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Review Article

The role of S100A8/A9 in cardiac rupture after myocardial infarction

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Abstract: S100A8/A9 signaling pathway is believed to be related with inflammation. Study found elevated S100A9/A9 level in patients with acute cardiac infarction. Due to the involvement of acute inflammation of cardiac tissues in the heart rupture after cardiac infarction, this study investigated the role of S100A8/A9 in cardiac rupture. Mouse model with cardiac infarction was established. Recombinant lentiviral vector over-expressing or interfering S100A8/A9 was injected into cardiac tissues. The morality rate of mice was observed within 1 week, along with the detection of myeloperoxidase (MPO) expression in cardiac tissues by immunohistochemical staining. Over-expression of S100A8/A9 effectively increased the mortality rate of model mice, while RNA interference against S100A8/A9 significantly decreased the mortality rate. Post-mortal examination revealed a positive correlation between cardiac rupture incidence and S100A8/A9 level, which was also directly correlated with MPO expression after cardiac infarction. Inhibition of S100A8/A9 can effectively reduce the inflammatory response after cardiac infarction. In conclusion, S100A8/A9 may enhance the incidence of heart rupture after myocardial infarction via facilitating inflammatory response.

Keywords: Inflammatory response, myocardial infarction, cardiac rupture

Introduction

Cardiac rupture is one severe complication after acute myocardial infarction. Although the development of re-perfusion approaches such as percutaneous transluminal coronary angioplasty (PTCA) has made major progresses in the treatment of cardiac infarction, the incidence of cardiac rupture is still up to 6% in the recent 20 years, with mortality rate being around 58% [1, 2]. Therefore studying the pathogenesis and countermeasures of cardiac rupture after myocardial infarction is of critical importance.

The major reason for cardiac rupture after acute myocardial infarction was believed to be the acute inflammation, leading to necrosis of cardiac tissues and higher tension of ventricle walls, and subsequent heart rupture [3]. Consistent with this, the over-infiltration of neutrophil can be observed in the mesenchymal tissue of cardiac muscles in the ventricle wall of those patients [3]. Moreover, myeloperoxidase (MPO) gene knockout mice had shown certain protection against heart rupture during myocardial infarction [4]. The anti-inflammatory growth transformation factor-15 was also known to inhibit the occurrence of heart rupture after cardiac infarction [5]. Therefore suppression of acute inflammatory response may effectively inhibit the occurrence of cardiac rupture after myocardial infarction.

Belonged to S100 protein family, S100A8 and S100A9 proteins are mainly secreted by myeloid cells and believed to be related with inflammation. The abnormal higher level of serum S100A8/A9 heterodimer is an indicator of inflammation and closely related with various inflammatory diseases such as peripheral nerve injury-induced inflammatory response [6] and chronic glomerulonephritis [7]. Therefore S100A8/A9 may work as biological marker for focal inflammation [8]. Recent study has suggested the elevated S100A8/A9 level in acute

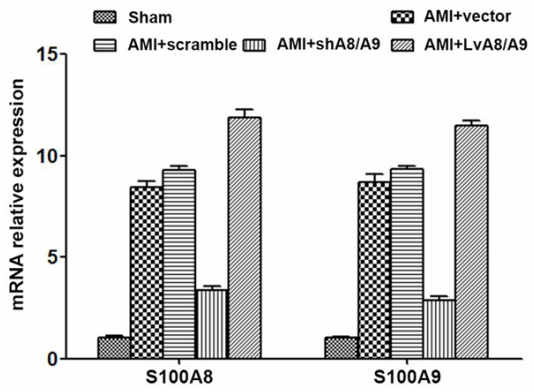

Figure 1. mRNA expression of S100A8 or S100A9 in cardiac tissues in different groups.

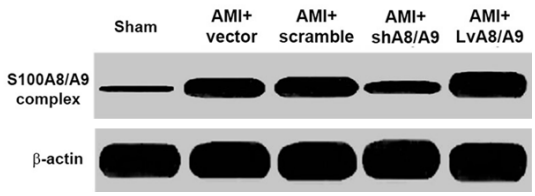

Figure 2. Protein expression of S100A8 or S100A9 in cardiac tissues in different groups.

myocardial infarction patients [9], indicating its potential involvement in inflammation after infarction. However, no direct study has been performed regarding the relationship between S100A8/A9 expression and heart rupture after acute myocardial infarction. We thus investigated the role of these two molecules in the occurrence of heart rupture.

