Potential Biomarkers of Acute Myocardial Infarction Based on Co-Expression Network Analysis

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Research article

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Abstract

**Background:** Acute myocardial infarction (AMI) is a common cause of death in many countries. Analyzing the potential biomarkers of AMI is crucial to understanding the molecular mechanism of disease. However, specific diagnostic biomarkers have not been fully elucidated, and candidate regulatory targets for AMI have not been determined.

**Methods:** In this study, AMI gene chip data GSE48060, blood samples from normal cardiac function controls (n = 21) and AMI patients (n = 26) were downloaded from Gene Expression Omnibus. The differentially expressed genes (DEGs) of AMI and control group were identified with Online tool GEO2R. the genes co-expressed with were found. The co-expression network of DEGs was analyzed by calculating the Pearson correlation coefficient of all gene pairs, MR screening and cutoff threshold screening. Then, GO database was used to analyze the function and pathway enrichment of genes in the most important modules. KEGG DISEASE and BioCyc were used to analyze the hub gene in the module to determine important sub-pathways. In addition, the expression of hub genes were certified by RT-qPCR in AMI and control specimens.

**Results:** This study identified 52 DEGs, including 26 up-regulated genes and 26 down-regulated genes. Co-expression network analysis of 52 DEGs revealed that there are mainly three up-regulated genes (AKR1C3, RPS24 and P2RY12) and three down-regulated genes (ACSL1, B3GNT5 and MGAM) as key hub genes in the co-expression network. Furthermore, GO enrichment analysis was performed on all AMI co-expression network genes and found to be functionally enriched mainly in RAGE receptor binding and negative regulation of T cell cytokine production. In addition, through KEGG DISEASE and BioCyc analysis, the functions of genes RPS24 and P2RY12 were enriched in cardiovascular diseases, AKR1C3 was enriched in cardenolide biosynthesis, MGAM was enriched in glycogenolysis, B3GNT5 was enriched in glycosphingolipids biosynthesis, and ACSL1 enriched in icosapentaenoate biosynthesis II.

**Conclusion:** The hub genes AKR1C3, RPS24, P2RY12, ACSL1, B3GNT5 and MGAM are potential targets of AMI and have potential application value in the diagnosis of AMI.

**Background**

Despite significant progress in vascular remodeling strategies, drug therapy, cardiac rehabilitation algorithms, and organ transplantation, AMI, as one of cardiovascular diseases, remains the main cause of morbidity and mortality in worldwide [1]. There are many risk factors for AMI, including smoking, obesity, high serum cholesterol, hypertension, and diabetes, which can be partially predicted in terms of disease prevention and prognosis, but it is not enough to provide an acute diagnosis [2, 3]. Despite many efforts, the prevention and treatment of this disease remains a major challenge for scientists. It is predicted that by 2020, this disease will be the main and most common threat to human life [4, 5]. Therefore, it is urgent to reveal the pathogenesis of AMI and develop new treatment strategies.
Early detection of AMI contributes to early treatment interventions and can significantly reduce mortality [6]. Many studies were looking for potential molecular biomarkers for AMI detection. As expected, some genes and proteins, such as monocyte-platelet aggregation, cardiac fatty acid binding protein, and troponin I, have been established as effective markers for the diagnosis of AMI [6–8]. In addition, a large number of miRNAs are considered to be key markers for AMI treatment, such as circulating miR-26a-1 [9], miR-17-5p [10] and miR-23a [11]. However, the interactions between these molecules the altered pathways were rarely reported. The pathogenesis of AMI remains unclear. Suresh et al. established gene expression profiles through microarrays and compared the DEGs between AMI samples and normal samples, as well as the deregulated pathways of these DEGs [12]. However, they only focus on pathways and recurring events. The regulatory correlation between these genes has not been investigated further. Therefore, a recent study by Gao et al. reanalyzed the GSE48060 microarray data and performed protein-protein interaction (PPI) network analysis and transcription factor network (TFN) analysis after identifying DEGs in AMI samples. Although they predicted several key genes in the progression of AMI, such as CCL5, BCL3, and NCOA7, subchannel analysis and co-expression network analysis were not involved [13].

