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Over-expressed Peroxiredoxin I Protects against Oxidative Damage in Mouse Embryonic Fibroblasts Lacking Peroxiredoxin II

Seonggon Kim[1], Jae-Young Kim[2], Zae Young Ryoo[1] and Sanggyu Lee[1,*]

[1]School of Life Science and Biotechnology, [2]Department of Biochemistry, School of Dentistry, IHBR, Kyungpook National University, Daegu 518-040, Republic of Korea

Abstract

Peroxiredoxins (Prxs) have a critical role in protecting cells against oxidative damage generated by reactive oxygen species (ROS). PrxI and PrxII are more than 90% homologous in their amino acid sequences, and both proteins reduce H_2O_2. In this study, an over-expression plasmid carrying PrxI was transfected into PrxII$^{-/-}$ mouse embryonic fibroblasts (MEFs) to investigate potential compensatory relationships between PrxI and PrxII. ROS levels induced by oxidative stress were increased in PrxII$^{-/-}$ MEFs as compared to wild-type MEFs. Moreover, exposure of PrxII$^{-/-}$ MEFs to H_2O_2 caused a reduction in cell viability of about 10%, and the proportion of cell death was increased compared to mock-treated PrxII$^{-/-}$ MEFs. However, transient over-expression of PrxI in PrxII$^{-/-}$ MEFs conferred increased resistance against the oxidative damage, as evidenced by increased cell viability and reduced intracellular ROS levels under H_2O_2 stress conditions. The findings suggest that over-expressed PrxI can partly compensate for the loss of PrxII function in PrxII-deficient MEFs.

Key Words: Peroxiredoxin I, Peroxiredoxin II, ROS, MEF

INTRODUCTION

Peroxiredoxins (Prxs) have attracted the notice of researchers in recent years as a family of thiol-specific antioxidant proteins (Kim *et al.*, 1988; Chae *et al.*, 1993). They have been identified in various organisms and there are six members in mammals (Seo *et al.*, 2000; Claiborne *et al.*, 2001). Prx-family proteins are abundant and widely distributed in various cell lines (Wood *et al.*, 2003; Rhee *et al.*, 2005b), and they can remove hydrogen peroxide (H_2O_2) through peroxidase action, utilizing thioredoxin or glutathione as the electron donor (Knoops *et al.*, 1999). Recent studies showed that human PrxI and PrxII have more than 90% homology at the amino acid sequence level (Rhee *et al.*, 2001; Wood *et al.*, 2003) and are the main candidates for regulating H_2O_2 signaling, because they have higher affinity for H_2O_2 than does catalase (Chae *et al.*, 1999). Moreover Prxs have various other functions, including cell differentiation and proliferation (Nemoto *et al.*, 1990; Tsuji *et al.*, 1995), cell-cycle control (Phalen *et al.*, 2006), molecular chaperon activity (Jang *et al.*, 2004), protection of cells from ionizing radiation-induced death (Zhang *et al.*, 2008), heme-binding (Iwahara *et al.*, 1995), induction of natural killer cell activity (Shau *et al.*, 1994), and intracellular signaling (Wen and Van Etten, 1997).

Previous studies showed that deficiency of Prxs led to increased ROS-induced damage in various organs. Elevated intracellular H_2O_2 levels induced by platelet-derived growth factor or epidermal growth factor in transfected endothelial cell lines were decreased by transient over-expression of the PrxI or PrxII gene. Moreover, over-expression of PrxII blocked the activation of nuclear factor κB (NFκB) induced by extracellularly added H_2O_2 or tumor necrosis factor-α (Kang *et al.*, 1998). And over-expressed PrxI or PrxII eliminated H_2O_2 that was generated by thyrotropin treatment in a thyroid cell-culture model (Kim *et al.*, 2000).

Furthermore, several Prx gene knockout mouse models were reported to exhibit severe defects. In PrxI knock-out mice, Neumann and colleagues observed a hemolytic anemia characterized by hemoglobin instability, Heinz body formation and decreased erythrocyte lifespan. An increase in the incidence of malignancies was also observed, as was the level of intracellular ROS (Neumann *et al.*, 2003). The higher incidence of malignancies suggests that PrxI protein functions as a tumor suppressor. Similarly, in PrxII-deficient mice, intracellular ROS levels were increased and erythrocyte life span was decreased, resulting in anemia and low hematocrit values (Lee *et al.*, 2003). In PrxVI$^{-/-}$ mice, macrophages exhibited increased H_2O_2 levels and decreased survival rates. PrxVI$^{-/-}$

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***Corresponding Author**

E-mail: slee@knu.ac.kr
Tel: +82-53-950-7363, Fax: +82-53-943-6925

mice had higher levels of protein oxidation, more severe tissue damage, and markedly lower survival rates (Wang *et al.*, 2003).

In the present study, PrxII-deficient mouse embryonic fibroblasts (MEFs) were used to investigate a possible compensatory mechanism between PrxI and PrxII, given that PrxI and PrxII are highly homologous proteins. In brief, PrxII$^{-/-}$ MEFs was transfected with PrxI over-expression vector and analyzed for intracellular ROS levels, cell viability, and cell death as compared to PrxII$^{+/+}$ MEFs. This study demonstrated that over-expression of PrxI conferred increased resistance to oxidative stress to PrxII$^{-/-}$ MEFs.

