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ORIGINAL RESEARCH

Screening for Regulatory Network of miRNA–Inflammation, Oxidative Stress and Prognosis-Related mRNA in Acute Myocardial Infarction: An in silico and Validation Study

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Background: Acute myocardial infarction (AMI), which commonly leads to heart failure, is among the leading causes of mortality worldwide. The aim of this study was to find potential regulatory network for miRNA-inflammation, oxidative stress and prognosis-related mRNA to uncover molecular mechanisms of AMI.

Methods: The expression profiles of miRNA and mRNA in the blood samples from AMI patients were downloaded from the Gene Expression Omnibus (GEO) dataset for differential expression analysis. Weighted gene co-expression network analysis (WGCNA) was used to further identify important mRNAs. The negatively regulatory network construction of miRNA–inflammation, oxidative stress and prognosis-related mRNAs was performed, followed by protein–protein interaction (PPI) and functional analysis of mRNAs.

Results: A total of three pairs of negatively regulatory network of miRNA–inflammation and prognosis-related mRNAs (hsa-miR-636/hsa-miR-491-3p/hsa-miR-188-5p/hsa-miR-188-3p-AQP9, hsa-miR-518a-3p-C5AR1 and hsa-miR-509-3-5p/hsa-miR-127-5p-PLAUR), two pairs of negatively regulatory network of miRNA–oxidative stress and prognosis-related mRNAs (hsa-miR-604-TLR4 and hsa-miR-139-5p-CXCL1) and three pairs of negatively regulatory network of miRNA-inflammation, oxidative stress and prognosis-related mRNA (hsa-miR-634/hsa-miR-591-TLR2, hsa-miR-938-NFKBIA and hsa-miR-520h/hsa-miR-450b-3p-ADM) were identified. In the KEGG analysis, some signaling pathways were identified, such as complement and coagulation cascades, pathogenic *Escherichia coli* infection, chemokine signaling pathway and cytokine–cytokine receptor interaction and Toll-like receptor signaling pathway.

Conclusion: Identified negatively regulatory network of miRNA-inflammation/oxidative stress and prognosis-related mRNA may be involved in the process of AMI. Those inflammation/oxidative stress and prognosis-related mRNAs may be diagnostic and prognostic biomarkers for AMI.

Keywords: acute myocardial infarction, miRNAs, mRNAs, prognosis, inflammation, oxidative stress

Introduction

Acute myocardial infarction (AMI), one of the major cardiovascular diseases that lead to a high morbidity and mortality, is commonly defined as a cardiomyocyte death due to the prolonged ischaemia.[1] In addition, other risk factors including age, size and location of infarct, and haemodynamic status are associated with AMI.[2] The improved survival rates have been observed in AMI patients.[3,4] However, recurrent patients with AMI, a higher risk subgroup of those with AMI, have worse outcomes.[5–7] Therefore, it is needed to identify potential prognostic indicators to prevent AMI patients from

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relapsing. Previous reports showed that the age, creatinine, ejection fraction score and SYNTAX Score II had a prognostic value for patients with ST-elevation myocardial infarction-related cardiogenic shock.[8,9]

MI is a primarily local event, which results in the activation of the acute systemic inflammatory response. Reactive oxygen species (ROS) play a crucial role in MI.[10] In heart cells, the ROS causes mitochondrial dysfunction and result in serious complications, such as remodeling of the left ventricle and infarct expansion after MI.[11] It is noted that oxidative stress also up-regulated synthesis of pro-inflammatory cytokine.[12] Therefore, novel therapeutic strategy against AMI intends to control both the inflammatory response and the oxidative stress.

MiRNAs regulate mRNA expression at the post-transcriptional level.[13,14] MiRNAs have emerged as a kind of promising tool associated with some pathophysiological processes, including cardiovascular diseases. In addition, weighted gene co-expression network analysis (WGCNA) is used to elucidate changes in transcriptome expression patterns in complex diseases.[15–17] In contrast to differential expression analysis, WGCNA aims to identify high-order correlations between mRNA products.[18] In the present study, we tried to find differentially expressed miRNAs and inflammation, oxidative stress and prognosis-related mRNAs through WGCNA to uncover the molecular regulatory mechanism of AMI.

Materials and Methods

Dataset Retrieval

The expression profiles of mRNA and miRNA were downloaded from Gene Expression Omnibus (GEO) datasets by searching keywords ("acute myocardial infarction" [All Fields]) AND "Homo sapiens"[porgn] AND "gse"[Filter]). Firstly, the following mRNA datasets were selected for differential expression analysis: 1) dataset must be genome-wide transcriptome data of mRNA; 2) data were obtained from blood of AMI group and normal control group; 3) both standardized and raw datasets were considered. Finally, two mRNA expression datasets (GSE34198 and GSE48060) were selected. Secondly, GSE66360 dataset was downloaded and used for WGCNA analysis. Thirdly, three datasets (GSE123342, GSE59867 and GSE62646), including follow-up data of AMI patients after cure and discharge, were downloaded and used to identify mRNAs associated with prognosis. The screening criteria of prognostic mRNAs were as follows: 1) the mRNA expression trend was consistent in the three data sets; 2) the mRNA expression after discharge was significantly different from that the onset of disease. Lastly, GSE31568 dataset was downloaded and used for identification of differentially expressed miRNAs. Detail information of above enrolled datasets is shown in Table 1.

Table 1 Detail Information of Enrolled Datasets of AMI

GEO ID	Platform	Samples (N:AMI)	Sample	Author	Year	Note
GSE34198	GPL 6102	N:AMI=48:49	Blood	Valenta Z	2014	mRNA
GSE48060	GPL 570	N:AMI=21:26	Blood	Suresh R	2014	mRNA
GSE66360	GPL 570	N:AMI=50:49	Blood	Kramer ER	2015	WGCNA
GSE123342	GPL17586	AMI: After 30 Days: After 1 Years= 65:64:37	Blood	Vanhaverbeke M	2019	Prognosis
GSE59867	GPL 6244	On the 1st day: after 4–6 days: after 1 month: after 6 months=111:101:95:83	Blood	Maciejak A	2015	Prognosis
GSE62646	GPL 6244	On the 1st day: after 4–6 days: after 6 months=28:28:28	Blood	Kiliszek M	2014	Prognosis
GSE31568	GPL9040	N:AMI=454:454	Blood	Andreas Keller	2011	miRNA

Abbreviations: N, normal control; AMI, acute myocardial infarction.

Screening of Differentially Expressed miRNAs and mRNAs

After scale standardization of the data set, MetaMA and Limma packages were used for differential expression analysis. The p value was combined by the inverse normal method. The false discovery rate (FDR) was calculated by Benjamini hochberg threshold. Differentially expressed miRNA and mRNA was identified under the threshold value of FDR < 0.05 and |log FC (fold change)| >1, and p value <0.05, respectively.

Construction of the WGCNA Co-Expression Network

Data in the GSE66360 dataset were preprocessed and annotated using the annotation file of the microarray platform. The average value of multiple probes corresponding to the same mRNA was taken. After pretreatment, the mRNA expression matrix was obtained for scale standardization. The WGCNA package in R was utilized for analysis of the top 25% mRNAs in the normalized mRNA expression matrix file to identify meaningful modules and mRNAs. Since gene co-expression analysis is extremely sensitive to the presence of abnormal samples, strict quality control procedures are needed to ensure the highest quality levels. The average linkage method in WGCNA was used to cluster the samples. To ensure the scale-free network, the scale-free fitting index and average connectivity were calculated (the β value was set to 7). A one-step method was adopted to build the network that covers modules. Interaction between modules was analyzed, followed by drawing of heat map. In addition, modules were clustered and the correlation between modules was calculated to explore the common expression similarity of all modules. It is worth mentioning that the common mRNAs in both key module in the WGCNA analysis and differential expression analysis were considered as hub mRNAs.

