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 using the edgeR package. DEGs were identified by the cutoff of 
P < .05 was used. The transcription factors-miRNA-gene network was constructed to identify potential biomarkers.

Result: A total of 913 upregulated genes and 1060 downregulated genes were identified in the AMI group. 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] According to the guidelines of the American heart association,[3] 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.[3,4] 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.
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. 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 $P$ 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 log2FC (fold change) value of a gene in each dataset was further calculated, with log2FC $>0$ as upregulated and log2FC $<0$ 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) ($P<.05$).

2.5. Construction of gene functional interaction network
The expression matrix data of DEGs was submitted to ReactomeFIViz to investigate gene–gene interaction based on known human pathway data. 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).

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, 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, 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, $4 \mu$g 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 $\mu$g of total RNA was applied to mRNA reverse transcription 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 used as a reference gene.
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 has04630: 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 genes (CBX4, CCR1, COL5A2, FGF12, FKBPS5, IGF1R, LAMP2, MEGF9, MMP2, OTUD1, TGFBI, UBE1, VAPA) and 26 downregulated genes (ADNP, CD47, CDK6, CKAP5, CLIP4, DAAM1, DOCK10, FAM20B, FAM3C, FBLX17, FOXN1, HPS5, LCORL, MTMR9, NBEA, NEK1, PER3, PHACTR2, POLR3B, PRCC1, SCR1, SEC63, SLK, SOCS5, TET1, 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 miRNA 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).
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 JAK/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 Azuaige 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...
| Module ID | Pathway term                          | Gene count | P-value     | Genes                                                                 |
|----------|---------------------------------------|------------|-------------|-----------------------------------------------------------------------|
| hsa04740 | Olfactory transduction                | 8          | 4.9E-06     | OR10A5, OR13C4, OR10A3, OR11A1, PRKACA, PRKACB, OR5S1, OR5S1         |
| hsa04020 | Calcium signaling pathway             | 6          | 1.6E-05     | ADCY4, ADCY9, PKH2, PKH2G4, PRKACA, PRKACB                            |
| hsa04011 | Insulin secretion                     | 5          | 1.96E-05    | ADCY4, ADCY9, PRKACA, RAPSEF4, PRKACB                                |
| hsa04713 | Circadian entrainment                | 5          | 3.05E-05    | ADCY4, ADCY9, PRKACA, PKH2, RSAD1                                   |
| hsa04913 | Ovarian steroidogenesis               | 4          | 0.001164    | ADCY4, ADCY9, PRKACA, PKH2                                          |
| hsa04261 | Adrenergic signaling in cardiomyocytes| 5          | 0.001841    | ADCY4, ADCY9, PRKACA, RAPSEF4, PRKACB                               |
| hsa04233 | Regulation of lipolysis in adipocytes | 4          | 0.001737    | ADCY4, ADCY9, PRKACA, PKH2                                          |
| hsa04017 | Thyroid hormone synthesis             | 4          | 0.002937    | ADCY4, ADCY9, PRKACA, PKH2                                          |
| hsa04018 | Gastric acid secretion                | 4          | 0.003818    | ADCY4, ADCY9, PRKACA, PKH2                                          |
| hsa04024 | cAMP signaling pathway                | 5          | 0.005178    | ADCY4, ADCY9, PRKACA, RAPSEF4, PKH2G4                               |
| hsa04025 | Aldosterone synthesis and secretion   | 4          | 0.005188    | ADCY4, ADCY9, PRKACA, PKH2                                          |
| hsa04727 | GABAergic synapse                     | 4          | 0.005188    | ADCY4, ADCY9, PRKACA, PKH2                                          |
