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Expression profiles of long noncoding RNAs and mRNAs in peripheral blood mononuclear cells of patients with acute myocardial infarction

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Abstract

Acute myocardial infarction (AMI) is the most serious type of coronary atherosclerotic diseases. The incidence of AMI in some countries increases year by year, and shows younger trend. Some study indicated that abnormal expression of lncRNAs was closely related to cardiovascular disease. The aim of this study was to examine the lncRNA expression profiles in peripheral blood mononuclear cells (PBMCs) of patients with AMI through controlled studies.

In the present study, we examined the lncRNA and mRNA expression profiles in 8 patients with AMI, with 7 NCA (noncoronary artery) subjects as controls using RNA sequencing protocol (RNA-seq) on the Illumina HiSeq 4000 platform. The differentially expressed lncRNAs were selected for bioinformatic analysis including gene ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes pathway (KEGG). Quantitative real time PCR (qRT-PCR) was used to confirm the differential expression of lncRNAs.

We kept about 11.29 gigabase (Gb) high-quality sequence data while the Q30 ranged from 94.39% to 95.19% for each sample. Compared to the lncRNA expression profiles of NCA controls, a total of 106 differentially expressed lncRNAs were discriminated in AMI patients, including 40 upregulated lncRNAs and 66 downregulated lncRNAs ($P < .05$). Among the genes corresponding to the identified mRNAs, 2905 genes are involved in biological processes, 339 in cellular components, and 501 in molecular functions. Based on the KEGG pathway analysis, the most enriched pathways corresponding to the differentially expressed lncRNAs were associated with systemic lupus erythematosus, alcoholism, oxidative phosphorylation, Parkinson's disease and viral carcinogenesis, and so on. Further, 3 upregulated and 3 downregulated lncRNAs were randomly selected for qRT-PCR verification and the results of qRT-PCR were consistent with the findings obtained from RNA sequencing analysis.

As a result, differential expression profiles of lncRNAs in AMI were identified in our study. The results suggested that lncRNAs may play important roles in the biological and pathological processes of AMI. These findings may provide useful reference for the early diagnosis and risk stratification of AMI patients. To enlarge the sample size in the next step will be needed for further research to confirm our results.

Abbreviations: AMI = acute myocardial infarction, ANOVA = one-way analysis of variance, CAD = coronary artery disease, DAVID = Database for Annotation Visualization and Integrated Discovery, FC = fold change, GAPDH = glyceraldehyde 3-phosphate dehydrogenase, GO = gene ontology, HDL-C = high-density lipoprotein cholesterol, KEGG = Kyoto Encyclopedia of Genes and Genomes, LDL-C = low-density lipoprotein cholesterol, lnRNAs = long noncoding RNAs, NCA = noncoronary artery, ncRNAs = noncoding RNAs, ncRNAs = noncoding RNAs, PBMCs = peripheral blood mononuclear cells, qRT-PCR = quantitative real time PCR, STEMI = ST-segment elevation myocardial infarction, UA = unstable angina.

Keywords: acute myocardial infarction, bioinformatics analyses, LncRNA, peripheral blood mononuclear cells

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PZ and HW contributed equally to this work.

The authors have no conflicts of interest to disclose.

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1. Introduction

Cardiovascular disease is one of the major diseases that threaten human health. Acute coronary syndrome (ACS) is a group of clinical syndromes characterized by rupture or invasion of coronary atherosclerotic plaques secondary to complete or incomplete occlusive thrombosis, including ST segment elevation myocardial infarction (STEMI), non-ST segment elevation myocardial infarction (NSTEMI) and unstable angina (UA). STEMI and NSTEMI are collectively referred to as acute myocardial infarction (AMI).[1,2]

AMI has the characteristics of rapid onset, rapid course and high mortality. It is the most serious coronary atherosclerotic disease and the main cause of death from nononcological diseases in some countries. In recent years, the incidence of AMI in some countries rises year by year, and shows younger trend.[3,4] At present, the pathogenesis of cardiovascular disease has not yet been fully elucidated. Abnormal expression of inflammatory, protease, and apoptotic molecules was associated with damage to cardiomyocytes and cardiovascular disease.[5] It is well known that many of the risk factors leading to cardiovascular disease have been identified, including age, gender, smoking, alcohol abuse and comorbidities such as diabetes, dyslipidemia, arterial hypertension, and peripheral vascular disease.[6] Despite the high risk of AMI, we lack the effective predictive diagnosis.[7–9]

The occurrence and development of cardiovascular disease include the occurrence of vascular wall inflammation, vascular injury and plaque formation, which involve the release of molecules in the immune system.[10,11] A peripheral blood mononuclear cell (PBMC) is any peripheral blood cell having a round nucleus.[12] PBMCs include lymphocytes (T cells, B cells, and NK cells), monocytes, and dendritic cells. In humans, the frequencies of these populations vary across individuals. These cells can be further classified into various functional subtypes based on the expression profiles of specific cytokines, surface markers, or transcription factors. Human immune system studies rely heavily on the phenotypic and functional assessments of PBMCs. In order to take advantage of PBMCs for human immune studies, it is important to know what populations are represented in peripheral blood and how PBMC populations differ in distribution and function from tissue immune cells. Also it is critical to become familiar with the identifying surface and intracellular markers and the types of assays best suited for human PBMC studies.[13–15] So the expression profiles in peripheral blood mononuclear cells can correlate with AMI progression.

Long noncoding RNAs (lncRNAs) are noncoding RNAs (ncRNAs) with a transcript length of 200 nt and without protein coding function. lncRNAs were initially considered as the "noise" of genome transcription. Recent studies have shown that lncRNAs are closely related to X chromosome silencing, genomic imprinting, chromatin modification, transcriptional activation, transcriptional interference, and nuclear transport.[16–20] It engages in the regulation of the growth and development of the individual, the differentiation, proliferation, apoptosis of cells, as well as other life activities. Although the specific functions of lncRNAs have not been clarified, the studies have shown that the abnormal expression of lncRNAs were highly correlated to cardiovascular diseases.[21–23]

The aim of this study was to examine the lncRNAs expression profiles in peripheral blood mononuclear cells (PBMCs) of patients with AMI through controlled studies. According to the comparison of the lncRNAs expression profiles among 15 subjects, we desire to obtain a correlation between lncRNAs and AMI. These results will provide a useful reference for further exploration of the role of lncRNAs in the progression of AMI.