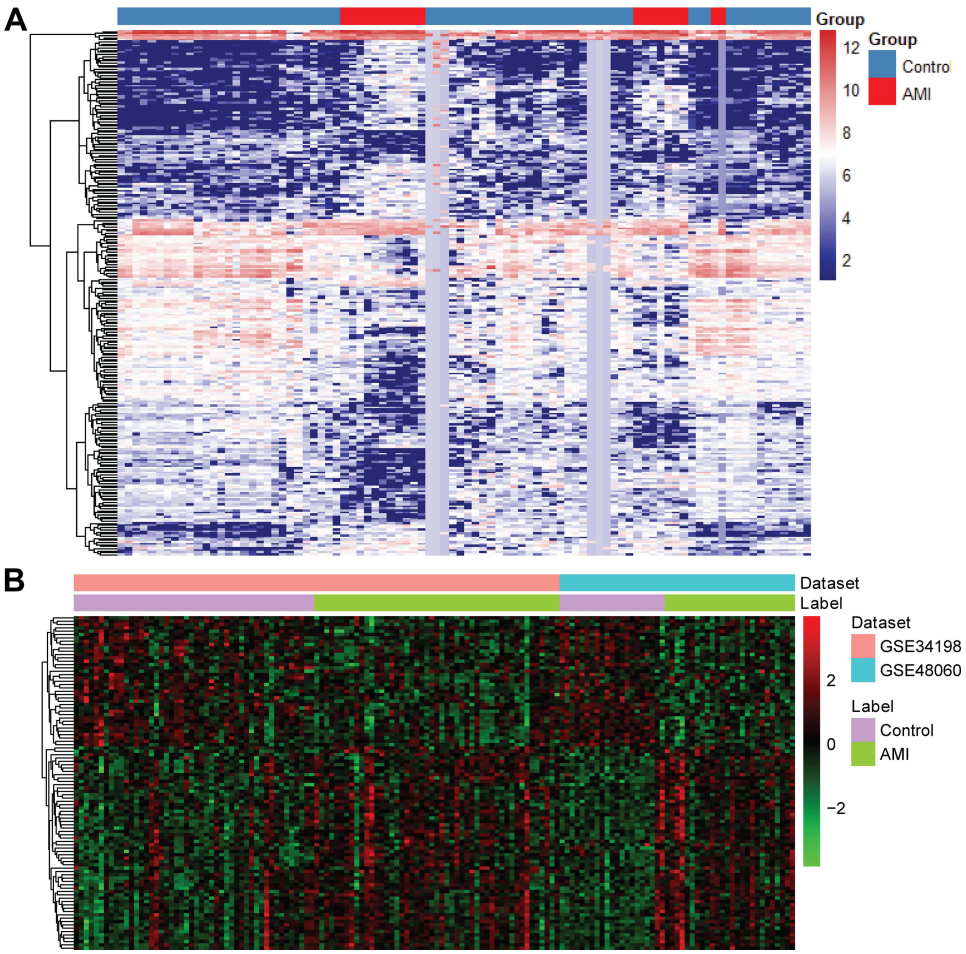

Figure 1 The heat map of all miRNAs (**A**) and top 100 mRNAs (**B**) in AMI.

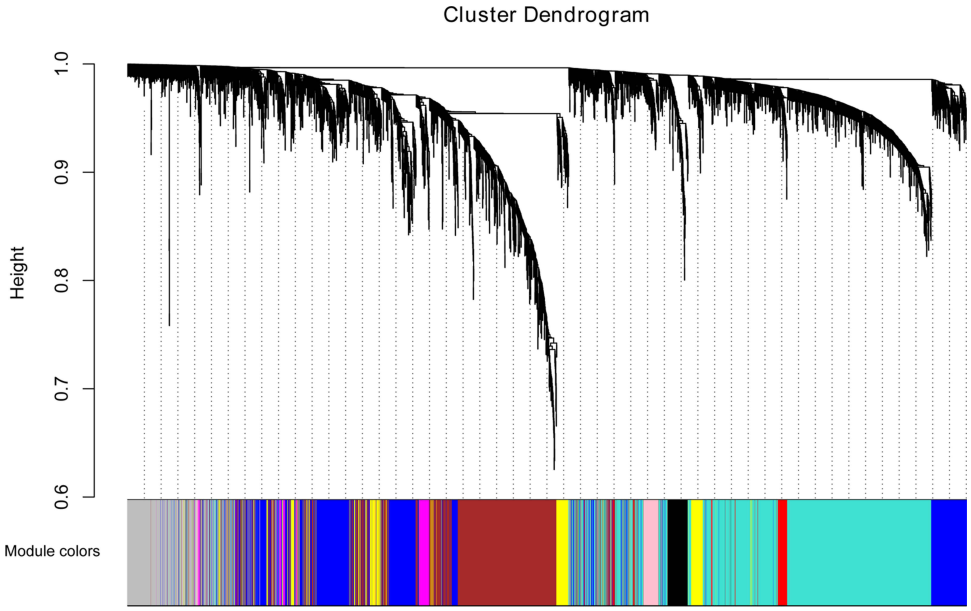

Figure 2 Modules identified by the WGCNA analysis. MRNAs in modules are marked with different colors.

Construction of miRNA–Inflammation and Oxidative Stress-Related mRNA Network

More and more evidence indicated that inflammation plays an important role in the pathophysiology of MI. Oxidative stress also plays a role in ventricular remodeling after MI. In order to investigate the relationship between inflammatory and oxidative stress and AMI, molecular characteristic database (1) and GeneCards (https://www.genecards.org) database (2) was respectively used to identify mRNAs associated with inflammation and oxidative stress among all differentially expressed mRNAs. MiRWalk (http://mirwalk.umm.uni-heidelberg.de/interactions/) was used to construct the negatively regulatory network between miRNAs and mRNAs associated with inflammation and oxidative stress.

PPI Analysis and Functional Enrichment of Hub mRNAs

To investigate the biological function of hub mRNAs, string database (https://string-db.org/) was firstly used to construct the PPI network, which was visualized by Cytoscape 3.6.1. In addition, DAVID 6.8 dataset (https://david.ncifcrf.gov/) was applied for functional analysis, including Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG). P value <0.05 was considered as statistical significance.

In vitro Validation of Inflammation and Oxidative Stress-Related mRNAs

In vitro validation QRT-PCR was performed. The inclusion criteria of AMI patients was as follows: 1) time of chest pain or distress >30 min within 24 hours and levels of cardiac enzymes creatine phosphokinase-MB type (CK-MB) and cardiac troponin T (cTnT) were higher than normal range; 2) the patient had AMI for the first time; 3) the patient did not receive medication or surgery prior to admission; 4) patient had blood samples at three time points, including before hospitalization, at discharge, and 6 months after AMI; 5) patient had complete clinical data. The exclusion criteria of AMI patients were as follows: 1) patient had myocarditis and other diseases caused by chest pain or distress; 2) patient with a history of kidney failure, malignant tumors, advanced liver disease, and other inflammatory diseases (psoriasis and rheumatoid arthritis); 3) recurrent patient; 4) patients with incomplete clinical data; 5) patients with missing blood samples at three time points of before hospitalization, at discharge, and 6 months after AMI. According to the above criteria, nine AMI patients and nine normal controls were enrolled. Blood samples from these patients were collected. All participating individuals provided informed

