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ORIGINAL RESEARCH

Screening and Analysis of Potential Critical Gene in Acute Myocardial Infarction Based on a miRNA-mRNA Regulatory Network

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Background: MicroRNAs (miRNAs) have been shown to be involved in the initiation, progression, and prevention of acute myocardial infarction (AMI), but the underlying mechanism remains unclear.

Objective: Through the GEO database, bioinformatics methods were used to explore the miRNA-mRNA regulatory relationship pairs associated with AMI and to elucidate the underlying mechanism.

Methods: Using the R software Limma package, differential expression analysis was performed using the AMI-related miRNA chip dataset (GSE31568) and mRNA chip dataset (GSE159657) from the GEO database. The miRDB, miRWalk, miRTarBase, and TargetScan databases were used to predict potential downstream target genes regulated by differentially expressed miRNAs, and a miRNA-mRNA regulatory network was built with Cytoscape; GO function and KEGG pathway enrichment analyses of target genes were done with Funrich software, and the protein interaction network of target genes in the regulatory network was built with the STRING database.

Results and Conclusions: A total of 187 differentially expressed miRNAs were experimentally screened, of which 91 were upregulated (such as hsa-miR-302b, hsa-miR-1299), and 96 were downregulated (such as hsa-miR-1201, hsa-miR-1283); 507 differentially expressed mRNAs were identified, of which 430 were upregulated (such as MRM1 and SFXN4), and 77 were downregulated (such as KCTD13 and CCDC134). And 16 miRNAs and 44 mRNAs were used for regulatory network construction. GO and KEGG enrichment analyses mainly focused on Integrins in angiogenesis, angiopoietin receptor Tie2-mediated signaling, and signaling events mediated by stem cell factor receptor (c-Kit). As hub genes in the PPI network, FGF2 and MMP2 may be key targets of AMI. The experimentally constructed miRNA-mRNA regulatory network found that hsa-miR-190b targets to inhibit FGF2, while hsa-miR-330-3p targets to regulate MMP2, which may mediate Integrins in angiogenesis, Angiopoietin receptor Tie2-mediated signaling pathway to induce AMI pathogenesis, providing strong data support and a research direction for the prevention and treatment of AMI.

Keywords: acute myocardial infarction, AMI, GEO database, bioinformatics, differential gene, regulatory networks, miRNA-mRNA, genes, protein interaction

Introduction

Acute myocardial infarction (AMI) is an important factor threatening human life worldwide. According to the existing epidemiological survey data in western countries, in 2017, an estimated 695,000 Americans had a new acute myocardial infarction (AMI), and another 325,000 had a recurrent event.[1] Statistical studies suggest reductions in hospital admissions and mortality in developed countries in recent years, but the global burden of CVD and acute myocardial infarction has shifted to low- and middle-income countries, as more than 80% of all CVD deaths currently occur in these countries.[2] It is well known that mortality from AMI is higher; although the case fatality rate has been reduced from 30% to approximately 5% in recent years due to the improved sensitivity of the detection of creatine kinase isozymes as well as biomarkers such as troponin,[3,4] as well as rapidly developing PCI and medical therapy, there is still a high risk of mortality. Currently, invasive coronary

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angiography remains the gold standard for the diagnosis of AMI; however, there is a great probability of misdiagnosis in the diagnosis of ECG, and blood-based biomarkers such as CK-MB and cTnT have certain deficiencies in the diagnosis of AMI.[5,6] Post-AMI onset progression is very rapid, with many patients dying within 1–2 h of onset, most deaths occurring within a week of onset, and there is a high rate of recurrence[1]. Some patients with acute myocardial infarction have premonitory symptoms before onset, such as chest pain, but a large part of them are asymptomatic and often can not attract attention. Once the disease occurs, it will cause very serious consequences. Therefore, it is crucial to improve criteria for early and accurate diagnostic workups, which will reduce mortality and improve outcomes. And more methods and biological markers need to be identified to develop diagnostics for AMI. Current research on AMI biomarkers has gradually become a hot spot, and there have been many studies related to gene markers for diagnosis as well as targeted therapy.[7,8] This study aimed to enrich the research on AMI biomarkers to perform the next advance work for definite clinical diagnosis and treatment.

MicroRNAs (miRNAs) constitute a class of small RNAs that are approximately 20–24 nucleotides in length and have various important regulatory roles in the cell. They are widely present in eukaryotic cells in various organisms and can be specifically expressed during cell differentiation and the tissue development stage. They do not encode proteins but can affect the transcriptional, posttranscriptional and epigenetic levels of genes and can inhibit protein translation or promote mRNA degradation. Studies have shown that miRNAs are of great clinical importance in the pathogenesis, prevention, and treatment of cardiovascular diseases and diseases such as diabetes.[9,10] However, there are few reports of in-depth exploration into the molecular mechanisms of miRNAs in AMI, and the molecular network involved in the miRNA-mediated regulatory mechanism during AMI disease development remains unclear.

The Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) is an international public repository where researchers may contribute high-throughput microarray and next-generation sequencing functional genomic data sets. The resource allows for the preservation of raw data, processed data, and metadata that is searchable, indexed, and cross-linked.[11] All data are freely available for download in a variety of formats. Therefore, an experiment was performed to construct a miRNA-mRNA regulatory network using bioinformatics and AMI-related miRNA and mRNA expression datasets in the GEO database to identify the key miRNA-mRNA regulatory relationship pairs, analyze the functions of targets and related signaling pathways, explore the mechanisms of action, and provide an important theoretical reference and scientific basis for the targeted therapy of AMI. The study flow chart was as follows (Figure 1).