Materials and methods

Animal model of myocardial infarction

Mice were provided by Shandong University. A total of 100 C57BL/6 male mice (aged 11~12 weeks) were randomly divided into sham group, scramble RNA group, Sh-A8/A9 group, vector group and LvA8/A9 group. Using previously documented method [10], the myocardial infarction model was established. In brief, mice were anesthetized with 50 mg/kg pentobarbital sodium via intraperitoneal injection. Animals were then fixed in a supine position, with tracheal intubation connected to artificial ventilation. An incision was made via the fourth rib left side of the sternum to expose the heart. The left coronary artery was ligated by 8-0 suture. The model was evaluated by the whitening color of myocardial tissues and persistent elevation of ST wave in electrocardiogram.

All procedures were approved by the Animal Ethics Committee of Shandong Provincial Hospital Affiliated to Shandong University.

Lentiviral vectors

Eukaryotic expression vector for shRNA of S100A8/A9 was generated to construct pLVX-shRNA2, pLVX-shRNA2-S100A8/A9 and empty vectors (pLVX-shRNA2-NC), all of which were amplified in 293T cells.

Vectors over-expression S100A8/A9 was also constructed as pLVX-Mrp8/14-IRES-ZsGreen1-IRES-Neo or empty vector (pLVX-IRES-ZsGreen1-IRES-Neo). Vectors were amplified in Lenti-X Lentiviral expression system.

Those vectors were injected in 5 different sites along the board line of infarction zone, using 30 G-needle. At each site, 10 μL viral vectors (scramble, vector, shA8/A9 or LvA8/A9) were applied (1×10^{11} viral genome copies per mL).

Immunohistochemical staining

One week after infarction, animals were sacrificed for collecting heart tissues, which were fixed in 10% neutral buffered formalin to prepare paraffin-based sections (5 μm thickness). Tissues sections were de-waxed, hydrated, processed for antigen retrieval in 0.01 M citric acid buffer (pH 6) in a microwave oven, and blocked with normal goat serum for 20-min incubation at room temperature. Rabbit anti-mouse MPO antibody (1:200 dilution, Abcam, UK) was then applied for overnight incubation at 4°C. On the next day, secondary antibody (1:1 000 dilution, Jackson, US) was applied for 1-hour incubation, followed by DAB substrate development for 10 min. Hematoxylin was used to counter-stain tissues. The expression of MPO was semi-quantitatively analyzed by Image-Pro Plus software.

qRT-PCR

Total RNA was extracted from cardiac tissues using Trizol reagent and reversely transcripted into cDNA using PrimeScript RT reagent Kit according to manufacturer's instructions. qRT-PCR was performed on ABI7500 in triplicates

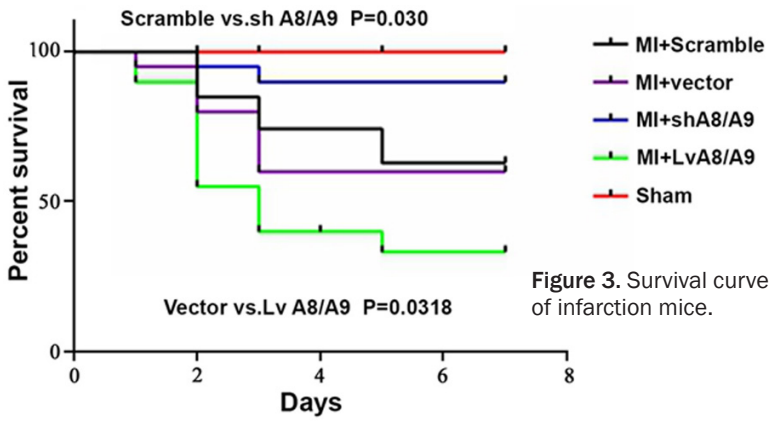

Figure 3. Survival curve of infarction mice.

with 10 L system including 4.5 L 2 × SYBR Green Mixture, 0.5 mL 2.5 m/L forward primer, 0.5 L 2.5 mm/L reverse primer, 1 L cDNA and 3.5 L ddH_2O. Primers sequences were designed as follows: S100A8 F: 5'-ATGCCGTCTACAGGGATGAC-3', S100A8 R: 5'-ACTGAGGACACTCGGTCTCTA-3'; S100A9 F: 5'-GGTCATAGAACACATCATGGAGG-3', S100A9 R: 5'-GGCCTGGCTTATGGTGGTG-3'; b-actin F: 5'-GAACCCTAAGGCCAAC-3', b-actin R: 5'-TGTCACGCACGATTTCC-3'. PCR conditions were: 95°C 15 sec, 60°C 30 sec, 74°C 30 sec for 40 cycles.

Western blot

Proteins were isolated from cardiac tissues using RIPA lysis buffer and concentration was measured by BCA assay. 50 mg protein was separated by SDS-PAGE followed by transferring to PVDF membrane and blocking with 5% milk for 1 h at room temperature. Then mouse anti-human S100A8 or S100A9 antibody (1:500 dilution) was added and incubated at 4°C overnight followed by wash three times by PBST and addition of HRP-conjugated secondary antibody (1:5000 dilution) for 1 h. After washing three times by PBST, positive band was detected by ECL.

