

Genes associated with inflammation and the cell cycle may serve as biomarkers for the diagnosis and prognosis of acute myocardial infarction in a Chinese population

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Abstract. The present study aimed to identify biomarkers for the clinical diagnosis of acute myocardial infarction (AMI) in a Chinese population using microarray data collected from the Gene Expression Omnibus database under accession number GSE97320. This included the peripheral blood samples of three patients with AMI and three controls. Differentially expressed genes (DEGs) were identified using the limma package and protein-protein interaction networks were constructed using data from the Search Tool for the Retrieval of Interacting Genes database, followed by module analysis to screen for hub genes. Functional enrichment analyses were performed using the Database for Annotation, Visualization and Integrated Discovery. The identified genes were verified by overlapping with the target genes of microRNAs (miRs) known to be associated with AMI, as well as the DEGs identified in other AMI datasets, including GSE24519, GSE34198 and GSE48060. As a result, 752 DEGs (449 upregulated and 303 downregulated) were identified in the GSE97320 dataset. The upregulated DEGs were predicted to participate in inflammatory pathways, including the toll-like receptor (TLR) signaling pathway, including ras-related C3 botulinum toxin substrate 1 (RAC1), TLR4, C-C motif chemokine receptor (CCR)1; cytokine-cytokine receptor interaction, including signal transducer and activator of transcription (STAT)3; chemokine signaling pathway, including CCR10; pathways associated with cancer, including colony stimulating factor 3 receptor (CSF3R); and leukocyte transendothelial migration, including matrix metalloproteinase 9 (MMP9). The downregulated DEGs were associated with the cell cycle, including alstrom syndrome protein 1 (ALMS1). These conclusions were made following functional analysis of the genes in the three identified modules. MMP9, TLR4, STAT3, CCR1 and ALMS1

were regulated by miR-21-5p, whereas RAC1 was regulated by miR-30c-5p. A comparison among the four datasets confirmed the roles of CSF3R and CCR10. HtrA serine peptidase 1 (HTRA1) was the only gene associated with both mortality and recurrence. In conclusion, inflammation-associated genes, including STAT3, CCR1, RAC1, MMP9, CCR10, CSF3R and HTRA1, as well as cell cycle-associated genes such as ALMS1, may be biomarkers for the diagnosis and prognosis of AMI in Chinese people.

Introduction

Acute myocardial infarction (AMI) is the most common and serious cardiovascular disease that can induce rapid heart failure and sudden cardiac mortality. Although a declining trend of AMI has been observed due to economic development, its incidence remains high at approximately 44.57 per 100,000 individuals in 2013 (1). In addition, the mortality rate in China was estimated to increase from 1987 to 2014 by 5.6-fold (2), particularly amongst young females (3). Thus, the development of strategies for the early diagnosis of AMI to prevent the occurrence of sudden mortality is increasingly important.

Other than ischemic symptoms, physical examination and electrocardiogram changes, cardiac troponins are the most commonly used biomarker for the clinical diagnosis of AMI (4,5). However, their sensitivity and specificity may be limited, as increased cardiac troponin levels are also observed in patients with non-acute coronary syndrome (6-8). Therefore, research has attempted to develop more sensitive and specific or combined type biomarkers for AMI. McMahon *et al* (9) evaluated the diagnostic efficacy of heart-type fatty acid-binding protein (H-FABP), cardiac troponin I (cTnI), creatine kinase-MB and myoglobin for the early detection of AMI and demonstrated that H-FABP has the greatest sensitivity at 0-3 h (64.3%) and 3-6 h (85.3%) following the onset of chest pain. The combination of cTnI and H-FABP measurements increases the levels of sensitivity to 71.4, 88.2, and 92.4% at 0-3, 3-6 and 6-12 h, respectively (9). Similarly, Pyati *et al* (10) indicated that H-FABP has additional diagnostic power for the early diagnosis of AMI, with its diagnostic efficiency markedly higher than that of CK-MB and myoglobin within the first 6 h of chest pain (10). Wang *et al* (11) used receiver operating characteristic curve analysis to reveal that the diagnostic accuracy of circulating monomeric C-reactive protein may be as

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high as 0.928 [95% confidence interval (CI), 0.887-0.969] for AMI (11), whereas Han *et al* (12) suggested that the plasma level of RING finger protein 207 may detect individuals with AMI with 90.5% sensitivity and 100% specificity (12). However, a limited number of genes were associated with the occurrence of AMI.

The aim of the current study was to further identify new biomarkers for the clinical diagnosis of AMI via an analysis of the gene-expression differences of peripheral blood samples between Chinese patients with AMI and healthy controls.

Materials and methods

AMI microarray data. Transcriptomics data were collected from the National Center of Biotechnology Information Gene Expression Omnibus database (NCBI GEO; www.ncbi.nlm.nih.gov/geo) under accession number GSE97320 (study not published). This database contained the peripheral blood samples of three patients with AMI (mean age, 53.0±13.1) and three healthy controls (mean age, 53.7±4.7). These participants were Han Chinese and were recruited from the Department of Cardiology at China-Japan Union Hospital, Jilin University of China (Changchun, China).

Data normalization and differentially expressed gene (DEG) identification. Raw Affymetrix Human Gene CEL files were preprocessed and normalized using the Robust Multichip Average algorithm (13) using the Bioconductor affy package (version 1.34.2; www.bioconductor.org/packages/release/bioc/html/affy.html) in R (version 3.4.1; <http://www.R-project.org/>) (14). The DEGs between patients with AMI and healthy controls were screened using the limma method (15) using the Bioconductor package in R (version 3.32.5; www.bioconductor.org/packages/release/bioc/html/limma.html). $P < 0.05$ and $|\log \text{ fold change (FC)}| > 1$ were set as the threshold values. A heatmap of the top 50 DEGs was constructed using the pheatmap package in R (Version 0.7.7; cran.r-project.org/web/packages/pheatmap/index.html).

