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Suppression of lysosomal-associated protein transmembrane 5 ameliorates cardiac function and inflammatory response by inhibiting the nuclear factor-kappa B (NF-κB) pathway after myocardial infarction in mice

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Abstract: Myocardial infarction (MI) as the remarkable presentation of coronary artery disease is still a reason for morbidity and mortality in worldwide. Lysosomal-associated protein transmembrane 5 (LAPTM5) is a lysosomal-related protein found in hematopoietic tissues and has been confirmed as a positive regulator of pro-inflammatory pathways in macrophages. However, the role of LAPTM5 in MI remains unknown. In this study, we found that both mRNA and protein expression levels of LAPTM5 were significantly elevated in MI mice. Suppression of LAPTM5 in myocardial tissues decreased cardiac fibrosis and improved cardiac function after MI. At the molecular level, downregulated LAPTM5 dramatically suppressed the macrophage activation and inflammatory response via inhibiting the activation of the nuclear factor-kappa B (NF-κB) pathway. Collectively, suppression of LAPTM5 in myocardial tissues inhibits the pro-inflammatory response and the cardiac dysfunction caused by MI. This study indicated that LAPTM5 as a pro-inflammatory factor plays a crucial role in MI disease.
Key words: inflammatory response, lysosomal-associated protein transmembrane 5 (LAPTM5), myocardial infarction, nuclear factor-kappa B (NF-κB) pathway

Introduction

Myocardial infarction (MI), a type of coronary atherosclerotic heart disease [1], is a series of clinical manifestations of myocardial necrosis caused by coronary artery occlusion, and about 7 million patients were diagnosed with MI each year [2]. MI leads to the dysfunction of cardiomyocytes, enhancement of vascular permeability, myocardial fibrosis, and left ventricular remodeling [3]. Besides that, MI usually causes an enhanced inflammatory response, which is of utmost essential for the process of cardiac wound healing [4]. Even though the mortality of MI is clearly decreased under the effective therapy strategies, the sufferers who survived MI still confronted an enhanced risk of heart failure as a consequence of reduced cardiac function, excessive cardiac fibrosis, and left ventricular remodeling [5]. Thus, it is crucial to find the rapid and exact diagnostic markers of MI as well as promote the clinical treatment and survival rate of MI patients.

Lysosomal-associated protein transmembrane 5 (LAPTM5), a kind of lysosomal-related protein found

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in hematopoietic tissues [6, 7], is firstly isolated from the hematopoietic and non-hematopoietic cells according to a subtractive hybridization strategy [8]. LAPTM5 is preferentially expressed in cells of lymphoid and myeloid origin [8]. LAPTM5 involves five hydrophobic transmembrane domains, with C-terminal tyrosine-based lysosomal targeting motifs [9]. It has been confirmed that it participates in the degradation of phagosomes through the lysosomal pathway and is related to cell apoptosis and its clearance [10, 11]. Besides that, some researchers have found that LAPTM5 is engaged in some signaling pathways regulation, containing nuclear factor-kappa B (NF-kB), transforming growth factor-β/Smads (TGF-β/Smads), mitogen-activated protein kinase kinase-extracellular regulated protein kinases 1/2 (MEK-ERK1/2), phosphatidylinositide 3-kinases-AKT (PI3K-AKT), and mitogen-activated protein kinase (MAPKs), which are related to cardiovascular diseases [12–14]. These studies suggest that LAPTM5 may play an important role in cardiovascular disease, while, the role of LAPTM5 in MI is not clear. Inflammation and fibrosis play a fundamental role in the development of MI [15]. After MI, for tissue integrity, a large number of immune cells will invade, including macrophages recruited from the circulatory system. M1 macrophages could produce pro-inflammatory cytokines to exacerbate the cardiac injury, promote the proliferation and activation of myofibroblasts, and recruit the circulating fibrocytes [16, 17]. Interestingly, it was reported that LAPTM5 is up-regulated during the differentiation of histiocyte lymphoma cells into monocytes/macrophages induced by 12-O-tetradecanoylphorbol 13-acetate (TPA) [10]. And a recent study has indicated that LAPTM5 acts as a positive regulator in pro-inflammatory pathways and pro-inflammatory cytokines generation in macrophages [18]. In addition, the gene expression omnibus (GEO) database (GSE161427) also showed that compared with the sham operation group, the expression of LAPTM5 is significantly upregulated in the myocardial tissues on 3 days after MI.

Accordingly, in this study, we delivered lentivirus carrying shRNA-LAPTM5 by using intra-myocardial injection to knock down LAPTM5 in a mice MI model. The purpose of this work was to explore whether LAPTM5 plays a vital role in cardiac function and could depress atrial inflammatory, profibrotic properties, and related signaling pathways in the mice MI model.

Materials and Methods

Animals

Approximately eight-week-old male C57BL/6 mice were used for animal studies. Mice were housed in 12 h light and 12 h dark surroundings with access to sufficient food and water. All experiments in this study were conformed to the Guide for the Care and Use of Laboratory Animals and approved by the Animal Experimental Ethical Inspection of Fushun central hospital of TCM (No. 2020001).

Myocardial infarction models

MI models were induced as follows. Briefly, mice were anesthetized by intraperitoneal injection with pentobarbital sodium (45 mg/kg, Xiya Reagent, Shandong, China), and intubated with mechanical ventilation during the experiment. The left anterior descending (LAD) coronary artery was permanently ligated using a 7-0 nylon suture. In the sham group, the mice underwent sham-operation, which comprised the same experimental procedures as the MI group, but without ligation of the LAD. The mice (n=24) were divided into four groups according to the different treatments: the sham, the MI, the MI+LV-shRNA-NC, and the MI+LV-shRNA-LAPTM5 groups. For LV-shRNA-NC and LV-shRNA-LAPTM5 groups, lentivectors were multipoint injected into the edge of infarcted area of myocardium after MI operation (2×10^6 TU in 10 μl).

