International Journal of Cardiology 278 (2019) 210–216

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

# The role of CD27-CD70 signaling in myocardial infarction and cardiac remodeling

Wei Li [1], Fengxiao Zhang [1], Chenhui Ju, Suying Lv, Kai Huang *,[2]

*Department of Cardiology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China*
*Clinic Center of Human Gene Research, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China*

ARTICLE INFO

*Article history:*
Received 9 September 2018
Received in revised form 16 November 2018
Accepted 28 November 2018
Available online 3 December 2018

*Keywords:*
CD27
CD70
Acute myocardial infarction
Inflammation
Ventricular remodeling

ABSTRACT

*Background:* $CD4^+$ T cells are key players in regulating the inflammatory processes and physiological repair mechanisms engaged after acute myocardial infarction (AMI). Although signaling through the CD27-CD70 co-stimulatory pathway are known to be important in $CD4^+$ T cell activation and proliferation in certain contexts, the role of the CD27-CD70 pathway in AMI remains unclear.

*Methods and results:* A total of 43 control subjects, 42 unstable angina patients, and 90 AMI patients were enrolled in the present study. The serum levels of soluble CD27 (sCD27) in patients were measured, revealing a significant increase in serum sCD27 levels in AMI patients within 24 h of the cardiac event, after which they decreased. Correlation analyses revealed that serum sCD27 was positively correlated with cardiac troponin I (c-TnI) ($r = 0.267$, $P = 0.011$). When anti-CD70 antibody was used to block the CD27-CD70 pathway in MI model mice, we found that this treatment increased left ventricular end-diastolic dimension (LVEDD) ($P < 0.01$) and left ventricular end-systolic dimension (LVESD) ($P < 0.01$), and decreased ejection fraction ($P < 0.01$). Flow cytometric analysis revealed that the percentage of regulatory T cells was lower in blocking antibody-treated mice ($P < 0.01$), while neutrophils levels were higher ($P < 0.01$). The number of CD31-positive endothelial cells ($P = 0.026$) and $\alpha$-smooth muscle actin-positive arterioles ($P < 0.01$) were significantly down-regulated in anti-CD70 treated-AMI mice. The formation of the extracellular matrix (ECM) was also impaired.

*Conclusion:* Serum sCD27 may be a potential biomarker for AMI. Blockade of the CD27-CD70 pathway worsens cardiac dysfunction, aggravates left ventricular remodeling, and impairs scar healing after AMI, resulting in heart failure.



## 1. Introduction

Acute myocardial infarction (AMI) is a major cause of mortality worldwide [1]. Given the high rate of mortality in individuals post-MI, it is clear that current therapies are insufficient to prevent AMI-induced cardiac dysfunction and subsequent heart failure. Further study is therefore needed in order to understand the mechanisms of cardiac injury and repair following AMI.

In recent years, several studies have found that the immune system plays a critical role in the pathological process of myocardial ischemia injury [2–6]. Ischemic cardiac injury induces immune activation, leads to the release of inflammatory mediators, and facilitates the activation and circulation of inflammatory cells including T lymphocytes, which are recruited into the injured myocardium, contributing to wound healing and ventricular remodeling [2,6–8]. Regulatory T cells (Tregs) are a CD4+ T cell subset capable of suppressing the immune response, with beneficial effects on wound healing after MI [9,10]. While these cells can be beneficial, T cell-mediated pathological autoimmune responses also often occur early after AMI, leading to further myocardial injury and ventricular remodeling [5,11].

CD27 (Gene ID. 939) is a member of tumor necrosis factor receptor (TNFR) family [12]. This protein is constitutively expressed on naive T cells at a steady state, and is also present on natural killer (NK) cells, activated B cells, and hematopoietic stem cells [13,14]. Upon ligation to CD70 (the only known CD27 ligand; Gene ID. 970), a soluble form of CD27 (sCD27) is released from activated T lymphocytes [14,15]. CD27-CD70 signaling is known to provide co-stimulatory signals required for T cell activation, expansion, survival, and memory formation [16–18]. In addition, CD27 signaling has been shown to rescue developing Treg cells in the thymus from undergoing apoptotic cell death [19], increase the frequency of Tregs [20], promote T helper (Th) 1 cell development [21], and stimulate collateral artery formation [22]. Previous studies suggest that CD27–CD70 signaling also contributes to $CD4^+$ T cell-mediated autoimmune pathophysiology [23–25]. However,

* Corresponding author at: 1277 Jiefang Ave, Wuhan 430000, Hubei, China.
*E-mail address:* huangkai1@hust.edu.cn (K. Huang).
[1] Dr. Wei Li and Dr. Fengxiao Zhang contributed equally to this work.
[2] This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

https://doi.org/10.1016/j.ijcard.2018.11.132
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the effects of the CD27-CD70 co-stimulation in the context of AMI remain unclear. Therefore, the purpose of our study was to evaluate the role of CD27-CD70 signaling in the inflammatory and reparative processes engaged after MI.