MATERIALS AND METHODS

Cell culture and transient transfection

For all studies, primary PrxII$^{+/+}$ and PrxII-deficient (PrxII$^{-/-}$) MEFs were used as described previously (Lee *et al.*, 2003). MEFs were prepared from day-13.5 embryos. The whole embryo was minced and dispersed in 0.25% trypsin and incubated for 2 hours at 4°C. 1×10^6 cells were then plated in 10-cm-diameter plates. MEFs were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% FBS (Fetal Bovine Serum) in the presence or absence of antioxidant, 5 mM *N*-acetyl-L-cysteine (NAC), and incubated in an atmosphere of 5% CO_2 at 37°C. C-terminal His tagged PrxI over-expression vector, pcDNA3.1-His-C+PrxI, was constructed by using specific primers (Supplementary Table 1). The transfection was conducted according to the methods provided by the manufacturer. MEF cells were plated at a density of 2×10^5 cells/ml in 6-well plates and allowed to recover for 24 hours. They were then incubated with 2 μg pcDNA3.1-His C+PrxI vector or empty vector (Mock transfection) and 6 μl of FUGENE HD reagent (Roche) in opti-MEM (Invitrogen) for 48 hours. Transfected cells were cultured in 2 ml of fresh DMEM containing 10% FBS for 24 hours. The efficacy and reproducibility of transfection were confirmed by western blot analysis and Real-Time PCR.

Quantitative real-time PCR and reverse-transcriptase PCR

The total RNA was extracted from various MEFs by using Trizol reagent (Invitrogen) according to the manufacturer's protocol. cDNA was synthesized by reverse-transcribing with 2 μg of total RNA, using a Moloney Murine Leukemia Virus reverse transcriptase (Invitrogen). The RT-PCR procedure was performed in a 50 μl volume containing 1 μl of the first-strand cDNA. The resulting cDNA was used as a template to amplify the Prx family transcripts with forward and reverse primers (Supplement Table 1). The expression levels of the Prx family were measured by real-time PCR (7300 Real-Time PCR System, Applied Biosystems, USA) using mouse Prx family-specific primers. The primer sequences for the Prx family are shown in Table S1. cDNA samples were mixed with primers and SYBR Master Mix (Applied Biosystems) in a total volume of 50 μl. The PCRs were performed in 96-well optical reaction plates (Applied Biosystems). The data from the quantitative real-time PCR analysis were normalized to the CT values of GAPDH mRNA isolated from the same cell line. Obtained data were analyzed by 7300 System SDS software version 1.3 (Applied Biosystems). Independent PCR experiments were performed in triplicate.

Treatment of cells with H_2O_2 and UV light, and ROS measurement

To generate ROS in MEF cells in culture, 30% H_2O_2 (Sigma-Aldrich Corporation, #216763), was used. To determine the most appropriate experimental conditions, the intracellular ROS levels were evaluated using three different concentrations of H_2O_2 in PBS buffer. MEF cells were treated by H_2O_2 for about 15 minutes and washed with PBS buffer. UV light treatments were carried out in PBS. UV light (8-watt 254 nm) doses in the range 0-100 J/m^2 were produced by using a Spectrolinker XL-1000 UV Crosslinker (Spectronics Corporation). Following exposure, the medium was replaced with fresh DMEM medium. Detection of total intracellular ROS was performed by the 2,7-dichlorofluorescein diacetate (DCF-DA, Molecular Probes, Eugene, USA) staining method. After exposure to H_2O_2 or UV light, MEF cells were incubated in pre-warmed PBS buffer containing 5 μM DCF-DA for 30 minutes at 37°C and washed twice with PBS buffer. The fluorescence intensity was quantified at 485 nm/535 nm excitation/emission wavelengths using a VICTOR3 plate reader (Perkin Elmer, Waltham, USA).

Cell viability assay

Cell viability was determined using a Cell Counting Kit-8 (Dojindo, Japan). Briefly, PrxII$^{+/+}$ MEFs and PrxII$^{-/-}$ MEFs transfected with either PrxI (over-expressed) or control vector were seeded in a 96-well plate and, after 6 hours of treatment with the different serum-free media containing different concentrations of H_2O_2, 10 μl of a solution of CCK-8 were added to each well and the plates were incubated at 37°C for 2 hours in a CO_2 incubator with protection from light. The O.D. of each well at 450 nm was then measured in an ELISA microplate reader.

Western blot and antibodies

MEF cells were lysed with lysis buffer (0.1% Triton X-100 in PBS) containing a protease inhibitor cocktail (P7005; BIOSESANG, Korea). Lysates were incubated for 30 minutes on ice before centrifugation at 13,000 rpm for 5 minutes at 4°C. Proteins in the supernatant were denatured by boiling for 5 minutes in SDS sample buffer. Proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to a PVDF membrane (Millipore, Bedford, MA, USA). Following transfer, equal loading of protein was verified by Coomassie Blue staining. The membranes were blocked with 5% skim milk in Tris-buffered saline with Tween 20 (TBST) and incubated with the indicated primary antibodies, polyclonal anti-PrxI (1:5000, LabFrontier, Seoul, Korea), anti-PrxII (1:2000, LabFrontier, Seoul, Korea), anti-Prx-SO_3 (1:2000, Abcam, Cambridge, UK), polyclonal anti-SAPK/JNK, anti-phospho-SAPK/JNK, anti-ERK, anti-phospho-ERK, anti-p38, anti-phospho-p38 (1:1000, Cell Signaling Technology, Inc.) and monoclonal β-actin (1:5000, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Bound antibodies were visualized with HRP-conjugated secondary antibodies with the use of enhanced chemiluminescence (ECL) (Elpis Biotech, Korea).

