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S100A8/A9 promotes MMP-9 expression in the fibroblasts from cardiac rupture after myocardial infarction by inducing macrophages secreting TNFα

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Abstract. – OBJECTIVE: Inflammation and extracellular matrix degradation play a role in cardiac rupture (CR) after myocardial infarction (MI). It has been found that the expression of inflammatory cytokine S100A8/A9 was elevated in acute MI patients, whereas its impact in CR after infarction remains unclear.

PATIENTS AND METHODS: Samples from cardiac tissue and peripheral blood of patients with CR after MI, MI, patients without CR, and healthy control (cardiotrauma) were collected to test the expressions of S100A8/A9, p-p65, and MMP-9. Co-culture system for HCF cells and macrophages were established to identify the impact of hypoxia-ischemia on the expressions of S100A8/A9 and TNFα. S100A9 and/or TNFα blocking agent were applied to examine the effect on macrophages migration, expressions of S100A8, S100A9, and TNFα. Western blot was adopted to determine levels of p-p65 and MMP-9 protein after the inhibition of S100A9 and/or TNFα.

RESULTS: Compared with healthy control and non-CR patients, serum S100A8/A9 and MMP-9 levels were elevated in cardiac tissues of CR patients, while S100A8/A9, p-p65, and MMP-9 were also overexpressed. Hypoxia-ischemia significantly caused the increasing levels of S100A8/A9 and TNFα in macrophages ($p < 0.05$). The blockade of S100A9 and/or TNFα suppressed the activation and migration of macrophages. The inhibition of S100A9 expression also decreased the secretion of TNFα in macrophages, while the suppression of TNFα showed no significant impact on S100A8 and S100A9 levels. Downregulation of TNFα or NF-κB markedly declined p-p65 and MMP-9 protein levels in HCF cells from co-culture system or single culture, whereas the blockade of S100A9 only reduced their expressions in co-cultured HCF cells.

CONCLUSIONS: The level of S100A8/A9 was upregulated in MI patients with CR. S100A8/A9 induced the activation of NF-κB and expression of MMP-9 protein in HCF cells through facilitating secretion of TNFα from macrophages, which may play a role in triggering extracellular matrix degradation and CR.

Key Words:
Acute myocardial infarction, Cardiac rupture, S100A8/A9, Macrophages, Cardiac fibroblasts, TNFα, NF-ÐB, MMP-9.

Introduction

Acute myocardial infarction (AMI) refers to myocardial necrosis caused by coronary artery blood acute reduction or interruption, persistent ischemia hypoxia on the basis of original coronary artery lesions[1]. Cardiac rupture (CR) is one of the most serious and common complications of AMI. It is also the leading cause of death in MI patients, accounting for about 20-30% of all deaths[2]. CR involves ventricular free wall, ventricular septum, and papillary muscles, and results in left ventricular free wall rupture, ventricular septal defect, and papillary muscle rupture. Although rapid development of medical science and improvement of technology greatly reduced the mortality of AMI in early stage, it failed to effectively control CR in late stage caused by ventricular remodeling, which gradually become the leading cause of death in MI patients[3]. Therefore, the prevention and treatment of CR after MI become an urgently problem to be solved all over the world. Inflammation participates in the whole process of coronary artery disease, AMI, cardiac remodeling after MI, and CR. The infiltration and aggregation of a large number of inflammatory cells, together with the release of inflammatory

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cytokines, play an important role in the occurrence of CR after AMI. CR is usually the result of the myocardial remodeling defects after MI. After infarction, excessive inflammatory response leads to imbalance between degradation and synthesis of extracellular matrix (ECM) and eventually results in CR. S100 calcium-binding protein A8/A9 complex (S100A8/A9), also known as myeloid related protein (MRP or MRP8/14), is secreted by activated neutrophils[4] and mononuclear macrophages[5] that plays a critical mediating role in inflammation. It was found that S100A8/A9 content was significantly elevated in serum from AMI patients, suggesting the role of S100A8/A9 in AMI[6-8]. However, its role in CR induced by MI has not been reported.

Patients and Methods

Main Reagents and Materials

Human cardiac fibroblasts (HCF) were bought from ATCC (Manassas, VA, USA). DMEM (Dulbecco's Modified Eagle's medium), α-MEM (Modified Eagle's medium), FBS (fetal bovine serum), and penicillin-streptomycin were from Gibco (Thermo Fisher Scientific, Waltham, MA, USA). Recombinant human S100A8/A9 Heterodimer and TNFα (tumor necrosis factor α) were bought from R&D Systems (Minneapolis, MN, USA). Human recombinant M-CSF (colony-stimulating factor) was bought from Peprotech (Rocky Hill, NJ, USA). Reverse transcription kit PrimeScript RT reagent Kit was got from TaKaRa (Otsu, Shiga, Japan). SYBR Green Real-time PCR (polymerase chain reaction) kit was bought from Toyobo (Osaka, Japan). PCR primers were designed and synthetized by Genepharma (Shanghai, China). Mouse anti-human S100 A8/A9 complex antibody and rabbit anti-human phospho-NF-κB p65 (S536) antibody were purchased from Abcam (Cambridge, MA, USA). Anti-TNFα blocking antibody was from R&D system (Minneapolis, MN, USA). S100A9 blocking peptide antibody was bought from GeneTex (Irvine, CA, USA). Mouse anti human MMP-9 antibody was from Santa Cruz (Santa Cruz, CA, USA). HRP (horseradish peroxidase) coupled goat anti-mouse and goat anti-rabbit secondary antibodies were from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA, USA). Human MMP-9 and TNFα ELISA kits were bought from eBioscience (Thermo Fisher Scientific, Waltham, MA, USA). Mouse anti-human CD68 antibody, Alexa Fluor 647 tagged S100A8/A9 flow antibody, and PE-tagged TNFα flow anti-body were got from BD Pharmingen (San Jose, CA, USA). Human S100 A8/A9 ELISA kit was from USCNK (Houston, TX, USA). BCA (bicinchoninic acid) protein quantification kit was from Beyotime (Beijing, China). NF-κB specific inhibitor PDTC was from Selleckchem (Houston, TX, USA).

