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Expression characteristics of neutrophil and mononuclear-phagocyte related genes mRNA in the stable angina pectoris and acute myocardial infarction stages of coronary artery disease

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Abstract

Objective To investigate expression differences of neutrophil and mononuclear phagocyte related gene mRNAs among acute myocardial infarction (AMI), stable angina (SA) and control groups, and then discuss their expression characteristics in the stable angina pectoris (SAP) and AMI stages of coronary artery disease (CAD). **Methods** Whole Human Genome Oligo Microarrays were applied to assess the differential expression characteristics of neutrophil and mononuclear phagocyte related mRNAs in patients with AMI (n = 20), SA (n = 20) and controls (n = 20). **Results** (1) Almost all colony-stimulating factors (CSF) and their receptors related mRNAs was up-regulated in AMI and SA groups compared with the control group, and the expression of granulocyte-macrophage colony stimulating factor receptor (GM-CSFR) and granulocyte colony stimulating factor receptor (G-CSFR) mRNAs in the AMI group was significantly up-regulated compared with the other two groups (P < 0.01). (2) The expression of mRNAs related to monocyte chemoattractant protein-1 (MCP-1), CCR2 (MCP-1 receptor) and CXCR2 (IL-8 receptor) was significantly up-regulated (P < 0.01) in AMI group compared with SA and control groups. IL-8 mRNA expression in the AMI group was clearly higher than the controls (P < 0.05). (3) All mRNAs expression related to opsonic receptors (IgG FcR and C3bR/C4bR) was significantly up-regulated in AMI group compared with SA and control group (P < 0.01), and the SA group showed an upward trend compared with controls. (4) Most pattern recognition receptor (PRR)-related mRNAs expression was up-regulated in AMI group compared with SA and control groups. Most toll-like receptor (TLR) mRNAs expression was significantly up-regulated (P < 0.01) than the SA and control groups; macrophage scavenger receptor (MSR) mRNA was significantly up-regulated in AMI group compared with the control group (P < 0.01), and the SA group showed an upward trend compared with the controls. **Conclusions** The expression of most neutrophil and mononuclear-macrophage function related genes mRNAs was significantly up-regulated by stages during the progression of CAD, suggesting that the adhesive, chemotactic and phagocytic functions of neutrophil and mononuclear-macrophage were strengthened in the occurrence and development of coronary atherosclerosis and AMI. This also showed a stepped upward trend as the disease progressed.

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Keywords: Acute myocardial infarction; Coronary atherosclerosis; Mononuclear-macrophage; Neutrophil

1 Introduction

Coronary artery disease (CAD) is one of the diseases with highest morbidity and mortality worldwide. Stable angina pectoris (SAP) and acute myocardial infarction (AMI) are the CADs with highest mortality and disability rate.[1] Inflammatory reaction goes through the entire occurrence, development and evolution of coronary atherosclerosis and affects the stability and natural proceeding of atherosclerotic plaque to some extent.[2] As the most important inflammatory cells, phagocytes including neutrophil and mononuclear directly participate in atherosclerosis and the occurrence and development of acute coronary events with a variety of inflammatory factors.[3]

It has been shown that absolute neutrophil count among patients with AMI was obviously higher than those among the stable angina (SA) and control group. Moreover, there existed striking expression of inflammatory factor, infiltration of neutrophils and macrophage in unstable plaque.[4,5] Activated neutrophils can prompt the progress of atheromatous plaque and increase its instability through chemotaxis, degranulation and oxidative stress and so on;[6] Monocyte adhesion to vascular endothelial cells is the initiation of

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atherosclerosis, and monocytes activate into macrophages, furthers the formation of accelerate foam cells and plaque.[7] There are many pattern recognition receptors (PRRs), opsonic receptors and cytokine receptors expressed on neutrophils and macrophages surface, which are closely related to the adhesive, chemotactic and phagocytic functions of neutrophil and mononuclear-macrophage, moreover. They can increase plaque instability by many immunoinflammatory factors.

Whole Human Genome Oligo Microarrays were applied to detect expression differences of neutrophil and mononuclear-macrophage function related genes mRNAs in the AMI, SA and control groups, then discuss their expression characteristics in the SA and AMI stages of CAD.