Flow cytometry analysis

To investigate the cell cycle, MEF cells treated with H_2O_2 or a control were harvested, fixed with cold 70% EtOH, and stored at 4°C. Fixed cells were resuspended in 500 μl of PI

(propidium iodide) staining solution (50 μg/ml of ribonuclease A, 50 μg/ml PI, and 0.05% Triton X-100 in double-distilled H_2O) and then incubated for 20 minutes at 37°C. These cells were analyzed by a FACS-Calibur system (Becton Dickinson, Mountain View, CA, USA).

Statistical analysis

The results are presented as mean ± standard deviation. Means, standard errors and *p* values were calculated using Student's *t*-test. $p<0.05$ was considered statistically significant.

RESULTS

Over-expression of PrxI does not affect the expression of other Prx family members in PrxII$^{-/-}$ MEFs

To increase the expression level of PrxI, an over-expression vector was constructed in which PrxI transcription is driven by a cytomegalovirus (CMV) promoter. This construct was transiently transfected into PrxII$^{-/-}$ MEFs and the mRNA and protein expression levels of PrxI were analyzed. As shown in Fig. 1A and B, the mRNA level of PrxI was increased by transient transfection. Nevertheless, significant differences in the expression levels of other Prx family members were not detected between mock-transfected and PrxI-over-expressing PrxII$^{-/-}$ MEFs. Moreover, the attenuated mRNA expression level of PrxV observed in PrxII$^{-/-}$ MEFs (compared to PrxII$^{+/+}$ MEFs) was not altered by over-expression of PrxI (Fig. 1A). Western blot analysis was performed to confirm the expected elevated level of PrxI protein in PrxII$^{-/-}$ MEFs (Fig. 1C and D). Thus, PrxII$^{-/-}$ MEFs transiently over-expressing PrxI were used in the next experiments.

Over-expression of PrxI reverses the cellular defects due to PrxII deficiency

Previous studies showed that PrxII$^{-/-}$ MEFs exhibit accelerated senescence (Han *et al.*, 2005) and are more sensitive to oxidative damage compared to PrxII$^{+/+}$ MEFs (Supplementary Fig. 2). To determine whether over-expression of PrxI can compensate for the cellular defects associated with PrxII deficiency, PrxI over-expression vector was transiently transfected into PrxII$^{-/-}$ MEFs. As a result, these cells displayed an increased cell proliferation rate (Fig. 2A) and reduced intracellular ROS levels in response to different oxidative conditions (i.e., exposure to H_2O_2 or ultraviolet light; Fig. 2C). Moreover, PrxI-over-expressing PrxII$^{-/-}$ MEFs treated with H_2O_2 or UV also showed increased cell viability (Fig. 2D) and decreased H_2O_2-induced sub-G1 rate compared with mock-transfected PrxII$^{-/-}$ MEFs (Fig. 2B). These data indicate that over-expression of PrxI rescues the defects which are induced by oxidative damage in PrxII$^{-/-}$ MEFs.

Although the PrxI protein level was increased by approximately twofold in transfected PrxII$^{-/-}$ MEFs (Fig. 1D), not all of the phenotypes were corrected to the wild-type level (Fig. 2). These results suggest that over-expression of PrxI in PrxII$^{-/-}$ MEFs only partially corrects the PrxII-deficiency-derived cellular defects. Therefore, further experiments were performed to determine whether over-expressed PrxI directly regulates ROS levels to effect rescue of the PrxII-deficiency-induced defects.

