Expression profiles of long noncoding RNAs in type 2 diabetes mellitus rat associated with the progression of ischemia-reperfursion injury

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Research article

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Abstract

Background

The aim of this study was to analyze the expressions of long noncoding RNA(lncRNA) in rat with type 2 diabetes mellitus(T2DM) complicated with acute myocardial ischemia reperfusion injury(IRI).

Methods

Type 2 diabetic rats were induced by high calorie diet combined with streptozotocin. IRI rats models were established by the ligation and release of left anterior descending coronary artery(LAD). The expression levels of lncRNA and mRNA in myocardial tissues of rats were detected via high-throughput sequencing technology, and Gene Ontology analysis and Kyoto Encyclopedia of Genes and Genomes pathway analysis were performed.

Result

Transcriptome analyses were performed to show expression profiles of mRNAs and lncRNAs in myocardial tissues of diabetic rats with IRI. A total of 2,476 lncRNAs and 710 mRNAs were differentially expressed between operation group and sham operation group. Then, an mRNA-lncRNA coexpression network was constructed. Finally, the present study verified that TCONS_00036439、TCONS_00151548、TCONS_00153276、TCONS_00344188、TCONS_00277692、TCONS_00236469、TCONS_00236468、TCONS_00153290、TCONS_00360941、TCONS_00142622 were associated with the initiation and development of ischemia reperfusion injury. Then, an lncRNA-mRNA coexpression network was constructed.

Conclusion

There is differential expression of lncRNAs in myocardial IRI tissues of diabetic rats. Building gene regulation networks to find the nodal gene and lncRNA is useful for understanding the pathogenesis of type 2 diabetes mellitus complicated with acute myocardial ischemia reperfusion injury and providing new therapy target.

Background

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia caused by insulin secretion deficiency and/or insulin action disorder. Its common complications include kidney, nerve, heart and blood vessel damage and dysfunction resulting in failure[1]. According to the latest data of the International Diabetes Federation, the number of adult diabetic patients worldwide reached 451 million in 2017, and the number of patients in 2045 will be 693 million[2]. Myocardial infarction is an important disease that endangers human health worldwide and is the most common cause of death[3]. Paradoxically, myocardial reperfusion itself causes myocardial cell death and increases infarct size, which is called myocardial ischemia reperfusion (I/R) injury[4]. Diabetes is an independent risk factor for all-cause death in myocardial infarction[5]. Compared with non-DM patients, DM patients have a higher incidence of I/R injury and more severe myocardial I/R damage[3]. Long non-coding RNAs (lncRNAs) are most commonly defined as the transcripts of more than 200 nucleotides that structurally resemble mRNAs but have no protein-coding capacity[6].The main functions of lncRNAs involve regulation of gene methylation, transcriptional activation and conjugation with mRNAs and miRNAs to affect translation progression[7].Multiple lines of evidence have linked lncRNA mutations and dysregulation with diverse human diseases, ranging from different types of cancer to cardiovascular diseases[8, 9]. However, the expression patterns, targets, and functions of lncRNAs involved in the pathogenesis of myocardial I/R injury remain to be elucidated. Therefore, further research is of great importance.

In the present study, we applied high-throughput sequencing technology to analyse the expression profiles of lncRNAs and messenger RNAs (mRNAs) in the myocardial tissues of diabetic rats in a validated IRI model. This may help to understand the initiation and development mechanism underlying diabetes mellitus complicated with IRI and provide the basis for further research.

Methods

Animal

All animal experimental procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Ethics Committee of the Zhongnan Hospital of Wuhan University (Wuhan, China). A total of 6

