Differential ceRNA Expression and Interaction Analysis in Coronary Artery Disease

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Abstract:

Previous studies had shown that mRNA, miRNA and lncRNA were associated with cardiovascular diseases. The study was aimed to explore the differential expressions of mRNA, lncRNA and miRNA between coronary artery disease (CAD) and healthy control, and their interaction in CAD. We investigated the differential expression of ceRNA between CAD and healthy control through data collected from Gene Expression Omnibus (GEO) microarrays. Furthermore, we investigated the biological function of these differential expressions of ceRNAs by Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses. Protein-protein interaction (PPI) network was created to identify the hub genes. Biosystems and literature search were performed for signaling pathways and their function of the included differential expression ceRNAs. A total of 456 miRNA expression profiles, 16,325 mRNA expression profiles, and 2,869 lncRNA expression profiles were obtained. Eleven Go and KEGG pathways (count ≥9), top 15 of PPI network node connectivity rank, and top 15 of ceRNA network node degree centrality rank were achieved at the statistical significance level (P<0.05). We further identified that several differential expressions of ceRNAs and their signaling pathways were associated with CAD through biosystems and literature search. Based on eleven Go and KEGG pathways, top 15 of PPI network node connectivity rank, and top 15 of ceRNA network node degree centrality rank in CAD population, our findings would contribute to further exploration for the molecular mechanism of CAD.

Key words: miRNA; lncRNA; mRNA; ceRNA; CAD
1. Introduction

Coronary artery disease (CAD) is a complex phenotype driven by genetic and environmental factors. However current therapies focus on addressing the role of cholesterol and lifestyle in CAD. Despite advances in the development of lipid-lowering therapies, clinical trials have shown that a substantial risk of cardiovascular disease persists after currently recommended medical therapy.\(^1\) Stratification for subsequent coronary events among patients with CAD is of considerable interest because of the potential to guide secondary preventive therapies. Recently, eight microRNAs (miRNAs) were identified to facilitate acute coronary syndrome diagnosis.\(^2\) Targeting Angptl3 messenger RNA (mRNA) retarded the progression of atherosclerosis and reduced levels of atherogenic lipoproteins.\(^3\) The expressing 9p21.3-associated long non-coding RNA ANRIL induces risk CAD phenotypes in non-risk vascular smooth muscle cells.\(^4\) So far, it is not clear the mechanism of these RNAs in CAD and their interaction.

Noticeably, the different types of RNA molecule competed to bind to miRNA, which reduced the inhibitory effect of miRNA targeting on its mRNA.\(^5\) These competitive endogenous RNA (ceRNA) included various types of RNA transcripts, such as circular RNA (circRNA), long-chain non-coding RNA (lncRNA), pseudogenes and protein-encoded mRNA, which competed for miRNA through the "language" mediated by the miRNA response element (MRE).\(^6\) After that, researchers used bioinformatics methods to predict ceRNA regulatory networks. The effect of ceRNA on the target gene and the dependence of ceRNA on miRNA would be verified at the experiments of proteins and RNAs, but the functional verification would be performed at the experiments of cells and animal models.

Thus, the study was aimed to explore the differential expressions of mRNA, lncRNA and miRNA between CAD and healthy control, and the
interaction of them, including constructure of ceRNA regulatory networks, which would contribute to the molecular mechanism of CAD.

2. Results

2.1. Basic Information Statistics of Differential Expression Analysis

As described in the Methods, a total of 456 miRNA expression profiles (supplementary Table 1), 16,325 mRNA expression profiles (supplementary Table 2), and 2,869 lncRNA expression profiles were obtained (supplementary Table 3).

According to the set threshold, 18 differentially expressed miRNAs were finally obtained, including 16 down-regulated and 2 up-regulated (supplementary Table 4). A total of 92 differential lncRNAs were obtained, including 46 down-regulated and 46 up-regulated (supplementary Table 5).

A total of 610 differential mRNAs were obtained, including 244 down-regulated and 366 up-regulated (supplementary Table 6).