| hsa04790 | Sallory secretion                     | 4          | 0.005773    | ADCY4, ADCY9, PKH2, PKH2G4                                          |
| hsa05414 | Dilated cardiomyopathy                | 4          | 0.005773    | ADCY4, ADCY9, PRKACA, PKH2                                          |
| hsa04914 | Progesterone-mediated oocyte maturation| 4          | 0.006399    | ADCY4, ADCY9, PRKACA, PKH2                                          |
| hsa04540 | Gap junction                          | 4          | 0.006617    | ADCY4, ADCY9, PRKACA, PKH2                                          |
| hsa04912 | GRH signaling pathway                 | 4          | 0.007299    | ADCY4, ADCY9, PRKACA, PKH2                                          |
| hsa05023 | Morphine addiction                   | 4          | 0.007299    | ADCY4, ADCY9, PRKACA, PKH2                                          |
| hsa04750 | Inflammatory mediator regulation of TRP channels| 4          | 0.008794    | ADCY4, ADCY9, PRKACA, PKH2                                          |
| hsa04723 | Retrograde endocannabinoid signaling  | 4          | 0.008794    | ADCY4, ADCY9, PRKACA, PKH2                                          |
| hsa04022 | Glucagon signaling pathway            | 4          | 0.009333    | PKH2, PKH2G4, PKH2, PKH2G4                                          |
| hsa04915 | Estrogen signaling pathway            | 4          | 0.009333    | ADCY4, ADCY9, PRKACA, PKH2                                          |
| hsa04016 | Melanogenesis                         | 4          | 0.009661    | ADCY4, ADCY9, PRKACA, PKH2                                          |
| hsa04114 | Oocyte meiosis                        | 4          | 0.012343    | ADCY4, ADCY9, PRKACA, PKH2                                          |
| hsa04725 | Cholinergic synapse                   | 4          | 0.013101    | ADCY4, ADCY9, PKH2, PKH2G4                                          |
| hsa05166 | HTLV-I infection                     | 5          | 0.013669    | ADCY4, ADCY9, XBP1, PRKACA, PKH2G4                                  |
| hsa04724 | Glutamatergic synapse                 | 4          | 0.014053    | ADCY4, ADCY9, PRKACA, PKH2                                          |
| hsa04270 | Vascular smooth muscle contraction    | 4          | 0.015006    | ADCY4, ADCY9, PRKACA, PKH2                                          |
| hsa04601 | Platelet activation                   | 4          | 0.015017    | ADCY4, ADCY9, PRKACA, PKH2                                          |
| hsa04010 | Insulin signaling pathway             | 4          | 0.015031    | PKH2, PKH2G4, PRKACA, PKH2                                          |
| hsa04962 | Vasopressin-regulated water reabsorption| 3          | 0.033005    | ADCY9, PRKACA, PKH2, PKH2G4                                          |
| hsa04742 | Taste transduction                    | 3          | 0.034537    | ADCY4, PKH2, PKACB                                                  |
| hsa04021 | Oxytocin signaling pathway            | 4          | 0.035747    | ADCY4, ADCY9, PRKACA, PKH2                                          |
| hsa04961 | Endocrine and other factor-regulated calcium reabsorption | 3          | 0.036101    | ADCY9, PKH2, PKH2G4, PKH2                                          |
| hsa05110 | Uvira cholerae infection              | 3          | 0.049786    | ADCY9, PRKACA, PKH2, PKH2G4                                        |
| hsa04062 | Chemokine signaling pathway           | 4          | 0.055702    | ADCY4, ADCY9, PRKACA, PKH2                                          |
| hsa05200 | Pathways in cancer                    | 4          | 0.041884    | ADCY4, ADCY9, PRKACA, PKH2                                          |
| hsa03050 | Protease                              | 9          | 5.80E-14    | PSA2, PSA2D1, PSA4, PSA5, PSA6, PSM2, PSM2D1, PSM2D2, PSM2D2, PSM2 |
| hsa03040 | Spliceosome                           | 11         | 1.18E-15    | NCBP2, HVNBPA3, PLG2, LMS8, RBM8A, LMS8, SRNP1, LMS8, SRNP4, LMS8, |
| hsa03015 | mRNA surveillance pathway             | 5          | 1.78E-05    | NCBP2, UFF1, RBM8A, NUD2T1, SMG7                                    |