SPF grade of 6 weeks male Wistar rats (215 ± 25 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. Type 2 diabetic group(n = 5)was fed with high calorie diet（10% lard, 20% sucrose, 2.5% cholesterol, 1.0% cholate, 66.5% routine feed）for 8 weeks, and non-diabetic group（n = 1）was fed with normal diet for 8 weeks. Type 2 diabetic group was treated with intraperitoneal injection of 1% STZ(45 mg/kg).Fasting blood glucose over 16.7 mmol/L proved successful Type 2 diabetic rats model. Type 2 diabetic group was then divided into 2 sub-groups: IRI group（n = 3）and sham group（n = 2）. The sham group include 2 T2DM rats and 1 non-diabetic rat. The rats were anesthetized by using intraperitoneal injection of chloral hydrate（10%）with an initial dose of 0.35 ml/100 g.The trachea was cannulated and the rats were ventilated with a ALC-V8S Rodent Ventilator (Shanghai Alcott Biotech CO., LTD.) and were electrocardiogram monitored with BL-420N information data acquisition and processing system（chengdu techman software co. ltd）before the thoracotomy was performed on the left side in the fourth inter-costal space to remove the pericardium. The IRI group was then subjected to 30 min of LAD ligation followed by 120 min of reperfusion. Myocardial ischemia was confirmed by changes in electrocardiogram and epicardial cyanosis. Sham operated animals underwent the same procedure without occlusion of the LAD. Animals were sacrificed via decapitation at reperfusion time (2 h after 30 min ischemia). Samples were collected for RNA analysis from infarct region and were stored in liquid nitrogen followed by storage at -80 °C prior to analysis.

RNA Extraction

Total RNA was extracted from each myocardial tissue sample using TRIzol Reagent(Invitrogen, Grand Island, NY, USA). RNA concentration and purity were estimated using NanodropTM OneC spectrophotometer(Thermo Fisher Scientific Inc), and RNA integrity was assessed via standard denaturing agarose gel electrophoresis.

High-throughput sequencing analysis

High-throughput sequencing was completed by Wuhan Seqhealth Tech Co. Ltd.After total RNA extraction, magnetic beads with Oligo(dT) was used to remove rRNA and Fragmentation Buffer added to achieve RNA fragmentation (average fragment length of approximately 200 nt). Double-stranded cDNA is synthesized and the adapters for sequencing are added to construct the final library. After the quality inspection of the library, six sets of samples (three vs. three) were sequenced using the Illumina HiSeq X Ten sequencing platform (Illumina, San Diego, CA, USA). The sequence data were processed by bioinformatics in the later stage.The datasets generated were mapped to the rat genome assembly Rnor_6.0 (ftp://ftp.ensembl.org/pub/release-87/fasta/rattus_norvegicus/dna/).

Gene Ontology and Kyoto Encyclopedia of Genes and Genomes analysis

Gene Ontology(GO) analysis was applied to annotate differentially expressed genes from cell composition, molecular function, and biological processes[10]. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis was performed to identify which biochemical metabolic pathways and signal transduction pathways were involved in differentially expressed genes[11]. KEGG analysis was also used to provide differentially expressed mRNAs with background knowledge of gene pathways and functional studies. In the GO and KEGG analyses, P value < 0.05 was used as the screening standard.

Construction of lncRNA–mRNA coexpression network

Gene co-expression networks were presented to identify interactions among differentially expressed lncRNAs and mRNAs. In brief, gene co-expression networks were based on the normalized signal intensity of mRNA and lncRNA expression levels. Pearson's correlation co-efficients (PCC) were used in this study to calculate gene pair correlation based on gene expression. Each gene corresponds to a node in this network. Pearson’s correlation coefficients equal to or greater than 0.8 were used to identify the lncRNAs and coding genes. Two genes connected by an edge indicate a strong correlation. A degree was the simplest and most important measure of the centrality of a gene within a network and determines the relative importance. A degree was defined as the number of directly linked neighbors. Then, the co-expression network was constructed using Cytoscape software (The Cytoscape Consortium, San Diego,CA, USA).

Statistical analysis

The data were analyzed using SPSS software, version 25.0 (SPSS, Inc., Chicago, IL, USA). Differential expression levels of lncRNAs were compared using an independent-samples t-test, between two groups. Student’s t test was performed for comparisons between two groups, whereas ANOVA was performed for repeated measures. Differences with $p < 0.05$ were considered statistically significant. Fold change (FC) and Student’s t test were used to analyse the statistical significance of the sequencing results. $|\log2(\text{fold change})| \geq 1$ and $p < 0.05$ were considered the threshold values for designating differentially expressed lncRNAs and mRNAs. Pearson correlation analysis was used to calculate the correlation coefficients between differentially expressed lncRNAs and mRNAs.