Based on the obtained differential miRNA, lncRNA and mRNA, the heat map was shown in Figure 1 and the volcano map was shown in Figure 2.

Figure 1. Heat map: Note: miRNA, lncRNA, and mRNA are presented from
left to right. Top red bar indicates the CAD samples, and blue bar indicates the control samples.

Figure 2. Volcano plot. Note: miRNA, IncRNA, and mRNA are displayed from left to right. Red indicates up-regulation, blue indicates down-regulation, grey indicates no significant difference.

2.2. Functional and Pathway Enrichment Analysis of Up- and Down-regulated mRNA

GOBP, GOMF, GOCC functional enrichment analysis and KEGG pathway enrichment analysis were performed on the obtained up-regulated and down-regulated mRNAs, respectively, and the results showed that a total of 36 GOBP, 3 GOCC, 15 GOMF and 2 KEGG pathways were significantly enriched (supplementary Table 7); Figure 3 presented only the TOP10 results, in accordance with ranking p value). The key results of GO and KEGG pathways enrichment analysis were displayed at Table 1.

Figure 3. GO and KEGG PATHWAY enrichment analysis. Note: black lines indicate -log10 (p value), bar length indicates the number of enriched
genes.

| Category          | Term                                                                 | Count | P Value       |
|-------------------|----------------------------------------------------------------------|-------|---------------|
| KEGG_PATHWAY      | hsa04380: Osteoclast differentiation                                  | 9     | 2.82E-02      |
| KEGG_PATHWAY      | hsa05200: Pathways in cancer                                         | 18    | 4.33E-02      |
| GOTERM_MF         | GO: 0003700--transcription factor activity, sequence-specific DNA binding | 50    | 1.77E-04      |
| GOTERM_MF         | GO: 0000978--RNA polymerase II core promoter proximal region sequence-specific DNA binding | 24    | 4.40E-04      |
| GOTERM_MF         | GO: 0001076--transcription factor activity, RNA polymerase II transcription factor binding | 4     | 7.53E-03      |
| GOTERM_MF         | GO: 0034711--inhibin binding                                         | 3     | 8.38E-03      |
| GOTERM_MF         | GO: 0046872--metal ion binding                                       | 81    | 8.79E-03      |
| GOTERM_MF         | GO: 0001077--transcriptional activator activity, RNA polymerase II core promoter proximal region sequence-specific binding | 15    | 1.16E-02      |
| GOTERM_MF         | GO: 0043565--sequence-specific DNA binding                           | 26    | 1.32E-02      |
| GOTERM_MF         | GO: 0008134--transcription factor binding                           | 16    | 2.39E-02      |
| GOTERM_MF         | GO: 0004675--transmembrane receptor protein serine/threonine kinase activity | 3     | 2.79E-02      |
| GOTERM_MF         | GO: 0045236--CXCR chemokine receptor binding                         | 3     | 2.79E-02      |
| GOTERM_CC         | GO: 0042582--azurophil granule                                       | 5     | 2.18E-04      |
| GOTERM_CC         | GO: 0042581--specific granule                                        | 5     | 4.50E-04      |
| GOTERM_CC         | GO: 0005622--intracellular                                           | 55    | 1.28E-02      |
| GOTERM_BP         | GO:0045944--positive regulation of transcription from RNA polymerase II promoter | 55    | 1.47E-05      |
| GOTERM_BP         | GO: 0006954--inflammatory response                                   | 27    | 9.45E-05      |
| GOTERM_BP         | GO: 0097191--extrinsic apoptotic signaling pathway                    | 7     | 1.55E-03      |
| GOTERM_BP         | GO: 0042832--defense response to protozoan                           | 5     | 2.23E-03      |
| GOTERM_BP         | GO: 0035284--brain segmentation                                      | 3     | 2.69E-03      |
| GOTERM_BP         | GO: 0090023--positive regulation of neutrophil chemotaxis           | 5     | 3.93E-03      |
| GOTERM_BP         | GO: 0000122--negative regulation of transcription from RNA polymerase II promoter | 35    | 7.00E-03      |
| GOTERM_BP         | GO: 0032024--positive regulation of insulin secretion               | 6     | 7.58E-03      |
| GOTERM_BP         | GO: 0045602--negative regulation of endothelial cell differentiation | 3     | 8.60E-03      |
| GOTERM_BP         | GO: 0048662--negative regulation of smooth muscle cell proliferation | 5     | 1.08E-02      |

