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Screening of Biomarkers Related to Myocardial Infarction Based on the Construction of a ceRNA Regulation Network

ABSTRACT

Background: This study aimed to identify the biomarkers related to myocardial infarction based on building lncRNA–miRNA–mRNA ceRNA regulation network.

Methods: The expression profile data were obtained from the Gene Expression Omnibus database. The differentially expressed RNAs (DElncRNAs, DEmiRNAs, and DEmRNAs) were analyzed using the limma package of R. In addition, the differential myocardial infarction-related genes were obtained and the Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway on the differential myocardial infarction-related genes were analyzed. The co-expression network of lncRNA–mRNA was constructed, and the Kyoto Encyclopedia of Genes and Genomes pathway of such a co-expression network was analyzed. Moreover, the lncRNA–miRNA–mRNA ceRNA network associated with myocardial infarction was built, and the key biomarkers of the ceRNA network were verified using the GSE141512 and GSE66752 datasets.

Results: In total, we acquired 51 DElncRNAs, 276 DEmiRNAs, and 1200 DEmRNAs as well as 291 differential myocardial infarction-related genes. Moreover, the co-expression network of lncRNA–mRNA was built, and mRNAs were found to be associated with 109 Kyoto Encyclopedia of Genes and Genomes pathways (the target gene of RP3-394A18.1 was significantly related to lipid and atherosclerosis). In addition, the ceRNA network related to myocardial infarction was constructed, and the dataset analyses of the RNAs were validated between the myocardial infarction and normal groups. It was also found that the expression levels of hsa-miR-1291 and Retinoid acid receptor-related orphan receptor α (RORA) in the myocardial infarction group were noticeably lower than those in the normal group, whereas the expression levels of ENTPD1, QSOX1, and TIMP2 in the myocardial infarction group were noticeably higher than those in the normal group.

Conclusion: In this study, we built a ceRNA regulation network of myocardial infarction. This study could help identify the biomarkers related to myocardial infarction.

Keywords: Myocardial infarction, ceRNA regulation network, biomarkers

ORIGINAL INVESTIGATION

INTRODUCTION

Myocardial infarction (MI), a type of cardiovascular disease (CVD), is a common acute disease associated with the coronary arteries. In both developed and developing countries, diseases due to coronary artery occlusion remain as the main causes of disability and death. Among the types of CVDs, MI has the highest mortality rate.[1,2] Although the current study on MI treatment has made certain achievements, the morbidity and mortality rates of MI are still high.[3] Thus, biomarkers with more characteristics and values need to be identified to facilitate the early prediction and treatment of MI at the molecular level.

Many noncoding RNAs (ncRNAs) have a significant influence on the regulation of biological processes (BPs) and development of diseases. Studies have proven that the mutual regulation between long noncoding RNA (lncRNA) and microRNA (miRNA) and their regulated mRNAs is strongly associated with MI development and can also be used as diagnostic and prognostic biomarkers for MI.[4,5] In previous studies, both lncRNAs and miRNAs were also found to be clearly associated with the development of MI. For example, the lncRNA ZFAS1 in the myocardium was

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found to be upregulated after the occurrence of MI.[6] In addition, the lncRNA GAS5 in the MI group was noticeably upregulated compared with that in the control group.[7] Although some MI-related biomarkers were identified, they were still insufficient for the study on MI. Therefore, an in-depth study of ceRNA-based regulatory mechanisms will provide a better understanding of MI development.

In this study, we first acquired the differentially expressed RNAs (DERs) and MI-related genes. Then, we conducted Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses of the MI-related genes. Next, we constructed the co-expression network of lncRNA–mRNA, and we conducted the KEGG pathway analysis of the co-expression network. We also built the lncRNA–miRNA–mRNA ceRNA network related to MI and verified the key biomarkers of the ceRNA network. All the results provide a reference for the study of MI biomarkers.

METHODS

Data Collection

By April 2021, we were able to acquire the expression profile data for the GSE60993 and GSE61741 datasets from the Gene Expression Omnibus (GEO) database (www.ncbi.nlm.nih.gov/geo/) based on the platforms of GPL6884 and GPL9040, respectively. The GSE60993 dataset includes the mRNA and lncRNA expression data, which contain the blood samples of 7 patients with ST-segment elevation MI and 7 healthy patients. On the other hand, the GSE61741 dataset includes the miRNA expression data, which contain the blood samples of 62 patients with MI and 94 healthy patients. The GSE60993 and GSE61741 datasets were used as the training datasets.

Meanwhile, the expression profile data of the GSE141512 and GSE66752 datasets were obtained from the GEO database based on the platforms of GPL17586 and GPL16770, respectively. The GSE141512 dataset includes the mRNA and lncRNA expression data, which contain the blood samples of 6 patients with MI and 6 healthy patients. On the other hand, the GSE66752 dataset includes the miRNA expression data, which contain the blood samples of 8 patients with MI and 8 healthy patients. The GSE141512 and GSE66752 datasets were used as the validation datasets.

