Original Article

Triggering receptor expressed on myeloid cells-2 promotes survival of cardiomyocytes after myocardial ischemic injury through PI3K/AKT pathway

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Background: Previous studies have already revealed that triggering receptor expressed on myeloid cells-2 (TREM2) plays a significant protective role during the pathogenesis of ischemia injury in both brain and liver. This study aims to investigate the effect of TREM2 in myocardial ischemic injury.

Methods: The mice myocardial infarction (MI) model was established via left anterior descending coronary artery ligation. TREM2 expression was examined with RT-PCR and Western blot. Whereafter, mice were randomly divided into control, sham, MI, Ad.TREM2 transfection group and Ad.Null transfection group. Recombinant adenovirus containing the gene coding full-length mouse TREM2 and EGFP (Ad.TREM2) or control vector containing *EGFP* gene only (Ad.Null) were immediately intramyocardial injected after left anterior descending ligated. After 7 days of MI, HE, Masson and TUNEL staining were performed to find the myocardial injury, infarcted size and cell apoptosis. Besides, echocardiography was performed to determine cardiac function. In addition, Western blot was performed to check the activity of PI3K/AKT signaling pathway in myocardial tissue. Furthermore, the plasma concentrations of TREM2 in 19 coronary artery disease (CAD) patients and 8 healthy controls were measured.

Results: Compared with the sham group, TREM2 expression was significantly up-regulated in cardiac tissue in mice with MI. Cardiac tissue in mice transfected with Ad.TREM2 was demonstrated with alleviated injury, reduced infarct size, and decreased number of apoptotic cells. Echocardiography revealed that heart function was significantly improved in Ad.TREM2 transfection mice. Also, TREM2 transfection significantly activated the phosphorylation of AKT. At last, the plasma concentration of TREM2 was significantly elevated in patients with CAD and correlated with the severity of CAD.

Conclusions: TREM2 may curb myocardial ischemia injury via activating PI3K/AKT signal pathway. Besides, plasma TREM2 may be treated as a potential biomarker in the diagnosis of CAD to reflect the severity of coronary stenosis.

Keywords: Triggering receptor expressed on myeloid cells-2 (TREM2); myocardial ischemic injury; cardiac function; apoptosis

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Introduction

Myocardial infarction (MI) is a major global public health problem (1). Although the development of interventional techniques remarkably decreased the mortality of MI, heart failure after MI is still a big challenge. Currently, researchers focused on the repair of cardiomyocytes and cardiac function recovery. Preventing heart failure after MI is still a problem that needed to be further studied (2).

Triggering receptor expressed on myeloid cells-2 (TREM2) is a classic cell surface receptor expressed on monocyte-derived cells which regulated differentiation of inflammatory cells and immune reaction (3-5). A great number of studies indicated that TREM2 is a key regulator of macrophage function and macrophage polarization (6,7). TREM2 was widely studied in Alzheimer's disease (AD) that the deficiency of TREM2 in microglia promotes the pathological progression of AD (8). Furthermore, plasma TREM2 could serve as a biomarker of neuronal injury in Parkinson's disease (9). Recently, the role of TREM2 in the organ protecting effect has been revealed (10). In sepsis, previous studies also revealed that TREM2 was the key molecular that elevated the survival and regulated the clearance of bacteria (11). However, in the circulation system, knowledge about TREM2 is still poor. Researchers have found that TREM2 may participate in atherosclerosis via single-cell RNA-Seq (12). According to The Human Protein Atlas (https://www.proteinatlas.org/), TREM2 was low expressed on cardiomyocytes. If the TREM2 functions in cell apoptosis or cell-protecting in ischemic heart injury was still unknown. Accordingly, we designed this study to identify if TREM2 has a protective effect in MI and preliminary revealed the mechanism using mice MI model and adenovirus transfection. We present the following article in accordance with the ARRIVE reporting checklist (available at https://cdt.amegroups.com/article/view/10.21037/cdt-21-490/rc).

Methods

Measurement of TREM2 expression in myocardial tissue in MI mice model

C57BL/6 mice were purchased from Qing Long Shan Animal Breeding Farm and fed in SPF animal house in Yi Ji Shan hospital. Mice were housed in individually ventilated cages under a 12:12 light: dark cycle in microisolator cages under static conditions with autoclaved rodent chow and autoclaved tap water ad libitum and bedding. The male C57BL6 mice (weight 20–22 g) were anesthetized with intraperitoneal pentobarbital (35 mg/kg) and intubated. The left anterior descending (LAD) coronary artery was ligated proximally with 7–0 silk suture via a left thoracotomy incision. After 1, 2, 3, 5 and 7 days, mice were received over-dose of intraperitoneal pentobarbital (130 mg/kg) and myocardial tissue was collected. mRNA was extracted using Trizol method and the expression of TREM2 mRNA was determined by RT-PCR (n=5 for each group). Additionally, to rule out the effects of macrophages, we used clodronate liposomes to delete cardiac macrophages after MI. The TREM2 mRNA expression and protein expression were analyzed using RT-PCR and western blot (shown in Appendix 1).

Neonatal mouse cardiomyocytes culture

Neonatal C57BL/6 mice were purchased from Qing Long Shan Animal Breeding Farm, Nanjing, China. Primary neonatal mouse cardiomyocytes were prepared according to the procedure described previously (13,14). After 48 hours of cell culture to achieve subconfluence, cardiomyocytes were used for immunofluorescence and harvested for real-time PCR analysis.

Characterization of cultured mouse cardiomyocytes

Neonatal mouse cardiomyocytes were primarily

characterized by immunofluorescence to evaluate the expression of TnI using rabbit anti-mouse TnI antibody (Proteintech Group, USA) and FITC labeled donkey anti-rabbit secondary antibody (Abcam, UK).

RT-PCR

The Mouse cardiomyocytes were lysate in TRIzol at −80 ℃. RNA from bone marrow cells was collected as a positive control. Femurs of the adult C57BL/6 mouse were flushed using standard PBS to collect bone marrow (BM) cells. FACSTM-Lysing solution (BD Biosciences, USA) was used to deplete erythrocytes. The RNA extraction, reverse transcriptrion and PCR were performed using Trizol method, PrimeScript™ RT reagent kit with gDNA Eraser (TAKARA, Japan) and TB Green Premix Ex Taq Kit (TAKARA, Japan) according to the manufacturer's protocol. TREM2 primers (forward: TATGACGCCTTGAAGCACTG, reverse: AGAGTGATGGTGACGGTTCC) and β-actin primers (forward: AGAGGGAAATCGTGCGTGAC, reverse: AGGAAGAGGATGCGGCAGT) were used.

TREM2 adenovirus

Recombinant adenovirus containing the gene coding full-length mouse TREM2 and enhanced green fluorescent protein (EGFP) and control vector was produced according to previous study (11). The detailed description was shown in Appendix 1.

Transfection of TREM2 adenovirus in cardiomyocytes

The male C57BL6 mice (weight 20–22 g) have received thoracotomy after intraperitoneal pentobarbital (35 mg/kg) and are intubated. Mice have received 50 μL of intramyocardial injections of 5×10^5 plaque-forming units (pfu) Ad.TREM2 and Ad.Null, respectively (grouping by random number method). The injection point was 1–2 mm above ligation point. After 7 days, the animals were euthanasia by over-dose of intraperitoneal pentobarbital (130 mg/kg) and GFP expression in cardiomyocytes was detected. Additionally, the myocardial tissue was devided into above injection, around injection and below injection according to injection point. The TREM2 expression in these three partes were determined using western blot (shown in Appendix 1).

Mice model of acute MI and transfection of TREM2

The male C57BL6 mice (weight 20–22 g) were anesthetized with intraperitoneal pentobarbital (35 mg/kg) and intubated. The MI moded was performed as described above. Animal models were randomly divided into three groups (n=5 in each group; grouping by random number method): (I) mice were subjected to LAD coronary ligation; (II) mice have received 50 μL of intramyocardial injections of 5×10^5 Ad.Null; (III) mice have received 50 μL of intramyocardial injections of 5×10^5 Ad.TREM2. The injection point was 1–2 mm above the ligation site. Non-operated control and sham surgery were performed.

Echocardiography

After 7 days of the MI model being established, 2-dimensional echocardiography was performed on the mice using a transthoracic echocardiogram (Visual Sonic, Canada) as previously described (15). The ejection fraction (EF), left ventricular internal dimension-end-systolic (LVIDs) and left ventricular internal dimension-end-diastolic (LVIDd) were measured and the shortening fraction (FS) was calculated (n=5 for each group).