Table 1. Go and KEGG pathway enrichment analysis of differential genes

2.3. Protein Interaction Network Construction (PPI) and Module Analysis

As described in the methods, we achieved a total of 388 protein interaction
relationship pairs, and the network construction was performed using Cytoscape software as shown in Figure 4. A total of 171 nodes were included in the network.

Figure 4. Protein interaction relationship network diagram (PPI). Note: red indicates up-regulated protein, green indicates down-regulated protein, gray line indicates protein interaction relationship, and node size indicates connectivity degree.

The network was analyzed for node connectivity according to the parameters set by the Method, the top15 of Degree Centrality (DC) of each node was ranked in Table 2. Notably, CXCL8, FPR2, IL6, and PPBP were ranked in Top15, which might be hub proteins in the network (supplementary Table 8). The top 15 of PPI network node connectivity rank were displayed at Table 2.

| Node  | Degree | P Value   | TYPE  | Name                                           |
|-------|--------|-----------|-------|------------------------------------------------|
| GNG13 | 22     | 0.017945  | DOWN  | G protein subunit gamma 13                     |
| Gene         | Rank | Score  | Direction | Description                                      |
|-------------|------|--------|-----------|--------------------------------------------------|
| GNG11       | 22   | 0.04065| DOWN      | G protein subunit gamma 11                       |
| CXCL1       | 18   | 0.000525| UP        | C-X-C motif chemokine ligand 1                   |
| CXCR4       | 14   | 0.000134| UP        | C-X-C motif chemokine receptor 4                 |
| CXCL8       | 14   | 0.001358| UP        | C-X-C motif chemokine ligand 8                   |
| SNAP23      | 14   | 0.006229| UP        | synaptosome associated protein 23                |
| FPR3        | 13   | 0.003603| DOWN      | formyl peptide receptor 3                        |
| RAB44       | 13   | 0.028049| UP        | RAB44, member RAS oncogene family                |
| SKP2        | 12   | 0.000943| UP        | S-phase kinase associated protein 2              |
| GPR18       | 12   | 0.001281| DOWN      | G protein-coupled receptor 18                    |
| GALR3       | 12   | 0.001499| UP        | galanin receptor 3                               |
| CXCL6       | 12   | 0.002672| UP        | C-X-C motif chemokine ligand 6                   |
| ITCH        | 12   | 0.007857| UP        | itchy E3 ubiquitin protein ligase                |
| S1PR3       | 12   | 0.010344| UP        | sphingosine-1-phosphate receptor 3               |
| GRM2        | 12   | 0.010472| UP        | glutamate metabotropic receptor 2                |

Table 2. PPI network node connectivity rank (TOP15)

2.4. mRNA and IncRNA Co-expression Analysis

We performed co-expression analysis of differentially expressed mRNAs and IncRNAs. According to the threshold set by the Methods, we screened a total of 1487 significantly coordinately expressed relationship pairs, including 381 mRNAs and 74 IncRNAs (supplementary Table 9).

2.5. miRNA Target Genes and Upstream IncRNA Prediction Analysis

Based on the differentially expressed miRNAs and differential IncRNAs, a total of 452 IncRNA-miRNA relationship pairs were predicted as described in the Methods (supplementary Table 10), including 18 miRNAs, and 72 IncRNAs.

Also based on differentially expressed miRNAs, target gene prediction was performed using mirWalk as described in the Methods, after taking the intersection with the differential mRNAs, 276 miRNA-mRNA relationship pairs were obtained, including 17 miRNAs, and 170 mRNAs (supplementary Table 11).