Protein-protein interaction (PPI) network construction. To screen for the crucial genes associated with AMI, DEGs were mapped into the PPI data retrieved from the Search Tool for the Retrieval of Interacting Genes database (version 10.0; www.string-db.org) (16) to construct the PPI network; the cut-off value was set as a combined score of > 0.9 . The PPI network was visualized using Cytoscape 2.8 (www.cytoscape.org) (17). The hub genes with the highest degree were selected and plotted using the ggplot2 package in R (version 1.0.0; cran.r-project.org/web/packages/ggplot2/index.html). To identify functionally related and highly interconnected clusters within the PPI network, a module analysis was subsequently performed using the Molecular Complex Detection (MCODE) plugin of Cytoscape with the following parameters: Degree cutoff, 6; node score cutoff, 0.2; k-core, 5; and maximum depth, 100 (version 2.2; www.baderlab.org/Software/MCODE) (18). Modules with MCODE scores of ≥ 5 and ≥ 6 nodes were considered significant.

microRNA (miRNA/miR) prediction. To further screen for the crucial genes associated with AMI, microRNAs

known to be associated with AMI were obtained from the mir2disease database (www.mir2disease.org) that documents 1,939 relationships between 349 human microRNAs and 163 human diseases based on a review of $> 3,273$ published papers (19). The target genes of the AMI-associated miRNAs were predicted using the miRWalk2 database (zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2) (20), which provides the largest collection of predicted and experimentally verified miR-target interactions using various miRNA databases. The predicted target genes were subsequently overlapped with the DEGs to obtain the miRNA-DEGs interaction relationships, which were used to construct the miRNA-DEG interaction network. These genes were visualized using Cytoscape 2.8 (www.cytoscape.org) (17).

Functional enrichment analysis. Kyoto Encyclopedia of Genes and genomes (KEGG) (21) pathway and Gene Ontology (GO) (22) enrichment analyses were performed to explore the underlying functions of all DEGs, genes in modules and miRNA-DEG networks using the Database for Annotation, Visualization and Integrated Discovery (DAVID) online tool (version 6.8; david.abcc.ncifcrf.gov). $P < 0.05$ was selected as the threshold for determining the significant enrichment for GO and KEGG analyses.

Validation analysis. AMI microarray data were also collected from other countries via the NCBI GEO database under accession number GSE24519 (study not published). This database includes the whole blood samples of 17 Italian patients with AMI and 2 healthy individuals. Two biological repeats were performed for each patient, resulting in 34 AMI and four control samples for analysis.

To investigate the relationship between the DEGs under investigation and various clinical characteristics, the microarray datasets of GSE34198 (23) and GSE48060 (24) were also downloaded from the NCBI GEO database. In the GSE34198 dataset (23), 97 peripheral whole blood samples were obtained, including 45 from 41 patients with AMI (four patients had two repeats) who were alive during the follow-up period, four from four patients with AMI who succumbed to the disease during the 6 month follow-up period, and 48 from 45 controls (three patients had two repeats) from the Czech Republic. In the GSE48060 dataset (24), 52 peripheral whole blood samples were available, including 21 controls and patients with AMI from the USA with ($n=5$) or without ($n=22$) any recurrent events over an 18-month follow-up period.

All of the validated datasets were preprocessed and the DEGs between the AMI and control groups as well as between the subgroups of patients with AMI who succumbed to the disease (recurrence) and those who lived (non-recurrence) were identified using the methods similar to those described above for the GSE97320 dataset. To visualize the shared DEGs between the validated datasets and the Chinese dataset GSE97320, a Venn diagram was generated using a web-based tool (bioinformatics.psb.ugent.be/webtools/Venn).

Results

Identification of the DEGs in GSE97320. Following data normalization, 752 genes were identified as DEGs between

the patients with AMI and healthy controls according to the thresholds of $P < 0.05$ and $|\log FC| > 1$, including 449 upregulated and 303 downregulated genes (Table I). Heatmap clustering analysis indicated that the identified DEGs easily distinguished patients with AMI from healthy controls (Fig. 1).

Functional enrichment analysis of DEGs. The potential functions of the DEGs were predicted using the online tool DAVID. It was demonstrated that 28 KEGG pathways were enriched in the upregulated DEGs, such as the nuclear factor (NF)- κ B signaling pathway, including toll-like receptor (TLR)4; the TLR signaling pathway, including ras-related C3 botulinum toxin substrate 1 (RAC1), TLR4 and C-C motif chemokine receptor 1 (CCR1); cytokine-cytokine receptor interaction, including RAC1, signal transducer and activator of transcription (STAT)3 and G protein subunit gamma 10 (GNG10), the hsa04062:chemokine signaling pathway, including CCR1, CCR10, and RAC1; pathways in cancer, including colony stimulating factor 3 receptor (CSF3R); and leukocyte trans-endothelial migration, including matrix metalloproteinase 9 (MMP9), as presented in Table II. No KEGG pathways were enriched in the downregulated DEGs. Subsequently, the GO term was determined, which included cell-cycle-related down-regulated gene alstrom syndrome protein 1 (ALMS1) and inflammatory associated upregulated genes TLR4, CCR1 and CCR10 (Table II).

PPI network construction and module analysis for DEGs. A PPI network was constructed once the DEGs were mapped into the PPI data, including 218 nodes (162 upregulated and 56 downregulated) and 477 edges (Fig. 2). By calculating their degrees, GNG10 (degree, 26), RAC1 (degree, 17), ubiquitin conjugating enzyme E2 D1 (UBE2D1; degree, 16), CCR1 (degree, 12), and CCR10 (degree, 12) were determined to be hub genes (Fig. 3). Furthermore, three significant modules were screened from the PPI network (Fig. 4). Module 1 was involved in ubiquitin-mediated proteolysis, including UBE2D1; module 2 was the chemokine signaling pathway cytokine-cytokine receptor interaction, including CCR1 and CCR10; and module 3 was associated with the cell cycle, including ALMS1 (Table III).

DEGs regulated by miRNA. By searching the mir2disease database, four miRNAs associated with AMI were identified, including hsa-miR-21, hsa-miR-133, hsa-miR-29 and hsa-miR-30c. A total of 1,134 genes were predicted to be target genes of these four miRNAs using the Mirwalk2 database, which were subsequently overlapped with the DEGs. As a result, 48 miRNA-DEG interaction pairs were obtained to construct a miRNA-DEG network that contained three miRNAs (hsa-miR-21-3p, hsa-miR-21-5p and hsa-miR-30c-5p) as well as 29 upregulated and 18 downregulated DEGs (Fig. 5). Examples include MMP9, TLR4, STAT3, CCR1 and ALMS1, which were regulated by hsa-miR-21-5p, whereas RAC1 was one gene regulated by hsa-miR-30c-5p.