2 Methods

2.1 Patient information

This prospective study included three groups of patients with a total of 60 subjects, 20 patients with AMI, 20 with SA, and 20 healthy volunteers. The baseline demographic data are displayed in Table 1. The AMI patients were admitted < 12 hours from the onset of symptoms to our coronary care unit between January and June 2013, included 18 males and 2 females, with an average age of 58 ± 12 years. All AMI subjects were diagnosed on the basis of following criteria: detection of a rise of cardiac biomarker values (preferably cardiac troponin) with at least one value above the 99th percentile upper reference limit and with at least one of the following: symptoms of ischemia, new or presumed new significant ST-segment-T wave changes or new left bundle branch block, development of pathological Q waves in the ECG, imaging evidence of new loss of viable myocardium or new regional wall motion abnormality, identification of an intracoronary thrombus by angiography.

In the SA group, 20 patients (18 males, 2 females, mean age 64 ± 10 years) with exclusively effort-related angina were studied, with a positive exercise stress test and at least one coronary stenosis was detected by angiography (> 70% reduction of lumen diameter). There were no significant differences between the AMI and SA patients in age, gender, smoking, body mass index (BMI), systolic blood pressure, diastolic blood pressure, low density lipoprotein-cholesterol (LDL-C), triglycerides, high density lipoprotein-cholesterol (HDL-C) and fasting plasma glucose (FPG) (Table 1).

The control group included 20 volunteers (17 males and 3 females, mean age 29 ± 3 years) enrolled during the same period with similar male/female ratio. Histories, physical examination, ECG, chest radiography and routine chemical analysis showed the controls had no evidence of CADs.

The exclusive criteria for the three groups were as follows: venous thrombosis, history of severe renal or hepatic diseases, haematological disorders, acute or chronic inflammatory diseases and malignancy.

The study protocol was approved by the local ethics committee of Tongji University and informed consent form was also obtained.

2.2 Gene expression clip

Agilent G4112F Whole Human Genome Oligo Microarrays were purchased from Agilent (USA). A microarray is composed of more than 41,000 genes or transcripts, including targeted 19,596 entrez gene RNAs. Sequence information used in the microarrays was derived from the latest databases of RefSeq, Goldenpath, Ensembl and Unigene. The functions of more than 70% of the genes in the microarray are already known. All patients were subjected to clip analysis.

Table 1. Comparison of the basic situation in each group.

Index	AMI, *n* = 20	SA, *n* = 20	Control, *n* = 20	P_{ALL}	$P_{AMI\ vs.\ SA}$
Age, yr	57.8 ± 11.9	63.6 ± 9.9	28.8 ± 3.3	0.000	0.251
Sex	18 M/2 F	18 M/ 2 F	17 M/2 F	0.853	1.000
BMI, kg/cm^2	23.6 ± 2.6	22.8 ± 2.7	21.3 ± 1.8	0.102	0.560
Smoking, per day	13.6 ± 12.2	9.8 ± 10.3	0	0.000	0.648
SBP, mmHg	128.6 ± 15.3	123.0 ± 12.1	120.8 ± 7.2	0.115	0.501
DBP, mmHg	67.0 ± 8.0	73.0 ± 8.0	71.6 ± 3.2	0.017	0.064
LDL-C, mmol/L	2.5 ± 1.0	2.1 ± 0.8	2.9 ± 0.5	0.327	0.548
TG, mmol/L	1.6 ± 1.1	1.5 ± 1.4	1.2 ± 0.4	0.730	0.762
HDL-C, mmol/L	0.8 ± 0.7	0.9 ± 0.2	1.3 ± 0.2	0.000	0.803
FPG	5.4 ± 0.9	5.0 ± 0.8	4.9 ± 0.5	0.61	0.082

Data are presented as mean ± SD unless other indicated. AMI: acute myocardial infarction; BMI: body mass index; DBP: diastolic blood pressure; FPG: fasting plasma glucose; HDL-C: high density lipoprotein-cholesterol; LDL-C: low density lipoprotein-cholesterol; SA: stable angina; SBP: systolic blood pressure; TG: triglyceride.