2.6. Pathway Enrichment Analysis of IncRNAs and MiRNAs

As described in the Methods, a total of 27 IncRNAs were enriched by
KEGG pathway (supplementary Table 12) and 12 miRNAs were enriched by KEGG pathway (supplementary Table 13), here we showed a part of the results in Figure 5.

Figure 5. Results of lncRNA and miRNA pathway enrichment analysis. Note: top: lncRNA; bottom: miRNA; the decrease of significant p-value is shown from blue to red color, and bubble size indicates the proportion of
enriched genes (the number of involved term genes accounts for the number of input genes).

2.7. CeRNA Network Analysis

As described in the Methods section, based on the obtained miRNA-LncRNA and miRNA-mRNA relationship pairs, miRNA-LncRNA-mRNA relationship pairs regulated by the same miRNA were firstly screened, along with the positive co-expression relationship between mRNA and LncRNA (correlation coefficient > 0.7), and LncRNA-miRNA-mRNA relationship pairs were further screened for network construction, i.e., the ceRNA network as shown in Figure 6.

Figure 6. ceRNA network diagram. Note: the red circles represent up-regulated mRNAs, and green circles represent down-regulated mRNAs; yellow triangles represent up-regulated miRNAs, and gray triangles represent down-regulated miRNAs; blue diamond’s represent
down-regulated lncRNAs, and pink diamonds represent up-regulated lncRNAs; The blue T-type lines represent the miRNA-lncRNA regulatory relationships, the yellow arrows represent the miRNA-mRNA regulatory relationships, and the green dotted lines represent the co-expression relationships of mRNA and lncRNA.

The network contained a total of 87 lncRNA-miRNA relationship pairs, 88 miRNA-mRNA relationship pairs, and 137 lncRNA-mRNA co-expression relationships (supplementary Table 14). There were a total of 36 lncRNAs, 64 mRNAs, and 15 miRNAs. Connectivity analysis was performed on each node of the ceRNA network to obtain mRNA, miRNA, and lncRNA connectivity as detailed in Table 3.

| Node          | Degree | Type    | logFC      | P Value  |
|---------------|--------|---------|------------|----------|
| hsa-miR-539-5p| 30     | mi_down | -0.31167   | 0.002388 |
| hsa-miR-654-5p| 27     | mi_down | -0.38709   | 0.001381 |
| hsa-miR-432-5p| 22     | mi_down | -0.29313   | 0.011733 |
| PSMA3-AS1     | 20     | lnc_up  | 0.629594   | 0.032669 |
| hsa-miR-136-5p| 19     | mi_down | -0.34012   | 0.010986 |
| MIR29A        | 18     | lnc_up  | 0.849701   | 0.001608 |
| RP11-420L9.4  | 17     | lnc_down| -0.69127   | 0.00077  |
| LINC01619     | 14     | lnc_up  | 0.610105   | 0.005996 |
| NACC2         | 14     | m_up    | 1.011445   | 7.23E-05 |
| hsa-miR-329-3p| 13     | mi_down | -0.28995   | 0.006895 |
| hsa-miR-1246  | 12     | mi_up   | 0.489247   | 0.000567 |
| hsa-miR-133b  | 11     | mi_down | -0.40401   | 0.045067 |
| hsa-miR-431-5p| 11     | mi_down | -0.37006   | 0.001259 |
| ST20-AS1      | 10     | lnc_up  | 1.167706   | 0.000122 |
| STK35         | 10     | m_up    | 1.147951   | 0.000764 |

Table 3. ceRNA network node degree centrality rank (TOP15)

3. Discussion

The study analyzed the differential genes at the statistical significance
level between CAD and healthy control, and found eleven Go and KEGG pathways (count ≥9), top 15 of PPI network node connectivity rank, and top 15 of ceRNA network node degree centrality rank, which would contribute to further exploration for the molecular mechanism of CAD.

