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Identifying key genes associated with acute myocardial infarction

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Abstract

Background: This study aimed to identify key genes associated with acute myocardial infarction (AMI) by reanalyzing microarray data.

Methods: Three gene expression profile datasets GSE66360, GSE34198, and GSE48060 were downloaded from GEO database. After data preprocessing, genes without heterogeneity across different platforms were subjected to differential expression analysis between the AMI group and the control group using metaDE package. $P < .05$ was used as the cutoff for a differentially expressed gene (DEG). The expression data matrices of DEGs were imported in ReactomeFIViz to construct a gene functional interaction (FI) network. Then, DEGs in each module were subjected to pathway enrichment analysis using DAVID. MiRNAs and transcription factors predicted to regulate target DEGs were identified. Quantitative real-time polymerase chain reaction (RT-PCR) was applied to verify the expression of genes.

Result: A total of 913 upregulated genes and 1060 downregulated genes were identified in the AMI group. A FI network consists of 21 modules and DEGs in 12 modules were significantly enriched in pathways. The transcription factor-miRNA-gene network contains 2 transcription factors FOXO3 and MYBL2, and 2 miRNAs hsa-miR-21-5p and hsa-miR-30c-5p. RT-PCR validations showed that expression levels of FOXO3 and MYBL2 were significantly increased in AMI, and expression levels of hsa-miR-21–5p and hsa-miR-30c-5p were obviously decreased in AMI.

Conclusion: A total of 41 DEGs, such as *SOCS3*, *VAPA*, and *COL5A2*, are speculated to have roles in the pathogenesis of AMI; 2 transcription factors *FOXO3* and *MYBL2*, and 2 miRNAs hsa-miR-21-5p and hsa-miR-30c-5p may be involved in the regulation of the expression of these DEGs.

Abbreviations: AMI = acute myocardial infarction, CTGF = connective tissue growth factor, FI = functional interaction, H-FABP = heart fatty acid binding protein, RT-PCR = real-time PCR.

Keywords: acute myocardial infarction, differentially expressed genes, gene functional interaction, pathway enrichment analysis, transcription factor-miRNA-gene network

1. Introduction

Acute myocardial infarction (AMI) is the world's leading cause of morbidity and mortality. A ruptured atherosclerotic plaque, causing thrombosis and occlusion of the coronary artery, is widely accepted for the occurrence of an AMI. Early reperfusion of the occluded artery after MI, including primary percutaneous coronary intervention (PCI) or thrombolytic therapy, will improve long-term prognosis of patients. Thrombolytic therapy has become the standard therapy for AMI since 1980s.[1] Recently, primary PCI seems to be more effective than fibrinolytic therapy in acute ST-segment elevation myocardial infarction.[2] However, approximately one-third of eligible patients failed to receive early reperfusion therapy because of late presentation.[3,4] Thus, an early diagnosis may benefit the survival remarkably.

Cardiac troponins (T/I) have been long considered as the "gold standard" biomarkers for early detection of AMI.[5,6] However, more sensitive and potent makers are preferred. Mccann et al[7] have proposed that heart fatty acid binding protein (H-FABP) is superior than cardiac troponin T. Several circulating microRNAs (miR-208a, miR-499, and miR-1) have also been recommended as potential biomarkers for early diagnosis of myocardial infarction.[8–10] Here, using 3 public gene expression profile datasets, we attempted to identify novel genes that may be useful for the early detection of AMI by bioinformatics methods.

2. Materials and methods

2.1. Source of microarray data

Three gene expression profile datasets GSE66360, GSE34198, and GSE48060 were downloaded from GEO (Gene Expression Omnibus, http://www.ncbi.nlm.nih.gov/geo/) database. The samples included in each dataset and annotation platform are listed in Table 1.

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Table 1

Information on platform and study subjects in each gene expression profile dataset.

	AML sample count	Control sample count	Platforms
GSE66360	49	50	Affymetrix Human Genome U133 Plus 2.0 Array
GSE34198	49	48	Illumina human-6 v2.0 expression beadchip
GSE48060	31	21	Affymetrix Human Genome U133 Plus 2.0 Array

2.2. Microarray data preprocessing

For the raw data in GSE66360 and GSE34198, they were first subjected to background correction and quantile normalization using the Affy package of R/Bioconductor.[11] For data in GSE34198, the Limma package of R/Bioconductor was used for background correction, normalization between arrays, and microarray data condensation.

2.3. Heterogeneity test and screening of characteristic genes

First, heterogeneity of each gene across different platforms was examined by measuring tau^2, Q value, and Q *P* value using MetaDE.ES function; tau^2 of 0 and Q pval >0.05 indicate no heterogeneity. Next, genes without heterogeneity were subjected to differential expression analysis of their expression levels between the AMI group and the control group using metaDE package; $P<.05$ was used as the cutoff for a differentially expressed gene (DEG). The $\log_2$FC (fold change) value of a gene in each dataset was further calculated, with $\log_2 FC > 0$ as upregulated and $\log_2 FC < 0$ as downregulated.

2.4. Pathway enrichment analysis of DEGs

The screened DEGs were submitted to DAVID (v6.8, Database for Annotation, Visualization and Integrated Discovery, https://david.ncifcrf.gov/) to examine the pathways in which these genes were enriched based on the KEGG database (Kyoto Encyclopedia of Genes and Genomes) ($P<.05$).[12]

2.5. Construction of gene functional interaction network

The expression data matrix of DEGs was submitted to ReactomeFIViz to investigate gene–gene interaction based on known human pathway data.[13] ReactomeFIViz first constructs a functional interaction (FI) network by merging interactions extracted from human curated pathways and the average Pearson correlation coefficient among genes involved in the same FIs are also calculated as weights for edges (i.e., FIs) in the whole FI network; next, using MCL (Monte Carlo Localization) graph clustering algorithm, subnetworks for a list of selected network modules were generated based on module size and average correlation. The FI network was finally visualized using Cytoscape 2.8.0 (National Institute of General Medical Sciences of the National Institutes of Health).[14]

2.6. Prediction of AMI-related microRNAs and construction of miRNA-gene network

First, microRNAs related to AMI were identified from the miR2disease database (http://www.mir2disease.org/). Next, the target genes that have been experimentally validated were downloaded from Mirwalk2,[15] which were further compared with the DEGs screened above. Next, these miRNAs and their target DEGs were used to construct a miRNA-gene network.