2. Materials and methods

2.1. Subjects

AMI was diagnosed by coronary angiography, dynamic evolution of electrocardiogram and dynamic changes of serum markers. The patients with ST segment elevation were diagnosed with ST segment elevation myocardial infarction, and those without ST segment elevation were diagnosed as non-ST segment elevation myocardial infarction. 15 subjects visited Meizhou People's Hospital located Guangdong province of China through February 2016 to April 2017 involved in this study, including 8 males and 7 females and aging from 43 to 68 years. Around 15 subjects were classified into 2 groups: NCA (noncoronary artery, 7 subjects) and AMI group (8 subjects). This study was performed in accordance with the Declaration of Helsinki, and was supported by the Ethics Committee of the Meizhou People's Hospital.

2.2. Samples collection and total RNA extraction

Around 3 mL of blood samples for the measurement of lipid levels were obtained from each subjects, plasma was separated and stored at −80 °C till further analysis. Plasma levels of triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were measured.

Whole blood samples (6 mL from peripheral venous blood) were collected from patients with AMI at the onset of symptoms and NCA controls. Blood samples were taken from antecubital vein and stored in vacuum tubes containing ethylenediaminetetraacetic acid (EDTA), on the upside down gently mix 10 times, immediately saved the blood in 4 °C. Plasma should be separated within one hour, and were centrifuged at 1500 r/min centrifugal 10 minutes to get the upper plasma samples, transferred the plasma to 1.5 mL RNA (RNasefree) centrifuge tube for extraction of RNA, packed stored at −80 °C.

Total RNA was extracted from the plasma using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The quantity and purity of total RNA were evaluated by Nanodrop 2000, and the RNA Nano 6000 Assay Kit of the Agilent Bioanalyzer 2100 system (Agilent Technologies, CA) was used to analyze RNA integrity.

2.3. Preparation for lncRNA sequencing library

A total amount of 3 μg RNA was utilized in the RNA sample preparations, strictly according to the manufacturer's protocol. Firstly, ribosomal RNA was removed by using an Epicentre RibozeroTM rRNA Removal Kit (Epicentre, Madison, WI), and residual RNAs were cleaned up using ethanol precipitation. Sequencing libraries were generated using the rRNA-depleted RNA with NEBNextUltraTM Directional RNA Library Prep Kit for Illumina (NEB). The RNA integrity was evaluated by using the RNA Nano 6000 Assay Kit of the Bioanalyser 2100 system (Agilent Technologies, CA). The libraries were sequenced on an Illumina Hiseq 4000 platform according to the commercially available protocols and 150 bp paired-end reads per sample were generated.

2.4. High throughput sequencing

Sequencing libraries were generated using NEBNext Multiplex Small RNA Library Prep Set for Illumina (NEB, USA.) according

to manufacturer's protocol. After the qualification of the library, the different libraries were sequenced in accordance with the requirement of the effective concentration and the amount of data of the machine under the target pooling, and then library sequencing was carried out on Illumina HiSeq 4000 platform according to the commercially available protocols in ShenZhen Realomics Inc.

2.5. Identification of differently expressed genes

The analysis of differences in lncRNA expression of 2 groups samples was performed using the DEGseq (2010) R package. *P*-value was adjusted using *q*-value. *q*-value < 0.05 and $|\log2(\text{foldchange})| > 1$ were set as the threshold for significantly differential expression by default.

2.6. Quantitative real-time polymerase chain reaction (qRT-PCR)

To validate the reliability of RNA sequencing data, differentially expressed lncRNAs were randomly selected and qRT-PCR was employed to examine the expression level of lncRNAs. Total RNA were extracted from the PBMCs using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. And qRT-PCR reactions were performed by using Luna Universal One-Step RT-qPCR kits (New England Biolabs, MA). The PCR reactions were carried out by the conditions: 15 s at 55 °C and 1 minute at 95 °C, followed with 40 cycles for 10 seconds at 95 °C and 30 seconds at 60 °C, and 30 seconds at 50 °C. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control for measurement of lncRNAs and the relative expression levels of candidate lncRNAs were calculated using the $2^{-\Delta\Delta CT}$ equation. At least triple experiments were subjected to qRT-PCR verification.

2.7. GO and KEGG enrichment analysis

The target mRNAs of lncRNAs were classified according to the principle of classification by Gene Ontology (http://www.geneontology.org/). GO gathers information from Gene Ontology and the NCBI database, annotates and classifies genes according to the biology process, molecular function and cellular location. KEGG (http://www.genome.jp/kegg/) is a comprehensive database for systematic analysis of gene function. It is based on the related knowledge of hand-painted metabolic pathways, mainly divided into categories: metabolism, genetic information processing, cellular processes, environmental information processing, organismal systems and human diseases. Each category is divided into some subitems.

2.8. Statistical analysis

SPSS statistical software version 19.0 was used for data analysis. Data were reported as the means ± SD. Chi-square and ANOVA tests were used to analyze the differences among the 2 groups. Statistical significance was set at a $P < .05$ (Fig. 1).