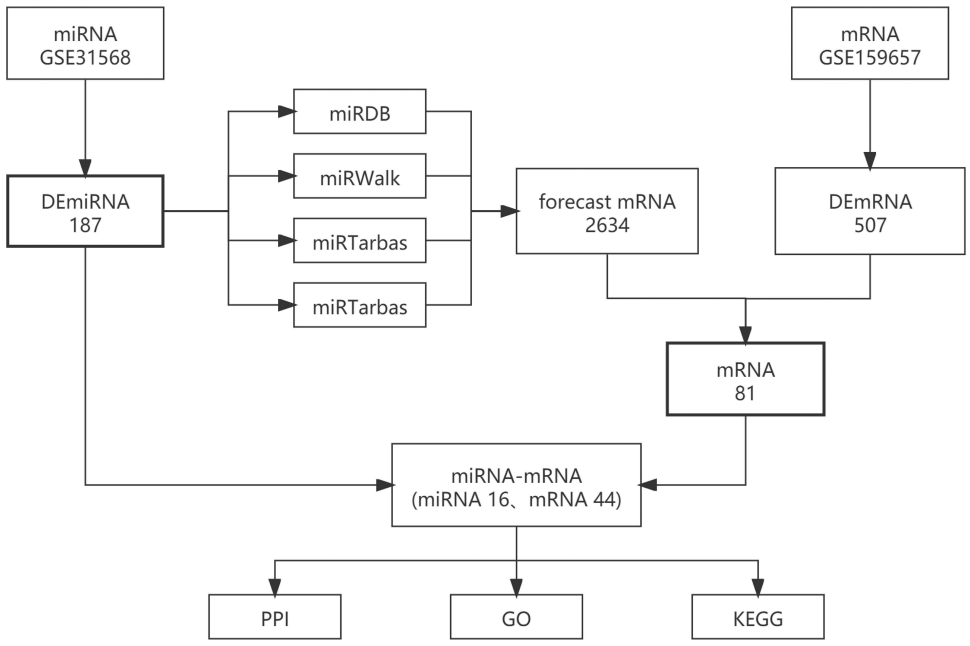

Figure 1 Flow chart.

Materials and Methods

Data Source

Chip data of miRNA expression profiles and mRNA expression profiles associated with AMI were retrieved from the NCBI GEO (Gene Expression Omnibus) database, and the screening criteria were samples of AMI patients and healthy populations. Cell lines or animal models were excluded. The time for this study to search data in geo database is from October 2021 to November 2021.The qualified miRNA expression chip dataset GSE31658 and mRNA expression high-throughput dataset GSE159657 were downloaded. The GSE31658 dataset is based on the GPL9040 platform (Febit Homo Sapiens miRBase 13.0) and contains 454 samples. Twenty whole blood samples from patients with acute myocardial infarction (AMI) and 20 whole blood samples from healthy controls (controls) were selected. The GSE159657 dataset is based on the GPL24676 platform (Illumina NovaSeq 6000 (Homo sapiens)) containing 28 samples selected, including plasma samples from 10 patients with acute myocardial infarction (AMI) and plasma samples from 10 healthy controls (controls). (Database or software for research in Table 1).

Methods

Data Processing and Differential Expression Analysis

The GSE31568 dataset was analyzed using the GEO2R function in the GEO database for differential expression using the Limma package in R software with the filtering criteria set at adj.p.Val<0.01, |log2-fold change (FC)| >1. Differential analysis of the GSE159657 dataset was performed using the NetworkAnalyst database, and the filtering criteria were set at P. Value <0.05, |log2-fold change (FC)| >1. The differential expression data were visualized and displayed with volcano plots and cluster plots.

Target Gene Prediction and miRNA-mRNA Regulatory Network Construction

Using the miRDB, miRWalk, miRTarBase, and TargetScan databases to predict the target genes of differentially expressed miRNAs, the differentially expressed mRNAs predicted by the simultaneous four databases and the GSE159657 analysis were intersected by Jvenn online, and the miRNA-mRNA relationship pairs were defined according to the regulatory relationship between miRNAs and mRNAs. MiRNA-mRNA regulatory network visualization was performed using Cytoscape software.

Functional Enrichment Analysis of Target Genes Regulated by miRNAs

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of target genes in the miRNA-mRNA regulatory network was performed using Funrich software, including biological process (BP), cellular component (CC) and molecular function (MF), setting the p value < 0.05.

Table 1 Database or Software for Research

Databases/Software	URLs/Versions
GEO database	https://www.ncbi.nlm.nih.gov/geo/
Targetscan database	http://www.targetscan.org/vert_72
miRTarBase database	https://mirtarbase.cuhk.edu.cn/
miRDB database	http://mirdb.org/
miRWalk database	mirwalk.umm.uni-heidelberg.de/
NetworkAnalyst database	https://www.networkanalyst.ca/
Jvenn	http://jvenn.toulouse.inra.fr/app/documentation.html
STRING database	https://string-db.org/
Weishengxin	http://www.bioinformatics.com.cn/login/
FunRich Software (3.1.3)	http://www.funrich.org/
Cytoscape Software (3.7.2)	https://cytoscape.org/
R Software (4.0.2)	https://www.r-project.org/

miRNA Regulated Target Gene Protein Interaction Network Construction

To further identify the relationship between target genes in the miRNA-mRNA regulatory network, protein–protein interaction analysis (PPI) was performed using the STRING (Search Tool for the Retrieval of Interacting Genes) database, setting the confidence score > 0.4. The PPI network was visualized in Cytoscape software. Each node in the PPI network was evaluated by the "degree" to screen core genes (hub genes). The higher the degree of a node is, the greater its significance in the PPI network.

Results

Differentially Expressed miRNAs

Compared with AMI and healthy control whole blood samples in the GSE31568 dataset, 243 differentially expressed miRNAs were obtained after removing duplicates, and 187 differentially expressed miRNAs, marked with *were obtained, including 91 genes with upregulated expression (eg, hsa-miR-302b, hsa-miR-1299, hsa-miR-613, hsa-miR-609, hsa-miR-190b, hsa-miR-1468, hsa-miR-1258, hsa-miR-508-3p, hsa-miR-1262, and hsa-miR-373) and 96 genes with downregulated expression (eg, hsa-miR-491-3p, hsa-miR-1201, hsa-miR-1283, hsa-miR-1245, hsa-miR-217, hsa-miR-518a-3p, hsa-miR-1291, hsa-miR-1271, hsa- miR-621, and hsa-miR-515-5p). The differential expression miRNA volcano plot is presented in Figure 2A, and the cluster plots of the top 50 differential miRNAs with larger fold differences are presented in Figure 2B.

Differentially Expressed mRNAs

Compared with AMI and healthy control plasma samples in the GSE159657 dataset, 507 differentially expressed mRNAs were acquired, including 430 upregulated and 77 downregulated mRNAs. Volcano plots of all differential mRNAs are shown in Figure 3A, and cluster plots of the top 50 differential mRNAs with large absolute values of fold difference are shown in Figure 3B.