Immunofluorescence staining

All the mice were received an over-dose of intraperitoneal pentobarbital (130 mg/kg) after echocardiography. Frozen sections of the heart were prepared. The GFP fluorescence was detected by fluorescence microscopy (Olympus, Tokyo, Japan) with an excitation wavelength of 470 nm. Further, for detecting the expression of TREM2 in myocardial tissue, the myocardial tissue paraffin section (2 μm thick) was stained with sheep anti-mouse TREM2 antibody (R&D, USA) and rabbit anti-mouse TnI antibody (Proteintech Group, USA) at 4 ℃ overnight and then stained with Alexa Flour 488 labeled donkey anti-sheep secondary antibody (Abcam, UK) and Alexa Flour 647 labeled Goat anti-rabbit secondary antibody (Beyotime, China) to measure the expression of TREM2. And TnI. The nucleus was stained by DAPI (Beyotime, China). Fluorescence microscopy (Olympus, Tokyo, Japan) was used to detect the fluorescence.

Cardiac histopathology

All heart samples with infarct site was not in the left

ventricle were excluded from the data analysis. The heart tissue was fixed in 10% formaldehyde and then embedded in paraffin. Tissue sections (2 μm thick) were stained with hematoxylin and eosin (HE) and Masson staining using a HE Staining Kit and Masson (Beyotime, China) and Masson stain kit (Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's protocol. Infarct size was calculated using ImageJ (Calculate the percentage of green-stained myocardium). Injury of myocardial tissue were observed using an optical microscope (Olympus, Japan) (n=5 for each group). The morphological evaluations were performed in a blinded manner by 2 independent pathologists blinded to the treatment group.

TUNEL immunohistochemistry

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) was performed to detect apoptotic nuclei by TUNEL Staining Kit (Abcam, UK) according to the manufacturer's protocol. The apoptotic cell number was counted by 2 independent observers blinded to the treatment group and expressed as a percentage of the total myocyte population.

Western blot

Protein was extracted from mouse myocardial tissue using a Tissue Protein Extraction Kit (Beyotime, China) according to the protocol provided by the manufacturer. Protein concentrations were measured using the BCA Assay Kit (Beyotime, China). Rabbit monoclonal anti-mouse AKT antibodies, rabbit monoclonal anti-mouse phosphorylated Ser^{473}AKT (P-AKT) antibody (Santa Cruz, USA)was used to detect AKT and P-AKT. GAPDH was used as a loading control (KangCheng Biotech, China). Horseradish peroxidase-conjugated goat anti-rabbit secondary antibody was obtained from Boster, China.

Blood sample collected

Nineteen coronary artery disease (CAD) patients who consecutively underwent coronary angiography (CAG) in the Department of cardiology, Yi Ji Shan Hospital affiliated to Wan Nan Medical College were involved in the study. The CAD was defined as previous described (16). Two experienced interventional cardiologists blinded to the clinical information analyzed the CAG. Three mL peripheral venous blood was collected after CAG in an EDTA-coated tube immediately. After being centrifugated at 500 ×g for 10 min, the plasma was collected and store and −80 ℃. The 8 sex and age-matched healthy controls were involved and the plasma sample was collected in the same way. The Gensini score in CAD patients were calculated according to the previous study (17).

Measurement of plasma concentrations of TREM2

The plasma concentrations of TREM2 were measure using Human TREM2 SimpleStep ELISA Kit (Abcam, UK) according to the manufacturer's protocol.

Statistical analysis

For all experiments, the data were analyzed using either a Student's *t*-test or Bonferroni's test, and values are expressed as the means ± SD. Student's *t*-test was used for the two sets of data. Bonferroni's test was used for more than two groups of data. All statistical analyses were performed using SPSS software (SAS Institute Inc., USA); P value <0.05 was considered to indicate significance (α=0.05, β=0.1). PASS was used to calculate the sample size of each group according to the experimental standard deviation of previous results of mouse MI model.

Ethical statement

Experiments were performed under a project license (No. 2021LSGY No. 31) granted by the Ethics Committee of Yi Ji Shan Hospital affiliated to Wannan Medical College, in compliance with Guidelines for the care and use of laboratory animals in biomedical research (18). The study was conducted ethically in accordance with the World Medical Association Declaration of Helsinki (as revised in 2013). Written informed consents were obtained from all subjects for the use of their urine and biopsy samples for research purposes. The protocol for this study was not registered.

Results

Expression of TREM2 in myocardial tissue after MI in mice model

To evaluated the level of TREM2 expression in infarcted myocardial tissue, we detected the TREM2 expression after MI using male C57BL/6 mice MI model. RT-

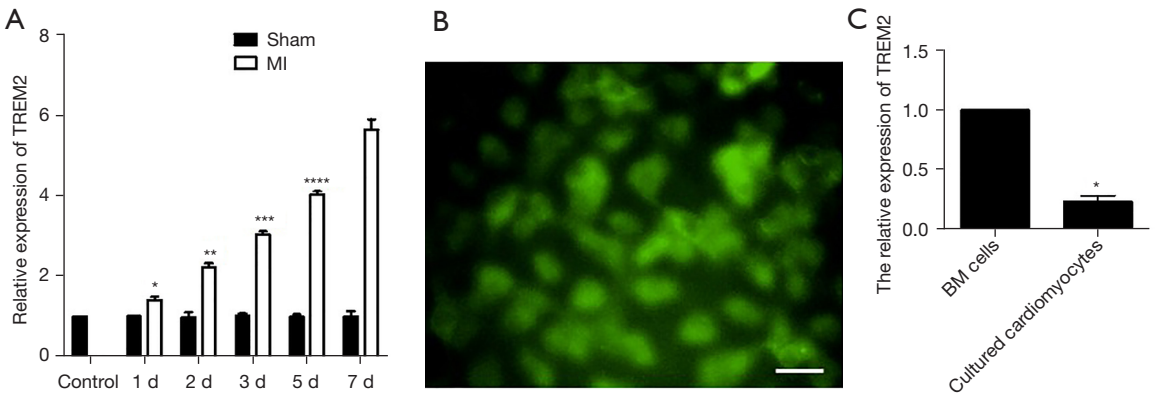

Figure 1 The expression of TREM2 mRNA in myocardial tissue was increased after MI and cultured neonatal mouse cardiomyocytes lowly expressed TREM2. (A) RT-PCR showed that after 1 d, 2 d, 3 d, 5 d and 7 d of MI, the expression of TREM2 mRNA in myocardial tissue gradually increased (n=5, *, P<0.05 *vs.* control; **, P<0.05 *vs.* control, 1 d, 3 d, 5 d and 7 d; ***, P<0.05 *vs.* control, 1 d, 2 d, 5 d and 7 d; ****, P<0.05 *vs.* control, 1 d, 2 d, 3 d and 7 d). The sham group showed no increased expression of TREM2. (B) Immunofluorescence showed that cultured neonatal mouse expressed TnI. (C) RT-PCR showed that cultured neonatal mouse cardiomyocytes lowly expressed TREM2 compared to mice BM cells. Magnification: ×400; bar: 50 μm; n=5, *, P<0.05 *vs.* BM cells. TREM2, triggering receptor expressed on myeloid cells-2; MI, myocardial infarction; RT-PCR, reverse transcription-polymerase chain reaction; BM, bone marrow.

PCR showed that after 1 d, 2 d, 3 d, 5 d and 7 d of MI, the expression of TREM2 mRNA in myocardial tissue gradually increased compared to control. The sham group showed no increased TREM2 expression compared to MI (*Figure 1A*). After clodronate liposomes treatment (the efficiency of macrophage clearance was shown in Figure S1), the TREM2 mRNA expression and protein expression were also increased after MI (Figure S2). Five heart samples where infarct site was not in the left ventricle were excluded. Adverse events were not observed in mice model.

Characterization of cultured neonatal mouse cardiomyocytes and the expression of TREM2

The cultured male C57BL/6 neonatal mice cardiomyocytes were charactered by detecting TnI expression. Immunofluorescence showed that cultured neonatal mouse cardiomyocytes expressed TnI (*Figure 1B*). Further, the mouse cardiomyocytes have low mRNA expression of TREM2 compared to mouse bone marrow cells (*Figure 1C*).