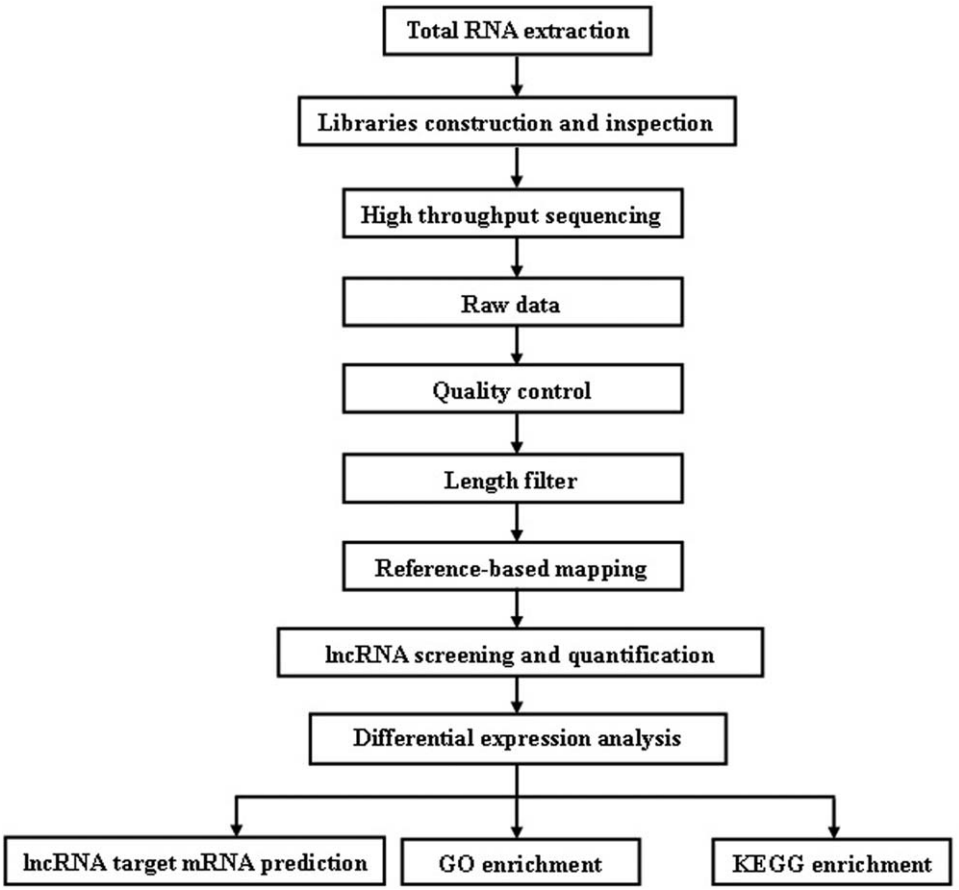

Figure 1. The workflow of the experiment.

Table 1

The baseline clinical characteristics.

Variable	NCA (n = 7)	AMI (n = 8)	*P* value
Age, years	55.14 ± 7.49	55.25 ± 7.59	.582
Sex (male)	2 (28.57%)	6 (75%)	.132
Smoking	1 (14.29%)	5 (62.5%)	.119
Drinking	0 (0%)	1 (12.5%)	.533
Systolic BP, mm Hg	130.43 ± 11.96	136.25 ± 26.71	.011
Diastolic BP, mm Hg	83.71 ± 12.89	78.00 ± 18.40	.217
Hypertension	1 (14.29%)	4 (50%)	.282
Diabetes	1 (14.29%)	1 (12.50%)	.919
Hyperlipidemia	2 (28.57%)	2 (25.0%)	.876
TC, mmol/L	4.60 ± 0.77	4.88 ± 1.55	.261
TG, mmol/L	1.26 ± 0.62	1.75 ± 0.97	.268
HDL-C, mmol/L	1.25 ± 0.43	1.09 ± 0.17	.065
LDL-C, mmol/L	2.35 ± 0.55	3.01 ± 1.36	.080

AMI = acute myocardial infarction, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, NCA = noncoronary artery, TC = total cholesterol, TG = triglycerides.

3. Results

3.1. The subjects' clinical characteristics

The clinical characteristics of the 15 subjects in this study were presented in Table 1. There were higher systolic BP ($P = .011$) in the AMI patients than in NCA controls. There were no statistical differences in age, sex, smoking, drinking, diastolic BP, hypertension, diabetes, hyperlipidemia, total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) between the AMI patients and non-AMI controls.

3.2. Overview of lncRNA sequencing data

In this study, a total of 15 cDNA libraries were constructed and sequenced on the Illumina HiSeq 4000 platform by using total RNA from each sample. After quality control, we kept about 11.29 gigabase (Gb) high-quality sequence data while the Q30 ranged from 94.39% to 95.19% for each sample. Obviously, these results indicated that the quality of the 15 libraries were suitable for subsequent analysis. Details of data quality and data characteristics were listed in Supplementary Table S1, http://links.lww.com/MD/C545 and Table S2, http://links.lww.com/MD/C545.

3.3. Differentially expressed lncRNAs and mRNAs in PBMCs

To systematically investigate the expression levels of lncRNAs and mRNAs associated with AMI, lncRNA and mRNA sequence analyses were performed on the PBMCs of 8 AMI patients and 7 NCA controls. The results of hierarchical clustering showed the differential expression of lncRNAs (Fig. 2A) and mRNAs (Fig. 2B) between AMI patients and NCA controls. Expression values are represented in red and blue, indicating expression above and below the median expression value in each group. These observations suggested that potential changes between normal and AMI state were identified by differences in the expression profile of either lncRNAs or mRNAs related to AMI (Table 2).

The volcano plots are presented as visualizations used to assess lncRNA and mRNA expressive variation between patients with AMI and NCA controls, respectively (Fig. 3). Compared to the lncRNA expression profiles of NCA controls, a total of 106 differential expression of lncRNAs were discriminated in AMI patients, including 40 upregulated lncRNAs and 66 down-regulated lncRNAs ($P < .05$).

3.4. GO and KEGG pathway analyses

To further understand the function and related pathways of mRNA identified in this study, we performed GO and KEGG