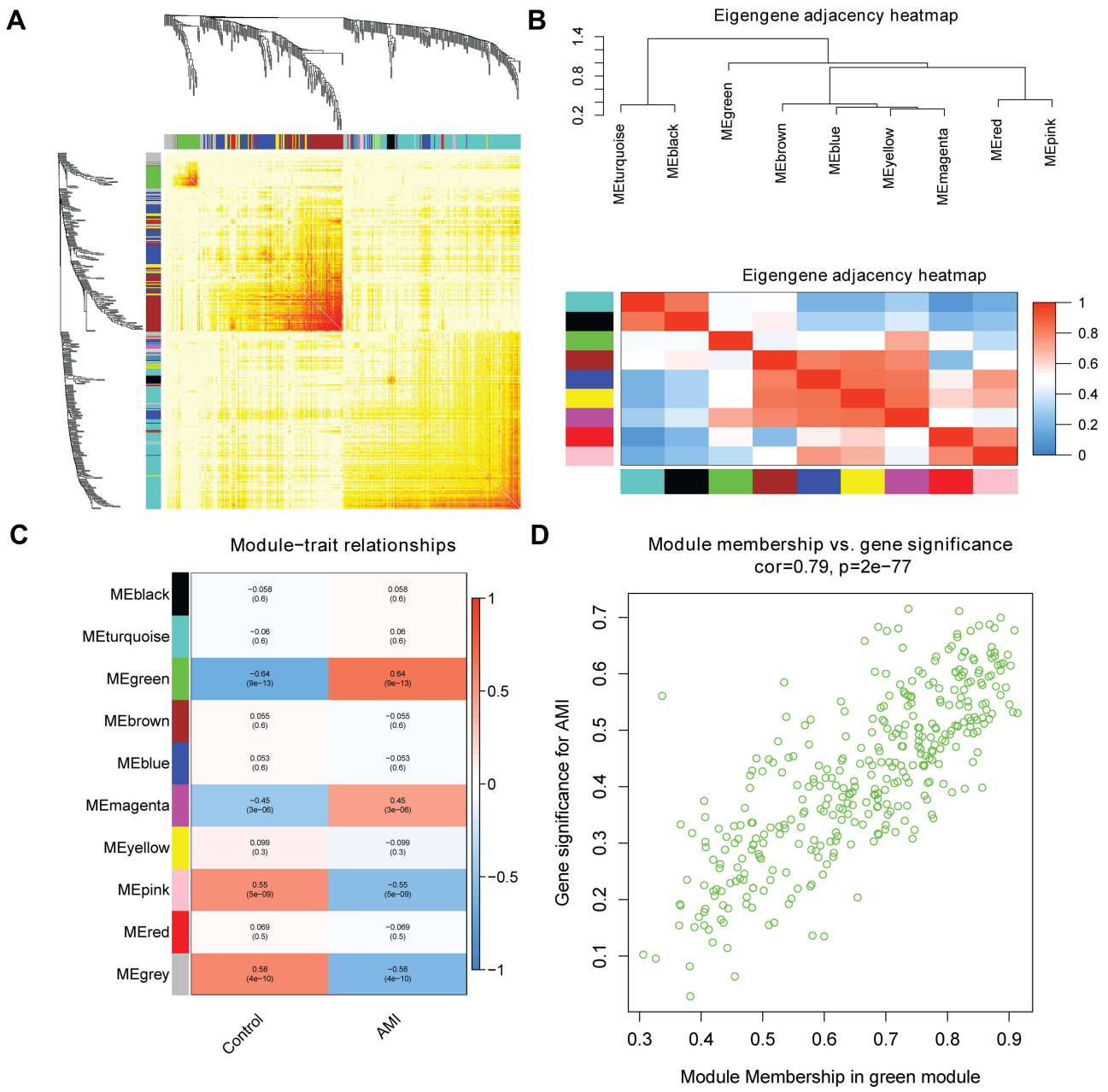

Figure 3 (**A**) The heat map of the first 400 mRNA in nine modules. The horizontal and vertical axes of different colors represent different modules. The brightness of yellow in the middle represents the connectivity of different modules. (**B**) Correlation between modules. (**C**) Heat map of the relationship between modules and AMI incidence. In each unit, the upper number and lower number refers to the correlation coefficient of each module and the corresponding p value, respectively. (**D**) Scatter plot of modular feature mRNAs in green module.

consent with the approval of the ethics committee of the Seventh People's Hospital of Jinan. In addition, this study was conducted in accordance with the Declaration of Helsinki.

Total RNA of the blood sample was extracted and synthesized DNA by FastQuant cDNA first strand synthesis kit. Real-time PCR was performed in the SuperReal PreMix Plus (SYBR Green analysis method). Relative mRNAs expression was analyzed by log2 (fold change) method and represented as fold change. FC > 1 and FC < 1 represented up-regulation and down-regulation, respectively.

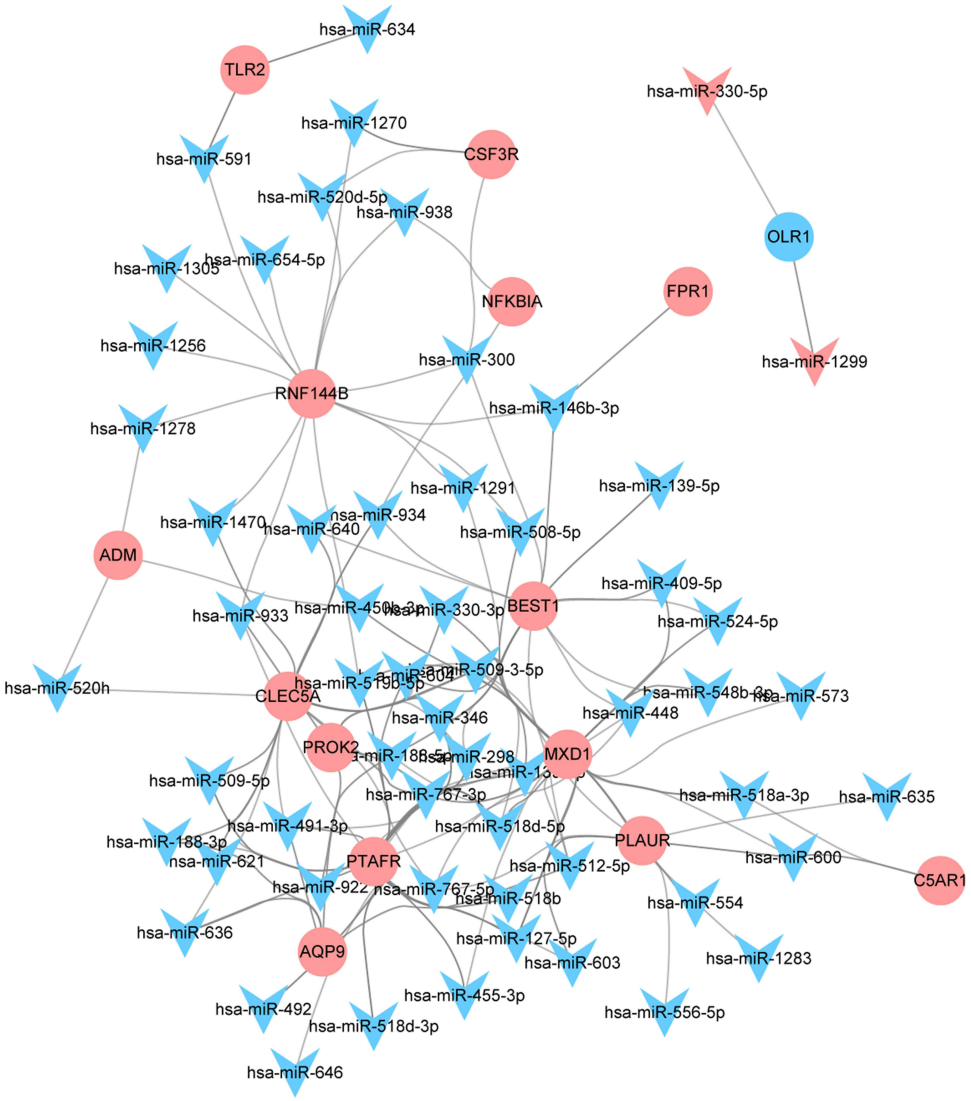

Figure 4 A total of 214 pairs of negatively regulatory network of miRNA-inflammation-related mRNA. Triangle and circle represents miRNA and mRNA, respectively.