Data Preprocessing

The probe sequences were extracted from the GEO database, and the mRNAs and lncRNAs were re-annotated using the reference genomes from the GENCODE database (www.gencodegenes.org/human/). The genes with annotation information of "protein coding" as mRNAs were retained, as well as those with annotation information of "antisense," "sense_intronic," "lincRNA," "sense_overlapping," "processed_transcript," "3prime_overlapping_ncRNA," or "non_coding" as lncRNAs. Subsequently, we removed the probes that were inconsistent with the gene symbol, and the average value of the probes was used as the final expression value of the gene.

Screening of Differentially Expressed RNAs

The limma package (version 3.10.3, www.bioconductor.org/packages/2.9/bioc/html/limma.html) of R was used to identify the DERs between the healthy and MI samples. $P < .05$ and $|\log_2 \text{fold change (FC)}| > 0.58$ were used as the significant standard.

Further Acquisition of Myocardial Infarction-Related Genes

Myocardial infarction-related genes were obtained from the Comparative Toxicogenomics Database (CTD) (http://ctdbase.org/)[8] by searching the keyword "myocardial infarction." The genes with inference score ≥50 were selected. The overlapping part of the MI-related genes and differentially expressed mRNAs (DEmRNAs) obtained in the previous step was retained as the differential MI-related genes.

Functional Enrichment Analysis of Differentially Expressed mRNAs

The GO and KEGG pathway analyses of the differential MI-related genes were conducted through the Database for Annotation, Visualization, and Integrated Discovery (DAVID) version 6.8 (https://david.ncifcrf.gov/)[9] ($P < .05$ and the enrichment score ≥ 2).

Construction of the ceRNA Network

We searched the miRWalk2.0 database (version 2.0, http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/)[10] to acquire mRNAs regulated by miRNAs as well as the miRWalk, Microt4, miRanda, miRDB, RNA22, and TargetScan databases to predict the target genes. If the miRNA–mRNA connection pair appeared in not less than 4 of the aforementioned databases, it was screened as the final miRNA–mRNA connection pair. The lncRNAs and DEmiRNAs of the lncRNA–mRNA co-expression network were screened using miRanda (version 3.3a) for the lncRNA–miRNA connection pairs with a score of ≥200 and energy of ≤ (–20).

Next, the lncRNA–miRNA–mRNA connection pairs regulated by the same miRNA were obtained, and the positive co-expression connections between mRNAs and

HIGHLIGHTS

- A total of 291 differential myocardial infarction (MI)-related genes were obtained, which were significantly enriched in 216 Gene Ontology biological processes (such as negative regulation of apoptotic process and response to drug) and 77 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (such as prostate cancer and cancer pathways).
- The lncRNA–mRNA co-expression network was constructed, and mRNAs were enriched in the 109 KEGG pathways (the target gene of RP3-394A18.1 was significantly related to lipid and atherosclerosis).
- The ceRNA network related to MI was constructed, and the dataset analyses of RNAs were validated between the MI and normal groups; the expression levels of hsa-miR-1291 and RORA in the MI group were noticeably lower than those in the normal group, whereas the expression levels of ENTPD1, QSOX1, and TIMP2 in the MI group were noticeably higher than those in the normal group.

lncRNAs were combined ($r > 0.8$). Finally, we constructed the lncRNA–miRNA–mRNA regulation network. We used the Cytoscape (version 3.4.0, http://chianti.ucsd.edu/cytoscape-3.4.0/)[11] software plug-in CytoNCA (version 2.1.6, http://apps.cytoscape.org/apps/cytonca) to determine the degree of node connectivity, and we set no weight as the parameter.

Construction of the lncRNA–mRNA Co-expression Network

Based on the calculation of the Pearson correlation coefficient (PCC) of the cor function in R language (version 3.4.1, http://77.66.12.57/R-help/cor.test.html), lncRNA–mRNA co-expression analysis was conducted. Moreover, to acquire the adjusted *P*-value, we employed the Benjamini–Hochberg method for the multiple testing correction. Finally, we