Co-expression network analysis is a useful method that has been widely used for gene expression to identify key disease-related modules [14, 15]. For example, using co-expression network analysis, Saris et al. selected two large co-expression modules related to amyotrophic lateral sclerosis [16]. By estimating the gene expression pattern, Azuaje et al. determined the WGCN in myocardial infarction to determine the potential role of Col5a2 and its transcription pattern [17]. Malki et al. constructed a gene co-expression network to identify the expression of nerve tumor abdominal wall antigen 1 (NOVA1) and ubiquitin-specific peptidase 9, X-linked (USP9X), and found that the most significant modules are related to depression and drug treatment response [18]. Co-expression network analysis identified spleen tyrosine kinase (SYK) as a candidate oncogene for small cell lung cancer [19]. Zhao et al. prompt cytokinesis factor 2 (DOCK2), cytokinesis factor 8 (DOCK8) and IgG Fc fragments, low-affinity IIa, receptor (FCGR2A) may represent potential therapeutic targets through co-expression network analysis combined with methylation data analysis [20]. Therefore, co-expression network analysis can be used for the analysis of AMI chip data.

In the present study, we reanalyzed Suresh's gene chip data GSE48060, and performed co-expression network analysis, enrichment analysis, and PPI analysis on gene expression. In addition, in terms of DEG screening, compared with Zhang et al. [21], in addition to co-expression network and PPI analysis, we also conducted KEGG DISEASE and BioCyc analysis on DEGs to further dig deep into gene functions. We aim to reveal the molecular basis of the occurrence and development of AMI and provide new and more accurate biomarkers for the detection and treatment of AMI.

**Methods**

Data resource and differential expression analysis
The data set GSE48060, consisting of 47 microarray expression profiles, is downloaded from the database of the Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/). All data were obtained from the peripheral blood tissues of 26 patients without recurrence (AMI samples) and 21 normal heart function controls (control samples). The platform of the data set is GPL570 (HG-u133plus2, Affymetrix human genome U133). The differentially expressed genes were analyzed by the GEO2R tool in AMI and the control group, which were selected according with a fold difference of 1.5 times or more.

AMI specimens

The whole blood collected from AMI patients and controls with a normal echocardiogram. These specimens were collected from Cardiovascular Department of Internal Medicine of Central Hospital of Karamay (Karamay, China).

Co-expression analysis

To construct a co-expression network, we calculated the PCC value of unique 28492 probes of all combinations in the GSE48060 data resource. Gene pairs \( p \leq 0.5 \) were selected and PCC threshold was set to 0.88 \([22]\). According to the previous report \([23]\), MR values between gene pairs were applied to further reduce false positives. Gene pairs with MR values less than 10 were considered to be important connections and selected for the co-expression network. The calculation was carried out by R/Bioconductor. To extract the oral and oropharyngeal cancer subnet dataset, we extracted the network vicinity from the guide gene by 2 steps according to the methods described previously by Mutwil et al. \([24]\). The cystoscope program was used to illustrate the network.

Gene ontology (GO) analysis

Cellular components (CC), molecular functions (MF), and biological processes (BP) were classified by GO analysis. Genes related with breast cancer were obtained according to the BP categories. The EC and GO enrichment analysis were retrieved from DAVID (https://david.ncifcrf.gov/). The significant differences for GO enrichment analysis of DEGs and genes in co-expression network were evaluated against background sets containing 52 and 256 genes, respectively. The \( P < 0.01 \) for BP and \( P < 0.1 \) for MF were set to be significance thresholds.

KEGG DISEASE and BioCyc analysis

KEGG Disease and BioCyc analysis of DEGs and co-expression network genes were analyzed through using the tool KOBAS, and the results of enrichment were analyzed by Fisher's exact test, \( p \leq 0.05 \).

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNAs were extracted from the whole blood with Trizol reagent (Sangon, Shanghai). The mRNAs were quantified and reverse transcribed to cDNAs with PrimeScript® RT Master Mix (TAKARA, Japan).
pursuant to the protocol. The qPCR reaction was detected with the SYBR Green Master Mix (Applied Biosystems, USA) and performed by a fluorescence quantitative PCR instrument (Applied Biosystems 7900HT, USA).

**Results**

Data preprocessing and DEGs screening

After data preprocessing, the expression matrix of 24277 genes was obtained from 47 samples (Supplemental Table 1). Under the threshold of $|\text{log}_{2} \text{FC}| \geq 0.5$, a total of 52 DEGs were selected for subsequent analysis, including 26 up-regulated genes and 26 down-regulated genes. The heat map results of gene expression cluster analysis show that these DEGs can distinguish these two samples well (Fig. 1 and Supplemental Table 2), indicating that DEGs can be used for further analysis.