Expression of TREM2 in cardiomyocytes after TREM2 adenovirus transfection

To determine the transfection efficiency, we analyzed the GFP and TREM2 expression on myocardial tissue of male C57BL/6 mice after Ad.TREM2 tansfection. Immunofluorescence showed that both Ad.TREM2 and Ad.Null successfully transfected in cardiomyocytes (*Figure 2A*). TREM2 expressed in cardiomyocytes after Ad.TREM2 transfection compared to Ad.Null transfection and control (*Figure 2B*). Additionally, the TREM2 protein expression around the adenovirus injection point and below the adenovirus injection point were analyzed by western blot. Both adenovirus injection point and below adenovirus injection point expressed TREM2 and no difference was found (Figure S3). Adverse events were not observed in mice.

Effects of TREM2 adenovirus transfection on cardiac function after MI

We analyzed the cardiac function to detect the effect of Ad.TREM2 transfection in male C57BL/6 mice MI model. Cardiac function was evaluated at 7 days after MI as shown in *Table 1* and *Figure 3*. MI group had lower LVEF (48.9%±2.1% *vs.* 68.4%±3.6%, 69.1%±2.3%, P<0.05, respectively) and LVFS (22.7%±3.1% *vs.* 43.3%±3.2%, 42.4%±1.9%, P<0.05, respectively) than the control and Sham. The LVEF (59.3%±4.4% *vs.* 48.9%±2.1%, 47.2%±2.1%, P<0.05, respectively) and LVFS (35.3%±3.1% *vs.* 22.7%±3.1%, 23.2%±3.8%, P<0.05, respectively) were significantly higher in Ad.TREM2 group than MI and Ad.Null group. Further, the LVIDd (4.52±0.28 *vs.* 3.97±0.18, 3.90±0.23 mm, P<0.05, respectively) and

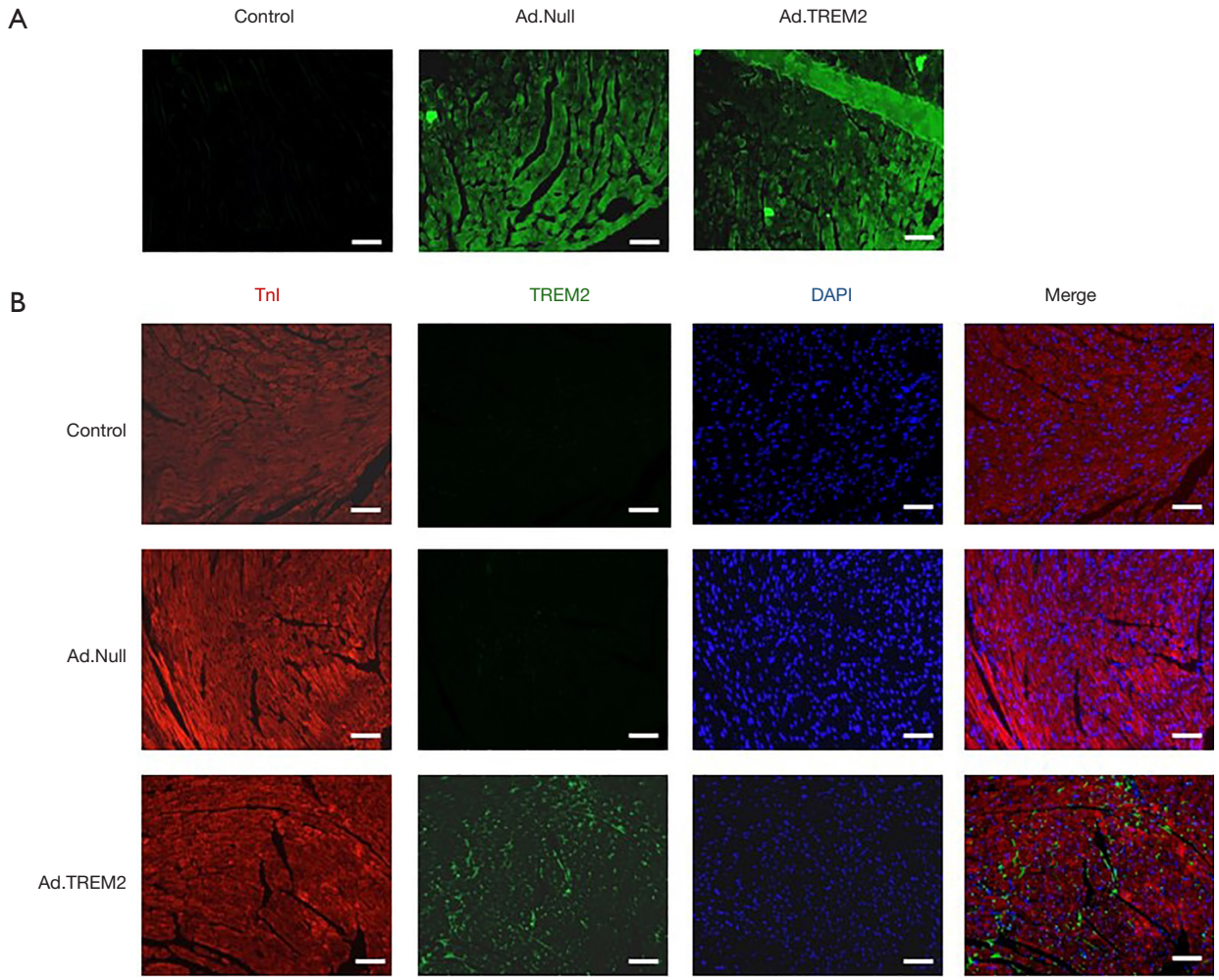

Figure 2 Transfection of TREM2 adenovirus in the heart tissue of mice. After Ad.TREM2 transfection, myocardial tissue expressed GFP and TREM2. (A) Immunofluorescence showed that TREM2 adenovirus and control adenovirus successfully transfected in heart tissue; (B) immunofluorescence showed that heart tissue expressed TREM2 after TREM2 adenovirus transfection. The control and control adenovirus transfection had no TREM2 expression. Magnification: ×200; bar: 100 μm. TREM2, triggering receptor expressed on myeloid cells-2; GFP, green fluorescent protein. DAPI, 4',6-diamidino-2-phenylindole; TnI, troponin I.

Table 1 Effects of TREM2 adenovirus on physiological parameters and cardiac function in MI

	Control	Sham	MI	Ad.Null	Ad.TREM2
LVEF, %	68.4±3.6	69.1±2.3	48.9±2.1[a]	47.2±2.1	59.3±4.4[b]
LVFS, %	43.3±3.2	42.4±1.9	22.7±3.1[a]	23.2±3.8	35.3±3.1[b]
LVIDd, mm	3.97±0.18	3.90±0.23	4.52±0.28[a]	4.51±0.36	4.12±0.25[b]
LVIDs, mm	2.40±0.10	2.31±0.34	3.20±0.24[a]	3.19±0.14	2.69±0.11[b]

[a], P<0.05 *vs.* control and MI; [b], P<0.05 *vs.* MI and Ad.Null. TREM2, triggering receptor expressed on myeloid cells-2; MI, myocardial infarction. LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening; LVIDd, left ventricular internal dimension-end-diastolic; LVIDs, left ventricular internal dimension-end-systolic.

