Biochemical and Biophysical Research Communications 464 (2015) 201–207

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Ablation of C/EBP homologous protein increases the acute phase mortality and doesn't attenuate cardiac remodeling in mice with myocardial infarction

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ARTICLE INFO

Article history:
Received 15 June 2015
Accepted 17 June 2015
Available online 23 June 2015

Keywords:
C/EBP homologous protein
Myocardial infarction
Heart failure

ABSTRACT

Endoplasmic reticulum stress is a proapoptotic and profibrotic stimulus. Ablation of C/EBP homologous protein (CHOP) is reported to reverse cardiac dysfunction by attenuating cardiac endoplasmic reticulum stress in mice with pressure overload or ischemia/reperfusion, but it is unclear whether loss of CHOP also inhibits cardiac remodeling induced by permanent-infarction. In mice with permanent ligation of left coronary artery, we found that ablation of CHOP increased the acute phase mortality. For the mice survived to 4 weeks, left ventricular anterior (LV) wall thickness was larger in CHOP knockout mice than in the wildtype littermates, while no difference was noted on posterior wall thickness, LV dimensions, LV fractional shortening and ejection fraction. Similarly, invasive assessment of LV hemodynamics, morphological analysis of heart and lung weight indexes, myocardial fibrosis and TUNEL-assessed apoptosis showed no significant differences between CHOP knockout mice and their wildtype ones, while in mice with ischemia for 45 min and reperfusion for 1 week, myocardial fibrosis and apoptosis in the infarct area were significantly attenuated in CHOP knockout mice. These findings indicate that ablation of CHOP doesn't ameliorate cardiac remodeling induced by permanent-myocardial infarction, which implicates that early reperfusion is a prerequisite for ischemic myocardium to benefit from CHOP inhibition.

Myocardial infarction (MI) is a leading cause of morbidity and mortality of coronary heart disease. Early reperfusion therapy including thrombolysis and primary percutaneous coronary intervention is the most practical and widely practiced approach for myocardial infarction [1]. For MI patients who have no opportunity to receive reperfusion therapy, attenuating post-infarction cardiac remodeling is a reasonable strategy.

Myocardial apoptosis and fibrosis greatly contribute to cardiac remodeling and dysfunction [2–7]. Endoplasmic reticulum (ER) stress has been demonstrated to be closely associated with apoptosis [2] and fibrosis [8,9]. ER stress is prolonged and aggravated in hearts subjected to pressure overload, ischemia/reperfusion injury or MI, whereas CHOP is up-regulated and contributes to cardiac dysfunction. Ablation of CHOP can attenuate cardiac ER stress in mice subjected to pressure overload [2], and alleviate myocardial reperfusion injury by inhibiting myocardial apoptosis and inflammation [10]. However, the role of CHOP deficiency in the heart suffering from MI without reperfusion remains unknown. Considering the clues aforementioned, we hypothesized that CHOP ablation would inhibit post-MI cardiac remodeling.

1. Materials and methods

1.1. MI and myocardial ischemia/reperfusion models

All procedures were performed in accordance with our institutional guidelines for animals for the Care and Use of Laboratory Animals (NIH Publication No. 85–23, revised 1996). Mice were kept at standard housing conditions with a light/dark cycle of 12 h and

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http://dx.doi.org/10.1016/j.bbrc.2015.06.117