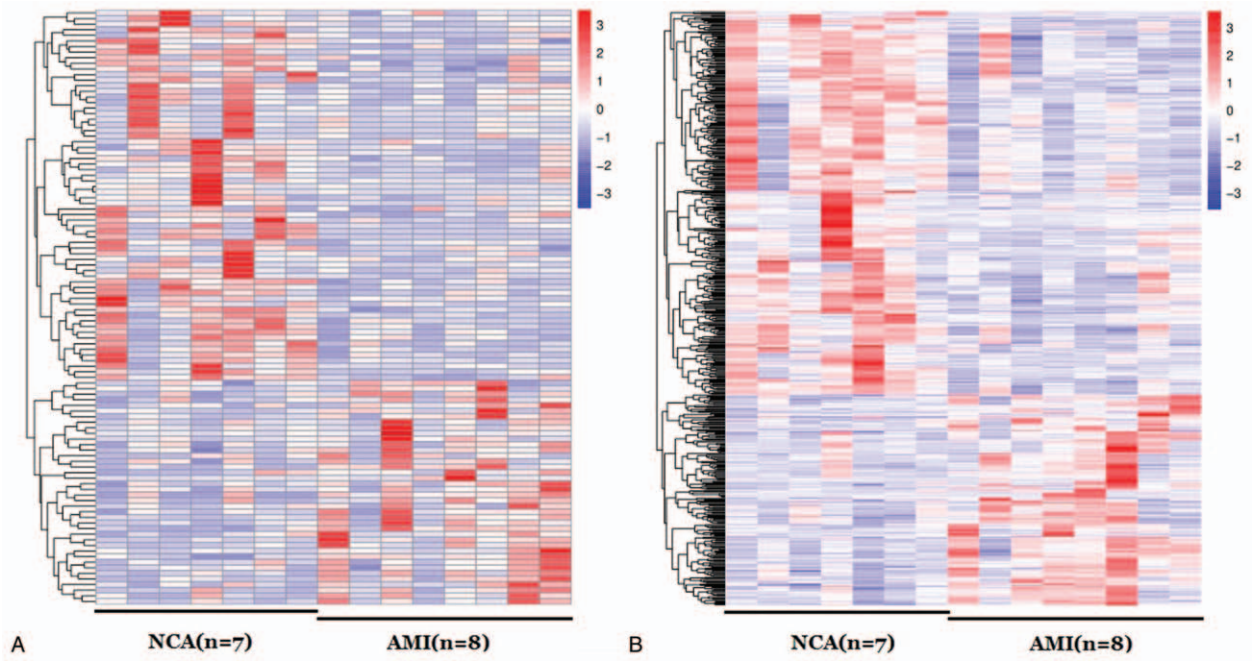

Figure 2. Hierarchical clustering of lncRNAs in AMI patients and NCA controls. The red and the green shades indicate an increase and a decrease in expression level, respectively, across all samples. (A) lncRNA; (B) mRNA. AMI = acute myocardial infarction, NCA = noncoronary artery.

Table 2

The most differentially expressed lncRNAs in AMI and NCA according |log2(foldchange)|>2.

Lnc RNA id	Genome location	log_2FoldChange	*P* value	Significant
ENSG00000229807.10	chrX:73820650-73852753	−10.5502	.0002	Down
ENSG00000235531.9	chr8:71828166-72118393	−4.10052	.0218	Down
ENSG00000276107.1	chr15:39581078-39599466	−3.69585	.03995	Down
ENSG00000278621.1	chr15:39581078-39599466	−2.9766	.04735	Down
XLOC_070339	chrY:11182357-11183043	2.78986	.00405	Up
XLOC_069933	chrY:3130963-3136968	2.77885	.00015	Up
XLOC_026336	chr17:19111244-19111752	−2.70725	.00025	Down
XLOC_040499	chr21:8436195-8436450	2.4289	5.00E−05	Up
XLOC_064417	chr9:65196497-65199424	−2.29207	.0064	Down
XLOC_070337	chrY:11177290-11178266	2.27431	.00355	Up
XLOC_052051	chr5:50135157-50135868	−2.15732	.02665	Down
XLOC_008906	chr10:91060123-91113137	−2.06816	.01335	Down
XLOC_064778	chr9:94314396-94317503	2.01384	.00695	Up

AMI = acute myocardial infarction, NCA = noncoronary artery.

pathway analysis. Genes are classified according to biological processes, cell components and molecular functional tissues to reveal gene regulatory networks. Of the genes corresponding to the identified mRNA, 2905 genes are involved in biological processes, 339 in cellular components and 501 in molecular functions (Table 3 and Fig. 4). Based on the KEGG pathway analysis, we found that the most enriched pathways corresponding to the differentially expressed lncRNAs were associated with systemic lupus erythematosus, alcoholism, oxidative phosphorylation, Parkinson's disease and viral carcinogenesis, and so on (Table 4 and Fig. 5).

3.5. qRT-PCR validation of lncRNA expression

To validate the sequencing data of lncRNA expression level, 3 upregulated lncRNAs (XLOC_040499, XLOC_067810, and XLOC_020735) and 3 downregulated lncRNAs (ENSG00000229807.10, ENSG00000278621.1 and XLOC_043118) with only one transcript were randomly selected. We verified the differential expression of these lncRNAs from PBMCs of AMI patients (n = 30) and NCA controls (n = 30) by qRT-PCR using GAPDH as the reference gene with the $2^{-\Delta\Delta CT}$ method. As shown in Figure 6, the results of qRT-PCR were consistent with the outcomes obtained from RNA sequencing analysis in the 6 differentially expressed lncRNAs of AMI patients compared with NCA controls.

4. Discussion

In China, deaths from cardiovascular diseases currently account for the top cause of total diseases deaths. The increasing burden of cardiovascular disease has become a major public health problem. In particular, with the aging of the population and the acceleration of urbanization, the prevalence of risk factors for cardiovascular diseases in China is significant, resulting in the continuous increase in the number of people suffering from cardiovascular diseases. The number of cardiovascular patients will continue to increase rapidly in the next 10 years.[24–28]

With the development of translational medicine, the role of biomarkers in cardiovascular diseases has attracted increasingly attentions. It has great application value in the early diagnosis, differential diagnosis, treatment response prediction and prognosis judgment of diseases.[29–33] The ideal biomarkers should have the following characteristics, which can reflect the onset and progression of diseases or conditions, have stability, simple and easy methods to detect, small individual injuries, have high sensitivity and specificity and high economics of the economy to contribute to popularization.[34] The biomarkers widely used in clinical practice including creatine kinase (CK), creatine kinase isoenzyme (CK-MB), type B natriuretic peptide (BNP) and troponin cTnI (cTnI) and so on, for they have important clinical significance in the diagnosis, treatment response and other aspects of myocardial injury, myocarditis, heart failure and other

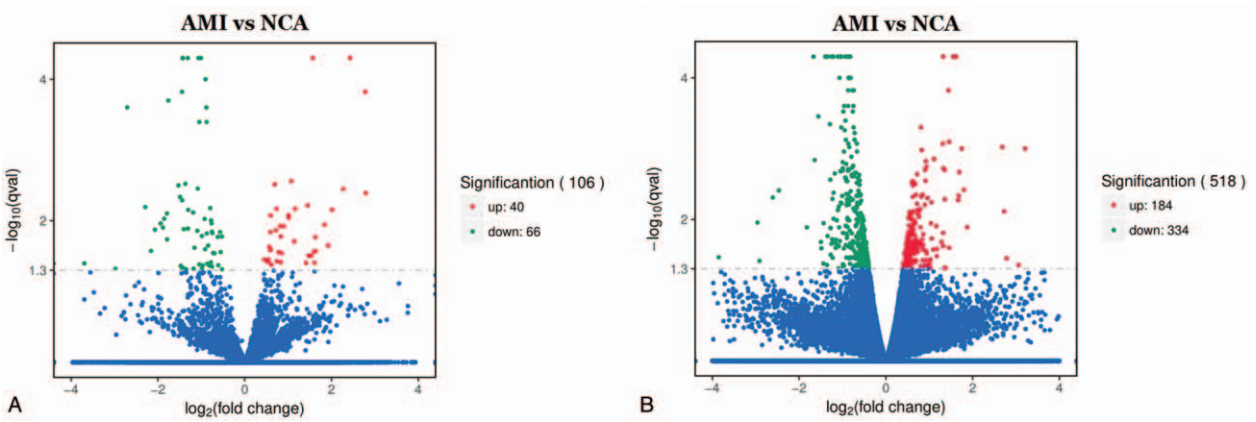

Figure 3. Volcano plot of differential lncRNA and mRNA expression. *X*-axis: log_2(fold change); Y-axis: $-1 \times \log_{10}$ (corrected *q*-value) for each probes. (A) AMI vs NCA in lncRNA; (B) AMI vs NCA in mRNA. AMI = acute myocardial infarction, NCA = noncoronary artery.