Results

Identification of Differentially Expressed miRNAs and mRNAs

A total of 250 differentially expressed (103 up-regulated and 147 down-regulated) miRNAs were identified in AMI. In addition, a total of 1981 differentially expressed (999 up-regulated and 982 down-regulated) mRNAs were identified in AMI. The heat map of all differentially expressed miRNAs and top 100 differentially expressed mRNAs is shown in Figure 1A and B, respectively.

WGCNA Co-Expression Network Analysis

Based on the WGCNA co-expression network analysis, a total of nine modules were identified (Figure 2). Interaction analysis between nine modules showed that each module had high independence and relative independence of mRNA expression. The heat map of the first 400 mRNA in nine modules is shown in Figure 3A. These modules were also clustered and the correlation between modules was calculated to explore the common expression similarity of all modules (Figure 3B). Compared with other modules, green module (involving 357 mRNAs) was found to be highly correlated with disease status, suggesting that mRNAs in green module may play a key role in

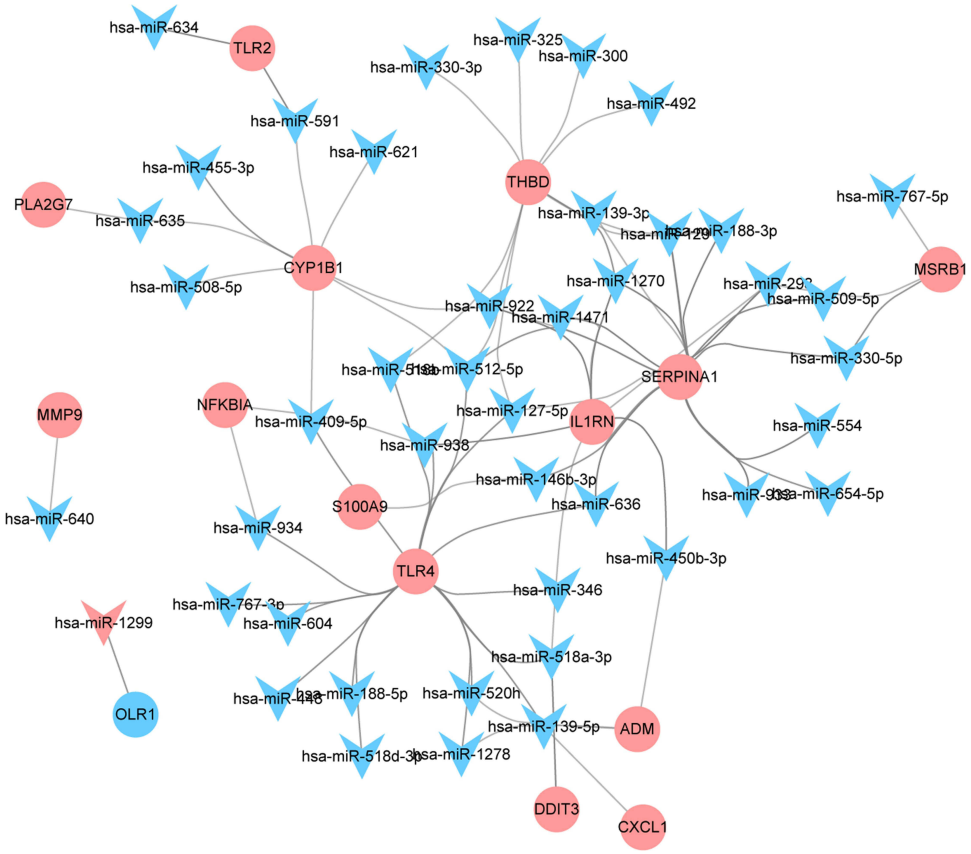

Figure 5 A total of 155 pairs of negatively regulatory network of miRNA-oxidative stress-related mRNA. Triangle and circle represents miRNA and mRNA, respectively.

the occurrence and development of AMI (Figure 3C). The relationship between the number of module membership and AMI in the green module is shown in Figure 3D. It is noted that there were 116 common mRNAs (hub mRNAs) between all 1981 differentially expressed mRNAs and 357 mRNAs in the green module, including 104 up-regulated and 12 down-regulated mRNAs in AMI.

Network of miRNA-Inflammation and Oxidative Stress Related mRNA

A total of 200 mRNAs were associated with inflammatory response in the molecular characteristic database. Among which, 16 common mRNAs were identified between 200 inflammation-related mRNAs and 116 hub mRNAs, such as NFKB inhibitor alpha (NFKB1A, up-regulation), adrenomedullin (ADM, up-regulation) and Toll-like receptor 2 (TLR2, up-regulation). MiRWalk analysis showed that a total of 11,427 miRNAs targeted the above 16 common mRNAs. Among which, 372 miRNAs were differentially expressed miRNAs in AMI. By analyzing the negative regulation of 372 miRNA and target mRNAs, 214 pairs of negatively regulatory network of miRNA-inflammation related mRNA were obtained, including 3 pairs of up-regulated miRNA-down-regulated mRNA and 211 pairs of down-regulated miRNA-up-regulated mRNA (Figure 4), such as down-regulated hsa-miR-636/hsa-miR-491-3p/hsa-miR-188-5p/hsa-miR-188-3p-up-regulated aquaporin 9 (AQP9), down-regulated hsa-miR-518a-3p-up-regulated complement C5a receptor 1 (C5AR1) and down-regulated hsa-miR-509-3-5p/hsa-miR-127-5p-up-regulated plasminogen activator, urokinase receptor (PLAUR). In addition, a total of 835 mRNAs were associated with oxidative stress in the GeneCards database. Among which, 16 common mRNAs were identified between 835 oxidative stress-related mRNAs and 116 hub mRNAs, such as NFKB1A, ADM and TLR2. MiRWalk analysis showed that a total of 9576 miRNAs targeted the above 16 common mRNAs. Among which, 284 miRNAs were differentially expressed miRNAs in AMI. By analyzing the negative regulation of 284 miRNA and target mRNAs, 155 pairs of negatively regulatory network of miRNA-oxidative stress-related mRNA were obtained, including 2 pairs of up-regulated