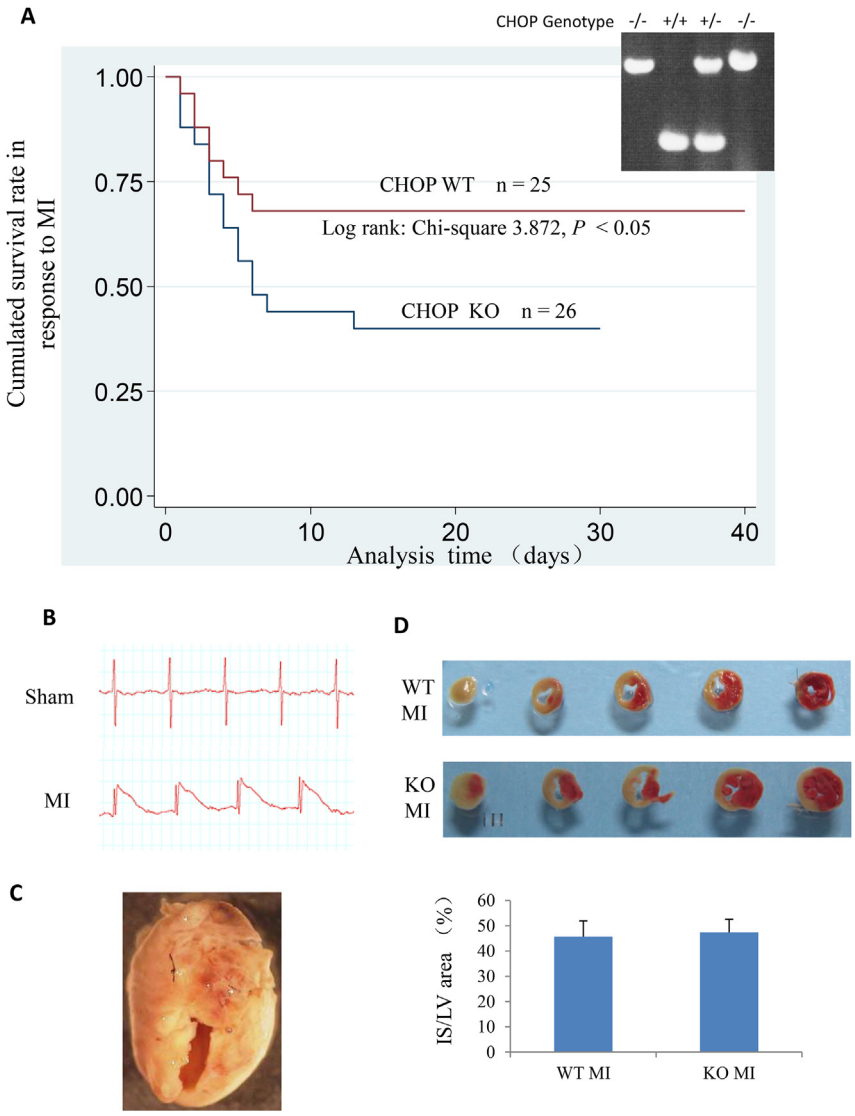

Fig. 1. Effects of C/EBP homologous protein (CHOP) deletion on survival of myocardial infarction (MI) mice. (A) Cumulated survival rate of CHOP knockout (KO) and wildtype (WT) mice in response to MI, insert picture indicates PCR results of genotyping. (B) Electrocardiogram before and after left coronary artery ligation. After ligation, the ST segment was significantly elevated, which was the sign of success of the MI surgery. (C) Examples of autopsy pictures from a dead mouse. Obvious rupture slits in the left ventricle free wall were identified. (D) Examples of TTC (triphenyltetrazolium chloride) stain in heart slices and myocardial infarct size (IS) (relative to left ventricular area (LV)) after MI for 24 h, n = 5 in each group, scale bar, 2 mm.

free access to food and water. CHOP$^{-/-}$ mice and CHOP$^{+/+}$ littermates were used for the experimental study. Heterozygous CHOP mice (purchased from the Jackson Laboratory) were intercrossed at our animal facility to obtain homozygous mice. Mice (aged 8–12 weeks, weighing 20–25 g) were used to generate MI and myocardial ischemia/reperfusion (IR) models by left coronary artery ligation as described elsewhere [3]. In brief, mice were anaesthetized with a mixture of xylazine (5 mg kg^{-1}, ip) and ketamine (100 mg kg^{-1}, ip), and the depth of anesthesia was assessed by monitoring the pedal withdrawal reflex. Mice were then ventilated and subjected to a left-sided thoracotomy and the left coronary artery ligation to induce MI with subsequent development of heart failure. For IR model, after 45 min of coronary occlusion, the ligature was removed and the heart was reperfused for 24 h or 1 week. Ischemia was judged by myocardial blanching and electrocardiogram ST-segment elevation. Sham operated mice underwent the same procedure without ligation of left coronary artery. The survival of mice was checked out twice a day to ensure that once a dead mouse is found, autopsy should be performed immediately to confirm the reason of death. Four weeks after surgery, the mice were sacrificed by overdose anesthesia with pentobarbital sodium (150 mg kg^{-1}, ip) and cervical dislocation, and their hearts and lungs were extracted for calculation of heart weight to body weight ratio (HW/BW) and lung weight to BW ratio (LW/BW) as well as histological examinations.