Firstly, the Go and KEGG pathways (count ≥9) showed their function in Table 1, and they contained a large number of differential genes at the statistical significance level in CAD, thus these Go and KEGG pathways might play a role of molecular level of CAD. Several further explored experiments related to these pathways would achieve the interesting and important findings in CAD field.

Secondly, in top 15 of PPI network node connectivity rank, we found that the extensive protein interaction relationship pairs at the statistical significance level in CAD (Table 2), which matched with their signaling pathways in the biosystems (supplementary Table 15). Some of them were identified to play a role in cardiovascular diseases. For example, GNG11 was a member of the gamma subunit family of heteromeric G-protein. Overexpression of GNG11 activated ERK1/2 of the MAP kinase family, but did not Ras.20 These findings provide clinically relevant biological insight into heritable variation in vagal heart rhythm regulation, with a key role for genetic variants (GNG1, RGS6) that influence G-protein heterotrimer action in GIRK-channel induced pacemaker membrane hyperpolarization (supplementary Table 15).21

CXCL1 was produced mainly by TNF-stimulated endothelial cells (ECs) and pericytes and supported luminal and sub-EC neutrophil crawling. CXCL1 and CXCL2 act in a sequential manner to guide neutrophils through venular walls as governed by their distinct cellular sources.22 Angiotensin II-induced infiltration of monocytes in the heart is largely mediated by CXCL1-CXCR2 signalling which initiates and aggravates cardiac remodelling. Inhibition of CXCL1 and/or CXCR2 may represent new therapeutic targets for treating hypertensive heart diseases (supplementary Table 15).23

Wnt-Cxcr4 (C-X-C motif chemokine receptor 4) signaling in regulation of oligodendrocyte precursor cells (OPCs)-endothelial interactions coordinates OPC
migration with differentiation. Many of the neutrophils reenter the vasculature and have a preprogrammed journey that entails a sojourn in the lungs to up-regulate CXCR4 before entering the bone marrow, where they undergo apoptosis. Vascular CXCR4 limits atherosclerosis by maintaining arterial integrity, preserving endothelial barrier function, and a normal contractile SMC phenotype. Enhancing these beneficial functions of arterial CXCR4 by selective modulators might open novel therapeutic options in atherosclerosis (supplementary Table 1).

Oleic acid treatment decreases the insulin sensitivity of heart muscle cells, and this sensitivity is completely restored by transfection with SNAP23. Thus, SNAP23 might be a link between insulin sensitivity and the inflow of fatty acids to the cell (supplementary Table 1).

Co-activator-associated arginine methyltransferase 1 (CARM1) is a crucial component of autophagy in mammals. CARM1-dependent histone arginine methylation is a crucial nuclear event in autophagy, and identify a new signalling axis of AMPK-SKP2-CARM1 in the regulation of autophagy induction after nutrient starvation (supplementary Table 1).

GPR18 is a cannabinoid-activated orphan G protein-coupled receptor (GPCR) that is selectively expressed on immune cells. A salutary cardiovascular role for GPR18, mediated, at least partly, via elevation in the levels of adiponectin (supplementary Table 1).

Thirdly, in top 15 of ceRNA network node degree centrality rank, the extensive ceRNA interaction relationship pairs at the statistical significance level in CAD (Table 3), which matched with their description in the literature search (supplementary Table 16). Certainly, we also found that several ceRNA was related to cardiovascular diseases. For example, by regulating CDKN2A and inhibiting G1- to S-phase transition STK35L1 may act as a central kinase linking the cell cycle and migration of endothelial cells. The interaction of STK35L1 with nuclear actin might be critical in the regulation of these fundamental endothelial functions. Serine/threonine kinase 35 (STK35) is a recently identified human kinase with an autophosphorylation function, linked functionally to actin stress fibers, cell
cycle progression and survival (supplementary Table 16).\(^4^5\) Nuclear-retained importin α2 binds with DNase I-sensitive nuclear component(s) and exhibits selective upregulation of mRNA encoding STK35 by microarray analysis. Chromatin immunoprecipitation and promoter analysis demonstrated that importin α2 can access to the promoter region of STK35 and accelerate its transcription in response to hydrogen peroxide exposure. Furthermore, constitutive overexpression of STK35 proteins enhances caspase-independent cell death under oxidative stress conditions (supplementary Table 16).\(^4^6\)