Real-time quantitative PCR

Total RNA was isolated from heart slice tissues using TRIpure (BioTeke, Beijing, China), and quantified by Nano 2000 spectrophotometry (Thermo, Waltham, MA, USA). The cDNA was prepared with the total RNA using BeyoRT II M-MLV reverse transcriptase (Beyotime, Shanghai, China). The level of mRNA was measured by qRT-PCR analysis based on SYBR Green kit (Solarbio, Beijing, China) and quantified with the Exicycler™ 96 apparatus (BIONEER, Daejeon, Korea). The primers were obtained from GenScript Biotechnology Co., Ltd., in Nanjing, China. *LAPTM5* primers: forward 5′ GAGTCGCCACCATAGCC 3′ and reverse 5′ TTGAAGGGACAGGAAGG 3′. *IL-1β* primers: forward 5′ CTCAACTGTGAAATGCCACC 3′ and reverse 5′ GAGTGATACTGCCTGCCTGA 3′. *IL-6* primers: forward 5′ ATGGCAATTCTGATTGTATG 3′and reverse 5′ GACTCTGGCTTTGTCTTTCT 3′. *TNF-α* primers: forward 5′ CAGGCGGTGCCTATGTCTCA 3′ and reverse 5′ GCTCCTCCACTTGGTGGTTT 3′. *β-actin* in this work was used as an internal standard and the gene expression level was calculated by the $2^{-\Delta\Delta Ct}$ method.

Western blot

As for Western blot, the whole proteins were extracted with lysate buffer (RIPA buffer, Beyotime). Quanti-

fication was accomplished by the BCA protein assay kit (Beyotime). After that, the proteins were electrophoresed in a 10% SDS-PAGE gels electrophoresis for separation and transferred onto the polyvinylidene fluoride membrane. Blocking buffer was prepared with 5% bovine serum albumin (Biosharp, Anhui, China). Then, they were immunoblotted with the primary antibodies (1:1,000) and detected with horseradish peroxidase-conjugated goat anti-rabbit-IgG (1:10,000, Proteintech, Wuhan, China). Then, the membranes were visualized by using Gel-Pro-Analyzer Software (Beijing, China). The primary antibodies involved in this procedure are listed as follows: LAPTM5 antibody, MCP1 antibody, and CD86 antibody were obtained from ABclonal (China). nuclear factor-kappa B (NF-κB) p65 antibody, NF-κB p-p65 antibody, p-IκBα antibody, and IκBα antibody were purchased from Affinity (Jiangsu, China).

Echocardiographic analysis

Echocardiography was used to evaluate cardiac function according to a VOLUSON E8 color doppler (GE, Fairfield, CT, USA). Twenty-eight days after MI, mice were anesthetized with pentobarbital sodium (Sinopharm Chemical Reagent Co., Ltd., Beijing, China) for the experiment. Left ventricular internal dimension at end-diastole (LVIDd) and left ventricular internal dimension at systole (LVIDs) were tested at the maximal and minimal diameters, respectively. Ejection fraction (EF) and fractional shortening (FS) were measured on the M-mode tracings and based on statistical analysis on an average of three cardiac cycles.

TTC staining

Twenty-eight days after LAD artery ligation, the mice were euthanized, and then the hearts were removed for this analysis. According to the method of 2, 3, 5-triphenyl tetrazolium (TTC) staining, myocardial tissues was isolated and cut into 5 slices and then immersed in 2 ml TTC staining solution (Solarbio) in phosphate buffer (pH 7.4) at 37°C for 15 min in the dark. TTC was dissolved beforehand with 0.01 M PBS and prepared into a 2% staining solution, which was shielded from light for reservation. Ipp6.0 software was used to calculate the infarct area and the percentage of infarct area. The mean percentage of infarct area of the last 5 pieces of myocardium was defined as the percentage of infarct area of this mouse.

Masson's trichrome staining

In Masson's trichrome staining, the myocardial tissues were embedded in paraffin and cut into 5-μm sections to demonstrate collagen. Paraffin-embedded sections were deparaffinized and dehydrated by xylene (Aladdin, Shanghai, China) and absolute alcohol (Sinopharm Chemical Reagent Co., Ltd., Beijing, China). Subsequently, the nuclei were stained with Regaud's hematoxylin solution (Solarbio) for 6 min. The resulting sections were washed successively with running water and distilled water. After fully washing with water, the sections were stained with ponceau-acid fuchsin solution (Sinopharm Chemical Reagent Co., Ltd.) for 1 min. Next, they were immersed in 0.2% glacial acetic acid solution for a moment, followed by 5 min of differentiation using 1% phosphomolybdic acid aqueous solution. After that, aniline blue was used for counterstaining at 5 min and immersed in 0.2% glacial acetic acid solution again. Finally, the sections were transparentized using 95% alcohol, absolute alcohol, and xylene, and mounted in neutral resin for further observation.

Hematoxylin and eosin (H&E) staining

Similar to Masson's staining; the myocardial tissues were also embedded in paraffin and cut into 5-μm sections for H&E staining. All slides were deparaffinized, rehydrated in xylene, 100%, 95%, 85%, 75% ethanol, and H_2O, and constantly stained with 10% hematoxylin (Solarbio) for 5 min, followed by 1% hydrochloric acid alcohol for 3 s. The resulting sections were washed successively with running water and distilled water. Next, they were immersed in eosin Y ethanol solution (Sangon, Shanghai, China) for 3 min. Finally, the sections were transparentized using 75%, 85%, 95% alcohol, absolute alcohol, and xylene, and mounted in neutral resin for further observation.