1.2. Echocardiographic measurements

Both left ventricular (LV) dimensions and function were evaluated by using echocardiography (a Sequoia 512 system with a 15L-8 probe, Siemens, Munich, Germany) at 4 weeks after surgery [5]. Mice were fixed in waking state to avoid the influence of anesthesia on the parameter measurements. Two-dimensional echocardiographic views of the mid-ventricular short axis were obtained at the level of the papillary muscle tips below the mitral valve. From M-mode tracings, LV anterior and posterior wall thickness at

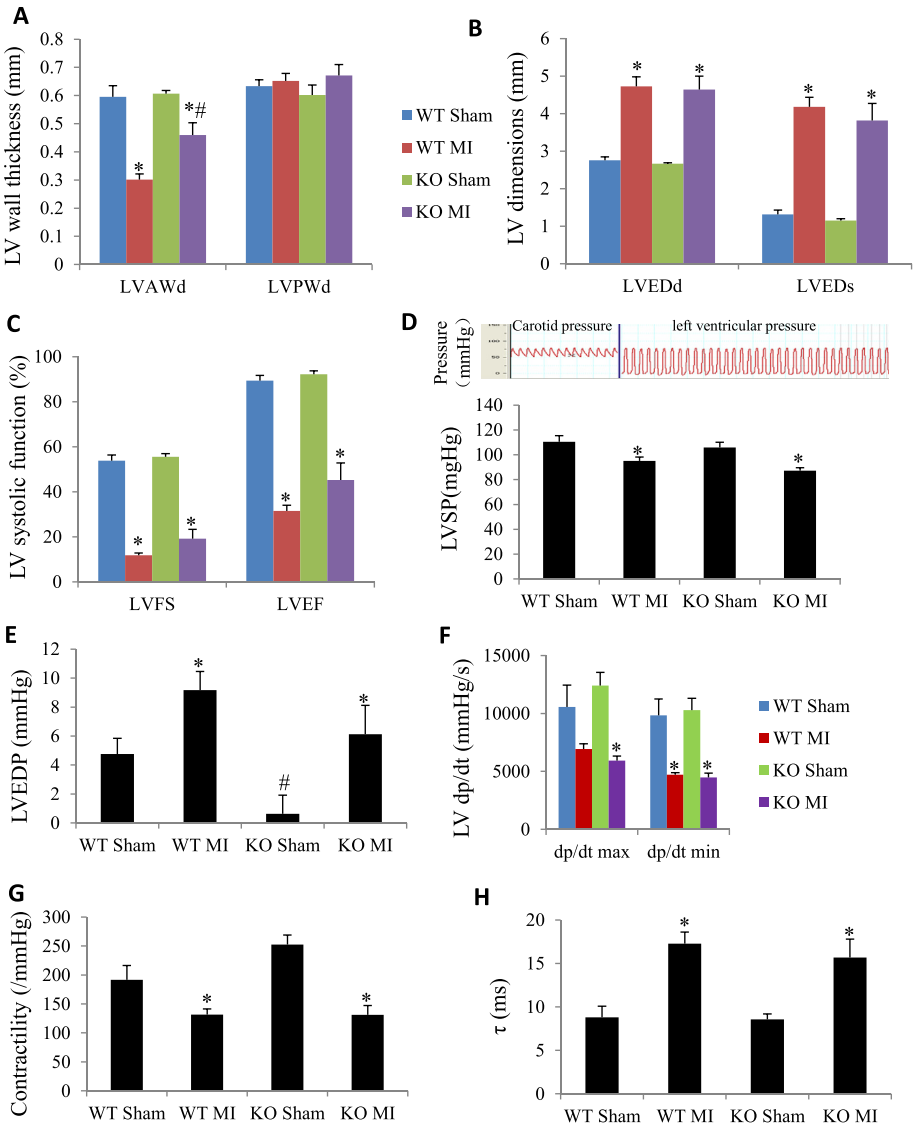

Fig. 2. Effect of CHOP deletion on echocardiographic cardiac remodeling and left ventricular hemodynamic at 4 weeks after myocardial infarction. (A) Left ventricular anterior and posterior wall thickness at end-diastolic stage (LVAWd and LVPWd). (B) Left ventricular end-diastolic diameter (LVEDd) and end-systolic diameter (LVESd). (C) Left ventricular fractional shortening (LVFS) and ejection fraction (LVEF). (D) Left ventricular systolic pressure (LVSP). The insert shows example of carotid arterial and left ventricular pressure curve recording. (E) Left ventricular end-diastolic pressure (LVEDP). (F) Maximum rates of left ventricular rising and descending (dp/dt max, dp/dt min). (G) Left ventricular contractility. (H) The exponential time constant of left ventricular relaxation (τ). $^{\#}P < 0.05$ vs. wildtype MI group (WT MI), $^{*}P < 0.05$ versus the corresponding sham group; n = 6 in each group. CHOP, C/EBP homologous protein; MI, myocardial infarction; KO, knockout; WT, wildtype.