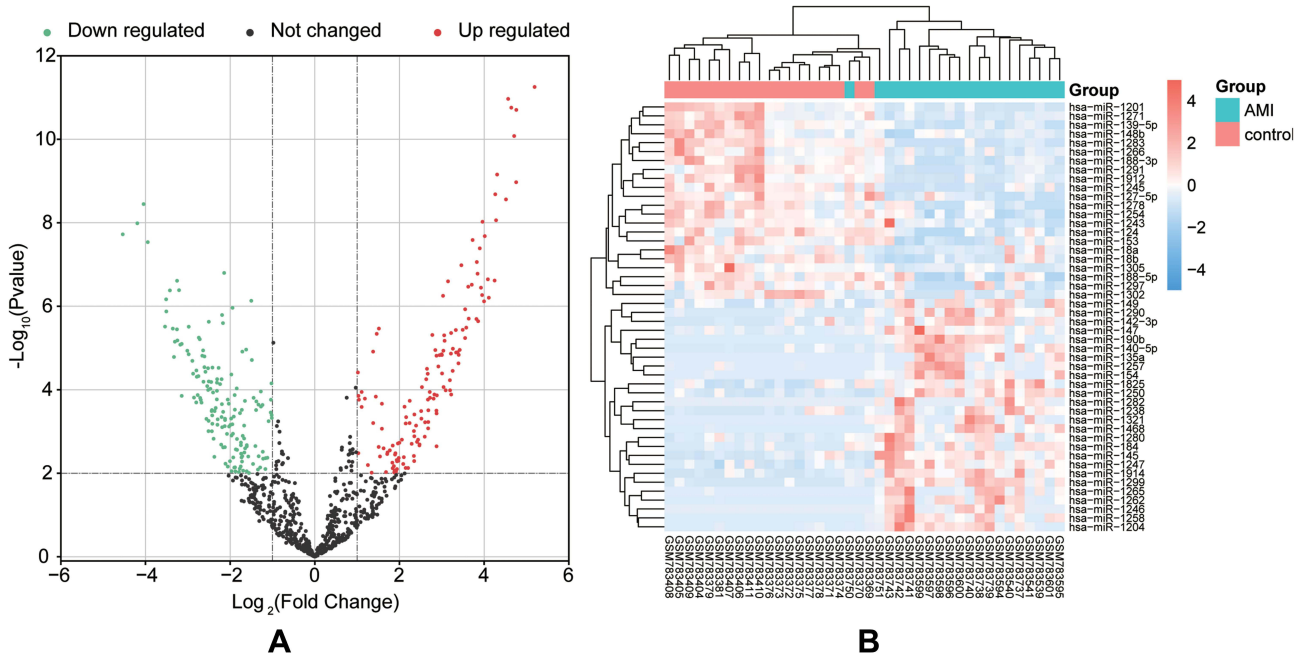

Figure 2 Differentially expressed miRNA levels of whole blood samples from patients with acute myocardial infarction and healthy controls.
Notes: (**A**) is a volcano plot for differential miRNA expression. Red dots represent upregulation, green dots represent downregulation, and black dots represent no differential expression. (**B**) is a differentially expressed miRNA cluster plot. Blue indicates upregulation, orange indicates downregulation, white indicates no differential expression; FC, fold change.

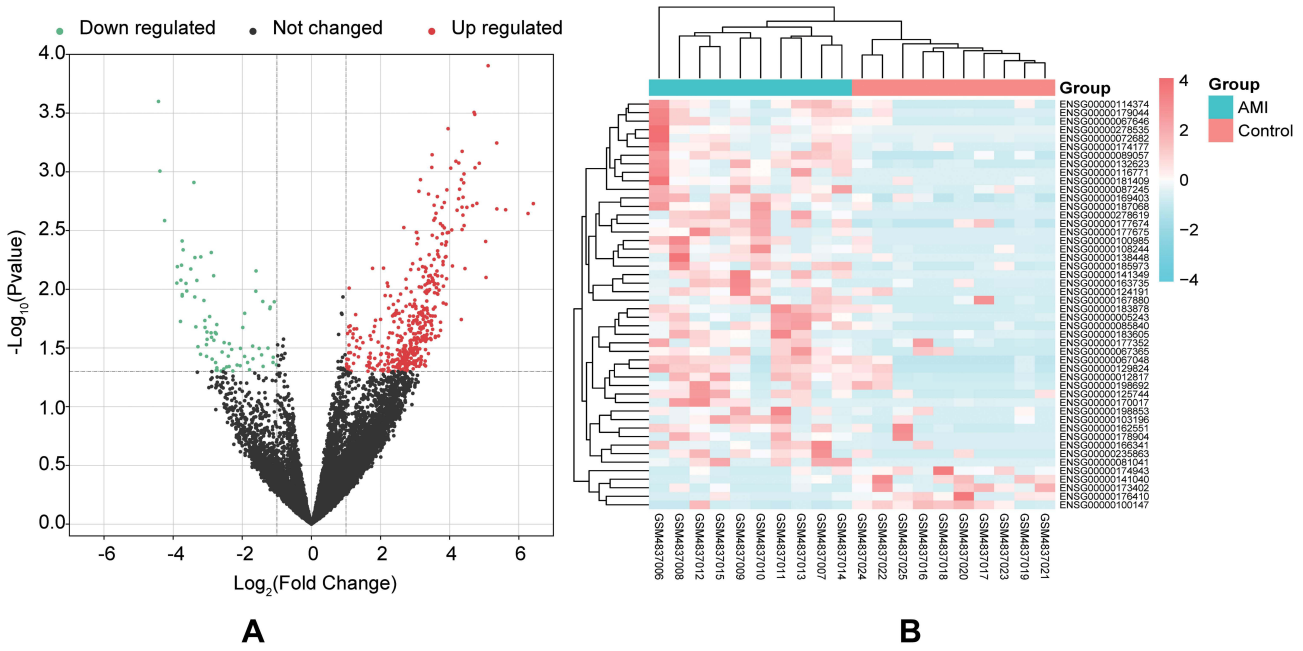

Figure 3 Differentially expressed mRNA levels in plasma samples of patients with AMI and healthy controls.
Notes: (**A**) is a volcano plot for differential mRNA expression. Red dots represent upregulation, green dots represent downregulation, and black dots represent no differential expression. (**B**) is a differentially expressed mRNA cluster plot. Blue represents upregulation, orange represents downregulation, and white represents no differential expression.

Target Gene Prediction and Regulatory Network Construction

Downstream target prediction of differentially expressed miRNAs was performed using the miRDB, miRWalk, miRTarBase and TargetScan databases. There were 2634 predicted targets in the four databases, and the predicted targets were intersected with the aforementioned 507 differentially expressed mRNAs in the dataset, resulting in a total of 81 candidate target genes (Figure 4, Table 2).