Clinical Sample Collection

A total of 22 cases of AMI patients with CR received treatment in the Fifth Affiliated Hospital, Xin-Jiang Medical University between May 2012 and Jun 2015 were enrolled as AMI CR group. A total of 25 cases of AMI patients without CR in the corresponding period were selected as AMI without CR group. Another 9 cases of normal cardiac tissue got from cardiotrauma surgery were set as normal control. MI diagnosis criteria contains Troponin I (TnI) elevation over 99% of the upper limit, together with one of the following clinical evidences of myocardial ischemia, including myocardial ischemia symptoms: chest pain persists for more than half an hour, electrocardiogram shows new myocardial ischemia change (new ST changes or new left bundle branch block), and electrocardiogram shows new pathologic Q wave, new appeared myocardial inactivation or new regional ventricular wall motion abnormalities. All the diagnosis of CR was confirmed by echocardiography and together with the corresponding clinical manifestations, such as chest pain, nausea and vomiting, sudden loss of consciousness, a sharp drop in blood pressure, and disappearance of artery wave. Cardiac tissue and peripheral blood specimens were collected within 12 h after MI onset, of which tissue samples were stored at -80°C immediately after liquid nitrogen, and the serum separated from peripheral blood was saved at -80°C. This study has been pre-approved by the Ethical Committee of the Fifth Affiliated Hospital, Xin-Jiang Medical University. All the patients have signed the consent forms before recruitment in this study.

HCF Cultivation and Hypoxia-Ischemia Treatment

HCF was cultured in DMEM (Dulbecco's Modified Eagle's medium) medium supplemented by 10% FBS (fetal bovine serum), 100 U/ml penicillin, and 100 μg/ml streptomycin and maintained at 37°C and 5% CO_2. The cells were passaged or applied for experiment when reached confluence

of 80%. Hypoxia-ischemia treatment: the cells in logarithmic phase were treated by low glucose serum free DMEM to simulate ischemia *in vivo*, and then maintained in incubator with 1% O_2, 5% CO_2, and 94% N_2 to simulate hypoxia[8].

Human Bone Marrow Macrophage Isolation and Induction Culture

A total of 10 ml bone marrow was extracted from the iliac bone graft patients without cardiac disease. After the bone marrow was diluted by equal volume phosphate-buffered solution (PBS), it was separated by density gradient centrifugation using Ficoll separating medium to isolate bone marrow mononuclear cells. The cells were maintained in α-MEM (Modified Eagle's medium) supplemented by 10% fetal bovine serum (FBS), 20 ng/ml M-CSF (colony-stimulating factor), 100 U/ml penicillin, and 100 μg/ml streptomycin. The un-adherent cells were removed after two days. After another 8 days cultivation, macrophages were fully differentiated and adopted for the following experiments.

HCF and Macrophages Co-Culture System Establishment

The macrophages were seeded in upper chamber, and the HCF was cultured in lower chamber of transwell chamber. After co-cultured in the abovementioned hypoxia-ischemia condition for 3, 6, and 12 h, the medium was collected to test TNFα, S100 A8/A9, and MMP-9 contents by ELISA, while the cells were collected for mRNA and protein expression detection.

Co-Culture System Grouping and Treatment

The co-culture system under hypoxia-ischemia condition was divided into four groups. Control group received no specific treatment. Blocking S100A9 group was treated by 3 μg/ml S100A9 blocking peptide. Blocking TNFα group was added with 0.2 μg/ml anti-TNFα blocking antibody. Blocking S100A9 + TNFα group was treated by both types of blocking reagents.

ELISA Detection

ELISA (enzyme linked immunosorbent assay) detection was performed according to the manual. Specially, a total of 100 μl coating antibody was added to 96-well plate at 4°C overnight. After washed by Wash Buffer for three times, a total of 200 μl blocking buffer was added at room temperature for 60 min. After washed by Wash Buffer for 1 time, 100 μl sample or standard substance was added to the plate at room temperature for 2 h. Next, 100 μl detection antibody was incubated at room temperature for 60 min. After washed for 4 times, 100 μl Avidin-HRP was added to the plate at room temperature for 30 min. At last, 100 μl TMB (3,3',5,5'-Tetramethylbenzidine) reaction liquid was treated at room temperature for 15 min and the reaction was terminated by 50 μl stop buffer. The plate was detected by microplate reader at 450 nm (BD, San Jose, CA, USA).

qRT-PCR (Quantitive Reverse Transcription Polymerase Chain Reaction)