2.3 Total RNA isolation

Five milliliters of peripheral blood samples with PAXgene tube were drawn from patients of AMI and SA, immediately after being admitted to the hospital, and did the same to the controls. Leucocytes were obtained through density gradient centrifugation with Ficoll solution and the remaining red blood cells were destroyed by erythrocyte lysis buffer (Qiagen, Hilden, Germany). Total RNA was extracted and purified using PAXgene^{TM} Blood RNA kit (Cat#762174, QIAGEN, GmBH, Germany) following the manufacturer's instructions and checked for a RIN number to inspect RNA integration by an Agilent Bioanalyzer 2100 (Agilent technologies, Santa Clara, CA, US). The sample was considered qualified when 2100 RIN $\geq$ 7.0 as well as 28S/18S $\geq$ 0.7.

2.4 RNA amplification and labeling

Total RNA was amplified and labeled by Low Input Quick Amp Labeling Kit, One-Color (Cat#5190-2305, Agilent technologies, Santa Clara, CA, US), following the manufacturer's instructions. Labeled cRNA were purified by RNeasy mini kit (Cat#74106, QIAGEN, GmBH, Germany).

2.5 Microarray hybridization

Each Slide was hybridized with 1.65μg Cy3-labeled cRNA using Gene Expression Hybridization Kit (Cat#5188-5242, Agilent technologies, Santa Clara, CA, US) in Hybridization Oven (Cat#G2545A, Agilent technologies, Santa Clara, CA, US), according to the manufacturer's instructions. After 17 hours hybridization, slides were washed in staining dishes (Cat#121, Thermo Shandon, Waltham, MA, US) with Gene Expression Wash Buffer Kit (Cat#5188-5327, Agilent technologies, Santa Clara, CA, US), according to the manufacturer's operation manual.

2.6 Chip scan and data acquisition

Slides were scanned by Agilent Microarray Scanner (Cat#G2565CA, Agilent technologies, Santa Clara, CA, US) with default settings. Dye channel: Green, Scan resolution=3μm, 20bit. Data were extracted with Feature Extraction software 10.7 (Agilent technologies, Santa Clara, CA, US). Raw data were normalized by Quantile algorithm, Gene Spring Software 11.0 (Agilent technologies, Santa Clara, CA, US).

2.7 RT-PCR

The spots in the microarray were randomly selected and their expressions were confirmed by RT-PCR. Among genes with differential expressions, 3 genes were randomly selected, and these genes and the house keeping genes, GAPDH, were subjected to RT-PCR. The relative expressions were indicated as the expression of the target genes normalized to the expression of GAPDH (2-ΔΔCt). The melting curve and the 2-ΔΔCt-method were used to compare the differences in the expressions among three groups. The results from RT-PCR were consistent with the microarray analysis.

2.8 Statistical analysis

Values were expressed as mean ± SD. Groups differences were examined by one-way analysis of variance (ANOVA). Pair-wise group comparisons after ANOVA were performed using Tukey's multiple comparison technique. Data were analyzed using SPSS 19.0, and *P*-values < 0.05 were considered statistically significant.

3 Results

3.1 Colony-stimulating factors and their receptors related mRNAs expression

Seven colony-stimulating factors (CSF) and their receptors related mRNAs were detected totally. Compared with the controls: (1) all CSFs and their receptors related mRNAs were up-regulated in AMI group, and the expression of granulocyte-macrophage colony stimulating factor receptor A (GM-CSFRA) and granulocyte colony stimulating factor receptor (G-CSFR) mRNAs was significantly up-regulated ($P < 0.01$); and (2) Six of all 7 mRNAs were up-regulated in SA group, and GM-CSFRA mRNA expression was significantly up-regulated ($P < 0.05$). Macrophage colony stimulating factor receptor (M-CSFR) mRNA was down-regulated without significant difference. Compared with the SA group, all CSFs and their receptors related mRNAs were up-regulated in AMI group, and the expression of GM-CSFRA and G-CSFR mRNAs was significantly up-regulated ($P < 0.01$) (Table 2 and Figure 1).

