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Protection of peroxiredoxin II on oxidative stress-induced cardiomyocyte death and apoptosis

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Abstract Peroxiredoxin II, a cytosolic isoform of the antioxidant enzyme family, has been implicated in cancer-associated cell death and apoptosis, but its functional role in the heart remains to be elucidated. Interestingly, the expression levels of peroxiredoxin II were decreased in mouse hearts upon ischemia-reperfusion, while they were elevated in two genetically modified hyperdynamic hearts with phospholamban ablation or protein phosphatase 1 inhibitor 1 overexpression. To delineate the functional significance of altered peroxiredoxin II expression, adenoviruses encoding sense or antisense peroxiredoxin II were generated; cardiomyocytes were infected, and then subjected to H_2O_2 treatment to mimic oxidative stress-induced cell death and apoptosis. H_2O_2 stimulation resulted in a significant decrease of endogenous peroxiredoxin II expression, along with reduced cell viability in control cells. However, overexpression of peroxiredoxin II significantly protected from H_2O_2-induced apoptosis and necrosis, while downregulation of this enzyme promoted the detrimental effects of oxidative stress in cardiomyocytes. The beneficial effects of peroxiredoxin II were associated with increased Bcl-2 expression, decreased expression of Bax and attenuated activity of caspases 3, 9 and 12. Furthermore, there were no significant alterations in the expression levels of the other five isoforms of peroxiredoxin, as well as active catalase or glutathione peroxidase-1 after ischemia-reperfusion or H_2O_2 treatment. These findings suggest that peroxiredoxin II may be a unique antioxidant in the cardiac system and may represent a potential target for cardiac protection from oxidative stress-induced injury.

Key words peroxiredoxin II – cardiomyocytes – protection – H_2O_2 – apoptosis

Introduction

Recent experimental and clinical studies suggest that generation of reactive oxygen species (ROS) or oxidative stress is enhanced in heart failure [47], the leading cause of morbidity and mortality in the United States. ROS are intermediates of the reduction of O_2 to water and include superoxide anion ($O^{2-\cdot}$), hydroxyl radical ($OH^{\cdot}$) and hydrogen peroxide (H_2O_2), which can cause the oxidation of membrane phospholipids, proteins and DNA. Growing evidence indicates that ROS play a critical role in many disorders of the cardiovascular system [4, 19, 46], such as ischemia-

BRC 764

reperfusion injury, myocardial stunning, apoptosis and arteriosclerosis [36]. Accumulation of ROS in mitochondria can lead to apoptotic cell death [55] and ROS may also have direct effects on cellular structure and function, including myocardial remodeling and failure [27, 47]. Under physiological conditions, the toxic effects of ROS can be prevented by such scavenging enzymes as superoxide dismutase (SOD), glutathione peroxidase (GHPx) and catalase, as well as other non-enzymatic antioxidants. However, when the production of ROS becomes excessive, oxidative stress might have a harmful effect on the functional and structural integrity of the heart [40, 47].

Recently, redox signaling and peroxiredoxin family enzymes have been suggested to play a role in mediating the antioxidant effects in the heart [11, 25, 37]. Ablation of peroxiredoxin VI, rendered the heart vulnerable to ischemia-reperfusion [32], while overexpression of the mitochondrial-specific peroxiredoxin III prevented left ventricular remodeling and failure after myocardial infarction in mice [29]. Interestingly, we have found that the expression levels of peroxiredoxin II, another member of the peroxiredoxin family, were increased in the hyperdynamic hearts of two genetically altered mouse models: phospholamban knockout (PLN KO) and protein phosphatase 1 inhibitor 1 overexpression (I-1 OE) [6, 34]. Taken together, these studies prompt us to further investigate the functional significance of peroxiredoxin II in the heart.

Peroxiredoxin II is a member of an antioxidant enzyme family (other members are peroxiredoxin I, III, IV, V and VI), which has the ability to reduce H_2O_2 and hydroperoxides into water and alcohol, respectively. Most of the known mammalian peroxiredoxins, except the peroxiredoxin VI isoform, utilize thioredoxin as an immediate electron donor; hence they are known as thioredoxin peroxidases. Peroxiredoxin II is a 25 kDa protein, which has a high affinity for H_2O_2 (*K*m for $H_2O_2 \leq 10$ μM), similar to GHPx (*K*m for H_2O_2 is approximately 1 μM) and is abundant in the cytosol from a wide range of tissues, making it a major regulator of the H_2O_2 signal in the cell, compared to catalase (*K*m for H_2O_2 around 1 mM) [49, 51]. Peroxiredoxin II knockout mice exhibited hemolytic anemia, indicating that peroxiredoxin II plays a major role in protecting red blood cells from oxidative stress in mice [24]. Peroxiredoxin II deficiency also resulted in increased production of H_2O_2, enhanced activation of platelet-derived growth factor (PDGF) receptor and phospholipase Cγ1, subsequently leading to increased cell proliferation and migration in response to PDGF [5]. Overexpression of peroxiredoxin II protected leukemia cells from apoptosis [54], while antisense peroxiredoxin II enhanced radiation-induced cancer cell death [33]. However, the functional significance of this isoform in the heart is not currently known. To determine whether peroxiredoxin II plays a role in cardiac oxidative stress, the levels of this enzyme were altered in isolated cardiomyocytes, which were then subjected to H_2O_2 treatment. Our results provide the first evidence that overexpression of peroxiredoxin II protects cardiomyocytes from oxidative stress-induced cell death and apoptosis, whereas downregulation of peroxiredoxin II greatly impairs these protective effects, suggesting a major role for this isoform in cardiac protection against oxidative stress.

Methods

Mouse models

Phospholamban (PLN) deficient and protein phosphatase 1 inhibitor 1 (I-1) overexpression mice were generated in our lab [26, 35]. All experimental procedures and protocols used in this study were reviewed and approved by the University of Cincinnati Institutional animal care and use committee and are in accordance with the National Institutes of Health "*Guide for the care and use of laboratory animals*" (NIH publication No. 85–23, revised 1996).

Global ischemia-reperfusion injury ex vivo

Global ischemia-reperfusion injury was performed in isolated perfused hearts by Langendorff mode, as previously described [14]. After a 20-min equilibration period, the hearts were subjected to 40 min of no-flow global ischemia, followed by 60 min of reperfusion. Hearts were then snap frozen in liquid nitrogen and homogenized in 1× Cell Lysis Buffer (Cell Signaling Technology, Danvers, MA) supplemented with 1 mM PMSF and complete protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN) for quantitative immunoblotting.

Isolation and culture of adult rat ventricular myocytes

Adult male Sprague-Dawley rats (8–10 weeks, 250–350 g) were anesthetized by pentobarbital (100 mg/kg) and heparinized (5,000 U/kg). Hearts were quickly removed and the aorta was cannulated by Langendorff mode and perfused with modified Krebs-Henseleit buffer (KHB: 118 mM NaCl, 4.8 mM KCl, 25 mM HEPES, 1.25 mM K_2HPO_4, 1.25 mM $MgSO_4$, 11 mM Glucose, 5 mM Taurine and 10 mM Butanedione Monoxime; pH 7.4) for 5–10 min [13]. Subse-

quently, hearts were perfused with an enzyme solution [KHB containing 0.7 mg/ml collagenase type II (278 U/mg), 0.2 mg/ml hyaluronidase, 0.1% BSA, and 25 μM Ca] for 15 min. Then, 25 μM Ca was added to the perfusion buffer, and hearts were perfused for another 5 min. The Ca concentration in the perfusion buffer was raised to 100 μM and perfusion continued for 5 min. Finally, the hearts became flaccid and left ventricular tissue was excised, minced, pipette dissociated and filtered through a 240 μm screen. Cells were quickly centrifuged at low speed (400 rpm/min), harvested and resuspended in 25 ml of 100 μM Ca-KHB with 1% BSA. The cells were allowed to settle down automatically and Ca concentration was gradually raised to 1.0 mM. Afterwards, the cells were washed two times with DMEM supplemented with ATCC (2 mg/ml BSA, 2 mM L-carnitine, 5 mM creatine, 5 mM taurine, 100 IU penicillin and 100 μg/ml streptomycin). Cells were then counted and plated on laminin-precoated dishes.

H_2O_2 treatment and analysis of cell death and apoptosis

After 24 h of adenoviral infection, different doses of H_2O_2 (0, 50, 100 and 200 μM) were added to cardiomyocytes and incubated for 2 h or for different time periods (0, 0.5, 1, 2, 4 to 8 h) [52, 53], as indicated. Cardiomyocytes were then examined for the occurrence of apoptosis by Hoechst staining [13]. Cell viability was calculated by incubating cells with MTT for 2 h [12].