## 2. Materials and methods

### 2.1. Patients and ethics statement

From May 2017 to February 2018, 175 subjects (43 control subjects, 42 unstable angina patients, and 90 AMI patients) admitted to Wuhan Union Hospital were enrolled in the present study. Subjects with <30% stenosis in a main coronary artery were enrolled into the control group. Patients in unstable angina (UA) group were considered when there is progressive increases in angina symptoms, angina at rest, and with ≥50% stenosis in at least one of the major coronary arteries as confirmed by coronary arteriography. AMI patients were diagnosed according to the American Heart Association/American Heart Association (ACC/AHA) guidelines [26]. Briefly, the inclusion criteria were: ischemic symptoms (>30 min), increased biochemical markers such as cardiac troponin I (c-TnI, Gene ID. 7137) and creatine kinase-MB (CK-MB, Gene ID. 1158), pathological Q waves, and ST-T segment changes. All patients had been diagnosed for the first time and underwent a primary percutaneous coronary intervention (PCI). The Gensini score was used to evaluate the severity of coronary artery stenosis [27]. Peripheral blood samples from all patients were collected within 24 h of admission.

Patients with current infections, malignant diseases, autoimmune diseases, chronic nephritis (estimated glomerular filtration rate < 60 ml/min for at least 3 months), severe liver dysfunction, or shock were excluded from the study.

Experiments with human samples were approved by the Ethical Committee of Huazhong University of Science and Technology (registration number ChiCTR-EOC-17011463) and informed consent was obtained from all study participants. This research was carried out according to the World Medical Association Declaration of Helsinki.

### 2.2. Animals

Male C57BL/6J mice aged 8–10 weeks were purchased from the Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). All procedures were approved by the Animal Care and Utilization Committee of Huazhong University of Science and Technology (project number 2017S743).

### 2.3. Surgical procedures and antibody treatments

Ligation of the left anterior descending (LAD) artery was performed as described previously [28]. Briefly, mice were anaesthetized by using pentobarbital sodium (50 mg/kg, Sigma-Aldrich, St Louis, MO, USA) *via* intraperitoneal injection and were ventilated with a rodent ventilator. The LAD coronary artery was visualized and ligated using a 6–0 prolene suture under a stereomicroscope. The distal end of myocardial discoloration was observed under the microscope to confirm regional ischemia. Sham operated mice were subjected to the same procedures without ligation of the LAD. Mice were sacrificed on days 1, 3, 7, and 14 post-operation and heart tissue was harvested and fixed in 4% paraformaldehyde overnight for histological staining. The freshly infarcted areas of the myocardium were collected for quantitative real-time polymerase chain reaction (PCR) and western blotting. Samples from sham group served as basal controls. To block CD27-CD70 interactions *in vivo*, we injected AMI mice intraperitoneally with anti-CD70 Ab (clone FR70; BioXCell, USA) or a rat IgG isotype control Ab (BE0090; BioXCell, USA). Mice received 500 μg of the appropriate antibody on the day of surgery, and 250 μg/dose on days 2, 4, 6, 8, 10, 12 post-MI [29]. Mice were therefore divided into four groups: sham, AMI, AMI + IgG, AMI + FR70.

### 2.4. Statistical analyses

Statistical analyses were performed using SPSS v20.0. Clinical variables are presented as mean ± SD, or as counts and percentages. Correlations between 2 variables were assessed *via* the Pearson correlation analysis. In animal experiments, the differences between 2 groups were analyzed using unpaired Student's *t*-test, and one-way or two-way ANOVAs were used for multiple comparisons, followed by a *post hoc* Student-Newmann-Keuls test. The threshold of statistical significance was set at $P < 0.05$. Experimental results are representative of 3 independent experiments. Additional materials and methods are available in the Supplementary material.

## 3. Results

### 3.1. Dynamic changes of sCD27 levels in AMI patients

Clinical characteristics and laboratory parameters of all participants are summarized in Table S1. Most of baseline clinical characteristics, including age, gender, body mass index (BMI), hypertension, diabetes mellitus, heart rate, total cholesterol (TC), and triglyceride (TG) levels showed no significant differences between groups. Compared to controls, serum levels of FBG (fasting blood glucose) and HDL-C (high-density lipoprotein cholesterol) were higher in AMI patients ($P < 0.01$; $P < 0.01$). In addition, AMI subjects were more likely to have a history of smoking ($P = 0.03$). Statistically, there was a significant difference between the AMI group and the control group with regard to the severity of coronary artery stenosis, as represented by the Gensini Score ($P < 0.01$).

We next conducted ELISA analyses of patient serum to investigate the levels of serum sCD27. In AMI patients, within first 24 h post-MI, serum sCD27 levels increased significantly ($P < 0.01$) but had declined back to baseline levels within 3 days ($P = 0.91$). Moreover, the serum sCD27 levels were significantly decreased on day 7 as compared with the control ($P < 0.01$) (Fig. 1A). We next investigated the relationship of serum sCD27 with various clinical parameters (Table S2). Serum sCD27 correlated with cardiac troponin I (c-TnI) ($r = 0.267$, $P = 0.011$) and CK-MB ($r = 0.318$, $P = 0.002$). It also correlated with the severity of coronary artery stenosis ($r = 0.240$, $P = 0.023$) (Fig. 1B).

