

# Identifying Serum Exosomal-Associated lncRNA/circRNA-miRNA-mRNA Network in Coronary Heart Disease

Jia Mao

The Affiliated Wuxi No.2 Peoples' Hospital of Nanjing Medical University

Yufei Zhou

The First Affiliated Hospital of Nanjing Medical University

Licheng Lu

Kunshan Hospital of Traditional Chinese Medicine

Ping Zhang

The Affiliated Wuxi No.2 Peoples' Hospital of Nanjing Medical University

Runhua Ren

The Affiliated Jiangning Hospital with Nanjing Medical University

Yaqiong Wang

The Affiliated Jiangning Hospital with Nanjing Medical University

Jing Wang (✉ [roubaobao09@163.com](mailto:roubaobao09@163.com))

The Affiliated Jiangning Hospital with Nanjing Medical University <https://orcid.org/0000-0003-2074-1028>

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## Research

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# Abstract

**Background:** Accumulating evidence has indicated that the importance of noncoding RNAs and exosomes in coronary heart disease (CHD). However, the exosomal-associated competing endogenous RNA (ceRNA)-mediated regulatory mechanism in CHD is still unknown. The present study aimed to explore exosomal-associated ceRNA network in CHD.

**Methods:** The dataset, including 6 CHD patients and 32 normal controls, were downloaded from the ExoRBase database. Differentially expressed mRNAs (DEMs), lncRNAs (DELs) and circRNAs (DECs) in the serum exosomes between CHD and normal controls were screened. MicroRNAs (miRNAs) targeting DEMs were predicted by TargetScan and miRanda, miRNAs targeting DELs and DECs were predicted with miRcode and starBase, respectively. The biological functions and related signal pathways of DEMs were analyzed using David and KOBAS database. Subsequently, the protein-protein interaction (PPI) network was established to screen out hub genes, enrichment analyses of hub genes were performed and the ceRNA network (lncRNA/circRNA-miRNA-mRNA) was constructed to elucidate ceRNA axes in CHD.

**Results:** A total of 312 DEMs, 43 DELs and 85 DECs were identified between CHD patients and normal controls. Functional enrichment analysis showed that DEMs were significantly enriched in “chromatin silencing at rDNA”, “telomere organization”, “negative regulation of gene expression, epigenetic”. The PPI network analysis showed that 25 hub DEMs were closely related to CHD, of which, ubiquitin C (UBC) was the most important. The biological function of hub genes showed that they were mainly enriched in “cellular protein metabolic process”. The exosomal-associated ceRNA regulatory network incorporated 48 DEMs, 72 predicted miRNAs, 10 DELs and 15 DECs. LncRNA/circRNA-miRNA-mRNA interaction axes (RPL7AP11/hsa-miR-17-5p/UBC, RPL7AP11/hsa-miR-20b-5p/UBC) were obtained from the network.

**Conclusions:** Our findings have provided a novel perspective on the potential roles of exosomal-associated ceRNA network regulating the pathogenesis of CHD.

## Introduction

Coronary heart disease (CHD) is a complex biological process accompanied by wide transcriptional changes, the mechanism of CHD is still complex and unclear [1].

Noncoding RNAs mainly consist of microRNAs (miRNAs/miRs), long noncoding RNAs (lncRNAs) and circular RNAs (circRNAs). MiRNAs are a class of small noncoding RNAs, which could block protein translation or induce degradation with the combination of specific region of target messenger RNA (mRNA) [2]. LncRNAs have more diverse functions acting as epigenetic regulators, molecular scaffolds, or decoys [3]. CircRNAs can function as templates for viroid and viral replication, as intermediates in RNA processing reactions, as regulators of transcription in cis, as small nucleolar RNAs, and as miRNA sponges [4]. With the development of sequencing technology and bioinformatics, it has been found that noncoding RNAs were involved in the pathophysiology of cardiovascular diseases [5]. Ahmadi, R et al [6] demonstrated that miR-342-5p could be a biomarker for diagnosis of CHD associated with inflammatory

cytokines. Wang, H et al [7] revealed that lncRNA BRAF-activated noncoding RNA is associated with the occurrence of CHD. Moreover, circRNAs, or lncRNAs have been found to interact with miRNAs as competitive RNAs (ceRNAs) to regulate target mRNAs activity and participate in CHD. For instance, circ YOD1 Deubiquitinase might be a novel target for diagnosing CHD from lncRNA/circRNA-miRNA-mRNA ceRNA network [8].

However, serum RNAs might often be degraded by RNA enzyme and may not accurately reflect the pathological differences. While exosomes could protect them from being degraded [9]. Exosomes are small vesicles with a diameter of approximately 30-150 nm containing proteins, nucleic acids, and lipids [10], which were related to diverse regulation processes of cardiovascular disorders, including myocardial injury, repair, regeneration and so on [11].

To better understand the underlying molecular regulatory mechanisms of CHD, we aimed to identify the differentially expressed exosomal-related lncRNAs, circRNAs, miRNAs and constructed the ceRNA network to discover more accurate and reliable candidate diagnostic biomarkers and therapeutic targets of CHD.

## Materials And Methods

### Ethics approval

This study was approved by the Ethics Committee of The First Affiliated Hospital of Nanjing Medical University. All methods were performed in accordance with the relevant guidelines and regulations.

### Data collection:

The study flowchart is shown in Fig. 1. Currently, the exoRBase database (<http://www.exorbase.org/>) is a repository of circRNAs, lncRNAs and mRNAs derived from RNA-seq data including human blood exosomes analysis. These samples come from different biological conditions, including normal persons (NP), CHD, colorectal cancer, hepatocellular carcinoma, pancreatic adenocarcinoma and breast cancer [12]. In the Study, data of NP and CHD blood samples were downloaded, including 6 patients with CHD and 32 normal controls.

### Identification of differently expressed mRNAs, lncRNAs and circRNAs:

The lists of differentially expressed circRNAs (DECs), lncRNAs (DEls), and mRNAs (DEMs) between controls and patients with CHD were generated using the LIMMA package in the R software. The values of  $|\log_2 \text{Fold Change (FC)}| > 0$  and  $P\text{-value} < 0.05$  were selected as the cut-off criteria.