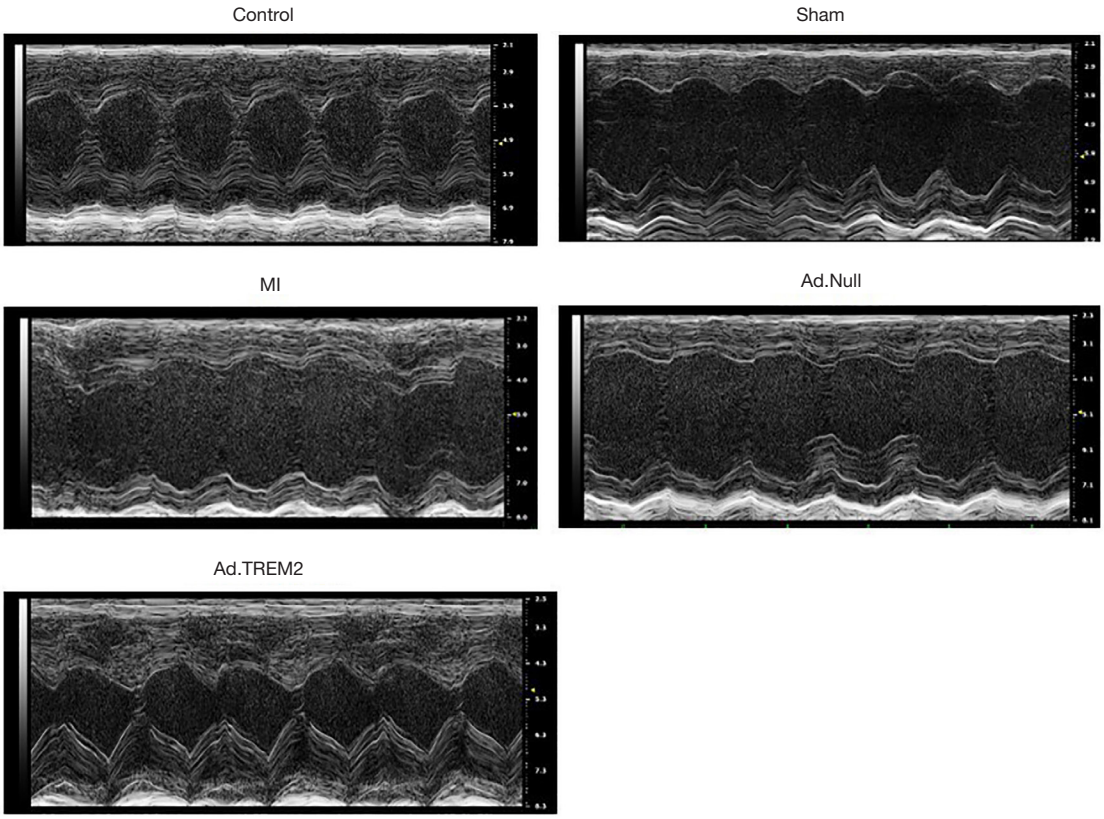

Figure 3 Effect of TREM2 on cardiac function after MI. Ad.TREM2 transfection increased cardiac function after myocardial infaction. The impairment of cardiac function occurred in the MI group. The expression of TREM2 prevented cardiac dysfunction after MI. The LVEF and LVFS were significantly higher in the TREM2 transfection group (n=5). TREM2, triggering receptor expressed on myeloid cells-2; MI, myocardial infarction; LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening.

LVIDs (3.20±0.24 *vs.* 2.40±0.10, 2.31±0.34 mm, P<0.05, respectively) were higher in the MI group than control and sham. Ad.TREM2 group had lower LVIDd (4.12±0.25 *vs.* 4.52±0.28, 4.51±0.36 mm, P<0.05, respectively) and LVIDs (2.69±0.11 *vs.* 3.20±0.24, 3.19±0.14 mm, P<0.05, respectively) than MI and Ad.Null. Adverse events were not observed in mice model.

Effects of TREM2 adenovirus transfection on the myocardial injury, infarction size and cell apoptosis

The effect of Ad.TREM2 transfection was determined by myocardial histopathology. The myocardial injury was determined by HE and TUNEL staining. Masson staining was used to detect the infarcted size. HE staining showed numerous inflammatory cells infiltration in infarct site, cardiomyolysis and cell apoptosis in the MI group compared to control and sham. In the Ad.TREM2 group, the myocardial injury was significantly alleviated compared to MI and Ad.Null (*Figure 4*). Masson staining showed the infarct size was rremarkably reduced after Ad.TREM2 transfection compared to MI and Ad.Null (16.7%±3.0% in Ad.TREM2 *vs.* 32.0%±2.6% in MI and 30.0%±2.0% in Ad.Null, P<0.05, respectively, *Figure 5*). TUNEL staining showed that the number of apoptotic cardiomyocytes markedly increased in the MI group compared to the control and sham. Ad.TREM2 transfection decreased the number of apoptotic cells compared to MI and Ad.Null (27±3/field in Ad.TREM2 *vs.* 55±6/field in MI and 56±4/field in Ad.Null, P<0.05, *Figure 6*). 3 heart samples where infarct site was not in the left ventricle were excluded. Adverse events were not observed in mice model.

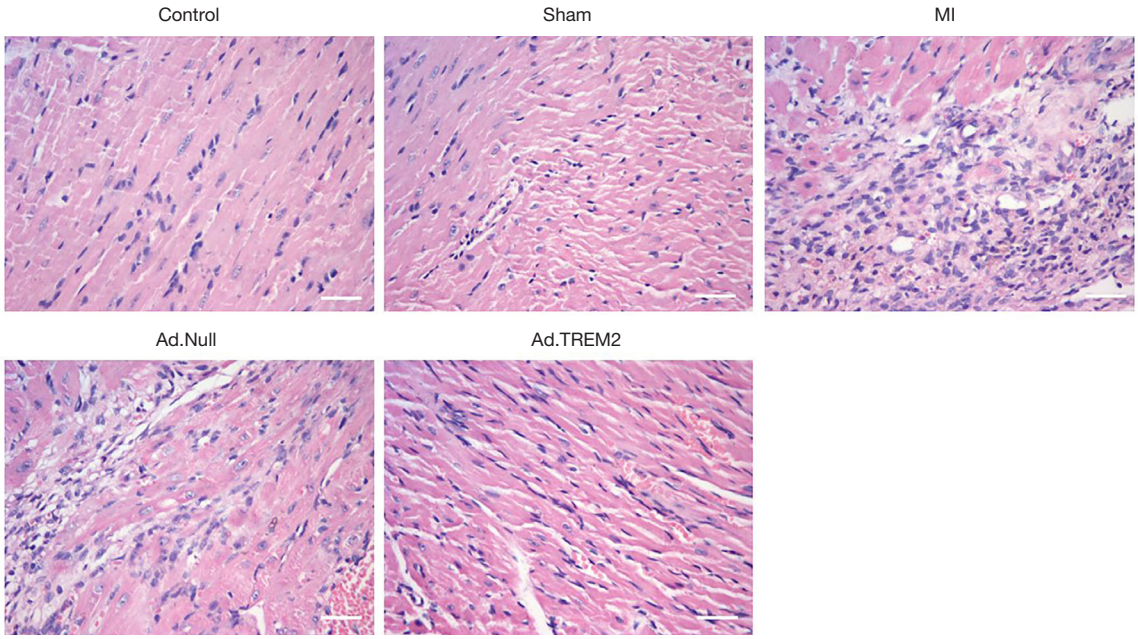

Figure 4 Effect of TREM2 expression on pathological change in myocardial tissue. Ad.TREM2 transfection alleviated myocardial injury after MI. HE staining showed that in the MI group, HE staining showed myocardial dissolving and numbers of inflammatory cells infiltration. TREM2 expression alleviated the myocardial injury after the MI group compared to MI and Ad.Null. Magnification: ×400; bar: 50 μm; n=5. TREM2, triggering receptor expressed on myeloid cells-2; MI, myocardial infarction.