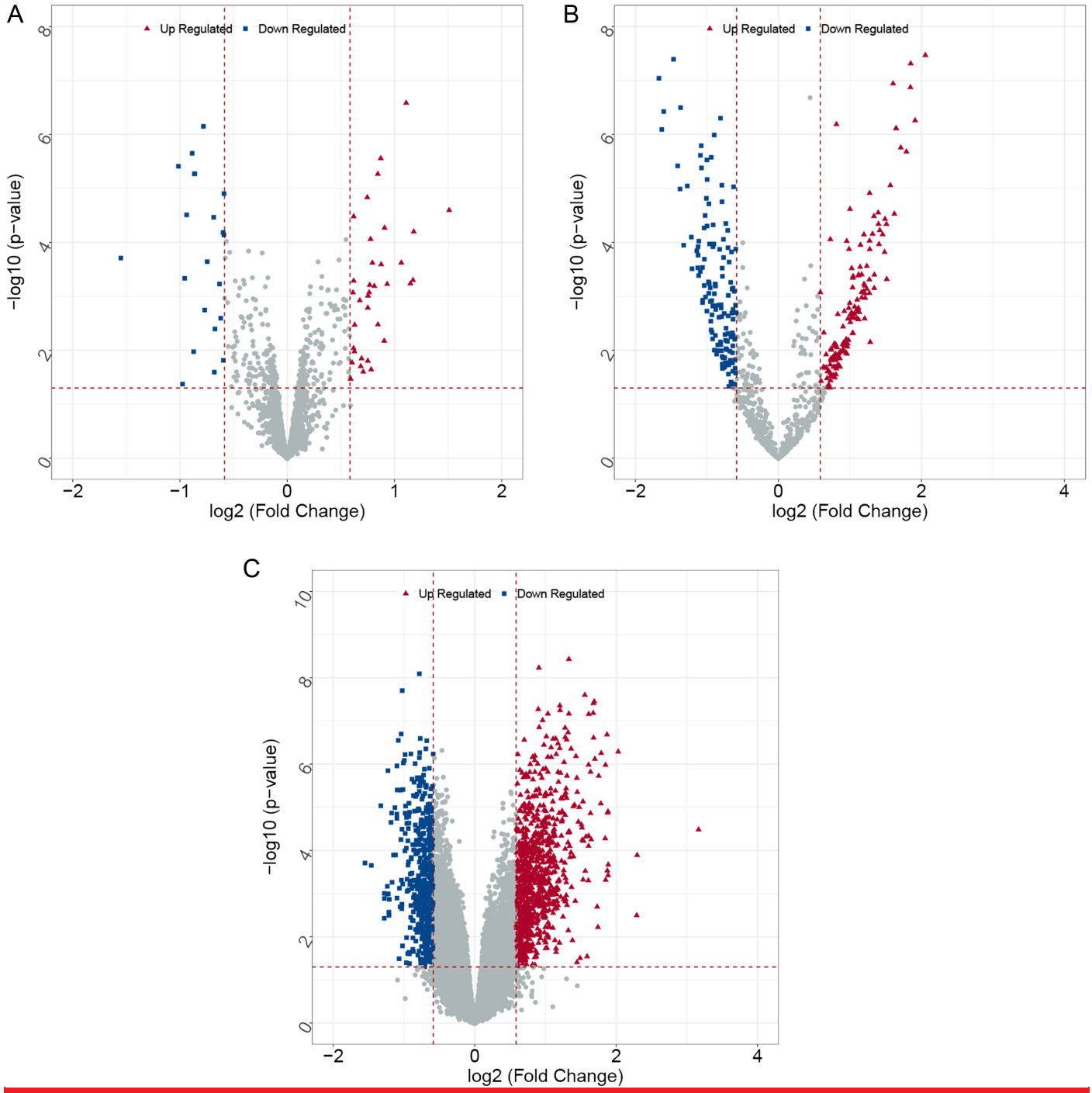

Figure 1. Volcano map of the DERs (A: DEmiRNAs, B: DElncRNAs, C: DEmRNAs). $P < .05$ and $|\log_2 FC| > 0.585$ were used as the cutoff values. The red and blue dots indicate the markedly upregulated and downregulated RNAs, respectively, and the gray dots represent the nonsignificant DERs. DERs, differentially expressed RNAs; FC, fold change.

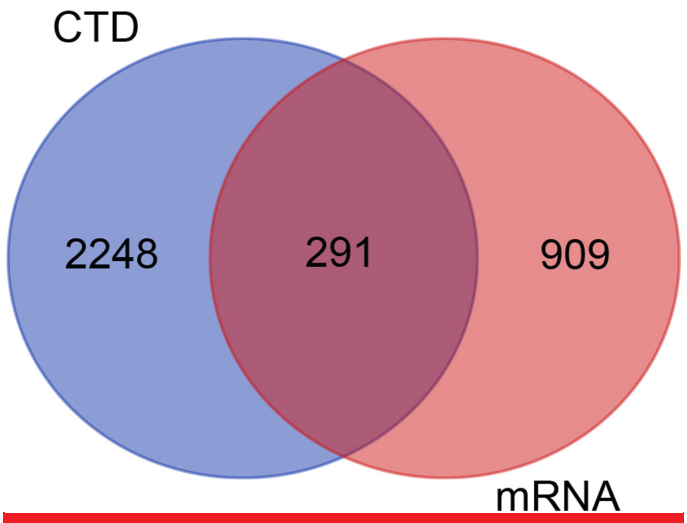

Figure 2. Venn diagram for screening MI-related mRNAs. CTD, Comparative Toxicogenomics Database; MI, myocardial infarction.

constructed the lncRNA–mRNA co-expression network ($r > 0.8$ and adjusted $P < .05$).