### Integration of PPI network and module analysis

The PPI network of DEMs was constructed by STRING (<https://string-db.org>) and visualized with Cytoscape software [13]. Furthermore, the Molecular Complex Detection (MCODE) application in Cytoscape was applied to select the PPI network modules, with a cut-off=2, node score cut-off=0.2, k-

core=2 and maximum depth=100 as the selection criteria. In addition, the nodes with degree  $\geq 5$  were identified as hub nodes in the PPI network.

## Functional enrichment analyses

Gene ontology (GO) analysis was used to annotate the DEMs and hub genes based on biological processes (BP), cellular components (CC) and molecular functions (MF) [14]. To investigate the biological function of DEMs and hub genes, the database for annotation, visualization, and integrated discovery (DAVID) online tool (version 6.8; [david.abcc.ncifcrf.gov](http://david.abcc.ncifcrf.gov)) was utilized to perform GO analysis [15]. In addition, the KOBAS 3.0 online analysis database was used to perform pathway enrichment analysis [16]. The significant enrichment for GO and KEGG analyses threshold was *p*-value <0.05 and count  $\geq 2$ .

## Prediction of microRNAs targeting mRNAs

Targeted miRNAs of the DEMs were predicted using TargetScan ([http://www.targetscan.org/vert\\_72/](http://www.targetscan.org/vert_72/)) [17] and miRanda database (<http://www.mirdb.org/>) [18]. To increase the accuracy of prediction, the targeted miRNAs predicted by both databases were retained. Targeted miRNAs of the DELs and DECs were predicted with miRcode (<http://www.mircode.org/>) [19] and starBase (<http://starbase.sysu.edu.cn/>) [20], respectively. The pairs of miRNAs-mRNAs, lncRNA-miRNA and circRNA-miRNA were constructed subsequently.

## Construction of the lncRNA/circRNA-miRNA-mRNA ceRNA network

CeRNA regulation has been reported to serve important roles in human disease, the circRNA or lncRNA-miRNA-mRNA interactions network was constructed to explore the association among circRNA, lncRNA, miRNA, and mRNA [21]. Finally, the lncRNA/circRNA-miRNA-mRNA network was built to visualize the interactions using Cytoscape.

## Statistical analysis

All data were expressed as the mean  $\pm$  standard error. Statistical analyses were performed using GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, CA, USA). *P*<0.05 was considered to indicate a statistically significant difference.

# Results

## Differential expression analysis

A total of 312 DEMs (55 up-regulated and 257 down-regulated), 43 DELs (24 up-regulated and 19 down-regulated), and 85 DECs (4 up-regulated and 81 down-regulated) were identified between the CHD patients and control individuals. The whole up-regulated and down-regulated DEMs, DELs, and DECs are listed in Additional file 1: Table S1-S3. Finally, based on the pre-set criteria of *P*-value < 0.05 and |

$\log_2(\text{fold change, FC}) | > 0.5$ , we plot the heat maps for DEMs, DELs and DECs, respectively, as shown in Fig. 2a-c.

## PPI network and module analyses

To further study the specific DEMs from an interactive respective, the PPI network was constructed using the STRING database. A PPI network with statistical significance consisted of 50 nodes and 189 edges. The nodes with a degree of  $\geq 5$  were regarded as hub mRNAs in the network. Two modules were formed in the PPI network with MCODE score  $\geq 7$ : module 1 with MCODE score of 16.875 (nodes =17), and module 2 with MCODE score of 7.429(nodes =8). Hub mRNAs, namely, ubiquitin C (UBC), 16 histone cluster (HIST) family genes were present in module 1, cathepsin G (CTSG), myeloperoxidase (MPO), cathelicidin antimicrobial peptide (CAMP), defensin alpha 1 (DEFA1), defensin alpha 3 (DEFA3), matrix metallopeptidase 8 (MMP8), azurocidin 1 (AZU1), defensin alpha 1B (DEFA1B) were present in module 2 (Fig. 3).

## Functional enrichment analyses

Functional enrichment analyses illustrated that the DEMs were mainly enriched “chromatin silencing at rDNA”, “telomere organization”, “negative regulation of gene expression, epigenetic” for the BP terms. CC analysis showed that the DEMs were significantly enriched for the “nucleosome”, “nuclear chromosome”, “nuclear chromosome”. For the MF category, the DEMs were enriched in “histone binding”, “protein heterodimerization activity”, “poly(A) RNA binding” (Fig. 4 and Additional file 1: Table S4). With the enrichment analyses for hub genes, we found biological function of UBC was enriched in “cellular protein metabolic process”, the location of UBC was abundantly enriched in “extracellular exosome”, and the molecular function comprised “poly(A) RNA binding” (Fig. 5 and Additional file 1: Table S5).

## LncRNA/circRNA-miRNA-mRNA ceRNA network

Accordingly, ceRNAs network analyses were performed to unravel the functions of identified differently expressed exosomal-associated ceRNA network in CHD patients. This ceRNA network consisting of 48 DEMs, 72 predicted miRNAs, 10 DELs, and 15 DECs. From the ceRNA network, we identified lncRNA RPL7AP11 competed for binding to hsa-miR-20a-5p and hsa-miR-17-5p, thereby affecting UBC expression (Fig. 6). These results suggested that the ceRNA networks we predicted in this paper might be a key factor underlying the pathogenesis of CHD.

## Discussion

Atherosclerotic disease and its thrombotic complication may lead to the development of CHD, and if untreated, it progresses into myocardial infarction. Exosomes are a type of extracellular vesicle that contain constituents (protein, DNA, and RNA) of the cells that secrete them [22].

Despite years of research, the underlying pathogenesis of coronary artery disease has not been fully defined. Recently, dysregulated expression of RNAs (lncRNAs, circRNAs, miRNAs, mRNAs) have been

partially found to be associated with CHD [8, 23]. However, serum RNAs might often be degraded by RNA enzyme and may not accurately reflect the pathological differences. While exosomes could protect them from being degraded [9]. Hence, we identified serum exosomal-associated RNAs and constructed the ceRNA network in CHD, revealing a new targeting axis in the pathogenesis of CHD. To our knowledge, this was the first to explore exosomal-associated ceRNA network in CHD.