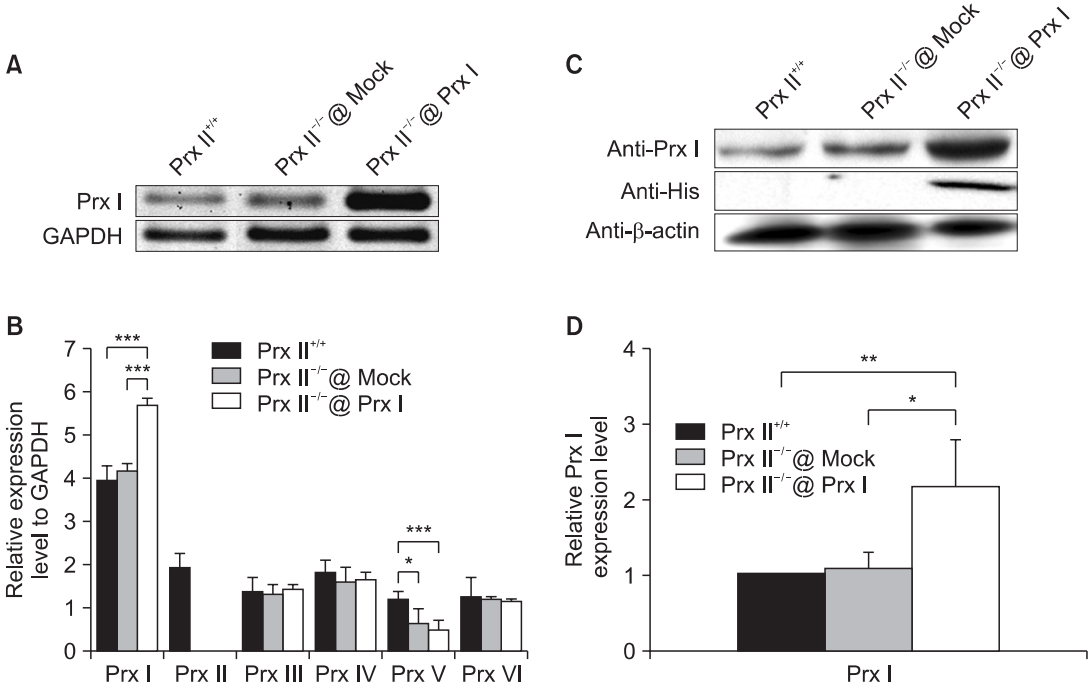

Fig. 1. Expression level of PrxI in transiently transfected cells. RT-PCR analysis. (A) and real-time PCR (B) were carried out to study the mRNA expression of PrxI. (C) Western blotting was performed by using PrxI-specific and His-specific antibodies. (D) PrxI protein level was quantified by densitometry. The quantitative results (B, D) shown are the mean ± SD of three independent experiments. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ (Student's *t*-test).