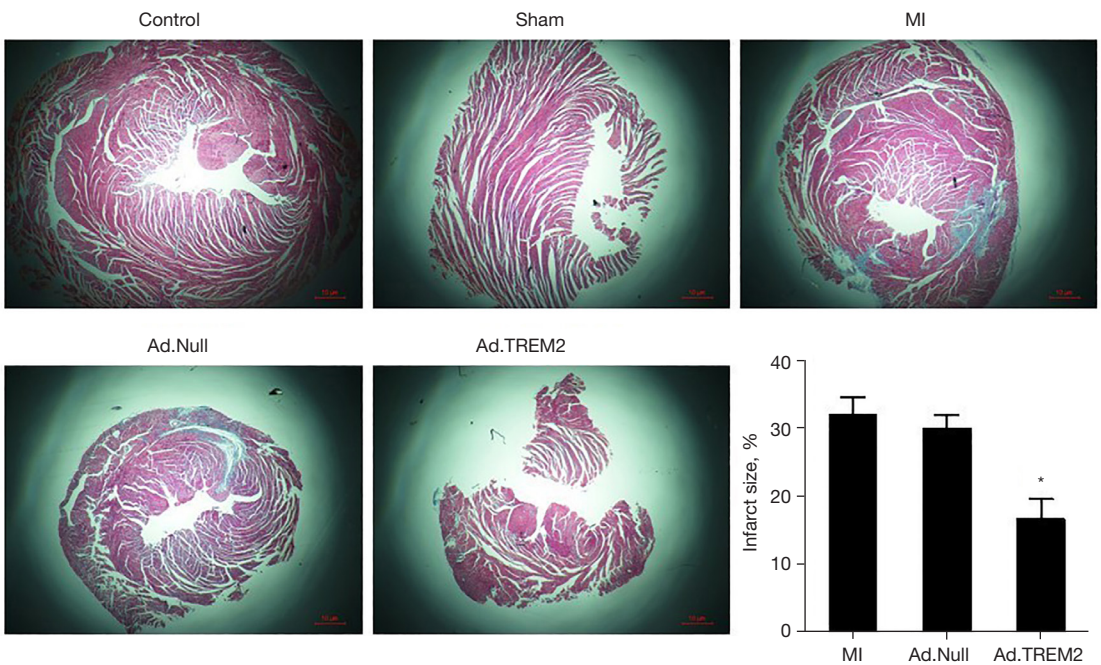

Figure 5 Effect of TREM2 expression on infarcted size after MI. The Ad.TREM2 transfection alleviated myocardial injury after MI, Masson staining showed that TREM2 adenovirus transfection significantly reduced the infarcted size after MI compared to Ad.Null transfection and MI group. Histogram showed the infarcted size in all groups. Magnification: 4×; n=5, *, P<0.05 *vs.* MI and Ad.Null group. TREM2, triggering receptor expressed on myeloid cells-2; MI, myocardial infarction.

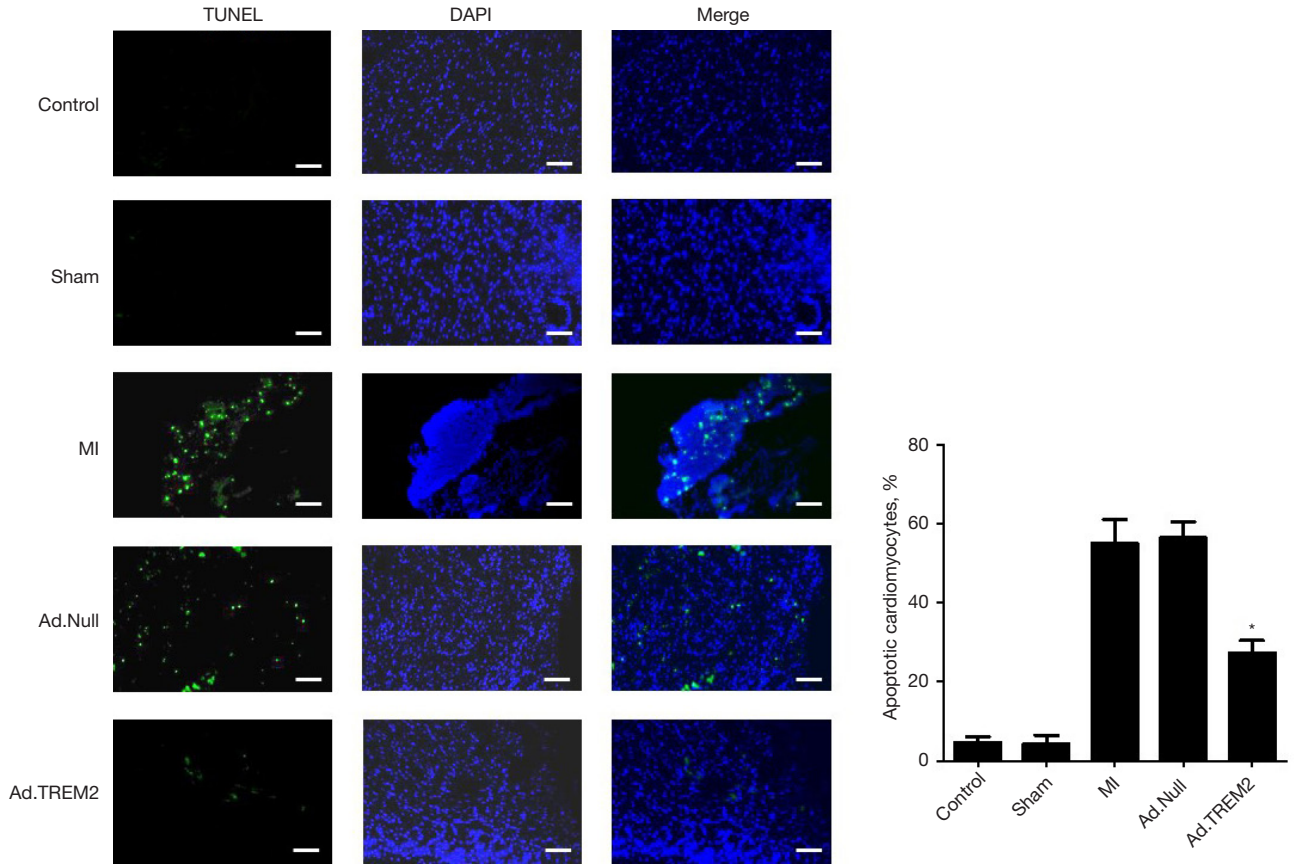

Figure 6 Effect of TREM2 on cardiomyocyte apoptosis after MI. The Ad.TREM2 transfection decreased cell apoptosis after myocardial infection. TUNEL staining showed that TREM2 adenovirus transfection significantly reduced the number of apoptotic cells after MI compared to Ad.Null transfection and MI group. Histogram showed the number of apoptotic cells in all groups. Magnification: ×200; bar: 100 μm; n=5, *, P<0.05 *vs.* MI and Ad.Null group. TREM2, triggering receptor expressed on myeloid cells-2; MI, myocardial infarction. DAPI, 4',6-diamidino-2-phenylindole; TnI, troponin I.

TREM2 adenovirus transfection activated PI3K/AKT signal pathway

We detected the expression of AKT and phosphorylated Akt in myocardial tissue to evaluate the effect of Ad.TREM2 transfection. In MI and Ad.Null group, the expression of phosphorylated AKT was significantly lower than the Ad.TREM2 group. TREM2 adenovirus transfection significantly activated the phosphorylation of AKT (*Figure 7*).

The plasma concentration of TREM2 was increased in CAD and correlated with the severity of coronary stenosis

To assessed the clinical application value of TREM2, we analyzed the plasma concentration of TREM2 in CAD patients and healthy controls. The plasma level of TREM2 was significantly higher in CAD patients compared with healthy control (10,713.6 pg/mL in CAD *vs.* 7,134.3 pg/mL in controls, P=0.008). Pearson correlation analysis showed that the plasma concentration of TREM2 significantly correlated with the Gensini score (r=0.532, P=0.004) (*Figure 8*).

Discussion

In this study, we firstly found that TREM2 protects against ischemic myocardial injury and promotes the survival of cardiomyocytes after MI. P13K/AKT signal pathway may regulate the protecting effect of TREM2. TREM2 has the potential to serve as the biomarker of CAD and the new therapeutic target of myocardial ischemic injury.

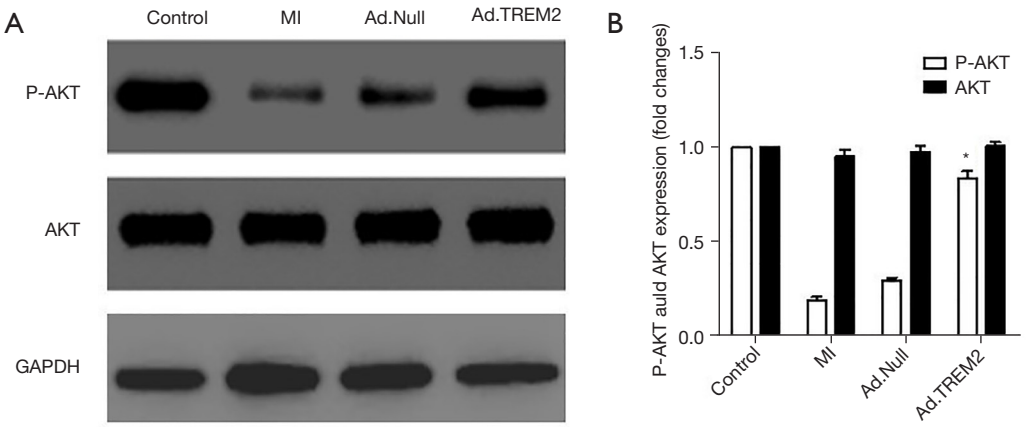

Figure 7 Effect of TREM2 expression on the expression of signal protein expression in heart tissue. The Ad.TREM2 transfection activated the phosphorylation of AKT. (A) Representative band showed that in MI, phosphorylation of AKT was inhibited and TREM2 expression increased the expression of phosphorylated AKT. TREM2 promoted the activation of phosphorylation of AKT compared to MI and Ad.Null. (B) Densitometry quantitation of protein expression levels are shown as fold changes in the histogram. n=5, *, P<0.05 *vs.* MI and Ad.Null group. TREM2, triggering receptor expressed on myeloid cells-2; MI, myocardial infarction. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