Immunofluorescence staining

Immunofluorescence (IF) staining was constructed by the method described in many studies. The tissue was embedded in paraffin and cut into 5-μm sections beforehand. All slides were deparaffinized, rehydrated in xylene, 100%, 95%, 85%, 75% ethanol, and PBS, and constantly stained with 10% antigen retrieval solution for 10 min. BSA (1%, Sangon) as the blocking buffer was incubated at room temperature for 15 min. The primary antibody was added and incubated at 4°C overnight. Then, the secondary antibody, and 4′,6-diamidino-2-phenylindole (DAPI) (Aladdin) were added to this procedure. The antibodies used in this section involved as follows: NF-κB p-p65 antibody (1:200, Affinity, Jiangsu, China), CD68 (1:50, Santa Cruz, Santa Cruz, CA, USA), iNOS (1:100, ABclonal), Cy3 labeled goat anti-mouse IgG (1:200, Invitrogen, Carlsbad, CA, USA), Cy3 labeled goat anti-rabbit IgG (1:200, Invitrogen), FITC labeled goat anti-mouse IgG (1:200, Abcam, Cambridge,

UK). The immunofluorescence images were taken and preserved using an OLYMPUS-BX53 microscope (OLYMPUS, Tokyo, Japan).

Statistical analysis

The results in this subject were subjected to statistical analysis using Graphpad Prism software version 8.0. Unpaired *t*-test and one-way analysis of variance with Tukey post hoc test was used for the results analysis in this work. Data were collected from independent experiments and $P<0.05$ was considered to be statistically significant.

Results

Expression of LAPTM5 is increased after MI

Results of the GEO database (GSE: 161427) in Fig. 1A obviously implied a high expression of *LAPTM5* in MI tissues (n=5) at 3 days as compared to the sham (n=4, Fig. 1A, $P<0.01$) samples. To assess the role of LAPTM5 played in MI, we first examined the expression of *LAPTM5* mRNA in myocardial tissues after MI. The results of qRT-PCR (Fig. 1B) and Western blot (Fig. 1C) analysis demonstrated that LAPTM5 in the infarct area of myocardial tissues at 3 and 28 days after MI was significantly increased. These results led us to examine what kinds of the mechanism are responsible for the increase of LAPTM5 after MI.

LAPTM5 inhibition improves cardiac function after MI

We first investigated the LAPTM5 expression of myocardial tissues in four different groups, the sham, MI, MI+LV-shRNA-NC, and the MI+LV-shRNA-LAPTM5. Results from qRT-PCR (Fig. 2A) and Western blot (Fig. 2B) exhibited significantly increased levels of LAPTM5 in the MI group as compared to sham, and obviously decreased levels of LAPTM5 was observed in MI+LV-shRNA-LAPTM5 when compared with the MI+LV-shRNA-NC group. There was no significant difference in LAPTM5 expression between the MI and MI+LV-shRNA-NC groups ($P>0.05$).

Cardiac function was examined using echocardiography 28 days after MI. Significant improvement in cardiac systolic function in the LAPTM5 suppressed group was presented after MI, and the values of EF and FS in this group were certainly higher than those of the shRNA-NC and the MI groups ($P<0.05$) (Fig. 2C). The values of LVIDd and LVIDIs were obviously increased after MI, while, knockdown of LAPTM5 alleviated the cardiac damage caused by MI. The TTC staining assay in this section was used to evaluate the myocardial infarct volume. The images in Fig. 2D indicated that the infarct volume in the MI mice was clearly increased compared with the sham one, but this increase was inhibited by the LAPTM5 shRNA injection. These results suggested that LAPTM5 suppression reduces the infarct volume in the MI mice model.

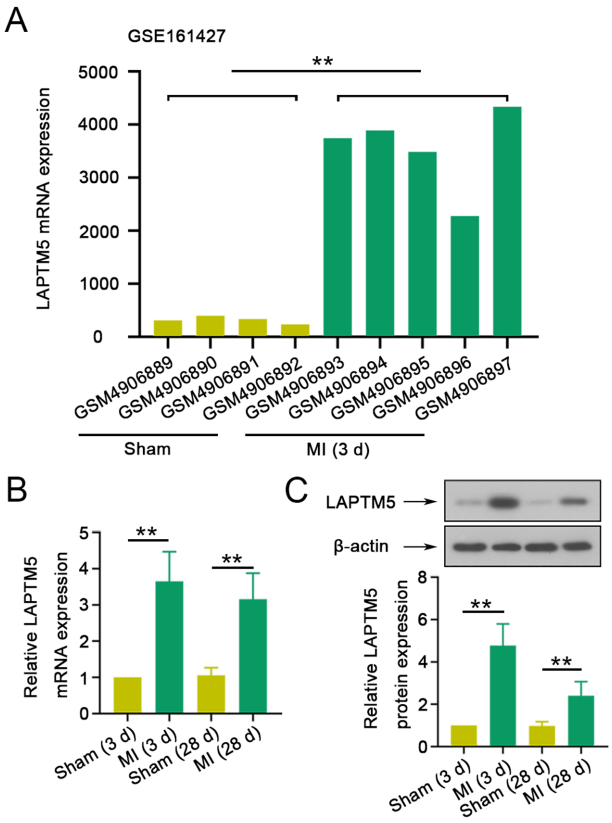

Fig. 1. Lysosomal-associated protein transmembrane 5 (LAPTM5) is overexpressed after myocardial infarction (MI). (A) The mRNA expression level of *LAPTM5* in 3 days after MI mice was obtained from gene expression omnibus (GEO) datasets. (B, C) The mRNA and protein expression levels of LAPTM5 in myocardial tissues at 3 and 28 days after MI. Unpaired *t*-tests are used to compare the differences. Data were expressed as mean ± SD. ns, no significant, $^{**}P<0.01$. n=6 for each group.