Total RNA was extracted using TRIzol method and was reverse transcripted to cDNA using PrimeScript RT reagent Kit. The cDNA was used for PCR reaction, and the primers used were as follows. MMP-9P_F: 5′-TGTACCGCTATGGTTACACTCG-3′, MMP-9P_R: 5′-GGCAGGGACAGTTGCTTCT-3′; TNF-αP_F: 5′-CCTCTCTCTAATCAGCCCTCTG-3′, TNF-αP_R: 5′-GAGGACCTGGGAGTAGATGAG-3′; S100A8P_F: 5′-ATGCCGTCTACAGGGATGAC-3′, S100A8P_R: 5′-ACTGAGGACACTCGGTCTCTA-3′; S100A9P_F: 5′-GGTCATAGAACACATCATGGAGG-3′, S100A9P_R: 5′-GGCCTGGCTTATGGTGGTG-3′; β-actinP_F: 5′-GAACCCTAAGGCCAAC-3′, β-actinP_R: 5′-TGTCACGCACGATTTCC-3′. The PCR reaction system in 10 μl contained 4.5 μL 2×SYBR Green Mixture, 0.5 μL primer (2.5 μm/L), 1 μL cDNA, and 3.5 μL ddH_2O. PCR reaction was performed on ABI ViiA7 amplifier at 40 cycles of 95°C for 15 s, 60°C for 30 s, and 74°C for 30 s. β-actin were adopted as internal references. The detection of each sample was repeated for three times. Comparative Ct method was applied for quantitative analysis (ABI, Waltham, MA, USA).

Western Blot

Total protein was extracted and quantified by BCA method. A total of 50 μg protein was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membrane. After blocked in 5% skim milk at room temperature for 1 h, the membrane was incubated in primary antibody at 4°C overnight (p-p65 1:200, MMP-9 1:300, β-actin 1:500). After washed by PBST for three times, the membrane was further incubated in HRP-tagged secondary antibody at room temperature for 60 min (1:10000). At last,

the membrane was treated by electrochemiluminescence (ECL) (Bio-Rad, Hercules, CA, USA) and scanned on Epson for data collection.

Flow Cytometry

At 12 h before flow cytometry detection, 1‰ volume of Brefeldin A solution was added to the cells to prevent cytoplasmic S100 A8/A9 protein secreted to extracellular space. HCF cells and macrophages were digested by enzyme and washed by PBS containing 0.5% BSA (bovine serum albumin). Next, the cells were fixed in 100 µl fixing solution at room temperature for 30 min and treated by 2 ml of perforation liquid. After centrifuged at 300 g, the cells were resuspended and added with 5 µl of Alexa Fluor 647 tagged S100 A8/A9 flow antibody or PE tagged TNFα flow antibody at room temperature in the dark for 40 min. After treated by 2 ml perforation liquid and centrifuged at 300 g for 5 min, the cells were washed by PBS containing 0.5% BSA and resuspended in 500 µl PBS containing 0.5% BSA for detection (BD, San Jose, CA, USA).

Transwell Assay Detection of Cell Chemotaxis

Type IV collagen was applied to coat maxi-cell-in-serts with 8 µm pore diameter for 24 h. Macrophages were seeded in upper chamber, while HCF cells were cultured in lower chamber for 12 h. After washed by PBS for two times, the transwell chamber was fixed by methanol for 30 min and stained by 0.1% crystal violet for 20 min. Five randomly visual fields were selected under 400× microscope (Olympus, Shinjuku, Tokyo, Japan) for calculation.

Statistical Analysis

All data analysis was performed on SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). Measurement data was presented as mean ± standard deviation. *t*-test was applied for group comparison. $p < 0.05$ was considered as statistical significance.

Results

S100A8/A9 and MMP-9 Expressions were Upregulated in CR Patients

ELISA detection showed that the levels of S100A8/A9 and MMP-9 in peripheral blood from AMI patients were significantly higher than that in normal control ($p < 0.05$), and the levels were even higher in CR patients, suggesting that the inflammation was more serious in AMI patients with CR with stronger degradation of extracellular matrix (Figure 1A and 1B). The results of qRT-PCR and Western blot demonstrated similar trends with ELISA data. The levels of S100A8, S100A9, and MMP-9 mRNA were significantly upregulated in AMI patients, especially with CR (Figure 1C and 1D). S100A8/A9 complex and MMP-9 protein expressions were also elevated, along with the remarkable increase of NF-κB p65 phosphorylation (Figure 1E).

Hypoxia-Ischemia Increased S100A8/A9 and TNFα Expression in Macrophages

Flow cytometry revealed that after M-CSF induction for 10 days, the macrophage specific marker, CD68, was expressed in more than 90% cells, indicating the successful induction of macrophage (Figure 2A). This study established hypoxia-ischemia model to investigate hypoxia-ischemia impact on S100A8/A9 expression. S100A8/A9 content was extremely low in co-culture system under normal condition, while it was elevated after hypoxia-ischemia treatment in a time dependent manner (Figure 2B). To explore the source of S100A8/A9, flow cytometry was applied to detect S100A8/A9 expression in HCF and macrophages. Almost no S100A8/A9 was expressed in macrophages under normal condition in co-culture system (Figure 2C). S100A8/A9 level was gradually elevated in macrophages following hypoxia-ischemia extension, revealing that hypoxia-ischemia may upregulate the synthesis and expression of S100A8/A9 in macrophages. On the contrary, no S100A8/A9 was detected in HCF cells under transwell co-culture (Figure 2D). The data demonstrated that S100A8/A9 was secreted from macrophages to the medium. In addition, hypoxia-ischemia significantly increased TNFα expression in macrophages (Figure 2E), and promoted its extracellular secretion (Figure 2B). However, hypoxia-ischemia showed no obvious impact on TNFα expression in HCF cells (Figure 2E).