Results

The present study compared the expression levels of lncRNAs and mRNAs in IRI rats relative to those in control rats by Second-generation sequencing technology. It was found that a total of 2,476 lncRNAs (1405 significantly upregulated and 1071 significantly upregulated) and 710

mRNAs (548 significantly upregulated and 162 significantly downregulated) were differentially expressed(≥ 2.0 fold, $p < 0.05$). The top 10 most differentially expressed lncRNAs are shown in Table 1. We used heat maps (Fig. 1)to show the relationships among lncRNA and mRNA expression patterns between IRI and the control groups.

Table 1
Top 10 aberrantly expressed lncRNAs (|log FC|>3.0,P Value<0.01)

Geneid	logFC	P
TCONS_00036439	3.867595	0.000631
TCONS_00151548	3.239359	0.003414
TCONS_00153276	3.239359	0.003414
TCONS_00344188	3.075179	0.000196
TCONS_00277692	3.026325	4.12E-05
TCONS_00236469	3.006901	0.000381
TCONS_00236468	3.000881	0.000394
TCONS_00153290	-3.241050	0.007422
TCONS_00360941	-3.351970	0.007429
TCONS_00142622	-3.469290	0.006219

Gene Ontology and KEGG Pathway Analyses

GO analysis was performed to annotate the transcripts with terms under the biological process, cellular component, and molecular function ontologies.The top 20 GO terms are listed in Fig. 2. The most enriched GO terms were inflammatory response (ontology: biological process, GO:0006954), sarcomere(ontology: cellular component, GO:0030017) and outward rectifier potassium channel activity (ontology:molecular function,GO:0015271). Additionally, The KEGG pathway analysis data are also listed in Fig. 3. Strikingly, The KEGG pathway revealed that most target genes were located in Cytokine-cytokine receptor interaction,JAK-STAT signaling pathway,TNF signaling pathway,glucolipid metabolism, among others.

lncRNA-mRNA coexpression network

As shown in Fig. 4, the network included the top 10 most differentially expressed lncRNAs and 444 mRNAs (PCC>0.8 or PCC<-0.8).Our date showed that the coexpression network was composed of 454 network nodes and 782 connections. There were 151 negative and 631 positive interactions within the network. It was found that one mRNA may bind to 1–5 lncRNAs and one lncRNA may bind to 1–114 mRNAs.

Discussion

Diabetes mellitus is an important risk factor to increase the risk of cardiovascular diseases. According to previous research[12–14],a series of pathophysiological changes caused by acute myocardial infarction in diabetic patients significantly increase the incidence and mortality of heart failure. The cause of this series of pathophysiological changes is still unclear, and studies have shown that different gene expression has a great impact on the prognosis of DM after acute myocardial ischemia[15].LncRNA, a subclass of non-coding RNA, participates in many physiological and pathological regulatory processes of the body, and has been widely studied and applied in oncology. However, the research on myocardial I/R injury have received relatively little attention, and the complex mechanism and signal pathway involved in lncRNA still need to be further clarified. Yang et al [16]confirmed that the expression of lncRNA Kcnq1ot1 was increased in cardiomyocytes induced by high glucose and cardiac tissues of DM mouse. Silencing Kcnq1ot1 moderated pyroptosis, reduced cell death, improved cytoskeleton structure abnormalities and calcium overload in vitro, and improved heart function and morphology in vivo. Another recent finding found that the expression of a novel lncRNA, myocardial infarction associated transcript 1(Mirt1) was upregulated in acute myocardial infarction. Knockdown of Mirt1 improved cardiac functions, decreased cardiomyocytes apoptosis, attenuated inflammatory cell infiltration in vivo, and inhibited NF-κB signaling pathway in vitro[17].