In this study, we first identified 312 DEMs, 85 DECs and 43 DELs involving in the pathogenesis of coronary heart disease. Enrichment analysis and PPI network were subsequently performed, of which, UBC (ubiquitin C) was one of the most important hub genes. After the prediction of miRNAs targeting mRNA, exosomal-associated circRNA/IncRNA-miRNA-mRNA ceRNA network was constructed. Our results suggest specific ceRNA axes in the pathogenesis of CHD, which may be promising targets for CHD diagnosis.

UBC (Ubiquitin C), belonging to the ubiquitin family, is associated with protein degradation, DNA repair, kinase modification, autophagy, regulation of inflammation and regulation of other cell signaling pathways [24, 25]. Our enrichment analysis both in DEMs and hub genes showed UBC participated in cellular protein metabolic process. Ji Y and his colleagues [26] proved that the expression level of ubiquitin was significantly higher in CHD patients than healthy individuals and the levels of ubiquitin were consistent with the severity of different classes of CHD. Our study further confirmed the function of UBC in the pathogenesis of CHD, which could be a non-invasive biomarker.

MiR-17-5p was reported to regulate cell cycle, proliferation, apoptosis. Board evidence has elucidated its profound function in regulating cardiovascular diseases. The deficiency of miR17 in neonatal mice is lethal and the over-expression of miR-17-5p could extend the life span of mice [27]. Liu G et al [28] confirmed the up-regulation of miR-17-5p could contribute to hypoxia-induced proliferation of human pulmonary artery smooth muscle cells, leading to pulmonary hypertension. Yang S et al [29] found miR-17-5p silencing protects heart function after AMI through decreasing the rate of apoptosis and repairing vascular injury. Moreover, recent studies have shown circulating miR-17-5p could be a novel biomarker for diagnosis of acute myocardial infarction [30].

MiR-20b-5p was found to attenuate hypoxia-induced apoptosis in cardiomyocytes [31]. Also, Zhen W et al [32] found the overexpression of miR-20b-5p could increase cell viability and repress autophagy and apoptosis in human umbilical vein endothelial cells with hypoxia-reoxygenation injury. Both hypoxia and hypoxia-reoxygenation models are similar to patients with MI and revascularization of MI, we considered its vital role in regulating CHD. However, there has been little research about the function of miR-20b-5p in CHD patients, which needs to be further validated.

The lncRNA RPL7AP11 (ribosomal protein L7a pseudogene 11) is a pseudogene of ribosomal protein L7a (RPL7A). Lou W et al [33] found RPL7A was down-regulated in high density lipoprotein induced vascular endothelial cell line ECV 304. Pseudogenes, abundant in the human genome, were considered as non-functional "junk genes traditionally [34]. Recent studies have proved its function in various diseases. However, there has been limited research on RPL7AP11. More evidence needs to be confirmed.

Our study demonstrated that RPL7AP11 could sponge hsa-miR-17-5p and hsa-miR-20b-5p to upregulate UBC, thus regulating the pathogenesis of CHD through cellular protein metabolic process.

There are some limitations to the present study. Firstly, the sample scale was not large. An additional validation cohort should be included in further studies to analyze the expression of these identified lncRNAs, circRNAs, miRNAs and mRNAs. Secondly, how these novel exosomal-associated ceRNA axes participate in the process of CHD development is still unclear. Further cell and animal experiments are needed to verify these findings.

## Conclusions

In conclusion, our comprehensive study identified several exosomal-associated lncRNA/circRNA-miRNA-mRNA interaction axes (RPL7AP11/hsa-miR-17-5p/UBC, RPL7AP11/hsa-miR-20b-5p/UBC) in the progression of CHD, which may be crucial targets for the disease treatment.

## Declarations

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### Authors' contributions

Conception and design of the study: JW, JM, and YFZ. Acquisition of data: JW and LCL; analyses and interpretation of data: JM and PZ; drafting of the manuscript: RHR, YQW and JW; All authors approved the final version of the manuscript.

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Our research did not receive specific funding, the whole work was guided by Dr. Jing Wang (Corresponding author). The duties of co-authors were described in Authors' Contributions section.

### Availability of data and materials

The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are accessible from the exoRBase database (<http://www.exorbase.org/>). Processed data are available from the corresponding author: Jing Wang (Email: [roubaobao09@163.com](mailto:roubaobao09@163.com)).

### Consent for publication

Not applicable.

### Conflicts of Interest

The authors declared that they have no competing interests.