S100A8/A9 Activated Macrophages and Upregulated TNFα Expression

Our study showed that hypoxia-ischemia markedly upregulated the expressions and secretion of S100A8/A9 and TNFα in macrophages, while no obvious impact was found in HCF cells, suggesting that hypoxia-ischemia may play a role in macrophage activation. We further discuss

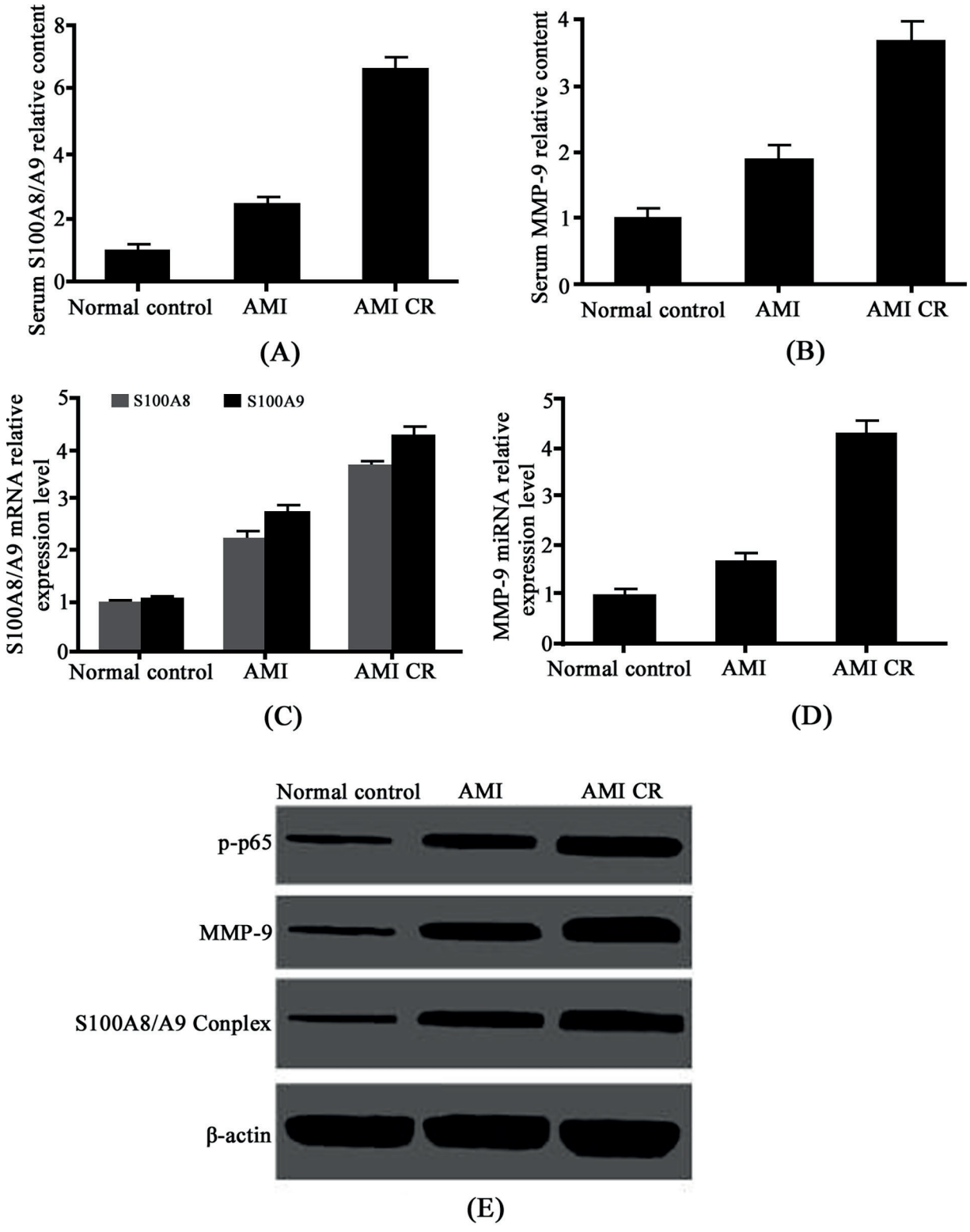

Figure 1. S100A8/A9 and MMP-9 expression upregulated in CR patients. ***(A)*** ELISA detection of peripheral blood S100A8/A9 complex content. ***(B)*** ELISA detection of peripheral blood MMP-9 content. ***(C)*** qRT-PCR detection of myocardial tissue S100A8 and S100A9 mRNA expression. ***(D)*** qRT-PCR detection of myocardial tissue MMP-9 mRNA expression. ***(E)*** Western Blot detection of myocardial tissue protein expression.