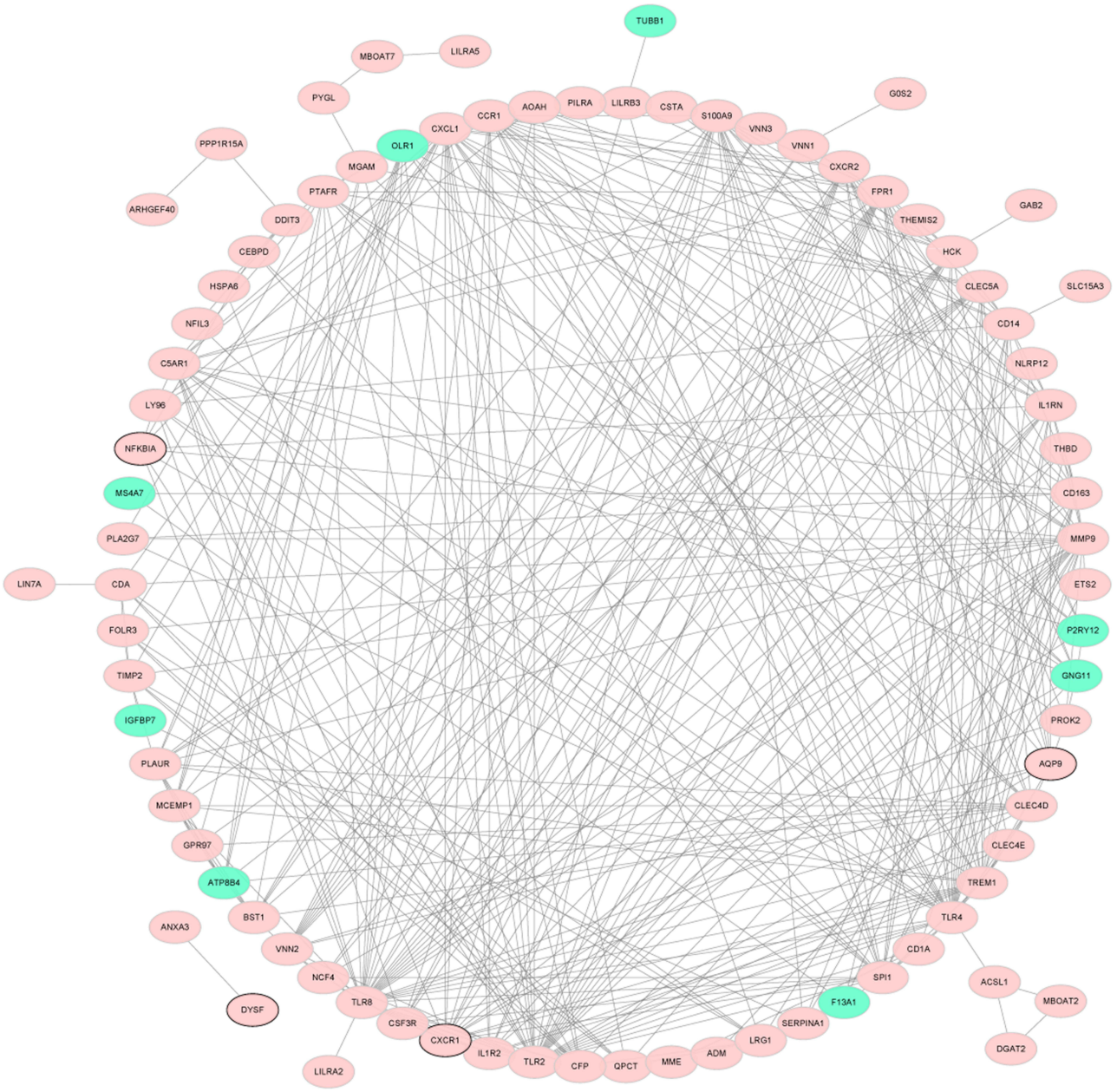

Figure 6 PPI network of 116 hub mRNAs. Red and green represents up-regulation and down-regulation, respectively. The mRNA with black borders represents top 10 up-regulation/down-regulation mRNA.

miRNA-down-regulated mRNA and 153 pairs of down-regulated miRNA-up-regulated mRNA (Figure 5), such as down-regulated hsa-miR-604-up-regulated Toll-like receptor 4 (TLR4) and down-regulated hsa-miR-139-5p-up-regulated C-X-C motif chemokine ligand 1 (CXCL1). It is worth mentioning that three pairs of negatively regulatory network of miRNA-inflammation and oxidative stress-related mRNA were obtained, including down-regulated hsa-miR-634/hsa-miR-591-up-regulated TLR2, down-regulated hsa-miR-938-up-regulated NFKBIA and down-regulated hsa-miR-520h/hsa-miR-450b-3p-up-regulated ADM.

PPI Analysis of Hub mRNAs

In order to investigate the interactions between 116 hub mRNAs, string database was used for construction of the PPI network. In the PPI network, there were 82 mRNAs (Figure 6). Some mRNAs with a high degree were

Table 2 Top 15 mRNAs with High Degree in the PPI Network in AMI

Name	ID	Combined ES	FDR	P value	Up/Down	Betweenness centrality	Closeness centrality	Degree
TLR8	51,311	0.373712566	0.345433	0.026313	Up	0.11528995	0.56834532	35
TLR2	7097	0.510199389	0.189571	0.002759	Up	0.08870119	0.56028369	33
TLR4	7099	0.445730841	0.240797	0.007922	Up	0.13604332	0.55633803	31
MMP9	4318	0.453781121	0.231706	0.00712	Up	0.10475912	0.51633987	28
CXCL1	2919	0.368494613	0.353592	0.027923	Up	0.06185563	0.5	24
FPR1	2357	0.521753016	0.179982	0.002295	Up	0.05211081	0.51973684	24
S100A9	6280	0.384638331	0.366569	0.030806	Up	0.05608539	0.51633987	23
TREM1	54,210	0.521228532	0.176172	0.001991	Up	0.01562858	0.5	23
SPI1	6688	0.442038996	0.249292	0.008873	Up	0.09324947	0.51298701	22
CCR1	1230	0.357902945	0.382853	0.034216	Up	0.01325911	0.48170732	20
CXCR1	3577	0.678452576	0.07628	6.87E-05	Up	0.00924253	0.4702381	18
IL1RN	3557	0.406991037	0.315708	0.019742	Up	0.01958485	0.45930233	18
HCK	3055	0.529607428	0.176968	0.002075	Up	0.05530631	0.46745562	18
CXCR2	3579	0.515525816	0.17894	0.002227	Up	0.00996122	0.45930233	17
CD14	929	0.408208618	0.303203	0.016625	Up	0.03612691	0.4702381	17

Abbreviations: ES, effect size; FDR, false discovery rate.

Table 3 Forty mRNAs Associated with Prognosis of AMI

ID	Name	Combined ES	FDR	P value	Up/Down
133	ADM	0.531012	0.17894	0.002262	Up
366	AQP9	0.701988	0.07628	4.64E-05	Up
683	BST1	0.528275	0.168898	0.001801	Up
728	C5AR1	0.464219	0.228003	0.005814	Up
978	CDA	0.594716	0.132653	4.54E-04	Up
1052	CEBPD	0.618119	0.109382	2.79E-04	Up
1441	CSF3R	0.494285	0.194173	0.003416	Up
1475	CSTA	0.417683	0.278341	0.012709	Up
2114	ETS2	0.451763	0.231706	0.007178	Up
2180	ACSL1	0.500418	0.194173	0.003344	Up
2352	FOLR3	0.39883	0.312914	0.019077	Up
2357	FPR1	0.521753	0.179982	0.002295	Up
2919	CXCL1	0.368495	0.353592	0.027923	Up
3055	HCK	0.529607	0.176968	0.002075	Up
3557	IL1RN	0.406991	0.315708	0.019742	Up
4689	NCF4	0.588623	0.139011	6.14E-04	Up
4783	NFIL3	0.513935	0.186062	0.002588	Up
4792	NFKBIA	0.680825	0.07628	6.49E-05	Up
5199	CFP	0.402163	0.302769	0.016441	Up
5265	SERPINA1	0.585844	0.139011	6.10E-04	Up
5329	PLAUR	0.648733	0.084117	1.46E-04	Up
5724	PTAFR	0.473841	0.220923	0.005147	Up
6280	S100A9	0.384638	0.366569	0.030806	Up
6688	SPI1	0.442039	0.249292	0.008873	Up
7056	THBD	0.463587	0.228003	0.005879	Up
7097	TLR2	0.510199	0.189571	0.002759	Up
7099	TLR4	0.445731	0.240797	0.007922	Up
8291	DYSF	0.685069	0.07628	6.85E-05	Up
8825	LIN7A	0.347027	0.398241	0.040136	Up
9332	CD163	0.433802	0.252755	0.009711	Up

(*Continued*)

Table 3 (Continued).

ID	Name	Combined ES	FDR	P value	Up/Down
23,645	PPP1R15A	0.558511	0.149415	9.53E-04	Up
25,797	QPCT	0.509773	0.186062	0.002574	Up
54,210	TREM1	0.521229	0.176172	0.001991	Up
55,701	ARHGEF40	0.376874	0.336257	0.024589	Up
79,143	MBOAT7	0.37571	0.336257	0.024769	Up
84,649	DGAT2	0.507738	0.194173	0.003101	Up
91,662	NLRP12	0.413637	0.291113	0.01387	Up
116,844	LRG1	0.516126	0.17894	0.002256	Up
199,675	MCEMP1	0.535062	0.166305	0.00165	Up
353,514	LILRA5	0.435528	0.267282	0.010967	Up

Abbreviations: ES, effect size; FDR, false discovery rate.