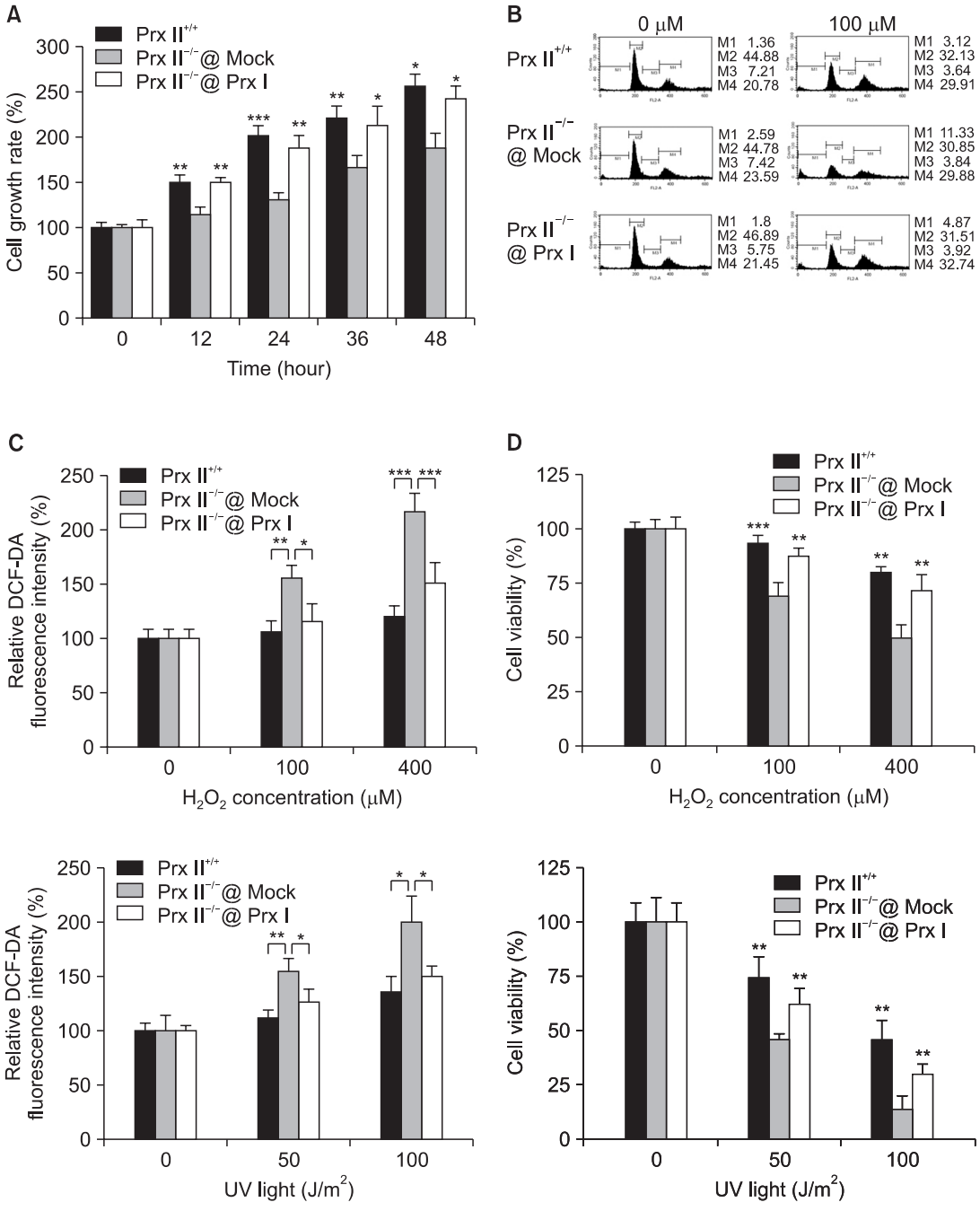

Fig. 2. Characterization of PrxI over-expressed in PrxII$^{-/-}$ MEF cells. (A) Measurement of cell growth rate every 12 hours after plating on dishes. (B) Cell-cycle analysis. After treatment with H_2O_2, MEFs were fixed in a 70% cold EtOH and incubated with 50 μg/ml of PI. PI-stained cells were analyzed by flow cytometry. M1=sub-G1, M2=G1, M3=S, and M4=G2/M phases. (C) Detection of intracellular ROS levels by DCF-DA staining. MEF cells were treated with various concentrations (100, 200 and 400 μM) of H_2O_2 or intensities (50, 100 J/m^2) of UV light. (D) Cell viability assay. The cell viability of MEFs was measured by using a CCK-8 kit with various concentrations of H_2O_2. The quantitative results shown are the mean ± SD of three independent experiments. *p<0.05, **p<0.01, and ***p<0.001: significant as compare to Mock transfected PrxII$^{-/-}$ MEFs (Student's t-test).

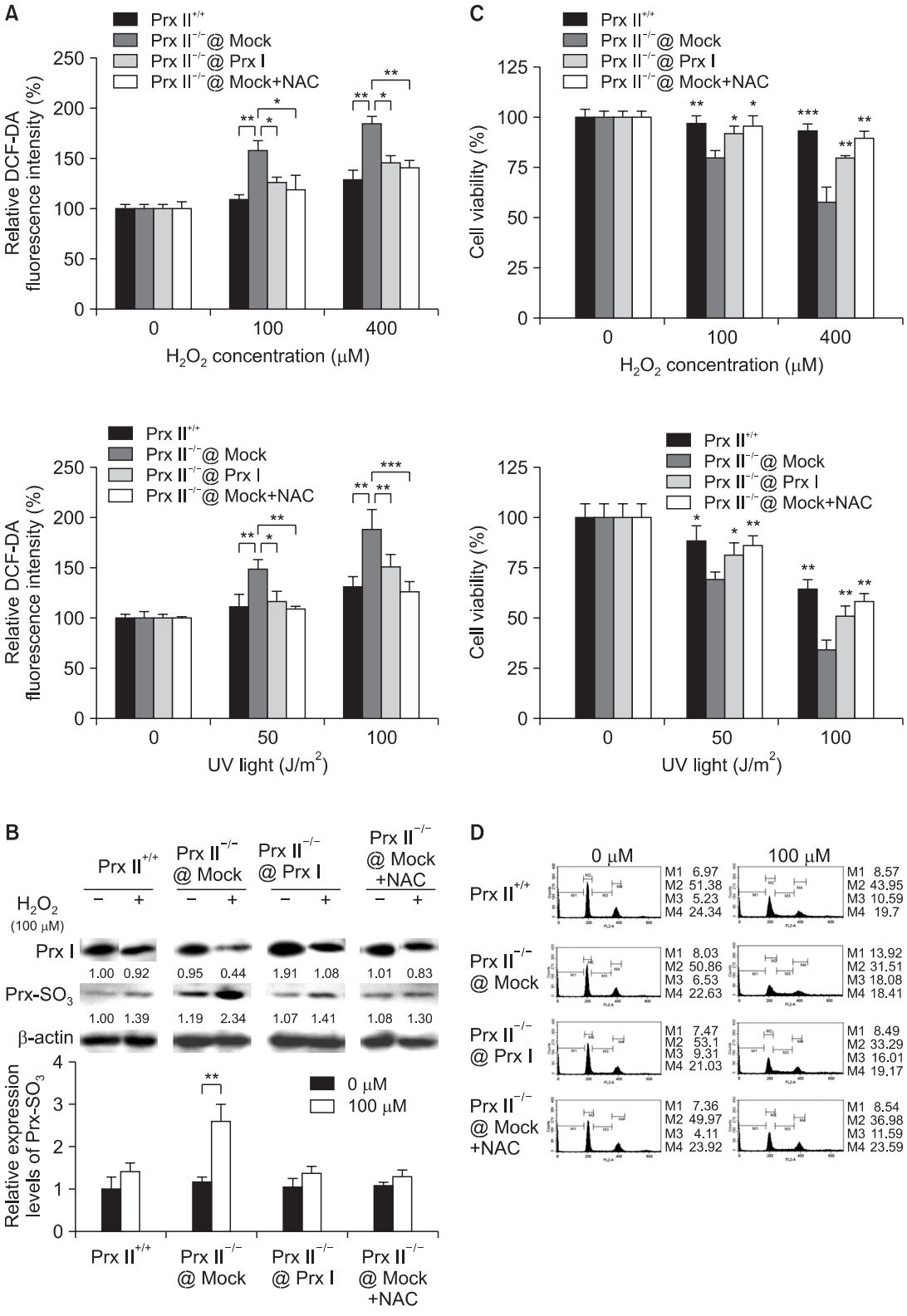

Fig. 3. Over-expression of PrxI protects against oxidative damage through regulation of ROS levels. (A) Detection of intracellular ROS levels by DCF-DA staining. H_2O_2- or UV-light-treated MEF cells were incubated with DCF-DA reagent for 30 minutes. To eliminate the intracellular H_2O_2, 5 mM *N*-acetyl-L-cysteine (NAC) was added to PrxII$^{-/-}$ MEFs. (B) Detection of oxidized PrxI by using Prx-SO_3 antibody. The graph shows the mean ± SD of the three separate experiments. (C) Cell viability was measured by using a CCK-8 kit. MEF cells were treated with H_2O_2 or UV light, and mitochondrial activity was determined by detection of fluorescence intensity. (D) H_2O_2-induced cell death was determined by using PI staining. The quantitative results shown are the mean ± SD of three independent experiments. **p*<0.05, ***p*<0.01, and ****p*<0.001: significant as compare to Mock transfected PrxII$^{-/-}$ MEFs (Student's *t*-test).