The functional enrichment analysis indicated that the DEGs in the network were involved in Hepatitis B (including TLR4 and STAT3), pancreatic cancer (including RAC1 and STAT3) and the proteoglycans associated with cancer (including RAC1, TLR4 and STAT3; Table IV).

Table I. Top 20 upregulated and downregulated differentially expressed genes between acute myocardial infarction patients and healthy controls.

A, Upregulated		
Gene	Log fold change	P-value
RNF19B	1.67	1.31x10 ⁻⁴
TNNT1	1.67	3.70x10 ⁻⁴
NFIL3	2.30	3.91x10 ⁻⁴
NCF4	1.69	5.86x10 ⁻⁴
RNASE2	1.29	6.91x10 ⁻⁴
GCA	1.84	7.04x10 ⁻⁴
CXCL1	1.96	8.77x10 ⁻⁴
WBP2	1.46	9.99x10 ⁻⁴
STEAP4	1.78	1.20x10 ⁻³
NINJ1	1.37	1.46x10 ⁻³
HCAR3	3.04	1.76x10 ⁻³
S100P	3.88	1.81x10 ⁻³
ATP6V0C	1.88	1.86x10 ⁻³
G6PD	1.27	1.96x10 ⁻³
PTGS2	2.44	2.07x10 ⁻³
NAMPT	3.35	2.23x10 ⁻³
PLIN3	1.20	2.27x10 ⁻³
ACSL1	2.47	2.32x10 ⁻³
TNFAIP6	3.23	2.35x10 ⁻³
LY96	1.54	2.72x10 ⁻³
B, Downregulated		
Gene	Log fold change	P-value
RP11-846E15.4	-1.85	6.13x10 ⁻⁴
MICU3	-1.62	7.23x10 ⁻⁴
NPTN-IT1	-1.12	1.01x10 ⁻³
FZD6	-1.37	1.27x10 ⁻³
LEF1-AS1	-1.99	1.53x10 ⁻³
PRO0471	-1.61	1.53x10 ⁻³
MAP2K6	-1.09	1.71x10 ⁻³
LOC151657	-1.33	1.79x10 ⁻³
GVINP1	-1.51	1.88x10 ⁻³
CTD-2035E11.5	-1.02	1.91x10 ⁻³
AC007401.2	-1.09	2.13x10 ⁻³
LINC01355	-1.14	2.21x10 ⁻³
C8orf37	-1.38	2.29x10 ⁻³
LOC202181	-1.67	2.30x10 ⁻³
RP11-846E15.2	-1.72	2.58x10 ⁻³
CD3D	-1.10	2.61x10 ⁻³
RPS27	-1.09	2.77x10 ⁻³
NDUFA12	-1.18	2.78x10 ⁻³
LOC102724356	-2.22	2.83x10 ⁻³
RPL3	-1.20	2.96x10 ⁻³

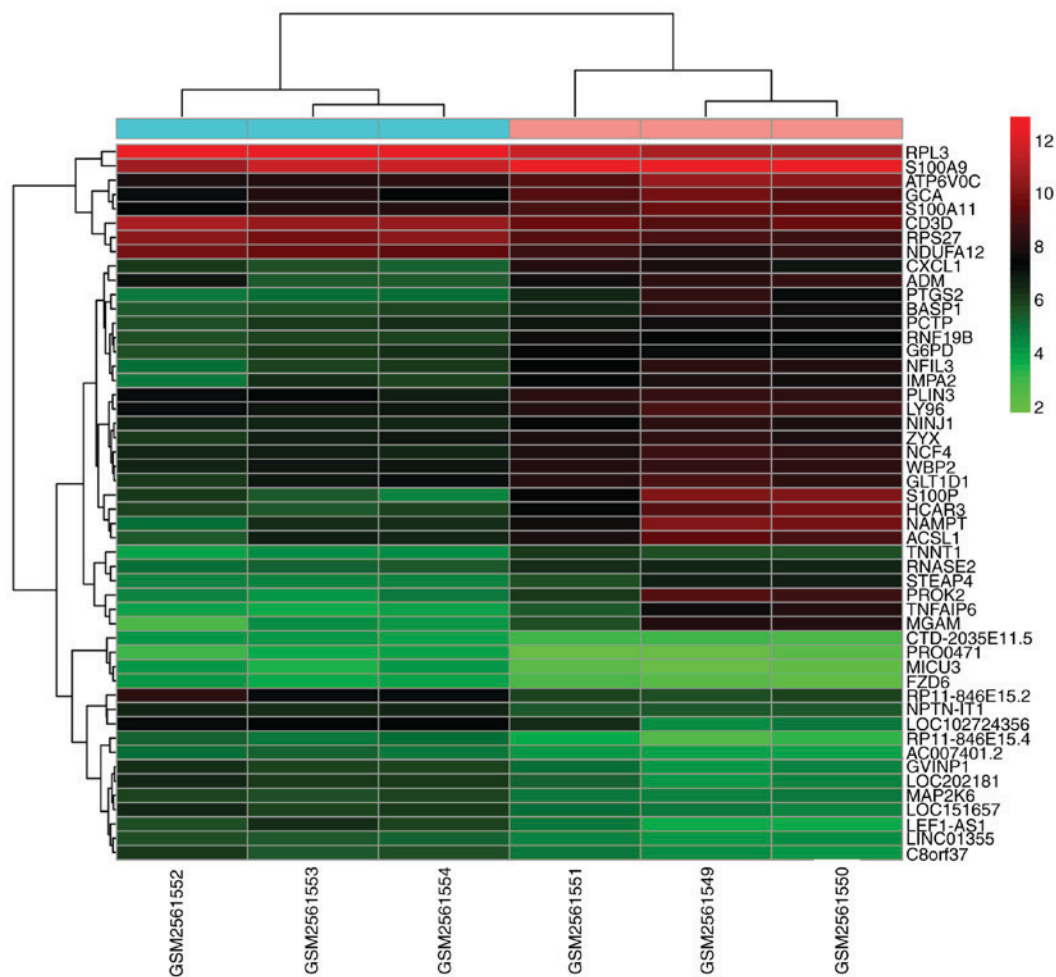


Figure 1. Heat map of the differentially expressed genes among the peripheral blood samples of three patients with acute myocardial infarction and three healthy controls from dataset GSE97320. Expression was presented as the z-score normalized within the samples. The horizontal coordinate represented the sample list. Blue groups represent healthy patients; pink groups represent patients with acute myocardial infarction. Red indicates upregulated expression and green indicates downregulated expression.