2.7. Prediction of transcription factor-miRNA-gene network

Transcription factors of the DEGs in the miRNA-gene network constructed above were predicted using a Cytoscape plug-in iRegulon,[16] which includes gene-transcription factor pairs in Transfac, Jaspar, Encode, etc. Minimum identity between orthologous genes was 0.05 and maximum false discovery rate on motif similarity: 0.001. The transcription factors predicted with normalized enrichment score (NES) >3 were retained.

2.8. Validation of DEGs

A total of 16 blood samples, including 8 normal control and 8 AMI blood samples, were collected from the Second Affiliated Hospital of Harbin Medical University to verify the expression levels of *FOXO3*, *MYBL2*, hsa-miR-30c-5p, and hsa-miR-21-5p identified in this study using quantitative real-time polymerase chain reaction (RT-PCR). Total RNAs were isolated using TRI pure LS Reagent Blood RNA Extraction Kit (Bioteke, Lot: RP1102, Beijing, China). Then, 4 μg of total RNA was utilized miRNA reverse transcription using Rayscript cDNA Synthesis Kit (GCK8030, GENEray, Shanghai, China) with neck-loop premiers instead of Oligo (dT). Amplification of miRNA was carried out on a ViiA7 real-time PCR instrument (ABI, Foster City, CA) using the following system: 50°C for 3 minutes, then 40 cycles of 95°C for 3 minutes, 95°C for 10 seconds, and 60°C for 30 seconds. Meanwhile, 0.5 μg of total RNA was applied to mRNA reverse transcription using using Rayscript cDNA Synthesis Kit (GCK8030, GENEray, Shanghai, China). Amplification of mRNA was performed using the following system: 95°C for 2 minutes, then 40 cycles of 95°C for 15 seconds, 60°C for 20 seconds, and 72°C for 20 seconds. Primers of RNAs are tabulated in Table 2. GAPDH was

Table 2

Primers of genes determined by quantitative real-time PCR.

Primers	Sequences
FOXO3 -F	TGATGGGCTGACTGAAAAC
FOXO3-R	AGATGAAGGTCCGAACACC
MYBL2 -F	GAGGAAAACAGTGAGGAGGA
MYBL2-R	GCAGGGATGAGGAGGTTAG
GAPDH-F	GGACCTGACCTGCCGTCTAG
GAPDH -R	GTAGCCCAGGATGCCCTTGA
hsa-miR-21-5p-neck-loop	CCTGTTGTCTCCAGCCACAAAAGAGC ACAATATTTCAGGAGACAACAGGTCAACA
hsa-miR-21-5p probe	FAM-TTCAGGAGACAACAGG-MGB
hsa-miR-21-5p-F	CAGCCACAAAAGAGCACAAT
hsa-miR-21-5p-R	GGGGGTAGCTTATCAGACTGA
hsa-miR-30c-5p-neck-loop	CCTGTTGTCTCCAGCCACAAAAGAGC ACAATATTTCAGGAGACAACAGGGCTGAG
hsa-miR-30c-5p probe	FAM-TTCAGGAGACAACAGG-MGB
hsa-miR-30c-5p-F	CAGCCACAAAAGAGCACAAT
hsa-miR-30c-5p-R	GGGGGTGTAAACATCCTACACT
5S-neck-loop	CCTGTTGTCTCCAGCCACAAAAGAG CACAATATTTCAGGAGACAACAGGAAAGCCTA
5S probe	FAM-TTCAGGAGACAACAGG-MGB
5S-F	CAGCCACAAAAGAGCACAAT
5S-R	GAGACCGCCTGGGAATAC

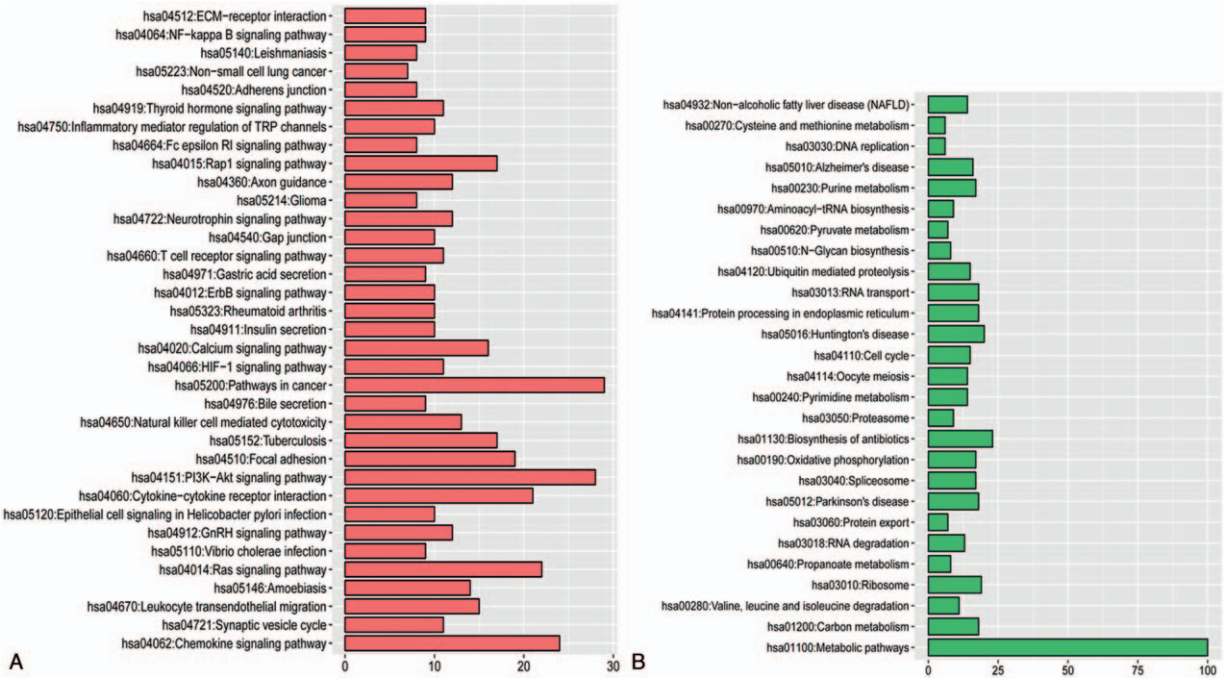

Figure 1. Pathway enrichment analysis of differentially expressed genes A, pathways enriched by the upregulated genes; B, pathways enriched by the downregulated genes.

utilized as the internal control for mRNA evaluation, and 5S was utilized as the internal reference for miRNA evaluation. The ethics committee of the Second Affiliated Hospital of Harbin Medical University approved this study, and informed written consents were obtained from the included subjects.