Adenoviral-mediated gene transfer

Peroxiredoxin II cDNA was purchased from Open Biosystem. Recombinant adenoviruses with sense or antisense of the peroxiredoxin II cDNA were generated, using the pAd.Track-CMV/pAdEasy-1 system [12]. The shuttle vector pAdTrack-CMV and the adenoviral backbone vector pAdEasy-1 were generously provided by Dr. Bert Vogelstein from the Johns Hopkins Oncology Center. Adenoviruses were amplified in HEK293 cells, purified with ViraKit from Virapur and titered according to the standard procedure of Adeno-X™ rapid titer kit from Clontech. After 2 h of plating, cardiomyocytes were infected with sense or antisense peroxiredoxin II adenoviruses (Ad.prxII or Ad.prxII-AS) or control Ad.GFP at a multiplicity of infection (MOI) of 200 for 2 h before the addition of a suitable volume of complete DMEM medium (DMEM containing 2 mg/ml BSA, 2 mM L-carnitine, 5 mM creatine, 5 mM taurine, 100 IU/ml penicillin and 100 μg/ml streptomycin) [22]. The efficiency of adenoviral gene transfer was evaluated in cultured adult rat myocytes with the use of Ad.GFP. Nearly 100% of myocytes appeared infected at 200 MOI by 24 h. The cell phenotype and morphology remained similar among non-infected and adenoviral-infected groups after 24 h of infection. The myocytes were then treated with 50 μM H_2O_2 for 2 h, washed with PBS and harvested for quantitative immunoblotting, or used in the experiments outlined in the results.

Intracellular TBARS and LDH release

The content of cardiomyocyte thiobarbituric acid substances (TBARS) was determined, as previously described [15]. Cardiomyocyte plasma membrane integrity was assessed by measuring lactate dehydrogenase (LDH) release into the culture medium with an in vitro LDH assay kit (Sigma–Aldrich).

Quantitative immunoblotting

Cultured myocytes were harvested and lysed for 20 min at 4°C in lysis buffer (20 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1% NP-40, 10% glycerol, 0.4 mM sodium orthovanadate, 10 mM sodium pyrophosphate, 10 mM sodium fluoride, 0.5 mM DTT, and 2 μl/ml protease inhibitor cocktail). After 10 min of centrifugation at 10,000*g*, the supernatants were obtained and used in subsequent experiments. For each protein, equal amounts of samples (5–120 μg) were analyzed by SDS-PAGE. After transfer to membranes, quantitative immunoblotting analysis was performed with the corresponding primary antibodies [peroxiredoxins I to IV from Alexis Biochemicals (Lausen, Switzerland), peroxiredoxins V and VI from Abcam (Cambridge, MA), catalase and GHPx-1 from Santa Cruz Biotechnology (Santa Cruz, CA), Bcl-2 and Bax from Invitrogen (South San Francisco, CA); cleaved caspase 3 (Asp175), caspase 9, and caspase 12 from Cell Signaling (Danvers, MA); and calsequestrin (CSQ) from Affinity Bioreagents (Golden, CO)] at a 1:500 to 1:5,000 dilution. This was followed by incubation with a secondary antibody conjugated with horseradish peroxidase at a 1:5,000 dilution. Visualization was achieved using SuperSignal West Pico chemiluminescent substrate (Pierce, Rockford, IL) or ECL-PLUS Western Blotting Detection kit (Amersham Pharmacia Biotech, Piscataway, NJ). The intensities of the bands were determined by the AlphaEaseFC™ software. For each protein, the densitometric values from non-treatment controls were arbitrarily converted to 100%, and the values of samples from the other groups were normalized accordingly and expressed as percentile changes. CSQ was used as an internal standard.

Statistics

Data are expressed as mean ± SE. Comparisons between two groups were performed by Student *t* test (from Microsoft Office, Excel), while one-way ANOVA (from GraphPad Prizm4) was used for multigroup comparison. Results were considered statistically significant at $P < 0.05$.

Results

Increased expression of peroxiredoxin II in the hyperdynamic hearts of two mouse models

Cardiac proteomics-based analysis of our two models with significantly enhanced cardiac function, the PLN KO and the protein phosphatase 1 inhibitor 1 overexpression (I-1 OE) mice, revealed increases in the levels of peroxiredoxin II [6, 34]. Further quantitative immunoblotting showed that the levels of peroxiredoxin II expression were enhanced by 2.5-fold in the PLN-KO and by 2.4-fold in the I-1 OE, compared to age-matched wild types (Fig. 1a and 1b). These results indicate that peroxiredoxin II, a relatively new antioxidant protein, may play an important functional role in the heart.

Alterations of cardiac peroxiredoxin II expression in ex vivo cardiac ischemia-reperfusion injury

It has been reported that ROS or oxidative stress are significantly increased upon cardiac ischemia-reperfusion injury. To investigate whether peroxiredoxin II expression is also altered, mouse hearts were perfused ex vivo in a Langendorff mode and subjected to 40 min of ischemia followed by 60 min of reperfusion. Interestingly, the peroxiredoxin II levels were significantly decreased to about 65% of pre-ischemic values, upon ischemia-reperfusion (Fig. 1c). These data suggest that decreased expression of peroxiredoxin II may contribute to the cardiac ischemic-reperfusion injury.

Dose-response and time-course of peroxiredoxin II expression upon H_2O_2 treatment of cardiomyocytes in vitro

The alterations of peroxiredoxin II expression in the hearts above promoted us to determine the functional significance of peroxiredoxin II, especially its antioxidant effects. To better understand this notion, H_2O_2 was chosen to treat isolated cardiomyocytes and mimic oxidative stress-induced cardiac cell injury. Briefly, cardiomyocytes were treated with different doses of H_2O_2 (0–200 μM) for 2 h and the levels of peroxiredoxin II were determined by quantitative immunoblotting. Consistent with our ex vivo ischemia-reperfusion findings (Fig. 1c), there was a H_2O_2 dose-dependent decrease in peroxiredoxin II expression (Fig. 1d). These decreases appeared stable up to 8 h of H_2O_2 treatment. Notably, treatment of cardiomyocytes with 50 μM H_2O_2 for 2 h resulted in a significant reduction of cell viability, compared to control non-treated myocytes (Fig. 1e). These results indicate that downregulation of peroxiredoxin II in cardiomyocytes may be associated with H_2O_2-induced cell injury.

Peroxiredoxin II overexpression protects myocytes from H_2O_2-induced cell death and apoptosis, while its downregulation eliminates this effect

To determine the role of peroxiredoxin II in cardiomyocyte death induced by H_2O_2, isolated cardiomyocytes were infected with Ad.GFP, Ad.prxII or Ad.prxII-AS for 24 h (Fig. 2a) and the levels of peroxiredoxin II were examined. In the cells infected with Ad.prxII, the level of peroxiredoxin II was increased by 1.5-fold, while this protein was decreased by 70% in the Ad.prxII-AS cells, compared to the Ad.GFP group (Fig. 2b). However, no apparent morphological alterations or differences in the number of adherent cells and rod-shaped cells were observed among the three groups. To determine the effect of peroxiredoxin II overexpression or downregulation on cell death and apoptosis, the infected cardiomyocytes were treated with H_2O_2 and cell viability as well as cell nuclear fragmentation were examined. Upon H_2O_2 treatment, cell viability was significantly decreased to the same extent in both control and Ad.GFP infected cells. More importantly, cell viability was restored upon overexpression of peroxiredoxin II. In contrast, downregulation of peroxiredoxin II significantly reduced the cell survival, compared to control cells (Fig. 2c). Accordingly, cell nuclear fragmentation was significantly increased by ~2.8-fold in both control and Ad.GFP infected cells. Overexpression of peroxiredoxin II reduced cell fragmentation to even lower levels than control or non-treated cells, while downregulation of peroxiredoxin II had opposite effects (Fig. 2d). These findings indicate that overexpression of peroxiredoxin II protects cardiomyocytes from H_2O_2-induced cell death and apoptosis, while downregulation of this protein eliminates the protective effect.