Table 4 Enriched Seven Signaling Pathways of 116 Hub mRNAs in AMI

Term	P value	mRNAs
Toll-like receptor signaling pathway	0.001644	LY96, TLR2, NFKBIA, TLR4, TLR8, CD14
Complement and coagulation cascades	0.002801	C5AR1, THBD, F13A1, SERPINA1, PLAUR
Chemokine signaling pathway	0.005028	CXCL1, CCR1, HCK, NFKBIA, CXCR1, GNG11, CXCR2
Hematopoietic cell lineage	0.006186	IL1R2, CSF3R, MME, CD1A, CD14
Pathogenic *Escherichia coli* infection	0.012779	LY96, TLR4, TUBB1, CD14
Epithelial cell signaling in *Helicobacter pylori* infection	0.020496	CXCL1, NFKBIA, CXCR1, CXCR2
Cytokine–cytokine receptor interaction	0.043822	CXCL1, IL1R2, CCR1, CXCR1, CSF3R, CXCR2

identified, including TLR2, TLR4 and CXCL1. Top 15 mRNAs with a high degree are listed in Table 2. After further screening of the above 82 mRNAs in datasets of GSE123342, GSE59867 and GSE62646, a total of 40 mRNAs were associated with prognosis of AMI, such as inflammation/oxidative stress-related mRNAs of AQP9, TLR2, TLR4, CXCL1, C5AR1, PLAUR, NFKBIA and ADM (Table 3). The expression box diagram of AQP9, TLR2, TLR4, CXCL1, C5AR1, PLAUR, NFKBIA and ADM in the GSE59867, GSE62646 and GSE123342 datasets is shown in Figure 7. It can be seen that these mRNAs were up-regulated in AMI, while down-regulated 4–6 days, 30 days, 1 month, 6 months and 1 year after AMI. It is indicated that these mRNAs could not only be used as biomarkers to detect AMI incidence but also as prognostic indicators of AMI patients.

Functional Enrichment of Hub mRNAs

To further study the biological function of 116 hub mRNAs, DAVID 6.8 dataset was used for functional analysis. According to the KEGG analysis, these 116 hub mRNAs were involved in only 7 signaling pathways (Figure 8, Table 4). Among which, inflammation-related C5AR1 and PLAUR were involved in complement and coagulation cascades; oxidative stress-related TLR4 and CXCL1 was respectively involved in pathogenic *Escherichia coli* infection, and chemokine signaling pathway and cytokine–cytokine receptor interaction; inflammation and oxidative stress-related TLR2 and NFKBIA were involved in Toll-like receptor signaling pathway.

In vitro Validation of Inflammation, Oxidative Stress and Prognosis-Related mRNAs

To validate the expression of inflammation, oxidative stress and prognosis-related mRNAs (AQP9, TLR2, TLR4, CXCL1, C5AR1 and PLAUR), blood samples from nine AMI patients and nine normal controls were collected for QRT-PCR (Figure 9). The clinical information of these individuals is listed in Table 5. The QRT-PCR result showed that AQP9, TLR2 and TLR4 were significantly up-regulated in AMI. The expression trend of

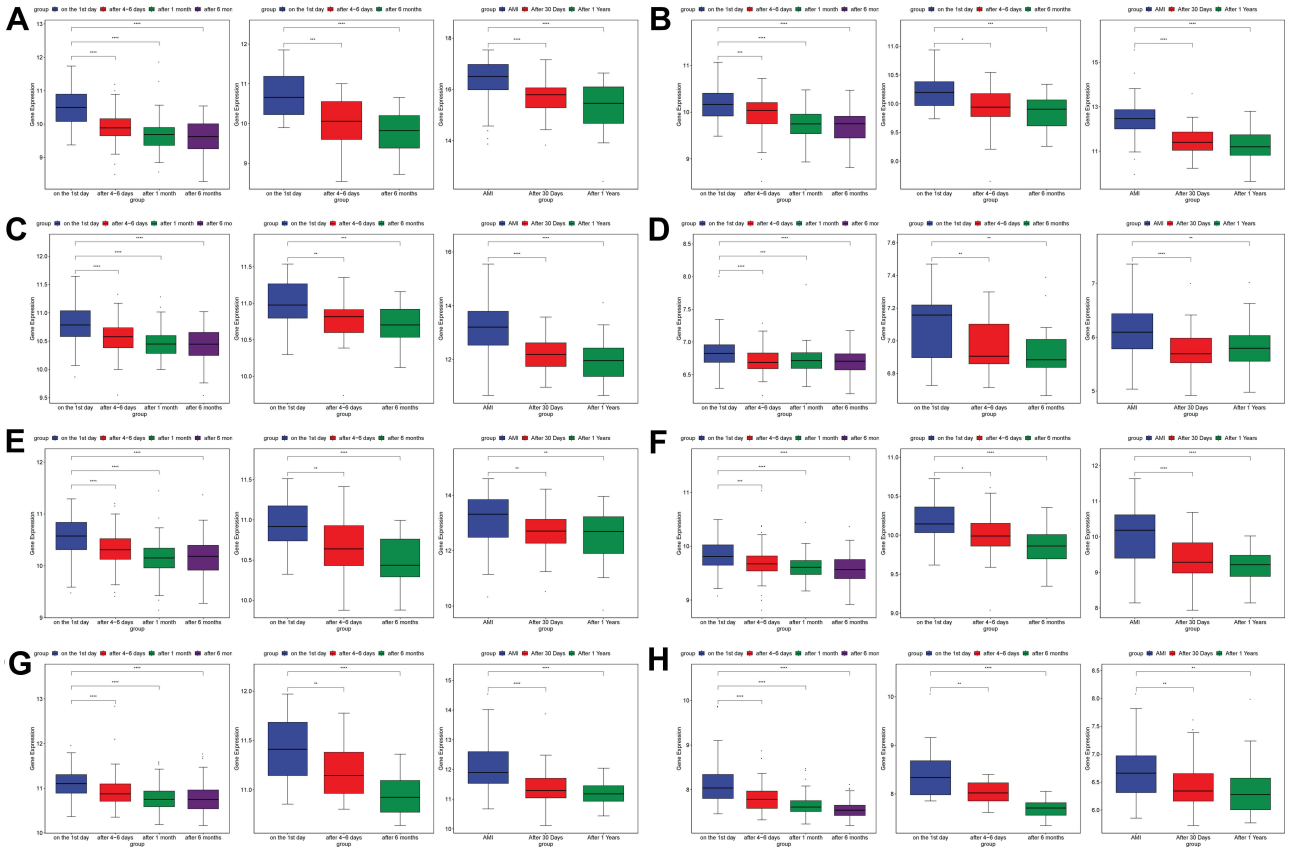

Figure 7 The expression box diagram of AQP9 (**A**), TLR2 (**B**), TLR4 (**C**), CXCL1 (**D**), C5AR1 (**E**), PLAUR (**F**), NFKBIA (**G**) and ADM (**H**) in the GSE59867, GSE62646 and GSE123342 datasets. *p value < 0.05; **p value < 0.01; ***p value < 0.001; ****p value < 0.0001.

CXCL1, C5AR1 and PLAUR was up-regulated without statistical significance. Larger numbers of samples are further needed.