Kyoto Encyclopedia of Genes and Genomes pathway analysis of the co-expression network was conducted using clusterProfiler (version 3.8.1, http://bioconductor.org/packages/release/bioc/html/clusterProfiler.html); the adjusted *P*-value correction method was found to be the same as the previous step (adjusted $P < .05$).

Verification of the Key Molecules

The mRNA–lncRNA expression matrix of GSE141512 and the miRNA expression matrix of GSE66752 were used to extract all lncRNAs of the ceRNA network and the mRNA–miRNA expression profiles of top 10 connectivity. The R package ggplot2 (version 3.3.0) was used to draw the box diagram of the expression values of the lncRNAs, miRNAs, and mRNAs between the MI and healthy groups, and the *t*-test was conducted to observe the differences in the expression of the RNAs between the 2 groups.

Statistical Analysis

We used R language (version 3.4.1, https://www.r-project.org/) for statistical analysis. The limma package (version 3.10.3, www.bioconductor.org/packages/2.9/bioc/html/limma.html) of R was used to identify the DERs between the healthy and MI samples with $P < .05$ and $|\log_2 FC| > 0.58$. The correlation was determined by Pearson correlation analysis with $r > 0.8$. The *t*-test was utilized to compare the differences of the key molecules between the healthy and MI samples. A *P*-value $< .05$ was considered statistically significant for all analyses.

RESULTS

Data Preprocessing and Differentially Expressed RNA Screening

Differential expression analysis of the circRNAs, mRNAs, and miRNAs was conducted, and a total of 1200 DEmRNAs, 51 DElncRNAs, and 276 DEmiRNAs were screened with values of $P < .05$ and $|\log_2 FC| > 0.585$. The DEmiRNAs, DElncRNAs, and DEmRNAs are presented in the volcano map (Figure 1A, B, and C).

Myocardial infarction-related genes were obtained from the CTD (http://ctdbase.org/) by searching the keywords "myocardial infarction." The genes with inference score ≥50 was selected, and a total of 2539 MI-related genes were obtained. After comparing the DEmRNAs obtained in the previous steps and the MI-related genes, 291 overlapping genes were acquired (Figure 2).

Functional Enrichment Analysis of DEmRNAs

We analyzed the differential MI-related genes using DAVID and obtained 216 GO BPs and 77 KEGG signaling pathways. The top 20 terms are presented (ranked according to *P*-value) in Figure 3. Among them, we found that the differential MI-related genes were significantly related to BPs, such as

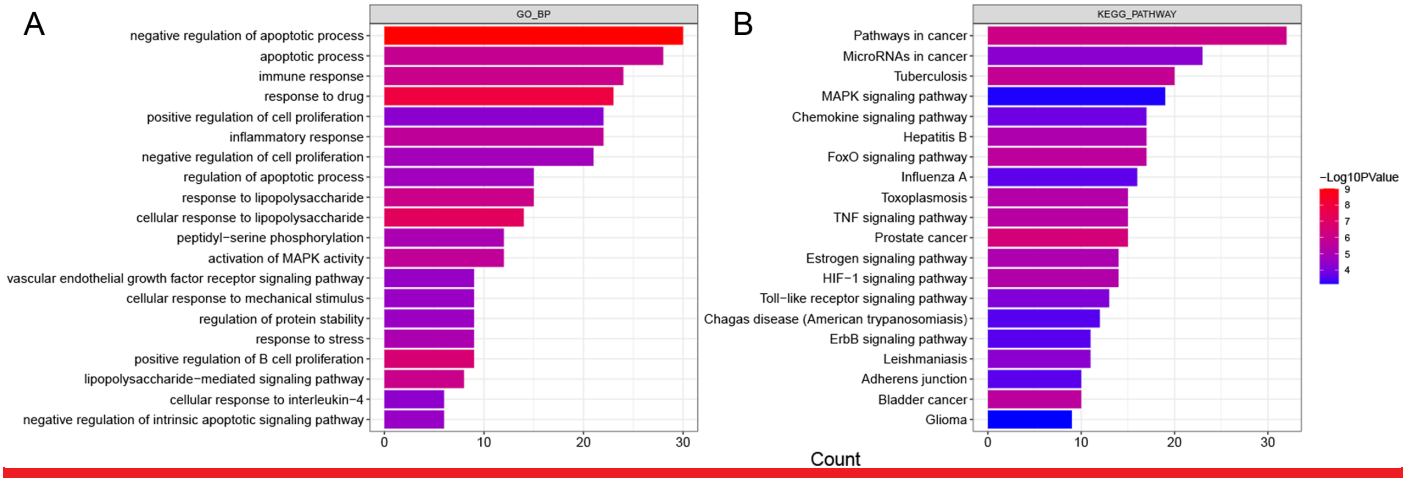

Figure 3. Top 20 (A) GO and (B) KEGG signaling pathway analyses of the differential MI-related genes. The horizontal axis indicates the gene counts and the vertical axis indicates the GO and KEGG entry names; the color indicates significance: the redder the color, the more significant it is. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; MI, myocardial infarction.