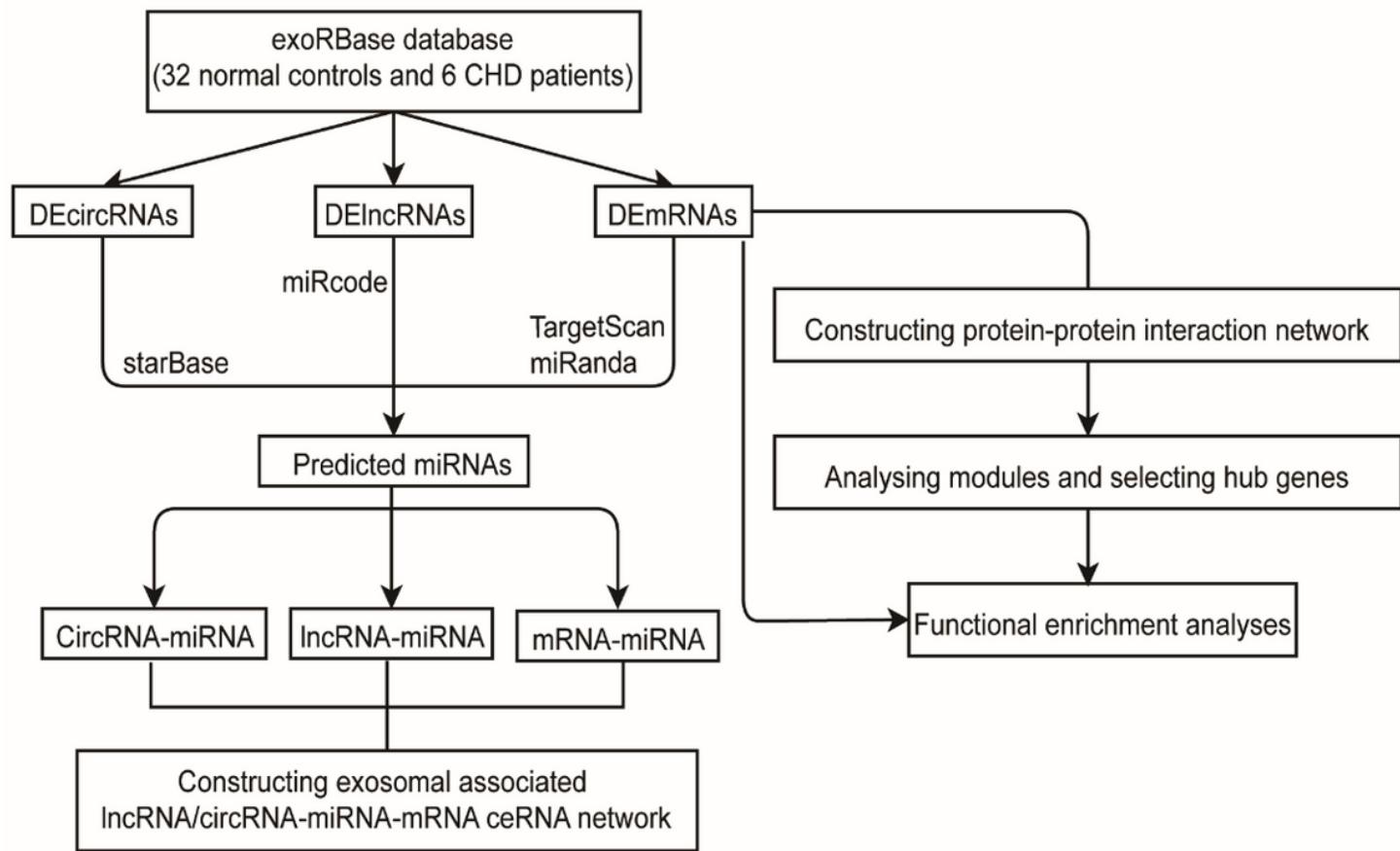
## References

1. Dalen JE, Alpert JS, Goldberg RJ, Weinstein RS. The epidemic of the 20(th) century: coronary heart disease. *Am J Med.* 2014;127:807-12.
2. Saliminejad K, Khorram Khorshid HR, Soleymani Fard S, Ghaffari SH. An overview of microRNAs: Biology, functions, therapeutics, and analysis methods. *J Cell Physiol.* 2019;234:5451-65.
3. Zhu J, Fu H, Wu Y, Zheng X. Function of lncRNAs and approaches to lncRNA-protein interactions. *Sci China Life Sci.* 2013;56:876-85.
4. Lasda E, Parker R. Circular RNAs: diversity of form and function. *Rna.* 2014;20:1829-42.
5. Jaquenod De Giusti C, Santalla M, Das S. Exosomal non-coding RNAs (Exo-ncRNAs) in cardiovascular health. *J Mol Cell Cardiol.* 2019;137:143-51.
6. Ahmadi R, Heidarian E, Fadaei R, Moradi N, Malek M, Fallah S. miR-342-5p Expression Levels in Coronary Artery Disease Patients and its Association with Inflammatory Cytokines. *Clin Lab.* 2018;64:603-9.
7. Wang H, Zhang N, Li G, Xu B. Proinflammatory cytokine IFN- $\gamma$ , lncRNA BANCR and the occurrence of coronary artery disease. *Life Sci.* 2019;231:116510.
8. Li L, Wang L, Li H, Han X, Chen S, Yang B, Hu Z, Zhu H, Cai C, Chen J, et al. Characterization of lncRNA expression profile and identification of novel lncRNA biomarkers to diagnose coronary artery disease. *Atherosclerosis.* 2018;275:359-67.
9. Ge Q, Zhou Y, Lu J, Bai Y, Xie X, Lu Z. miRNA in plasma exosome is stable under different storage conditions. *Molecules.* 2014;19:1568-75.
10. Jan AT, Rahman S, Khan S, Tasduq SA, Choi I. Biology, Pathophysiological Role, and Clinical Implications of Exosomes: A Critical Appraisal. *Cells.* 2019;8.
11. Zhang Y, Hu YW, Zheng L, Wang Q. Characteristics and Roles of Exosomes in Cardiovascular Disease. *DNA Cell Biol.* 2017;36:202-11.
12. Li S, Li Y, Chen B, Zhao J, Yu S, Tang Y, Zheng Q, Li Y, Wang P, He X, et al. exoRBase: a database of circRNA, lncRNA and mRNA in human blood exosomes. *Nucleic Acids Res.* 2018;46:D106-d12.
13. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* 2003;13:2498-504.
14. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet.* 2000;25:25-9.