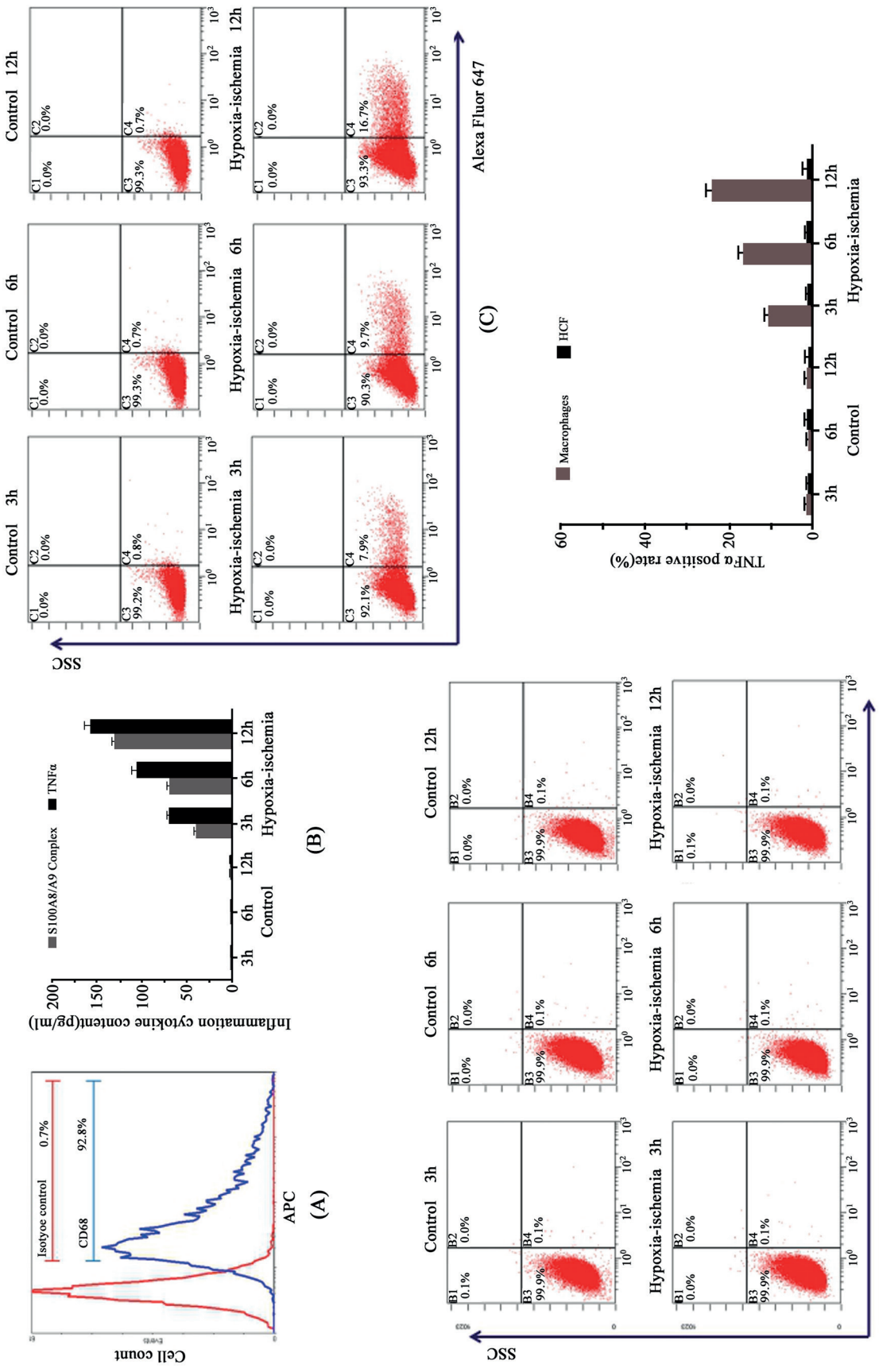

Figure 2. Hypoxia-ischemia increased S100A8/A9 and TNFα expression in macrophages. ***(A)*** Flow cytometry detection of macrophage differentiation marker CD68. ***(B)*** ELISA detection of S100A8/A9 content in medium. ***(C)*** Flow cytometry detection of S100A8/A9 expression in macrophages. ***(D)*** Flow cytometry detection of S100A8/A9 expression in HCF cells. ***(E)*** Flow cytometry detection of TNFα expression in HCF cells and macrophages.

whether S100A8/A9 and TNFα play roles in macrophage activation induced by hypoxia-ischemia. Transwell assay revealed that hypoxia-ischemia treatment significantly activated macrophages and promoted macrophages migration after 12 h (Figure 3A). However, the activation and migration were weakened after the blockade of S100A8/A9 and/or TNFα (Figure 3A). qRT-PCR results showed that blocking S100A9 significantly reduced TNFα gene expression in macrophages, whereas blocking TNFα had no obvious influence on S100A8 and S100A9 expressions. The result indicated that under hypoxia-ischemia condition, S100A8/A9 served as an upstream regulator for TNFα. Hypoxia-ischemia promoted macrophages activation and upregulated TNFα expression through elevating the expression of inflammatory factor S100A8/A9 (Figure 3B).