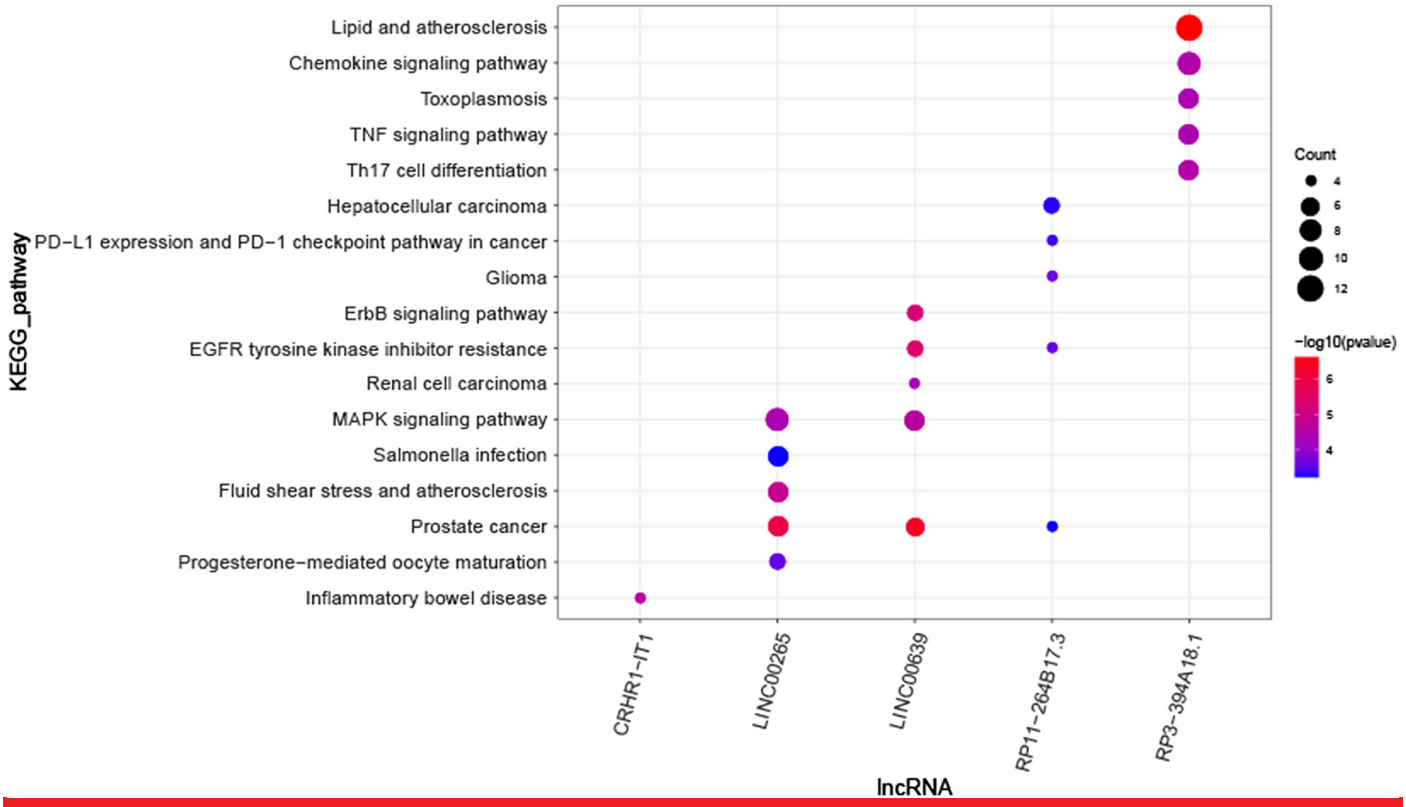

Figure 4. Top 5 KEGG pathway analyses of the lncRNA target genes. KEGG, Kyoto Encyclopedia of Genes and Genomes.

negative regulation of apoptotic process and response to drug (Figure 3A). The differential MI-related genes were significantly associated with the KEGG signaling pathways, such as prostate cancer and cancer pathways (Figure 3B).