Discussion

Hsa-miR-636 is down-regulated and has a potential diagnostic value in the plasma of AMI patients.[19] Hsa-miR-491-3p is involved in regulation of cardiac regeneration after AMI.[20,21] Hsa-miR-188-5p is down-regulated in hyperhomocysteinemia cardiomyocytes.[22,23] The expression levels of hsa-miR-188-3p are decreased upon MI.[24] Hsa-miR-518a-3p is down-regulated in the development of coronary artery disease.[25] Hsa-miR-509-3-5p is involved in human atrial aging.[26] The expression levels of hsa-miR-127-5p are associated with cardiac score and cardiac function in AMI patients.[27] In addition, increased expression levels of AQP9 are related to atherosclerotic lesions.[28] AQP9 is up-regulated in AMI.[29] AQP9 is associated with recovery from MI.[30] Huang et al found that silencing of the AQP9 gene improved cardiac function following MI.[31] C5AR1 is involved in complement and coagulation cascades in AMI.[32] PLAUR, a conserved gene, is up-regulated in AMI.[32,33] In this study, we found regulatory networks of miRNA–inflammation and prognosis-related mRNAs (hsa-miR-636/hsa-miR-491-3p/hsa-miR-188-5p/hsa-miR-188-3p-AQP9, hsa-miR-518a-3p-C5AR1 and hsa-miR-509-3-5p/hsa-miR-127-5p-PLAUR) in AMI, which suggested that these molecules may play an important role in myocardial function of AMI. In addition, inflammation-related AQP9, C5AR1 and PLAUR can be considered as potential diagnostic and prognostic biomarkers for AMI.

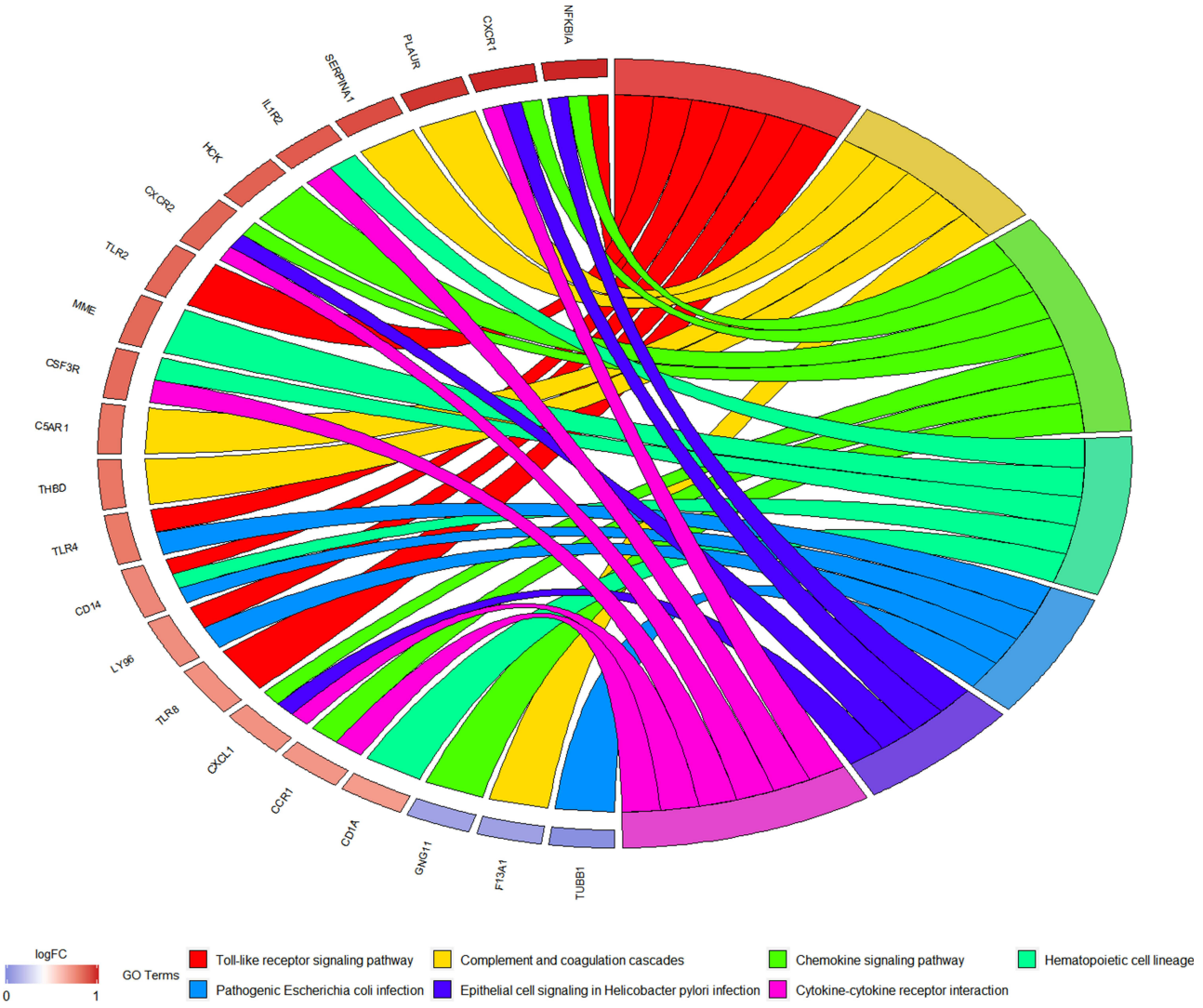

Figure 8 KEGG analysis of 116 hub mRNAs in AMI.

Hsa-miR-604 is over expressed in the serum of paracoccidioidomycosis patients.[34] Hsa-miR-139-5p is affected by ischemic pre- or post-conditioning in the heart.[35] Yuan et al found that hsa-miR-139-5p was a potential biomarker of atherosclerosis.[36] The expression levels of hsa-miR-139-5p are decreased earlier, within just 7 days following MI.[37] In AMI, TLR4 mediates the synthesis of cytokines in circulating monocytes and is associated with the bad disease outcome.[38–40] Plasma levels of CXCL1 are significantly increased in patients with AMI.[41] In the present study, we found negatively regulatory networks of miRNA–oxidative stress and prognosis-related mRNAs (hsa-miR-604-TLR4 and hsa-miR-139-5p-CXCL1) in AMI. This indicated that these miRNAs and targets play a crucial role in angiogenesis of AMI. Oxidative stress-related TLR4 and CXCL1 may be regarded as potential prognostic biomarkers for AMI.

Hsa-miR-634 is up-regulated in atrial fibrillation and coronary artery disease.[13,42] In patients with idiopathic dilated cardiomyopathy, hsa-miR-591 is used to predict time-dependent heart remodeling.[43] The expression of hsa-miR-520h is found in human heart disease.[44] Hsa-miR-450b-3p is regulated by ischemia.[45] TLR2 has been reported as a critical regulator in AMI and identified as a potential biomarker for AMI detection.[46–48] Blocking NFKBIA-mediated nuclear factor of kappa B (NF-κB) signaling pathway protects against MI.[49] In patients with AMI, high