LAPTM5 affects myocardial tissues histopathology changes after MI

H&E staining was used to examine the pathological changes in the myocardial tissues. The images in Fig. 3A exhibited the results of H&E staining on day 3 after MI. It was obvious to see that normal cardiac myofibrils structure runs horizontally with intact centrally located nuclei shown in the myocardium from the sham group while myocardial cells from the MI group displayed the damaged myofibrils (red arrow), increased spaces between the myofibrils (star), and infiltrated inflammatory cells (black arrow). Also, there was no significant difference between the MI and MI+LV-shRNA-NC. How-

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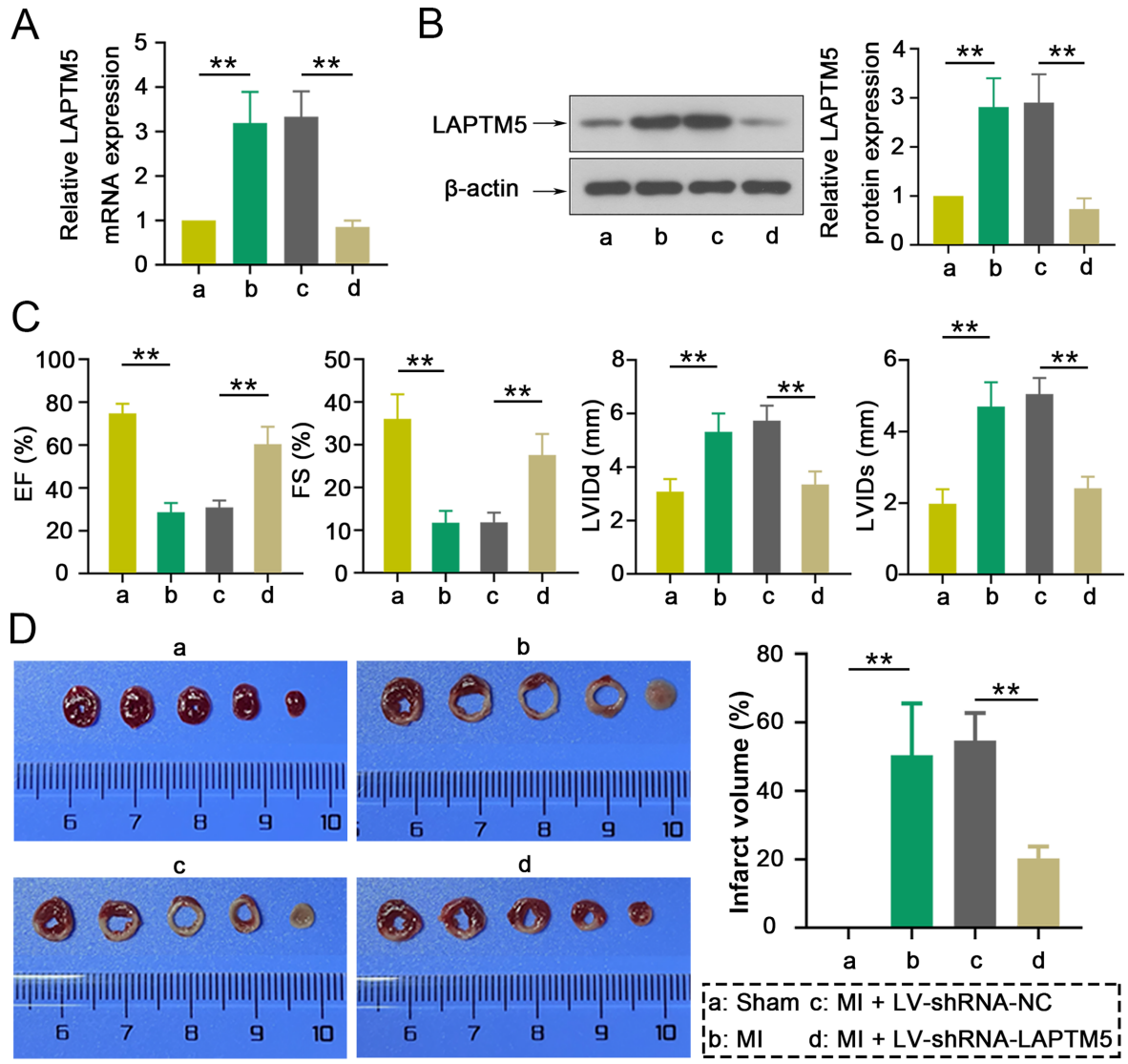

Fig. 2. Lysosomal-associated protein transmembrane 5 (LAPTM5) inhibition improves cardiac function at 28 days after myocardial infarction (MI). The mRNA (A) and protein (B) expression levels of LAPTM5 in myocardial tissues of all groups of mice. (C) Cardiac functional parameters include ejection fraction (EF%), fractional shortening (FS%), left ventricular internal dimension at end-diastole (LVIDd), and left ventricular internal dimension at systole (LVIDs) were measured after MI by using echocardiography assay. (D) Representative images of the 2,3,5-triphenyl tetrazolium (TTC)-stained myocardial tissues after MI in all groups of mice and quantification of the infarct size. a, b, c, d means sham, MI, MI+LV-shRNA-NC and MI+LV-shRNA-LAPTM5, respectively. Statistical evaluation was performed by one-way analysis of variance with Tukey post hoc test. Data were expressed as mean ± SD. $^{**}P<0.01$. n=6 for each group.

ever, the knockdown of LAPTM5 in Fig. 3A ameliorated the breakage of myocardial fiber and the infiltration of inflammatory cells in some way. According to the results of pathological changes of H&E staining in the myocardial tissues, we determined the effects of LAPTM5 on pro-inflammatory cytokines by qRT-PCR analysis. As shown in Fig. 3B, MI increased the expression level of pro-inflammatory cytokines including *IL-1β, IL-6,* and tumor necrosis factor-a (*TNF-a*) in both MI and MI+LV-shRNA-NC mice. However, downregulation of LAPTM5 significantly attenuated the increase of *TNF-α, IL-6*, and *IL-1β* caused by MI. For further investigation, histological sections were also stained with Masson's trichrome to explore the effect of LAPTM5 on cardiac fibrosis. As shown in Fig. 3C, MI caused an increase in cardiac fibrosis as compared to the sham and MI+LV-shRNA-NC groups, which was significantly diminished by LAPTM5 inhibition.