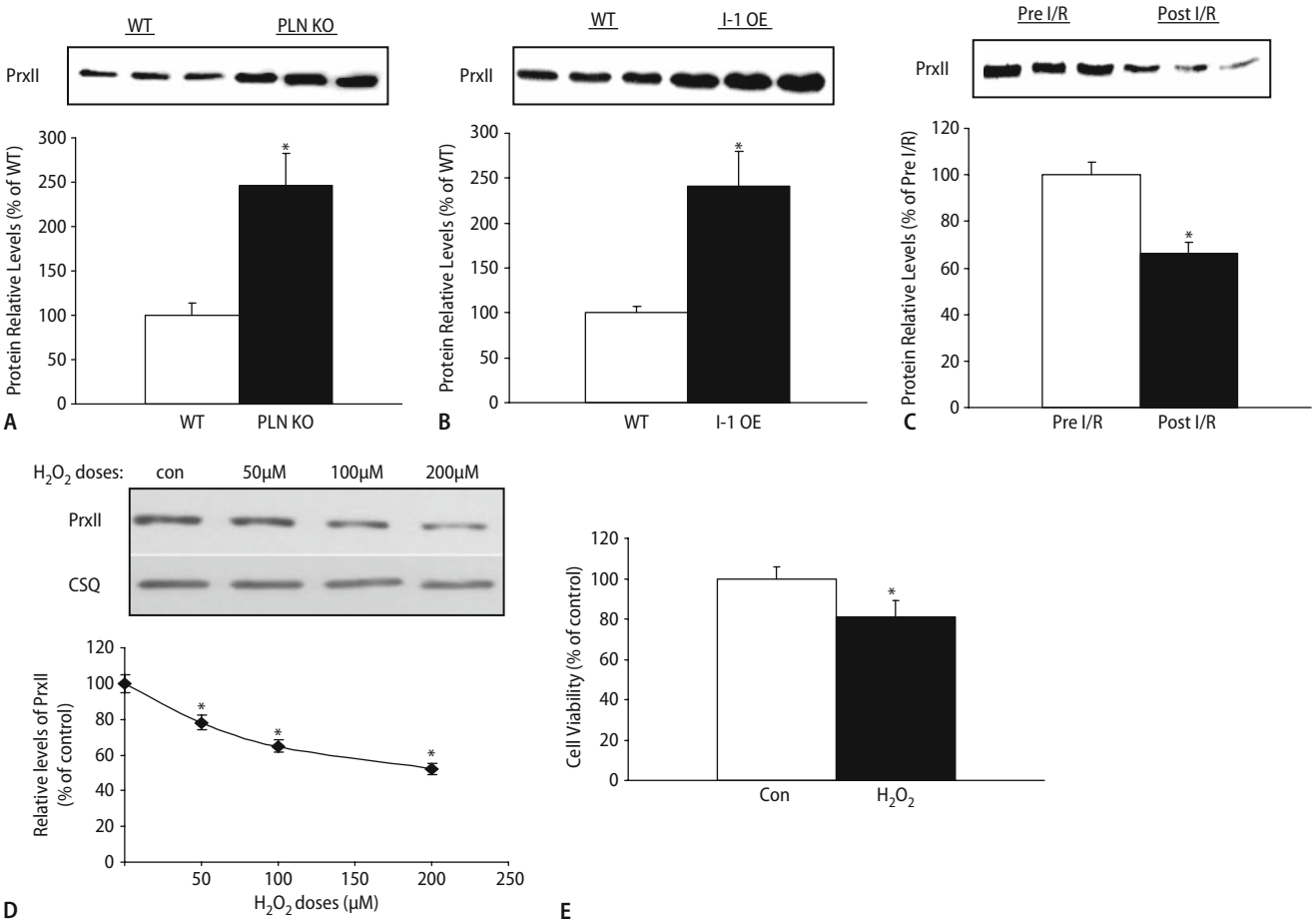

Fig. 1 Alterations of peroxiredoxin II expression in the hearts and isolated cardiomyocytes as well as cell viability upon H_2O_2 treatment. Hearts from phospholamban deficient (**a**: PLN KO) and protein phosphatase 1 inhibitor-1 overexpression (**b**: I-1 OE) mice were homogenized and processed for quantitative immunoblotting for the expression of peroxiredoxin II. **c** Wild type hearts were subjected to ex vivo Langendorff perfusion, consisting of 40 min ischemia (pre I/R) followed by 60 min reperfusion (post I/R) and the levels of peroxiredoxin II were determined; $n = 6$ hearts for each group. Values are mean ± SE, $^*P < 0.05$, compared to pre I/R or wild type values. **d** Quantitative immunoblotting and relative expressions of peroxiredoxin II (prxII) in cultured cardiomyocytes (24 h) in response to treatment with various H_2O_2 doses for 2 h. Calsequestrin was used as a loading control ($n = 7$ hearts for each group). **e** Cardiomyocyte viability was analyzed by MTT assay after H_2O_2 (50 μM) treatment for 2 h; $n = 6$ hearts for each group. Values are mean ± SE, $^*P < 0.05$, compared to control

Peroxiredoxin II alters thiobarbituric acid substances (TBARS) and lactate dehydrogenase (LDH) in cardiomyocytes upon H_2O_2 treatment

To further determine the effect of overexpression or downregulation of peroxiredoxin II on cardiomyocyte membrane integrity before and after 50 μM H_2O_2 treatment, the levels of TBARS were assessed. We found that overexpression or downregulation of peroxiredoxin II did not influence the intracellular TBARS under basal condition (data now shown). However, after H_2O_2 treatment, the levels of TBARS were increased by 14 or 15-fold in the control and Ad.GFP infected myocytes, compared to untreated cardiomyocytes (Fig. 3a). Overexpression of peroxiredoxin II significantly attenuated this increase, while downregulation of peroxiredoxin II resulted in further increases of TBARS (Fig. 3a). Accordingly, evaluation of the levels of lactate dehydrogenase (LDH) release, another parameter in evaluating cell membrane integrity, revealed similar results. Specifically, overexpression or downregulation of peroxiredoxin II did not play a role in LDH release under basal condition (data now shown). However, H_2O_2 treatment increased the LDH levels by threefold in control groups (Fig. 3b), and this effect was attenuated by overexpression of peroxiredoxin II, while downregulation of this enzyme resulted in increased LDH release (Fig. 3b). Thus, increases in peroxiredoxin II expression in cardiomyocytes decrease TBARS and LDH levels, while downregulation of peroxiredoxin II has opposite effects in H_2O_2-treated cardiomyocytes.

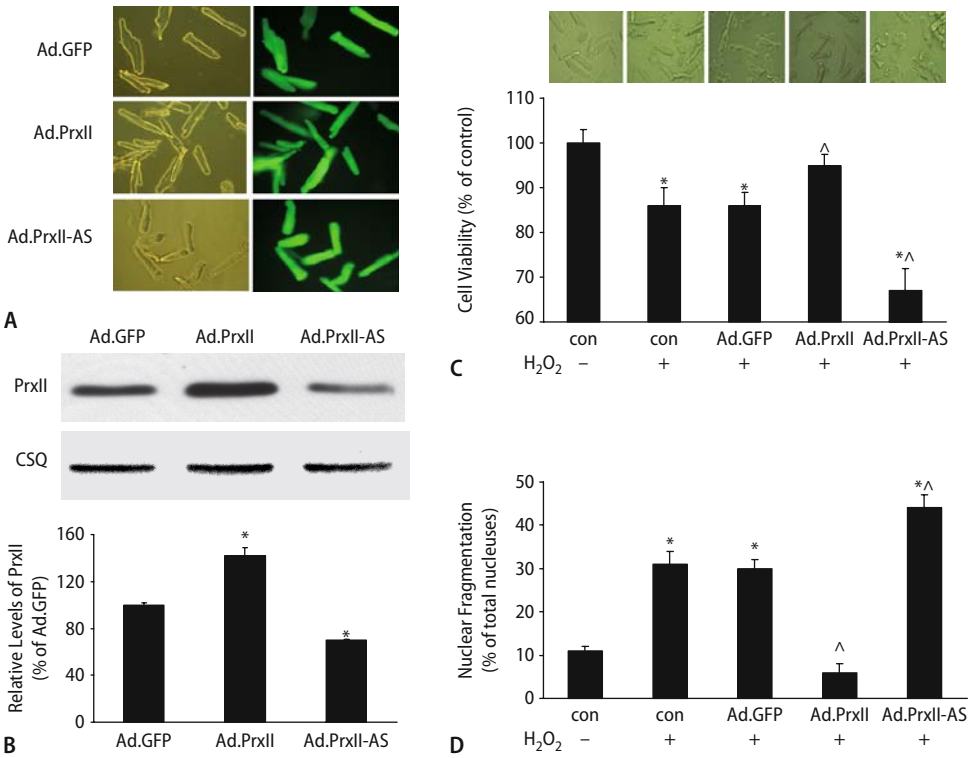

Fig. 2 Effects of peroxiredoxin II (prxII) overexpression and downregulation on cardiomyocyte viability and nuclear fragmentation upon H_2O_2 treatment. **a** Cardiomyocytes infected with adenoviruses: Ad.GFP, Ad.prxII (sense) or Ad.prxII-AS (antisense), under light (×40, *left panel*) and fluorescence (×40, *right panel*) microscope. **b** Quantitative immunobloting of peroxiredoxin II expression levels in infected cardiomyocytes. Calsequestrin was used as a loading control; $n = 8$ hearts for each group. Values are mean ± SE, $^*P < 0.05$, Vs. Ad.GFP group. Cardiomyocytes were treated with H_2O_2 (50 μM) for 2 h after 24 h viral infection, then (**c**) MTT assay was used to analyze the cell viability, and (**d**) cell nuclear fragmentation was determined by Hoechst staining. $n = 4$ hearts for each group. Values are mean ± SE, $^*P < 0.05$, Vs. control (control without H_2O_2 treatment). $^\wedge P < 0.05$, Vs. Ad.GFP