Table 3

Significantly enriched gene ontology (GO) terms.

GO_accession	Description	Corrected_*P* Value	Total number of genes
GO:0000786	Nucleosome	4.75E−18	21
GO:0032993	Protein–DNA complex	1.23E−15	23
GO:0006334	Nucleosome assembly	5.07E−13	19
GO:0034728	Nucleosome organization	6.16E−13	20
GO:0000785	Chromatin	8.10E−13	29
GO:0006333	Chromatin assembly or disassembly	1.06E−12	20
GO:0031497	Chromatin assembly	1.25E−12	19
GO:0071824	Protein–DNA complex subunit organization	1.66E−11	22
GO:0065004	Protein–DNA complex assembly	1.66E−11	21
GO:0006323	DNA packaging	5.86E−11	19
GO:0034622	Cellular macromolecular complex assembly	2.20E−10	38
GO:0044427	Chromosomal part	3.63E−10	33
GO:0005694	Chromosome	2.33E−09	34
GO:0071103	DNA conformation change	1.26E−08	19
GO:0065003	Macromolecular complex assembly	5.42E−08	46
GO:0000790	Nuclear chromatin	7.67E−08	19
GO:0000788	Nuclear nucleosome	1.25E−07	9
GO:0070469	Respiratory chain	7.75E−07	12
GO:0044454	Nuclear chromosome part	1.15E−06	22
GO:0042775	Mitochondrial ATP synthesis coupled electron transport	2.05E−06	11
GO:0042773	ATP synthesis coupled electron transport	2.18E−06	11
GO:0043933	Macromolecular complex subunit organization	2.33E−06	48
GO:0000228	Nuclear chromosome	2.63E−06	22
GO:0006342	Chromatin silencing	3.65E−06	11
GO:0005743	Mitochondrial inner membrane	8.02E−06	21
GO:0006119	Oxidative phosphorylation	8.61E−06	11
GO:0022904	Respiratory electron transport chain	8.80E−06	11
GO:0006325	Chromatin organization	8.87E−06	24
GO:0006335	DNA replication-dependent Nucleosome assembly	8.87E−06	7
GO:0034723	DNA replication-dependent nucleosome organization	8.87E−06	7
GO:0022900	Electron transport chain	8.93E−06	11
GO:0070062	Extracellular vesicular exosome	1.04E−05	54
GO:0043230	Extracellular organelle	1.04E−05	54
GO:0065010	Extracellular membrane-bounded organelle	1.04E−05	54
GO:0005740	Mitochondrial envelope	1.25E−05	25
GO:0045814	Negative regulation of gene expression, epigenetic	1.28E−05	11
GO:0000183	Chromatin silencing at rDNA	2.36E−05	7
GO:0046982	Protein heterodimerization activity	2.99E−05	19
GO:0019866	Organelle inner membrane	2.99E−05	21
GO:0005746	Mitochondrial respiratory chain	2.99E−05	10
GO:0022607	Cellular component assembly	3.51E−05	48
GO:0044429	Mitochondrial part	5.90E−05	28
GO:0031966	Mitochondrial membrane	6.57E−05	23
GO:0044455	Mitochondrial membrane part	8.47E−05	13
GO:0051290	Protein heterotetramerization	.00011165	7
GO:0008137	NADH dehydrogenase (ubiquinone) activity	.00018132	7
GO:0050136	NADH dehydrogenase (quinone) activity	.00018132	7
GO:0003954	NADH dehydrogenase activity	.00023061	7
GO:0060968	Regulation of gene silencing	.00025679	9
GO:0045333	Cellular respiration	.00025756	11
GO:0006120	Mitochondrial electron transport, NADH to ubiquinone	.00028468	7
GO:0003677	DNA binding	.00041673	44
GO:0006461	Protein complex assembly	.00049335	32
GO:0070271	Protein complex biogenesis	.00050521	32
GO:0060964	Regulation of gene silencing by miRNA	.00055712	8
GO:0006259	DNA metabolic process	.00055712	27
GO:0051276	Chromosome organization	.00056916	26
GO:0032991	Macromolecular complex	.00058617	77
GO:0060147	Regulation of posttranscriptional gene silencing	.00058617	8
GO:0060966	Regulation of gene silencing by RNA	.00058617	8
GO:0005747	Mitochondrial respiratory chain complex I	.00059103	7
GO:0030964	NADH dehydrogenase complex	.00059103	7
GO:0045271	Respiratory chain complex I	.00059103	7
GO:0016655	Oxidoreductase activity, acting on NAD(P)H, quinone or similar compound as acceptor	.0005982	7
GO:0044085	Cellular component biogenesis	.00061853	48

(*continued*)

Table 3

(continued).