Validation analysis. Following data normalization of the GSE24519 dataset, 1,933 genes were identified as DEGs between patients with AMI and controls, including 1,242 upregulated and 691 downregulated genes. For GSE34198, 329 DEGs were screened, consisting of 166 upregulated and 163 downregulated genes; for GSE48060, 1,171 DEGs were obtained, containing 236 upregulated and 935 downregulated genes. A Venn diagram analysis indicated that there were 235 overlapped DEGs between GSE97320 and GSE24519, of which 153 had a consistent expression trend (Fig. 6A). The DEGs of all four datasets were compared, only four common genes were identified, including aldehyde dehydrogenase 8 family member A1 (ALDH8A1), CSF3R, regulator of G protein signaling 10, and CCR10 (Fig. 6B). Among these, the expression levels of ALDH8A1, CSF3R and CCR10 were consistent across the four datasets (Table V).

To investigate the relationship between the 153 DEGs exhibiting a consistent expression trend and clinical characteristics, a subgroup analysis of GSE34198 and GSE48060 was also performed to screen for genes associated with mortality or recurrence. As a result, 1,039 DEGs (864 downregulated and 175 upregulated) were identified between the mortality and non-mortality groups. A total of 1,216 (629

downregulated and 587 upregulated) were screened between the recurrence and non-recurrence groups. Considering the small number of common genes obtained across the four datasets, the shared 153 genes between GSE97320 and GSE24519 were used to overlap with the mortality- or recurrence-associated genes screened in GSE34198 and GSE48060, respectively. Consequently, four (Fig. 6C) and 14 (Fig. 6D) overlaps were separately obtained. However, only three were ultimately suggested as underlying biomarkers for the prediction of mortality [exostosin like glycosyltransferase 2, chromosome 11 open reading frame 80, HtrA serine peptidase 1 (HTRA1)] or recurrence (MAP2K2, HTRA1, and MAF1, homolog, negative regulator of RNA polymerase III) in Chinese patients with AMI, due to their consistent expression trends with GSE97320 (Table V).

Discussion

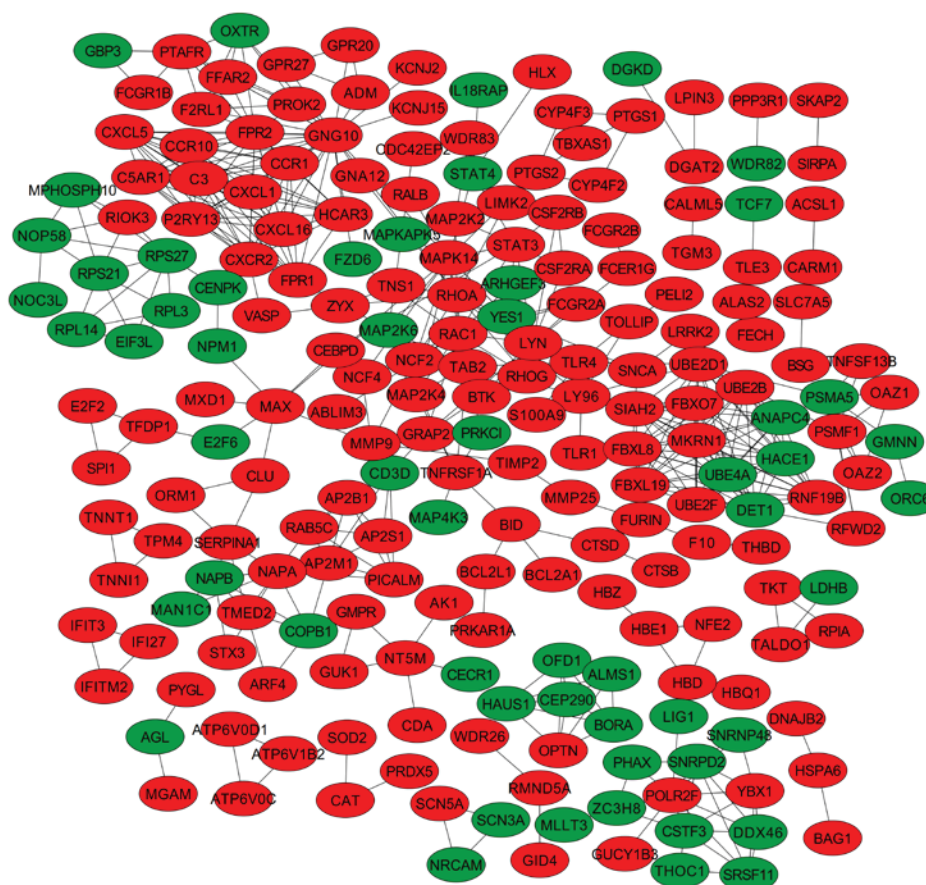
In the present study, several underlying biomarkers for the diagnosis of AMI in Chinese people were suggested prior to analyses. ALMS1 was demonstrated as a key gene in the PPI module analysis and regulated by miRNAs associated with AMI hsa-miR-21-5p (25,26). In addition, TLR4, MMP9,

Table II. Significantly enriched functions for differentially expressed genes between acute myocardial infarction patients and healthy controls. Upregulated genes were enriched in Kyoto encyclopedia of Genes and Genomes pathways, whereas the downregulated genes were enriched in Gene ontology biological process terms.