Over-expression of PrxI protects against PrxII-deficiency-associated oxidative damage through regulation of ROS levels

PrxI and PrxII are antioxidant enzymes containing essential catalytic cysteine residues that use thioredoxin to scavenge H_2O_2 (Rhee *et al.*, 2005a). The above data (Fig. 2) showed that over-expression of PrxI at least partially rescued the cellular defects associated with deficiency of PrxII. To evaluate whether over-expression of PrxI can actually regulate intracellular ROS levels in PrxII-deficient MEFs, ROS levels were compared in PrxI-over-expressing PrxII$^{-/-}$ MEFs versus *N*-acetyl-L-cysteine (NAC)-treated PrxII$^{-/-}$ MEFs. NAC is a well-known antioxidants, meaning it protects cells from being damaged by ROS. Because, the cysteine residues of the active sites of Prx under oxidative stress condition are more readily oxidized to sulfenic acid (Cys-SOH) than are other cysteines because of their molecular environment. The unstable sulfenic acid undergoes further hyperoxidation to sulfinic (Cys-SO_2H) and sulfonic (Cys-SO_3H) acids. The over-oxidative forms (sulfinic and sulfonic acids) of Prx protein are inactive (Rhee *et al.*, 2005a). These inactive forms were detected by anti-Prx-SO_3 antibody.