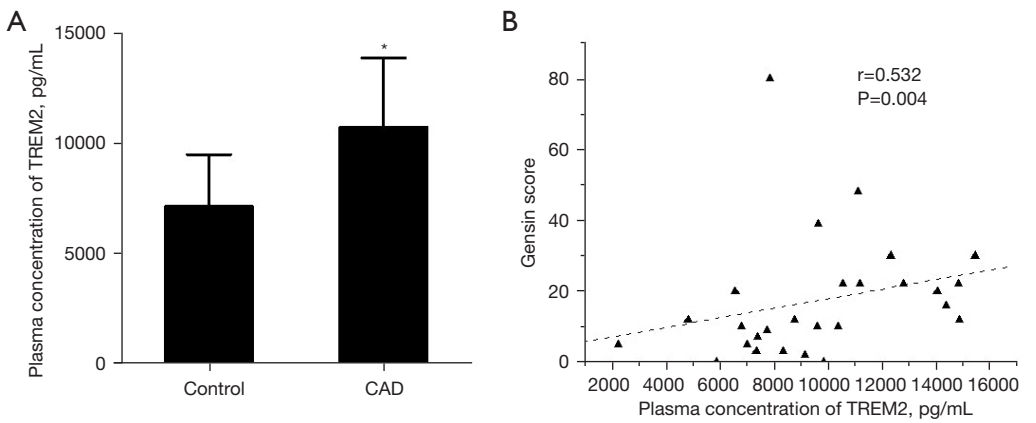

Figure 8 The plasma concentration of TREM2 in CAD patients and controls. The plasma concentration of TREM2 in CAD patients was significantly higher than controls. (A) Plasma concentration of TREM2 were elevated in CAD patients (*, P=0.008 *vs.* control); (B) plasma concentration of TREM2 were correlated with severity coronary stenosis (r=0.532, P=0.004). TREM2, triggering receptor expressed on myeloid cells-2; CAD, coronary artery disease.

Heart failure is the major complication of MI due to a large number of cells apoptosis. The prevention of ischemic myocardial injury is a big change for clinicians and researchers. In nearly ten years, the therapeutic potential of stem cells attracted more attention (19). Clinical trials showed that cardiac progenitor cells have the potential to improve ventricular function (20). However, the credibility of cardiac progenitor cells has been questioned that the mechanism of cardiac progenitor cells protecting effect has not been well demonstrated and the stem cell character of cardiac progenitor cells has not been proved (21). Although other pluripotent stem cells have been found the potential candidate for cardiac cell therapy (22), the clinical application of stem cells in treating ischemic or other myocardial injuries are need to be further demonstrated.

TREM2 is a cell surface receptor and express on monocyte-derived cells, such as macrophages. In some other cells, the expression of TREM2 was not yet

determined. So far, there was no research reporting if cardiomyocytes could express TREM2. Our data firstly showed that mouse cardiomyocytes expressed a low level of TREM2. TREM2 was widely studied in the nervous system. In cardiovascular disease, the knowledge of TREM2 islimited. In sepsis, TREM2 had the protecting effect and regulated the immune reaction (23,24). Further, the organ protecting effect has been reported. In immune-mediated hepatocellular damage and acute lung injury, the protecting role of TREM2 has been revealed (25,26). In this study, we found that over-expression of TREM2 in cardiomyocytes reduced the infarcted size and improved cardiomyocytes survival after acute MI in the mice model. These data indicated that TREM2 may directly regulate the anti-apoptosis ability in cardiac ischemic injury independent of the regulation of macrophages. TREM2 has the potential to serve as a target that could reverse cardiac function after MI.

Now, we are known more and more about the ligand of TREM2. It is known that DNAX adaptor protein 12 (DAP12) is the ligand of TREM2 which contains an immunoreceptor tyrosine-based activation motif (3). The previous study has focused on regulating macrophages polarization of TREM2, and auto-phage in neuroinflammation (27). Classic PI3K/AKT signal pathway is also involved in TREM2 signaling. Various studies indicated that PI3K/AKT was the key regulator that mediated the effect of TREM2 in bacterial infection and cancer (28), and also in regulating the microglia in the nervous system (29). In this study, our data firstly showed that the over-expression of TREM2 in cardiomyocytes couldincrease the ability of anti-apoptosis and protected against acute myocardial ischemic injury. TREM2 further activated the PI3K/AKT pathways. However, if other key signal pathway molecules participated in signal transduction is still need further study.

In this study, we further suggested that plasma TREM2 concentration was elevated in CAD patients. The knowledge of the relationship between CAD and TREM2 was limited. Previous study showed that peripheral blood mononuclear cells from CAD patients have high level expression of TREM2 (30). Our data showed that over expression of TREM2 in cardiac myocytes can promote the survival of cardiac myocytes after MI. These data revealed the clinical application value of TREM2 in ischemic heart disease. Plasma TREM2 level may indicate the progression of CAD and elevated expression of TREM2 in cardiac myocytes may have therapeutic effects in MI.

However, this research have several limitations. Firstly, the mechanisms of signaling are need to be further demonstrated through gene knockdown animal models. Secondly, the detailed signal pathways that participated as the downstream of PI3K/AKT were not well studied. Thirdly, Large sample size is required to confirm the clinical value of TREM2 as a biomarker of coronary heart disease.

Conclusions

TREM2 may curb myocardial ischemia injury via activating PI3K/AKT signal pathway. Besides, plasma TREM2 may be treated as a potential biomarker in the diagnosis of CAD to reflect the severity of coronary stenosis.

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Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at https://cdt.amegroups.com/article/view/10.21037/cdt-21-490/rc

Data Sharing Statement: Available at https://cdt.amegroups.com/article/view/10.21037/cdt-21-490/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://cdt.amegroups.com/article/view/10.21037/cdt-21-490/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Experiments were performed under a project license (No. 2021LSGY No. 31) granted by the Ethics Committee of Yi Ji Shan Hospital affiliated to Wannan Medical College, in compliance with

Guidelines for the care and use of laboratory animals in biomedical research for the care and use of animals. The study was conducted ethically in accordance with the World Medical Association Declaration of Helsinki (as revised in 2013). Written informed consents were obtained from all subjects for the use of their urine and biopsy samples for research purposes. The protocol of the study was not registered.