In the present study, we found 2,476 lncRNAs and 710 mRNAs using high-throughput sequencing analysis. The differentially expressed lncRNAs were selected as described previously,TCONS_00036439,TCONS_00151548,TCONS_00153276,TCONS_00344188,TCONS_00277692,TCONS_00236469,TCONS_00236468 were markedly upregulated. Conversely, TCONS_00153290,TCONS_00360941,TCONS_00142622 were downregulated. To predict the potential

roles of the differentially expressed lncRNAs,we performed GO and pathway analysis to identify the biological functions and mechanisms of the coding genes associated with the significantly differentially expressed lncRNAs. It revealed that the dysregulated transcripts were most highly enriched in inflammatory response, immune response, response to hypoxia, sarcomere, outward rectifier potassium channel activity GO terms. Additionally, we used KEGG pathway analysis and found that these lncRNAs are involved in Cytokine-cytokine receptor interaction, JAK-STAT signaling pathway ,TNF signaling pathway, glucolipid metabolism. Furthermore, a gene co-expression network was constructed to identify correlated targets of highly-regulated lncRNAs. Some of the targets mentioned here have been reported to be related to ischemia-reperfusion injury: as for Sestrin2 has been redefined as an LKB1-AMPK scaffold to initiate AMPK activation in the ischemic heart;AGO1 was targeted by hypoxia-responsive microRNAs to enhance angiogenesis in vitro and in vivo. To further investigate the inter-regulation of lncRNAs and mRNAs involved in IRI, function gain or loss experiments are needed.

However,there are some disadvantages in our study. First, we only predicted lncRNA functions through lncRNA-mRNA co-expression network analysis. Second,high-throughput sequencing technology faces several challenges,including complex library construction and efficient methods to store, retrieve and process large amounts of data. Third,larger sample sizes are required to verify the results in future research.

Conclusion

In this study, we have performed an exploratory analysis on the expression of lncRNAs in myocardial IRI tissues and investigated some of their potential roles. Further research are required to elucidate the biological functions, molecular mechanisms and signaling pathways of the significantly dysregulated lncRNAs.

Abbreviations

lncRNA：long noncoding RNA ；T2DM：type 2 diabetes mellitus；IRI: ischemia reperfusion injury；LAD: left anterior descending coronary artery； DM:diabetes mellitus; I/R: ischemia reperfusion;mRNAs:messenger RNAs;STZ: Streptozocin;GO: Gene Ontology;KEGG: Kyoto Encyclopedia of Genes and Genomes;FC: fold change; PCC:Pearson's correlation co-efficients;Mrit1: myocardial infarction associated transcript 1;

Declarations

Ethics approval and consent to participate

All animal experimental procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals .All rats care and procedures were approved by the Ethics Committee for experimental animals, Zhongnan Hospital of Wuhan University，Hubei,China Grant/Award Number:2018050．

Consent for Publication

Not applicable.

Availability of data and materials

The data from this study was deposited in NCBI Sequence Read Archive under accession SRA: PRJNA627896.

Competing interests

The authors have no conflicts of interest to disclose.

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Authors' contributions

WS, PT, YG and HW conceived and designed the study. W S, PT, Y G analyzed the results. WS and YG performed the experiments. LZ and ZJ contributed analysis tools. WS wrote the manuscript. All authors have read and approved the final manuscript.

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Not applicable.