### 3.2. Blockade of CD27-CD70 pathway worsens cardiac dysfunction and promotes cardiac apoptosis

To further investigate the role of the CD27-CD70 pathway in MI development, we took advantage of a commonly used anti-CD70 antibody (FR70) to block this pathway. MI mice were intraperitoneally injected with rat FR70 or an IgG2b isotype control. As shown in Fig. 2A, 23 out of 28 isotype-treated mice survived 7 days after MI (82.1%), while 20 out of 28 FR70-treated mice survived 7 days after MI (71.4%; $P = 0.50$). Furthermore, 20 out of 28 isotype-treated mice survived 14 days after MI (71.4%), while 12 out of 28 FR70-treated mice survived 14 days after MI (42.9%; $P = 0.038$). These data indicated that blockade of the CD27-CD70 pathway did not impact early mortality in infarcted mice, but contributed to the mortality associated with long-term ischemia. Masson's trichrome staining was then performed to evaluate infarct size. Results showed that FR70 treatment significantly increased the infarct size following MI at day 7 or day 14 ($P = 0.019$; $P = 0.03$) (Fig. 2B, C). Besides, there was no significant difference in area at risk (AAR) between the AMI groups at day 1 (Fig. 2D). Together these results thus showed that blockade of CD27-CD70 signaling had a deleterious effect on ischemic responses post-MI.

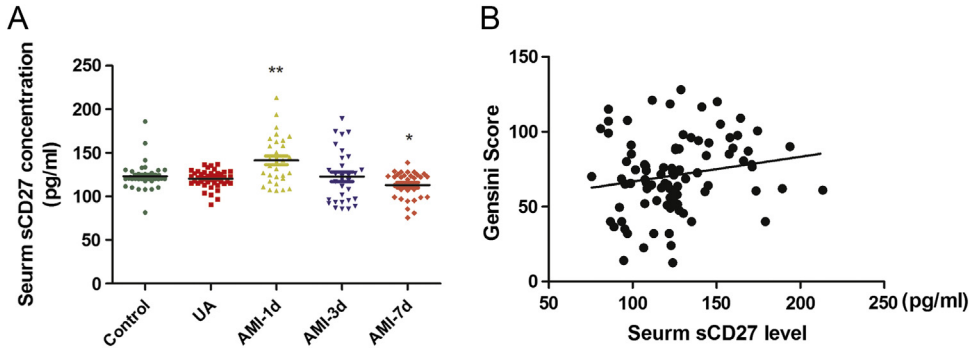



**Fig. 1.** Dynamic changes in sCD27 levels in AMI patients. A. The expression levels of serum sCD27 in the control group, unstable angina (UA) group, and acute myocardial infarction (AMI) group. B, The correlation between Gensini score and circulating sCD27 levels. $^{*}P < 0.05$, $^{**}P < 0.01$ *versus* control group.

Due to the anticipated marked variation in infarct size in mice that underwent AMI, TTE was performed at day 7 and day 14 post-MI in these animals (Fig. 2E, F). As described previously [30], *in vivo* coronary ligation lead to heart failure. Compared to the isotype-treated group, the LVEDD and LVESD were significantly increased and the EF and FS were significantly decreased in the FR70-treated group on day 7 ($P < 0.01$) and day 14 ($P < 0.01$) post-MI (Fig. 2E, F). These results indicated that activation of the CD27-CD70 pathway could prevent cardiac dysfunction at early time points post-MI.

To further determine the mechanism of blocking CD27-CD70 pathway in AMI mice, cell apoptosis was measured by TUNEL staining on day 14 after MI. As shown in Fig. 2G and H, the ratio of apoptotic cells in peri-infarct area were significantly increased in anti-CD70 antibody treated group on day 14 post-MI. The protein level of apoptosis related factor cleaved caspase-3 was also up-regulated in the FR70 treated group (Fig. 2I). These data suggest that blockade of CD27-CD70 pathway may indirectly worsen MI induced myocardial dysfunction by promoting cardiac apoptosis.