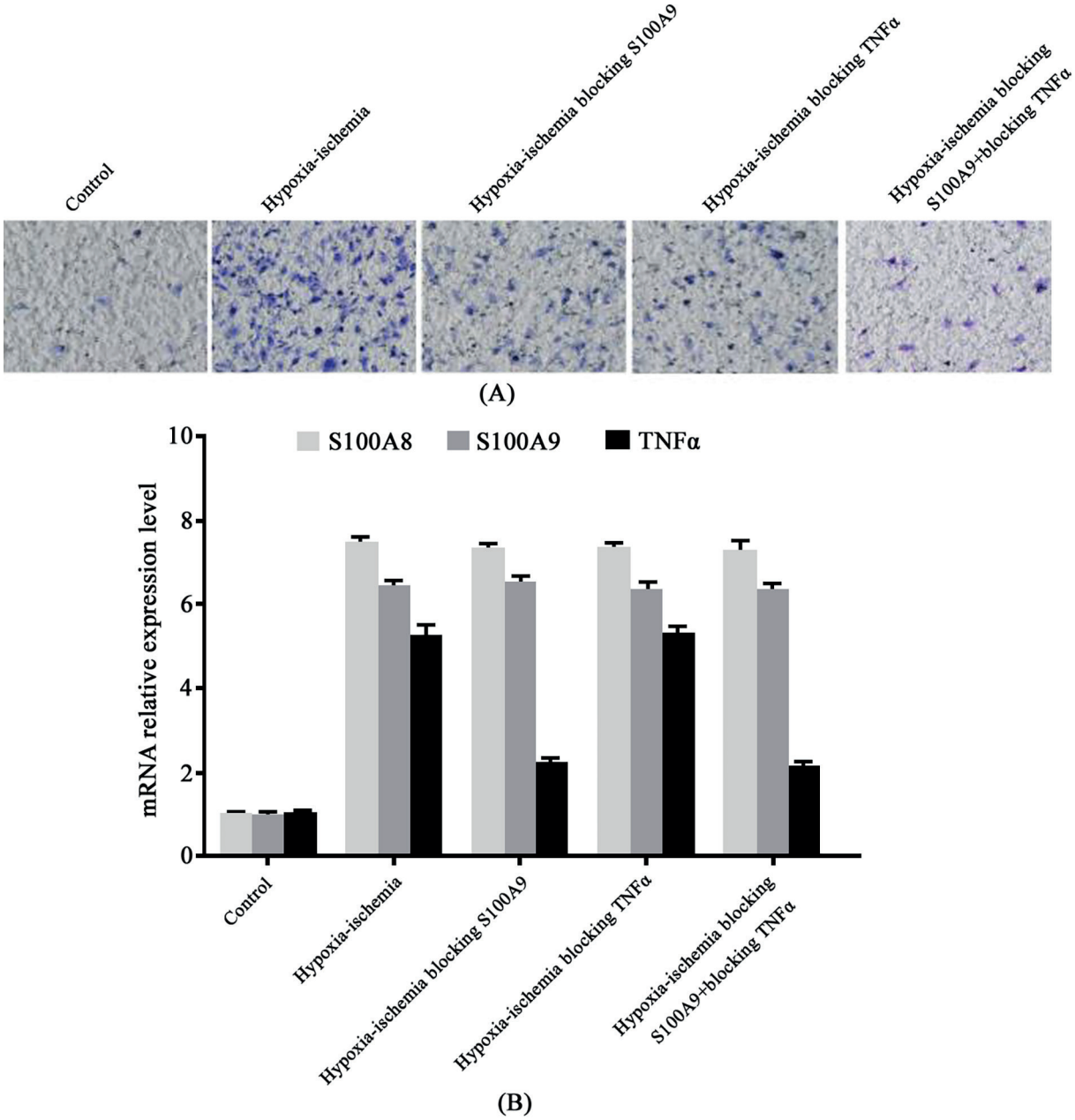

Figure 3. S100A8/A9 activated macrophages and upregulated TNFα expression. ***(A)*** Transwell detection of macrophages migration. ***(B)*** qRT-PCR detection of mRNA expression in macrophages.

TNFα Activated NF-κB Signaling Pathway and Upregulated MMP-9 Expression in HCF Cells

The integrity of the cardiac structure and function is largely determined by the myocardial interstitium. The key factor of myocardial remodeling defects after MI induced CR is the damage and loss of extracellular matrix. Cardiac fibroblasts make up about 90-95% of the heart cells, which play a critical role in regulating myocardial interstitial tissue and affecting the synthesis and metabolism of extracellular matrix. This study observed the impact of hypoxia-ischemia on MMP-9 expression in HCF cells, and discussed the related mechanism. In normal condition, almost no MMP-9 protein and extremely low level of NF-κB p65 phosphorylation were detected in HCF cells (Figure 4A). The level of MMP-9 protein was significantly elevated over time, and the phosphorylation of NF-κB p65 protein was enhanced in HCF cells under hypoxia-ischemia condition, suggesting that hypoxia-ischemia induced MMP-9 expression in cardiac HCF cells. The elevation of MMP-9 may play a role in hypoxia-ischemia induced CR after MI. This study found that hypoxia-ischemia promoted macrophages activation and upregulated TNFα level through enhancing the expression and function of S100A8/A9. We also found that, in co-culture system, MMP-9 protein and NF-κB p65 phosphorylation in HCF cells were inhibited after blocking S100A8/A9 or TNFα, and the suppression effect became strongest after blocking the both (Figure 4B), revealing that both S100A8/A9 and TNFα may affect NF-κB activation and MMP-9 expression in HCF cells in co-culture system. In single HCF cell culture system, hypoxia-ischemia failed to cause MMP-9 and p-p65 protein elevation in HCF cells, suggesting that the NF-κB activation and MMP-9 overexpression in HCF cells were dependent on S100A8/A9 and/or TNFα secreted by macrophages (Figure 4C). We added recombinant S100A8/A9 Heterodimer and/or TNFα