LAPTM5 promotes inflammatory response after MI

To illustrate the relation responsible for the effects of LAPTM5 on MI inflammation, the position and expression level of CD68 and inducible nitric oxide synthase (iNOS) were visualized by a double immunofluorescence

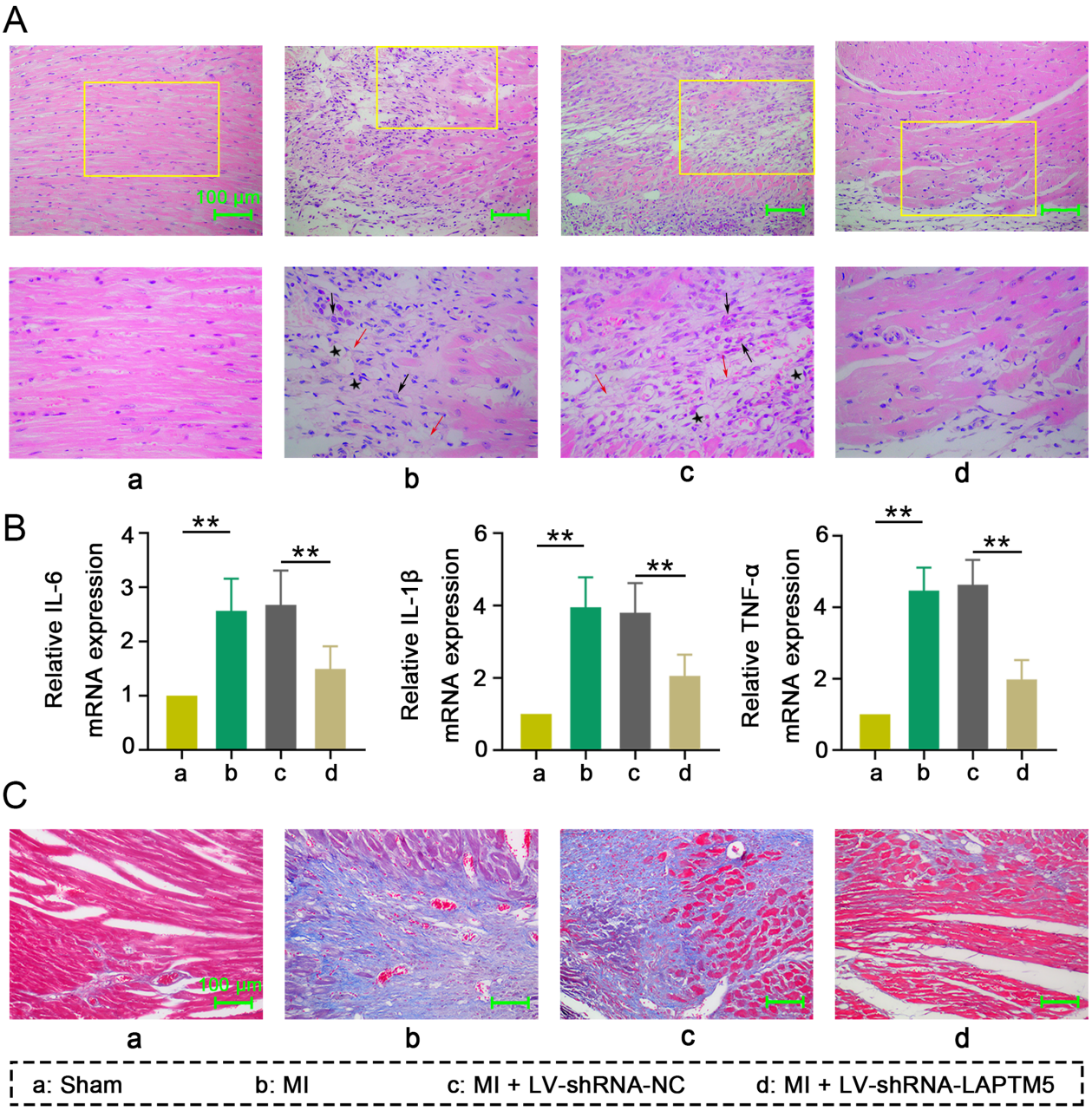

Fig. 3. Histological changes of the myocardial tissues after myocardial infarction (MI). Histological changes evaluation of the myocardial tissues in all groups of mice. (A) Haematoxylin and eosin (H&E) staining was performed at 3 days after MI. Red arrow: damaged myofibrils, star: increased spaces between the myofibrils, black arrow: infiltrated inflammatory cells. (B) The mRNA expression level of *IL-6, IL-1β*, and TNF-α in myocardial tissue in all groups of mice at 3 days after MI. (C) Masson's trichrome staining was performed at 28 days after MI. a, b, c, d means sham, MI, MI+LV-shRNA-NC and MI+LV-shRNA-lysosomal-associated protein transmembrane 5 (LAPTM5), respectively. Statistical evaluation was performed by one-way analysis of variance with Tukey post hoc test. Data were expressed as mean ± SD. $^{**}P<0.01$. n=6 for each group.

staining assay according to a confocal laser scanning microscopy. In Fig. 4A, we found that both iNOS (red fluorescence) and CD68 (green fluorescence) were increased and almost localized in the same region of MI and MI+LV-shRNA-NC groups compared with the sham one. Suppression of LAPTM5 reduced the accumulation of CD68 and iNOS cells in MI. Similar results were obtained in Fig. 4B from the quantitative analysis of protein expression of MCP1 and CD86, the chemokine and the pro-inflammatory macrophage marker. The enhancements of MCP1 and CD86 expression were seen in the MI and MI+LV-shRNA-NC mice. Nevertheless, the inhibition of LAPTM5 could suppress the enhancement of MCP1 and CD86 caused by MI.