4. Materials and Methods

4.1. Data Preprocessing

miRNA expression profiling data were obtained from NCBI GEO (Gene Expression Omnibus, GEO, http://www.ncbi.nlm.nih.gov/geo/) database\(^7\) to download the expression profile data after normalization of the dataset serial number GSE59421. A total of 96 samples with the subjects’ characteristics (63 healthy controls (CTRL), 33 CAD blood samples), which were detected using the Agilent - 021827 Human miRNA MicroArray (V3) platform (miRBase release 12.0 miRNA ID version).

The mRNA/lncRNA data were also obtained from the NCBI GEO database to download the expression profile data after normalization of the dataset serial number GSE42148. A total of 24 samples with the subjects’ characteristics (11 CTRL, 13 CAD blood samples), which were detected using the Agilent - 028004 SurePrintG3 Human GE 8 x 60K Microarray platform (Feature Number version).

4.2. mRNA and lncRNA Annotation

The sequences matched the probes of Agilent-028004 were obtained from the platform annotation file, and the human reference genome (GRCh38) sequences was downloaded from the GENCODE database\(^8\) (https://www.gencodegenes.org/releases/current.html), and the probe sequences were aligned onto the reference genome using the seqmap software.\(^9\) Firstly, we retained the uniquely aligned (unique map) probes,
and secondly, we referred their position to the chromosome with positive and negative strand information, the gene matched each probe was obtained according to the human gene annotation file (Release 25) provided by GENCODE.

We kept the probe with the annotating information "protein_coding" as the matching probe for mRNA, the probes with the annotating information with "antisense", "sense_intronic", "lincRNA", "sense_overlapping" or "processed_transcript" were considered to the matching lncRNA probe.

Finally, the probe numbers and mRNA/lncRNA (Gene symbol) were matched one by one to remove probes that did not match to Gene symbol. For different probes mapping to the same gene, we used the average of different probes as the final expression value of the mRNA/lncRNA.

4.3. Differential MRNA, lncRNA and MiRNA Screening

We took the R software limma package with the classical Bayesian method\textsuperscript{10} (version 3.10.3, http://www.bioconductor.org/packages/2.9/bioc/html/limma.html). The differential analysis was performed between CAD and CTRL. Importantly, the miRNAs, mRNAs, and lncRNAs were analyzed to obtain their p values and logFC values, which were evaluated at the levels of both fold difference and statistical significance. The threshold of differential expression was set as miRNA: p value < 0.05 and |logFC| > 0.263 (>1.2 times).

4.4. Functional Enrichment and Pathway Analysis of Differentially Expressed MRNA

Enrichment analysis was performed with the common enrichment analysis tool DAVID\textsuperscript{11} (version 6.8, https://david-d.ncifcrf.gov/) to analyze the up- and down- regulated genes, which were involved in the pathways of Gene Ontology BP (biological process),\textsuperscript{12} CC (cellular component), MF (molecular function) and KEGG.\textsuperscript{13} Significant enrichment results were considered to a significance threshold p value < 0.05 and an at least 2 of
enrichment number (count).

