Overexpression of histidine-rich Ca-binding protein protects against ischemia/reperfusion-induced cardiac injury

Xiaoyang Zhou\textsuperscript{a,b}, Guo-Chang Fan\textsuperscript{a}, Xiaoping Ren\textsuperscript{a}, Jason R. Waggoner\textsuperscript{a}, Kimberly N. Gregory\textsuperscript{a}, Guoli Chen\textsuperscript{a}, W. Keith Jones\textsuperscript{a}, Evangelia G. Kranias\textsuperscript{a,c,*}

\textsuperscript{a} Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, Cincinnati, OH 45267-0575, USA
\textsuperscript{b} Department of Cardiology, Renmin Hospital of Wuhan University, Wuhan 430060, China
\textsuperscript{c} Molecular Biology Division, Center for Basic Research, Foundation for Biomedical Research of the Academy of Athens, Athens 11527, Greece

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Abstract

Objective: The histidine-rich Ca-binding protein (HRC) is a Ca-storage protein in cardiac sarcoplasmic reticulum. Recent transgenic studies revealed that this protein inhibits the maximal rates of sarcoplasmic reticulum Ca-transport, leading to cardiac dysfunction. In view of the role of sarcoplasmic reticulum Ca-cycling in myocardial ischemia/reperfusion injury, we designed this study to gain further insight into the role of HRC during ischemia/reperfusion.

Methods and results: The transgenic mouse model with cardiac-specific overexpression of HRC was utilized and cardiac contractile parameters were assessed before and after ischemia/reperfusion injury by Langendorff perfusion. After a 20-min stabilization period, the hearts were subjected to 40 min of global ischemia, followed by 60 min of reperfusion. We found that although transgenic (TG) hearts showed depressed cardiac function (25%) compared to wild types (WTs) at baseline, they exhibited better recovery of left ventricular developed pressure (86.6±2.6% in TGs vs. 58.3±4.0% in WTs of pre-ischemic values, \( P<0.05 \)) and higher rates of contraction and relaxation after ischemia/reperfusion than WTs. This improvement was accompanied by smaller infarcts (23.1±1.7% in TGs vs. 41.1±2.5% in WTs of infarct region-to-risk region ratio, \( P<0.05 \)) and lower creatine kinase release. Notably, the extent of apoptotic cell death was significantly attenuated, as evidenced by decreased DNA fragmentation, upregulation of the antiapoptotic protein Bcl-2, and downregulation of the active caspases (3, 9 and 12) following ischemia/reperfusion in TG hearts, compared with WTs. Extension of these studies to an \textit{in vivo} model of 30-min myocardial ischemia, via coronary artery occlusion, followed by 24-h reperfusion, showed that the infarct region-to-risk region ratio was 9±0.9% in TGs, compared with 20.4±2.9% in WTs (\( P<0.05 \)).

Conclusions: Our findings suggest that increased cardiac HRC expression protects against ischemia/reperfusion injury in the heart, resulting in improved recovery of function and reduced infarction.

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Keywords: Histidine-rich Ca-binding protein; Transgenic; Ischemia/reperfusion; Apoptosis; Ca cycling

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1. Introduction

Acute coronary syndrome including myocardial infarction is currently the leading cause of death worldwide [1,2]. In this scenario, the best hope of limiting the size of the myocardial infarct/ischemia is the timely restoration of coronary blood flow, by either thrombolysis or percutaneous transluminal coronary angioplasty. However, the
restoration of blood flow to ischemic tissues causes additional damage, referred to as ischemia/reperfusion (I/R) injury. In addition to necrosis, apoptosis has recently been recognized to be involved in I/R-induced myocardial injury [3]. Over the past two decades, a great body of studies have shown that intracellular Ca overload plays a crucial role in I/R injury by inducing activation of various proteases and mitochondria-mediated cellular apoptosis and necrosis [4,5]. Moreover, recent observations have demonstrated that increased cytosolic Ca significantly increased susceptibility to apoptosis via activation of caspase-12, which is responsible for sarcoplasmic/endoplasmic reticulum (SR/ER) stress-induced apoptosis [6,7]. Therefore, intracellular Ca overload may be a trigger for both mitochondria-and SR/ER-mediated myocyte cell death.