GO_accession	Description	Corrected_*P* Value	Total number of genes
GO:0031490	Chromatin DNA binding	.00071625	8
GO:0043227	Membrane-bounded organelle	.00071625	138
GO:0045652	Regulation of megakaryocyte differentiation	.00087002	7
GO:0010257	NADH dehydrogenase complex assembly	.00088896	7
GO:0032981	Mitochondrial respiratory chain complex I assembly	.00088896	7
GO:0097031	Mitochondrial respiratory chain complex I biogenesis	.00088896	7
GO:0000784	Nuclear chromosome, telomeric region	.001151	9
GO:0042393	Histone binding	.0012928	10
GO:0006336	DNA replication-independent nucleosome assembly	.0015218	6
GO:0034724	DNA replication-independent nucleosome organization	.0016871	6
GO:0047961	Glycine *N*-acyltransferase activity	.0018469	3
GO:0034508	Centromere complex assembly	.0019103	6
GO:0032200	Telomere organization	.0020685	9
GO:1902036	Regulation of hematopoietic stem cell differentiation	.0021321	8
GO:0045815	Positive regulation of gene expression, epigenetic	.0021339	7
GO:0044237	Cellular metabolic process	.0026532	121
GO:0003988	Acetyl-CoA C-acyltransferase activity	.0031707	3
GO:0006807	Nitrogen compound metabolic process	.0031707	90
GO:0044422	Organelle part	.0033478	108
GO:0060218	Hematopoietic stem cell differentiation	.0033478	8
GO:0044446	Intracellular organelle part	.0034176	107
GO:0030219	Megakaryocyte differentiation	.0035802	7
GO:0031981	Nuclear lumen	.0035802	59
GO:0031982	Vesicle	.0039707	59
GO:0031967	Organelle envelope	.0039707	26
GO:0031975	Envelope	.0039707	26
GO:0045653	Negative regulation of megakaryocyte differentiation	.0043143	4
GO:0031491	Nucleosome binding	.0043143	6
GO:0046983	Protein dimerization activity	.0044534	26
GO:0015980	Energy derivation by oxidation of organic compounds	.0044534	11
GO:0016408	C-acyltransferase activity	.0045747	4
GO:0044421	Extracellular region part	.0046921	62
GO:0000781	Chromosome, telomeric region	.0047447	9
GO:0006139	Nucleobase-containing compound metabolic process	.0049824	81
GO:0043228	Nonmembrane-bounded organelle	.005247	59
GO:0043232	Intracellular non-membrane-bounded organelle	.005247	59
GO:0006950	Response to stress	.0058715	60
GO:0034080	CENP-A containing nucleosome assembly at centromere	.0058715	5
GO:0003676	Nucleic acid binding	.0062425	58
GO:0033108	Mitochondrial respiratory chain complex assembly	.0063893	7
GO:0071822	Protein complex subunit organization	.0069593	33
GO:0006996	Organelle organization	.0071552	50
GO:0046483	Heterocycle metabolic process	.0072491	82
GO:0090304	Nucleic acid metabolic process	.007393	70
GO:0006725	Cellular aromatic compound metabolic process	.0075669	82
GO:0002227	Innate immune response in mucosa	.0077457	4
GO:0005739	Mitochondrion	.0083387	32
GO:1901360	Organic cyclic compound metabolic process	.0084236	84
GO:0031055	Chromatin remodeling at centromere	.0084613	5
GO:0031492	Nucleosomal DNA binding	.009007	5
GO:0043044	ATP-dependent chromatin remodeling	.0096123	6
GO:0043226	Organelle	.010291	140
GO:0016651	Oxidoreductase activity, acting on NAD(P)H	.011457	7
GO:0031988	Membrane-bounded vesicle	.01172	55
GO:0034641	Cellular nitrogen compound metabolic process	.014483	83
GO:0051291	Protein heterooligomerization	.015243	7
GO:0051253	Negative regulation of RNA metabolic process	.01599	24
GO:0045892	Negative regulation of transcription, DNA-dependent	.016939	23
GO:0005576	Extracellular region	.01946	68
GO:0006091	Generation of precursor metabolites and energy	.020316	12
GO:0070013	Intracellular organelle lumen	.020629	65
GO:0010558	Negative regulation of macromolecule biosynthetic process	.022281	26
GO:0043623	Cellular protein complex assembly	.023134	16
GO:0043486	Histone exchange	.023134	5
GO:0016458	Gene silencing	.024164	12

(*continued*)

Table 3

(continued).

GO_accession	Description	Corrected_*P* Value	Total number of genes
GO:0016829	Lyase activity	.025067	8
GO:0002385	Mucosal immune response	.025067	4
GO:0016233	Telomere capping	.025695	4
GO:2000113	Negative regulation of cellular macromolecule biosynthetic process	.027396	25
GO:0044428	Nuclear part	.027648	59
GO:0003985	Acetyl-CoA C-acetyltransferase activity	.030194	2
GO:0002251	Organ or tissue specific immune response	.030905	4
GO:0043231	Intracellular membrane-bounded organelle	.031058	124
GO:0051259	Protein oligomerization	.031997	14
GO:0045637	Regulation of myeloid cell differentiation	.033117	9
GO:1901362	Organic cyclic compound biosynthetic process	.033856	60
GO:0016509	Long-chain-3-hydroxyacyl-CoA dehydrogenase activity	.03415	2
GO:0031974	Membrane-enclosed lumen	.03415	66
GO:2000736	Regulation of stem cell differentiation	.034815	9
GO:0051119	Sugar transmembrane transporter activity	.037607	4
GO:0043566	Structure-specific DNA binding	.04207	10
GO:0018130	Heterocycle biosynthetic process	.043119	58
GO:0051262	Protein tetramerization	.043119	7
GO:0019438	Aromatic compound biosynthetic process	.043119	58
GO:0043233	Organelle lumen	.043327	65
GO:0031327	Negative regulation of cellular biosynthetic process	.047794	26

NADH = Nicotinamide adenine dinucleotide.