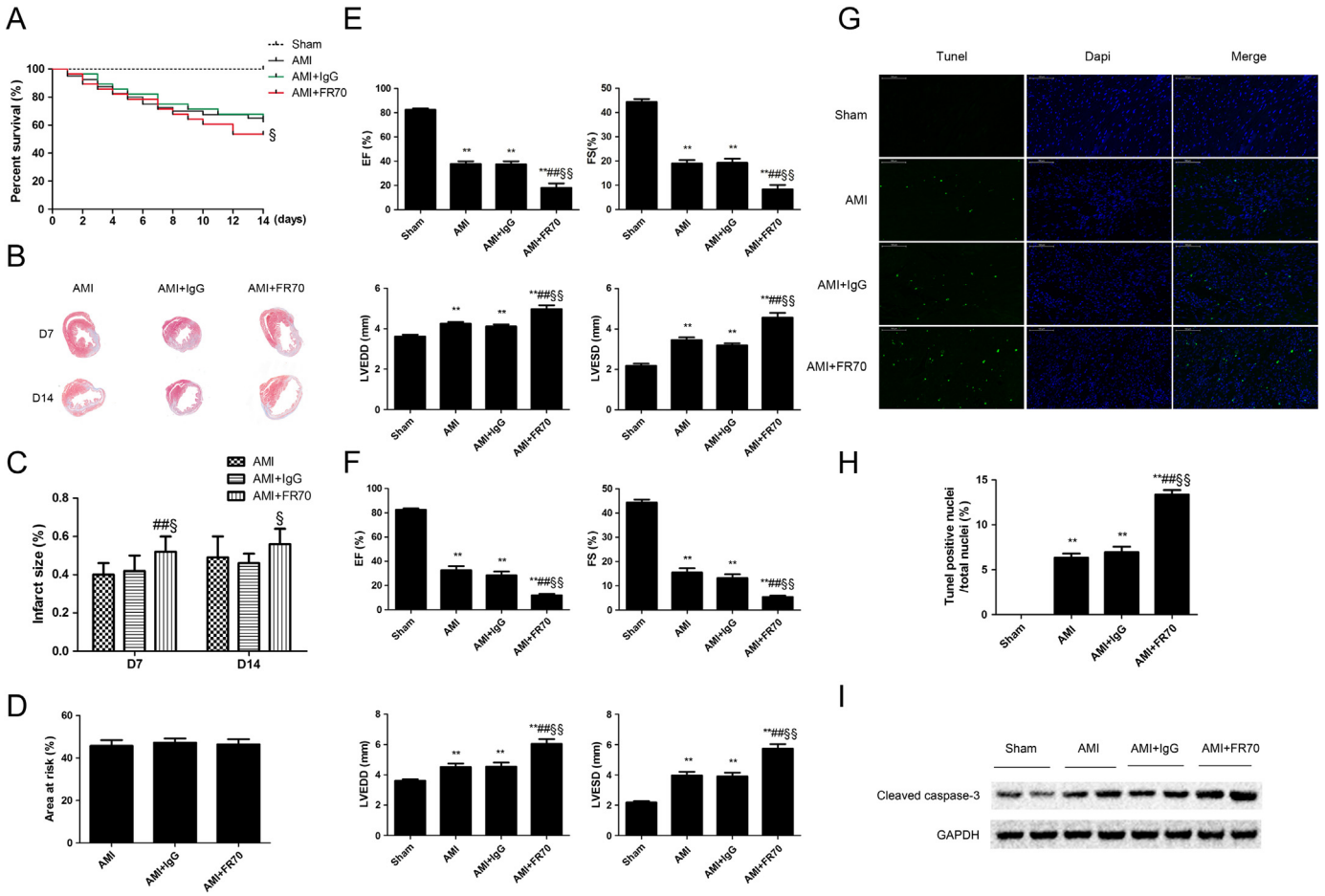
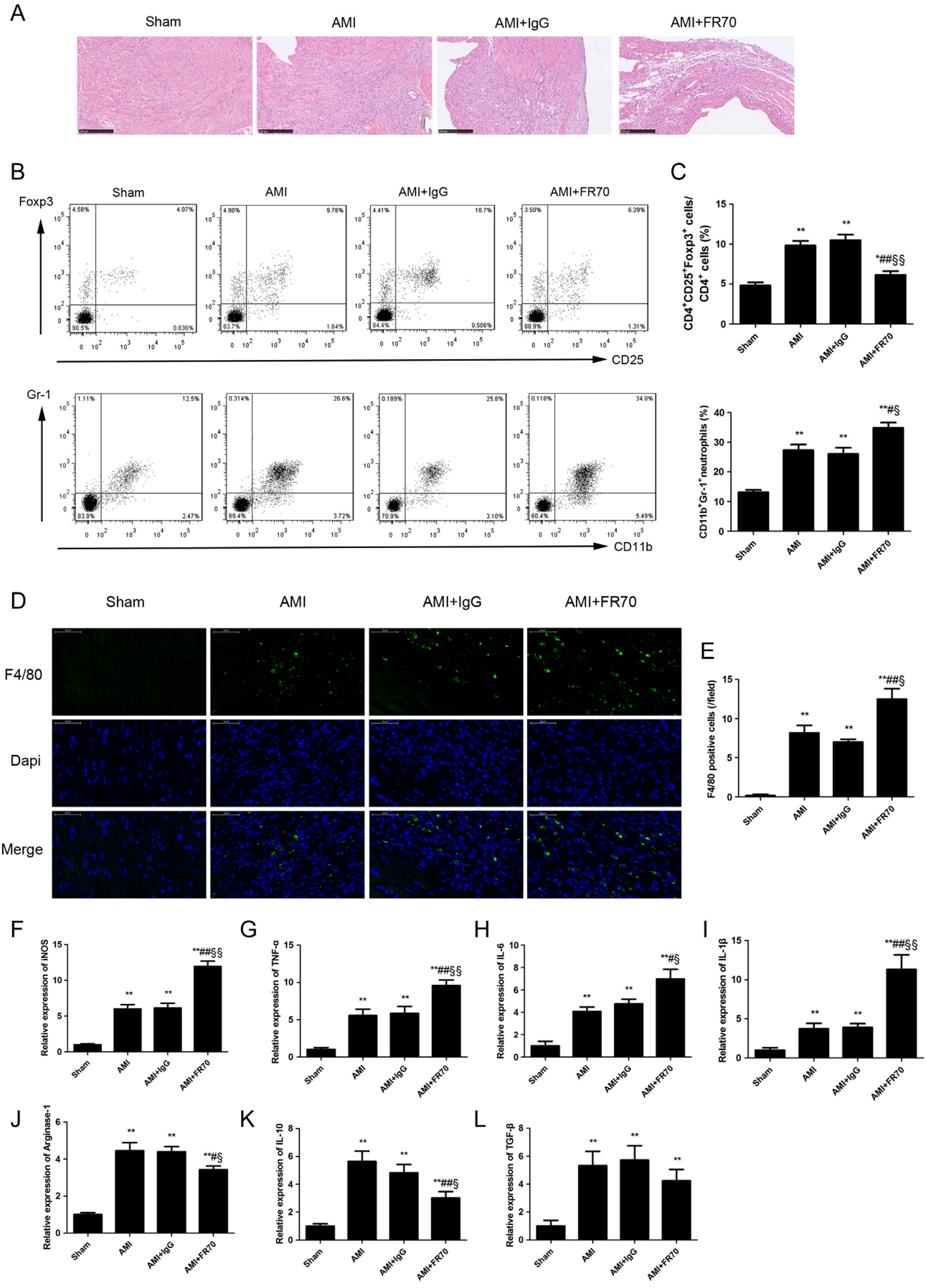