diastole (LVAWd and LVPWd), the LV end-diastolic diameter (LVEDd) and LV end-systolic diameter (LVESd) were measured, while LV shortening (LVFS) and ejection fraction (LVEF) were calculated by the following equations: LVFS = (LVEDd − LVESd)/LVEDd × 100. LVEF = [(LV end-diastolic volume − LV systolic volume)/LV end-diastolic volume] × 100.

1.3. Invasive assessment of hemodynamics

LV hemodynamics was evaluated before sacrifice of the animals as described elsewhere [7]. Briefly, mice from each group were anesthetized with a combination of xylazine and ketamine (light anesthesia for MI mice), and were ventilated. A Millar catheter was inserted via the right carotid artery and carefully introduced into the LV to measure the heart rate (HR), systolic pressure (LVSP), end-diastolic pressure (LVEDP), maximum and minimum rates of change in the LV pressure (dp/dt max and dp/dt min, respectively). Both the contractility index (max dp/dt divided by the pressure at the time of max dp/dt) and the exponential time constant of relaxation (τ) were calculated using a software program (Blood Pressure Module).

1.4. Histological examinations and apoptosis assay

Myocardial infarct size was determined by using TTC (triphenyltetrazolium chloride) staining. Myocardial fibrosis as well as old myocardial infarct size was stained with Masson's trichrome. Apoptosis in myocardium was determined with TUNEL assay by using an In situ cell death Detection kit, TMR red (Roche, Switzerland). The positive rate of TUNEL-labeled nuclei was calculated from four different and randomly selected areas under confocal microscopy.