References

1. Anderson JL, Morrow DA. Acute Myocardial Infarction. N Engl J Med 2017;376:2053-64.
2. Ritsinger V, Nyström T, Saleh N, et al. Heart failure is a common complication after acute myocardial infarction in patients with diabetes: A nationwide study in the SWEDEHEART registry. Eur J Prev Cardiol 2020;27:1890-901.
3. Daws MR, Lanier LL, Seaman WE, et al. Cloning and characterization of a novel mouse myeloid DAP12-associated receptor family. Eur J Immunol 2001;31:783-91.
4. Colonna M. TREMs in the immune system and beyond. Nat Rev Immunol 2003;3:445-53.
5. Ford JW, McVicar DW. TREM and TREM-like receptors in inflammation and disease. Curr Opin Immunol 2009;21:38-46.
6. Sieber MW, Jaenisch N, Brehm M, et al. Attenuated inflammatory response in triggering receptor expressed on myeloid cells 2 (TREM2) knock-out mice following stroke. PLoS One 2013;8:e52982.
7. Kawabori M, Kacimi R, Kauppinen T, et al. Triggering receptor expressed on myeloid cells 2 (TREM2) deficiency attenuates phagocytic activities of microglia and exacerbates ischemic damage in experimental stroke. J Neurosci 2015;35:3384-96.
8. Krasemann S, Madore C, Cialic R, et al. The TREM2-APOE Pathway Drives the Transcriptional Phenotype of Dysfunctional Microglia in Neurodegenerative Diseases. Immunity 2017;47:566-581.e9.
9. Wilson EN, Swarovski MS, Linortner P, et al. Soluble TREM2 is elevated in Parkinson's disease subgroups with increased CSF tau. Brain 2020;143:932-43.
10. Wu R, Li X, Xu P, et al. TREM2 protects against cerebral ischemia/reperfusion injury. Mol Brain 2017;10:20.
11. Chen Q, Zhang K, Jin Y, et al. Triggering receptor expressed on myeloid cells-2 protects against polymicrobial sepsis by enhancing bacterial clearance. Am J Respir Crit Care Med 2013;188:201-12.
12. Cochain C, Vafadarnejad E, Arampatzi P, et al. Single-Cell RNA-Seq Reveals the Transcriptional Landscape and Heterogeneity of Aortic Macrophages in Murine Atherosclerosis. Circ Res 2018;122:1661-74.
13. Zhai T, Zhang J, Zhang Y, et al. Cathelicidin deficiency exacerbates cardiac dysfunction in lipopolysaccharide-induced endotoxaemic mice. Clin Exp Pharmacol Physiol 2020;47:677-86.
14. Sheng Z, Yao Y, Li Y, et al. Bradykinin preconditioning improves therapeutic potential of human endothelial progenitor cells in infarcted myocardium. PLoS One 2013;8:e81505.
15. Goradel NH, Hour FG, Negahdari B, et al. Stem Cell Therapy: A New Therapeutic Option for Cardiovascular Diseases. J Cell Biochem 2018;119:95-104.
16. Yao YY, Fu C, Ma GS, et al. Tissue kallikrein is related to the severity of coronary artery disease. Clin Chim Acta 2013;423:90-8.
17. Wu Y, Lu X, Xiang FL, et al. North American ginseng protects the heart from ischemia and reperfusion injury via upregulation of endothelial nitric oxide synthase. Pharmacol Res 2011;64:195-202.
18. Jones-Bolin S. Guidelines for the care and use of laboratory animals in biomedical research. Curr Protoc Pharmacol 2012;Appendix 4:Appendix 4B.
19. Sano T, Ousaka D, Goto T, et al. Impact of Cardiac Progenitor Cells on Heart Failure and Survival in Single Ventricle Congenital Heart Disease. Circ Res 2018;122:994-1005.
20. van Berlo JH, Kanisicak O, Maillet M, et al. c-kit+ cells minimally contribute cardiomyocytes to the heart. Nature 2014;509:337-41.
21. Duran AG, Reidell O, Stachelscheid H, et al. Regenerative Medicine/Cardiac Cell Therapy: Pluripotent Stem Cells. Thorac Cardiovasc Surg 2018;66:53-62.
22. Molloy EJ. Triggering Receptor Expressed on Myeloid Cells (TREM) family and the application of its antagonists. Recent Pat Antiinfect Drug Discov 2009;4:51-6.

23. Gawish R, Martins R, Böhm B, et al. Triggering receptor expressed on myeloid cells-2 fine-tunes inflammatory responses in murine Gram-negative sepsis. FASEB J 2015;29:1247-57.
24. Perugorria MJ, Esparza-Baquer A, Oakley F, et al. Non-parenchymal TREM-2 protects the liver from immune-mediated hepatocellular damage. Gut 2019;68:533-46.
25. Liu D, Dong Y, Liu Z, et al. Impact of TREM-2 gene silencing on inflammatory response of endotoxin-induced acute lung injury in mice. Mol Cell Biochem 2014;394:155-61.
26. Zhuang X, Yu Y, Jiang Y, et al. Molecular hydrogen attenuates sepsis-induced neuroinflammation through regulation of microglia polarization through an mTOR-autophagy-dependent pathway. Int Immunopharmacol 2020;81:106287.
27. Zhang H, Sheng L, Tao J, et al. Depletion of the triggering receptor expressed on myeloid cells 2 inhibits progression of renal cell carcinoma via regulating related protein expression and PTEN-PI3K/Akt pathway. Int J Oncol 2016;49:2498-506.
28. Harry GJ. Microglia during development and aging. Pharmacol Ther 2013;139:313-26.
29. Selman C, Withers DJ. Mammalian models of extended healthy lifespan. Philos Trans R Soc Lond B Biol Sci 2011;366:99-107.
30. Li X, He X, Wang J, et al. The Regulation of Exosome-Derived miRNA on Heterogeneity of Macrophages in Atherosclerotic Plaques. Front Immunol 2020;11:2175.

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Methods

Clodronate liposome treatment

To demonstrate if the elevated expression of TREM2 was independent on macrophage, the macrophage scavenger clodronate liposome (Target Technology, China) was used according to previous study (31). Briefly, clodronate liposome was administered i.p. (10 μL/g) 4 hours before MI and again at 1, 3, and 6 days after MI. Due to limitations in IV access, the final dose was administered by intraperitoneal injection. 7 days after MI, the heart samples were collected and myocardial tissue was ground into cell suspension using gentleMACS (Miltenyi Biotec, German). The cells were stained with FITC-F4/80 mouse antibody (Bio-Rad, USA) and the ratio of F4/80 positive macrophage was analysis by FACS (BD science, USA).

RT-PCR

To determine the expression of TREM2 after clodronate liposome treatment in MI, the heart samples were collected after 1 d, 2 d, 3 d, 5 d and 7 d after MI and clodronate liposome treatment. Total RNA was extracted using TRIZOL method according to the manufacturer's protocol (Ambion, Life Technologies, USA). Furthermore, the concentration and purity of RNA were assessed using the relative absorbance ratio at 260/280 in a NanoDrop 2000 (Thermo, USA). β-actin RNA was measured as a control. cDNA was synthesized using the PrimeScriptTM RT reagent kit with gDNA Eraser (TAKARA, Japan). RT-PCR was performed using TREM2 primers (forward: TATGACGCCTTGAAGCACTG, Reverse: AGAGTGATGGTGACGGTTCC), β-actin (forward: AGAGGGAAATCGTGCGTGAC, Reverse: AGGAAGAGGATGCGGCAGT). After RT (50 ℃, 30 min), hot start (94 ℃, 15 min), and 40–42 cycles of PCR (94 ℃, 1 min; 52.5 ℃, 1 min; 72 ℃, 1 min), TREM2 mRNA expression was normalized to β-actin and calculated as $2^{-\Delta\Delta Ct}$.

Adenoviral vector system and adenovirus transfection

Recombinant adenovirus was produced using the ViraPower Adenoviral Expression System (Life Technologies, Grand Island, NY), according to the manufacturer's instructions. Briefly, the recombination region of pIRES2-EGFP expression vector containing the gene coding full-length mouse TREM2 was transferred to the Gateway Vector pAd/CMV/V5-DEST using the transfer vector pDONR221. The recombined adenoviral plasmids generated in this manner were then transformed into competent DH5α *E.coli* (Life Technologies, Grand Island, NY) to be amplified. After digested by endonuclease PacI (New England Biolabs, Ipswich, MA), the recombinant adenoviral plasmids were transfected into 293A cells. The harvested adenovirus was concentrated and purified by CsCl gradient centrifugation. Viral titer was determined using Adeno-X Rapid Titer Kit (Clontech, Mountain View, CA). The virus titer was determined using TCID50. A stock titer ranged from 10^{11} to 10^{12} plaque-forming units (pfu)/mL was applied in the following experiments. A control vector containing EGFP gene while without any transgene, was constructed in the same way.

Western blot analysis

To determine the expression of TREM2 after clodronate liposome treatment in MI, heart samples were collected after 1 d, 2 d, 3 d, 5 d and 7 d of MI. The proteins were extracted using Tissue Protein Extraction Kit (Beyotime, China) according to the protocol provided by the manufacturer. The sheep anti-mouse TREM2 antibody (R&D systems, USA) and HRP-rabbit anti-sheep IgG antibody (Amyjet Scientific, China) were used. GAPDH was used as a loading control (KangCheng Biotech, China).