LAPTM5 knockdown abates the activation of the NF-κB pathway

Furthermore, we investigated the alteration of the NF-

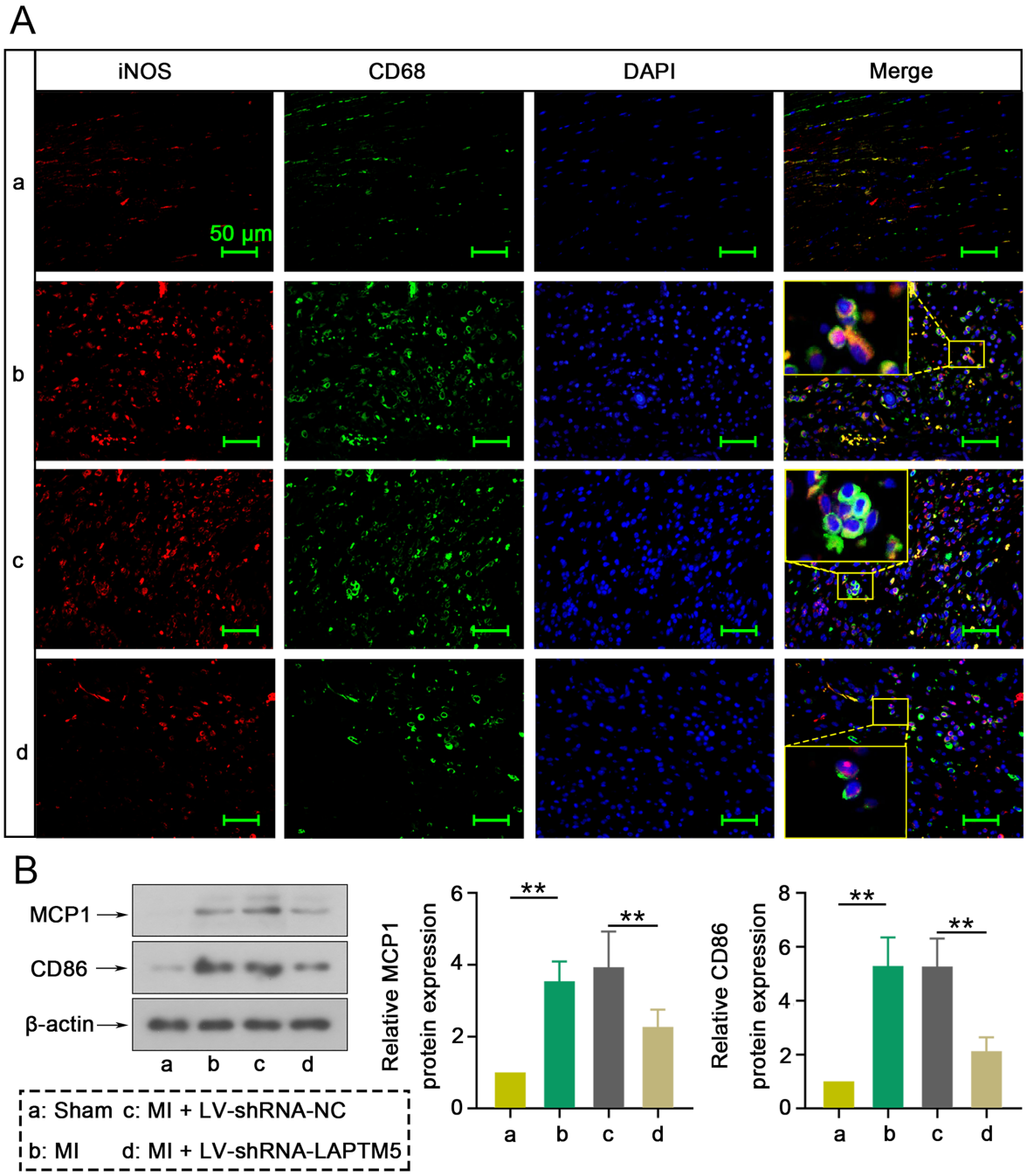

Fig. 4. Lysosomal-associated protein transmembrane 5 (LAPTM5) promotes inflammatory response at 3 days after myocardial infarction (MI). (A) The immunofluorescence staining images of iNOS and CD68 in macrophages and the nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI). (B) The protein expression level of MCP1 and CD86 in myocardial tissues in all groups of mice. a, b, c, d means sham, MI, MI+LV-shRNA-NC and MI+LV-shRNA-LAPTM5, respectively. Statistical evaluation was performed by one-way analysis of variance with Tukey post hoc test. Data were expressed as mean ± SD. ***P*<0.01. n=6 for each group.

κB pathway which plays a critical role in inflammation. The expression of NF-κB related proteins detected by Western blot analysis showed that the expression levels of p-p65 and p-IκBα in the MI and MI+LV-shRNA-NC were increased significantly compared with that in the sham group (Fig. 5A). However, after being treated with LAPTM5 suppression, the expression of p-p65 and p-IκBα in MI mice was decreased significantly, which suggests that LAPTM5 may inhibit the activation of the NF-κB signaling pathway.

We further utilized the immunofluorescence staining to explore LAPTM5 regulation in NF-κB p65 nuclear translocation. Red fluorescence indicated the localization of p-p65. As shown in Fig. 5B, the level of p-p65 was