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Figures

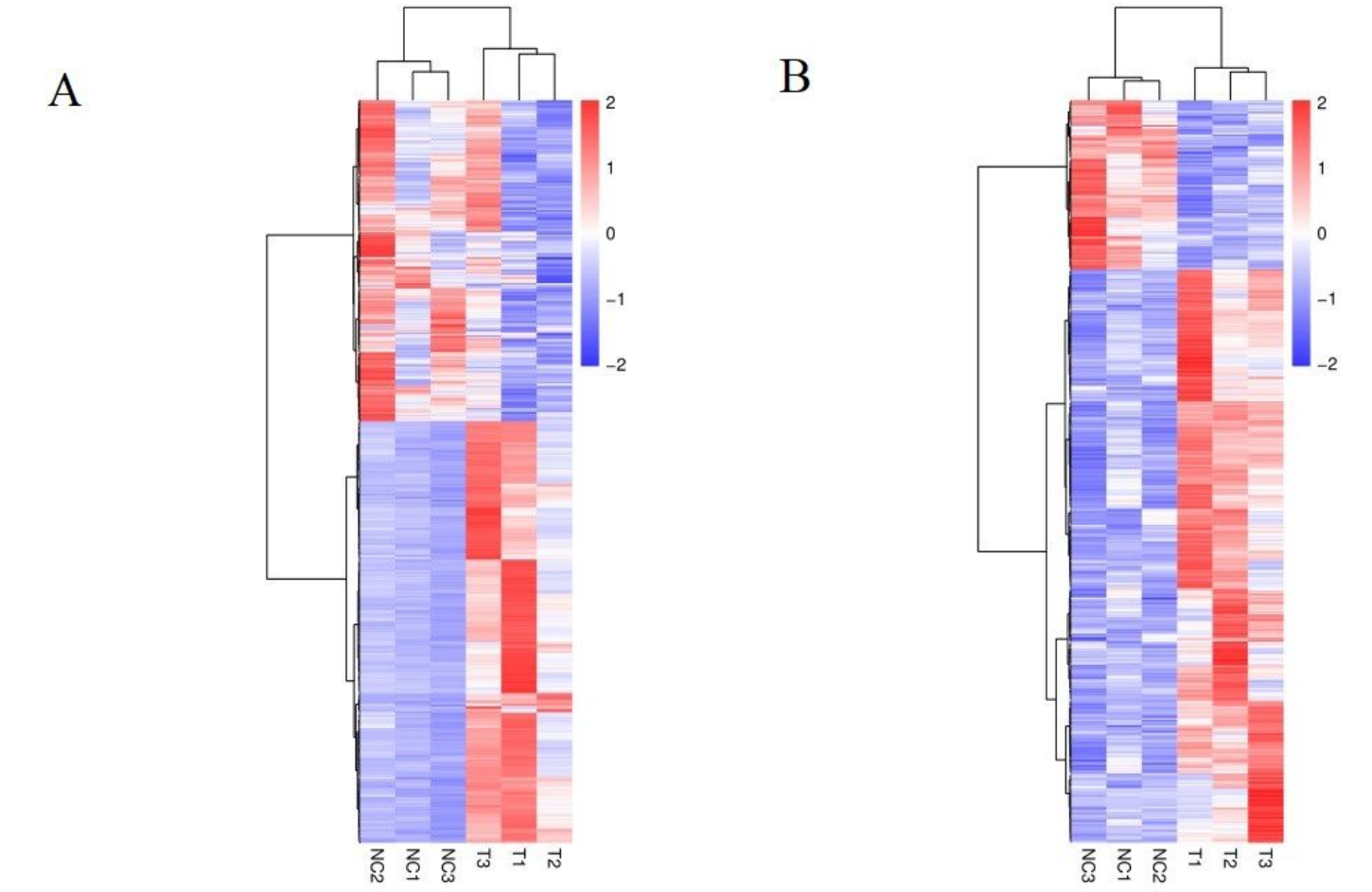

Figure 1

The heat map and hierarchical clustering of differentially expressed lncRNAs (A) and mRNAs (B) between IRI group and sham group. “Red” denotes high relative expression and “Green” denotes low relative expression. −2.0, 0 and 2.0 are FCs in the corresponding spectrum.