Ca homeostasis in the cardiomyocyte is a complex process regulated prominently by a well-developed sarcoplasmic reticulum (SR), which plays three major functional roles in cardiomyocyte Ca cycling: (a) Ca uptake from the cytosol to induce relaxation; (b) Ca storage in the SR lumen during cardiac filling; and (c) Ca release from the SR to induce contraction. The function of the SR may be dysregulated at the levels of the SR Ca-ATPase (SERCA) and its regulatory protein phospholamban (PLN), Ca-binding protein calsequestrin (CSQ), or Ca-release channel ryanodine receptor (RyR) [8,9]. Recent studies on the histidine-rich Ca-binding protein (HRC), structurally analogous to CSQ, suggest that HRC may play a similar role to CSQ in intracellular Ca cycling, as a Ca storage protein [10,11]. Consistent with this notion, acute overexpression of HRC enhanced SR Ca storage capacity in both neonatal and adult rat cardiomyocytes [12,13]. However, transgenic mice with cardiac-specific overexpression of HRC showed left ventricular dysfunction, which was associated with depressed maximal SR Ca uptake rates, indicating that HRC may be also a regulator of SERCA activity [14]. Taken together, these findings suggest that HRC is a potentially important regulator of SR Ca cycling, but the pathophysiological role of HRC in the heart, especially in response to I/R, remains to be elucidated.

Although there is a large amount of evidence suggesting that SR dysfunction may underlie the detrimental intracellular Ca overload during I/R [15–18], the final outcome of I/R appears to depend critically on the level of Ca overload at the end of ischemia and the extent of SR dysfunction [16]. As far as post-ischemic recovery is concerned, alteration of SERCA activity could have two extreme consequences. Increased activation of SERCA could result in increased SR Ca load, which may lead to increased oscillatory Ca release from the SR, ultimately contributing to worsening of intracellular Ca overload and post-ischemic injury [16]. On the other hand, SERCA is the chief system involved in removal of Ca from the cytosol, and increased activity of this pump may favor cytosolic Ca removal, attenuating Ca overload and the subsequent cardiac injury [15]. Currently, the role of SERCA function in I/R injury remains controversial [17,18]. Importantly, HRC is emerging as a new regulator of SERCA [14], as pointed out above, and it was of special interest to investigate the susceptibility of HRC-overexpressing hearts to I/R injury. Here, we observed that HRC-overexpressing hearts demonstrated better recovery of contractile function and smaller infarct size in both ex vivo and in vivo studies, associated with attenuation of apoptosis and necrosis.

2. Methods

2.1. Animal model

As previously described [14], Line B of HRC transgenic (TG) mice (FVB/N) and their wild-type (WT) littermates were used in this study. The TG mice have an approximate 3-fold overexpression of HRC protein level relative to WT (Fig. 4A and B). To avoid the complication of gender differences, only male mice at 3 months of age were used for these studies, which were performed according to the National Institutes of Health Publication No. 8523: Guide for the Care and Use of Laboratory Animals.

2.2. Global ischemia ex vivo

The cellular and functional responses to I/R were assessed in mice by using an isolated perfused heart model, as previously described [19]. Briefly, Hearts from TG and WT mice were mounted on a Langendorff apparatus, and perfused with Krebs–Henseleit buffer. Temperature was maintained constant at 37 °C by water-jacketed glassware for a heart chamber, buffer reservoirs, and perfusion lines. In addition, an overhead light source was used to ensure maintenance of temperature during ischemia, which was monitored by a thermometer placed close to the perfused heart in the glass chamber. A water-filled balloon made of plastic film was inserted into the left ventricle and adjusted to achieve a left ventricular end-diastolic pressure (LVEDP) of 5 to 10 mm Hg. The distal end of the catheter was connected to a Heart Performance Analyzer (Micro-Med) via a pressure transducer. Hearts were paced at 400 bpm except during ischemia, and pacing was reinitiated 2 min after reperfusion. After a 20-min equilibration period, hearts were subjected to 40 min of no-flow global ischemia, followed by 60 min of reperfusion. The LVEDP, left ventricular developed pressure (LVDP) (peak systolic pressure minus LVEDP), maximum rate of contraction (+dP/dt), and maximum rate of relaxation (−dP/dt) were monitored during this process.