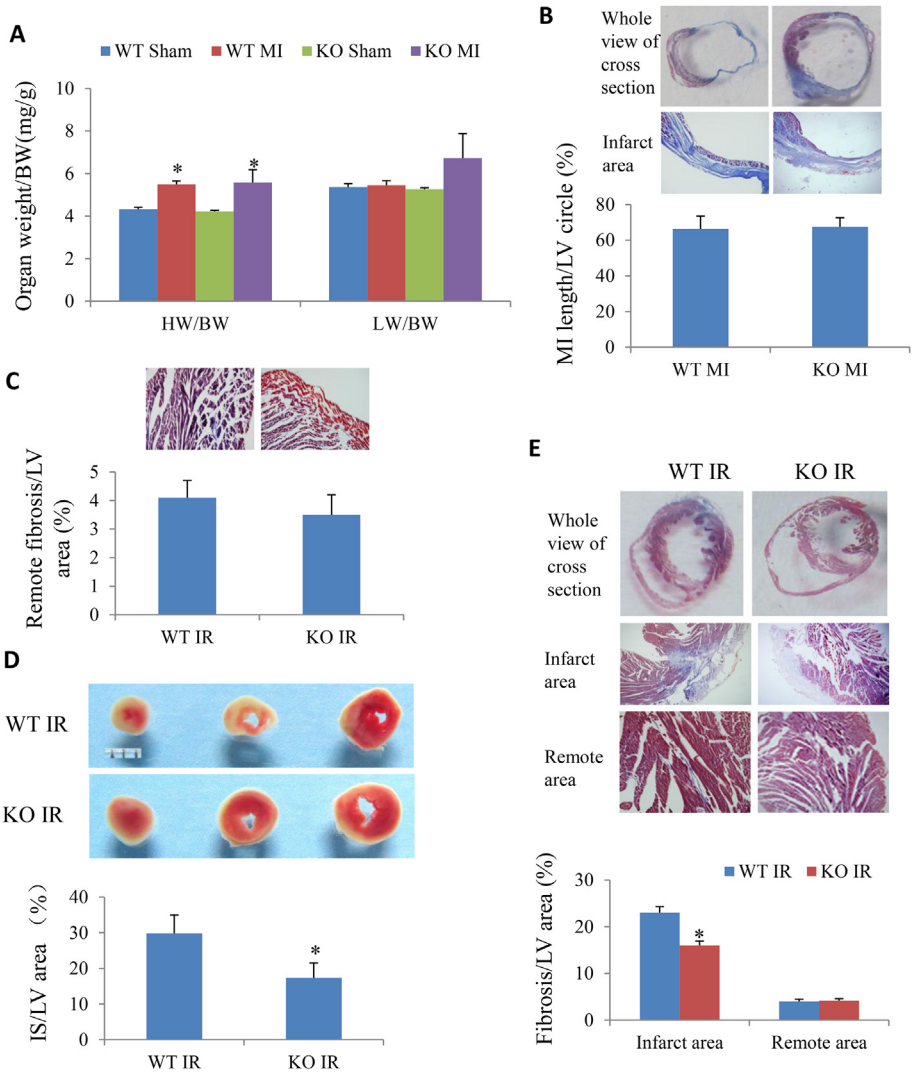

Fig. 3. Effect of CHOP knockout on cardiac morphology. (A) Heart weight/body weight (HW/BW) and lung weight/body weight (LW/BW) ratios in response to sham or myocardial infarction (MI) for 4 weeks. (B) Masson stained old MI size in response to sham or MI for 4 weeks, the insert figures show the representative pictures of cross section slice of heart (×5) and magnified infarct area (×50). (C) Fibrosis in remote area after MI for 4 weeks, the insert figures show the representative pictures of magnified remote area (×100). (D) Examples of TTC (triphenyltetrazolium chloride) stain in heart slices and myocardial infarct size (IS) (relative to left ventricular area (LV)) after ischemia for 45 min and reperfusion for 24 h, scale bar, 2 mm. (E) Percent fibrosis in both infarct and remote area of mice subjected to ischemia for 45 min and reperfusion for 1 week, the insert figures show the representative pictures of cross section slice of heart (×5) and magnified infarct area (×100) and remote area (×100). $^{*}P < 0.05$ versus the corresponding sham or wildtype group; n = 5–6 in each group. CHOP, C/EBP homologous protein; MI, myocardial infarction; KO, knockout; WT, wildtype.

1.5. Statistical analysis

Statistical significance was calculated using SPSS16.0. Numerical data were expressed as mean ± standard error of mean. Statistical differences were analyzed using Student's unpaired t-test. The overall survival of MI mice for 4 weeks was evaluated using Kaplan–Meier survival analysis and groups were compared by log-rank test. *P* values lower than 0.05 were considered to be statistically significant.

2. Results

2.1. Ablation of CHOP increased MI-induced mortality

We found that the overall mortality of CHOP knockout mice with MI was significantly higher than in wildtype group (68% vs 38.5%, $P < 0.05$; Fig. 1A). Most of them died in the first week. There was clear ST segment elevation after left coronary artery ligation in all the MI mice introduced into the research (Fig. 1B). Autopsy on dead mice showed that cardiac rupture was the principal cause of death during the first 10 days of MI (Fig. 1C). The myocardial infarct size after MI for 24 h was similar between CHOP knockout mice and wildtype ones (Fig. 1D). No death occurred in both wildtype and CHOP knockout mice subjected to ischemia for 45 min and reperfusion for 1 week. These results indicate that ablation of CHOP increased post-MI rupture in mice without prompt reperfusion.

2.2. Ablation of CHOP didn't attenuate post-MI cardiac remodeling and LV dysfunction

Four weeks after MI, no significant differences were noted on LV diameter and systolic function except that LVAWd (infarcted wall) was significantly thicker in CHOP knockout MI mice than in wildtype ones (Fig. 2A). When compared with the corresponding sham group, it was similar to wildtype mice that CHOP knockout mice with MI showed enlarged left ventricle (increased LVESd and