Statistical analysis

SPSS 13.0 software was used to process all collected data, which were firstly tested for normality. Measurement data were presented as mean ± standard deviation (SD). Analysis of variance (ANOVA) was used to compare means across groups. Between-group-comparison was performed by SNK test. The survival analysis was performed by Kaplan-Meier method. A statistical significance was defined when $P < 0.05$.

Results

Overexpression of S100A8/A9 facilitated the occurrence of cardiac rupture

As most of heart rupture occurred within one week after primary onset of myocardial infarction, we analyzed the survival rate of mice within one week by twice daily check. Those dead mice were immediately examined to confirm the mortality reason. The expressions of S100A8 and S100A9 (mRNA and protein levels) in cardiac tissues were upregulated and downregulated after overexpression of S100A8/A9 or interference against S100A8/A9 in mice as demonstrated by qRT-PCR (**Figure 1**) and western blot (**Figure 2**), suggesting the success of manipulation of S100A8/19 expression in vivo. Survival analysis showed elevated mortality rate in animals with over-expression of S100A8/A9 ($P < 0.05$ in comparing vector vs. LvA8/A9 group), and decreased mortality rate after interference against S100A8/A9 ($P < 0.05$ as scramble vs. ShA8/A9, **Figure 3**). Post-mortal examination showed more frequent heart rupture after overexpression S100A8/A9 (11 vs. 6, $P < 0.05$), while RNA interference against S100A8/A9 effectively decreased cardiac rupture cases (1 in shA8/A9 vs. 6 in scramble group, $P < 0.05$, **Figure 4**).

S100A8/A9 enhanced inflammatory response after myocardial infarction

Immunohistochemical staining showed significant elevation of MPO expression in myocardial tissues in mice with acute myocardial infarction, suggesting the enhanced acute inflammatory response. The over-expression of S100A8/A9 effectively increased the MPO expression (vector vs. LvA8/A9 group, $P < 0.05$). The RNA interference against S100A8/A9 significantly depressed MPO expression (scramble vs. ShA8/A9 group, $P < 0.05$, **Figure 5**). These results suggested that inhibition of S100A8/A9 expression might suppress the inflammatory response after myocardial infarction.

Discussion

The body's immune system will be activated after severe ischemia of myocardial tissues

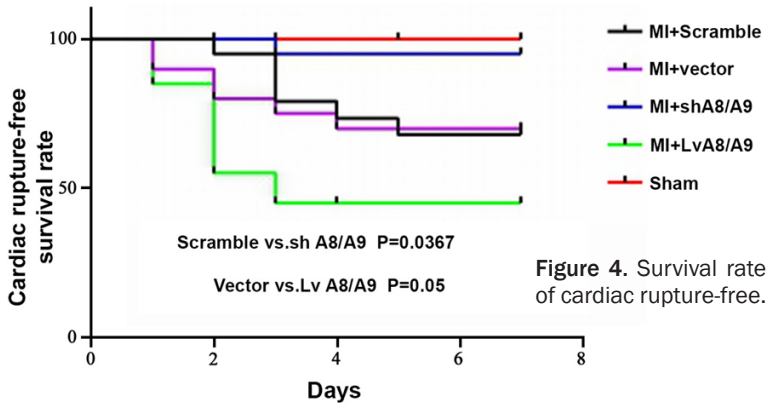

Figure 4. Survival rate of cardiac rupture-free.