Construction of the ceRNA Network

In total, 117 lncRNA–miRNA and 6403 miRNA–mRNA connection pairs were obtained, as well as 392 lncRNA–miRNA–mRNA connection pairs. Based on the lncRNA–miRNA and miRNA–mRNA connection pairs, the ceRNA network was constructed (Figure 4). The ceRNA network contains 17 lncRNAs, 50 miRNAs, and 95 mRNAs, including 92 lncRNA–miRNA connection pairs, 330 miRNA–mRNA connection pairs, and 148 lncRNA–mRNA co-expression connection pairs. The connectivity degree of each node in the network was analyzed to determine the degree of the mRNAs, miRNAs, and lncRNAs. We found that hsa-miR-1291 has the highest connectivity degree among the miRNAs.

Construction of the lncRNA–mRNA Co-expression Network

The PCCs of 291 MI-related genes and 51 differential lncRNAs were calculated. In total, we obtained 2043 lncRNA–mRNA connection pairs, and the KEGG pathway analysis of the co-expression network was conducted. Finally, we obtained 109 KEGG pathways. In this study, we only presented the KEGG pathways that appeared in the top 5 lncRNA target genes of the ceRNA network in terms of connectivity, e.g., the target gene of RP3-394A18.1 was significantly related to lipid and atherosclerosis, that of LINC00639 to prostate cancer, and that of LINC00265 to prostate cancer as well (Figure 5).

Verification of the Key Molecules

The GSE141512 and GSE66752 expression matrices were used to extract all lncRNAs in the ceRNA network and the top 10 connectivity of the mRNA/miRNA expression profiles. We found 3 downregulated and 3 upregulated lncRNAs. Although the differences were not significant, the regulation directions were consistent with those in the screening datasets. In addition, we found 6 downregulated miRNAs, and the regulation direction was consistent with that in the screening datasets (1 was significant at $P < .05$); the expression level of hsa-miR-1291 in the MI group was noticeably lower than that in the healthy group (Figure 6A). We also found 8 downregulated and 2 upregulated mRNAs, and the regulation directions were consistent with those in the screening datasets (4 were significant at $P < .05$); the expression level of RORA in the MI group was noticeably lower than that in the healthy group (Figure 6B), whereas the expression levels of ENTPD1 (Figure 6C), QSOX1 (Figure 6D), and TIMP2 (Figure 6E) in the MI group were noticeably higher than those in the healthy group.

DISCUSSION

Myocardial infarction has been regarded as one of the most common and fatal CVDs. It causes huge economic burden every year, especially acute MI.[12] At present, although there have been certain achievements in the diagnosis and treatment of MI, the best way to treat MI is to dissolve the thrombus using drugs; however, this method cannot reduce the risk of heart failure nor can it increase the 5-year survival rate.[13]