3. Results

3.1. Screening of candidate characteristic genes

According to the analytical results, a total of 913 upregulated genes and 1060 downregulated genes were identified in the AMI group compared with the control group. Then, KEGG enrichment analyses for upregulated and downregulated genes were carried out. The enrichment results performed that the upregulated genes and downregulated genes were enriched in 35 and 27 KEGG pathways, respectively (Fig. 1).

3.2. Construction of FI network and pathway enrichment analysis

On the basis of the expression data matrices, a FI network was constructed, which contained 249 nodes forming 1071 interaction pairs (Fig. 2). The network includes 21 modules, of which 12 modules had Pearson correlation coefficient > 0.9.

KEGG pathway enrichment of DEGs in each module shows that DEGs in Module 1 were significantly enriched in 38 KEGG pathways, such as DEGs in Module 5 and 13 were enriched in 7 pathways each; DEGs in Module 20 were enriched in 6 pathways; DEGs in Module 3 were enriched in 4 pathways; DEGs in Module 9 were enriched in 3 pathways, such as hsa04630: Jak-STAT signaling pathway; DEGs in Module 6 and 14 were enriched in 2 pathways each; DEGs in Module 2, 4, 7, 11, and 19 were enriched in 1 pathway each (Table 3).

3.3. Construction of miRNA-gene network

The miRNA-gene network consists of 9 miRNAs (hsa-miR-21, hsa-miR-133, hsa-miR-29, hsa-miR-30c, hsa-miR-1, hsa-miR-133a, hsa-miR-133b, hsa-miR-208, hsa-miR-499) and 191 genes, forming 213 miRNA–gene pairs (Fig. 3). Among the DEGs in this network, 28 were also found in the FI network.

3.4. Prediction of transcription factor-miRNA-gene network

The transcription factor-miRNA-gene network contains 40 DEGs, including 14 upregulated gens (*CBX4*, *CCR1*, *COL5A2*, *FGF12*, *FKBP5*, *IGF1R*, *LAMP2*, *MEGF9*, *MMP2*, *OTUD1*, *TGFB2*, *NDEL1*, *UBN1*, and *VAPA*) and 26 downregulated genes (*ADNP*, *CD47*, *CDK6*, *CKAP5*, *CLIP4*, *DAAM1*, *DOCK10*, *FAM20B*, *FAM3C*, *FBXL17*, *FOXN2*, *HPS5*, *LCORL*, *MTMR9*, *NBEA*, *NEK1*, *PER3*, *PHACTR2*, *POLR3B*, *PRRC1*, *SCRN1*, *SEC63*, *SLK*, *SOCS5*, *TET1*, and *ZBTB20*), 2 upregulated transcription factors FOXO3 and MYBL2, and 2 downregulated miRNAs hsa-miR-21-5p and hsa-miR-30c-5p (Fig. 4).

3.5. Experimental validations of DEGs

To further confirm our identifications, the expression levels of *FOXO3* and *MYBL2* and 2 miRNAs hsa-miR-21-5p and hsa-miR-30c-5p were determined in AMI patients and normal controls. The RT-PCR results presented that the relative mRNA expression levels of *FOXO3* and *MYBL2* were significantly increased in AMI patients compared with the normal controls (Fig. 5A and B). Meanwhile, the expression levels of hsa-miR-30c-5p and hsa-miR-21-5p were significantly decreased in AMI patients compared with the normal controls (Fig. 5C and D).