3.2 Main chemokines and their receptors related mRNAs expression

Six chemokines and their receptors related mRNAs were detected. Compared with the controls: (1) Four mRNAs were significantly up-regulated in AMI group (CCR2 (chemokine (C-C motif) receptor 2, MCP-1 receptor), CCL2/MCP-1 (chemokine (C-C motif) ligand/monocyte chemoattractant protein-1), and CXCR2 (chemokine (C-X-C motif) receptor 2, IL-8 receptor) mRNAs expression $P < 0.01$; IL8 mRNA expression $P < 0.05$), and 2 MIP-1 mRNAs (CCL3, CCL4) expression was down-regulated without significant difference. (2) All chemokines and their receptors related mRNAs

Table 2. CSFs and their receptors related mRNAs expression.

Gene	AMI, $n = 20$	SA, $n = 20$	Control, $n = 20$	P_{ALL}	$P_{AMI\ vs.\ SA}$	$P_{AMI\ vs.\ Control}$	$P_{SA\ vs.\ Control}$
M-CSF	2.45 ± 0.50	2.39 ± 0.60	2.25 ± 0.48	0.500			
M-CSFR	13.73 ± 0.52	13.43 ± 0.36	13.44 ± 0.37	0.052			
GM-CSF	2.47 ± 0.86	2.22 ± 0.11	2.19 ± 0.07	0.165			
GM-CSFRA	9.41 ± 0.44	8.71 ± 0.31	8.42 ± 0.36	**0.000	**0.000	**0.000	*0.049
GM-CSFRB	8.64 ± 0.47	8.34 ± 0.54	8.30 ± 0.39	0.056			
G-CSF	2.68 ± 0.20	2.77 ± 0.37	2.61 ± 0.20	0.180			
G-CSFR	13.12 ± 0.39	12.51 ± 0.40	12.29 ± 0.38	**0.000	**0.000	**0.000	

Data are presented as mean ± SD. P_{ALL}: the *P*-value compared among the 3 groups. *$P < 0.05$; **$P < 0.01$. AMI: acute myocardial infarction; G-CSF: granulocyte colony stimulating factor; G-CSFR: granulocyte colony stimulating factor receptor; GM-CSF: granulocyte-macrophage colony stimulating factor; GM-CSFRA: granulocyte-macrophage colony stimulating factor receptor A; GM-CSFRB: granulocyte-macrophage colony stimulating factor receptor B; M-CSF: macrophage colony stimulating factor; M-CSFR: macrophage colony stimulating factor receptor; SA: stable angina.

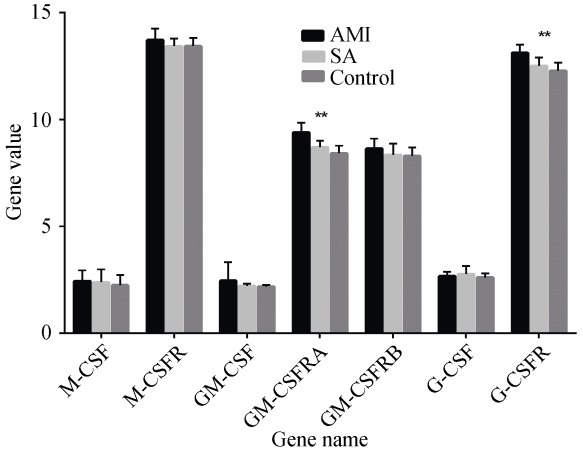

Figure 1. CSFs and their receptor related mRNAs expression (*$P_{ALL} < 0.05$, **$P_{ALL} < 0.01$). AMI: acute myocardial infarction; G-CSF: granulocyte colony stimulating factor; G-CSFR: granulocyte colony stimulating factor receptor; GM-CSF: granulocyte-macrophage colony stimulating factor; GM-CSFRA: granulocyte-macrophage colony stimulating factor receptor A; GM-CSFRB: granulocyte-macrophage colony stimulating factor receptor B; M-CSF: macrophage colony stimulating factor; M-CSFR: macrophage colony stimulating factor receptor; SA: stable angina.

expression in SA group showed an upward trend, and CCR2 mRNA was significantly up-regulated ($P < 0.01$). Compared with the SA group, the expression of CCR2, CCL2/MCP-1 and CXCR2 mRNAs was significantly up-regulated ($P < 0.01$) (Table 3 and Figure 2).