Term	P-value	Genes
Upregulated		
hsa04380: Osteoclast differentiation	4.57x10 ⁻⁷	NCF2, NCF4, PPP3R1, SPI1, SIRPA, TAB2, BTK, TNFRSF1A, FCGR2B, LILRA2, FCGR2C, MAPK14, LILRA5, RAC1, JUND, FCGR2A, FCGR3B
hsa04145: Phagosome	7.75x10 ⁻⁷	DYNC1LI1, RILP, NCF2, C3, TUBB2A, RAB5C, NCF4, TLR4, ATP6V1B2, ATP6V0C, LAMP2, FCGR2B, FCGR2C, RAC1, FCGR2A, ATP6V0D1, FCGR3B, TUBB3
hsa05152: Tuberculosis	1.35x10 ⁻⁶	BID, C3, RAB5C, TLR1, PPP3R1, TLR4, ATP6V0C, TNFRSF1A, LAMP2, FCGR2B, FCGR2C, MAPK14, RHOA, FCER1G, CTSD, FCGR2A, CALML5, ATP6V0D1, FCGR3B
hsa05150: Staphylococcus aureus infection	1.30x10 ⁻⁵	C5AR1, FCGR2B, C3, FCGR2C, FPR1, FCGR2A, FPR2, FCGR3B, CIQC, PTAFR
hsa04064: NF-κB signaling pathway	1.19x10 ⁻⁴	TNFRSF1A, TNFSF13B, PTGS2, LYN, LY96, BCL2A1, CXCL8, TLR4, BCL2L1, TAB2, BTK
hsa05140: Leishmaniasis	1.22x10 ⁻⁴	PTGS2, NCF2, C3, FCGR2C, MAPK14, NCF4, TLR4, FCGR2A, FCGR3B, TAB2
hsa05120: Epithelial cell signaling in Helicobacter pylori infection	4.43x10 ⁻⁴	ATP6V0C, LYN, MAPK14, RAC1, MAP2K4, CXCL8, CXCR2, ATP6V1B2, ATP6V0D1
hsa04620: Toll-like receptor signaling pathway	6.03x10 ⁻⁴	LY96, MAP2K2, TOLLIP, MAPK14, TLR1, RAC1, MAP2K4, CXCL8, TLR4, TAB2, TLR8
hsa05133: Pertussis	9.50x10 ⁻⁴	CXCL5, C3, LY96, MAPK14, RHOA, CXCL8, TLR4, CALML5, CIQC
hsa04721: Synaptic vesicle cycle	1.55x10 ⁻³	ATP6V0C, AP2B1, STX3, AP2S1, NAPA, ATP6V1B2, ATP6V0D1, AP2M1
hsa05014: Amyotrophic lateral sclerosis (ALS)	2.26x10 ⁻³	BID, TNFRSF1A, MAPK14, RAC1, PPP3R1, CAT, BCL2L1
hsa05132: Salmonella infection	7.38x10 ⁻³	CXCL1, DYNC1LI1, RILP, MAPK14, RAC1, CXCL8, TLR4, RHOG
hsa04611: Platelet activation	9.07x10 ⁻³	TBXAS1, LYN, MAPK14, PTGS1, RHOA, FCER1G, GUCY1B3, FCGR2A, VASP, BTK
hsa04664: Fc epsilon RI signaling pathway	1.04x10 ⁻²	LYN, MAP2K2, MAPK14, RAC1, MAP2K4, FCER1G, BTK
hsa04062: Chemokine signaling pathway	1.28x10 ⁻²	CXCL1, CXCL5, LYN, GNG10, CCR1, CXCL16, CCR10, RAC1, RHOA, CXCL8, CXCR2, STAT3
hsa05145: Toxoplasmosis	1.53x10 ⁻²	PPIF, TNFRSF1A, LY96, MAPK14, HSPA6, TLR4, BCL2L1, TAB2, STAT3
hsa04071: Sphingolipid signaling pathway	1.67x10 ⁻²	BID, TNFRSF1A, MAP2K2, MAPK14, GNA12, RAC1, RHOA, FCER1G, CTSD
hsa05200: Pathways in cancer	2.14x10 ⁻²	BID, E2F2, PTGS2, MAP2K2, MMP9, GNA12, SPI1, CXCL8, EGLN1, BCL2L1, DAPK3, STAT3, MAX, GNG10, RAC1, RHOA, RALB, CSF3R, CSF2RA
hsa05219: Bladder cancer	2.20x10 ⁻²	E2F2, MAP2K2, MMP9, CXCL8, DAPK3
hsa04666: Fc gamma R-mediated phagocytosis	2.70x10 ⁻²	FCGR2B, LIMK2, LYN, RAC1, MARCKS, FCGR2A, VASP
hsa05323: Rheumatoid arthritis	3.30x10 ⁻²	ATP6V0C, TNFSF13B, CXCL5, CXCL8, TLR4, ATP6V1B2, ATP6V0D1
hsa04610: Complement and coagulation cascades	3.99x10 ⁻²	THBD, F10, C5AR1, C3, SERPINA1, CIQC
hsa04662: B cell receptor signaling pathway	3.99x10 ⁻²	FCGR2B, LYN, MAP2K2, RAC1, PPP3R1, BTK
hsa04670: Leukocyte transendothelial migration	4.25x10 ⁻²	CLDN9, NCF2, MAPK14, MMP9, NCF4, RAC1, RHOA, VASP
hsa00030: Pentose phosphate pathway	4.37x10 ⁻²	G6PD, TALDO1, TKT, RPIA
hsa00760: Nicotinate and nicotinamide metabolism	4.37x10 ⁻²	NAMPT, NT5M, BST1, NAPRT
hsa05164: Influenza A	4.89x10 ⁻²	TNFRSF1A, TNFRSF10C, EIF2AK1, MAP2K2, MAPK14, MAP2K4, HSPA6, CXCL8, TLR4, FURIN
hsa05130: Pathogenic Escherichia coli infection	4.99x10 ⁻²	TUBB2A, LY96, RHOA, TLR4, TUBB3

Table II. Continued.