To determine the expression of TREM2 after transfection, heart samples were collected after transfection for 7 days. Additionally, myocardial tissue around injection point and below the injection point were collected. When the heart sample was collected and the injection point and ligation point were determined, the heart is crosscut into three parts according to the position of the point. The around injection myocardium tissue was from 1 to 2 mm above injection point to 1–2 mm below injection point. The rest two parts of myocardium tissue were above injection myocardium tissue and below injection myocardium tissue. The proteins were extracted using Tissue Protein Extraction Kit (Beyotime, China) according to the protocol provided by the manufacturer. The protein in Ad.Null transfection heart sample and none transfection heart sample were extracted from whole heart homogenate. The sheep anti-mouse TREM2 antibody (R&D systems, USA) and HRP-rabbit anti-sheep IgG antibody (Amyjet Scientific, China) were used. GAPDH was used as a loading control (KangCheng Biotech, China).

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Results

The macrophage clearance efficiency

The FACS showed that after clodronate liposome treatment, the ratio of F4/80 positive macrophage was significantly lower than no clodronate liposome treatment after MI. The ratio was decreased from 54.7%±2.3% to 8.2%±1.0% (*Figure S1*, P<0.05).

The protein expression of TREM2 after clodronate liposome treatment in MI

The RT-PCR showed that the TREM2 mRNA expression was increased gradually from after MI although the clodronate liposome treated (*Figure S2A*). Similarly, the TREM2 protein expression was also increased gradually (*Figure S2B*).

The protein expression of TREM2 after adenovirus transfection

The data showed that above injection point, around injection point and below injection point expressed TREM2 compared to control and Ad.Null transfection. The three parts have the similar TREM2 protein expression (*Figure S3*).

References

31. Aurora AB, Porrello ER, Tan W, et al. Macrophages are required for neonatal heart regeneration. J Clin Invest 2014;124:1382-92.

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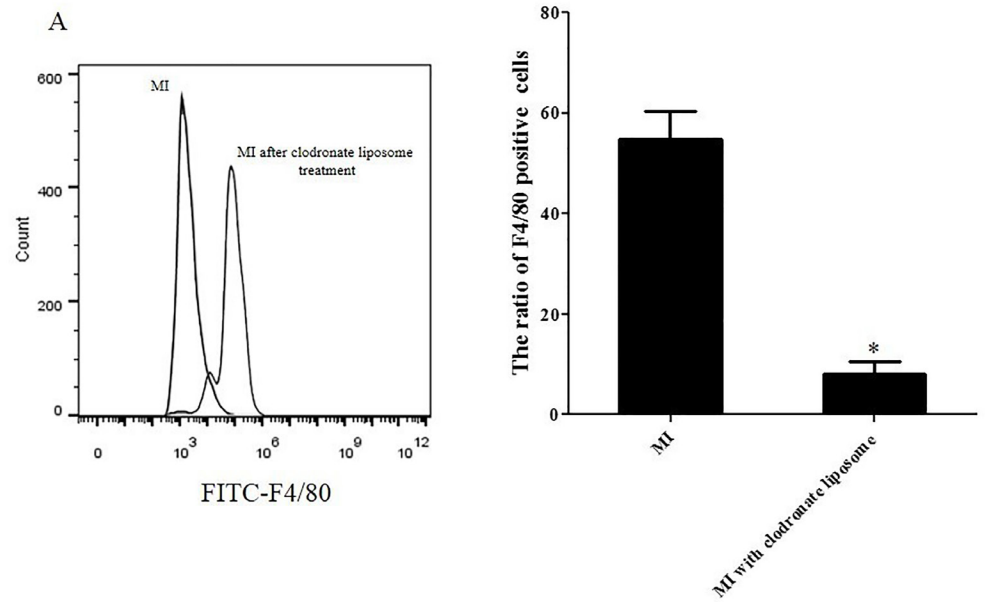

Figure S1 The efficiency of macrophage clearance after clodronate liposome treatment. (A) FACS showed that clodronate liposome effectively eliminated macrophage in myocardial tissue after MI. Left unimodal diagram showed the F4/80 positive cells in heart after MI. Right unimodal diagram showed the F4/80 positive cells in heart after MI and clodronate liposome treatment; (B) histogram showed the ratio of macrophage was decreased from 54.7%±2.3% to 8.2%±1.0% (n=5, *P<0.05 *vs.* MI).

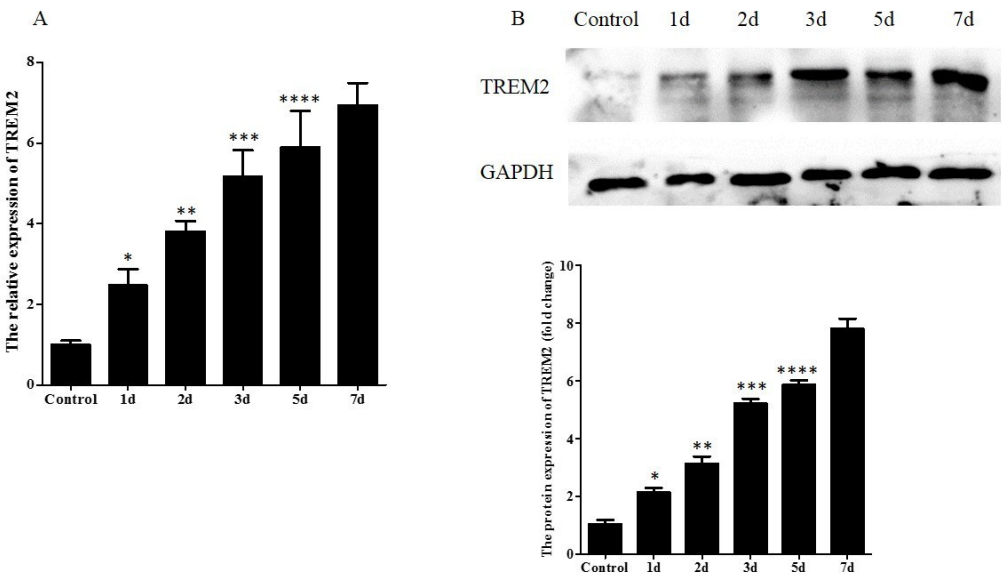

Figure S2 The expression of TREM2 in myocardial tissue after MI and clodronate liposome treatment. (A) The TREM2 mRNA expression in myocardial tissue after MI significantly higher than control in case of clodronate liposome treatment; (B) Western blot showed that TREM2 protein expression in myocardial tissue after MI significantly higher than control in case of clodronate liposome treatment. n=5, *P<0.05 *vs.* control; **P<0.05 *vs.* control, 1 d, 3 d, 5 d and 7 d; ***P<0.05 *vs.* control, 1 d, 2 d, 5 d and 7 d; ****P<0.05 *vs.* control, 1 d, 2 d, 3 d and 7 d.

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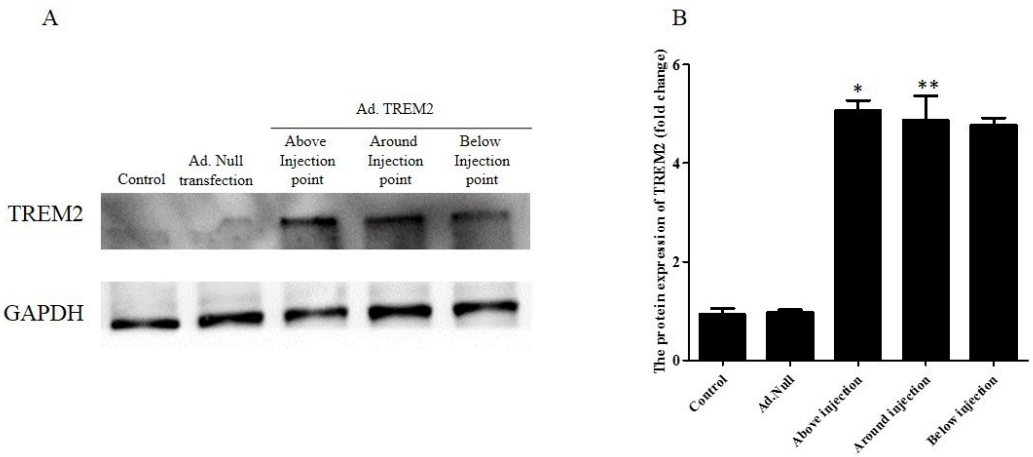

Figure S3 The TREM2 protein expression after adenovirus transfection. (A) The representative western blot band showed that TREM2 expressed in above injection point, around injection point and below injection point; (B) histogram showed the fold change of TREM2 expression. n=5, *P<0.05 *vs.* control and Ad.Null; **P>0.05 *vs.* above injection point and below injection point.

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