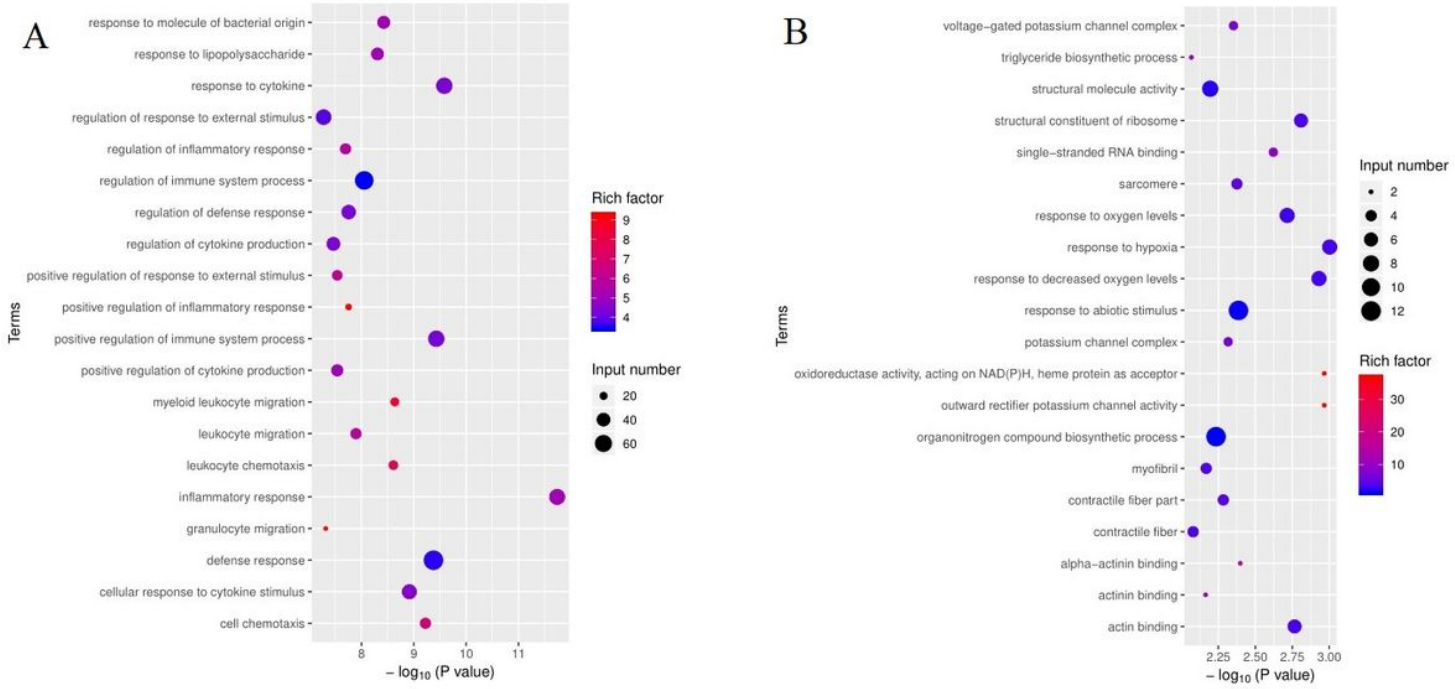

Figure 2

Top 20 significantly upregulated (A)and downregulated (B) terms.