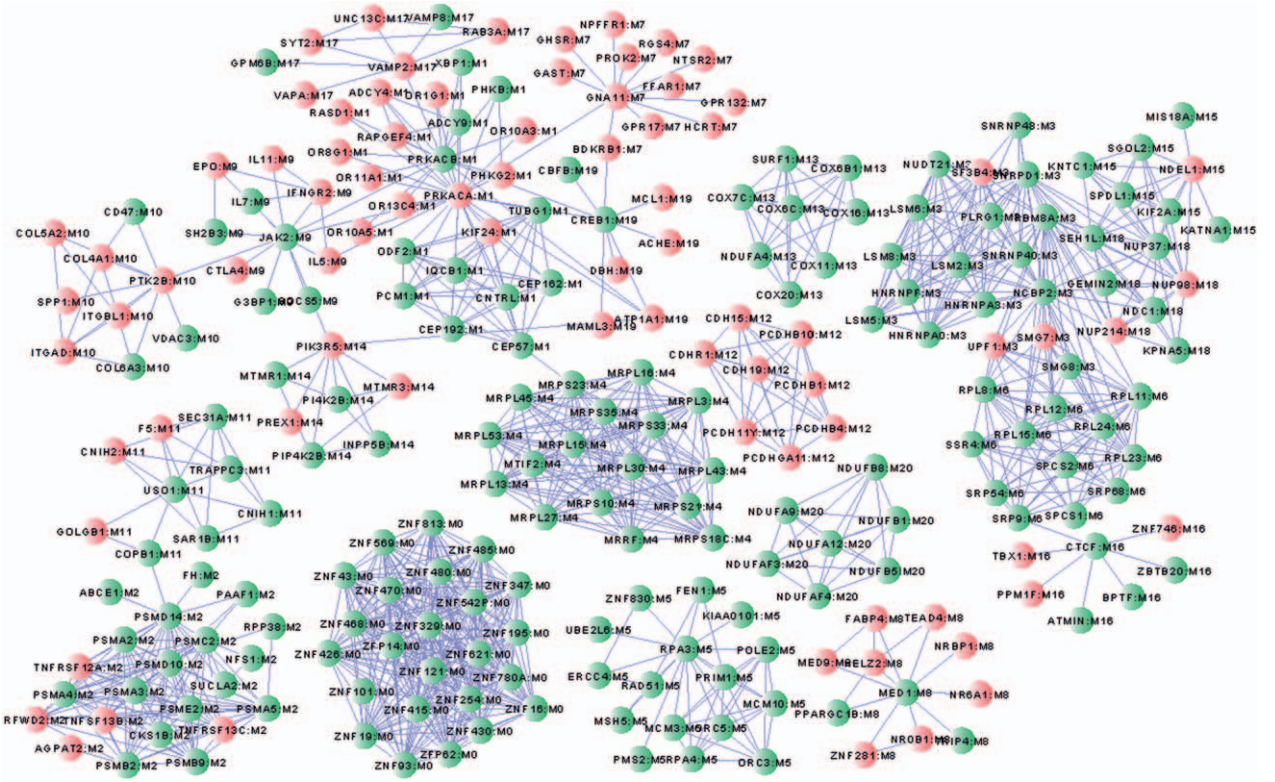

Figure 2. Modules of the gene functional interaction network.

4. Discussion

In the present study, we first identified genes differentially expressed in patients with AMI across 3 different platforms using an R package MetaDE; then, we constructed a FI network and also subnetworks consisting of genes with close interaction; finally, miRNAs and transcription factors which might regulate these DEGs were predicted. Consequently, we found out 41 DEGs that were speculated to have critical roles in AMI, as well as 2 transcription factors FOXO3 and MYBL2, and 2 miRNAs hsa-miR-21-5p and hsa-miR-30c-5p that were predicted to regulate them.

An integrated analysis of the 3 gene expression profile datasets found that the downregulated DEGs were slightly more than the upregulated ones in patients with AMI. From the perspective of gene–gene interaction based on known human pathway data, 41 DEGs that were speculated to have important roles in AMI were further identified. Among them, *SOCS5* encodes suppressor of cytokine signaling 5 protein belonging to the suppressor of cytokine signaling (SOCS) family, each member containing a central SH2 domain and a carboxyl-terminal 40-residue SOCS box. Many studies have reported that SOCS proteins (especially SOCS1 and SOCS3) have a negative regulation of JAKJ/STAT pathway.[17,18] Actually, *SOCS5* was predicted to function in AMI with some other genes in Module 9 (*IL5*, *IL7*, *JAK2*, *IFNGR2*, *IL11*, and *EPO*) via the Jak-STAT signaling pathway. Seki et al[19] have demonstrated that SOCS5 can bind to the interleukin 4 receptor α chain via the first 100 residues of its N-terminal region to suppresses the interaction of JAK1 and its corresponding receptor, by which to inhibit the downstream signaling transduction of JAK1. A further study has specified that SOCS5 can directly bind to the JAK1 via N-terminal residues 175 to 244 inhibiting the phosphorylations of JAK1 and JAK2, as well as their downstream signaling pathway.[20] A recent research has documented that SOCS5 is downregulated in the blood of multiple sclerosis patients,[21] while JAK/STAT signaling pathway has been revealed to involve in the onset of AMI and the ventricular remodeling after AMI.[22] Thus, decreased SOCS5 expression may have an adverse effect on the onset of AMI and the left ventricular remodeling after AMI, which seems to agree with its downregulation observed in the AMI patients here. By contrast, *COL5A2* encoding the alpha chain of collagen type V showed an increased expression in AMI patients. Moreover, this gene was found to be highly expressed in the left ventricle after MI by Azuaje et al[23] using a network-based discovery strategy. Wang et al[24] have recently revealed that the expression of COL5A2 is significantly elevated in AMI patients, and its expression can be significantly reduced by a Chinese Herbal Medicine Qishenyiqi. These identifications might suggest that COL5A2 served a critical role in the pathogenesis of AMI, but the detailed mechanism was still needed to be further analyzed.

Interestingly, both *SOCS3* and *COL5A2* were found to be regulated by both *FOXO3* and hsa-miR-21-5p. Actually, *FOXO3* and hsa-miR-21-5p seem to regulate most of the DEGs identified in AMI here. *FoxO3* encodes an evolutionarily conserved transcription factor belonging to the forkhead family of transcriptional regulators. This family members negatively regulate cardiomyocyte proliferation and promote neonatal cell cycle withdrawal during heart development.[25] Furthermore, FoxO1 and FoxO3 promote cardiomyocyte survival via inducing antioxidants and cell survival pathways upon induction of oxidative stress,[26] which seemed consistent with the upregulation expression identified in this study. Thus, we concluded that FoxO3 may also have a role in AMI via regulating SOCS3 and

Table 3

Pathway enrichment analysis of differentially expressed genes in the modules of the gene functional interaction network.