Term	P-value	Genes
Downregulated		
GO: 0006355~regulation of transcription, DNA-templated	1.89x10 ⁻⁵	ZNF611, NACA, ZNF823, ZNF558, HINT1, ZNF638, STAT4, ZNF776, ZNF738, ZNF300, ZNF540, ZNF721, KRBOX4, ZNF493, MAP2K6, MLLT3, ZNF563, ZNF529, ZNF548, ZNF566, ZNF565, ZNF354A, ZNF337, HACE1, ZFP3, HLTf, ZNF662, ZNF585B, ZRANB2, ZZZ3, ZSCAN16, ZNF550, ZNF765, ZNF766, ZNF571, PHF6
GO: 0008380~RNA splicing	9.84x10 ⁻⁵	SNRNP48, RBM4, SRSF11, ZRANB2, MPHOSPH10, SNRNP22, ZNF638, NSRPI, LUC7L3, THOC1
GO: 0006351~transcription, DNA-templated	3.70x10 ⁻⁴	ZNF611, NACA, ZNF823, ZNF558, E2F6, HINT1, ZNF638, TCEAL1, ZXDA, STAT4, ZNF776, ZNF738, ZNF300, ZNF540, ZNF721, ZNF493, MAP2K6, MLLT3, PLAG1, ZNF563, NFKBIZ, ZNF529, ZNF548, ZNF566, TCF7, ZNF565, ZNF354A, ZNF337, HACE1, ZFP3, ZNF662, ZNF585B, ZZZ3, ZSCAN16, ZNF550, ZNF765, ZNF766, ZNF571, PHF6
GO: 0000086~G2/M transition of mitotic cell cycle	1.83x10 ⁻²	OFD1, BORA, HAUS1, CEP290, ALMS1, MASTL
Upregulated		
GO:0006954~inflammatory response	9.36 x10 ⁻⁹	CXCL1, CXCL5, PTGS2, TOLLIP, C3, CCR1, PTGS1, S100A9, F2RL1, TLR1, FPR1, CXCL8, PRDX5, CXCR2, TLR4, FPR2, MMP25, TLR8, TNFRSF1A, RAC1, BCL6, C5AR1, LYN, LY96, CHI3L1, ORM1, TNFAIP6, PROK2, TNFRSF10C, PTAFR
GO:0006935~chemotaxis	6.18 x10 ⁻⁷	CXCL1, PROK2, CMTM2, C5AR1, RNASE2, CXCL5, CXCL16, CCR1, MAPK14, CCR10, FPR1, CXCL8, CXCR2, FPR2, PTAFR
GO:0032496~response to lipopolysaccharide	9.09 x10 ⁻⁷	CXCL1, ALPL, C5AR1, CXCL5, PTGS2, LY96, SNCA, TLR4, SOD2, TNFRSF1A, TNFRSF10C, THBD, ADM, JUND, CSF2RB, SLPI, AKIRIN2
GO:0006955~immune response	1.03 x10 ⁻⁵	CXCL1, IL1R2, CXCL5, AQP9, IFITM2, C3, CCR1, TLR1, PGLYRP1, CXCL8, TLR4, CIQC, TNFRSF1A, CCR10, FCGR1B, NFIL3, FCGR3B, C5AR1, NCF4, TNFRSF10C, FCGR2B, TNFSF13B, FCGR2C, AIRE, SLPI, PTAFR
GO:0050900~leukocyte migration	9.56 x10 ⁻⁵	BSG, THBD, C5AR1, LYN, MMP9, F2RL1, FPR1, FCER1G, FPR2, TREM1, SLC7A5, SIRPA



Gene Cluster	Number of Genes
UBE4A	12
UBE2F	12
UBE2B	12
RNF19B	12
P2RY13	12
MKRN1	12
LYN	12
HCAR3	12
HACE1	12
FBXO7	12
FBXL8	12
FBXL19	12
CXCL5	12
CXCL16	12
CXCL1	12
CCR10	12
CCR1	12
C5AR1	12
C3	12
SIAH2	13
RHOA	13
FPR1	13
DET1	13
CXCR2	13
ANAPC4	14
MAPK14	15
UBE2D1	16
RAC1	17
FPR2	17
GNG10	26

AMI may occur predominantly as a result of atherosclerotic plaque rupture and thrombosis that occludes the

Table III. Significantly enriched functions for module genes screened from the protein-protein interaction network.

Module	Term	P-value	Genes
1	hsa04120: Ubiquitin mediated proteolysis	2.85x10 ⁻⁹	UBE4A, ANAPC4, DET1, UBE2F, UBE2D1, UBE2B
2	hsa04062: Chemokine signaling pathway	2.84x10 ⁻⁷	CXCL1, CXCL5, GNG10, CCR1, CXCL16, CCR10, CXCR2
	hsa05150: Staphylococcus aureus infection	9.44x10 ⁻⁵	C5AR1, C3, FPR1, FPR2
	hsa04060: Cytokine-cytokine receptor interaction	6.401x10 ⁻³	CCR1, CXCL16, CCR10, CXCR2
	hsa04080: Neuroactive ligand-receptor interaction	1.07x10 ⁻²	P2RY13, C5AR1, FPR1, FPR2
3	GO: 0000086~G2/M transition of mitotic cell cycle	3.36x10 ⁻¹¹	OFD1, BORA, HAUS1, CEP290, ALMS1, OPTN
	GO: 0007067~mitotic nuclear division	2.11x10 ⁻³	OFD1, BORA, HAUS1
	GO: 0042384~cilium assembly	3.64x10 ⁻²	CEP290, ALMS1
	GO: 0060271~cilium morphogenesis	3.98x10 ⁻²	OFD1, CEP290

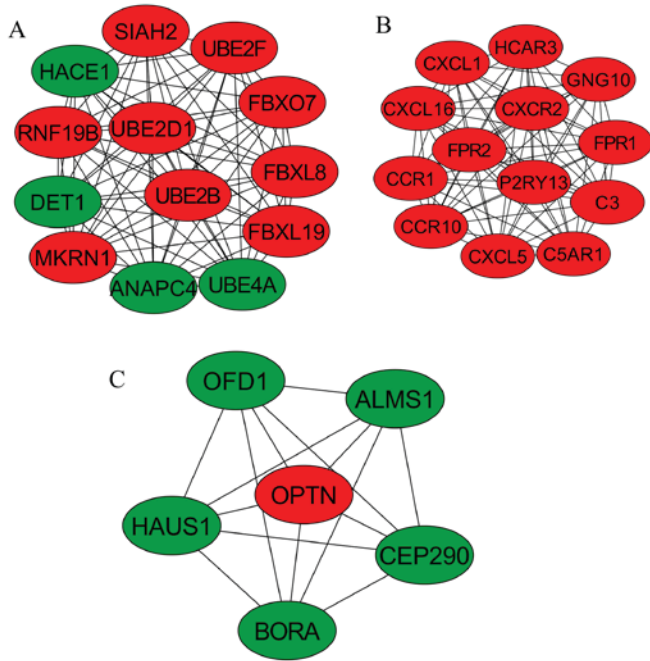


Figure 4. Modules obtained from protein-protein interaction network of differentially expressed genes among the peripheral blood samples of three patients with acute myocardial infarction and three healthy controls. (A) Module 1, (B) module 2 and (C) module 3. Red indicates the upregulated genes and green indicates the downregulated genes.