Peroxiredoxin II overexpression increases Bcl-2, inhibits Bax and decreases active caspases 3, 9 and 12 expression in cardiomyocytes

To further investigate the possible mechanisms underlying the protection of cell death and apoptosis by peroxiredoxin II overexpression, the expression levels of key apoptotic-related proteins were quantitatively assessed. H_2O_2 treatment was associated with significant decreases in the expression of the anti-apoptotic protein Bcl-2 in non-infected or Ad.GFP infected myocytes. However, overexpression of peroxiredoxin II prevented the Bcl-2 decrease, rather than further increased its levels. Accordingly, downregulation of peroxiredoxin II resulted in augmented decreases of Bcl-2, compared to the control treated groups (Fig. 4a, b). Moreover, the well-known pro-apoptotic proteins Bax, active caspases 3, 9 and 12 were significantly increased upon H_2O_2 treatment (Fig. 4a, b), which were prevented by peroxiredoxin II overexpression. Consequently, reduction in peroxiredoxin II levels had opposite results and promoted further increases in these pro-apoptotic proteins, compared to controls (Fig. 4a, b). These results suggest that the mechanisms underlying the protection of peroxiredoxin II are associated with increased expression of the anti-apoptotic protein Bcl-2, and decreased expression of the pro-apoptotic proteins Bax and active caspases 3, 9 and 12.

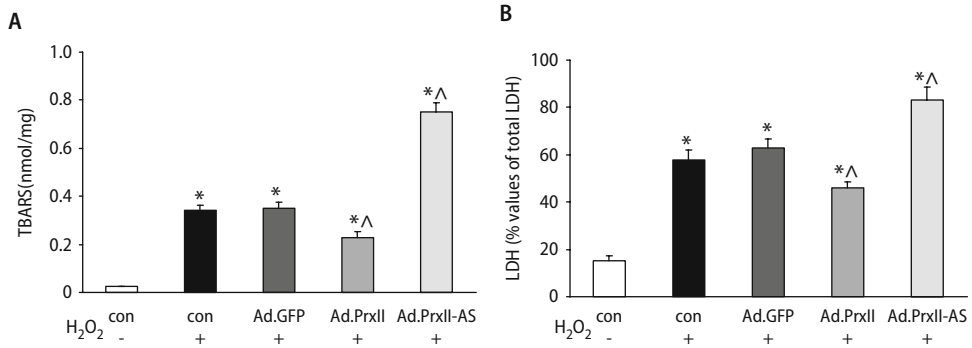

Fig. 3 Effects of peroxiredoxin II (prxII) overexpression and downregulation on intracellular lipid peroxidation (TBARS) and lactate dehydrogenase (LDH) release upon H_2O_2 treatment. Cardiomyocytes were isolated, infected with Ad.GFP, Ad.prxII and Ad.prxII-AS for 24 h, and then treated with 50 μM H_2O_2 for 2 h. Cells were harvested, intracellular lipid peroxidation, TBARS (**a**) and LDH release (**b**) were determined; $n = 4$ hearts for each group. Values are mean ± SE, $^*P < 0.05$, Vs. control (control without H_2O_2 treatment), $^\wedge P < 0.05$, Vs. Ad.GFP

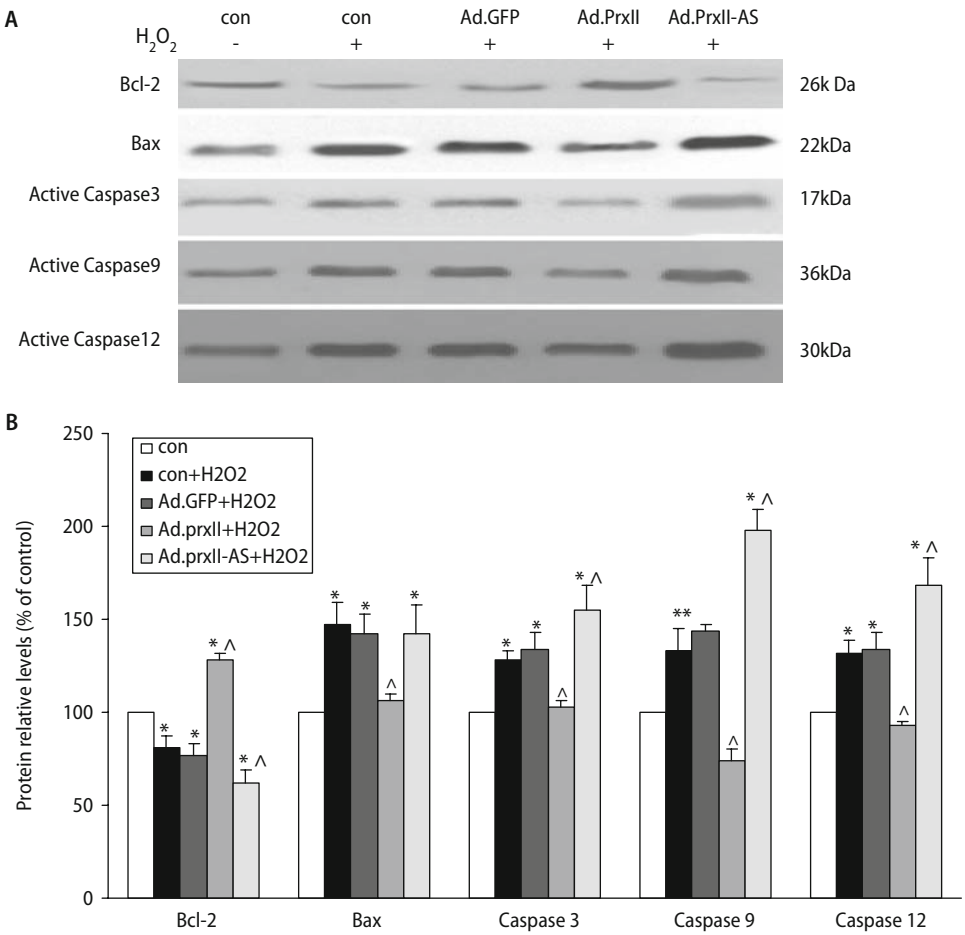

Fig. 4 Effects of peroxiredoxin II (prxII) overexpression and downregulation on apoptosis-related proteins in cardiomyocytes after H_2O_2 treatment. Cardiomyocytes were isolated, infected with Ad.GFP, Ad.prxII and Ad.prxII-AS for 24 h, and then treated with 50 μM H_2O_2 for 2 h. Cells were harvested, lysed and processed for quantitative immunobloting to determine the expression levels of Bcl-2, Bax, active caspase 3, active caspase 9 and active caspase 12. **a** Representative immunoblots for Bcl-2, Bax, active caspases 3, 9 and 12. **b** Quantitation of these proteins; $n = 4$–6 hearts for each group. Values are mean ± SE, $^*P < 0.05$, Vs. control (control without H_2O_2 treatment), $^\wedge P < 0.05$, Vs. Ad.GFP

Expression of the other peroxiredoxin family members, catalase and glutathione peroxidase (GHPx-1)

To further determine the specificity of decreased expression of peroxiredoxin II in the heart upon ischemia-reperfusion injury or in the cardiomyocytes with H_2O_2 treatment, protein levels of the other five peroxiredoxin isoforms, including: peroxiredoxin I, III, IV, V and VI were examined. As shown in Fig. 5a–d, we did not find any significant differences in these proteins, except peroxiredoxin I, which was decreased to 80% in the hearts after ischemia-reperfusion, compared to pre-ischemia-reperfused hearts. Similarly, the peroxiredoxin I expression was downregulated to 72% in H_2O_2-treated cardiomyocytes, compared to controls. In addition, the active forms of two representative cytosolic house-keeping proteins, catalase and GHPx-1, were examined. There was no significant difference in the expression of catalase and GHPx-1 after ischemia-reperfusion injury. In cardiomyocytes treated with H_2O_2, the levels of catalase were not altered, while GHPx-1 levels were significantly depressed (Fig. 5c–d). Although it is not currently clear why the levels of GHPx-1 were not consistently decreased in isolated hearts or isolated cardiomyocytes, these findings suggest that peroxiredoxin I and GHPx-1 may also play a role in cardiac ischemia-reperfusion or H_2O_2-induced injury.