Module ID	Pathway term	Gene count	*P*	Genes	FDR
Module1	hsa04740:Olfactory transduction	8	4.99E-06	*OR10A5*, *OR13C4*, *OR10A3*, *OR11A1*, *PRKACA*, *PRKACB*, *OR8G1*, *OR1G1*	0.0050308
	hsa04020: Calcium signaling pathway	6	1.66E-05	*ADCY4*, *ADCY9*, *PHKB*, *PHKG2*, *PRKACA*, *PRKACB*	0.0167367
	hsa04911: Insulin secretion	5	1.96E-05	*ADCY4*, *ADCY9*, *PRKACA*, *RAPGEF4*, *PRKACB*	0.0198237
	hsa04713: Circadian entrainment	5	3.05E-05	*ADCY4*, *ADCY9*, *PRKACA*, *PRKACB*, *RASD1*	0.0308047
	hsa04913: Ovarian steroidogenesis	4	.0001164	*ADCY4*, *ADCY9*, *PRKACA*, *PRKACB*	0.1174204
	hsa04261: Adrenergic signaling in cardiomyocytes	5	.0001641	*ADCY4*, *ADCY9*, *PRKACA*, *RAPGEF4*, *PRKACB*	0.16546
	hsa04923: Regulation of lipolysis in adipocytes	4	.0001737	*ADCY4*, *ADCY9*, *PRKACA*, *PRKACB*	0.1751419
	hsa04976: Bile secretion	4	.0003232	*ADCY4*, *ADCY9*, *PRKACA*, *PRKACB*	0.325689
	hsa04918: Thyroid hormone synthesis	4	.0003373	*ADCY4*, *ADCY9*, *PRKACA*, *PRKACB*	0.3398419
	hsa04971: Gastric acid secretion	4	.0003818	*ADCY4*, *ADCY9*, *PRKACA*, *PRKACB*	0.384664
	hsa04024: cAMP signaling pathway	5	.0005178	*ADCY4*, *ADCY9*, *PRKACA*, *RAPGEF4*, *PRKACB*	0.5212951
	hsa04925: Aldosterone synthesis and secretion	4	.0005188	*ADCY4*, *ADCY9*, *PRKACA*, *PRKACB*	0.5223062
	hsa04727: GABAergic synapse	4	.0005188	*ADCY4*, *ADCY9*, *PRKACA*, *PRKACB*	0.5223062
	hsa04970: Salivary secretion	4	.0005773	*ADCY4*, ADCY9, *PRKACA*, *PRKACB*	0.5810495
	hsa05414: Dilated cardiomyopathy	4	.0005773	*ADCY4*, *ADCY9*, *PRKACA*, *PRKACB*	0.5810495
	hsa04914: Progesterone-mediated oocyte maturation	4	.0006399	*ADCY4*, *ADCY9*, *PRKACA*, *PRKACB*	0.6438567
	hsa04540: Gap junction	4	.0006617	*ADCY4*, *ADCY9*, *PRKACA*, *PRKACB*	0.6657144
	hsa04912: GnRH signaling pathway	4	.0007299	*ADCY4*, *ADCY9*, *PRKACA*, *PRKACB*	0.7341095
	hsa05032: Morphine addiction	4	.0007299	*ADCY4*, *ADCY9*, *PRKACA*, *PRKACB*	0.7341095
	hsa04750: Inflammatory mediator regulation of TRP channels	4	.0008794	*ADCY4*, *ADCY9*, *PRKACA*, *PRKACB*	0.8839462
	hsa04723: Retrograde endocannabinoid signaling	4	.0008794	*ADCY4*, *ADCY9*, *PRKACA*, *PRKACB*	0.8839462
	hsa04922: Glucagon signaling pathway	4	.0009333	*PHKB*, *PHKG2*, *PRKACA*, *PRKACB*	0.9378678
	hsa04915: Estrogen signaling pathway	4	.0009333	*ADCY4*, *ADCY9*, *PRKACA*, *PRKACB*	0.9378678
	hsa04916: Melanogenesis	4	.000961	*ADCY4*, *ADCY9*, *PRKACA*, *PRKACB*	0.9655907
	hsa04114: Oocyte meiosis	4	.0012343	*ADCY4*, *ADCY9*, *PRKACA*, *PRKACB*	1.2385867
	hsa04725: Cholinergic synapse	4	.001301	*ADCY4*, *ADCY9*, *PRKACA*, *PRKACB*	1.3051434
	hsa05166: HTLV-I infection	5	.0013669	*ADCY4*, *ADCY9*, *XBP1*, *PRKACA*, *PRKACB*	1.3709053
	hsa04724: Glutamatergic synapse	4	.0014053	*ADCY4*, *ADCY9*, *PRKACA*, *PRKACB*	1.4091087
	hsa04270: Vascular smooth muscle contraction	4	.0015906	*ADCY4*, *ADCY9*, *PRKACA*, *PRKACB*	1.5936033
	hsa04611: Platelet activation	4	.0019617	*ADCY4*, *ADCY9*, *PRKACA*, *PRKACB*	1.9620717
	hsa04910: Insulin signaling pathway	4	.0024331	*PHKB*, *PHKG2*, *PRKACA*, *PRKACB*	2.428359
	hsa04962: Vasopressin-regulated water reabsorption	3	.0033005	*ADCY9*, *PRKACA*, *PRKACB*	3.2811996
	hsa04742: Taste transduction	3	.0034537	*ADCY4*, *PRKACA*, *PRKACB*	3.4310789
	hsa04921: Oxytocin signaling pathway	4	.0035747	*ADCY4*, *ADCY9*, *PRKACA*, *PRKACB*	3.549344
	hsa04961: Endocrine and other factor-regulated calcium reabsorption	3	.0036101	*ADCY9*, *PRKACA*, *PRKACB*	3.583959
	hsa05110: *Vibrio cholerae* infection	3	.0049786	*ADCY9*, *PRKACA*, *PRKACB*	4.9119684
	hsa04062: Chemokine signaling pathway	4	.0055702	*ADCY4*, *ADCY9*, *PRKACA*, *PRKACB*	5.4809168
	hsa05200: Pathways in cancer	4	.0418894	*ADCY4*, *ADCY9*, *PRKACA*, *PRKACB*	35.06845
Module2	hsa03050: Proteasome	9	5.89E-14	*PSMA2*, *PSMD14*, *PSMA5*, *PSME2*, *PSMC2*, *PSMA4*, *PSMA3*, *PSMB2*, *PSMB9*	4.88E-11
Module3	hsa03040:Spliceosome	11	1.18E-15	*NCBP2*, *HNRNPA3*, *PLRG1*, *LSM8*, *RBM8A*, *LSM6*, *SNRPD1*, *LSM5*, *SNRNP40*, *LSM2*, *SF3B4*	5.33E-13
	hsa03015: mRNA surveillance pathway	5	1.78E-05	*NCBP2*, *UPF1*, *RBM8A*, *NUDT21*, *SMG7*	0.0077335
	hsa03018: RNA degradation	4	.000354	*LSM8*, *LSM6*, *LSM5*, *LSM2*	0.153867
	hsa03013: RNA transport	3	.0399075	*NCBP2*, *UPF1*, *RBM8A*	16.231928
Module4	hsa03010: Ribosome	9	1.66E-14	*MRPL13*, *MRPL3*, *MRPS18C*, *MRPL15*, *MRPL27*, *MRPL16*, *MRPS10*, *MRPS21*, *MRPL30*	1.65E-12
Module5	hsa03030: DNA replication	6	1.32E-09	*PRIM1*, *POLE2*, *MCM3*, *RPA4*, *FEN1*, *RPA3*	9.35E-07
	hsa03460: Fanconi anemia pathway	5	9.89E-07	*PMS2*, *ERCC4*, *RPA4*, *RAD51*, *RPA3*	0.0007024
	hsa03420: Nucleotide excision repair	4	4.72E-05	*POLE2*, *ERCC4*, *RPA4*, *RPA3*	0.0335351
	hsa03430: Mismatch repair	3	.0005755	*PMS2*, *RPA4*, *RPA3*	0.407984
	hsa03440: Homologous recombination	3	.0009188	*RPA4*, *RAD51*, *RPA3*	0.6506143
	hsa04110: Cell cycle	3	.0158865	*ORC5*, *MCM3*, *ORC3*	10.749309
	hsa03410: Base excision repair	2	.0499469	*POLE2*, *FEN1*	30.500744
Module6	hsa03060: Protein export	5	3.07E-08	*SRP54*, *SRP68*, *SPCS1*, *SPCS2*, *SRP9*	9.65E-06
	hsa03010: Ribosome	6	1.09E-06	*RPL23*, *RPL15*, *RPL8*, *RPL24*, *RPL11*, *RPL12*	0.0003417