**Fig. 2.** Blockade of the CD27-CD70 pathway worsens cardiac dysfunction and promote cardiac apoptosis. A. A Kaplan-Meier survival analysis of the Sham group ($n = 20$), AMI group ($n = 40$), AMI + IgG group ($n = 28$), and AMI + FR70 group (n = 28). B. Representative images of Masson's trichrome staining of cardiac sections. C. Quantification of infarct sizes at 7 and 14 days after MI ($n = 6$–8). D. Quantification of area at risk (AAR) on day 1 after MI (n = 6–8). Left ventricular end-diastolic dimension (LVEDD), left ventricular end-systolic dimension (LVESD), ejection fraction (EF), and fractional shortening (FS) on day 7 (E) and day 14 (F) post-MI ($n = 7$–10) were measured. G. Representative images of terminal deoxynucleotide transferase dUTP nick end labelling (TUNEL) staining showing cardiac apoptosis on day 14 after AMI. Original magnification ×200, $n = 5$ per group. H. The percentages of TUNEL-positive cells. I. The protein levels of cleaved caspase-3 in infarcted hearts at 14 days after AMI. $^{**}P < 0.01$ *versus* sham group, $^{\#\#}P < 0.01$ *versus* AMI group, $^{\S}P < 0.05$, $^{\S\S}P < 0.01$ *versus* AMI + IgG group.


A
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Relative expression of IL-6
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Relative expression of IL-1β
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Relative expression of Arginase-1
K
Relative expression of IL-10
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Relative expression of TGF-β

### 3.3. Blockade of the CD27-CD70 pathway enhances MI-induced inflammation

Acute myocardial injury-induced cardiomyocyte death triggers the activation of innate immune mechanisms initiating inflammatory responses, ultimately leading to the formation of a collagen-based scar. When this inflammatory response is excessive, it can lead to sustained myocardial damage and adverse healing, enlarging the infarct area and thereby inducing cardiac dysfunction. Relative to sham controls, after surgery mice in the AMI model group exhibited increased separation of cardiac muscle fibers and necrosis with inflammatory cell infiltration, as detected in H&E-stained sections (Fig. 3A). Treatment with anti-CD70 antibody aggravated this separation of cardiac muscle fibers, and was associated with increased inflammatory cell infiltration (Fig. 3A).