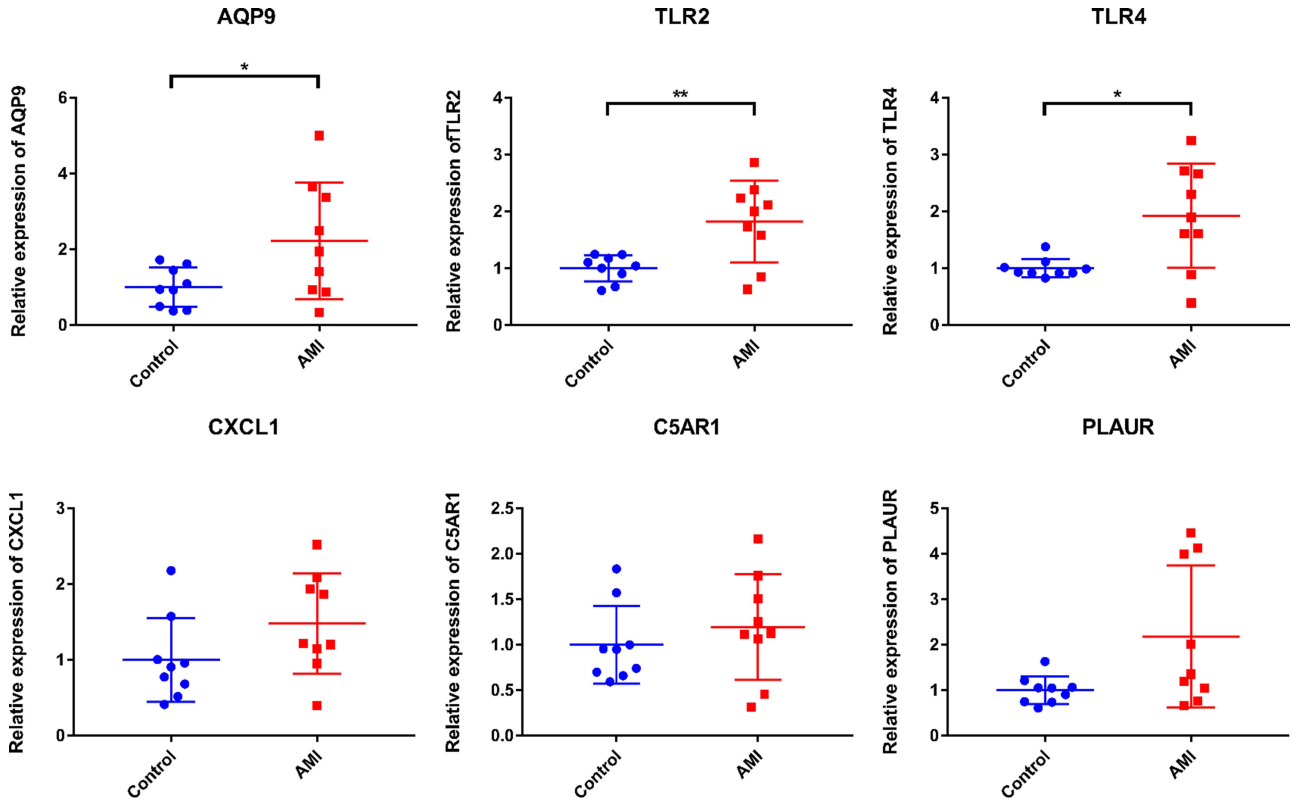

Figure 9 In vitro validation of inflammation and oxidative stress-related AQP9, TLR2, TLR4, CXCL1, C5AR1 and PLAUR. *p value < 0.05; **p value < 0.01.

ADM levels are related to impaired left ventricular function and death.[50–52] Herein, we found that inflammation and oxidative related TLR2, NFKBIA and ADM could be involved in the pathological process of AMI under the regulation of miRNAs.

The above inflammation/oxidative stress and prognosis-related mRNAs were not only involved in miRNA regulatory network but also some signaling pathways. For example, inflammation-related C5AR1 and PLAUR were involved in complement and coagulation cascades; oxidative stress-related TLR4 and CXCL1 was respectively involved in pathogenic E*scherichia coli* infection, and chemokine signaling pathway and cytokine–cytokine receptor interaction; inflammation and oxidative stress-related TLR2 and NFKBIA were involved in Toll-like receptor signaling pathway. In blood circulation, the complement and coagulation cascades, evolutionarily related enzymatic cascades, are involved in post-MI responses.[53] Gram-negative *Escherichia coli* has an activating or aggregating effect on platelets.[54,55] In the infarcted myocardium, production of chemokines provides exit cues for circulating leukocytes expressing cognate receptors.[56–58] Cytokine-cytokine receptor interaction is involved in stable coronary artery disease.[59] In the infarcted heart, Toll-like receptor signaling pathway prompts NF-κB triggering and the up-regulation of cytokine.[60]

Conclusion

Our study found several negatively regulatory networks of miRNA–inflammation/oxidative stress and prognosis-related mRNAs, including hsa-miR-636/hsa-miR-491-3p/hsa-miR-188-5p/hsa-miR-188-3p-AQP9, hsa-miR-518a-3p-C5AR1, hsa-miR-509-3-5p/hsa-miR-127-5p-PLAUR, hsa-miR-604-TLR4, hsa-miR-139-5p-CXCL1, hsa-miR-634/hsa-miR-591-TLR2, hsa-miR-938-NFKBIA and hsa-miR-520h/hsa-miR-450b-3p-ADM) in AMI. In addition, some signaling pathways were also identified. Our study may provide a new field in understanding pathological mechanisms of AMI of the inflammation and oxidative stress levels. Nonetheless, our study has some limitations.

Table 5 Clinical Information of Enrolled Individuals in QRT-PCR

Group	Gender	Age (years)	Height (m)	Weight (kg)	BMI (kg/m^2)	Hypertension History	Diabetes History	Smoking History	Drinking History	LDL (mmol/L)	HDL (mmol/L)	CHO (mmol/L)	TG (mmol/L)	Atherosclerosis History	CTnT (ng/mL)	CK-MB	ST segment elevation
Control	Male	73	1.7	81	28.02768166	Yes	No	No	No	2.04	1.02	3.29	2.14	No	NA	NA	NA
	Male	58	1.71	71	24.28097534	No	Yes	No	No	No	1.18	3.47	1.39	No	NA	NA	NA
	Male	57	1.78	71	22.40878677	Yes	No	Yes	No	2.24	0.89	3.81	2.25	No	NA	NA	NA
	Male	57	1.75	81	26.44897959	No	No	No	Yes	3.09	0.89	3.81	1.34	No	NA	NA	NA
	Male	60	1.61	61	23.53304271	No	No	No	No	2.51	1.14	4.35	1.89	No	NA	NA	NA
	Female	56	1.60	62	24.21875	No	No	No	No	2.39	0.95	3.86	1.07	No	NA	NA	NA
	Female	62	1.58	61	24.43518667	Yes	No	No	Yes	1.06	1.21	2.79	1.54	No	NA	NA	NA
	Female	54	1.71	61	20.86111966	No	No	No	No	1.59	1.12	3.51	1.24	No	NA	NA	NA
	Female	68	1.73	81	27.06405159	No	No	No	No	2.67	1.02	4.09	1.16	No	NA	NA	NA
AMI	Male	57	1.68	80	28	No	Yes	No	No	2.44	0.62	3.99	2.04	No	0.187	10.3U/L	Yes
	Male	60	1.7	72	25	Yes	No	Yes	Yes	2.18	1.08	3.87	1.34	No	23	>80ng/mL	Yes
	Male	57	1.75	70	23	Yes	No	Yes	2	2.16	0.71	3.80	2.05	No	2.84	16.8U/L	Yes
	Male	55	1.74	90	30	No	No	No	Yes	3.49	0.87	4.88	1.14	No	0.410	21.3U/L	Yes
	Female	62	160	62	32	No	No	No	No	3.55	0.94	5.34	1.86	No	22.77	30.99ng/mL	Yes
	Female	69	158	63	25	Yes	No	No	No	1.39	1.15	2.86	0.7	No	0.28	23.1ng/mL	No
	Female	73	156	60	24	Yes	Yes	No	No	1.26	1.24	2.75	0.55	No	0.395	7.8U/L	No
	Male	56	172	62	21	No	No	Yes	Yes	2.59	1.16	4.31	1.24	No	0.893	78.8U/L	No
	Male	49	170	80	27	No	Yes	Yes	Yes	2.68	0.97	5.09	3.16	No	0.468	36.8U/L	Yes

Abbreviations: BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; CHO, cholesterol; TG, triglyceride; CTnT, cardiac troponin T; CK-MB, cardiac enzymes creatine phosphokinase-MB type; NA, not applicable.

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In vitro validation of miRNA-mRNA regulatory network is needed. In addition, in vivo animal model is further needed to investigate the potential biological function of identified miRNAs and mRNAs.

Disclosure

The authors report no conflicts of interest in this work.

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