Functional enrichment of DEGs

Though performing gene ontology (GO) analysis on 52 DEGs, we found that MHC class I protein complex binding, cellular response to prostaglandin D stimulus, and natural killer cell mediated immunity were significantly enriched (Supplemental Fig. 1 and Supplemental Table 3). Furthermore, we conducted KEGG DISEASE analysis on the 52 obtained DEGs and found that RPS24, P2RY12 and HLA-DQA1 are involved in cardiovascular diseases (Supplemental Table 4). In addition, through BioCyc analysis, the results revealed that these DEGs are mainly related to cardenolide biosynthesis (such as AKR1C3), glycosphingolipids biosynthesis (such as B3GNT5), fatty acid activation (such as AKR1C3) and glycogenolysis (such as MGAM) (Supplemental Table 4).

Co-expression network analysis network

According to the character string database, the genes pairs were selected with p value less than 0.05, by calculating the Pearson correlation coefficient of all genes. Finally, 256 genes co-expressed with DEGs were obtained through calculating the rank and median of each pair of genes (as cutoff), MR $\leq 10$ and cutoff $\geq 0.88$. The results of co-expression network analysis revealed that 8 gene modules were up-regulated (Fig. 2A, red mark) and 8 gene modules were down-regulated (Fig. 2B, blue mark). Among them, the expression levels of AKR1C3, RPS24 and P2RY12 as key hub genes were up-regulated (Fig. 2A and Supplemental table 2) and ACSL1, B3GNT5 and MGAM were down-regulated (Fig. 2B and Supplemental table 2).

Functional enrichment of genes in co-expression networks

Through analysis of the genes in this module by GO, we were surprised to find that RAGE receptor binding, positive regulation of killing of cells of other organism, leukocyte aggregation and negative regulation of T cell cytokine production were significantly enriched (Fig. 3 and Supplemental Table 5).
Furthermore, KEGG DISEASE and BioCyc analysis was constructed containing 256 genes in co-expression network. Three co-expression subnets centered on up-regulated genes (AKR1C3, RPS24 and P2RY12), and three co-expression subnets centered on down-regulated genes (ACSL1, B3GNT5 and MGAM) were obtained. These genes with up-regulated expression have significant correlations with functions such as cardiovascular diseases and cardenolide biosynthesis (Table 1 and Supplemental Fig. 2). The down-regulated genes have significant correlation with functions such as glycogenolysis, glycosphingolipids biosynthesis and fatty acid activation (Table 1 and Supplemental Fig. 3). Interestingly, all of them were also involved in the enrichment of those genes obtained by KEGG DISEASE and BioCyc analysis of 52 DEGs in this study. This shows that some genes that have not previously reported AMI can be mined from existing data, and new interactions between these genes can also be identified from the connections in the network. AMI development is an extremely special biological event, so co-expression network analysis may have the greatest potential for identifying gene interactions in AMI.

The expression of hub genes in AMI specimens

To further investigate the expression level of hub genes in AMI patients, AKR1C3, RPS24, P2RY12, ACSL1, B3GNT5 and MGAM expression were investigated in 31 couplings of the whole blood collected from AMI and normal groups using qRT-PCR. As depicted in Fig. 4, the $2^{-\Delta\Delta Ct}$ value of AKR1C3, RPS24 and P2RY12 was significantly increased in AMI patients relative to that of normal adjacent group ($p < 0.01$) (Fig. 4A to 4C). On the contrary, the $2^{-\Delta\Delta Ct}$ value of ACSL1, B3GNT5 and MGAM was significantly decreased in AMI patients ($p < 0.01$) (Fig. 4D to 4F). This finding was consistent with the data of microarray expression profiles.

Discussions

AMI is a serious cardiovascular disease that threatens human lives. It may cause malignant arrhythmias and congestive heart failure, resulting in high morbidity and mortality [25]. Although percutaneous coronary intervention (PCI) and thrombolysis can efficiently improve the prognosis of AMI, many AMI patients eventually develop heart failure or arrhythmia due to unknown etiology [26]. The discovery of potential AMI diagnostic biomarkers and possible regulatory targets will help to reduce AMI mortality. Recently, systematic biological analysis based on gene expression profiles provides better strategies to elucidate mechanisms of AMI from the perspective of gene interaction [27]. In this study, we obtained sufficient information about genes changed expressions and associated with disease from gene expression profiles. Previously microarray analysis has elucidated that a series of genes, such as those related with platelet function, atherosclerotic plaque stability, and glucose/lipid metabolism, changed expressions when ST-elevation myocardial infarction (STEMI) happened [28]. Based on the systematisms of gene expression profiling, gene co-expression network analysis can be an alternative means to gain a deeper understanding of the molecular regulatory mechanisms of heart disease due to myocardial infarction [29, 30]. This study used gene ontology (GO), pathway enrichment, KEGG DISEASE and BioCyc analysis methods to explore the molecular mechanism of myocardial infarction-induced heart disease.
Gene co-expression network analysis has also been applied to identify changes in transcriptome expression patterns in complex diseases [31, 32]. Unlike DEG’s standardized analysis detects individual genes related with diseases, co-expression network analysis aims to determine a higher correlation between gene products. In addition, its algorithm can greatly simplify multiple unavoidable testing problems lying in the gene-centric standard microarray analysis for expression profiling [33]. Thus, it should be a powerful systematic analysis tool focusing on the relevant functions of network modules.