After 60 min of reperfusion, the heart was infused into the coronary vasculature through the sidearm of the aortic cannula with a 1% solution of 2,3,5-triphenyl tetrazolium chloride (TTC) in phosphate buffer at 37 °C and then frozen. Subsequently, the tissue was sectioned into 5 to 6 transverse slices. TTC stained the viable tissue red, whereas the necrotic tissue remained discolored (pale). The area of infarction
For each protein, equal amounts of samples (5 μg of inhibitor cocktail (Roche Applied Science, Indianapolis, IN). Cell Lysis Buffer (Cell Signaling Technology, Danvers, MA) were added to the Langendorff perfusion period and homogenized in 1× phosphate-buffered saline (PBS) and 0.5 mmol/L DTT. Oxalate-supported Ca uptake in cardiac homogenates was measured by a modified Millipore filtration technique, using 45CaCl2 under conditions that restrict Ca-pumping to the SR. Briefly, 200–400 μg of homogenate protein was incubated at 37 °C in reaction buffer containing 40 mmol/L imidazole (pH=7.0), 95 mmol/L KCl, 5 mmol/L NaN3, 5 mmol/L MgCl2, 0.5 mmol/L EGTA, and 5 mmol/L K2C2O4. The initial uptake rate was determined at a Ca concentration of 6.0 μmol/L. Ca uptake into the cardiomyocytes was initiated by an addition of 5 mmol/L ATP, and aliquots were filtered through a 0.45 μm Millipore filter after 0, 30, 60, and 90 s to terminate the reaction.

2.6. Regional ischemia in vivo

In vivo myocardial I/R was performed by ligating the left anterior descending coronary artery (LAD) and releasing the ligation as previously described [21]. ECG electrodes were placed subcutaneously, and data were recorded by PowerLab system (Australia AD Instruments). The LAD was ligated at 2 mm distal from the tip of the left appendix for a period of 30 min, and this was followed by releasing the ligation and closing the chest. Ischemia was confirmed by visual observation (cyanosis) and continuous ECG monitoring. After 24 h of reperfusion, the aorta was reoccluded, and the heart was perfused with 1% TTC to stain viable myocardium. The LAD was then reoccluded, and each heart was perfused with 5% phthalo blue to delineate the area at risk, i.e., perfusion area of the LAD. The hearts were frozen and cut into 5 to 6 transverse slices. The images of the slices were digitally analyzed for infarct area, ischemic area (area at risk), and total left ventricular (LV) area using NIH image software. The ratio of area at risk to total LV and the ratio of infarct area to total LV were calculated and expressed as percentages, as previously described [21].

2.7. Statistical analysis

Data were expressed as the mean±SEM. Comparisons between the means of two groups were performed by unpaired Student's t-test. Differences were considered significant at p-values <0.05.
Student’s t-test. Multiple groups were analyzed using a one-way ANOVA with a Bonferroni test for post hoc analysis. For multiple comparisons involving repeated measures over time (Figs. 1 and 2E), a two-way repeated-measure ANOVA was used with a Newman–Keul’s post hoc test. Results were considered statistically significant at $P<0.05$.

3. Results

3.1. Animal phenotype and basal contractile performance

Table 1 shows basal characteristics and contractile function in Langendorff-perfused TG hearts and their WT littermates. WT and TG mice showed no significant differences in body, heart, or lung weights. However, LVDP, $+dP/dt$, and $-dP/dt$ were significantly lower in TG than WT hearts. LVEDP and coronary flow rates were similar in both groups. These findings are consistent with the recently published echocardiographic data in this transgenic model [14].

3.2. Overexpression of HRC improves post-ischemic recovery of function

To further determine the role of HRC in I/R injury, WT and TG hearts were subjected to 40 min of global no-flow ischemia, followed by 60 min of reperfusion. As shown in Fig. 1, although TG hearts had depressed basal function, they exhibited significantly better contractile function during recovery, with increased LVDP (Fig. 1A), almost complete recovery of $+dP/dt$ (Fig. 1B), and significantly higher $-dP/dt$ (Fig. 1C), compared to WTs. LVEDP was also markedly decreased (Fig. 1D) relative to WT hearts.

3.3. Decreased myocardial infarction size in TG mice ex vivo and in vivo

After 40 min of global no-flow ischemia, followed by 60 min of reperfusion ex vivo, we determined myocardial infarct size by TTC staining. As shown in Fig. 2A and B, myocardial infarct size as a percentage of the area at risk was significantly reduced in TG hearts (23.1±1.7%), compared with WT hearts (41.1±2.5%, $P<0.05$). Furthermore, we assessed the activity of creatine kinase, an index of myocyte injury, released from cardiac effluent before ischemia and during reperfusion. The levels of creatine kinase were comparable between TG and WT groups at baseline; whereas they were markedly lower in TG hearts than in WTs at the corresponding time points during reperfusion (Fig. 2E).