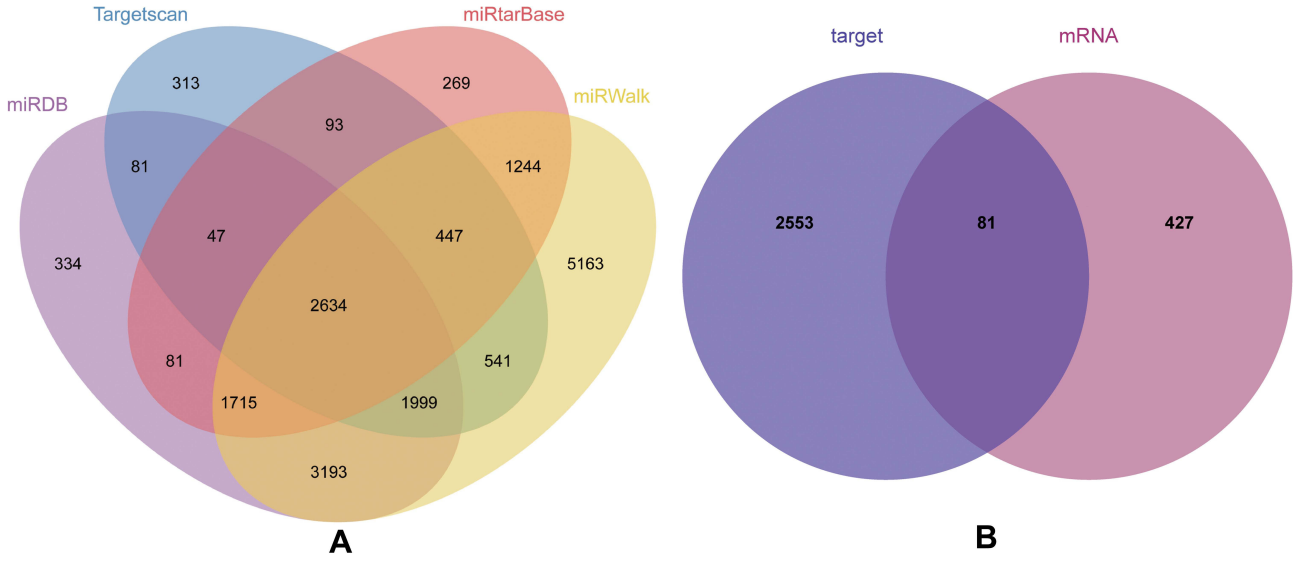

Figure 4 Screening target genes of interest.
Notes: (**A**) presents a Venn diagram of predicted miRNA corresponding downstream target genes for the miRDB, miRWalk, miRTarBase and TargetScan databases. (**B**) shows a Venn diagram of miRNA downstream target genes and differentially expressed mRNAs.

Table 2 Intersection of Differentially miRNA Predicted Target Genes and Differentially Expressed mRNAs Candidate Target Genes in AMI

mRNA	Gene Name	Log FC
ACSL1	Acyl-CoA synthetase long-chain family member 1	1.1469
ADAM19	ADAM metallopeptidase domain 19	1.7034
ADAMTS5	ADAM metallopeptidase with thrombospondin type 1 motif 5	3.5411
ADH1B	Alcohol dehydrogenase 1B	2.8785
AGTRAP	Angiotensin II receptor associated protein	4.7329
ALCAM	Activated leukocyte cell adhesion molecule	3.8997
AMOT	Angiomotin	2.8446
AP4E1	Adaptor related protein complex 4 epsilon 1 subunit	3.0825
ARNTL2	Aryl hydrocarbon receptor nuclear translocator like 2	3.0021
BTBD9	BTB domain containing 9	3.2351
C3orf70	Chromosome 3 open reading frame 70	4.3384
CCNJ	Cyclin J	−3.3705
CDC37L1	Cell division cycle 37 like 1	2.2299
CEP44	Centrosomal protein 44	3.6043
CNNM2	Cyclin and CBS domain divalent metal cation transport mediator 2	3.0244
CPM	Carboxypeptidase M	3.0804
DAG1	Dystroglycan 1	−4.2471
DNAJC16	DnaJ heat shock protein family	2.267
DPY19L3	dpy-19 like 3	4.4967
E2F6	E2F transcription factor 6	2.5
EDEM3	ER degradation enhancing alpha-mannosidase like protein 3	2.2134
EFNA1	Ephrin A1	2.4028
ENTPD7	Ectonucleoside triphosphate diphosphohydrolase 7	3.169
ERMP1	Endoplasmic reticulum metallopeptidase 1	−3.7399
EZH2	Enhancer of zeste 2 polycomb repressive complex 2 subunit	3.2865
FBXL7	F-box and leucine rich repeat protein 7	2.8207
FGF2	Fibroblast growth factor 2	−2.7522
FOSL2	FOS like 2, AP-1 transcription factor subunit	1.0603
FUT4	Fucosyltransferase 4	2.881
GRB10	Growth factor receptor bound protein 10	1.0221
HCFC2	Host cell factor C2	−1.1045
HECTD2	HECT domain E3 ubiquitin protein ligase 2	2.9185
HOMER2	Homer scaffolding protein 2	2.5622
HOXB13	Homeobox B13	2.2269
IFNLR1	Interferon lambda receptor 1	−3.3753
ITGAV	Integrin subunit alpha V	3.881
KLF8	Kruppel like factor 8	2.8849
LAYN	Layilin	−3.7881
LEPROTL1	Leptin receptor overlapping transcript-like 1	2.4694
LPAR2	Lysophosphatidic acid receptor 2	−3.3437
LRP5	LDL receptor related protein 5	3.0585
LYPLAL1	Lysophospholipase like 1	−1.5123
MITF	Melanogenesis associated transcription factor	3.7745
MMP2	Matrix metallopeptidase 2	3.9271
MTMR9	Myotubularin related protein 9	2.1334
MXD1	Max dimerization protein 1	1.0783
NAMPT	Nicotinamide phosphoribosyltransferase	1.0848
NBEA	Neurobeachin	2.9283
NDUFA2	NADH:ubiquinone oxidoreductase subunit A2	−2.8965
NUP35	Nucleoporin 35	−3.7113

(*Continued*)

Table 2 (Continued).