15. Huang DW, Sherman BT, Tan Q, Collins JR, Alvord WG, Roayaei J, Stephens R, Baseler MW, Lane HC, Lempicki RA. The DAVID Gene Functional Classification Tool: a novel biological module-centric algorithm to functionally analyze large gene lists. *Genome Biol.* 2007;8:R183.
16. Xie C, Mao X, Huang J, Ding Y, Wu J, Dong S, Kong L, Gao G, Li CY, Wei L. KOBAS 2.0: a web server for annotation and identification of enriched pathways and diseases. *Nucleic Acids Res.* 2011;39:W316-22.
17. Agarwal V, Bell GW, Nam JW, Bartel DP. Predicting effective microRNA target sites in mammalian mRNAs. *Elife.* 2015;4.
18. Chen Y, Wang X. miRDB: an online database for prediction of functional microRNA targets. *Nucleic Acids Res.* 2020;48:D127-d31.
19. Jeggari A, Marks DS, Larsson E. miRcode: a map of putative microRNA target sites in the long non-coding transcriptome. *Bioinformatics.* 2012;28:2062-3.
20. Yang J-H, Li J-H, Shao P, Zhou H, Chen Y-Q, Qu L-H. starBase: a database for exploring microRNA-mRNA interaction maps from Argonaute CLIP-Seq and Degradome-Seq data. *Nucleic acids research.* 2011;39:D202-D9.
21. Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? *Cell.* 2011;146:353-8.
22. Kalluri R, Lebleu VS. The biology, function, and biomedical applications of exosomes. *Science.* 2020;367.
23. Wang L, Shen C, Wang Y, Zou T, Zhu H, Lu X, Li L, Yang B, Chen J, Chen S, et al. Identification of circular RNA Hsa\_circ\_0001879 and Hsa\_circ\_0004104 as novel biomarkers for coronary artery disease. *Atherosclerosis.* 2019;286:88-96.
24. Lopez-Castejon G. Control of the inflammasome by the ubiquitin system. *Febs j.* 2020;287:11-26.
25. Grumati P, Dikic I. Ubiquitin signaling and autophagy. *J Biol Chem.* 2018;293:5404-13.
26. Ji Y, Yao J, Zhao Y, Zhai J, Weng Z, He Y. Extracellular ubiquitin levels are increased in coronary heart disease and associated with the severity of the disease. *Scand J Clin Lab Invest.* 2020;80:256-64.
27. Dellago H, Bobbili MR, Grillari J. MicroRNA-17-5p: At the Crossroads of Cancer and Aging - A Mini-Review. *Gerontology.* 2017;63:20-8.
28. Liu G, Hao P, Xu J, Wang L, Wang Y, Han R, Ying M, Sui S, Liu J, Li X. Upregulation of microRNA-17-5p contributes to hypoxia-induced proliferation in human pulmonary artery smooth muscle cells through modulation of p21 and PTEN. *Respir Res.* 2018;19:200.
29. Yang S, Fan T, Hu Q, Xu W, Yang J, Xu C, Zhang B, Chen J, Jiang H. Downregulation of microRNA-17-5p improves cardiac function after myocardial infarction via attenuation of apoptosis in endothelial cells. *Mol Genet Genomics.* 2018;293:883-94.
30. Xue S, Liu D, Zhu W, Su Z, Zhang L, Zhou C, Li P. Circulating MiR-17-5p, MiR-126-5p and MiR-145-3p Are Novel Biomarkers for Diagnosis of Acute Myocardial Infarction. *Front Physiol.* 2019;10:123.

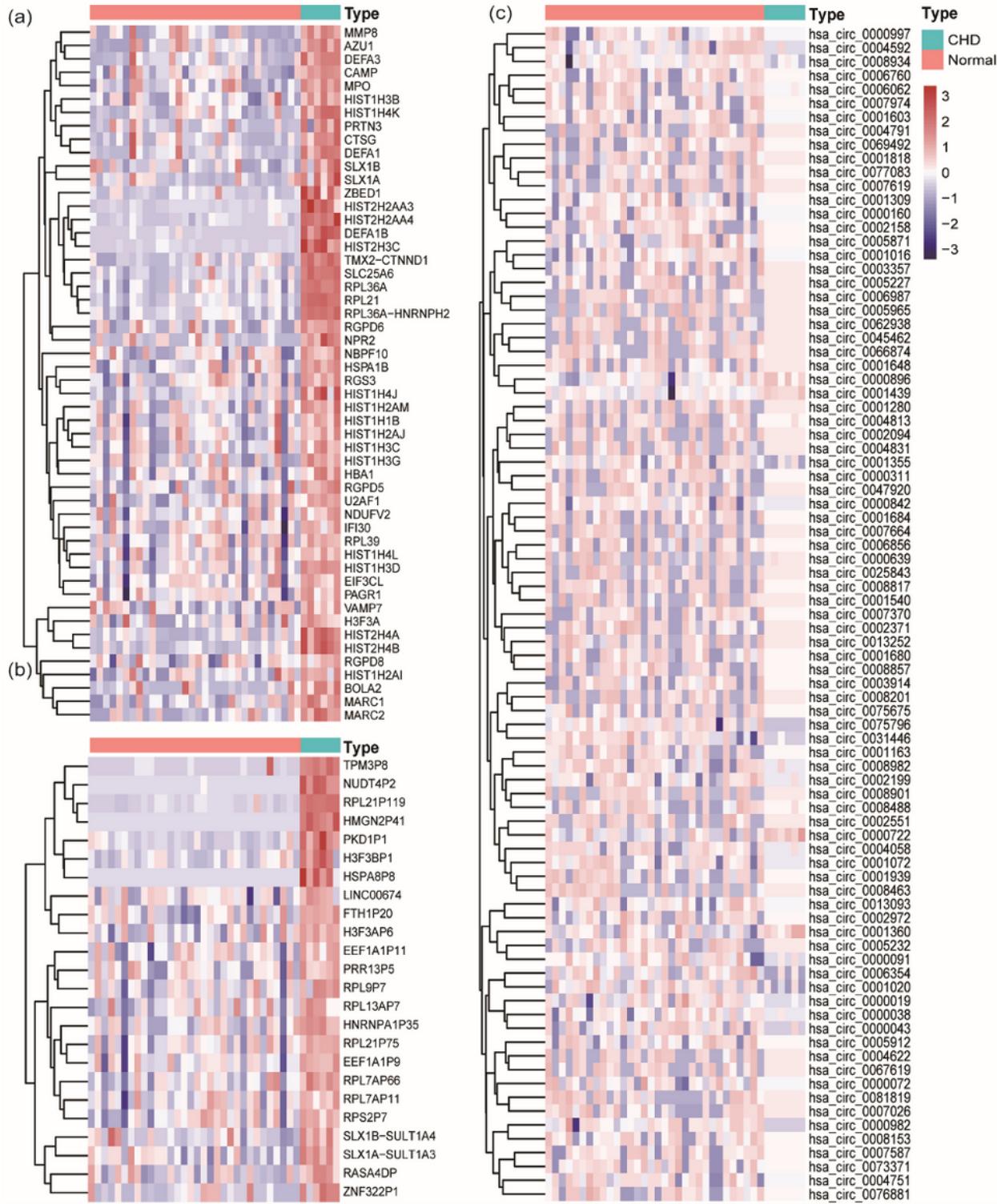
31. Zhou Z, Chen S, Tian Z, Deng S, Yi X, Yang S, Yang X, Jin L, Shi W. miR-20b-5p attenuates hypoxia-induced apoptosis in cardiomyocytes via the HIF-1 $\alpha$ /NF- $\kappa$ B pathway. *Acta Biochim Biophys Sin (Shanghai)*. 2020;52:927-34.
32. Zhen W, Hui D, Wenyi S, Yulong S. MicroRNA-20b-5p regulates propofol-preconditioning-induced inhibition of autophagy in hypoxia-and-reoxygenation-stimulated endothelial cells. *J Biosci*. 2020;45.
33. Zhang W, Chen B, Zeng W, Bai L, Xue H, Wu G. [Differentially expressed genes in vascular endothelial cell line ECV 304 induced by high density lipoprotein]. *Zhonghua Yi Xue Za Zhi*. 2002;82:921-3.
34. Lou W, Ding B, Fu P. Pseudogene-Derived lncRNAs and Their miRNA Sponging Mechanism in Human Cancer. *Front Cell Dev Biol*. 2020;8:85.