In order to assess which immune cells subsets infiltrating the heart of MI mice are affected by CD27-CD70 signaling, we performed a flow cytometric analysis of cells harvested from infarcted hearts. The results demonstrated that FR70 treatment significantly decreased the number of $CD4^+CD25^+Foxp3^+$ Tregs ($P < 0.01$) in the infarcted heart at day 3 post-ischemia, while levels of $CD11b^+Gr1^+$ neutrophils were clearly up-regulated ($P < 0.01$) (Fig. 3B, C). The percentages of $CD4^+$ T cells and $CD8^+$ T cells did not change (Fig. S1). As immunofluorescence staining demonstrated, the F4/80 macrophages infiltrated in the peri-infarcted area markedly increased in FR70 treated group, compared to other MI groups (Fig. 3D, E). Furthermore, real-time PCR assay showed that, FR70 treatment resulted in an increase in the cardiac expression of M1 macrophage markers inducible nitric oxide synthase (iNOS), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-1β (IL-1β) ($P < 0.01$; $P < 0.01$; $P = 0.042$; $P < 0.01$) (Fig. 3F–I), whereas expression of Arginase-1 which is associated with M2 macrophage polarization was down-regulated ($P = 0.014$) (Fig. 3J). We also found that the anti-inflammatory cytokine interleukin-10 (IL-10), which is known to be released primarily by Tregs, was decreased ($P = 0.030$) in these FR70-treated mice (Fig. 3K). Furthermore, no significant differences were observed in transforming growth factor-β (TGF-β) mRNA levels between isotype- and FR70-treated groups ($P = 0.278$) (Fig. 3L). These results suggested that the anti-CD70 treatment might have led to M1 macrophage accumulation in MI heart, thus resulting in a prolonged inflammatory period and impaired wound healing.

### 3.4. Blockade of the CD27-CD70 pathway suppresses ECM formation and angiogenesis after MI

ECM remodeling had been demonstrated to play a key role in tissue repair and scar healing post-infarct [31]. ECM formation was impaired in the FR70 treated group post-MI, as indicated by decreased mRNA and protein levels of collagen I and III (Fig. 4A, B). Masson's trichrome staining further demonstrated that the collagen deposition in the infarct area was reduced in FR70 treated mice compared with the isotype controls at 14 days post-MI ($P = 0.01$) (Fig. 4C, D). In addition, protein levels of MMP-9 in the FR70 group were lower than in the isotype group (Fig. 4B).

Angiogenesis has the potential to protect the ischemic myocardium during early stages after MI, and is also essential for long-term left ventricular remodeling to prevent the transition to heart failure [32,33]. Simons et al. have shown that inhibition of CD27-CD70 co-stimulation impairs post-ischemic blood flow recovery in a hind limb ischemia mouse model [22]. To further study how angiogenesis is affected in our experimental system, we performed immunofluorescence staining for CD31 and α-SMA on day 14 post-MI (Fig. 4E, G). We observed that FR70 treatment significantly reduced capillary density in infarct area ($P < 0.01$) and peri-infarct area ($P = 0.026$) but not remote area ($P = 0.87$) (expressed as the number of CD31-positive cells per field) in MI mice (Fig. 4E, F, Fig. S2A, B). As shown in Fig. 4H, we also found that α-SMA mRNA expression was down-regulated in anti-CD70-treated mice compared to the isotype-treated group at day 7 ($P < 0.01$) and day 14 ($P < 0.01$) post-MI. There were only few α-SMA positive arterioles found in sham group, with no obvious differences observed between the AMI and isotype-treated groups (Fig. 4G, I). However, the α-SMA positive arterioles were also decreased in the FR70-treated group as compared with the isotype-treated group in the infarct ($P = 0.04$) and peri-infarct area ($P < 0.01$) (Fig. 4G, I, Fig. S2C, D). All these results showed that anti-CD70 antibody inhibits ECM formation and angiogenesis of MI hearts.

## 4. Discussion

In the present study, we demonstrated that serum sCD27 levels were positively correlated with the severity of coronary artery stenosis in MI patients. In MI model mice, anti-CD70 blocking antibody administration aggravated neutrophil and macrophage infiltration but decreased Treg infiltration into the cardiac tissue, thus accentuating the resultant inflammation. Blockade of CD27-CD70 further inhibited angiogenesis and ECM formation after MI, resulting in impaired wound healing and cardiac dysfunction. Together, these data from MI patients and a mouse model of MI have for the first time thus identified the important role of the CD27-CD70 pathway in MI.

We found that serum sCD27 levels were significantly higher in the early stages of MI, suggesting that AMI promoted the release of sCD27 from PBMCs. This serum level of sCD27 was correlated with c-TnI and Gensini scores in patients. Furthermore, Masson's trichrome staining revealed that the blockade of CD27-CD70 led to an expansion of the necrotic area post-MI in mice, which may in turn increase immune cell infiltration and cytokines production in the infarcted heart, ultimately leading to a poor prognosis. These data therefore suggest that sCD27 quantification may be helpful in assessing severity of MI.