mRNA	Gene Name	Log FC
OPHN1	Oligophrenin 1	2.541
PAX8	Paired box 8	3.2019
PDE12	Phosphodiesterase 12	3.1091
PLXNA2	Plexin A2	3.3247
POLQ	DNA polymerase theta	3.065
PXDN	Peroxidasin	2.891
RAB3IP	RAB3A interacting protein	2.2313
RBM48	RNA binding motif protein 48	2.657
SACS	Sacsin molecular chaperone	2.0848
SERAC1	Serine active site containing 1	−3.0831
SGK3	Serum/glucocorticoid regulated kinase family member 3	3.2908
SLC30A7	Solute carrier family 30 member 7	3.5754
SLC43A2	Solute carrier family 43 member 2	2.8318
SOCS7	Suppressor of cytokine signaling 7	2.5097
SPRY1	Sprouty RTK signaling antagonist 1	3.3414
SRGAP2	SLIT-ROBO Rho GTPase activating protein 2	1.2791
STARD3	StAR related lipid transfer domain containing 3	1.6353
SULF1	Sulfatase 1	−2.8889
TBC1D24	TBC1 domain family member 24	3.7759
THBS1	Thrombospondin 1	2.5793
TM7SF3	Transmembrane 7 superfamily member 3	2.7458
TMEM68	Transmembrane protein 68	3.0191
TTC26	Tetratricopeptide repeat domain 26	3.492
UBE2F	Ubiquitin conjugating enzyme E2 F	3.2148
USP31	Ubiquitin specific peptidase 31	3.0492
VCL	Vinculin	1.0919
VEGFA	Vascular endothelial growth factor A	3.2343
YIPF4	Yip1 domain family member 4	2.5612
ZC3H12C	Zinc finger CCCH-type containing 12C	3.5523
ZNF281	Zinc finger protein 281	1.8282
ZNF470	Zinc finger protein 470	−1.9333

According to the negative regulatory relationships of miRNAs and mRNAs, 44 miRNA-mRNA relationship pairs consisting of 16 differentially expressed miRNAs and 44 differentially expressed mRNAs were ultimately screened out. The miRNA-mRNA regulatory network was constructed and visualized using Cytoscape software (Figure 5, Table 3).

Functional Analysis of Network Target Gene

GO function and KEGG pathway enrichment analysis of target genes in the miRNA-mRNA regulatory network were performed in Funrich software. After screening according to p value <0.05, 25 GO enrichment entries, including 2 BP entries, 17 CC entries and 6 MF entries, were obtained. According to the magnitude of enrichment values, BP was mainly enriched in Protein metabolism and Signal transduction; CC was mainly enriched in Cell-substrate junction, Cell body fiber, Interleukin-28 receptor complex, Vehicle, Costamere, and Microtubule basal body; MF was mainly enriched in Fucosyltransferase activity, Peroxidase activity, Carboxypeptidase activity, Transmembrane receptor activity, Metallopeptidase activity and Receptor signaling complex scaffold activity. The most significantly enriched

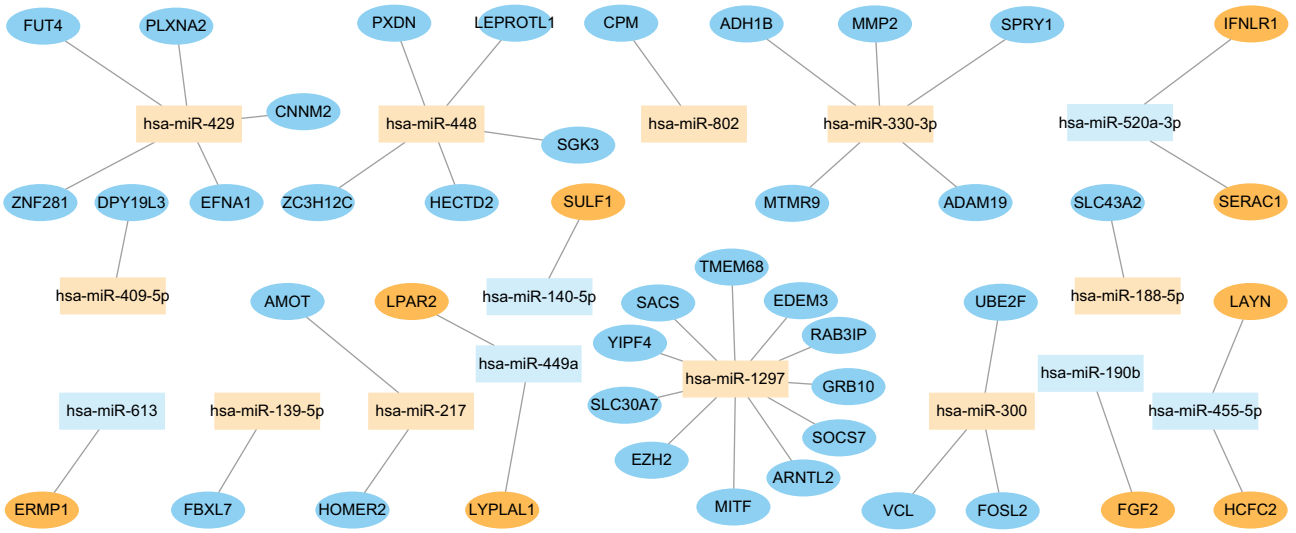

Figure 5 AMI related miRNA-mRNA regulatory network.
Notes: Blue indicates upregulated expression, and orange indicates downregulated expression. MiRNAs and mRNAs are represented by rectangles and circles, respectively.

BP, CC, and MF terms are shown in Figure 6A. KEGG pathway enrichment analysis resulted in 29 signaling pathways, mainly Integrins in angiogenesis, Angiopoietin receptor Tie2-mediated signaling, Signaling events mediated by Stem cell factor receptor (c-Kit), Stabilization and expansion of the E-cadherin adherens junction, E-cadherin signaling in the nascent adherens junction, and E-cadherin signaling events. The most significantly enriched pathways are shown in Figure 6B, and the relationships between the major pathways and the enriched genes are shown in Table 4.

Target Gene PPI Network Construction

The 44-candidate target gene PPI network in the miRNA mRNA regulatory network was predicted and constructed using the STRING database (see Figure 7), and the network consisted of 10 nodes and 17 edges. Of these, FGF2 and MMP2 had higher degree values, and it was coincident that they all had the highest absolute values of fold difference, while all had more than three direct connections to other nodes and thus were of significance in the PPI network. Among these nodes, FGF2 and SULF1 were downregulated genes, while the others were upregulated genes.

Discussion

In this study, both data sets were compared between patients with AMI and healthy people. The key step of this study is to find out the differential mRNA and miRNA between patients with AMI and healthy people, and then construct the corresponding regulatory relationship. Then, PPI network construction, GO and KEGG enrichment analysis were carried out for miRNAs in the regulatory relationship. Further analyze the miRNAs closely related to the disease. These miRNAs may affect the occurrence and development of AMI through multiple targets, multiple pathways and multiple pathways, and provide new ideas and strategies for the early diagnosis and treatment of AMI.