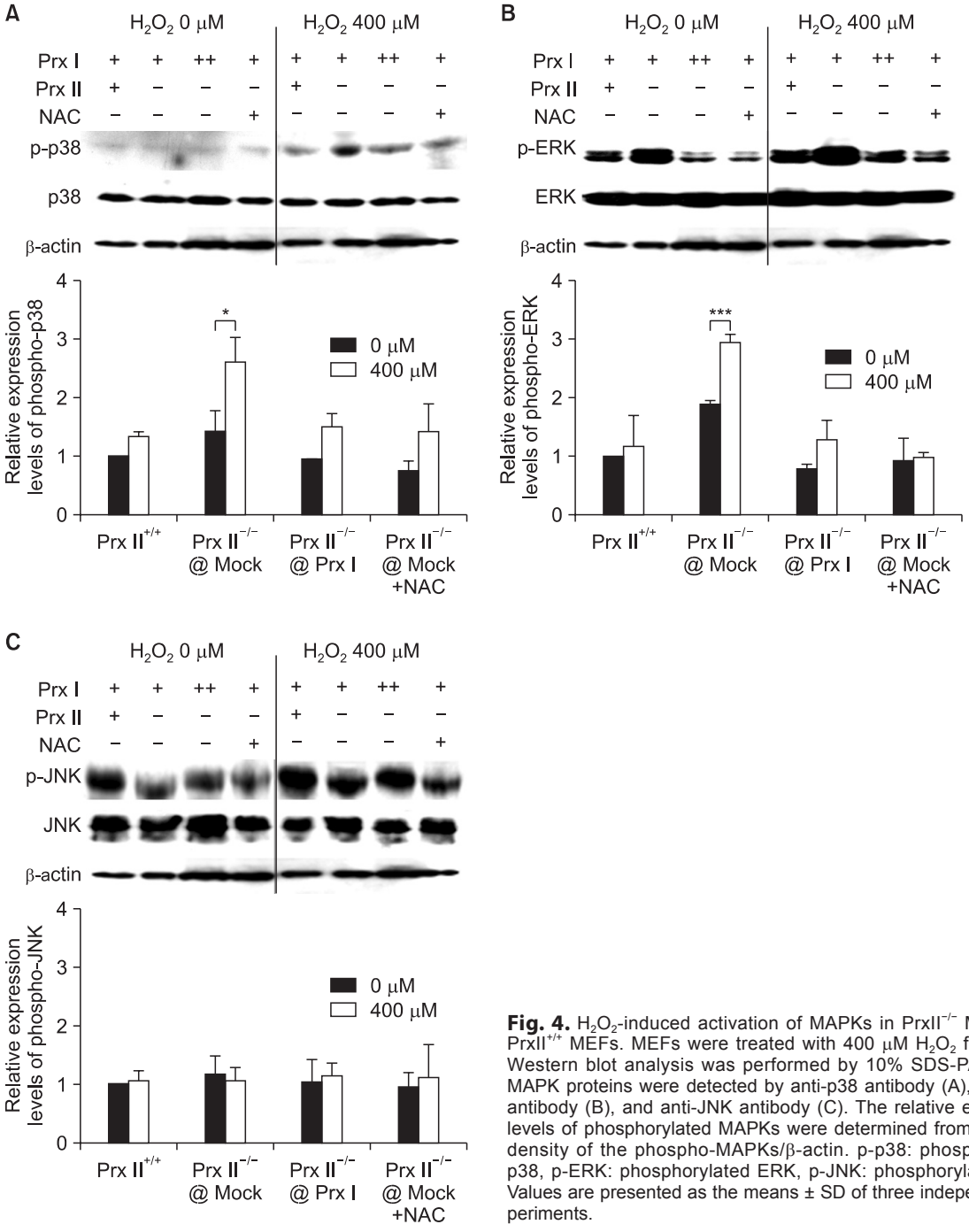

Fig. 4. H_2O_2-induced activation of MAPKs in PrxII$^{-/-}$ MEFs and PrxII$^{+/+}$ MEFs. MEFs were treated with 400 μM H_2O_2 for 1 hour. Western blot analysis was performed by 10% SDS-PAGE, and MAPK proteins were detected by anti-p38 antibody (A), anti-ERK antibody (B), and anti-JNK antibody (C). The relative expression levels of phosphorylated MAPKs were determined from the band density of the phospho-MAPKs/β-actin. p-p38: phosphorylated p38, p-ERK: phosphorylated ERK, p-JNK: phosphorylated JNK. Values are presented as the means ± SD of three independent experiments.

As shown in Fig. 3A, PrxII-deficient MEFs showed high intracellular ROS levels after exposure to H_2O_2 or UV light. Both PrxI-over-expressing and NAC-treated PrxII$^{-/-}$ MEFs had lower ROS levels than mock-transfected PrxII$^{-/-}$ MEFs. Also, the level of Prx-SO_3 was substantially enhanced in PrxII-deficient MEFs as compared with PrxII$^{+/+}$ MEFs (Fig. 3B), which confirms that PrxII$^{-/-}$ MEFs have greater intracellular ROS levels than do wild-type cells. The high levels of Prx-SO_3, which were induced by oxidative conditions, were attenuated in both PrxI-over-expressing and NAC-treated PrxII$^{-/-}$ MEFs. These results indicate that over-expressed PrxI may have an essential role in scavenging intracellular ROS produced under oxidative conditions.

To examine the cell growth and the cell-death rate, PrxII$^{+/+}$ and PrxII-deficient MEFs were exposed to various concentrations of H_2O_2 for 6 hours or to various intensities of UV light. Cell viability was determined by CCK-8 assay. Fig. 3C shows the cell viability of the H_2O_2- or UV light-treated samples. The cell viability of PrxI-over-expressing and NAC-treated PrxII$^{-/-}$ MEFs was increased under both of the oxidative-stress conditions compared to that of mock-transfected PrxII$^{-/-}$ MEFs.

Additionally, cell-cycle analysis was performed by flow cytometry to evaluate the effect of intracellular ROS levels on the cell cycle of PrxII-deficient MEFs. As shown in Fig. 3D, after exposure to 100 μM H_2O_2 for 6 hours, 13.9% of PrxII$^{-/-}$ MEF cells were dead, compared to 8.6% of PrxII$^{+/+}$. PrxI-over-expressing and NAC-treated PrxII$^{-/-}$ MEFs, which exhibited lower ROS levels than PrxII$^{-/-}$ MEFs, showed cell-death rates of 8.5% and 8.5%, respectively.

These results indicate that over-expression of PrxI abrogates the effects of PrxII$^{-/-}$-induced oxidative damage through directly regulating the ROS levels in PrxII$^{-/-}$ MEFs.

Activation of the MAPK pathway is modulated by over-expressed PrxI in PrxII$^{-/-}$ MEFs

Previous reports indicated that mitogen-activated protein kinase (MAPK) signaling is altered by various stimuli in PrxI- or PrxII-deficient MEFs (Han *et al.*, 2005; Ma *et al.*, 2009). Therefore, we hypothesized that expressions of PrxI and PrxII may effect on MAPK signaling. To test this hypothesis, we then determined to whether the over-expression of PrxI can regulate the activation of MAPKs induced by PrxII-deficiency through their ROS regulartory function. PrxII$^{-/-}$ MEFs were stimulated with exposure of H_2O_2 to encourage the activation of MAPKs. As shown in Fig. 4, p38 phosphorylation (Fig. 4A) was enhanced by H_2O_2 stimulation in PrxII$^{-/-}$ MEFs compared to PrxII$^{+/+}$ MEFs, whereas the total p38 expression level (phosphorylated and non-phosphorylated) remained similar between the two groups. Activation of ERKs (Fig. 4B) was also elevated in PrxII$^{-/-}$ MEFs, and total ERK levels were not changed under H_2O_2 exposure. And also, PrxI-over-expression and NAC treatment reduced JNK phosphorylation in PrxII$^{-/-}$ MEFs under both oxidative- and non-oxidative conditions. However, there were no statistically significant changes of JNK phosphorylation (Fig. 4C). Thus, these results indicate that PrxII-deficiency in MEFs is associated with increased activation of p38, ERK, but not JNK. Furthermore, H_2O_2-induced activation of both p38 and ERK were attenuated in both PrxI-over-expressing and NAC-treated PrxII$^{-/-}$ MEFs compared to PrxII$^{+/+}$ MEFs. These results also suggest that p38 and ERK MAPK-signaling, but not JNK-signaling, is associated with the reduction in intracellular ROS levels in PrxI-over-expressing PrxII$^{-/-}$ MEFs.