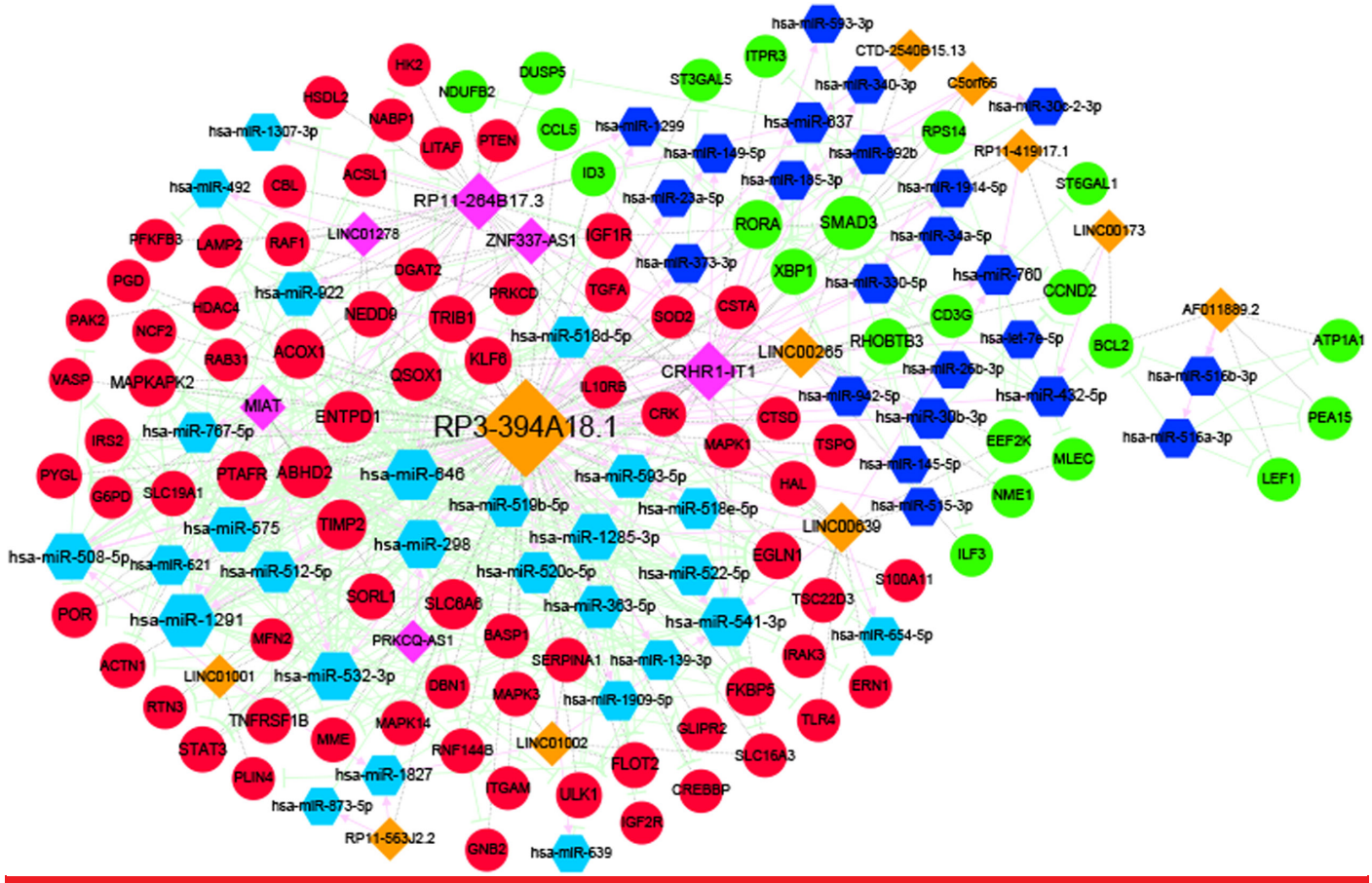

Figure 5. ceRNA regulation network. The red circles, orange diamonds, and navy blue triangles indicate the upregulated mRNAs, lncRNAs, and miRNAs, respectively; the green circles, purple diamonds, and light blue triangles indicate the downregulated mRNAs, lncRNAs, and miRNAs, respectively; the red arrow line denotes lncRNA competitive binding to miRNA; the green T-shaped line denotes the miRNA–mRNA regulatory relationship; and the gray dotted line denotes the lncRNA–mRNA co-expression network.

Thus, specific biomarkers related to MI for diagnosis and treatment need to be identified.

ceRNA is a novel mechanism for exploring the interactions of RNAs. For example, mRNA, miRNA, and lncRNA are known as RNA interaction molecules that have biological significance. miRNA has been shown to be involved in many major biological functions and can act as an excellent biomarker for CVD therapy. It also affects the development of CVD. For instance, a previous study has proven that miR-19a plays a role in the regulation of cardiomyocyte function.[14] A previous study demonstrated that miR-26b-5p is involved in the regulation of microvascular function after the occurrence of MI[15]; in addition, hsa-miR-1285-3p was found to be linked to valvular heart disease.[16] Therefore, a study on the ceRNA network of MI and the identification of significant biomarkers are important for understanding the development of MI.

In this study, bioinformatics analysis was conducted to screen differential MI-related genes; then, the lncRNA–mRNA co-expression network was constructed, and the KEGG pathway of the co-expression network was analyzed. We found that the target gene of lncRNA RP3-394A18.1 was significantly related to lipid and atherosclerosis. A study has demonstrated that atherosclerosis is closely associated with MI[17]; it indirectly indicated that RP3-394A18.1 is associated with MI.

Furthermore, we constructed the lncRNA–miRNA–mRNA ceRNA regulation network and verified the key RNAs; the expression levels of hsa-miR-1291 and RORA in the MI group were noticeably lower than those in the normal group, whereas the expression levels of ENTPD1, QSOX1, and TIMP2 in the MI group were noticeably higher than those in the normal group. RORA, ENTPD1, QSOX1, and TIMP2 were the target genes of hsa-miR-1291. It has been proven that hsa-miR-1291 could be a biomarker of acute MI[18,19] and that miR-1291 is related to the pathology of MI.[20] RORA was also found to play a significant role in coronary heart disease,[21] atrial fibrillation,[22] and hypoxia damage of cardiomyocytes.[23] In addition, ENTPD1 has been proven to inhibit CVD due to venous thrombosis.[24] Studies have demonstrated that QSOX1 is related to the development of CVD[25] and that QSOX1 plays a key role in the pathogenesis of CVD.[26] TIMP2 was also found to be involved in atherosclerosis[27] and heart failure.[28]