Since the first microRNA (miRNA) was identified in Caenorhabditis elegans by Lee et al[12] in 1993, miRNAs have been widely studied in physiology and pathophysiology. A large body of evidence indicates that miRNAs play key roles in cardiovascular diseases, such as cardiac remodeling, nonischemic heart disease, atherosclerosis, myocardial repair, apoptosis and angiogenesis after AMI, and these findings may change our traditional understanding of the cardiovascular field, regulate the levels of some miRNAs after AMI, and help limit tissue damage, promote neovascularization and inhibit ventricular remodeling, thereby improving long-term prognosis. Numerous studies have provided a biological basis for miRNAs as early AMI biomarkers, such as miRNA-1, miRNA-133a7, and miR-181a, with significantly increased concentrations in the short term of onset. Most of the above studies focus on the upstream and downstream

Table 3 AMI Related miRNA mRNA Regulatory Relationship Pairs

miRNA	mRNA	miRNAlogFC	mRNAlogFC
hsa-miR-613	ERMP1	4.75986	−3.7399
hsa-miR-520a-3p	IFNLR1	3.19067	−3.3753
hsa-miR-520a-3p	SERAC1	3.19067	−3.0831
hsa-miR-455–5p	LAYN	3.21972	−3.7881
hsa-miR-449a	LPAR2	2.60442	−3.3437
hsa-miR-140–5p	SULF1	3.99819	−2.8889
hsa-miR-449a	LYPLAL1	2.60442	−1.5123
hsa-miR-455–5p	HCFC2	3.21972	−1.1045
hsa-miR-190b	FGF2	4.75658	−2.7522
hsa-miR-409–5p	DPY19L3	−1.83895	4.4967
hsa-miR-1297	SLC30A7	−2.16574	3.5754
hsa-miR-330–3p	MMP2	−2.23822	3.9271
hsa-miR-300	UBE2F	−2.05723	3.2148
hsa-miR-1297	SACS	−2.16574	2.0848
hsa-miR-1297	MITF	−2.16574	3.7745
hsa-miR-448	ZC3H12C	−2.12139	3.5523
hsa-miR-1297	EZH2	−2.16574	3.2865
hsa-miR-1297	EDEM3	−2.16574	2.2134
hsa-miR-300	VCL	−2.05723	1.0919
hsa-miR-330–3p	MTMR9	−2.23822	2.1334
hsa-miR-1297	SOCS7	−2.16574	2.5097
hsa-miR-1297	TMEM68	−2.16574	3.0191
hsa-miR-448	LEPROTL1	−2.12139	2.4694
hsa-miR-217	AMOT	−3.20746	2.8446
hsa-miR-1297	ARNTL2	−2.16574	3.0021
hsa-miR-802	CPM	−3.32669	3.0804
hsa-miR-448	SGK3	−2.12139	3.2908
hsa-miR-429	ZNF281	−2.70799	1.8282
hsa-miR-188–5p	SLC43A2	−2.43207	2.8318
hsa-miR-330–3p	SPRY1	−2.23822	3.3414
hsa-miR-429	PLXNA2	−2.70799	3.3247
hsa-miR-429	CNNM2	−2.70799	3.0244
hsa-miR-429	FUT4	−2.70799	2.881
hsa-miR-330–3p	ADH1B	−2.23822	2.8785
hsa-miR-448	HECTD2	−2.12139	2.9185
hsa-miR-217	HOMER2	−3.20746	2.5622
hsa-miR-330–3p	ADAM19	−2.23822	1.7034
hsa-miR-1297	GRB10	−2.16574	1.0221
hsa-miR-448	PXDN	−2.12139	2.891
hsa-miR-1297	RAB3IP	−2.16574	2.2313
hsa-miR-139–5p	FBXL7	−3.24619	2.8207
hsa-miR-1297	YIPF4	−2.16574	2.5612
hsa-miR-300	FOSL2	−2.05723	1.0603
hsa-miR-429	EFNA1	−2.70799	2.4028

interaction between single or several miRNAs, genes or pathways, but the occurrence and development of the disease is the result of the synergy of multiple targets, multiple pathways, multiple pathways and multiple links. If we only study the relationship between a single miRNA or gene and AMI, the study of the mechanism of AMI will be limited to a certain extent.