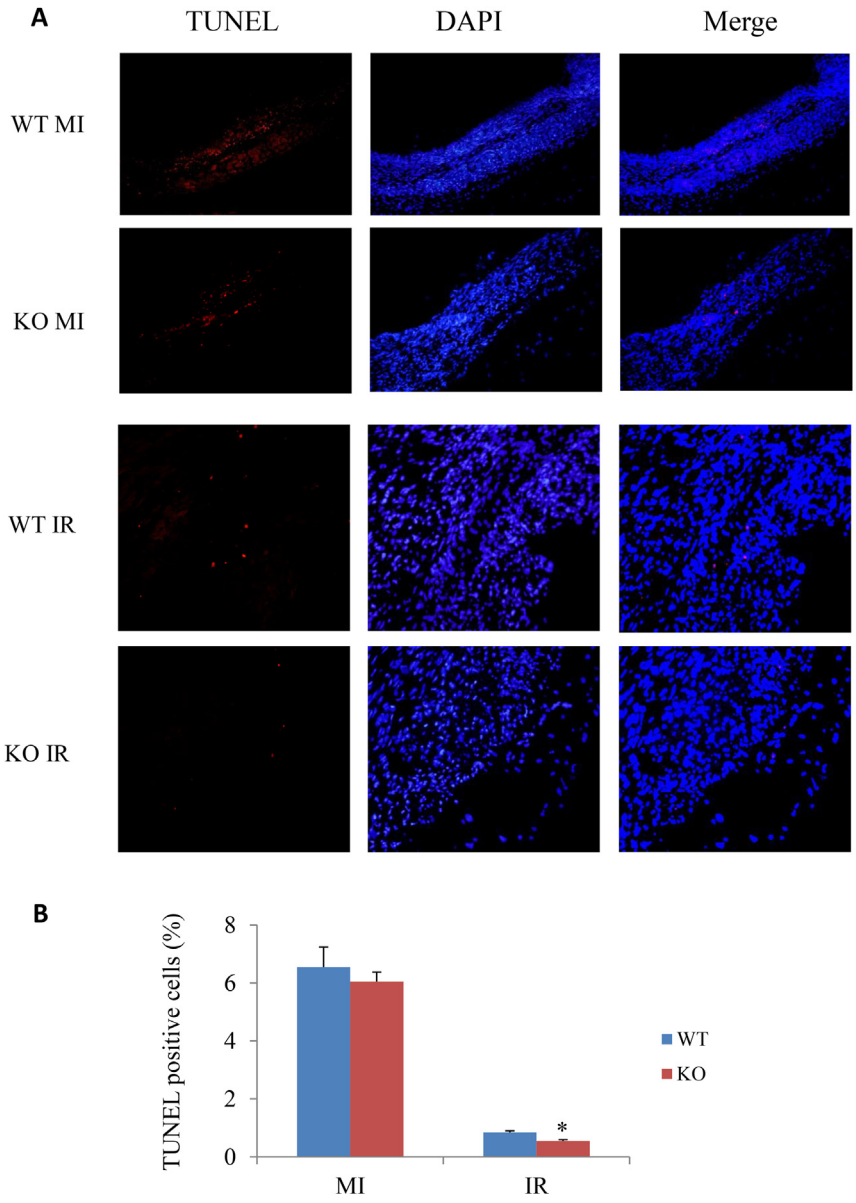

Fig. 4. Effect of CHOP knockout on cardiomyocyte apoptosis determined by TUNEL assay. (A) Representative pictures of apoptosis in the infarct area. (B) Quantitation of apoptosis in the infarct area of heart with myocardial infarction (MI) or myocardial ischemia for 45 min and reperfusion for 1 week. $^{*}P < 0.05$ versus the wildtype group. KO, knockout; WT, wildtype; IR, ischemia/reperfusion.

LVEDd), decreased LVFS and LVEF, (Fig. 2B and C). These findings revealed that deficiency of CHOP can't significantly ameliorate post-MI cardiac remodeling.

2.3. Ablation of CHOP didn't improve post-MI left ventricular hemodynamic

The LV hemodynamic assessment showed that MI for 4 weeks resulted in a significant reduction of LVSP, max dp/dt, min dp/dt, and contractility as well as a significant increase of LVEDP and τ when compared with the corresponding sham groups, but ablation of CHOP didn't exert significant influences on those indexes (Fig. 2D–H).

2.4. Ablation of CHOP didn't change cardiac morphology and apoptosis after permanent ischemia

At 4 weeks after MI, both CHOP knockout mice and their wildtype littermates exhibited increased heart weight to body weight ratio (HW/BW). However, there was no significant difference on HW/BW, LW/BW, old myocardial infarct size (fibrosis) and myocardial fibrosis in the remote area between CHOP knockout mice and wildtype ones (Fig. 3A–C). In mice with ischemia for 45 min and reperfusion for 24 h, myocardial infarct size was significantly smaller in CHOP knockout mice than in their wildtype littermates (Fig. 3D). Fibrosis was checked 1 week after reperfusion. We noted that fibrosis in the infarct area was significantly less in CHOP knockout mice than in wildtype ones, while it was similar in the remote area (Fig. 3E). These results suggest that ablation of CHOP fails to reverse cardiac remodeling in mice after MI without early reperfusion therapy.