DISCUSSION

Previous studies showed that PrxII$^{-/-}$ MEFs are more sensitive to oxidative damage than are PrxII$^{+/+}$ MEFs. Especially, Lee and his colleagues discovered that PrxII knockout mice exhibit hemolytic anemia, Heinz body formation in red blood cells (Lee *et al.*, 2003), and have increased thymus size (Moon *et al.*, 2004). Moreover, in the present study, we found that PrxII$^{-/-}$ MEFs have low cell viability, increased levels of ROS, and a higher percentage of cells in sub-G1 phase than PrxII$^{+/+}$ MEFs. These phenomena may be due to increased intracellular ROS levels (Supplementary Fig. 2).

PrxI and PrxII play critical roles in the elimination of H_2O_2, an important ROS (Knoops *et al.*, 1999). PrxI and PrxII are a major typical 2-Cys Prx-family members, sharing more than 90% amino-acid sequence homology (Rhee *et al.*, 2001; Wood *et al.*, 2003). Proteins belonging to the typical 2-Cys Prx group including the *N*-terminal peroxidatic Cys residue go through a cycle of peroxide-dependent oxidation to sulfenic acid and thiol-dependent reduction during H_2O_2 catalysis. Typical 2-Cys Prxs are obligate homodimers containing two redox-active cysteines (Rhee *et al.*, 2005a). However, PrxI and PrxII comprise unique functions and regulatory mechanisms. PrxI involves additional cysteine (Cys^{83}) at the putative dimer-dimer interface, which is absent in PrxII. Lee and his colleague suggest that the role of Cys^{83} in regulating the peroxidase and chaperone activities of PrxI, because the redox status of Cys^{83} might influence the oligomeric structure and consequently the functions of PrxI. PrxI is more efficient as a molecular chaperone, whereas PrxII is better suited as a peroxidase enzyme (Lee *et al.*, 2007). Although PrxI and PrxII have been studied independently in various cell lines and animal models, there is only little information available for compensatory relationships between PrxI and PrxII.

We showed here that transient over-expression of PrxI is able to at least partially compensate for the loss of PrxII in PrxII$^{-/-}$ MEFs. A previous study showed that PrxII$^{-/-}$ MEFs present a reduced level of expression of PrxV compared to PrxII$^{+/+}$ MEFs (Han *et al.*, 2005). We determined the expression levels of other Prx-family members, including PrxV, in PrxI over-expressing PrxII$^{-/-}$ MEFs and found no significant expression changes in the other Prxs, with the exception of PrxV, which was lower. However, the mechanism leading to reduced expression of PrxV in PrxII$^{-/-}$ MEFs has yet to be elucidated. This result suggests that over-expression of PrxI had no effect on the observed pattern of expression of Prx-family members in PrxII$^{-/-}$ MEFs.

We further showed that over-expression of PrxI plays a protective role by reducing the levels of oxidative damage induced by intracellular ROS in MEFs. Two oxidative conditions were used in this study: exposure of MEFs to varying concentration of H_2O_2 and exposure to UV light. It is well known that both of these treatments generate intracellular ROS. Here, the levels of intracellular ROS were decreased by over-expression of PrxI in PrxII$^{-/-}$ MEFs. These protective effects may have been mediated by the catalytic cycle of PrxI.

The main targets of PrxI and PrxII in the proliferation and apoptotic pathways of the cell are not yet clear. However, recent studies identified PrxI and PrxII as important factors of

the MAPK-associated signaling pathway in MEFs. Cisplatin, a chemotherapy drug used to treat various types of cancers through induction of ROS, regulated the three major MAPKs (JNK, ERK and p38) in PrxI$^{-/-}$ MEFs (Ma *et al.*, 2009). Furthermore, another study showed that the observed increase in the rate of cellular senescence in PrxII$^{-/-}$ MEFs is mediated by p38 and ERK activation (Han *et al.*, 2005). These results emphasize that PrxI and PrxII are important factors in JNK, ERK and p38 MAPK signaling pathways. Therefore, we determined whether over-expressed PrxI might modulate the activation of components of these MAPK pathways in PrxII$^{-/-}$ MEFs through regulation of intracellular ROS levels. The levels of phosphorylated p38 and ERK, but not JNK, were increased in PrxII$^{-/-}$ MEFs by exposure to H_2O_2, and the increased activation of p38 and ERK was attenuated by over-expressing PrxI and by treatment with NAC. The findings suggest that over-expressed PrxI can modulate the activation of MAPK pathway constituents through regulation of intracellular ROS levels in PrxII$^{-/-}$ MEFs.

In conclusion, we showed that over-expressed PrxI can at least partially compensate for the loss of PrxII in PrxII$^{-/-}$ MEFs, as evidenced by rescue of a variety of cellular defects. Both catalytic and non-catalytic activities of PrxI are important factors in cell proliferation and cell death under oxidative stress. Previous research showed that PrxII-deficient mice have defects in hematic cells, including shorter life span of erythrocytes, anemia, and low hematocrit values. Therefore, this paper theorized that over-expressed PrxI may rescue the PrxII-deficiency-induced defects in hematic cells.

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