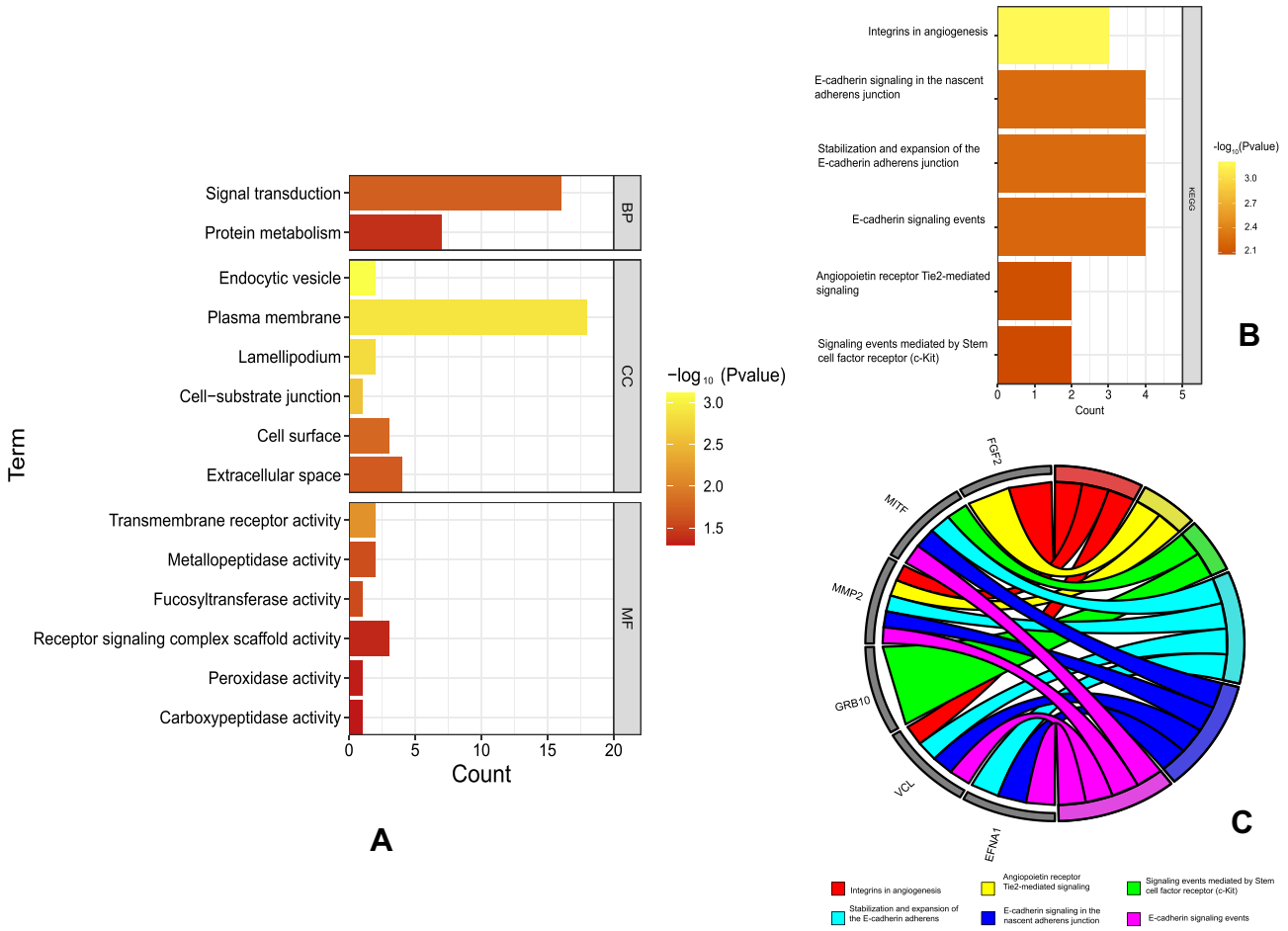

Figure 6 GO function and KEGG enrichment analysis plots of the 44 target genes in the miRNA-mRNA regulatory network.
Notes: (**A**) shows a bar chart of BP entries, CC entries, and MF entries for GO enrichment analysis. (**B**) shows the KEGG enrichment analysis plot. The color of the nodes is displayed from yellow to red in descending order of p values. (**C**) shows the relationships between the major pathways and the main target genes.

With the development of transcriptomics, the molecular network patterns of diseases during occurrence and development can be studied using bioinformatics. The GEO database is one of the central resources important for bioinformatics research, where large amounts of high-throughput data are deposited, but most of the data are under-utilized. Domestic and foreign scholars apply high-throughput data because of the differences in research purposes and the limitation of data independence, and the gene information of miRNAs and mRNAs in AMI research has not been integrated and compared. For other diseases, some people have done relevant research with this method and draw effective conclusions. Such as using miRNA-mRNA Regulatory Networks to study the pathogenesis of Osteonecrosis of the Femoral Head.[13] However, this research method has not been applied to study the pathogenesis of AMI. In this study, the differentially expressed genes were analyzed by mining the chip data of miRNAs and mRNAs associated

Table 4 Main KEGG Pathways and Enriched Genes of the 44 Target Genes in the miRNA-mRNA Regulatory Network

Biological Pathway	Fold Enrichment	P-value	Genes Mapped
Integrins in angiogenesis	17.38864	0.000617	FGF2 MMP2 VCL
Angiopoietin receptor Tie2-mediated signaling	14.86231	0.007805	FGF2 MMP2
Signaling events mediated by Stem cell factor receptor (c-Kit)	14.29079	0.008421	MITF GRB10
Stabilization and expansion of the E-cadherin adherens junction	5.391913	0.005417	MMP2 MITF VCL EFNA1
E-cadherin signaling in the nascent adherens junction	5.391913	0.005417	MMP2 MITF VCL EFNA1
E-cadherin signaling events	5.295633	0.005775	MMP2 MITF VCL EFNA1

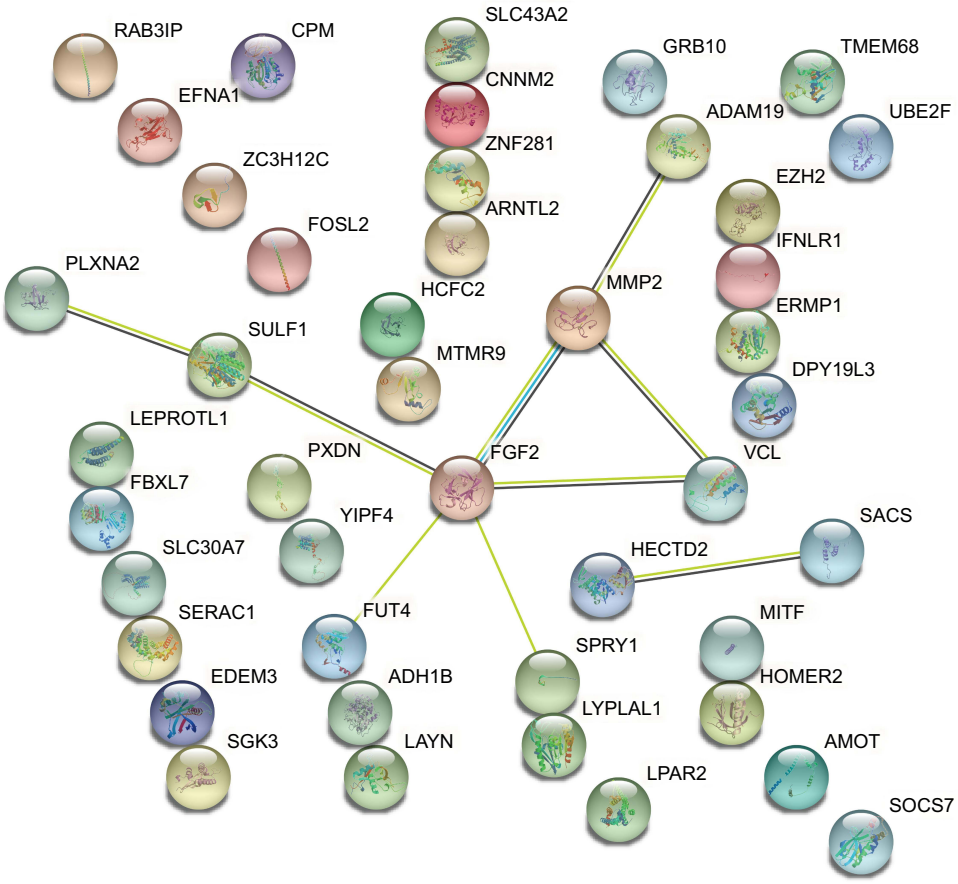

Figure 7 PPI network of target genes in the miRNA-mRNA regulatory network.

with AMI from the GEO database, and then a miRNA-mRNA regulatory relationship network was constructed in the hope of providing data support and a theoretical basis for the occurrence, development and prevention of AMI.

