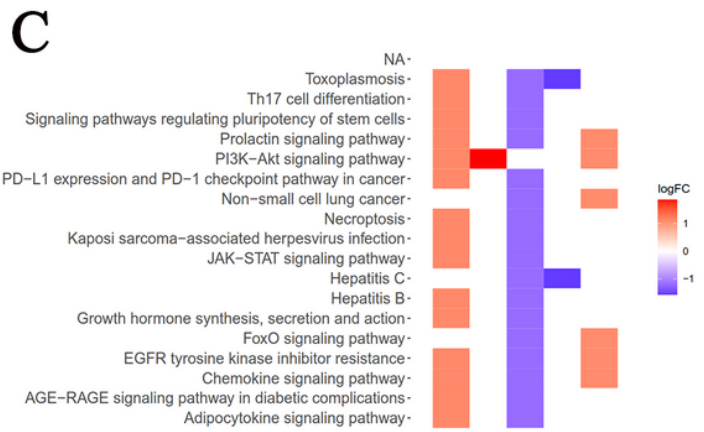
GO functional enrichment of differentially expressed genes.(A) GO analysis shows the biological processes and molecular functions involved in differential genes.(B) GO analysis revealed the biological processes and molecular functions involved in the 9 differential genes.(C) Heat map based on differential expression of 9 genes in biological processes and molecular functions.(D) The chord diagram shows the involvement of differential genes in biological processes and molecular functions.

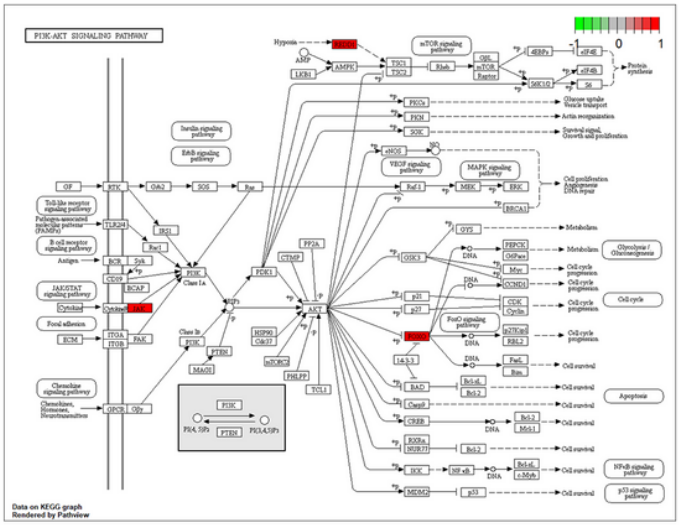




B

ID	Description
hsa04917	Prolactin signaling pathway
hsa01521	EGFR tyrosine kinase inhibitor resistance
hsa05145	Toxoplasmosis
hsa04062	Chemokine signaling pathway
hsa04920	Adipocytokine signaling pathway
hsa05223	Non-small cell lung cancer
hsa05235	PD-L1 expression and PD-1 checkpoint pathway in cancer
hsa04151	PI3K-Akt signaling pathway
hsa04933	AGE-RAGE signaling pathway in diabetic complications
hsa04659	Th17 cell differentiation
hsa04935	Growth hormone synthesis, secretion and action
hsa04068	FoxO signaling pathway
hsa04550	Signaling pathways regulating pluripotency of stem cells
hsa05160	Hepatitis C
hsa04217	Necroptosis
hsa04630	JAK-STAT signaling pathway
hsa05161	Hepatitis B
hsa05167	Kaposi sarcoma-associated herpesvirus infection





E

ID	Description	geneID
hsa04917	Prolactin signaling pathway	JAK2/STAT3/FOXO3
hsa01521	EGFR tyrosine kinase inhibitor resistance	JAK2/STAT3/FOXO3
hsa05145	Toxoplasmosis	JAK2/LDLR/STAT3
hsa04062	Chemokine signaling pathway	JAK2/STAT3/FOXO3
hsa04151	PI3K-Akt signaling pathway	JAK2/DDIT4/FOXO3

Figure 4

KEGG functional enrichment of differentially expressed genes.(A) KEGG shows the signaling pathway involved in differential genes.(B) KEGG shows the signaling pathways involved in the 9 differential genes. (C) Heat map based on differential expression of 9 genes in signaling pathways.(D) Several signaling pathways enumerated and the differential genes involved. (E) PI3K-Akt signaling pathway and the genes involved. Red box plot represents upregulated genes.