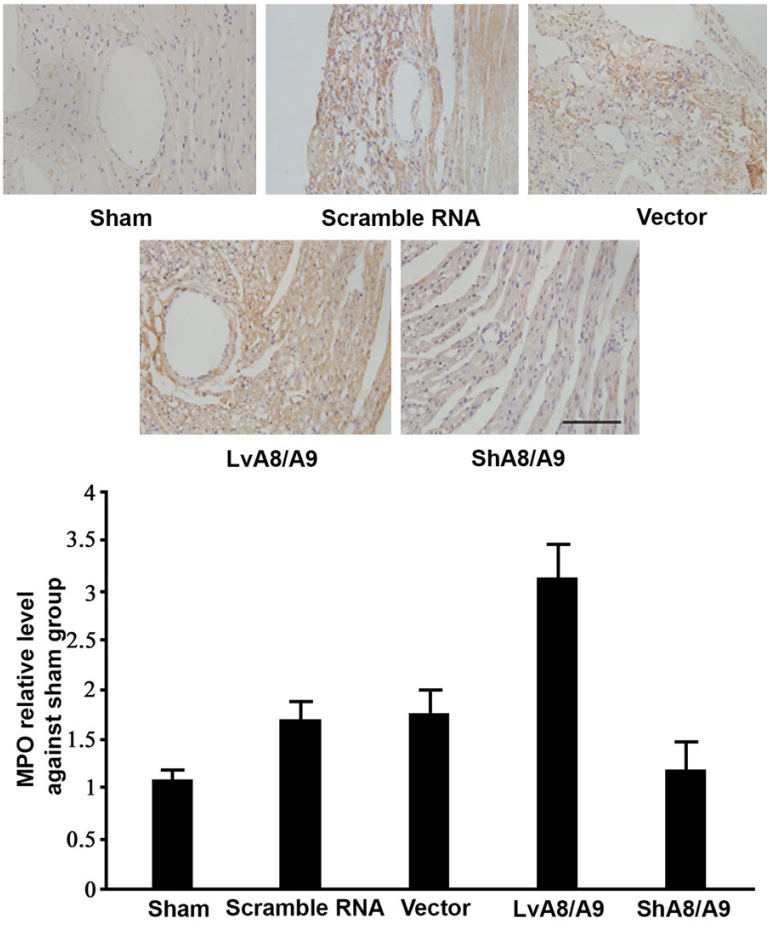

Figure 5. MPO expressions in all groups. Scale bar, 100 μm.

during acute cardiac infarction, to recruit several inflammatory cells toward the infarction lesion for releasing cytokines which participate in the inflammatory response [11, 12]. Previous studies have revealed the adverse effects and cytotoxicity of over-expressing inflammatory cytokines on cardiomyocytes to facilitate cell apoptosis and eventually cardiac dysfunction [13-15]. The infiltration of large amounts of inflammatory cells may also release inflammatory mediators such as MPO to participate in the inflammation. Those factors enhance the necrosis and apoptosis of cardiomyocytes, as well as cellular dysfunction of endothelial cells [16]. MPO is secreted by neutrophils, monocytes and some macrophages, and is mainly derived from polymorphic nuclear cells (PMNs). As the marker for neutrophil activation, MPO level reflects the status and functional role of PMN [16]. Previous study has suggested MPO enzyme as one independent predictive factor for acute coronary syndrome and is closely related with acute cardiac infarction [16].

Belonged to S100 protein family, S100A8 and S100A9 are mainly secreted by myeloid cells. Serum level of S100A8/A9 heterodimer is an indicator of inflammation and closely related with various inflammatory diseases such as peripheral nerve injury-induced inflammatory response [6] and chronic glomerulonephritis [7]. Therefore S100A8/A9 may work as biological marker for focal inflammation [8]. More importantly, recent study has suggested the elevated S100A8/A9 level in acute myocardial infarction patients [9]. The peak level of S100A8/A9 is correlated with the peak level of peripheral leukocytes and neutrophil, in addition to inflammatory protein C reactive protein and myocardial enzyme. Immunohistochemical staining results showed the expression of S100A8/A9 complex in neutrophil and macrophage that are infiltrated in myocardial tissues at infarction site [17, 18].

Although previous studies have revealed the correlation between S100A8/A9 complex and myocardial infarction, its detailed role in the pathogenesis of myocardial infarction is still unclear. Our results showed that over-expression of S100A8/A9 could effectively enhance the MPO expression, while RNA interference can decrease MPO level, suggesting the inhibition of inflammation after infarction by S100A8/A9 down-regulation. The acute inflammatory response is of critical importance in the occurrence of cardiac rupture after acute myocardial infarction. Previous studies have reported over-infiltration of neutrophil in the mesenchymal tissue of cardiac muscles in the ventricle wall of those patients [3]. Moreover, myeloperoxidase (MPO) gene knockout mice had shown certain protection against heart rupture in myocardial infarction [4]. The anti-inflammatory growth transformation factor-15 was also known to inhibit the occurrence of heart rupture after cardiac infarction [5]. Results from this study showed the inhibition of post-infarction inflammatory response by suppressing S100A8/A9 expression, indicating the possible role of S100A8/A9 in the occurrence of cardiac rupture.

In acute myocardial infarction patients with elevated ST wave, the rupture of ventricles often occur within 10 days after the primary infarction [12, 19]. Similar patterns can be observed in mice, which had the peak time of heart rupture at day 3~day 5 after infarction [19, 20]. We thus observed the survival rate and mortality reason of myocardial infarction mice. Post-mortal examination showed the positive correlation between S100A8/A9 expression and the incidence of cardiac rupture, suggesting the potential involvement of S100A8/A9 in the occurrence of cardiac rupture after acute myocardial infarction by mediating related inflammatory response.

In summary, this study confirmed the role of S100A8/A9 in heart rupture after acute myocardial infarction, providing more evidence for clinical prevention of heart rupture in myocardial infarction patients.

Disclosure of conflict of interest

None.

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