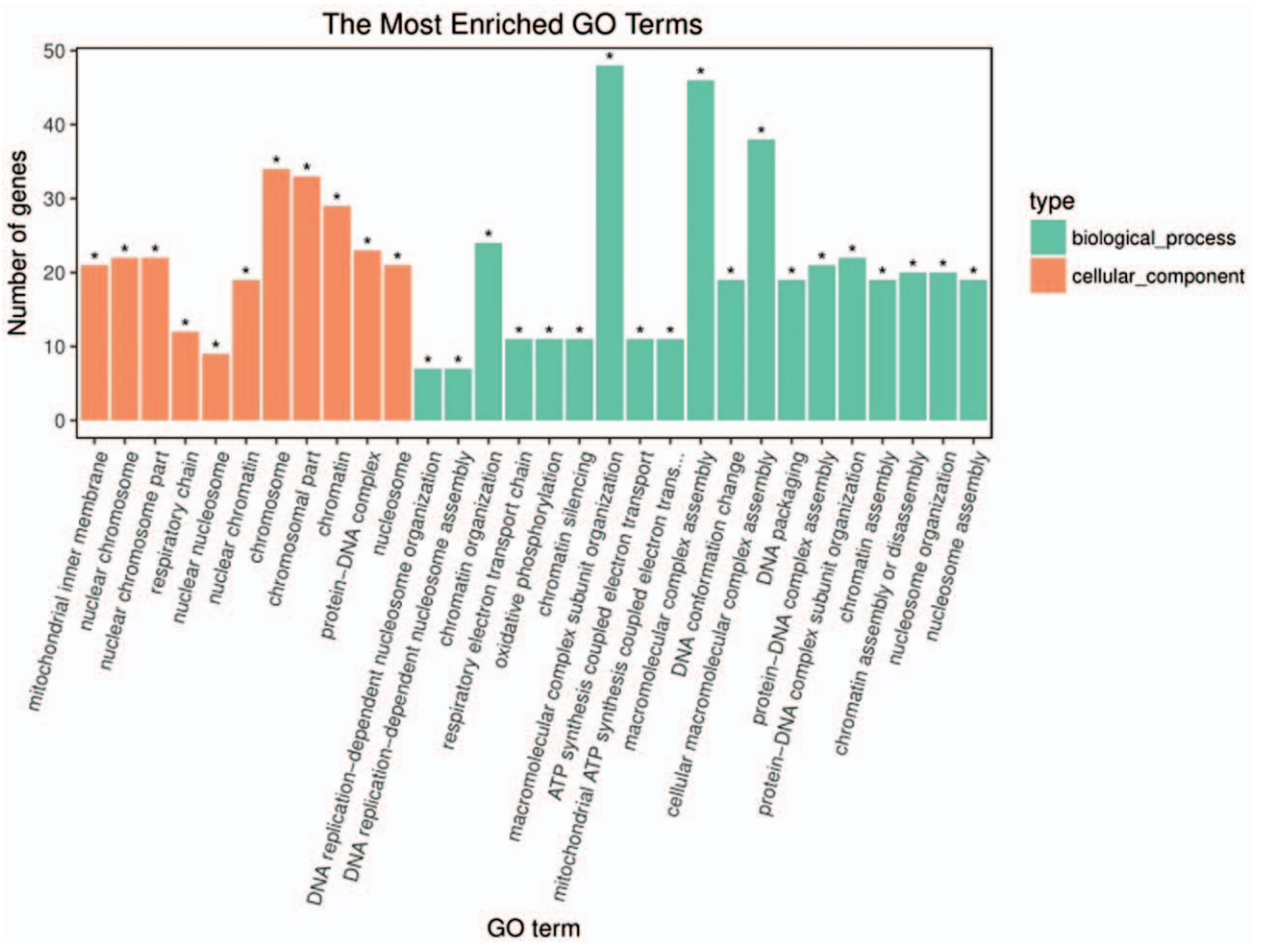

Figure 4. GO analysis of differentially expressed lncRNAs which covers 3 domains: biological process, cellular component and molecular function. *X*-axis: GO terms of biological process, cellular component and molecular function. The green column indicates biological process, the red column indicates cellular component and the blue column indicates molecular function. *Y*-axis on the left: numbers of genes (lncRNAs).

Table 4

Significantly enriched KEGG pathways.

ID	Pathway name	Total number of genes	*P* value
hsa05322	Systemic lupus erythematosus	19	2.62E−14
hsa05034	Alcoholism	18	1.99E−11
hsa00190	Oxidative phosphorylation	12	1.41E−07
hsa05012	Parkinson's disease	12	2.72E−07
hsa05203	Viral carcinogenesis	11	4.94E−05
hsa00071	Fatty acid degradation	4	.002336
hsa01212	Fatty acid metabolism	4	.003135
hsa04260	Cardiac muscle contraction	4	.015457
hsa00360	Phenylalanine metabolism	2	.020246
hsa00280	Valine, leucine, and isoleucine degradation	3	.021529
hsa05202	Transcriptional misregulation in cancer	6	.022032
hsa00900	Terpenoid backbone biosynthesis	2	.031461
hsa00062	Fatty acid elongation	2	.039112
hsa01100	Metabolic pathways	22	.040133
hsa00650	Butanoate metabolism	2	.047388
hsa00630	Glyoxylate and dicarboxylate metabolism	2	.047388

KEGG = Kyoto Encyclopedia of Genes and Genomes.