4.5. **Protein Interaction Network (PPI) Construction and Node Connectivity Analysis**

In combination with STRING (version: 10.0, http://www.string-db.org/), the database predicted whether there was an interaction relationship between the proteins encoded by the analyzed genes. The differential mRNAs were inputted into gene sets, and homo was inputted into species. The parameter of PPI score was set to 0.9 (highest confidence), the interactional protein nodes were required in the up- and down-regulated genes. After the PPI relationship pairs were obtained, the data were analyzed using Cytoscape software (version 3.4.0, http://chianti.ucsd.edu/cytoscape-3.4.0/), for which a network map was constructed. The node connectivity analysis was performed with parameters of no weigh by using the CytoNCA plugin (Version 2.1.6, http://apps.cytoscape.org/apps/cytonca). The results obtained the important nodes in the PPI network that were involved in protein interaction relationships, i.e., hub proteins, through the connectivity Degree Centrality (DC) rank of individual nodes.

4.6. **lncRNA and mRNA Co-expression Analysis**

The correlation test was performed and their pearson correlation coefficients of the differential mRNA and lncRNA were respectively calculated by using the matched sample of mRNA and lncRNA data. The relationship pairs with \( r > 0.7 \) (coordinate expression) and \( p \) value < 0.05 were focused on screening for the subsequent ceRNA network construction, and these mRNAs were considered to be significantly correlated with lncRNAs, while mRNAs were considered as potential target genes of lncRNAs.

4.7. **Target Genes MiRNA and The Prediction for Their Upstream lncRNA**

Based on the differential miRNAs obtained from the differential analysis, miRWalk2.0 (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/)
database was used, which integrated the four typical databases including miRWalk, miRanda, RNA22, and TargetScan. If the predicted target genes were presented in each of four databases, the marching mRNA was considered to be regulated by the miRNA. After the predicted miRNA-mRNA relationship pairs were obtained, the mRNAs were further intersected with the differential mRNAs to obtain the differential miRNA-differential mRNA relationship pairs.

With regard to differential lncRNAs versus differential miRNAs, we used the local software miRanda (v3.3a) to predict differential miRNA-differential lncRNA relationship pairs through software parameters (-sc140, -en-20, i.e., screen score ≥ 140, energy ≤ -20).

4.8. Pathway Enrichment Analysis of lncRNAs and MiRNAs

Based on the obtained lncRNA-mRNA co-expression relationship pairs and miRNA-mRNA relationship pairs, mRNAs were used as potential target genes of matching lncRNAs and miRNAs, respectively. KEGG Pathway enrichment analysis was performed by using the R package clusterProfiler (version: 3.8.1, http://bioconductor.org/packages/release/bioc/html/clusterProfiler.html), and its results indirectly predicted the functions of lncRNAs and miRNAs. The threshold was set at p value < 0.05.

4.9. CeRNA Network Construction

Based on the obtained mRNA-miRNA and lncRNA-miRNA relationship pairs, we firstly screened the miRNA-lncRNA-mRNA relationship pairs regulated by the same miRNA, then combined the positive co-expression relationship between mRNA and lncRNA (correlation coefficient > 0.7), and further screened the miRNA-lncRNA-mRNA relationship pairs for network construction, i.e., the ceRNA network. Thus, the lncRNAs and mRNAs with positive co-expression relationship regulated by the same miRNA in the ceRNA network were each other ceRNAs.

Finally, the node connectivity (degree) analysis was also performed
using the Cytoscape plugin CytoNCA with the parameter set to no weight. The higher the connectivity, the higher the importance of this node in the network.

5. Conclusions

Based on eleven Go and KEGG pathways, top 15 of PPI network node connectivity rank, and top 15 of ceRNA network node degree centrality rank in CAD population, our findings would contribute to further exploration for the molecular mechanism of CAD.

Supplementary Materials: Supplementary tables can be found in supplementary Material

Authors' Contributions: Shen Kang conceived and designed the study, Shen Kang analyzed data and drafted the manuscript, Yong Ye participated in the data collection. Guang Xia was responsible for quality control and revised the manuscript, Haibo Liu analysis the data and participated in the fund support of the study.

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Abbreviations

| Abbreviation | Description                        |
|--------------|------------------------------------|
| BP           | biological process                 |
| CAD          | coronary artery disease            |
| CARM1        | Co-activator-associated arginine methyltransferase 1 |
| CC           | cellular component                 |
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