vessel lumen and significantly lowers the supply of oxygen and metabolites to the myocardium, ultimately inducing cardiomyocyte death (33,34). Inflammation due to bacterial infection (35) or conditions including hypercholesterolemia or hyperlipidemia, are important contributors to atherosclerotic plaque rupture, including both local and systemic inflammation (36). Ishikawa *et al* (37) performed immunostaining analysis and demonstrated that TLR4 was positively expressed in infiltrated macrophages in ruptured plaque material. In addition, local and systemic levels of TLR4 and TLR2 were significantly higher in patients with AMI, particularly those with cardiovascular events, compared with

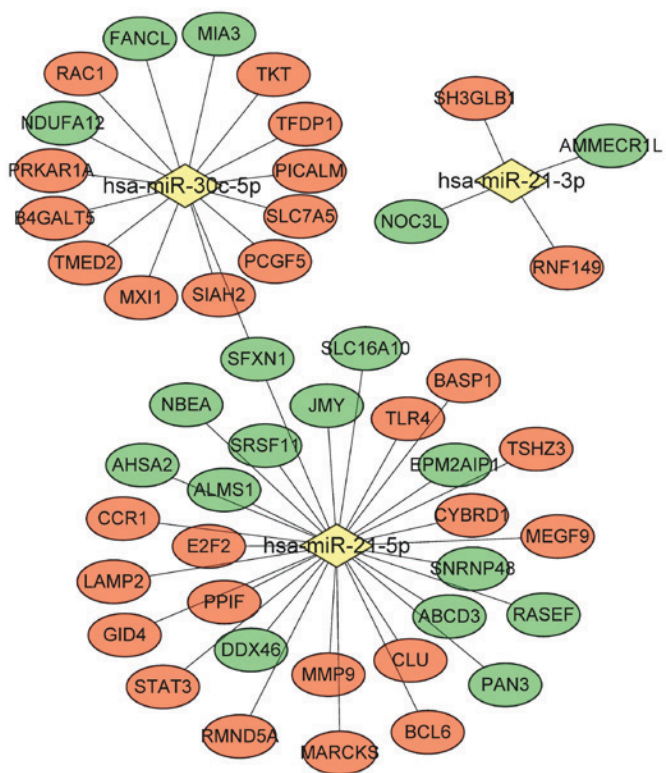


Figure 5. miRNA-gene interaction network. Orange indicates the upregulated genes and green indicates the downregulated genes. All miRNAs were associated with acute myocardial infarction. miRNA/miR, microRNA.

patients with stable angina or controls (37,38). Activated TLRs may weaken the fibrous cap and predispose the plaque to rupture by mediating the high expression of inflammatory genes, including interleukin (IL)-18 receptor (R)1, IL-18R2, IL-8, CCR1, CCR10 and MMP9, via the myeloid differentiation primary response protein MyD88/RAC1/NF- κ B pathway (39-42). Thus, TLRs and their downstream genes may be underlying biomarkers for the early diagnosis of AMI. This theory is supported by the current results and those of a previous study, that revealed the extent of TLR

Table IV. Significantly enriched pathways for genes in the microRNA-target gene network.

Term	P-value	Genes
hsa05161: Hepatitis B	1.18×10^{-2}	E2F2, MMP9, TLR4, STAT3
hsa05212: Pancreatic cancer	1.94×10^{-2}	E2F2, RAC1, STAT3
hsa05205: Proteoglycans in cancer	2.76×10^{-2}	MMP9, RAC1, TLR4, STAT3

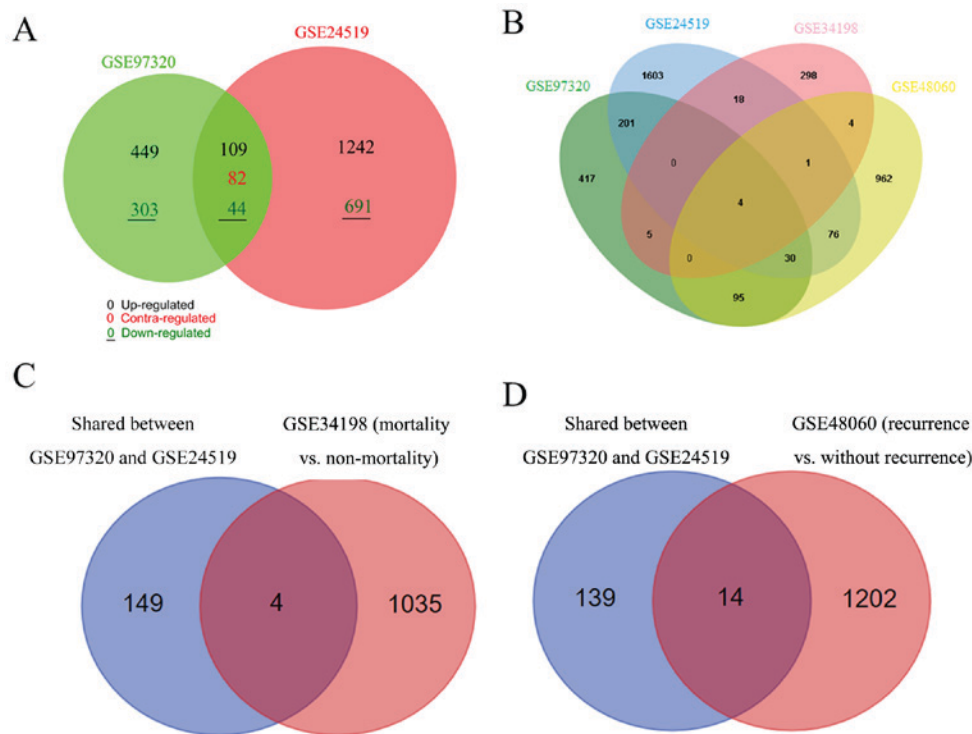


Figure 6. Venn diagram analyses of the common differentially expressed genes among the different datasets. Overlap between differentially expressed genes in (A) GSE97320 and GSE24519 datasets; (B) GSE97320, GSE24519, GSE34198 and GSE48060 datasets; (C) GSE97320 and GSE24519, and GSE34198 datasets; (D) and GSE97320 and GSE24519, and GSE48060 datasets.