Myocardial infarction triggers an intense inflammatory response that is indispensable for cardiac repair. Inflammatory cell activation can alter the process of post-infarction remodeling and heart failure [34,35]. It has been reported that Treg cells ameliorate cardiac remodeling and that insufficient recruitment of Tregs results in adverse ventricular remodeling after MI [36,37]. In addition, Coquet et al. demonstrated that CD27-CD70 interactions can effectively promote the survival and development of Tregs, and that a deficiency of CD27 or its ligand CD70 decreased Treg cell numbers [19]. One recent study has indicated that CD70 expressed on T cells can ameliorate inflammatory diseases *via* a regulatory T cell-independent mechanism [38]. In our study, we observed that anti-CD70 treatment leads to the decreased infiltration of Tregs into infarcted hearts, with a corresponding decrease in IL-10 expression. However, TGF-β, which produced by several types of cells [29], did not differ significantly among the AMI, isotype-treated, and FR70-treated groups. Moreover, the levels of neutrophils, macrophages and pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6 were increased in anti-CD70 treated mice. iNOS and Arginase-1 are knowns as the phenotype marker of M1 and M2 macrophages, respectively. Our data suggest that the CD27-CD70 signaling may play a crucial role in regulating macrophage polarization. Taken together, our results

**Fig. 3.** Blockade of the CD27-CD70 pathway enhances the MI-induced inflammatory response. A. Representative haematoxylin and eosin (H & E) staining images of the peri-infarct area of the infarcted myocardium at 7 days after AMI. Original magnification ×100, n = 5 per group. B. Representative flow cytometric images of $CD25^+$ $Foxp3^+$ Tregs (gated on $CD4^+$ T cells) and $Gr\text{-}1^+$ $CD11b^+$ neutrophils infiltrating the myocardium at 3 days after MI. C. Quantitative analysis of the percentage of Treg cells and neutrophils in the infarcted heart (n = 7 per group). D. Representative images of F4/80-positive cells in the peri-infarct area on day 3 after MI. Original magnification ×400. n = 6 per group. E. Quantification of the number of F4/80-positive cells. F–L. The mRNA levels of inducible nitric oxide synthase (iNOS), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-1β (IL-1β), Arginase-1, interleukin-10 (IL-10), and transforming growth factor-β (TGF-β) in infarcted myocardial samples at 7 days after MI (n = 6–8). $^*P < 0.05$, $^{**}P < 0.01$ *versus* sham group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *versus* AMI group, $^{\S}P < 0.05$, $^{\S\S}P < 0.01$ *versus* AMI + IgG group.