We further evaluated the role of overexpression or downregulation of peroxiredoxin II on the expression of peroxiredoxin I, catalase and GHPx-1 in cardiomyocytes with or without H_2O_2 treatment. Under basal condition, these proteins' expression showed no significant difference among Ad.GFP, Ad.prxII and Ad.prxII-AS-infected cardiomyocytes (Fig. 6a, b). Furthermore, H_2O_2 treatment did not elicit any significant alterations in the levels of catalase, compared to control cells (Fig. 6a, b). In addition, the decreased expression levels of peroxiredoxin I and GHPx-1 remained the same among the cells infected with Ad.-prxII or Ad.prxII-AS, compared to controls (Fig. 6a, b). These data indicate that overexpression or downregulation of peroxiredoxin II does not alter the expression levels of peroxiredoxin I, catalase and GHPx-1 before and after H_2O_2 treatment, suggesting a

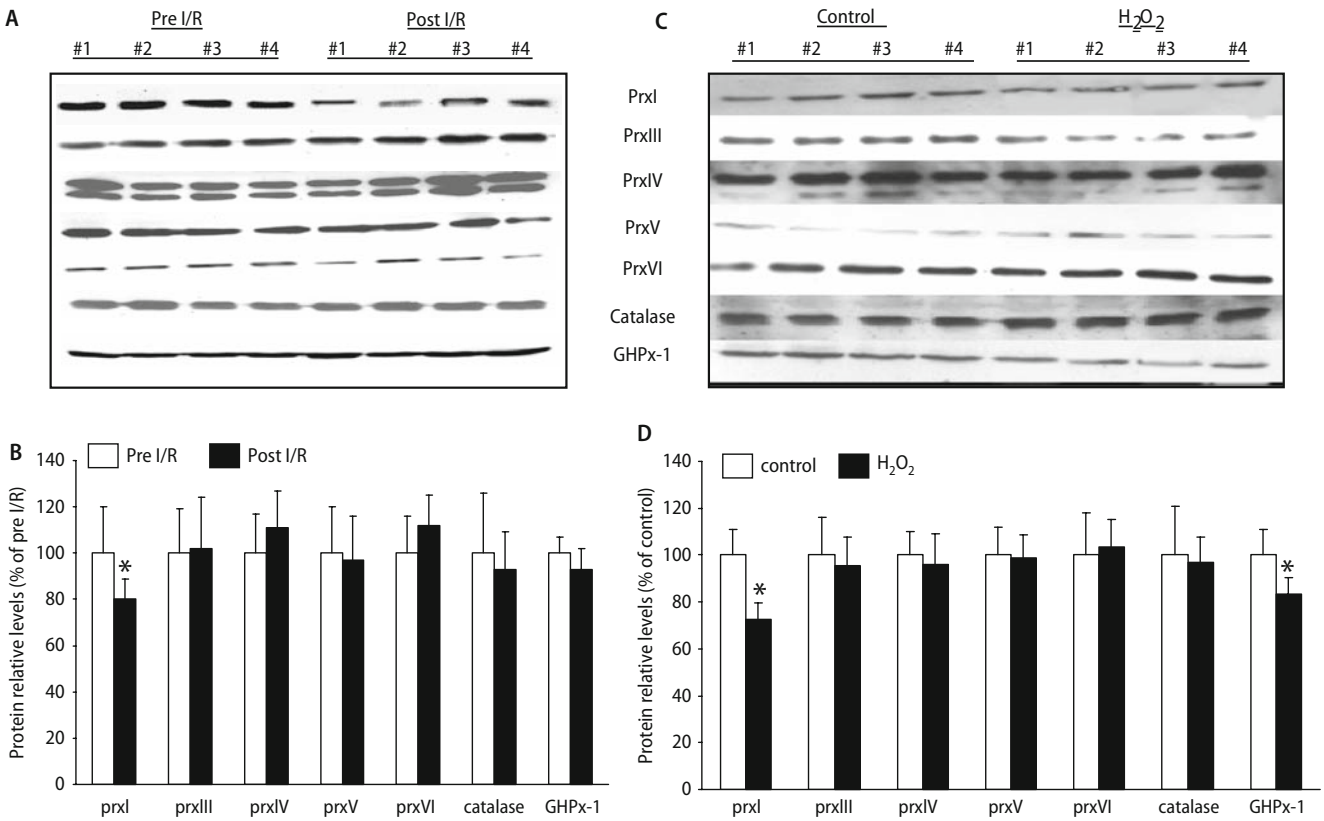

Fig. 5 Effect of cardiac ischemia-reperfusion injury and H_2O_2 stimulation on the expression of peroxiredoxin family members, catalase and glutathione peroxidase (GHPx-1). **a** and **b** WT hearts were subjected to ex vivo Langendorff perfusion, as described in Fig. 1. The levels of the other five peroxiredoxin family members, including peroxiredoxin I, III, IV, V and VI, as well as two representative cytosolic house keeping proteins: catalase and GHPx-1 were determined. **c** and **d** Cardiomyocytes were isolated and cultured for 24 h, then treated with 50 μM H_2O_2 for 2 h. Cells were harvested, lysed and processed for western blot to examine the expression levels of the same proteins as those in (**a**) and (**b**); $n = 4$ hearts for each group, Values are mean ± SE, $^*P \leq 0.05$, Vs. per I/R or control cells

specific effect of peroxiredoxin II in mediating oxidative-stress-induced cell injury.

Discussion

The present study provides the first evidence that peroxiredoxin II may play an important protective role in cardiac oxidative stress. Increases in the levels of peroxiredoxin II protected the cardiomyocytes against oxidative stress-induced apoptosis and cell death by H_2O_2, the major source of ROS in cardiomyocytes under stress conditions [23]. These beneficial effects of peroxiredoxin II in cardiomyocytes were associated with increases in the anti-apoptotic protein Bcl-2 and decreases in the pro-apoptotic protein Bax expression, as well as attenuation of the activity of active caspases 3, 9 and 12. Figure 7 summarizes the possible effects of peroxiredoxin II in oxidative-stress-induced cardiac injury. Consistent with our findings, overexpression of peroxiredoxin I and peroxiredoxin II protected thyroid cells from H_2O_2-induced apoptosis, which was also associated with reduced Bax levels [23]. Furthermore, overexpression of human peroxiredoxin II in Molt-4 leukemia cells inhibited release of cytochrome c from mitochondria to cytosol and lipid peroxidation, therefore protecting the cells from apoptosis in a similar manner as Bcl-2 [54]. Interestingly, peroxiredoxin II could also prevent H_2O_2 accumulation in these leukemic cells, suggesting that it may function upstream of Bcl-2 [54].

To further investigate the specificity of the loss of peroxiredoxin II during cardiac ischemia-reperfusion injury and in response to H_2O_2, we examined the expression levels of the other 5 isoforms of the peroxiredoxin family, as well as two representative cytosolic house keeping proteins: catalase and GHPx-1. Consistent with other previous reports [29, 32, 50], there were no significant differences in the expression levels of most of these proteins. Furthermore, overexpression or downregulation of peroxiredoxin II did not change the expression of the cytosolic house keeping proteins, catalase and GHPx-1, with or

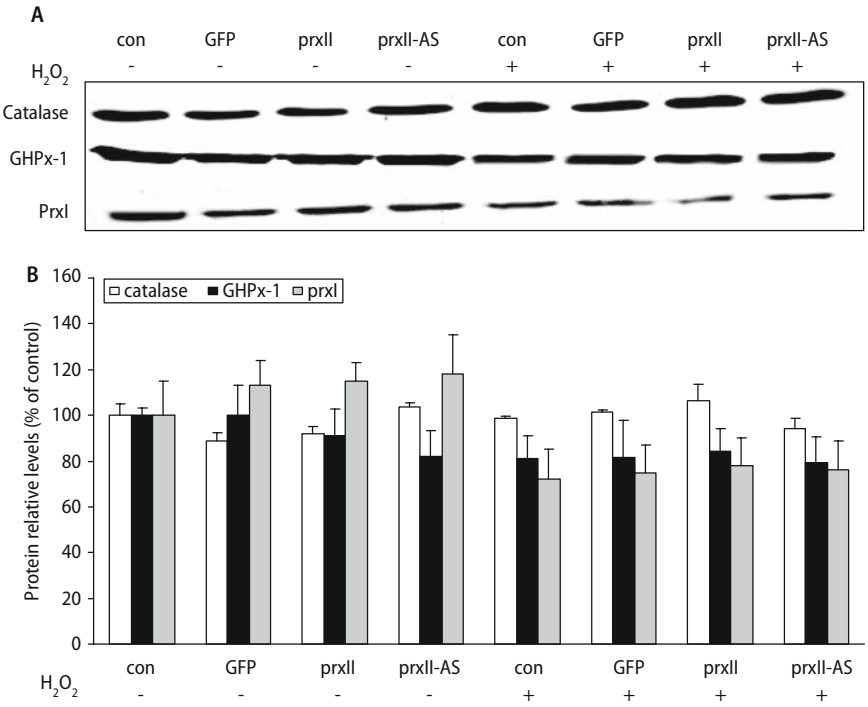

Fig. 6 Effect of overexpression or downregulation of peroxiredoxin II on the expression levels of catalase, GHPx-1 and peroxiredoxin I. Cardiomyocytes were isolated, infected with Ad.GFP, Ad.prxII and Ad.prxII-AS for 24 h, and then some of the cells were treated with 50 μM H_2O_2 for 2 h. Cells were then harvested, lysed and processed for western blot to examine the expression levels of catalase, GHPx-1 and peroxiredoxin I. **a** Representative immunoblots and **b** quantitation of the expressions of catalase, GHPx-1 and peroxiredoxin I; $n = 4$ hearts for each group. Values are mean ± SE