| hsa03018 | RNA degradation                       | 4          | 0.003554    | LMS8, LMS6, LMS5, LMS2                                             |
| hsa03013 | RNA transport                         | 3          | 0.009075    | NCBP2, UFF1, RBM8A                                                   |
| hsa03010 | Ribosome                              | 9          | 1.66E-14    | MRPL13, MRPL3, MRPS18C, MRPL15, MRPL27, MRPL16, MRPS10, MRPL30     |
| hsa03030 | DNA replication                       | 6          | 1.32E-09    | PRM1, POLE2, MCM3, RP4, FEN1, RP4                                    |
| hsa03460 | Fanconi anemia pathway                | 5          | 9.89E-07    | PMS2, ERC4, RP4, RP4, RAS51, RP4                                    |
| hsa03420 | Nucleotide excision repair            | 4          | 4.72E-05    | POLE2, ERC4, RP4, RP4                                              |
| hsa03430 | Mismatch repair                       | 3          | 0.005755    | PMS2, RP4, RP4                                                     |
| hsa03440 | Homologous recombination              | 3          | 0.009188    | RP4, RAS51, RP4                                                    |
| hsa04110 | Cell cycle                            | 3          | 0.015866    | ORC5, ORC10, ORC3                                                  |
| hsa03410 | Base excision repair                  | 2          | 0.049469    | POLE2, FEN1                                                       |
| hsa03060 | Protein export                        | 5          | 3.09E-06    | SRP54, SRP68, SRPS1, SRPS2, SRP9                                   |
| hsa03101 | Ribosome                              | 6          | 1.05E-06    | RPL23, RPL15, RPL8, RPL24, RPL11, RPL12                             |

(continued)
| Module ID | Pathway term                                      | Gene count | P        | Genes                          | FDR    |
|-----------|--------------------------------------------------|------------|----------|--------------------------------|--------|
| Module 7  | hsa04080: Neuroactive ligand–receptor interaction | 4          | .0011741 | BDKRB1, NPFFR1, GHSR, NTSR2   | 0.8622339 |
| Module 9  | hsa04650: Jak-STAT signaling pathway             | 7          | 2.12E-09 | IL5, IL7, JAK2, SOCS5, IFNGR2, IL11, EPO | 1.79E-06 |
|           | hsa04640: Cytokine–cytokine receptor interaction  | 5          | 7.48E-05 | IL5, IL7, IFNGR2, IL11, EPO    | 0.0631994 |
|           | hsa04640: Hematopoietic cell lineage             | 4          | 9.70E-05 | IL5, IL7, IL11, EPO            | 0.0818866 |
| Module 11 | hsa04141: Protein processing in endoplasmic reticulum | 2          | .0478851 | SEC31A, SAR1B                    |        |
| Module 13 | 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 |
|           | hsa05101: 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            | 0.3599762 |
|           | hsa01100: Metabolic pathways                     | 5          | .0009689 | NDUFA4, COX11, COX7C, COX6B1, COX6C | 0.4984845 |
| Module 14 | hsa04070: Phosphatidylinositol signaling system  | 6          | 3.12E-09 | MTMR3, MTMR1, PKC3R, PKC2B, NPP5B, PIP5K | 3.25E-06 |
|           | hsa00562: Inositol phosphate metabolism          | 5          | 1.44E-07 | MTMR3, MTMR1, PKC2B, NPP5B, PIP5K | 0.0001506 |
| Module 19 | hsa04918: Thyroid hormone synthesis              | 2          | .0497836 | CREB1, ATP1A1                   | 38.43176 |
| Module 20 | 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 | 8.26E-05 |
|           | hsa04932: Nonalcoholic fatty liver disease (NAFLD) | 5          | 2.10E-07 | NDUFB5, NDUFB8, NDUFA9, NDUFA12, NDUFB1 | 0.0001005 |
|           | hsa05101: Alzheimer disease                      | 5          | 3.41E-07 | NDUFB5, NDUFB8, NDUFA9, NDUFA12, NDUFB1 | 0.0001632 |
|           | hsa05016: Huntington’s 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 oxytoster-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 predicted 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 be 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 thiold-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 bioinformatic 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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