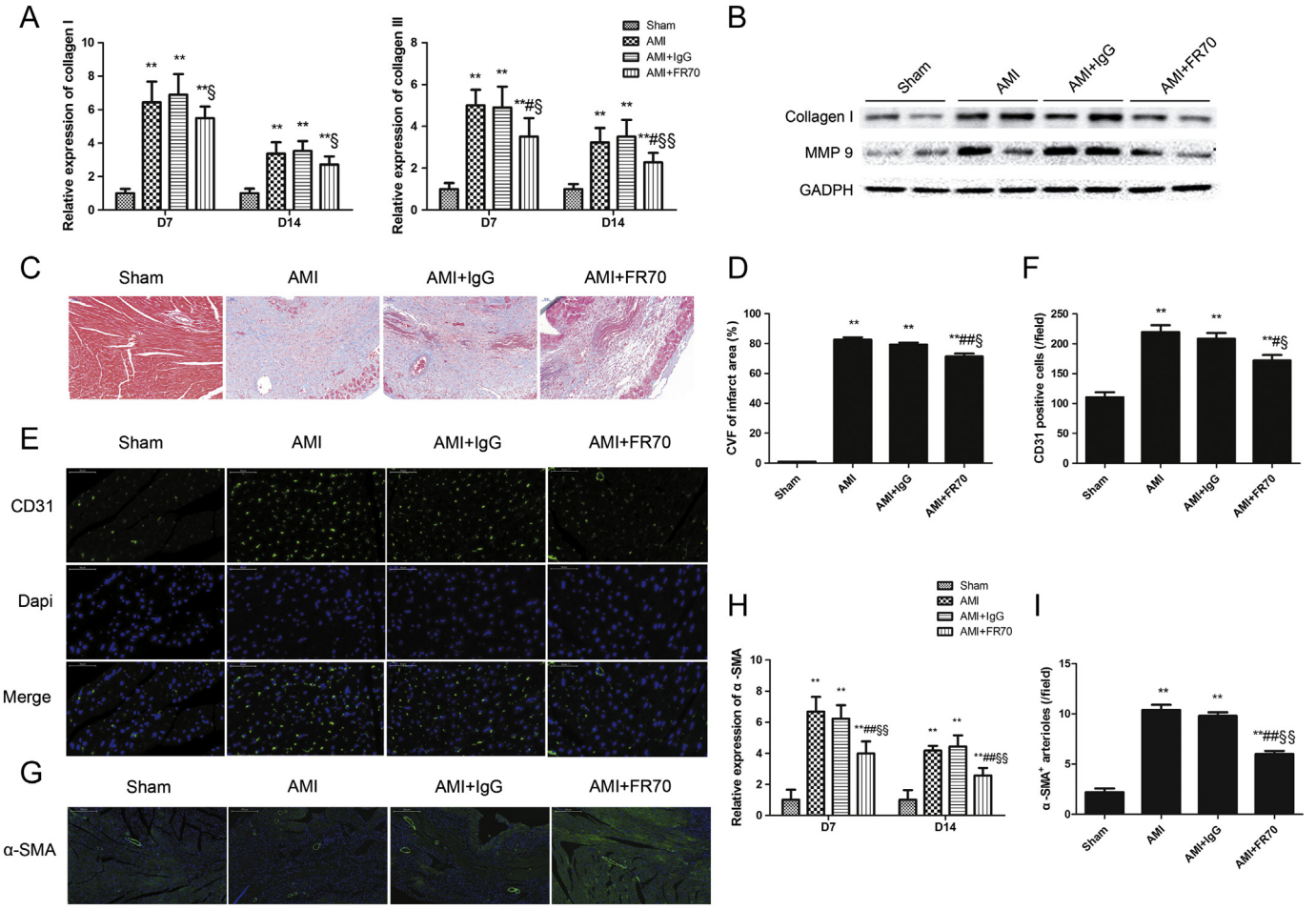


**Fig. 4.** Blockade of the CD27-CD70 pathway suppressed extracellular matrix (ECM) formation and angiogenesis after MI. A. The mRNA levels of collagen I and collagen III at 7 and 14 days after MI (n = 6 per group). B. Collagen I and MMP-9 protein levels in the infarct area on day 7 post-MI. C. Representative images of Masson's trichrome staining of collagen deposition (blue) in the infarct area on day 14 post-MI. Original magnification ×200, n = 5 per group. D. The extent of fibrosis as determined based on collagen volume fraction (CVF) in the infarct area on day 14 after MI (n = 5 per group). E. Representative images of CD31-positive endothelial cells in the peri-infarct area on day 14 after MI. Original magnification ×400. n = 6 per group. F. Quantification of the number of CD31-positive cells. G. Representative images of α-SMA positive arterioles in the peri-infarct area on day 14 after MI. Original magnification ×100, n = 5 per group. H. α-SMA mRNA expression in the infarcted myocardium at 7 and 14 days after MI (n = 6 per group). I. Quantification of the number of α-SMA positive arterioles. $^{**}P < 0.01$ *versus* sham group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *versus* AMI group, $^{\S}P < 0.05$, $^{\S\S}P < 0.01$ *versus* AMI + IgG group.

suggest that CD27-CD70 signaling plays an anti-inflammatory role in the context of MI.

AMI triggers undesirable changes in myocardial ECM formation and homeostasis, including break down of collagen fibers, resulting in adverse myocardial remodeling [39–41]. Wound healing post-ischemia requires the expression of ECM proteins, such as collagen, which help to form a stable scar. Our study revealed that anti-CD70 treated mice exhibit reduced expression of types I and III collagen, indicating that blocking CD27-CD70 inhibited ECM production. Traditionally, MMPs are thought to be the dominant proteases responsible for degrading ECM proteins and contributing to poor prognosis after MI [42,43]. MMP-mediated proteolysis triggers neutrophil migration into injured tissue soon after AMI [35,44]. Neutrophils have been identified to be the primary source of MMP-9 during the early inflammation [45,46]. Our data suggest that in anti-CD70 treated mice, the increased infiltration of neutrophils in turn lead to increased MMP-9 production, aggravating the localized reduction in ECM. These results therefore suggest that CD27-CD70 signaling may contribute to the formation of a stable collagenous scar after MI.

Angiogenesis occurs in the granulation tissue that will ultimately form the infarct scar, as efficient perfusion provided by micro vessels is required to prevent cardiomyocyte death, which can lead to infarct expansion and cardiac dysfunction. In our AMI mouse model, we also observed reduced angiogenesis in anti-CD70 treated group. This also contributed to undesirable healing and cardiac dysfunction.

There are some limitations of the present study. For one, the clinical sample sizes were small for analyses in subgroups. Subsequent studies will be needed in order to extend these findings to later time-points.

In conclusion, we confirmed for the first time that CD27-CD70 co-stimulation appears to protect myocardial infarction induced ventricular remodeling and promote wound healing. This provides evidence that the CD27-CD70 pathway may be therapeutic target for related cardiovascular diseases.

## Acknowledgements

This work was supported by National Natural Science Foundation of China (No: 81400303, No: 81570405, No: 81570257), Major key technology research project of Department of Science and Technology, Hubei Province (2016ACA151), National Key Research and Development Program (2016YFA0101100) and Key projects of Huazhong University of Science and Technology (2016JCTD107).

Kai Huang, Fengxiao Zhang designed the experiments and wrote the manuscript. Wei Li performed the experiments. Wei Li, Chenhui Ju and Suying Lv performed the surgical experiments in mice. Wei Li and Fengxiao Zhang analyzed the data.

## Conflict of interest statement

None.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2018.11.132.

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