without oxidative stress stimulation. Interestingly, the expression levels of peroxiredoxin I were found to be significantly decreased after ischemia-reperfusion injury and after H_2O_2 treatment, indicating the potential effect of this protein involved in the process of oxidative stress-related cardiac injury. However, peroxiredoxin II overexpression or downregulation did not further influence the expression of preoxiredoxin I. These findings indicate that the antioxidant properties of peroxiredoxin II in the heart might be independent of other antioxidant enzymes, suggesting a unique effect of peroxiredoxin II in the process of cardiac ischemia-reperfusion injury or H_2O_2-induced cell death.

The decreased expression of peroxiredoxin II found in the hearts of ischemia-reperfusion injury or cardiomyocytes after H_2O_2 treatment may involve leakage of this enzyme into the extracellular fluid and then elimination during reperfusion of the hearts or culturing of the cells [49]. Although the exact mechanisms and interactions among various antioxidants are not fully understood, it is possible that one antioxidant may equilibrate with another to establish a cellular redox potential and that all endogenous antioxidants may work in conjunction with one another to protect against oxidative stress [8]. It is also possible that certain antioxidants act as the first line of defense against oxidative stress in ischemia-reperfusion, while other antioxidants may only act later during severe oxidative stress [8]. It is unclear if the loss of peroxiredoxin II is based on its oxidation by H_2O_2 or excessive ROS and subsequent degradation by the proteasome. However, it has been reported that silica, one of the fibrogenic agents, induces rapid degradation of peroxiredoxin I and peroxiredoxin II in Rat2 cells along with significant production of ROS in the cells. The degradation of peroxiredoxin enzymes was insensitive to the proteasome inhibitors: MG132 and lactacystin [43]. Another report showed opposite results, as cardiomyocytes treated with MG132 exhibited induced antioxidant protein expression, including peroxiredoxin I and SOD-1 [10]. Although experimental studies with proteasome inhibitor strongly suggest the role of proteasomes in the process of ischemia-reperfusion injury [1, 2, 16, 39], there are still some controversial reports. One study has shown that reperfusion of isolated hearts with MG132 improves posthypoxic function of excised isolated papillary muscles [45]. However, this occurred after a prehypoxic delay of at least 30 minutes with demonstrable increases in heat shock proteins, but no determination of myocardial proteasome activity. The same group also showed that incubation of vascular smooth muscle cells with low concentrations of proteasome inhibitors results in upregulation of proteasome subunit transcription and translation [30], indicating the possibility that the previous results were related to increases in proteasome activity. Conversely, it was reported that preischemic treatment of isolated rat hearts with MG132 results in a

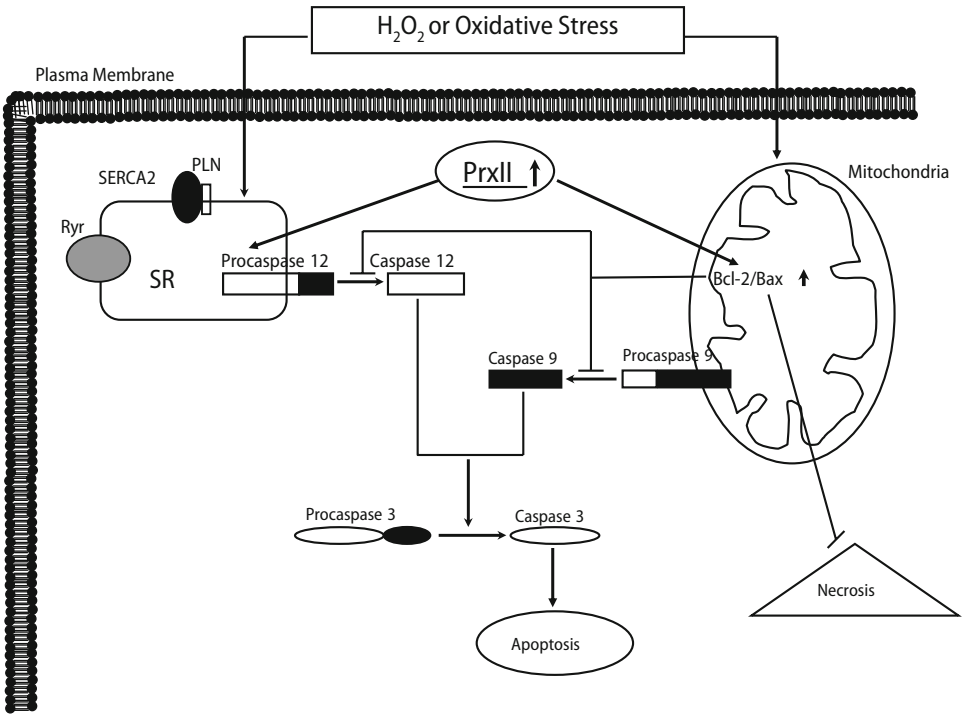

Fig. 7 Proposed scheme for the effects of peroxiredoxin II (prx II) in H_2O_2 or oxidative stress-induced myocyte apoptosis and necrosis

dose-dependent decrease in postischemic function, but increased levels of ubiquitinated proteins [38]. In addition, pretreatment with the more specific inhibitor, lactacystin, according to a protocol that decreased preischemic proteasome activity by 40%, failed to have any effects on postischemic function [9]. Taken together, these studies indicate the complicated regulation for cardiac ischemia-reperfusion injury with multiple factors involved. Furthermore, several studies have demonstrated the complex modification of cardiac peroxiredoxins during oxidative stress. Schroder et al. [41] reported that H_2O_2 induces an array of changes in the myocardium, including formation of disulfide bonds that were intermolecular for peroxiredoxin I, II and III, but intramolecular within peroxiredoxin V. Peroxiredoxin oxidation was also associated with movement from the cytosol to the membrane and myofilament-enriched fraction. Jang et al. [21] suggested that two cytosolic yeast peroxiredoxins: peroxiredoxin I and peroxiredoxin II, which display diversity in structure and apparent molecular weight (MW), act alternatively as peroxidases and molecular chaperones. The peroxidase function predominates in the lower MW forms, whereas the chaperone function predominates in the higher MW complexes. Oxidative stress and heat shock exposure of yeast cause the protein structures of cytosolic peroxiredoxin I and II to shift from low MW species to high MW complexes. Moon et al. [31] also indicated that human peroxiredoxin II assumes a high MW complex structure, which has a highly efficient chaperone function upon exposure to oxidative stress. However, the subsequent removal of stressors induces the dissociation of this protein structure into low MW proteins and triggers a chaperone-to-peroxidase functional switch. These studies indicate a complicated intracellular molecular interaction, which needs to be further investigated.

It has been reported that short term exposure of cardiomyocytes to H_2O_2 results in cardiomyocyte death due to necrosis as well as apoptosis [28]. Cardiomyocyte necrosis induced by H_2O_2 is associated with cell membrane phospholipid peroxidation by ROS. Hydroxyl radicals generated from the decomposition of H_2O_2 through the iron dependent Fenton reaction initiate lipid peroxidation by reacting with methylene groups of polyunsaturated fatty acids [3]. Our results suggest that hydroxyl radical-mediated lipid peroxidation and LDH release, may contribute to H_2O_2-induced cell necrosis. Increases in peroxiredoxin II appear to prevent cell necrosis by attenuating TBARS and LDH release, while reduction in peroxiredoxin II levels have opposite effects. The anti-apoptotic effects of peroxiredoxin II overexpression were associated with increases in Bcl-2 and preserved Bax levels after H_2O_2 treatment, leading to inhibition of caspases 3 and 9 activation (Fig. 7). Consequently, there was reduced cell death and apoptosis, suggesting that the cardioprotective effects of peroxiredoxin II are at least partially associated with the mito-

chondrial signaling pathway. Furthermore, caspase 12 was inhibited in peroxiredoxin II-overexpressing cardiomyocytes, indicating that protection was also associated with reduced H_2O_2-triggered ER/SR stress [20] (Fig. 7).