To more thoroughly evaluate the association between HRC overexpression and cardiac survival under pathophysiologic conditions, these studies were extended to an in vivo model. TG mice and WT controls were subjected...
to 30 min of LAD coronary artery occlusion followed by 24 h of reperfusion. The area at risk, expressed as a percentage of the LV area, was comparable between the TG and WT hearts (63.9±6.2% in TGs vs. 62.8±7.1% in WTs; P >0.05). Infarct sizes of the TG and WT hearts were 5.8±1.5% vs. 12.8±4.9% of the LV, respectively. Consistent with the above ex vivo results, the myocardial infarct size normalized to area at risk was decreased by 56% in TGs, compared to WTs (Fig. 2C and D; 12.8±4.9% in WTs; *P<0.05 vs. WTs). E shows creatine kinase activity, expressed as units per min per gram of wet heart weight (U/min/g), indicating significantly lower values during reperfusion in TG than in WT hearts (n=8, TG; n=7, WT; *P<0.05 vs. WTs). All data are expressed as mean±SEM.

3.4. Attenuation of ischemia/reperfusion-induced apoptosis in TG mice

To further investigate whether the function of protection in the TG hearts is related to antiapoptotic mechanisms, we assessed the extent of apoptosis in both TG and WT hearts after I/R ex vivo, using two relative quantitative apoptosis assays: TUNEL-staining and nucleosome assay. Apoptosis is characterized by the formation of DNA fragmentation in the nucleus. In situ end-labeling of DNA fragmentation (TUNEL-staining) showed a significantly decreased proportion of TUNEL-positive nuclei (green fluorescence) in the myocardium of TG mice after I/R injury, compared with WTs (Fig. 3A and B). Furthermore, heart lysates from a subset of experimental WT
and TG hearts were assayed for DNA fragmentation by the use of a quantitative nucleosome assay to more accurately assess the incidence of apoptotic cell death. Levels of mono- and oligonucleosomes were significantly lower in TGs than in WTs after I/R (Fig. 3C). Thus, both apoptosis assays consistently demonstrate significant attenuation of DNA fragmentation in TGs than in WTs, suggesting that antiapoptosis mechanisms could, at least partly, contribute to the protection of HRC-overexpression against cardiac I/R injury.

3.5. Mechanism(s) of cardioprotection against ischemia/reperfusion in TG mice

In order to determine whether the ischemic procedure had modified the baseline abnormalities in SR Ca cycling absorbed in TG hearts [14], the levels of the SR Ca-cycling proteins (before and after I/R) and SR Ca uptake (before and after ischemia) were measured, respectively. Consistent with our previous report [14], although a 3-fold overexpression of HRC protein in TG hearts was accompanied by a marked upregulation of triadin protein, it did not alter the expression of the other SR Ca-cycling proteins, such as RyR, SERCA, PLN, CSQ and junctin at baseline (Fig. 4A and B). Following I/R, the expression levels of SERCA, PLN and RyR were slightly decreased in both WT and TG hearts, but the decrease was not statistically significant. Similarly, expression of SERCA, PLN, RyR, CSQ, as well as the ratio of PLN to SERCA, exhibited no significant differences
between TG and WT hearts at the end of I/R, while the protein levels of HRC and triadin remained significantly higher in TG hearts than WTs (Fig. 4, A and B). Moreover, there was no significant difference in SR Ca transport in WT hearts before (67.79 ± 4.79 nmol/mg/min) and after (64.54 ± 3.48 nmol/mg/min) ischemia (Fig. 4C), as well as in TG hearts before (47.54 ± 2.64 nmol/mg/min) and after (41.84 ± 3.20 nmol/mg/min) ischemia (Fig. 4C). Consistent with previous observations [14], the SR Ca-uptake rates were significantly lower in TG hearts in comparison to WT hearts, both before and after ischemia. Thus, our results suggest that ischemia did not modify the baseline abnormalities in SR Ca cycling between the WT and TG hearts.