## Figures



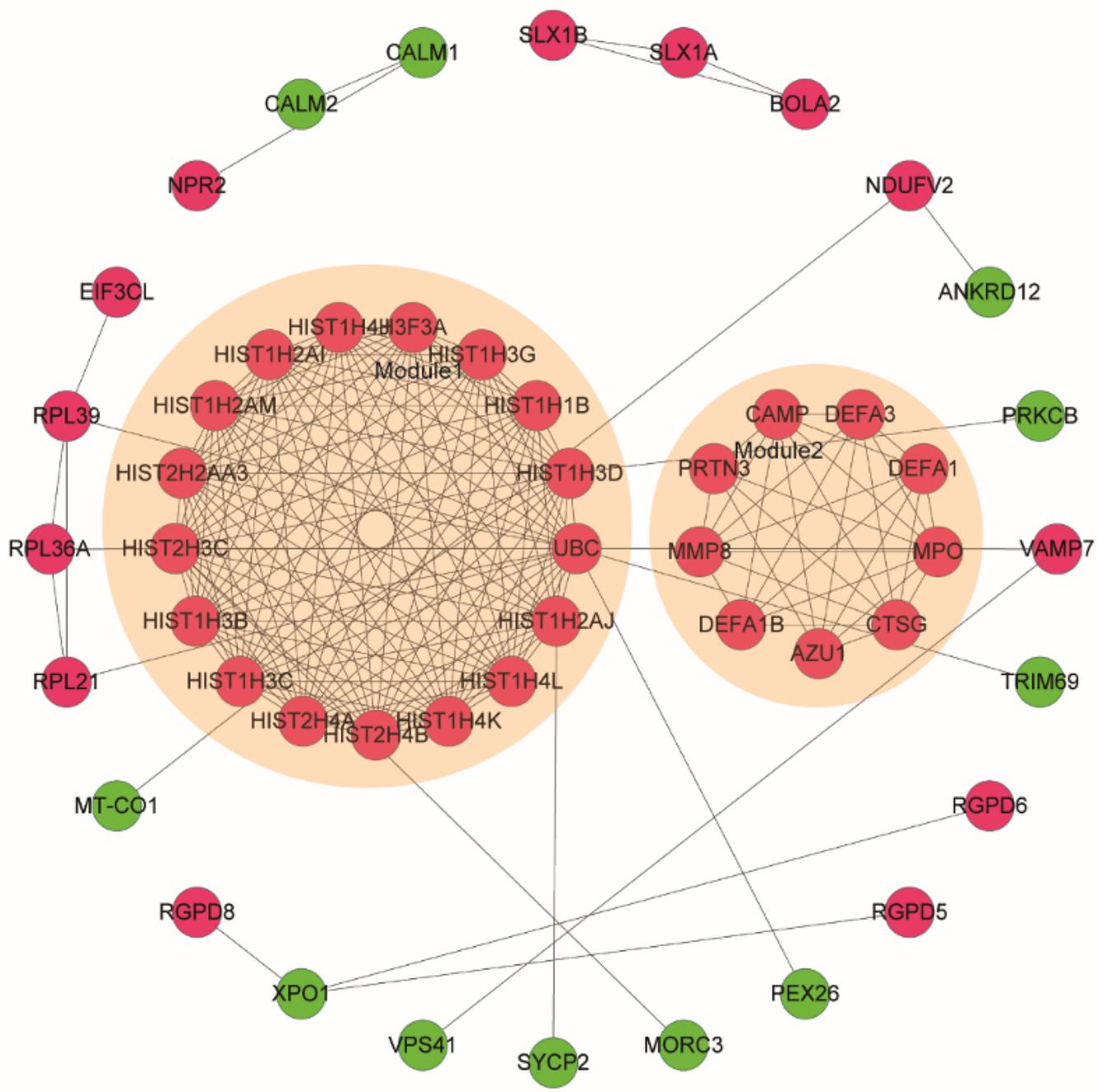
**Figure 1**

Study flowchart. Abbreviations: CHD, coronary artery disease; mRNAs, messenger RNAs; miRNAs, microRNAs; lncRNAs, long noncoding RNAs; circRNAs, circular RNAs; DEMs, differentially expressed mRNAs; DELs, differentially expressed lncRNAs; DECs, differentially expressed circRNAs; ceRNA, competing endogenous RNA.