The in vivo role of peroxiredoxin II in oxidative stress-induced cardiomyocyte death and apoptosis is currently unclear. Neonatal cardiomyocytes exposed to H_2O_2 for 2 hours exhibited increased transcripts of peroxiredoxin II and V, which shifted to lower pI values upon two-dimensional gel electrophoresis, suggesting complex regulation of these two enzymatic activities in cardiomyocytes [7]. It could be hypothesized that overexpression of peroxiredoxin II in the hyperdynamic PLN KO and protein phosphatase 1 inhibitor-1 overexpressing (I-1 OE) hearts may serve as an important cardioprotective mechanism, allowing for normal life-span in these models [44]. Interestingly, in vitro studies have shown that co-transfection of PLN and HAX-1, another anti-apoptotic protein, in HEK293 cells enhances protection from hypoxia-reoxygenation-induced cell death and apoptosis [48], which suggest a potential involvement of PLN in cardiomyocyte apoptosis. However, the potential association between peroxiredoxin II and PLN in the cardiac anti-apoptotic pathways needs to be further examined.

A major consequence of increased cardiac metabolism associated with PLN KO and I-1 OE mice is the significant increase in formation of oxidative radicals at the level of the cellular NAD(P)H oxidases in the mitochondrial electron transport chain [18], which represents a major source of free radical generation in the cell. The increased expression of peroxiredoxin II (the cytosolic form of the peroxiredoxin family) in these hyperdynamic mouse hearts may represent an essential adaptation to neutralize oxidative stress generated by increased oxide-reduction activity. This energetic adaptation may also be important in heart failure, where multiple anatomic and molecular factors converge to increase the net energetic demands on the heart. Growing evidence suggests an important role for increased oxidative stress in adverse left ventricular remodeling after myocardial infarction [17]. It has been shown that various antioxidant approaches (vitamin C, vitamin E, probucol, dimethylthiourea or genetic manipulation) can ameliorate this adverse remodeling. The beneficial effects of these experimental approaches extend to improved contractile function, reduced left ventricular dilatation and lower mortality, especially in a short term. However, long term effects of these treatments seem disappointing [42]. A better understanding of the precise roles of oxidative stress and redox signaling pathways may therefore provide the basis for new therapeutic strategies [42]. The protective effects of peroxiredoxin II from oxidative stress-induced cell injury observed in this study may yield new insights in the treatment of cardiac oxidative stress.

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References

1. Bao J, Sato K, Li M, Gao Y, Abid R, Aird W, Simons M, Post MJ (2001) PR-39 and PR-11 peptides inhibit ischemia-reperfusion injury by blocking proteasome-mediated I kappa B alpha degradation. Am J Physiol Heart Circ Physiol 281:H2612–2618
2. Campbell B, Adams J, Shin YK, Lefer AM (1999) Cardioprotective effects of a novel proteasome inhibitor following ischemia and reperfusion in the isolated perfused rat heart. J Mol Cell Cardiol 31:467–476
3. Casey TM, Arthur PG, Bogoyevitch MA (2007) Necrotic death without mitochondrial dysfunction-delayed death of cardiac myocytes following oxidative stress. Biochim Biophys Acta 1773:342–351
4. Chen HW, Chien CT, Yu SL, Lee YT, Chen WJ (2002) Cyclosporine A regulate oxidative stress-induced apoptosis in cardiomyocytes: mechanisms via ROS generation, iNOS and Hsp70. Br J Pharmacol 137:771–781
5. Choi MH, Lee IK, Kim GW, Kim BU, Han YH, Yu DY, Park HS, Kim KY, Lee JS, Choi C, Bae YS, Lee BI, Rhee SG, Kang SW (2005) Regulation of PDGF signalling and vascular remodelling by peroxiredoxin II. Nature 435:347–353
6. Chu G, Kerr JP, Mitton B, Egnaczyk GF, Vazquez JA, Shen M, Kilby GW, Stevenson TI, Maggio JE, Vockley J, Rapundalo ST, Kranias EG (2004) Proteomic analysis of hyperdynamic mouse hearts with enhanced sarcoplasmic reticulum calcium cycling. FASEB J 18:1725–1727
7. Cullingford TE, Wait R, Clerk A, Sugden PH (2006) Effects of oxidative stress on the cardiac myocyte proteome: modifications to peroxiredoxins and small heat shock proteins. J Mol Cell Cardiol 40:157–172
8. Dhalla NS, Elmoselhi AB, Hata T, Makino N (2000) Status of myocardial antioxidants in ischemia-reperfusion injury. Cardiovasc Res 47:446–456
9. Divald A, Powell SR (2006) Proteasome mediates removal of proteins oxidized during myocardial ischemia. Free Radic Biol Med 40:156–164
10. Doll D, Sarikas A, Krajcik R, Zolk O (2007) Proteomic expression analysis of cardiomyocytes subjected to proteasome inhibition. Biochem Biophys Res Commun 353:436–442