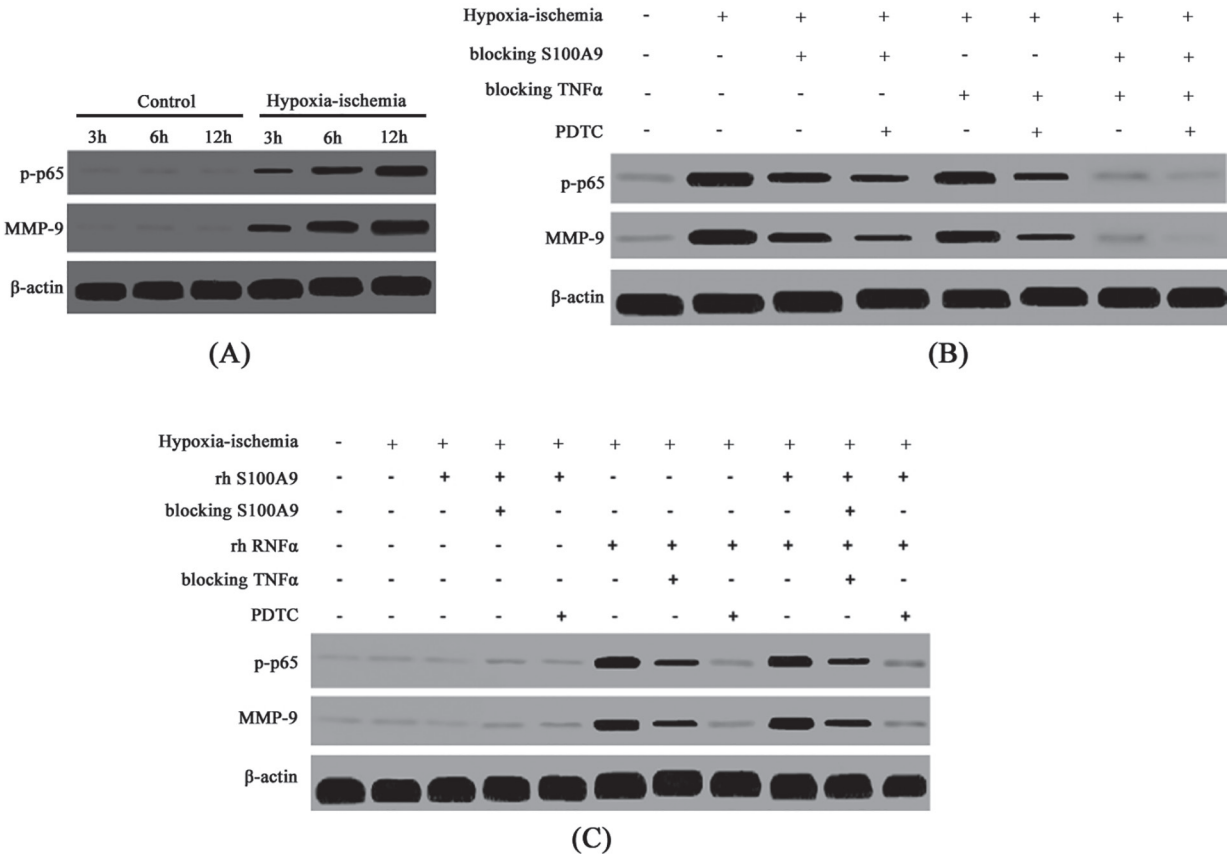

Figure 4. TNFα activated NF-κB signaling pathway and upregulated MMP-9 expression in HCF cells. ***(A)*** Western Blot detection of hypoxia-ischemia impact on protein expression in HCF cells. ***(B)*** Western Blot detection of protein expression in co-cultured HCF cells. ***(C)*** Western Blot detection of protein expression in single cultured HCF cells.

protein to single cultured HCF cells, and found that single S100A8/A9 Heterodimer showed no obvious impact on NF-κB activity and MMP-9 expression in HCF cells treated by hypoxia-ischemia. Blocking S100A9 or adding NF-κB inhibitor PTDC also failed to affect NF-κB activity and MMP-9 expression in HCF cells. It suggested that under hypoxia-ischemia condition, NF-κB activity and MMP-9 expression in HCF cells did not directly rely on S100A8/A9. Single TNFα treatment significantly enhanced NF-κB activity and facilitated MMP-9 expression, whereas blocking TNFα or PTDC application significantly antagonized TNFα impact on NF-κB activity and MMP-9 expression, revealing that NF-κB activation under hypoxia-ischemia condition depended on TNFα effect, while MMP-9 upregulation requires the involvement of NF-κB activity. The combined treatment of S100A8/A9 Heterodimer and TNFα showed similar effect on NF-κB activity and MMP-9 expression in HCF cells with single TNFα treatment, further confirming that S100A8/A9 did not affect HCF cells biological process directly, without co-culture of macrophages (Figure 4C). The results demonstrated that under hypoxia-ischemia condition, TNFα secreted by macrophages can directly enhance NF-κB activity and upregulate MMP-9 protein expression in HCF cells.