A total of 187 differentially expressed miRNAs were screened in this experiment, of which 91 were upregulated and 96 were downregulated; and 507 differentially expressed mRNAs were screened out, of which 430 were upregulated and 77 were downregulated. Using the miRDB, miRWalk, miRTarBase and TargetScan databases, 2634 downstream target genes were predicted to be present in 4 databases simultaneously, 16 miRNAs and 44 mRNAs were collated for regulatory network construction, and 44 regulatory network relationship pairs were constructed. These differential miRNAs and mRNAs may be the key nodes in the pathophysiology of AMI, and the PPI network of the 44 candidate target genes in the miRNA-mRNA regulatory network showed that the degree values of FGF2 and MMP2 were high, indicating that they were the core nodes in the regulatory network.

FGF2 is an important protein of the fibroblast growth factor family. FGF2, activated by binding to FGFR1, regulates the differentiation and proliferation of cells, playing an important role in the inflammatory response.[14] FGFR1 has a high affinity for FGF2 and is mainly responsible for endothelial cell signaling, which can directly affect vascular endothelial cell growth, migration, and angiogenesis, among others. Hsa-miR-190b upregulated and thereby regulated FGF2 down-regulation, thereby alleviating the inflammatory response and vascular endothelial injury and activating FGF2 and FGFR1 protein expression. MMP-2 is a zinc ion-dependent protease secreted and activated by macrophages/foam cells and T lymphocytes in a prozymogen form. Macrophage invasion into atherosclerotic lesions promotes the secretion of a large number of factors, such as MMP-2, which injures endothelial cells when acting to cleave extracellular matrix components, collagen and elastase, and accelerates the formation of intravascular thrombi, thus accelerating atherosclerosis and plaque formation.[15,16]

GO functional annotation and KEGG pathway enrichment analyses of the 44 target genes in the miRNA-mRNA regulatory network were performed and highlighted Integrins in angiogenesis, Angiopoietin receptor Tie2-mediated signaling, Signaling events mediated by Stem cell factor receptor (c-Kit), Stabilization and expansion of the E-cadherin adherens junction, E-cadherin signaling in the nascent adherens junction, and E-cadherin signaling events.

Integrins are heterodimeric transmembrane cell adhesion molecules consisting of alpha (α) and beta (β) subunits arranged in numerous dimeric pairings. These complexes have varying affinities for extracellular ligands. Integrins regulate cellular growth, proliferation, migration, signaling, and cytokine activation and release and thereby play important roles in cell proliferation and migration, apoptosis, tissue repair, and all processes critical to inflammation, infection, and angiogenesis. Several studies have shown that soluble fragments of ECM proteins suppress angiogenesis. Many of these proteins appear to bind to and suppress the function of endothelial cell integrins. These include but are not limited to Stupack and Cheresh proteolytic cleavage fragments of plasminogen (angiostatin), MMP-2 (PEX), collagen 18 (endostatin), and the NC domains from the alpha 2, alpha 3 (tumstatin), and alpha 6 chains of type IV collagen. These proteins may act to block interactions with immobilized ECM components and may therefore elicit apoptosis through a variety of mechanisms.[17] It was suggested that this inhibition is due to blocked MMP2 binding to avb3. The organic molecule TSRI265 can disrupt avb3/MMP2 formation, and although it does not block vitronectin binding, it does block tumor angiogenesis in vivo.[18] Thus, the importance of MMPs in angiogenesis has been established, but a direct interaction of MMP2 with avb3 is still somewhat debated.[19]

Regarding angiopoietin receptor Tie2-mediated signaling, the current study suggests that the development of a functional cardiovascular system is dependent on the regulated proliferation, migration and differentiation of endothelial cells in two discrete processes known as vasculogenesis and angiogenesis. Angiogenesis is the formation of new capillaries from pre-existing vessels, whereas vasculogenesis is de novo capillary formation from EPCs (endothelial precursor cells). New capillaries arise from pre-existing larger vessels to give rise to a more complex vascular network with a hierarchy of both large and small vessels.[20] These sequential vascular developments are tightly regulated by a range of pro- and antiangiogenic factors, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), thrombospondin, angiopoietins and, more recently, angiopoietin-like proteins (eg, Angptl).[21] Angiopoietins are a new family of growth factor ligands that bind specifically to TIE2/Tek RTK (Receptor Tyrosine Kinase). To date, four angiopoietins (Ang1 to 4) bind Tek and behave as either agonists (Ang1 and Ang4) or context-dependent antagonists (Ang2 and Ang3) of Tek kinase activity.[22] Angiopoietin mainly regulates two pathways that mediate cell motility, the first being through activation of the Phosphatidylinositol-3 Kinase pathway and the second involving the Ras pathway.[23] However, there are currently no studies to clarify the mechanism of FGF2 and MMP2 in angiopoietin receptor Tie2-mediated signaling, which can be investigated as a new direction for future research.

Despite the key role of miRNAs in cardiovascular diseases, the early release and ultrasensitivity of miRNAs in myocardial infarction give them the advantage of competing with the traditional myocardial biomarker CTN, but their specificity still needs validation in a large number of clinical trials. The diverse regulatory functions of miRNAs in the field of CVD revolutionize the current understanding of CVD, which may provide a novel approach in predicting, diagnosing, and treating CVD. Although the experiments constructed a potential miRNA-mRNA regulatory network based on bioinformatics, there were certain limitations. The experiments did not distinguish between different types of AMI, and the high-throughput datasets of AMI included in the GEO database and the number of samples were small, especially datasets that lacked documents from the same population and the same platform. More studies, such as dual-luciferase reporter assays, will be designed in the future to verify the biological functions of miRNA-mRNA regulatory network patterns in vitro and in vivo.

Conclusions

In summary, based on GEO high-throughput datasets and with the aid of bioinformatics, 44 miRNA-mRNA regulatory relationship pairs related to AMI were experimentally constructed, and the complex net regulation of AMI multiple targets and multiple pathways was elucidated. The experimentally constructed miRNA-mRNA regulatory network found that hsa-miR-190b targets to inhibit FGF2, while hsa-miR-330-3p targets to regulate MMP2, which may mediate

Integrins in angiogenesis, Angiopoietin receptor Tie2-mediated signaling pathway to induce AMI pathogenesis, providing strong data support and a research direction for the prevention and treatment of AMI.

Ethical Review

The ethics committee of the Affiliated Hospital of Shandong University of Chinese Medicine certifies that the study belongs to the data mining class of papers, and all data are derived from publicly available databases, Permission to use the database does not need to be obtained. The study did not involve animal or human experimentation and did not involve ethical issues. Therefore, it is hereby declared that the ethics committee of the Affiliated Hospital of Shandong University of Chinese medicine reviewed the study and certified that the study does not require ethical review.

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Disclosure

The authors declare that they have no conflicts of interest in this work.

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