3.3 Opsonic receptors related mRNAs expression

Six opsonic receptors related mRNAs were detected. Compared with the controls: (1) all opsonic receptors (IgG FcR (immunoglobulin G fragment crystallizable receptor) and C3bR/C4bR) mRNAs were significantly up-regulated ($P < 0.01$) in AMI group. (2) All mRNAs in the SA group were also up-regulated (FCGR2B(Fc fragment of IgG receptor IIb) mRNA expression, $P < 0.05$). Compared with the controls, all opsonic receptors mRNAs were significantly up-regulated (FCGR2A, FCGR2B, FCGR3A, FCGR3B and CR1 mRNAs expression $P < 0.01$) in AMI group (Table 4 and Figure 3).

3.4 PRR related mRNAs expression

Twelve PRR related mRNAs were detected totally. Compared with the controls: (1) Eleven PRR related mRNAs

Table 3. Main chemokines and their receptors related mRNAs expression.

Gene	AMI, $n = 20$	SA, $n = 20$	Control, $n = 20$	P_{ALL}	$P_{AMI\ vs.\ Control}$	$P_{AMI\ vs.\ SA}$	$P_{SA\ vs.\ Control}$
CCR2	11.69 ± 0.45	10.97 ± 0.36	10.9 ± 0.36	**0.000	**0.000	**0.000	**0.000
CCL2/MCP-1	4.41 ± 1.03	3.79 ± 1.04	2.85 ± 0.55	**0.000	**0.000	**0.005	
CCL3/MIP-1a	9.63 ± 1.35	9.47 ± 1.18	9.87 ± 1.17	0.582			
CCL4/MIP-1b	11.28 ± 0.60	11.65 ± 0.48	11.60 ± 0.57	0.087			
CXCR2	15.14 ± 0.49	14.30 ± 0.59	14.18 ± 0.36	**0.000	**0.000	**0.000	
IL-8	3.34 ± 1.30	2.80 ± 0.86	2.63 ± 0.41	**0.048	**0.048		

Data are presented as mean ± SD. P_{ALL}: the *P*-value compared among the 3 groups. **$P < 0.01$. AMI: acute myocardial infarction group; CCL2/MCP-1: chemokine (C-C motif) ligand 2/monocyte chemoattractant protein-1; CCL3/MIP-1a: chemokine (C-C motif) ligand 3/macrophage inflammatory protein-1a; CCL4/MIP-1a: chemokine (C-C motif) ligand 4/macrophage inflammatory protein-1b; CCR2: chemokine (C-C motif) receptor 2; CXCR2: chemokine (C-X-C motif) receptor 2; IL-8: interleukin-8; SA: stable angina group.

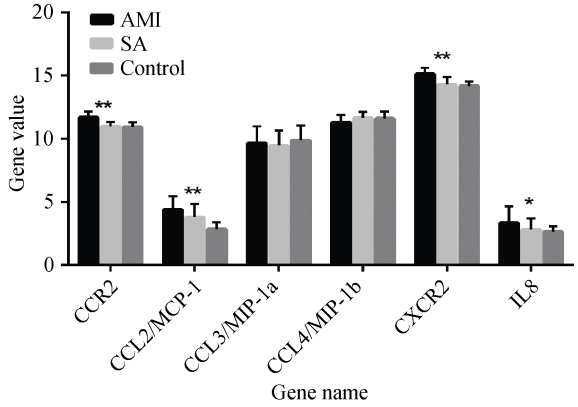

Figure 2. Main chemokines and their receptors related mRNAs expression (*P_{ALL} < 0.05, **P_{ALL} < 0.01). AMI: acute myocardial infarction group; CCL2/MCP-1: chemokine (C-C motif) ligand 2/monocyte chemoattractant protein-1; CCL3/MIP- 1a: chemokine (C-C motif) ligand 3/macrophage inflammatory protein-1a; CCL4/MIP-1a: chemokine (C-C motif) ligand 4/macrophage inflammatory protein-1b; CCR2: chemokine (C-C motif) receptor 2; CXCR2: chemokine (C-X-C motif) receptor 2; IL-8: interleukin-8; SA: stable angina group.

were up-regulated in AMI group, and 9 (MSR (macrophage scavenger receptor), TLR (toll-like receptor) 1, 2, 4-6, 8-10) of them were significantly up-regulated ($P < 0.01$); (2) Ten PRR related mRNAs were up-regulated in SA group (TLR2, 5 mRNAs expression, $P < 0.01$; TLR6 mRNA expression $P < 0.05$), MRC (mannose receptor C) and TLR9 mRNA expression was down-regulated without significant difference. Compared with SA group, 11 mRNAs expression was significantly up-regulated (TLR1, 2, 4-6, 8-10 mRNA expression, $P < 0.01$; MRC mRNA expression $P < 0.05$) (Table 5 and Figure 4).