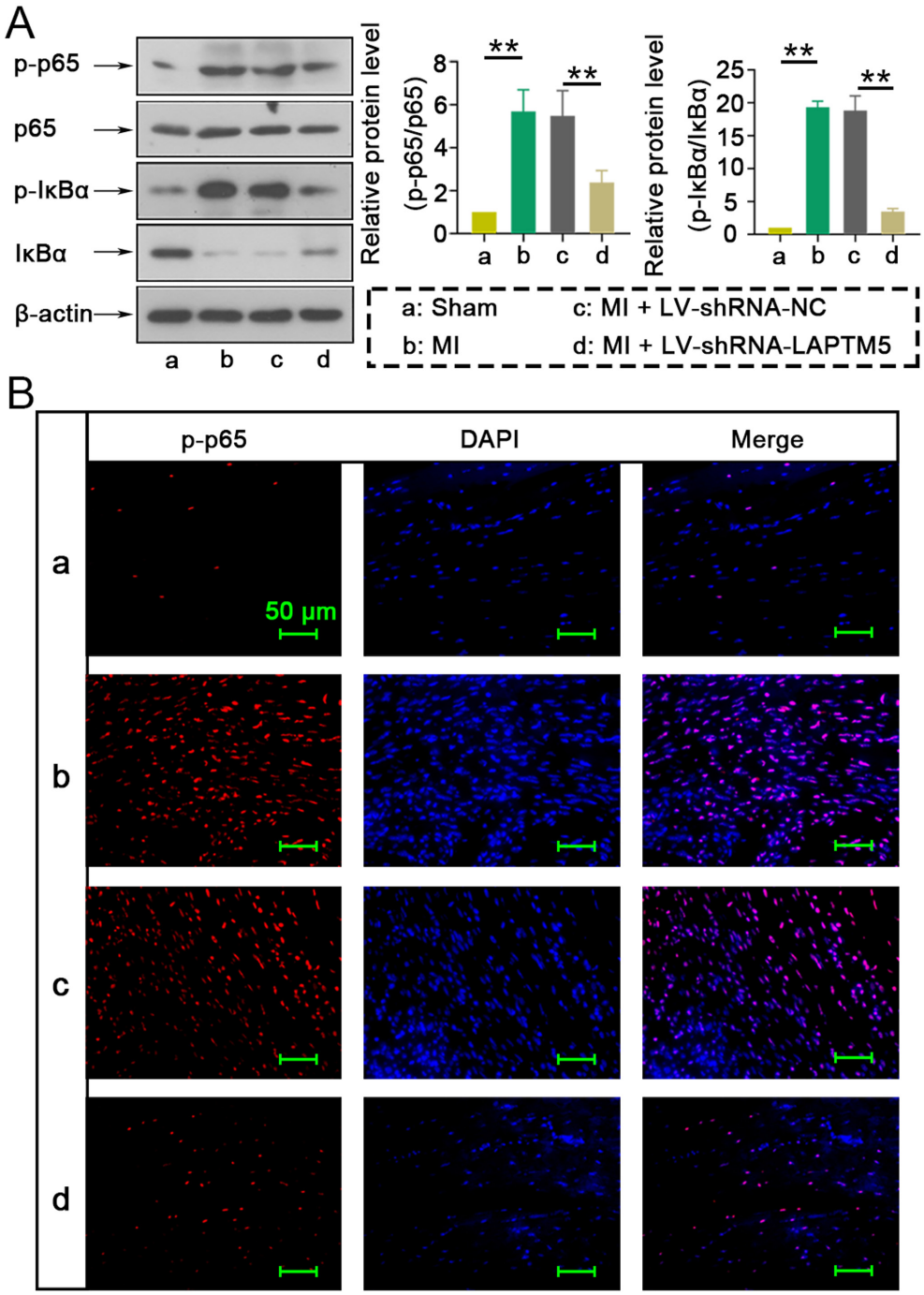

Fig. 5. Lysosomal-associated protein transmembrane 5 (LAPTM5) knockdown abates the activation of the NF-κB pathway at 3 days after myocardial infarction (MI). (A) The protein expression level of NF-κB p-p65, NF-κB p65, p-IκBα (Ser32) and IκBα in myocardial tissues in all groups of mice. (B) The immunofluorescence staining images of NF-κB p65 nuclear translocation. Red fluorescence indicates localization of p-p65, with the nuclei counterstained with 4′,6-diamidino-2-phenylindole (DAPI). a, b, c, d means sham, MI, MI+LV-shRNA-NC and MI+LV-shRNA-LAPTM5, respectively. Statistical evaluation was performed by one-way analysis of variance with Tukey post hoc test. Data were expressed as mean ± SD. ***P*<0.01. n=6 for each group.

predominately higher in the MI and MI+LV-shRNA-NC groups, which means the activation of the NF-κB signaling pathway. Conversely, knockdown of LAPTM5 decreased the level of p-p65, thus suppressing the activation of the NF-κB signaling pathway.

Discussion

MI is one of the major causes of death worldwide, and most MI survivors will gradually develop to heart failure. In recent decades, the increasing uses of novel therapies

and lifestyle changes have greatly reduced the mortality rate of heart disease. Although considerable progress in systemic treatment of immunosuppressive agent or antibody treatment, there are still huge challenges in clinical treatment of MI due to limited therapeutic effect and serious adverse events. For example, IL-6 inhibitor tocilizumab can increase myocardial salvage in ST-segment elevation MI patients, however serious adverse events such as infections requiring hospitalization, new malignancy, and chest pain may occur in patients allocated to tocilizumab [19]. Another study has reported that TNF-α inhibitor (CAS1049741-03-8) administration to mice with MI injury daily for 7 days by intraperitoneal injection can reduced the levels of inflammatory cytokines and the inflammatory cell infiltration around the infarct area, however, myocyte apoptosis and cardiac functions fractional shortening and ejection fraction worsened, indicating an adverse effect of anti-TNF-α therapy [20]. Thus, it is still needed to explore molecular targets for MI.

We investigate whether LAPTM5 acts on MI. Results of the GEO database showed a high expression of *LAPTM5* in 3 days after the MI model. We further detected the LAPTM5 expression by qRT-PCR and Western blot analysis, and the result is the same as the database that LAPTM5 was overexpressed in myocardium tissues after MI. LV mediated shRNA delivery has been shown to induce stable and long-term gene silencing [21, 22]. Furthermore, LV infection of local tissues only causes gene knockdown in specific locations, which is relatively safe. Considering the advantages of local LV infection, including safety, efficacy, and stability, LV-vector mediated shRNA targeting *LAPTM5* was established and injected at the border between the ischemic area and the healthy area of the myocardium in the current study.