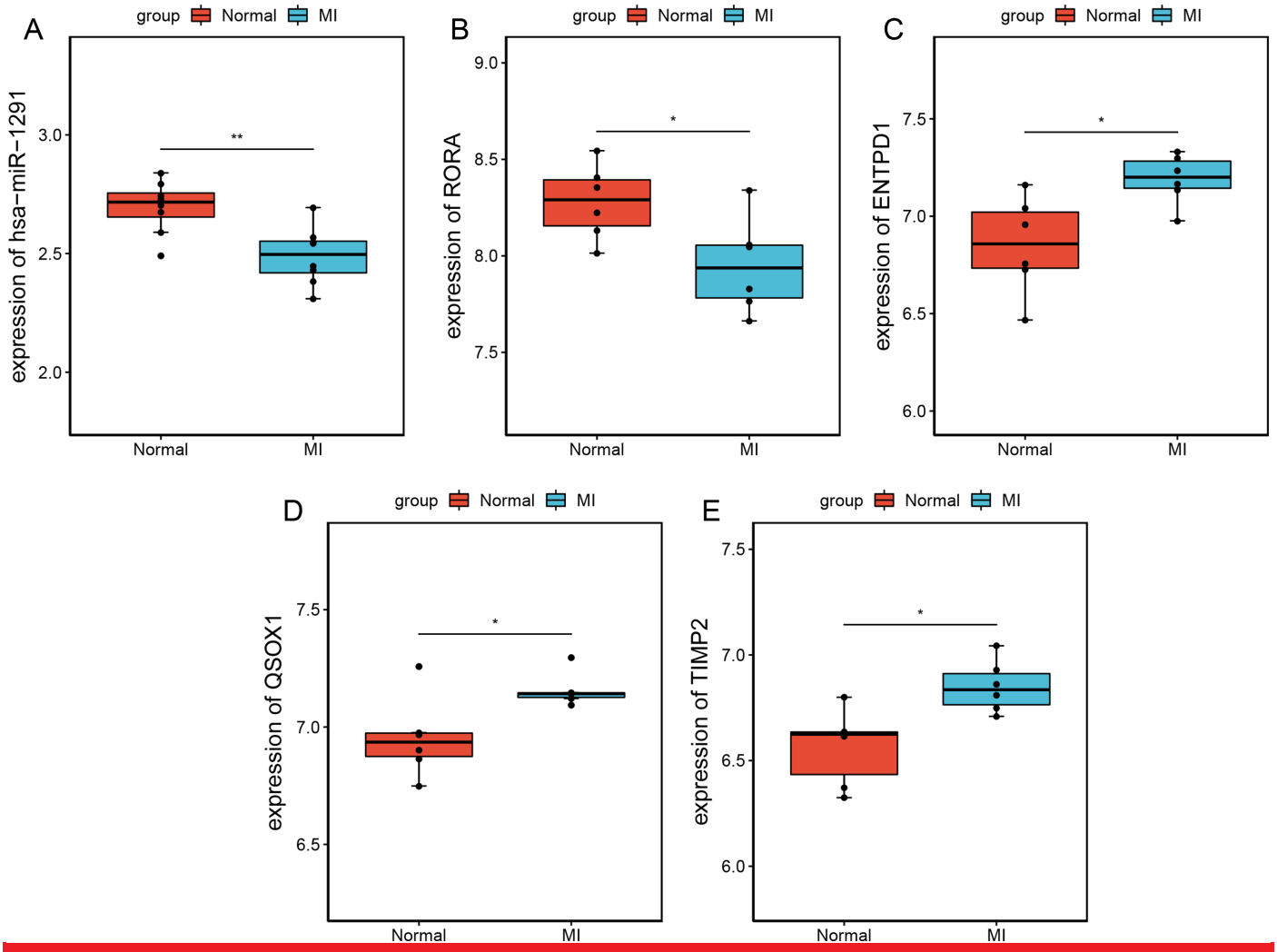

Figure 6. Box diagrams of (A) hsa-miR-1291, (B) RORA, (C) ENTPD1, (D) QSOX1, and (E) TIMP2.

Study Limitations

This study still has some limitations. First, the sample size of our datasets is not large enough. Second, the functional experiments on the targets are needed to increase the significance of this research. Finally, we need to do some protein-level experiments to validate our results in the future.

CONCLUSIONS

In summary, we constructed a ceRNA network associated with MI according to the DERs; the expression levels of hsa-miR-1291 and RORA in the MI group were noticeably lower than those in the normal group, whereas the expression levels of ENTPD1, QSOX1, and TIMP2 in the MI group were noticeably higher than those in the healthy group. These results can help us identify the biomarkers and better understand the underlying molecular mechanisms of MI.

Availability of Data and Materials: The datasets generated during and analyzed during the current study are available in the [GPL6884 and GPL9040] repository [http://www.ncbi.nlm.nih.gov/geo/].

Ethics Committee Approval: This study did not involve human and animal experiments and therefore does not require ethical approval.

Peer-review: Externally peer-reviewed.

Author Contributions: M.C. conceived and the study, P-W. M. and J-F.L. conducted the experiments, P-W.M. and J-F.L. analyzed the data, and P-W.M. and M.C. wrote the manuscript. All authors read and approved the final version.

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Declaration of Interests: The authors declare that there is no conflict of interest.

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