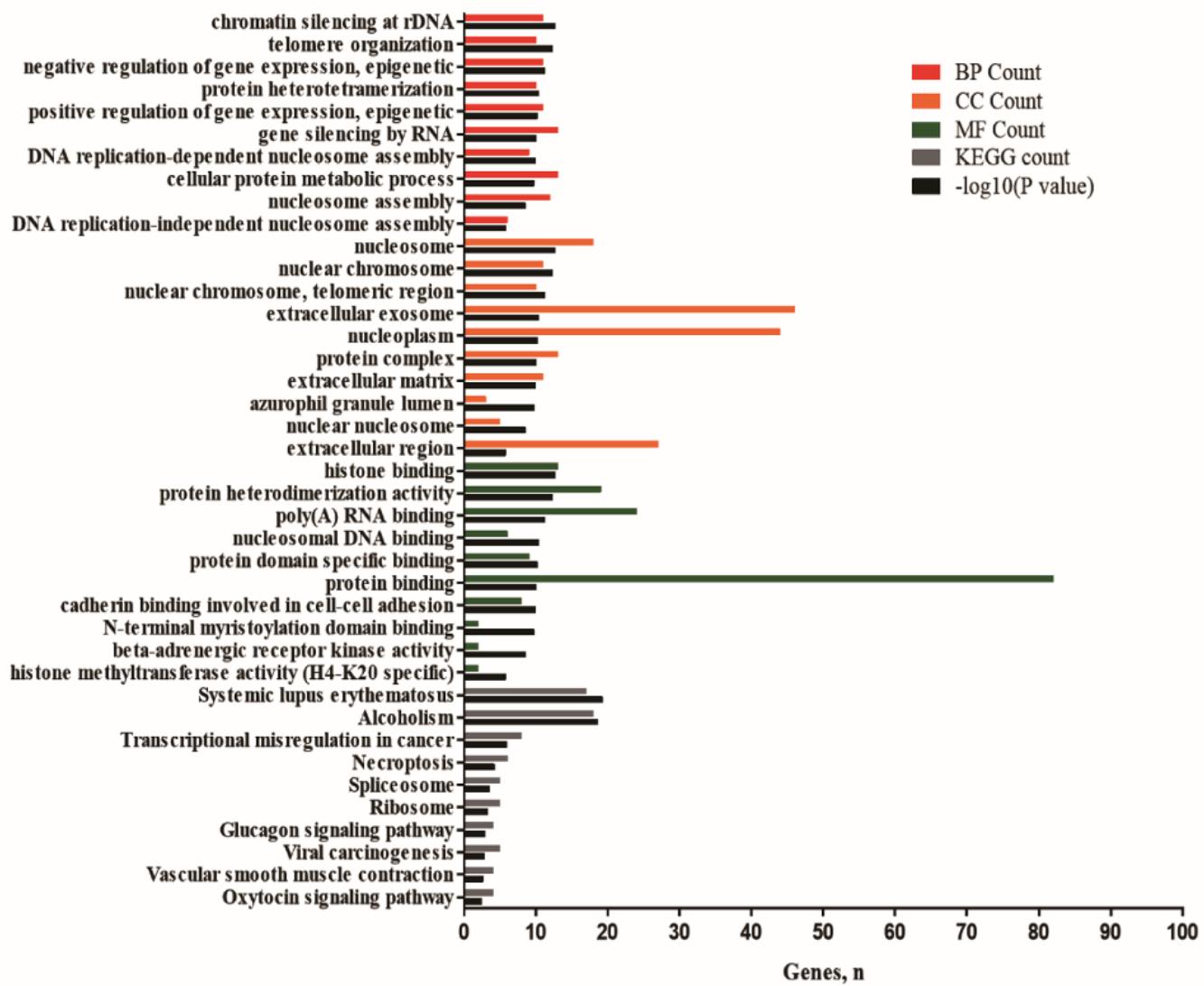
**Figure 2**

Hierarchical clustering and heat map analysis of differentially expressed profiles of exosomal RNAs. (a)mRNAs, (b) lncRNAs, (c) circRNAs. The color scale indicates the expression of differentially expressed exosomal RNAs. Red and blue indicate up- and down-regulation, respectively. Abbreviations: See Fig. 1.