4 Discussion

Inflammatory reaction plays an important role in different stages of coronary atherosclerosis, including the formation, stability, progress, and rupture of plaque and acute thrombosis.[2] As the most important inflammatory cells, neutrophil and mononuclear-macrophage and inflammatory factors work together promote atherosclerosis and the occurrence and development of acute coronary events.[3] It has been shown that the adhesive, chemotactic and phagocytic functions of neutrophil and mononuclear-macrophage are closely related to many cytokines and receptors, which express on their own surface.[8,9]

4.1 CSFs and their receptors related mRNAs expression

CSF is a multifunctional cytokines, which can stimulate the proliferation and differentiation of pluripotent hematopoietic stem cells and hemopoietic progenitor cells in the different development stage. It includes GM-CSF, M-CSF, G-CSF, erythropoietin, thrombopoietin and so on. M-CSF can induce hematopoietic progenitor cells to differentiate into mononuclear and macrophage, and increase the expression of scavenger receptor on macrophages surface to promote the atherosclerosis process.[10] GM-CSF mainly promotes the proliferation and differentiation of granulocyte and macrophage, enhances the adhesive, chemotactic and phagocytic functions of neutrophil and mononuclear, strengthens monocyte-macrophages adhesion and infiltration to endothelial cells, and then promotes the atherosclerosis inflammatory reaction.[11] G-CSF can adjust specifically the proliferation and differentiation of granulocyte, strengthen the function of mature granulocytes, increase the number of neutrophils, and promote inflammation.[12] The results of this study showed that: almost all CSFs and their receptors related mRNAs were up-regulated in AMI and SA groups compared with the control group, and the expression of GM-CSFR and G-CSFR mRNAs in the AMI group was significantly up-regulated than the SA and control groups ($P < 0.01$), suggesting that CSF is expressed obviously in coronary AS inflammatory reaction, and the expression of CSF showed a stepped upward trend as the disease progressed.

Table 4. Opsonic receptors related mRNAs expression.

Gene	AMI, n = 20	SA, n = 20	Control, n = 20	P_{ALL}	$P_{AMI\ vs.\ Control}$	$P_{AMI\ vs.\ SA}$	$P_{SA\ vs.\ Control}$
FCGR1	11.99 ± 0.76	11.41 ± 0.43	10.99 ± 0.69	**0.000	**0.000	*0.017	
FCGR2A	13.42 ± 0.50	12.68 ± 0.39	12.39 ± 0.29	**0.000	**0.000	**0.000	
FCGR2B	8.75 ± 0.49	8.52 ± 0.58	8.10 ± 0.41	**0.001	**0.000		*0.028
FCGR3A	16.48 ± 0.60	15.70 ± 0.56	15.53 ± 0.43	**0.000	**0.000	**0.000	
FCGR3B	13.39 ± 0.74	12.58 ± 0.69	12.34 ± 0.47	**0.000	**0.000	**0.001	
CR1	8.75 ± 0.55	7.71 ± 0.71	7.51 ± 0.62	**0.000	**0.000	**0.000	

Data are presented as mean ± SD. P_{ALL}: the P-value compared among the 3 groups. *$P < 0.05$, **$P < 0.01$. AMI: acute myocardial infarction group; CR1: complement ceceptor 1; FCGR1: Fc fragment of IgG receptor I; FCGR2A: Fc fragment of IgG receptor IIa; FCGR2B: Fc fragment of IgG receptor IIb; FCGR3A: Fc fragment of IgG receptor IIIa; FCGR3B: Fc fragment of IgG receptor IIIb; SA: stable angina group.