activation and IL-18R1/2 expression in circulating cells preceding the excessive release of troponin-T (39), which indicates a potential advantage for their use as biomarkers for early diagnosis compared with troponin-T. Furthermore, high serum MMP-9 levels are associated with an increased risk of AMI (odds ratio, 1.06; 95% CI, 0.87-1.28) (43), and significantly higher levels of CCR1 are detected in patients with AMI compared with controls (3.76 ± 0.85 vs. 0.66 ± 0.19 ng/ml, $P < 0.05$) (44). In addition to TLR, the activation of the tyrosine-protein kinase JAK1 (JAK)/STAT signaling pathway has an important role in the onset of AMI by transducing the intracellular signals of various cytokines, such as tumor necrosis factor- α (TNF- α) (45). Inhibition of the JAK/STAT signaling pathway by AG490 inhibits NF- κ B protein expression in cardiomyocytes and reduces plasma TNF- α concentrations to prevent the development of AMI (45). However, no studies have directly investigated the diagnostic efficiency of STAT3 in patients with AMI, which may be a future research direction.

Granulocyte colony-stimulating factor (G-CSF) is a multifunctional cytokine that interacts with its receptor G-CSFR, which is encoded by CSF3R. G-CSF promotes inflammation by

enhancing the function of mature granulocytes and increasing the number of neutrophils (46). These effects suggest that the expression levels of G-CSF and CSF3R increase with disease progression. This hypothesis was supported by the current study and Li *et al* (47), who demonstrated that G-CSF and its receptor CSF3R were significantly upregulated in patients with AMI compared with controls and patients with stable angina. However, other studies have suggested that G-CSF and CSF3R improve cardiac function and reduce mortality following AMI by inducing the regeneration of cardiac myocytes and blood vessels through the mobilization of bone marrow stem cells (48), which suggests that high CSF3R expression is a protective response.

HTRA1 is also a gene associated with oxidative stress and the inflammation response. HTRA1 is highly expressed in activated macrophages and interacts with growth differentiation factor 15 to mediate inflammation and promote cell senescence through the p38 mitogen-activated protein kinase pathway, ultimately predisposing the individual to high risk for the development of age-related macular degeneration (49,50). AMI is particularly prevalent in elderly people (51). Thus, HTRA1 may be important with regard to AMI. In line with

Table V. Shared differentially expressed genes among different datasets.

A, Differentially expressed genes screened in four datasets								
Gene	GSE97320 ^a		GSE24519 ^a		GSE34198 ^a		GSE48060 ^a	
	logFC	P-value	logFC	P-value	logFC	P-value	logFC	P-value
ALDH8A1	-1.60	1.20x10 ⁻²	-2.67	2.94x10 ⁻⁴	-1.94	1.03x10 ⁻²	-1.97	7.88x10 ⁻³
CSF3R	1.06	1.22x10 ⁻²	1.11	3.38x10 ⁻³	1.59	1.85x10 ⁻²	1.20	1.6x10 ⁻⁴
CCR10	1.58	8.81x10 ⁻³	1.53	7.24x10 ⁻³	1.18	3.86x10 ⁻²	1.95	2.4x10 ⁻²

B, Mortality-related differentially expressed genes						
Gene	GSE97320 ^a		GSE24519 ^a		GSE34198 ^b	
	logFC	P-value	logFC	P-value	logFC	P-value
EXTL2	-2.17	3.54x10 ⁻²	-1.01	8.24x10 ⁻³	-1.70	2.75x10 ⁻³
C11orf80	-1.35	1.50x10 ⁻²	-1.85	2.92x10 ⁻²	-1.98	2.31x10 ⁻⁴
HTRA1	1.06	1.18x10 ⁻²	1.11	6.42x10 ⁻³	2.56	1.09x10 ⁻²

C, Recurrence-related differentially expressed genes						
Gene	GSE97320 ^a		GSE24519 ^a		GSE48060 ^c	
	logFC	P-value	logFC	P-value	logFC	P-value
MAP2K2	1.17	1.74x10 ⁻²	1.23	3.46x10 ⁻²	1.10	2.65x10 ⁻²
HTRA1	1.06	1.18x10 ⁻²	1.11	6.42x10 ⁻³	1.81	1.36x10 ⁻²
MAF1	1.05	6.78x10 ⁻³	1.10	9.42x10 ⁻³	1.84	1.42x10 ⁻²

FC, fold-change. ^aAMI vs. control; ^bmortality vs. non-mortality; ^crecurrence vs. non-recurrence.

FC, fold-change. ^aAMI vs. control; ^bmortality vs. non-mortality; ^crecurrence vs. non-recurrence.

the above study, the present study demonstrated that HTRA1 was significantly upregulated in the AMI group, particularly in patients who succumbed to the disease following a recurrence.

In conclusion, the present study preliminarily suggests that inflammation markers, including STAT3, CCR1, RAC1, MMP9, CCR10, CSF3R and HTRA1, as well as cell-cycle-associated genes including ALMS1, have a potential role that is crucial for the diagnosis and prognosis of AMI in Chinese people. However, additional clinical studies are required to confirm the above findings, which represents a limitation of the present study.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the NCBI database repository [<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE97320>; <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE24519>; <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE34198>; <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE48060>].

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE97320>; <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE24519>; <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE34198>; <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE48060>].

Author's contributions

JS and CG participated in the design of this study. JS and CG performed the statistical and bioinformatics analyses. RW, CX and MY contributed to the acquisition and interpretation of data. JS and CG drafted and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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