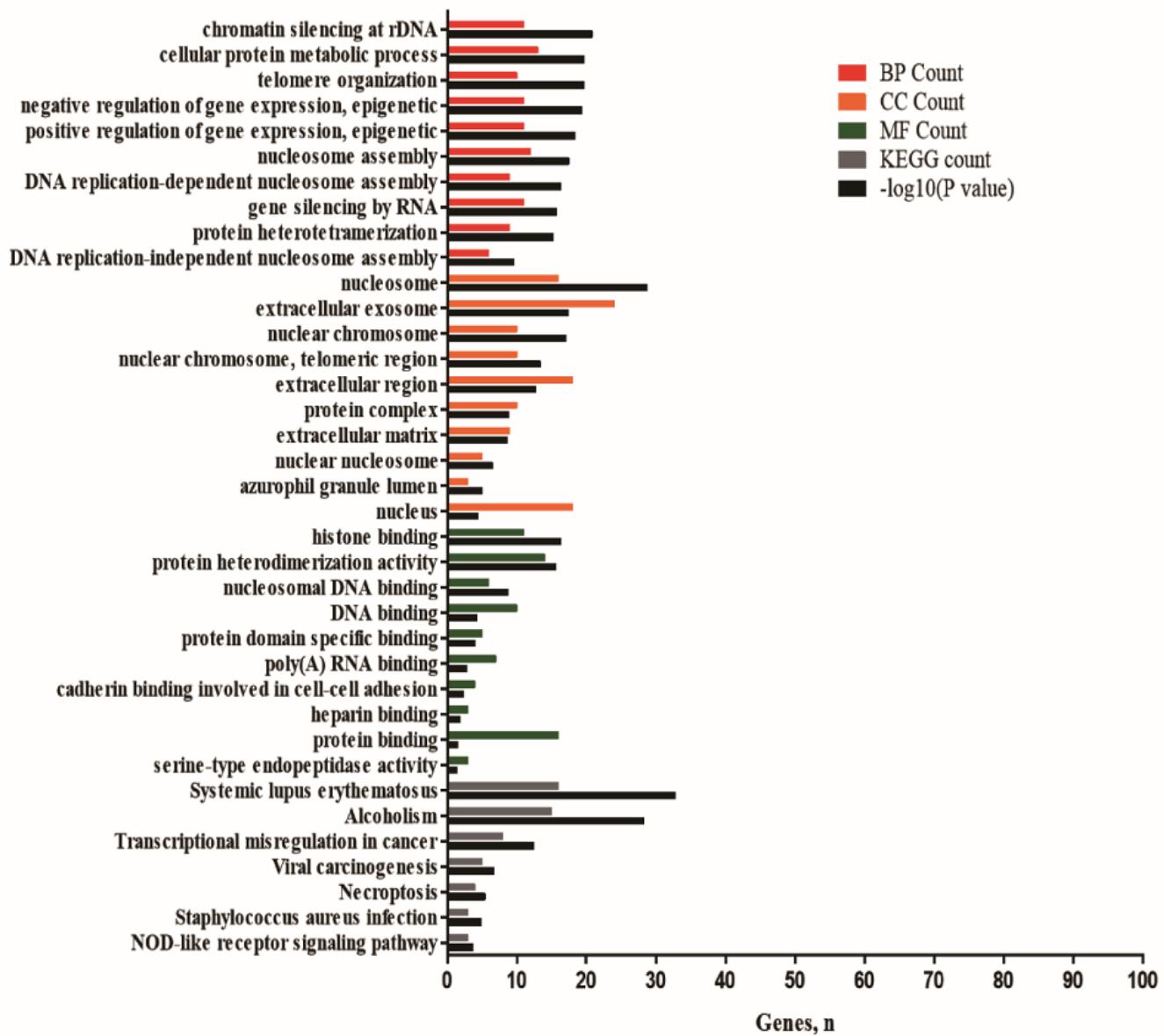
**Figure 3**

Protein-protein interaction (PPI) network and the significant modules. Notes: The red nodes represent the up-regulated mRNAs and the green nodes represent the down-regulated mRNAs. The most significant module identified by MCODE (score = 16.875). The second significant module identified by MCODE (score = 7.429). Abbreviations: MCODE: molecular complex detection; Others: See Fig. 1.



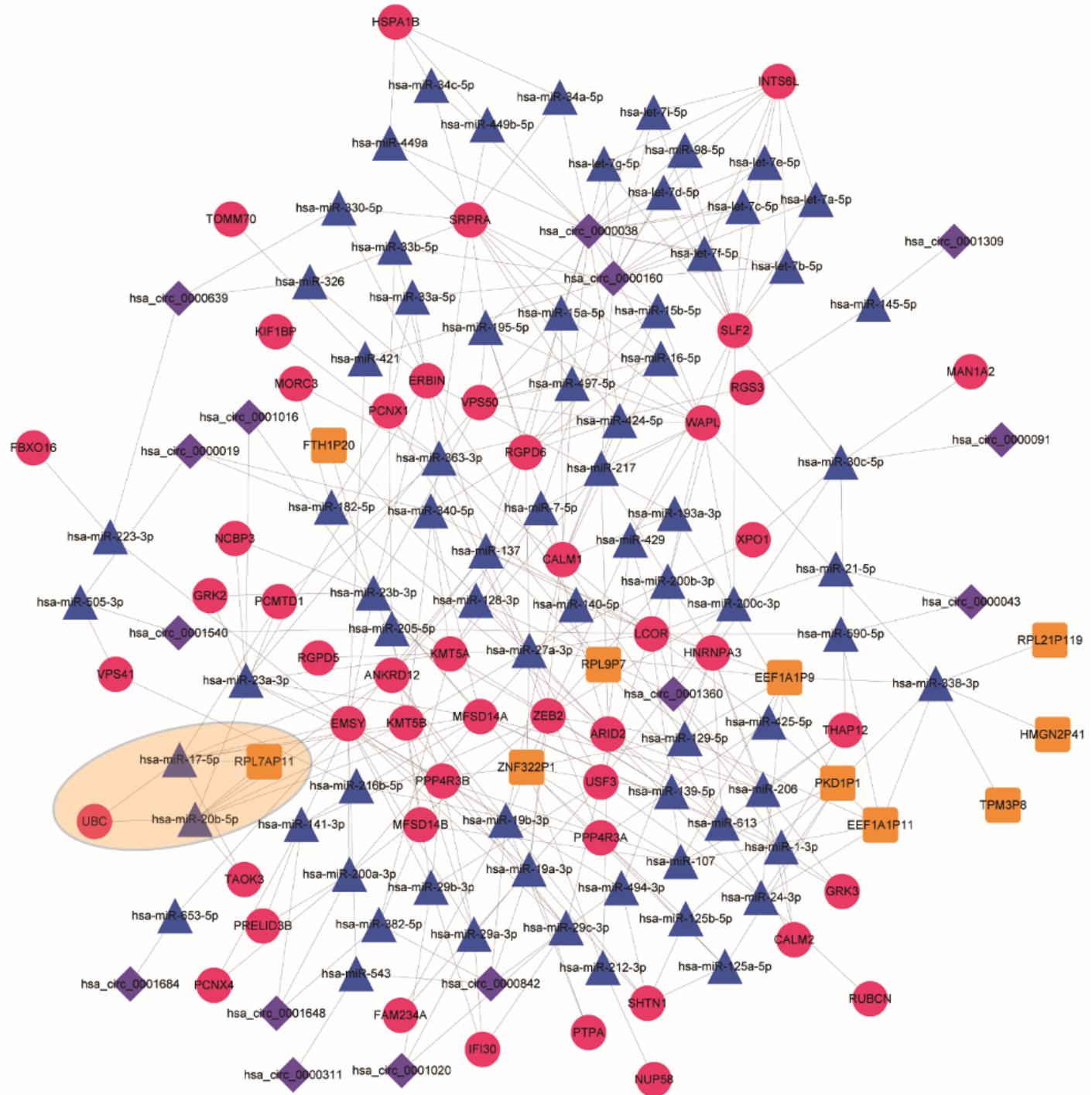
**Figure 4**

The functional terms and pathways enriched for the differentially expressed mRNAs. Notes: The red lines represent biological process; The orange lines represent cellular component; The green lines represent molecular function; The black lines represent KEGG pathway; The gray lines represent  $-\log_{10}(P\text{-value})$ . Abbreviations: KEGG, Kyoto Encyclopedia of Genes and Genomes.



**Figure 5**

Functional and pathway enrichment analyses of hub genes selected from protein-protein interaction network. Notes: The red lines represent biological process; The orange lines represent cellular component; The green lines represent molecular function; The black lines represent KEGG pathway; The gray lines represent  $-\log_{10}(P\text{-value})$ . Abbreviation: See Fig. 5.



**Figure 6**

Competing endogenous RNA (ceRNA) interaction network. Notes: Red circle represents mRNAs; Blue triangle represents miRNAs; Brown hexagon represents lncRNAs. Purple diamond represents circRNAs. Abbreviations: See Fig. 1.

# Supplementary Files

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