Discussion

CR is a kind of serious fatal complication of AMI, which has become the second cause of AMI hospital death[9]. AMI results in a series of inflammatory cells activation and the release of a large number of inflammatory cytokines, and triggers the inflammatory repair process of the infarction myocardial tissue, including dead cells clearance, extracellular matrix degradation, and collagen fibrous tissue replacement[10]. Excessive inflammation plays a critical role in CR occurrence. It was found that the incidence of CR was positively correlated with the strength of the inflammatory response, and CR patients after AMI showed more serious inflammation than non-CR patients[11]. CR is usually the result of myocardial remodeling defect after MI.S100A8/A9, produced by activated neutrophils[4] and mononuclear macrophage[5], plays an important role in mediating inflammation. This study found that S100A8/A9 level in the peripheral blood and myocardial tissue from AMI patients was significantly increased compared with normal control group, which was in accordance with Katashima et al[6] and Du et al[7] findings.

Matrix metalloproteinases (MMPs), a zinc ion-dependent extra-cellular proteinase in the human body, could take part in the physiological processes, including the regulation of the generation of cancers[12]. MMPs play a vital role in extracellular matrix degradation, of which, MMP-9 upregulation not only occurred in AMI patients[13], also related to the occurrence of CR after MI[14]. We observed higher MMP-9 expression in CR patients than non-CR patients, which was in consistent with Kameda's report[14]. Macrophage activation and migration towards the infarcted myocardium area is an important process of inflammatory repair initiation after MI[15, 16]. Therefore, this study established *in vitro* co-culture model of hypoxia-ischemia HCF-macrophages to explore the function of inflammatory response in HCF and macrophages. The results showed that hypoxia-ischemia can significantly elevate S100A8/A9 content in the medium, which was in accordance with the phenomena of S100A8/A9 content elevation in the peripheral blood from AMI patients. Aochi et al[8] found S100A8/A9 was expressed and secreted in both macrophages and HCF. Interestingly, in our research, flow cytometry further found that secreted S100A8/A9 was not from HCF but from activated macrophages, which might be similar with the phenomenon observed by previous finding that S100A8/A9 upregulation was not derived from myocardial cells[7]. After inflammatory reaction, macrophages are activated and migrate to the inflammatory injured part to secrete S100A8 and S100A9. Thus, S100A8/A9 is considered to be the biomarker of macrophages activation[17]. S100A8/A9 can activate downstream signaling pathways by binding the receptors such as advanced glycation end products (RAGE)[18] and Toll-like receptors 4 (TLR-4)[19], thus inducing the migration of inflammatory cells to inflammatory site and to release a large number of inflammatory cytokines[20].

It has been demonstrated that MMP-9 leads to myocardial remodeling by fracturing interstitial collagen and changing the permeability between blood vessels and myocardial bundles basement membrane[21,22]. The expression and function of MMP-9 is regulated by NF-κB. It was reported that the inactivity of MMP-9 can significantly reduced the incidence of CR[23]. Timmers et al[24] showed that the transcription

activity of NF-κB was markedly increased after MI, while the knockout of NF-κB p50 subunit can significantly reduce the risk of CR after MI. These results suggested the possible role of NF-κB transcription activity and MMP-9 expression in MI and CR. This study found that myocardial tissue phosphor-NF-κB p65 protein level was significantly enhanced in CR patients, which was in accordance with the change of MMP-9 expression, indicating that the transcription activity of NF-κB may contribute to elevating MMP-9 level and inducing CR. TNFα is a well-known activator of NF-κB signaling pathway and is involved in the regulation of the transcriptional activity of NF-κB, cell proliferation, apoptosis, migration, and inflammatory response[25, 26]. Sun et al[27] discovered that NF-κB activity and MMP-9 expression in infarcted myocardial tissue was significantly suppressed in TNFα knockout mouse, and the incidence of CR was also significantly reduced, revealing the role of TNFα/NF-κB/MMP-9 axis in CR. Xue et al[28] also found the critical role of TNFα in regulating the transcriptional activity of NF-κB and expression of MMP-9, as well as affecting vascular remodeling. We found that hypoxia-ischemia significantly promoted NF-κB activity and MMP-9 expression in HCF cells. In the hypoxia-ischemia co-culture system, both blocking S100A8/A9 and/or TNFα suppressed NF-κB activity and MMP-9 expression in HCF cells. We further found that NF-κB activity and MMP-9 expression upregulation in HCF cells did not rely on S100A8/A9 directly but TNFα activation. The results showed that hypoxia-ischemia significantly upregulated S100A8/A9 expression, activated macrophages, and promoted TNFα secretion.

Conclusions

We showed that S100A8/A9 expression was significantly enhanced in CR patients after MI. The up-regulation of S100A8/A9 induced NF-κB activity and MMP-9 expression in HCF cells by activating macrophages and promoting secretion of TNFα, which participates in extracellular matrix degradation, myocardial remodeling defects, and CR.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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