11. Dost T, Cohen MV, Downey JM (2008) Redox signaling triggers protection during the reperfusion rather than the ischemic phase of preconditioning. Basic Res Cardiol 103:378–384
12. Fan GC, Chu G, Mitton B, Song Q, Yuan Q, Kranias EG (2004) Small heat-shock protein Hsp20 phosphorylation inhibits beta-agonist-induced cardiac apoptosis. Circ Res 94:1474–1482
13. Fan GC, Gregory KN, Zhao W, Park WJ, Kranias EG (2004) Regulation of myocardial function by histidine-rich, calcium-binding protein. Am J Physiol Heart Circ Physiol 287:H1705–1711
14. Fan GC, Ren X, Qian J, Yuan Q, Nicolaou P, Wang Y, Jones WK, Chu G, Kranias EG (2005) Novel cardioprotective role of a small heat-shock protein, Hsp20, against ischemia/reperfusion injury. Circulation 111:1792–1799
15. Fan GC, Yuan Q, Song G, Wang Y, Chen G, Qian J, Zhou X, Lee YJ, Ashraf M, Kranias EG (2006) Small heat-shock protein Hsp20 attenuates beta-agonist-mediated cardiac remodeling through apoptosis signal-regulating kinase 1. Circ Res 99:1233–1242
16. Gao Y, Lecker S, Post MJ, Hietaranta AJ, Li J, Volk R, Li M, Sato K, Saluja AK, Steer ML, Goldberg AL, Simons M (2000) Inhibition of ubiquitin-proteasome pathway-mediated I kappa B alpha degradation by a naturally occurring antibacterial peptide. J Clin Invest 106:439–448
17. Giordano FJ (2005) Oxygen, oxidative stress, hypoxia, and heart failure. J Clin Invest 115:500–508
18. Griendling KK, FitzGerald GA (2003) Oxidative stress and cardiovascular injury: Part I: basic mechanisms and in vivo monitoring of ROS. Circulation 108:1912–1916
19. Hess ML, Manson NH (1984) Molecular oxygen: friend and foe. The role of the oxygen free radical system in the calcium paradox, the oxygen paradox and ischemia/reperfusion injury. J Mol Cell Cardiol 16:969–985
20. Ihara Y, Urata Y, Goto S, Kondo T (2006) Role of calreticulin in the sensitivity of myocardiac H9c2 cells to oxidative stress caused by hydrogen peroxide. Am J Physiol Cell Physiol 290:C208–221
21. Jang HH, Lee KO, Chi YH, Jung BG, Park SK, Park JH, Lee JR, Lee SS, Moon JC, Yun JW, Choi YO, Kim WY, Kang JS, Cheong GW, Yun DJ, Rhee SG, Cho MJ, Lee SY (2004) Two enzymes in one; two yeast peroxiredoxins display oxidative stress-dependent switching from a peroxidase to a molecular chaperone function. Cell 117:625–635
22. Kang PM, Haunstetter A, Aoki H, Usheva A, Izumo S (2000) Morphological and molecular characterization of adult cardiomyocyte apoptosis during hypoxia and reoxygenation. Circ Res 87:118–125
23. Kim H, Lee TH, Park ES, Suh JM, Park SJ, Chung HK, Kwon OY, Kim YK, Ro HK, Shong M (2000) Role of peroxiredoxins in regulating intracellular hydrogen peroxide and hydrogen peroxide-induced apoptosis in thyroid cells. J Biol Chem 275:18266–18270
24. Lee TH, Kim SU, Yu SL, Kim SH, Park DS, Moon HB, Dho SH, Kwon KS, Kwon HJ, Han YH, Jeong S, Kang SW, Shin HS, Lee KK, Rhee SG, Yu DY (2003) Peroxiredoxin II is essential for sustaining life span of erythrocytes in mice. Blood 101:5033–5038
25. Liu Y, Yang XM, Iliodromitis EK, Kremastinos DT, Dost T, Cohen MV, Downey JM (2008) Redox signaling at reperfusion is required for protection from ischemic preconditioning but not from a direct PKC activator. Basic Res Cardiol 103:54–59
26. Luo W, Grupp IL, Harrer J, Ponniah S, Grupp G, Duffy JJ, Doetschman T, Kranias EG (1994) Targeted ablation of the phospholamban gene is associated with markedly enhanced myocardial contractility and loss of beta-agonist stimulation. Circ Res 75:401–409
27. Maack C, O'Rourke B (2007) Excitation-contraction coupling and mitochondrial energetics. Basic Res Cardiol 102:369–392
28. Mangat R, Singal T, Dhalla NS, Tappia PS (2006) Inhibition of phospholipase C-gamma 1 augments the decrease in cardiomyocyte viability by H2O2. Am J Physiol Heart Circ Physiol 291:H854–860
29. Matsushima S, Ide T, Yamato M, Matsusaka H, Hattori F, Ikeuchi M, Kubota T, Sunagawa K, Hasegawa Y, Kurihara T, Oikawa S, Kinugawa S, Tsutsui H (2006) Overexpression of mitochondrial peroxiredoxin-3 prevents left ventricular remodeling and failure after myocardial infarction in mice. Circulation 113:1779–1786
30. Meiners S, Heyken D, Weller A, Ludwig A, Stangl K, Kloetzel PM, Kruger E (2003) Inhibition of proteasome activity induces concerted expression of proteasome genes and de novo formation of Mammalian proteasomes. J Biol Chem 278:21517–21525
31. Moon JC, Hah YS, Kim WY, Jung BG, Jang HH, Lee JR, Kim SY, Lee YM, Jeon MG, Kim CW, Cho MJ, Lee SY (2005) Oxidative stress-dependent structural and functional switching of a human 2-Cys peroxiredoxin isotype II that enhances HeLa cell resistance to H2O2-induced cell death. J Biol Chem 280:28775–28784
32. Nagy N, Malik G, Fisher AB, Das DK (2006) Targeted disruption of peroxiredoxin 6 gene renders the heart vulnerable to ischemia-reperfusion injury. Am J Physiol Heart Circ Physiol 291:H2636–2640
33. Park SH, Chung YM, Lee YS, Kim HJ, Kim JS, Chae HZ, Yoo YD (2000) Antisense of human peroxiredoxin II enhances radiation-induced cell death. Clin Cancer Res 6:4915–4920
34. Pathak A, Baldwin B, Kranias EG (2007) Key protein alterations associated with hyperdynamic cardiac function: insights based on proteomic analysis of the protein phosphatase 1 inhibitor-1 overexpressing hearts. Hellenic J Cardiol 48:30–36
35. Pathak A, del Monte F, Zhao W, Schultz JE, Lorenz JN, Bodi I, Weiser D, Hahn H, Carr AN, Syed F, Mavila N, Jha L, Qian J, Marreez Y, Chen G, McGraw DW, Heist EK, Guerrero JL, DePaoli-Roach AA, Hajjar RJ, Kranias EG (2005) Enhancement of cardiac function and suppression of heart failure progression by inhibition of protein phosphatase 1. Circ Res 96:756–766
36. Penna C, Mancardi D, Tullio F, Pagliaro P (2008) Postconditioning and intermittent bradykinin induced cardioprotection require cyclooxygenase activation and prostacyclin release during reperfusion. Basic Res Cardiol 103:368–377
37. Penna C, Rastaldo R, Mancardi D, Raimondo S, Cappello S, Gattullo D, Losano G, Pagliaro P (2006) Post-conditioning induced cardioprotection requires signaling through a redox-sensitive mechanism, mitochondrial ATP-sensitive K + channel and protein kinase C activation. Basic Res Cardiol 101:180–189
38. Powell SR, Wang P, Katzeff H, Shringarpure R, Teoh C, Khaliulin I, Das DK, Davies KJ, Schwalb H (2005) Oxidized and ubiquitinated proteins may predict recovery of postischemic cardiac function: essential role of the proteasome. Antioxid Redox Signal 7:538–546

39. Pye J, Ardeshirpour F, McCain A, Bellinger DA, Merricks E, Adams J, Elliott PJ, Pien C, Fischer TH, Baldwin AS, Jr., Nichols TC (2003) Proteasome inhibition ablates activation of NF-kappa B in myocardial reperfusion and reduces reperfusion injury. Am J Physiol Heart Circ Physiol 284:H919–926
40. Ruusalepp A, Czibik G, Flatebo T, Vaage J, Valen G (2007) Myocardial protection evoked by hyperoxic exposure involves signaling through nitric oxide and mitogen activated protein kinases. Basic Res Cardiol 102:318–326
41. Schroder E, Brennan JP, Eaton P (2008) Cardiac peroxiredoxins undergo complex modifications during cardiac oxidant stress. Am J Physiol Heart Circ Physiol 295:H425–433
42. Seddon M, Looi YH, Shah AM (2007) Oxidative stress and redox signalling in cardiac hypertrophy and heart failure. Heart 93:903–907
43. Seo MS, Kim JK, Lim Y, Kang SW, Cho YJ, Lee WK, Kim HJ, Cho KK, Lee KH, Rhee SG (1999) Rapid degradation of PrxI and PrxII induced by silica in Rat2 cells. Biochem Biophys Res Commun 265:541–544
44. Slack JP, Grupp IL, Dash R, Holder D, Schmidt A, Gerst MJ, Tamura T, Tilgmann C, James PF, Johnson R, Gerdes AM, Kranias EG (2001) The enhanced contractility of the phospholamban-deficient mouse heart persists with aging. J Mol Cell Cardiol 33:1031–1040
45. Stangl K, Gunther C, Frank T, Lorenz M, Meiners S, Ropke T, Stelter L, Moobed M, Baumann G, Kloetzel PM, Stangl V (2002) Inhibition of the ubiquitin-proteasome pathway induces differential heat-shock protein response in cardiomyocytes and renders early cardiac protection. Biochem Biophys Res Commun 291:542–549
46. Suzuki YJ, Ford GD (1999) Redox regulation of signal transduction in cardiac and smooth muscle. J Mol Cell Cardiol 31:345–353
47. Tsutsui H, Ide T, Kinugawa S (2006) Mitochondrial oxidative stress, DNA damage, and heart failure. Antioxid Redox Signal 8:1737–1744
48. Vafiadaki E, Sanoudou D, Arvanitis DA, Catino DH, Kranias EG, Kontrogianni-Konstantopoulos A (2007) Phospholamban interacts with HAX-1, a mitochondrial protein with anti-apoptotic function. J Mol Biol 367:65–79
49. Venardos KM, Perkins A, Headrick J, Kaye DM (2007) Myocardial ischemia-reperfusion injury, antioxidant enzyme systems, and selenium: a review. Curr Med Chem 14:1539–1549
50. Wang X, Phelan SA, Forsman-Semb K, Taylor EF, Petros C, Brown A, Lerner CP, Paigen B (2003) Mice with targeted mutation of peroxiredoxin 6 develop normally but are susceptible to oxidative stress. J Biol Chem 278:25179–25190
51. Watabe S, Hasegawa H, Takimoto K, Yamamoto Y, Takahashi SY (1995) Possible function of SP-22, a substrate of mitochondrial ATP-dependent protease, as a radical scavenger. Biochem Biophys Res Commun 213:1010–1016
52. Witting PK, Liao WQ, Matthew JH, Neuzil J (2006) Expression of human myoglobin in H9c2 cells enhances toxicity to added hydrogen peroxide. Biochem Biophys Res Commun 348:485–493
53. Yaniv G, Shilkrut M, Larisch S, Binah O (2005) Hydrogen peroxide predisposes neonatal rat ventricular myocytes to Fas-mediated apoptosis. Biochem Biophys Res Commun 336:740–746
54. Zhang P, Liu B, Kang SW, Seo MS, Rhee SG, Obeid LM (1997) Thioredoxin peroxidase is a novel inhibitor of apoptosis with a mechanism distinct from that of Bcl-2. J Biol Chem 272:30615–30618
55. Zorov DB, Filburn CR, Klotz LO, Zweier JL, Sollott SJ (2000) Reactive oxygen species (ROS)-induced ROS release: a new phenomenon accompanying induction of the mitochondrial permeability transition in cardiac myocytes. J Exp Med 192:1001–1014