Using the above-mentioned bioinformatics methods, the GO analysis, pathway function network and gene module changes of AMI patients were analyzed to explore the potential AMI diagnostic biomarkers and regulatory targets. We successfully constructed the gene co-expression network based on the expression profile [12]. Co-expression network analysis was used to detect peripheral blood gene modules when AMI occurred. We found that the up-regulated genes AKR1C3, RPS24 and P2RY12 were mainly involved in cardenolide biosynthesis and cardiovascular diseases. Cardiac glycosides have been considered as the main medical treatment for congestive heart failure and arrhythmia, because the effects of increasing muscle contractility while reduce the burden of the heart. The three genes whose expression is down-regulated, ACSL1, B3GNT5 and MGAM, are associated with glycogenolysis, glycosphingolipids biosynthesis and fatty acid activation. This finding implies that the etiology of AMI is related to genes related to intravascular disease, immune response and brain-derived factor regulation system, suggesting that ACSL1, B3GNT5 and MGAM may be also used as biomarkers for gene expression in early AMI.

**Conclusions**

Our findings provide important values in prediction of molecular events related to AMI as well as potential biomarkers for detection and prevention. The hub genes, AKR1C3, RPS24, P2RY12, ACSL1, B3GNT5 and MGAM were considered to be biomarkers to assess the severity of heart disease, and might be a promising therapeutic target to promote new treatment plans. In conclusion, our research identified key genes involving in the regulation of median development through bioinformatics analysis, providing potential targets for AMI diagnosis and treatment.

**Abbreviations**

AMI: Acute myocardial infarction; DEGs: Differentially expressed genes; NOVA1: Nerve tumor abdominal wall antigen 1; USP9X: Ubiquitin-specific peptidase 9, X-linked; PPI: Protein-protein interaction; TFN: Transcription factor network; SYK: Spleen tyrosine kinase; DOCK8: Cytokinesis factor 8; GO: Gene ontology; PCI: Percutaneous coronary intervention; STEMI: ST-elevation myocardial infarction; CC: Cellular components; BP: Biological processes; MF: Molecular functions.

**Declarations**

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**Authors' contributions**

ZHH and XHL designed and analyzed the work. XJD and YYJ participated in the GO analysis and data interpretation; GYL and YPW participated in the KEGG DISEASE and BioCyc analysis; SQX performed the qRT-PCR experiments. ZWD wrote the manuscript. All authors have read and approved the manuscript.

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**Availability of data and materials**

The datasets used and analyzed during the current study are available in the manuscript and its additional files.

**Ethics approval and consent to participate**

This study was approved by the Ethics Committee of Internal Medicine of Central Hospital of Karamay. Written informed consent was collected from all patients.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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Tables
Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures
Figure 1

Columns of heat map represent individual samples with AMI patients on left-hand side and controls on right-hand side while rows represent all differentially expressed probe-sets. Heat map color gradient represents relative expression, with up-regulated genes shown in red and down-regulated genes in blue.

Figure 2

The AMI co-expression network in humans. (A) The co-expression subnetwork was constructed using three upregulated AMI genes as guide genes (AKR1C3, RPS24 and P2RY12). (B) The co-expression subnetwork was constructed using three downregulated AMI genes as guide genes (ACSL1, B3GNT5 and MGAM). A link between two nodes indicates a direct interaction with \( p < 0.05, \) \( PCC \geq 0.88 \) and \( MR \leq 10. \) The subnetwork vicinity is extracted by taking two steps out from each guide gene.
Figure 3

The GO term analysis of BP, MF, and CC for the AMI co-expression network genes. The minus logarithm of the P-value (x-axis) indicates the significance of the gene set belonging to predefined categories under the co-expression network genes background. The y-axis represents each GO category. The gene number and EC value of the category in the subnetwork are shown in brackets.
Figure 4

The co-expression network genes were analysed in whole blood collected from AMI patients and controls with a normal echocardiogram via RT-qPCR. Expression of three upregulated genes (A) AKR1C3, (B) P2RY12 (C) RPS24 and three downregulated genes (D) ACSL1, (E) B3GNT5 and (F) MGAM were validated by RT-qPCR, respectively.

Supplementary Files

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