To elucidate the potential antiapoptotic mechanism(s) of HRC overexpression, we further assessed the expression levels of the antiapoptotic Bcl-2, proapoptotic Bax, active caspase-3, caspase-9 and caspase-12. Caspase-3, the final executor of apoptosis, is inactive at rest, and when an apoptotic stimulus occurs it is cleaved into a biologically active peptide of 17 kDa and an inactive 12 kDa fragment. As shown in Fig. 5, there were no significant differences in the protein levels of Bcl-2, Bax, active caspase-3, caspase-9, and caspase-12 between TG and WT hearts before I/R, suggesting that overexpression of HRC does not modify the apoptosis-related proteins at baseline. Even after 40 min of global ischemia, quantitative immunoblotting revealed no significant alterations of these apoptosis-related proteins between TG and WT hearts. However, following 60 min of reperfusion, the expression of Bcl-2 was significantly increased, whereas the levels of Bax were unaltered in TG hearts, compared to WT hearts. Consequently, the relative
ratio of Bcl-2 to Bax expression was increased in the TG hearts after I/R. Furthermore, the levels of the active caspase-3 (17 kDa), caspase-9 (35 kDa) and caspase-12 (30 kDa) were markedly decreased in TG hearts than in WTs after I/R. These findings suggest that cardiac-specific overexpression of HRC protects against I/R injury, at least in part, by inhibition of apoptotic cell death. In addition, the actin expression levels were comparable not only between TG and WT hearts, but also over time within the two groups.

4. Discussion

The HRC protein is first identified from rabbit skeletal muscle, and it is shown to be located in the SR lumen [10]. In vitro studies in cardiomyocytes have shown that HRC-overexpression was associated with increased SR Ca load but decreased Ca-induced Ca release, resulting in impaired Ca cycling and depressed contractility [13]. Recently, we demonstrated that cardiac-specific overexpression of HRC depressed SERCA function, resulting in an impaired maximal SR Ca uptake rate [14]. Moreover, the HRC-overexpressing mice exhibited left ventricular dysfunction in vivo, which became more pronounced with aging [14]. Consistently, results from the present study show depressed cardiac function in perfused HRC-overexpressing hearts. However, the HRC-overexpressing hearts exhibited better recovery of LVDP and ±dP/dt and less creatine kinase release secondary to I/R, compared to WTs. Furthermore, both ex vivo global- and in vivo regional-infarction were significantly reduced by overexpression of HRC, indicating that HRC is protective against I/R injury. These findings are similar to a recent report on cardiac-specific ablation of the Na–Ca exchanger, which decreased baseline contractility but significantly protected against I/R injury [22].

Recently, apoptosis and necrosis have been recognized as two independent contributors to I/R-induced myocardial infarction [3]. Accumulating evidence suggests that cardiomyocyte apoptosis is one of the important mechanisms of cell death, following I/R [3,23–26]. Kajastura et al. [27] even reported that apoptosis was the predominant mode of cardiac

Fig. 5. Effects of HRC overexpression on apoptosis-related proteins upon ischemia/reperfusion (I/R) injury. A, representative Western blots illustrating Bcl-2, Bax, active caspase-3, caspase-9, and caspase-12, and actin protein levels from baseline (pre-I/R), after ischemia (post-ischemia), or at the end of I/R (post-I/R) in TG and WT hearts. Actin was used as an internal standard. B, quantitative analyses of these apoptosis-related protein levels, which were expressed as fold changes relative to pre-I/R levels in WT hearts (n=4 in each subgroup; data expressed as mean±SEM; *P<0.05 vs. WTs under similar conditions, #P<0.05 vs. pre-I/R corresponding hearts). Cas indicates caspase.
cell death induced by coronary artery occlusion. Consistent with this notion, our data demonstrate that the improved cardiac recovery in HRC-overexpressing hearts may be partly attributed to attenuation of apoptosis, which was clearly revealed by decreased DNA fragmentation, attenuation of activation of caspase cascade, and increased ratio of the antiapoptotic Bcl-2 to proapoptotic Bax expression following I/R. The apoptosis process is believed to be initiated shortly after the onset of ischemia and becomes markedly enhanced during reperfusion due to cytosolic and intramitochondrial Ca overload, oxygen free radical production, as well as replenishment of high-energy phosphates [28]. Caspases, a family of Ca-dependent proteases, are critical mediators of apoptosis [29]. So far, three pathways have been known to activate the caspase-dependent apoptosis: (a) the mitochondria-initiated caspase-9-dependent pathway; (b) the SR/ER stress-induced caspase-12-dependent pathway; and (c) the extrinsic receptor-mediated caspase-8 activation pathway [30]. Activation of caspase-3 has been well accepted as the final common pathway by which a variety of pathologic stimuli induce caspase-dependent apoptosis.