(*continued*)

Table 3
(continued).

Module ID	Pathway term	Gene count	*P*	Genes	FDR
Module7	hsa04080: Neuroactive ligand–receptor interaction	4	.0011741	*BDKRB1, NPFFR1, GHSR, NTSR2*	0.8622339
Module9	hsa04630: Jak-STAT signaling pathway	7	2.12E-09	*IL5, IL7, JAK2, SOCS5, IFNGR2, IL11, EPO*	1.79E-06
	hsa04060: Cytokine-cytokine receptor interaction	5	7.49E-05	*IL5, IL7, IFNGR2, IL11, EPO*	0.0631994
	hsa04640: Hematopoietic cell lineage	4	9.70E-05	*IL5, IL7, IL11, EPO*	0.0818866
Module11	hsa04141: Protein processing in endoplasmic reticulum	2	.0478851	*SEC31A, SAR1B*	14.312831
Module13	hsa00190: Oxidative phosphorylation	5	1.33E-07	*NDUFA4, COX11, COX7C, COX6B1, COX6C*	6.84E-05
	hsa05012: Parkinson disease	4	3.38E-05	*NDUFA4, COX7C, COX6B1, COX6C*	0.0174037
	hsa04932: Nonalcoholic fatty liver disease (NAFLD)	4	3.90E-05	*NDUFA4, COX7C, COX6B1, COX6C*	0.0201109
	hsa05010: Alzheimer disease	4	5.59E-05	*NDUFA4, COX7C, COX6B1, COX6C*	0.0288317
	hsa05016: Huntington's disease	4	8.22E-05	*NDUFA4, COX7C, COX6B1, COX6C*	0.0423499
	hsa04260: Cardiac muscle contraction	3	.0006915	*COX7C, COX6B1, COX6C*	0.3559762
	hsa01100: Metabolic pathways	5	.0009689	NDUFA4, COX11, COX7C, COX6B1, COX6C	0.4984845
Module14	hsa04070: Phosphatidylinositol signaling system	6	3.12E-09	*MTMR3, MTMR1, PIK3R5, PI4K2B, INPP5B, PIP4K2B*	3.25E-06
	hsa00562:Inositol phosphate metabolism	5	1.44E-07	*MTMR3, MTMR1, PI4K2B, INPP5B, PIP4K2B*	0.0001506
Module19	hsa04918: Thyroid hormone synthesis	2	.0497836	*CREB1, ATP1A1*	38.432176
Module20	hsa00190: Oxidative phosphorylation	5	1.33E-07	*NDUFB5, NDUFB8, NDUFA9, NDUFA12, NDUFB1*	6.35E-05
	hsa05012: Parkinson disease	5	1.73E-07	*NDUFB5, NDUFB8, NDUFA9, NDUFA12, NDUFB1*	8.28E-05
	hsa04932: Nonalcoholic fatty liver disease (NAFLD)	5	2.10E-07	*NDUFB5, NDUFB8, NDUFA9, NDUFA12, NDUFB1*	0.0001005
	hsa05010: Alzheimer disease	5	3.41E-07	*NDUFB5, NDUFB8, NDUFA9, NDUFA12, NDUFB1*	0.0001632
	hsa05016: Huntington disease	5	5.72E-07	*NDUFB5, NDUFB8, NDUFA9, NDUFA12, NDUFB1*	0.0002739
	hsa01100: Metabolic pathways	5	.0009689	*NDUFB5, NDUFB8, NDUFA9, NDUFA12, NDUFB1*	0.4628423