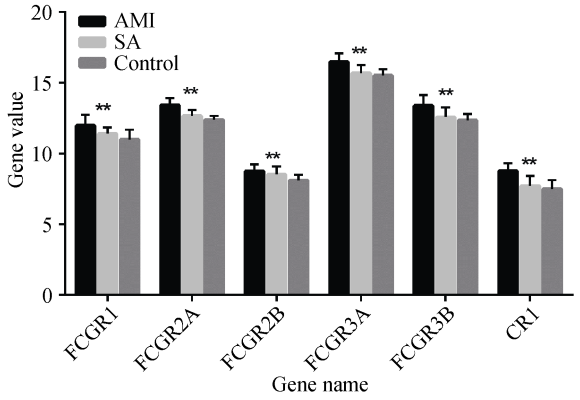

Figure 3. Opsonic receptors related mRNAs expression (P_{ALL} < 0.01).** AMI: acute myocardial infarction group; CR1: complement ceceptor 1; FCGR1: Fc fragment of IgG receptor I; FCGR2A: Fc fragment of IgG receptor IIa; FCGR2B: Fc fragment of IgG receptor IIb; FCGR3A: Fc fragment of IgG receptor IIIa; FCGR3B: Fc fragment of IgG receptor IIIb; SA: stable angina group.

4.2 Main chemokines and their receptors related mRNAs expression

Chemokines and their receptors play important roles in activation and migration of inflammatory cells and are contributing factors in the progression of AS. Studies have shown that the occurrence and development of coronary atherosclerotic heart disease are closely related to main chemokines such as MCP-1, MIP-1 and IL-8.[13–15] These chemokines can enhance the chemotaxis and activation of neutrophil and monocyte, induce macrophages to release inflammatory mediators, promote endothelial cell activation and the expression of adhesion molecules, promote monocyte adhesion and infiltration on vascular endothelial cells and then transform into macrophages to swallow lipid, finally lead to foam cell and plaque formation. In this study, we found that the expression of mRNAs related to MCP-1, CCR2 and CXCR2 was significantly up-regulated in AMI group compared with SA and control groups ($P < 0.01$). IL-8 mRNA expression in the AMI group was clearly higher ($P < 0.05$) than the controls. The result indicated that the chemokines MCP-1 and IL-8 are closely related to the occurrence and development of atherosclerosis and acute ischemic events, moreover, the expression of MCP-1 and IL-8 showed a stepped upward trend as the disease progressed.

4.3 Opsonic receptors and PRR related mRNAs expression

Epidemiological and clinicopathologic studies have suggested that infection factors such as bacteria, viruses and chlamydia are associated with the occurrence and development of atherosclerosis and CAD.[16,17] Infection factors may initiate and maintain inflammatory response through innate immune and play roles in the formation and development of atherosclerosis. Studies have suggested that in innate immune, opsonic receptors (IgG FcR and C3bR/C4bR) and pattern recognition receptors (MR, MSR, TLR) in the surface of neutrophil and mononuclear-macrophage play important roles in the progression of atherosclerosis and CAD through mediating Inflammation.[18–20] The results of this study showed that: (1) all the mRNAs related to opsonic receptors (IgG FcR and C3bR/C4bR) expression was significantly up-regulated ($P < 0.01$) in AMI group compared with SA and control group. The SA group showed an

Table 5. PRRs related mRNAs expression.