After MI for 4 weeks, the infarct area was replaced by fibrotic scar tissues, and apoptosis in this area was similar between CHOP knockout mice and wildtype ones (Fig. 4A and B). In response to ischemia for 45 min and reperfusion for 1 week, apoptosis in the infarct area was significantly less in CHOP knockout mice than in wildtype ones (Fig. 4A and B).

3. Discussion

The endoplasmic reticulum, an organelle that participates in folding of secretory and membrane proteins, plays an important role in regulating apoptosis [11]. CHOP is an ER stress-associated transcription factor that can either promote expression of pro-apoptotic factors or suppress anti-apoptotic gene transcription and is also a profibrotic factor [9,12]. Previous studies have demonstrated that myocardial apoptosis and fibrosis were attenuated in CHOP-deficient mice subjected to pressure overload or ischemia/reperfusion [2,10]. Both fibrosis and apoptosis are important factors influencing post-MI cardiac rupture [13,14]. It seems that ablation of CHOP should produce inhibitory effects on myocardial apoptosis and fibrosis in mice with MI, then how to explain our finding in this study that CHOP deficiency resulted in more post-MI rupture? Although inhibition of apoptosis was reported to be able to prevent cardiac rupture [15], simultaneous inhibition of fibrosis would be detrimental for cardiac healing because recruitment of activated fibroblasts is necessary for prevention of post-MI rupture [14]. Loss of the profibrotic ability of CHOP in CHOP knockout mice might be the leading cause of higher incidence of post-MI rupture.

Accumulated evidence has indicated that myocardial ischemia/reperfusion or MI or pressure overload can induce the up-regulation of CHOP and then lead to ER stress-mediated apoptosis and cardiac dysfunction [2,11,16], while pharmacological interventions and genetic approaches for inhibiting the expression of CHOP can attenuate cardiac remodeling in animals subjected to pressure overload or myocardial ischemia/reperfusion [10,11,17,18]. These lines of evidence suggest that CHOP deficiency should also be beneficial for post-MI cardiac remodeling mediated by inhibition of apoptosis and fibrosis in human or animals with MI but without receiving prompt reperfusion therapy. Unexpectedly, we noted that neither the morphological remodeling nor functional deterioration in mice with permanent coronary ligation could be prevented by deletion of CHOP. Our findings support the idea that early reperfusion treatment is a prerequisite to obtain benefit from CHOP inhibition in MI heart. In clinical practice, there has been an increased emphasis on ensuring timely access of patients to percutaneous coronary intervention.

It is well-known that unrelieved ischemia (usually longer than 6 h in human) would cause complete damage to the myocardium previously supplied by the occluded artery. Permanent ischemia-destroyed myocardium is replaced by fibrous scar tissue and finally develops to progressive chronic heart failure. It is a consensus that “time is muscle”, thus timely reperfusion is the standard treatment for acute MI so as to salvage jeopardized ischemic but still viable myocardium and prevent or slow the cardiac remodeling. Drugs or genetic manipulations slowing the progression of ischemic damage should limit infarct size if timely reperfusion is guaranteed. Effects of β-blockers on ST-elevation MI patients without undergoing reperfusion were widely evaluated in many clinical trials, but no clear reduction in infarct size was detected [19], in agreement with our findings in this study that ablation of pro-fibrotic and pro-apoptotic CHOP didn't attenuate permanent MI-induced cardiac remodeling. Emerging evidence is supporting the idea that early reperfusion treatment is a prerequisite to obtain benefit from pharmacological or genetic interventions because the chances of myocardium salvage are negligible without prompt reperfusion, lending to explain why CHOP deficiency is beneficial for myocardial ischemia/reperfusion injury but not chronic MI.

In conclusion, although inhibition of CHOP is known to exert anti-apoptotic and anti-fibrotic effects in heart, deletion of CHOP increases the incidence of post-MI cardiac rupture and doesn't prevent cardiac remodeling in the case of permanent coronary ligation without early reperfusion.

Funding

This work was supported by grants from the National Natural Science Foundation of China (31271513 to Dr Liao), President Foundation of Nanfang Hospital, Southern Medical University (2014B019 to Dr Huang).

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.06.117.

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