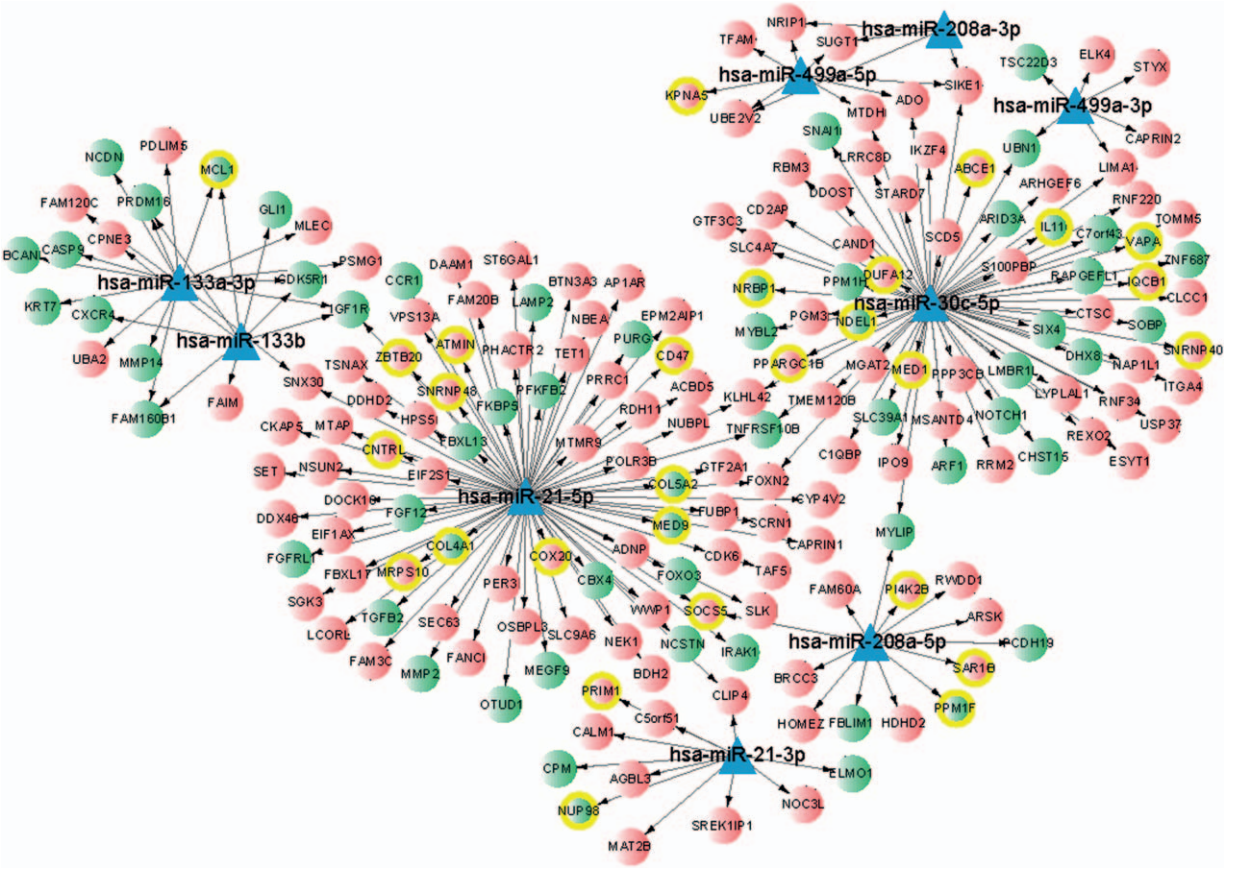

Figure 3. A microRNA-gene network consisting of differentially expressed genes and the miRNAs regulating them, A, triangle represents a microRNA, B, a circle represents a differentially expressed gene.

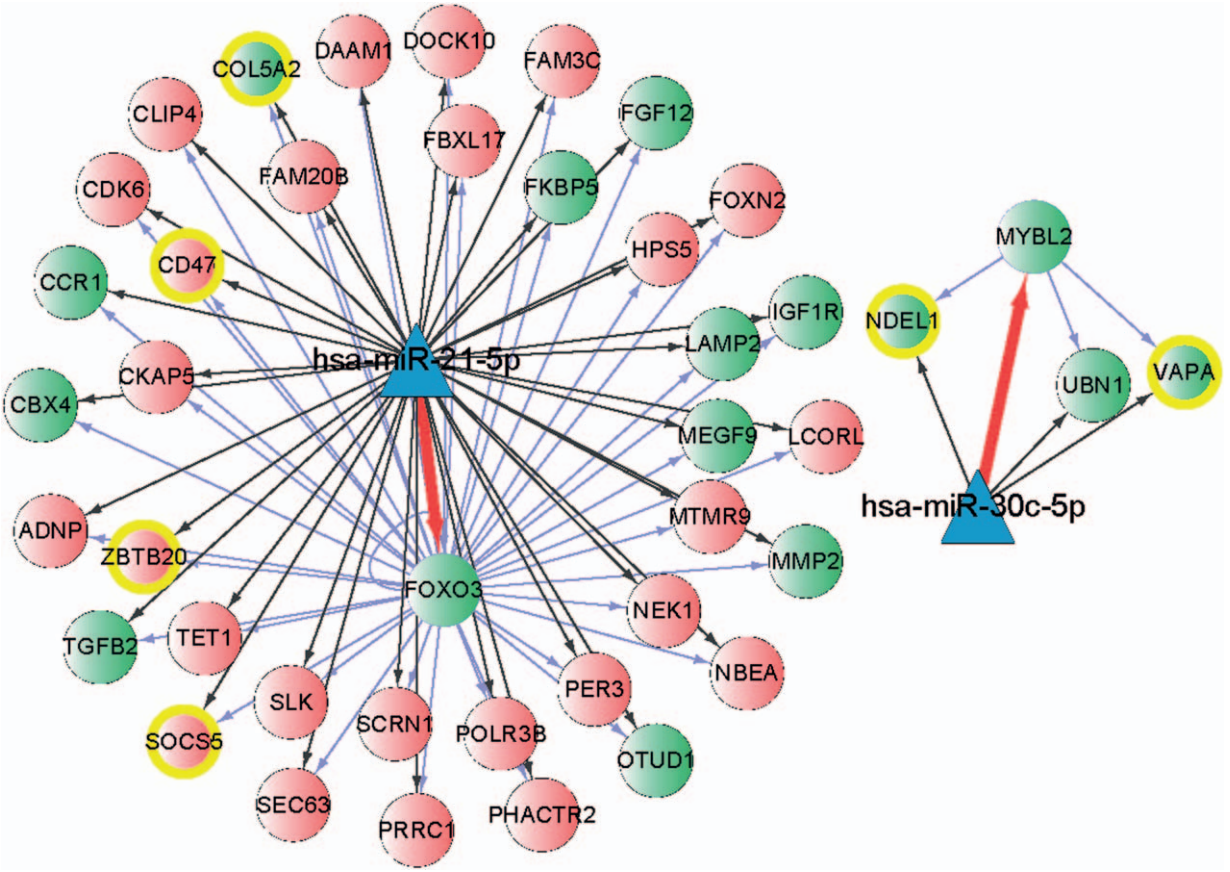

Figure 4. The transcription factor-miRNA-gene network, A, triangle represents a microRNA, B, green circle represents the upregulated differentially expressed gene or upregulated transcription factor, and red circle represents the downregulated differentially expressed gene.