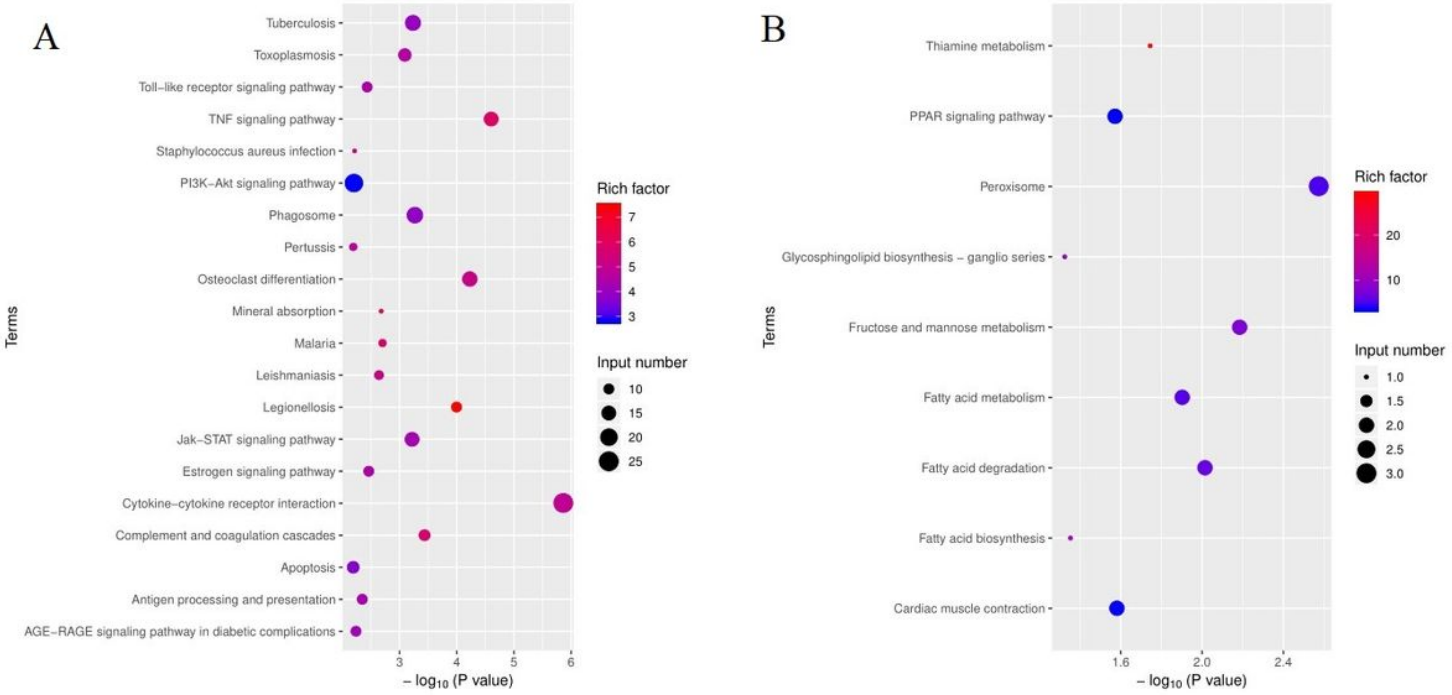

Figure 3

KEGG pathway analysis of differentially expressed mRNAs. Top 20 significantly upregulated (A)and top 9 downresulated (B)pathways.

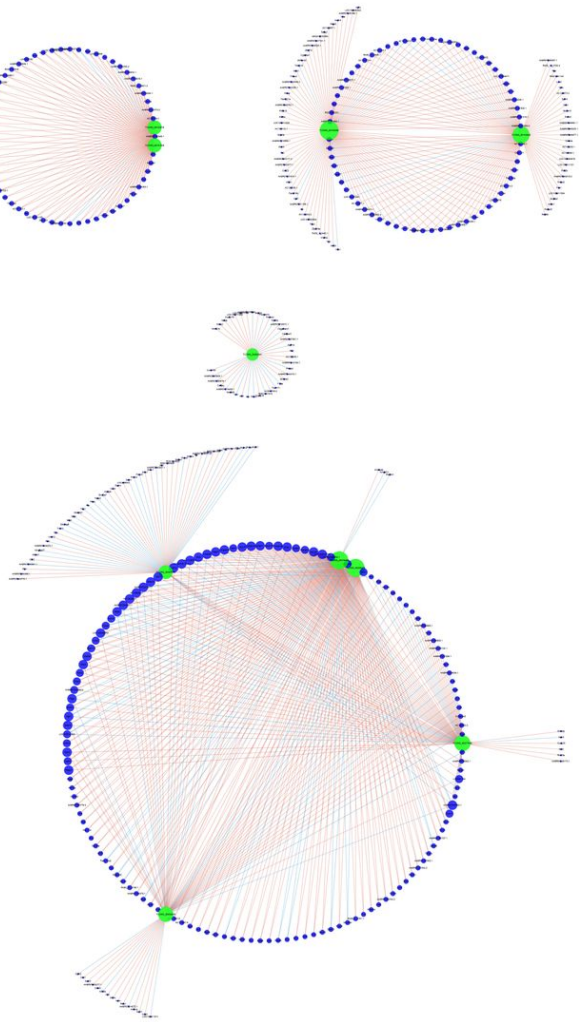

Figure 4

lncRNA-mRNA-network analysis. Blue nodes represent dysregulated mRNAs, green nodes represent dysregulated lncRNAs. The cyan lines between lncRNAs and mRNAs indicate a negative correlation, while the pink lines indicate a positive correlation.

Supplementary Files

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