In the present study, we demonstrated that suppression of LAPTM5 expression was effective in decreasing myocardial fibrosis and improving cardiac function in MI model. Cardiac function was examined using echocardiography, and results showed that MI led to significantly decreased EF and FS, and significantly increased LVIDd and LVIDs. Whereas these effects were blunted by LAPTM5 inhibition. Accumulating evidence suggests that LAPTM5 is critical regulators in the development of various cell types and organs, including the heart. Gao *et al.* concluded that LAPTM5 was involved in pathological cardiac hypertrophy and LAPTM5 deletion aggravated pressure load-induced myocardial hypertrophy and heart failure through MEK-ERK1/2 pathway in mice, as evidenced by changed echocardiographic parameters [14]. We analyze that different animal models, disease duration, and signaling pathways involved are the reasons for the differential role of LAPTM5 in the heart diseases. Another study reported that LAPTM5 as the potential diagnostic marker of hypertensive left ventricular hypertrophy demonstrated a positive association with the left ventricle wall thickness as well as electrocardiogram parameters widths (QRS complex and QTc interval) [23]. In regard to fibrosis, Ramadori *et al.* reported that LAPTM5 was remarkably increased in the THP-1 cells induced by profibrogenic TGF-β1 and LAPTM5 was identified as a novel cell-specific putative fibrosis marker associated with liver fibrosis [24]. Similarly, the results of Masson staining in our work convinced that LAPTM5 suppression reduced the cardiac fibrosis caused by MI.

Frangogiannis and his coworkers [25–27] have confirmed that inflammation does play an important role in cardiac remodeling and heart failure after MI. The inflammatory response that occurs after tissue damage is an indispensable physiological response in the healing process, but excessive inflammatory response usually inhibits repair fibrosis. We first measured the influences of LAPTM5 on pro-inflammatory cytokines, *IL-6*, and *IL-1β* by qRT-PCR analysis. According to our results, on day 3 after MI, the expression levels of *IL-1β* and *IL-6* were significantly increased, which is the same as the previous studies. The research also convinced that the increase of pro-inflammatory cytokines, IL-6 and IL-1β, could induce endothelial cell adhesion molecule synthesis and activate leukocyte integrins, causing the supplement of inflammatory cells into the infarcted areas, thereby exacerbating MI [26]. However, when treated with LAPTM5 knockdown, the enhancement of *IL-1β* and caused by MI was significantly attenuated, which means that LAPTM5 played a pro-inflammatory role after MI. LV-shLAPTM5 was thought to knock down the expression of LAPTM5 in myocardial tissue which includes cardiomyocytes fibroblasts, smooth muscle cells and immune cells, such as mast cells and macrophages. Circulating blood monocytes migrate into the infarcted myocardium, and differentiate into macrophages via the circulation post MI. Macrophages have been confirmed as the most dominant immune cells in MI [28]. Furthermore, it is clear that LAPTM5 plays a positive role in regulating proinflammatory signaling pathways in macrophages [18]. Therefore, we focused on the possibility that LAPTM5-knockdown macrophages were involved in mitigating MI. The position and expression level of CD68 and iNOS, the marker of M1 macrophage, were visualized by a double immunofluorescence staining assay in this work. The images reflected that suppression of LAPTM5 significantly reduced the accumulation of CD68 and iNOS cells in MI.

Similar results were observed from the quantitative analysis of protein expression of MCP1 and CD86, the chemokine, and the pro-inflammatory macrophage marker. Chemokine expression is a key characteristic of the inflammatory response after MI and may take a great part in inflammatory leukocyte recruitment [29–31]. Our result showed that the inhibition of LAPTM5 could suppress the enhancement of MCP-1 and CD86 caused by MI. However, except for macrophages, LAPTM5 was also shown to be present in cardiomyocytes, indicating its widespread expression [14]. We speculated that the mechanism of LAPTM5 knockdown alleviating MI might be associated with a variety of cells. More in-depth mechanisms need to be explored in the future.

And a recent study has indicated that LAPTM5 acts as a positive regulator in pro-inflammatory pathways and pro-inflammatory cytokine generation in macrophages [18]. The key element in this process is the activation of the nuclear NF-kB family of transcription factors [32]. Importantly, NF-κB has been recognized as a redox-sensitive transcription factor associated with immune responses [33]. The NF-κB related protein levels of p-p65 and p-IκBα were increased significantly after MI. However, after being treated with LAPTM5 suppression, the expression of both p-p65 and p-IκBα was decreased significantly, which suggests that LAPTM5 may exert an effect in the MI model through inhibition of the NF-κB signaling pathway. After activation, NF-κB translocates to the nucleus to regulate the transcription of pro-inflammatory cytokines [34]. The immunofluorescence staining of LAPTM5 regulation in NF-κB p-p65 nuclear translocation also demonstrated that LAPTM5 positively regulated NF-κB pathway after MI. Mechanisms about the regulatory effect of LAPTM5 on the NF-κB signaling pathway, Glowacka *et al.* reported that LAPTM5 deficiency markedly reduced the phosphorylation of NF-κB p65, which was a process achieved through a protein-protein interaction of LAPTM5 and A20 [18]. The C terminus of LAPTM5 could bind to A20, subsequently A20 restricted NF-κB activation by directly removing Lys63-linked polyubiquitin chains from key signal-transducing proteins, resulting in destabilization of IKK activation complex [18].

In conclusion, the present study demonstrated that LAPTM5 is overexpressed after MI and the suppression of LAPTM5 could ameliorate cardiac function and attenuate the cardiac fibrosis caused by MI. Meanwhile, downregulation of LAPTM5 inhibits the inflammatory response in the MI mice model via inhibiting the activation of the NF-κB pathway. Consequently, these results imply that LAPTM5 may play a pro-inflammatory role in MI.

Conflict of Interests

The authors declare that they have no competing interests.

Author Contribution

Zhanchun Song designed the project. Zhanchun Song and Xiaozeng Wang collected data. Zhanchun Song, Lianqi He, and Zhichao Ren were involved in data analysis and literature conduction. Zhanchun Song did almost all the experiments and drafted the manuscript. Liang Chen and Siyu Song reviewed the manuscript and made significant revisions to the drafts. All authors read and approved the final manuscript.

Ethics Approval Statement

This study was approved by the Animal Experimental Ethical Inspection of Fushun central hospital of TCM.

Data Availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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