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 TREM-2 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).

https://dx.doi.org/10.21037/cdt-21-490

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.

https://dx.doi.org/10.21037/cdt-21-490

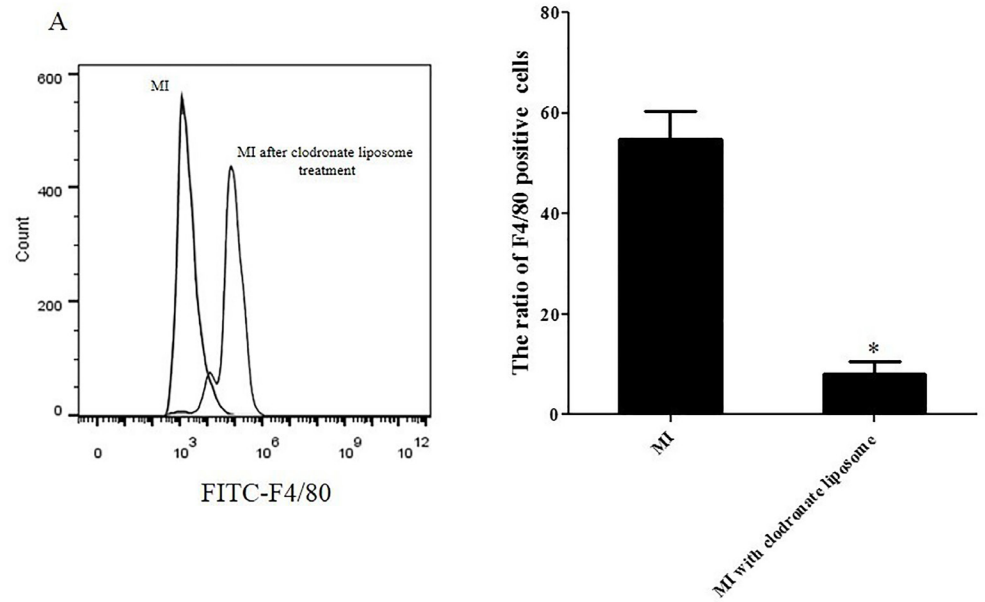

Figure S1 The efficiency of macrophage clearance after clodronate liposome treatment. (A) FACS showed that clodronate liposome effectively eliminated macrophage in myocardial tissue after MI. Left unimodal diagram showed the F4/80 positive cells in heart after MI. Right unimodal diagram showed the F4/80 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).

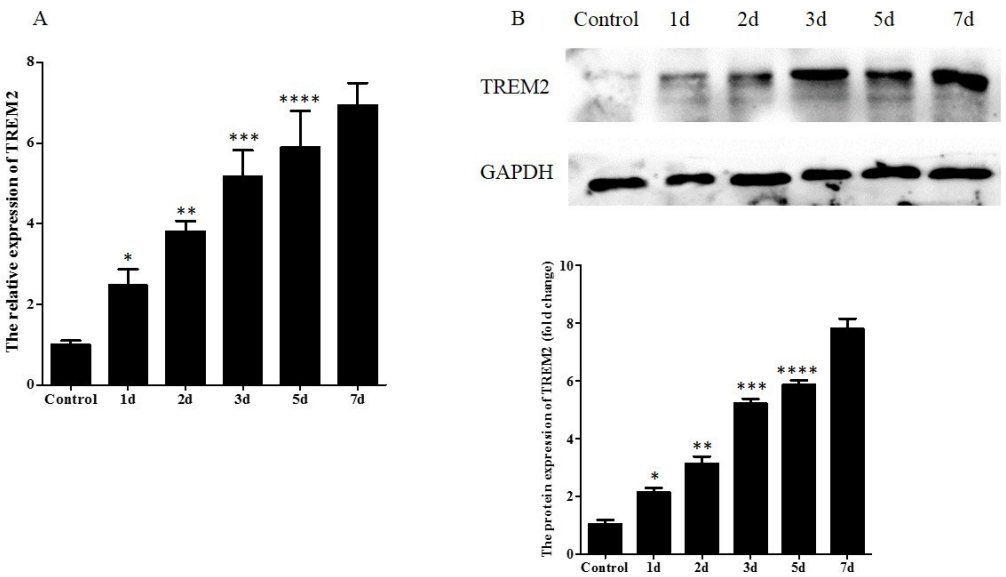

Figure S2 The expression of TREM2 in myocardial tissue after MI and clodronate liposome treatment. (A) The TREM2 mRNA expression in myocardial tissue after MI significantly higher than control in case of clodronate liposome treatment; (B) Western blot showed that TREM2 protein expression in myocardial 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.

https://dx.doi.org/10.21037/cdt-21-490

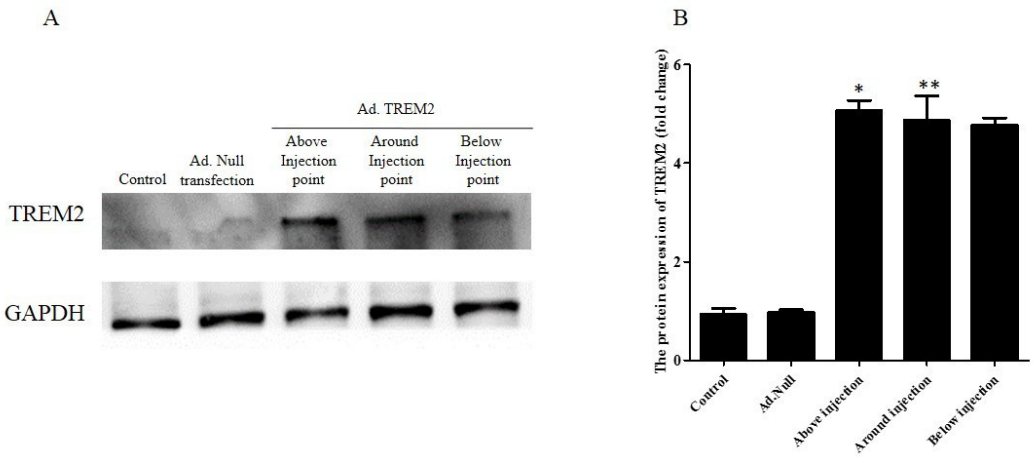

Figure S3 The TREM2 protein expression after adenovirus transfection. (A) The representative western blot band showed that TREM2 expressed in above injection point, around injection point and below injection point; (B) histogram showed the fold change of TREM2 expression. n=5, *P<0.05 *vs.* control and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

https://dx.doi.org/10.21037/cdt-21-490