Gene	AMI, n = 20	SA, n = 20	Control, n = 20	P_{ALL}	$P_{AMI\ vs.\ Control}$	$P_{AMI\ vs.\ SA}$	$P_{SA\ vs.\ Control}$
MRC	4.60 ± 0.52	3.98 ± 0.81	4.00 ± 1.01	*0.030		*0.050	
MSR	4.49 ± 0.64	4.49 ± 0.72	3.70 ± 0.85	**0.005	**0.004		
TLR1	12.26 ± 0.52	11.70 ± 0.30	11.41 ± 0.34	**0.000	**0.000	**0.000	
TLR2	13.54 ± 0.55	12.78 ± 0.47	12.26 ± 0.22	**0.000	**0.000	**0.000	**0.001
TLR3	4.86 ± 0.72	4.98 ± 0.59	4.90 ± 0.65	0.837			
TLR4	10.26 ± 0.51	9.39 ± 0.42	9.13 ± 0.32	**0.000	**0.000	**0.000	
TLR5	9.80 ± 0.65	8.61 ± 0.43	8.13 ± 0.34	**0.000	**0.000	**0.000	**0.010
TLR6	11.12 ± 0.46	10.56 ± 0.32	10.22 ± 0.32	**0.000	**0.000	**0.000	*0.016
TLR7	5.58 ± 0.52	5.38 ± 0.38	5.37 ± 0.46	0.253			
TLR8	9.77 ± 0.51	9.01 ± 0.38	8.88 ± 0.32	**0.000	**0.000	**0.000	
TLR9	7.14 ± 0.36	6.71 ± 0.32	6.75 ± 0.26	**0.000	**0.001	**0.000	
TLR10	6.38 ± 0.47	5.98 ± 0.39	5.79 ± 0.37	**0.000	**0.000	**0.008	

Data are presented as mean ± SD. P_{ALL}: the P-value compared among the 3 groups. *$P < 0.05$, **$P < 0.01$. AMI: acute myocardial infarction group; MRC: mannose receptor C; MSR: macrophage scavenger receptor; TLR 1-10: Toll like receptor 1-10; SA: stable angina group.

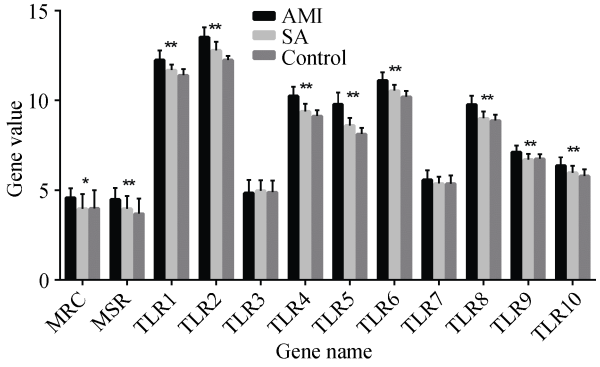

Figure 4. PRRs related mRNAs expression (*P_{ALL}< 0.05, **P_{ALL} < 0.01). AMI: acute myocardial infarction group; MRC: mannose receptor C; MSR: macrophage scavenger receptor; TLR 1-10: toll like receptor 1-10; SA: stable angina group.

upward trend compared with the controls. (2) Most PRR related mRNAs expression was up-regulated in AMI group compared with SA and control groups. Most TLR mRNAs expression was significantly up-regulated than the SA and control groups except TLR3 and TLR7 ($P < 0.01$); TLR2, 5, 6 mRNAs were significantly up-regulated ($P < 0.05$) in SA group compared with the control group. MSR mRNA was significantly up-regulated ($P < 0.01$) in AMI group compared with the control group. The result indicated that in the progression of CAD, especially AMI, (1) opsonic receptors mRNAs expression is significantly up-regulated in neutrophil and mononuclear-macrophage, which can enhance the phagocytosis of phagocytes and promote inflammatory response obviously; (2) PRR mRNAs expression in mononuclear-macrophage is significantly up-regulated. MSR can strengthen the adhesion and aggregation of monocyte to vascular endothelial cell, and then promote the inflammation in atherosclerosis area. Besides, MSR plays an important role in macrophage foam cell formation by promoting macrophage to swallow oxidized low density lipoprotein (ox LDL).[21] TLRs activate signal transduction pathways such as the pathways of NF-κβ and mitogen-activated protein kinase after binding related ligands, and initiate mRNA transcription of inflammatory cytokines and promote inflammation through inducing mononuclear cells gathered to atherosclerosis.[18]

4.4 Conclusions

In the process of coronary atherosclerosis and AMI, Functionally related genes mRNAs expression in neutrophil and mononuclear-macrophage was up-regulated and significantly up-regulated, including CSF and their receptors, chemokines and their receptors, opsonic receptors, PRR. This result suggested that the enhancement of adhesive, chemotactic and phagocytic functions in neutrophil and mononuclear-macrophage goes through the entire occurrence, development of CAD. Moreover, this functional enhancement will become more remarkable with the deterioration of coronary atherosclerosis disease.

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