The mitochondria-mediated apoptotic pathway has been shown to be an important mechanism in myocardial I/R injury. It is believed that reperfusion of the heart after a period of ischemia leads to the opening of the permeability transition pore (PTP) in the inner mitochondrial membrane, which enables the rapid accumulation of Ca within the mitochondria, leading to Ca overload [31]. Opening of the mitochondrial PTP is associated with a decrease of the Bcl-2/Bax ratio [32–34]. In the present study, TG hearts exhibited an increased ratio of Bcl-2 to Bax after I/R, and a reduction in active caspase-9 and caspase-3, suggesting that overexpression of HRC may inhibit I/R-induced apoptosis through the mitochondria-mediated pathway.

Caspase-12 is proposed to be responsible for SR/ER stress-induced but not mitochondria-mediated apoptosis [6,7]. Increasing evidence indicates that increased cytosolic Ca concentrations significantly enhance susceptibility to apoptosis via activation of caspase-12, localized on the cytoplasmic side of the SR [7]. Recently, Nakagawa et al. [6] reported that cells from caspase-12-deficient mice are resistant to apoptosis triggered by the known SR/ER stress agents. Furthermore, Jang et al. [35] provided in vivo evidence that doxorubicin-induced apoptosis is mediated by the SR to a greater extent than other apoptotic pathways. In this study, HRC-overexpressing hearts exhibited lower expression levels of active caspase-12 than WTs after I/R. Hence, our findings indicate that the SR/ER-mediated apoptotic pathway might be also involved in the protective effect of HRC-overexpression to I/R injury.

In addition, the mitochondrial PTP opening is believed to be important in both necrosis and apoptosis, depending on the severity of the ischemic damage. PTP opening and ATP maintenance lead to apoptosis, whereas excessive pore opening and ATP depletion result in necrosis [36,37]. Our present data indicate that the infarct size (TTC-negative area) vs. TUNEL-positive nuclei in WT hearts is 41% vs. 5%, secondary to I/R injury. Furthermore, the TTC-negative area was reduced to 23% in TGs, whereas myocardial apoptotic nuclei were decreased to 1.4% in TGs after I/R. These results suggest that HRC may protect the heart through suppression of necrosis.

Fig. 6. Proposed scheme for the role of HRC in ischemia/reperfusion (I/R)-induced myocyte cell death.
Finally, overexpression of SERCA in nonmyocytes has been shown to be proapoptotic, whereas cells devoid of the SR/ER Ca release channels are resistant to apoptotic stimuli [38,39]. Cross et al. [18] recently reported that enhancing SR Ca uptake, via ablation of PLN’s inhibitory effect on SERCA, renders hearts more susceptible to I/R injury. A more recently study demonstrated that increased Ca influx causes apoptosis by inducing SR Ca overload in adult feline cardiomyocytes [40]. Taken together, these investigations support a crucial role for SR Ca overload in contributions of apoptosis and necrosis to I/R injury [41]. Based on the Ca-binding properties of the HRC protein and our previous observations in vitro and in vivo [11,13,14], we propose a sequence of events, depicted in Fig. 6. Upon an I/R insult, overexpression of HRC decreases the free Ca content in SR and subsequently oscillatory Ca release from SR via depression of SR Ca uptake. Consequently, the relative rise in cytosolic Ca is attenuated, leading to reduced intramitochondrial Ca accumulation. This in turn favors upregulation of the Bel-2/Bax ratio, and thus attenuates opening of the PTP. As a result, overexpression of HRC may favor the integrity of mitochondria, and thereby repress mitochondrial-mediated apoptotic and necrotic cell death pathways secondary to I/R. Furthermore, attenuation of SR Ca load and subsequent cytosolic Ca rise prevents caspase-12 activation during I/R, which in turn depresses downstream SR/ER-mediated apoptotic caspase cascade. Collectively, a more plausible explanation is that overexpression of HRC confers simultaneous protection against apoptosis and necrosis induced by I/R.

Nevertheless, compensatory mechanisms, accompanying overexpression of HRC in transgenic mouse hearts [14], may also contribute to the observed protective effects of HRC in I/R. Future studies, using conditional transgenesis to increase HRC expression in a tissue- and time-specific manner, may further clarify the beneficial effects of this protein under I/R insults.

In summary, hearts from mice overexpressing HRC have significantly less I/R injury than WT hearts. The protection observed in HRC-overexpressing hearts is possibly attributed to the attenuation of SR and cytosolic Ca rise during I/R, which alleviates I/R-induced cardiomyocyte apoptosis and necrosis. These data not only further support the hypothesis that HRC is an important regulator of SR Ca cycling but also suggest that interventions affecting SR Ca function may have promising therapeutic potential in myocardial I/R injury.

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References