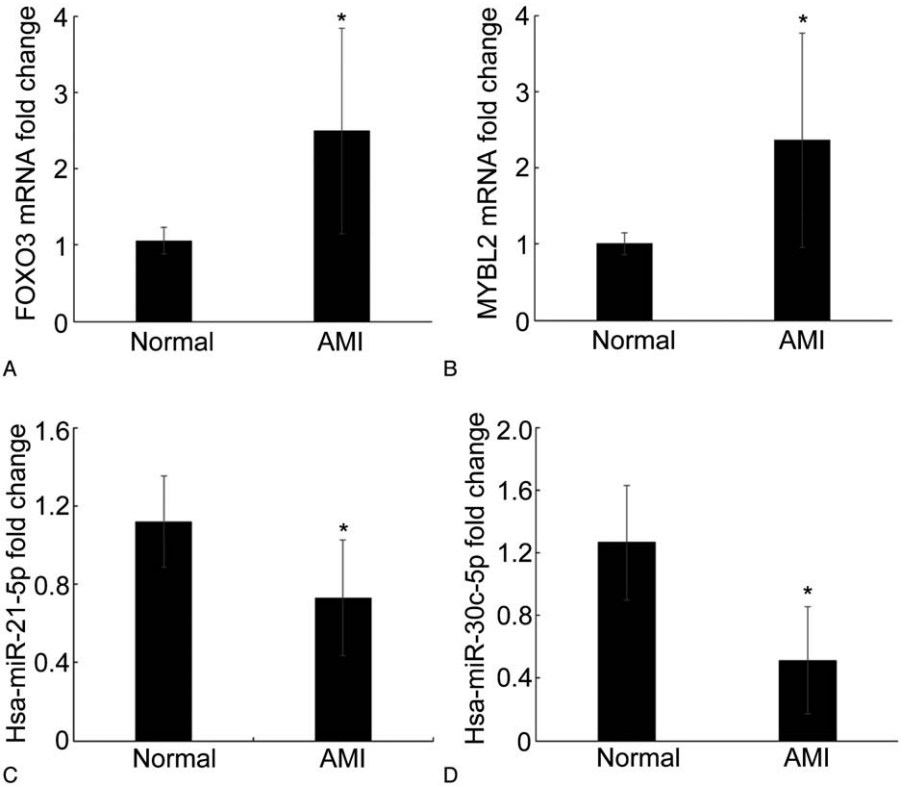

Figure 5. Expression levels of *FOXO3*, *MYBL2*, hsa-miR-21-5p, and hsa-miR-30c-5p determined using quantitative real-time PCR.

COL5A2 expression. Previously, Dong et al[27] reported that miR-21 expression was significantly decreased in infarcted areas of rat hearts rat hearts 6 hours after AMI. Dong et al[27] have confirmed that miR-21 has a protective effect against cardiac cell apoptosis via its target gene *PDCD4* in the early phase of AMI. An elevated expression of hsa-miR-21-5p was also identified in this study. Thus, hsa-miR-21-5p may also be involved in AMI via regulating various DEGs, such as *SOCS3* and *COL5A2*.

VAPA encoding vesicle-associated membrane protein associated protein-A showed an upregulated expression in AMI patients here. According to the previously published literatures, VAPA is commonly involved in the regulation of endoplasmic reticulum to Golgi transportation via binding to oxysterol-binding protein, by which to regulate vesicle transport and sterol homeostasis.[28–30] However, the biofunction of VAPA in AMI is rarely reported, and only Zhao et al[31] have reported that VAPA is markedly decreased in the infarcted myocardium of rats, which is contrary to our identification. Therefore, further investigation of VAPA in AMI was still required. Here, VAPA expression was predicated to be regulated by *MYBL2* and *hsa-miR-30c-5p*. *MYBL2* encodes a member of the MYB family member Myb-related protein B. Increased MYBL2 expression was reported in the peripheral blood leukocytes of humans with acute ischemic stroke[32] and it is also implicated to participate in the regulation of genes downregulated in left ventricular remodeling following myocardial infarction.[33] Taken together, *VAPA* upregulation observed here may be elevated by transcription factor MYBL2. Hsa-miR-30c-5p has been reported as an apoptosis-related microRNA in myocardial infarction.[34] Duisters et al[35] further reported that miR-30 directly downregulated connective tissue growth factor (CTGF), which thus was considered to have an important role in the control of structural changes in the extracellular matrix of the myocardium, which was consistent with the result validated in this study. Thus, this miRNA may perform an adverse effect in the regulation of *VAPA* expression in AMI. Another 2 upregulated genes *UBN1* and *NDEL1* were also speculated to be regulated by *MYBL2* and hsa-miR-30c-5p. *UBN1* encodes the shutting protein ubinuclein 1, which was an interacting partner of RACK1 protein, and the latter is confirmed to have a role in the process of myocardial damage.[36] Thus, we assume that UBN1 may be involved in the pathogenesis of AMI. However, *NDEL1* encoding a thiol-activated oligopeptidase that is involved in the regulation of cytoplasmic dynein function and microtubule organization during mitotic cell division has never been reported in AMI.

However, there were also some limitations that should be strengthened in this study. First, because most of the results in this study were obtained from in silico analysis, thus, further experimental validations should be required. Second, although expression levels of several DEGs and predicted miRNA were verified to be consistent with the bioinfomatic analysis results, the regulatory ships between them were still not be confirmed, as well as the enrichment analytical results. Third, some of the results identified in this study were not consistent with previously published results in other diseases. Therefore, further validations in both in vivo and in vitro were still needed. Despite these limitations, our study also provided some new insights in the mechanism of AMI.

In summary, we identified 41 DEGs, such as *SOCS3*, *VAPA*, and *COL5A2*, that were speculated to have a role in the pathogenesis of AMI, as well as 2 transcription factors *FOXO3* and *MYBL2*, and 2 miRNAs hsa-miR-21-5p and hsa-miR-30c-5p that might regulate the expression of these DEGs. Our work provides some potential genes for the targeted therapy of AMI and its early detection. However, as some of our findings are not consistent with the published references, we have to further validate them by other means.

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