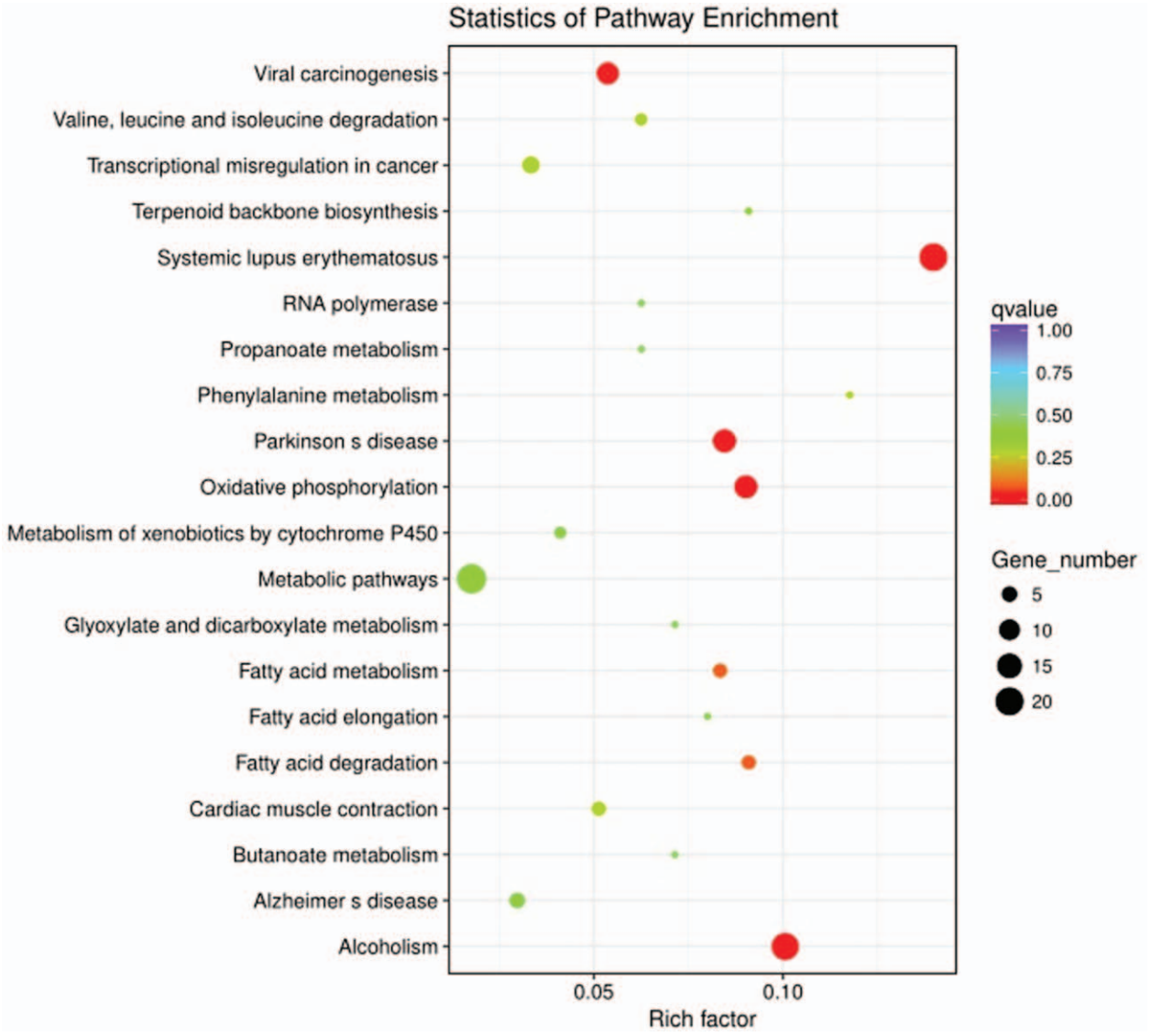

Figure 5. Pathway analysis of differentially expressed lncRNAs. Pathway analysis is a functional analysis mapping genes to KEGG pathway and other pathway databases. The lower the *P*-value, the more significant the pathway.

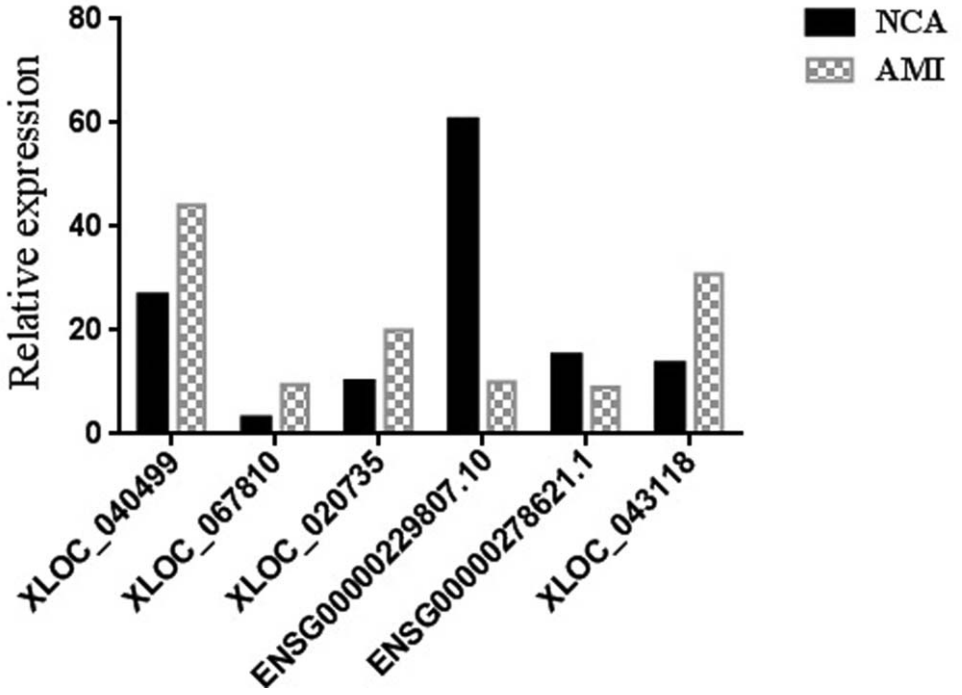

Figure 6. Validation of RNA-seq results by using quantitative qRT-PCR. The data are expressed as the mean ± SD (n = 30).

diseases.[35–38] Therefore, discovering more biomarkers and giving full play to their role in precision medicine are important directions for basic research and clinical work in the future.

lncRNAs are noncoding RNAs with a length of more than 200 nt in the nucleus or cytoplasm and relatively long nucleotide chains. They have a specific and complex secondary space structure inside the molecule and can provide multiple sites for protein binding or interactions with DNA and RNA, occurring through specific and dynamic interactions, forming a complex, precise, and delicate network of gene expression and regulation. lncRNA has the characteristics of tissue specificity, cell specificity, development stage specificity, spatiotemporal specificity and disease specificity. It is widely involved in cell differentiation, metabolism and proliferation, and closely related to various diseases, including AMI. In this study, a total of 106 differentially expressed lncRNAs were discriminated in AMI patients, including 40 upregulated lncRNAs and 66 downregulated lncRNAs.

In this study, in the significantly enriched KEGG signaling pathways, systemic lupus erythematosus signaling pathway is involved in the development of immune complex deposition, vasculitis, and vascular lesions.[39] Cardiac muscle contraction signaling pathway[40] is associated with the onset and progression of myocardial infarction. In addition, in the significantly enriched KEGG signaling pathway, several fatty acid signaling pathways[41,42] are involved in the metabolism of triglycerides, which may also be associated with the occurrence and development of myocardial infarction.

The regulation mechanism of microRNA and lncRNA in vascular injury, remodeling and aging has attracted more and more attentions. They regulate various aspects of gene expression through multiple targets, multiple pathways, such as chromatin remodeling, transcription, processing, and post-transcriptional modification.[43–46] Vascular system diseases include a series of common diseases such as atherosclerosis, hypertension, myocardial infarction, stroke, pulmonary hypertension and diabetic vascular disease. Therefore, it is very important to explore the relationship between microRNA and lncRNA and vascular system diseases and clinical diagnosis. However, the understanding of the regulation mechanism of microRNA and lncRNA is still superficial, and their interaction with other regulatory mechanisms needs to be further studied. This study may help to understand that microRNA and lncRNA play an important role in maintaining the complex structure and function of blood vessels.

5. Conclusions

Our study used RNA sequencing to describe the comprehensive identifications and analysis of lncRNA expression profiles in AMI patients and compared them with corresponding NCA controls. The results provided differences in lncRNA expression profiles between AMI and NCA, and some of the differentially expressed lncRNAs may play a key role in various biological and pathological processes of AMI. These findings may provide useful biological information in early diagnosis and risk stratification of AMI patients. Of course, further research will be required to reveal the functional significance of abnormally expressed lncRNAs in AMI. This is one of the main research contents in our next step.

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Author contributions

Pingsen Zhao conceived and designed the experiments; Heming Wu and Pingsen Zhao recruited subjects and collected clinical data. Heming Wu conducted the laboratory testing. Zhixiong Zhong, Qifeng Zhang, Wei Zhong, Bin Li, Cunren Li, Zhidong Liu and Min Yang helped to analyze the data. Pingsen Zhao and Heming Wu prepared the manuscript.

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