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Differential Expression and Function of Peroxiredoxin 1 and Peroxiredoxin 6 in Cancerous MCF-7 and Noncancerous MCF-10A Breast Epithelial Cells

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Peroxiredoxins are thiol-specific antioxidant proteins whose expression is elevated in several cancers. We compared the expression and function of Prdx1 and Prdx6 between the MCF-7 mammary adenocarcinoma cell line and the noncancerous MCF-10A cell line. We found elevated Prdx1 expression in MCF-7 cells and comparable expression of Prdx6. Suppression of Prdx1 and/or Prdx6 resulted in a modest increase in peroxide-induced cytotoxicity of MCF-7 cells, and a dramatic increase in MCF-10A cytotoxicity with and without hydrogen peroxide treatment. Our data confirm a cytoprotective role for peroxiredoxins and suggest a synergistic role for Prdx1 and Prdx6 in MCF-10A cells.

Keywords: Breast cancers; Gene expression; Molecular biology

INTRODUCTION

Cancer cells possess many properties that are characteristic of oxidative stress, including increased levels of highly reactive oxygen species (ROS) such as hydrogen peroxide and superoxide. Elevated ROS have been implicated in both cancer initiation, through induction of DNA damage, as well as cancer promotion, likely through aberrant redox signaling (1, 2). While high ROS levels are cytotoxic to normal cells, cancer cells are able to tolerate increased oxidative stress and evade cell death. This ability may involve increased activity of cellular antioxidants, limiting ROS to nonlethal levels, and/or inactivation of apoptotic pathways. Regardless of the precise mechanism, it is clear that the oxidant/antioxidant balance in cancer cells is shifted to allow increased proliferation and cell survival (3).

Peroxiredoxins (Prdxs) are a family of evolutionarily conserved antioxidant proteins that reduce a wide variety of cellular substrates, including aqueous and lipid peroxides (4–6). Unlike other antioxidant proteins such as superoxide dismutase and catalase, peroxiredoxins do not rely on the use of cofactors for their enzymatic activity, but instead use internal active site cysteine residues for target reduction. There are six members of the mammalian peroxiredoxin family, which can be subdivided into three classes: the 2-Cys Prdxs (Prdx1–4), the atypical 2-Cys Prdx (Prdx5), and the 1-Cys Prdx (Prdx6). Besides serving a general protective role for the cell, peroxiredoxins are also important in many redox-sensitive cellular activities including the regulation of cell signaling, cell proliferation, and apoptosis (5, 7, 8). Over the past several years, studies have found peroxiredoxins to be overexpressed in several types of cancer (9–14), and there is mounting evidence that peroxiredoxins play a role in carcinogenesis (15–17). Given these findings, it is likely that overexpression of peroxiredoxins in cancer cells is a cellular adaptation to elevated oxidative stress, providing a protective function for cancer cell survival.

Several studies have reported overexpression of Prdx1 and Prdx6 in breast cancer (9, 11, 14, 18–20); however, the functional significance of this upregulation is not known. Prdx1, first isolated as a stress-inducible macrophage protein (21), is found in nearly all mammalian tissues and localizes to the cytoplasm and nucleus of cells (4). Suppression of Prdx1 in various cell types increases susceptibility to oxidant-induced apoptosis while overexpression confers protection (21–28). Prdx6 possesses only one conserved cysteine residue and is functionally unique, in that it is the only peroxiredoxin capable of reducing lipid peroxides and additionally possesses phospholipase A2 activity (29). The protein is expressed primarily in epithelial tissue with predominant localization to the cytoplasm. Studies in mice and various cell lines have demonstrated that Prdx6 overexpression reduces cellular H$_2$O$_2$ levels and oxidative stress-induced apoptosis (29–33), while loss of Prdx6 increases oxidative stress-induced cell death (34–38). On the basis of the fact that Prdx1 and Prdx6 are two functionally and structurally unique peroxiredoxins overexpressed in breast cancer, we sought to compare their levels in noncancerous MCF-10A (MCF, Michigan Cancer Foundation) cells and the cancerous MCF-7 mammary adenocarcinoma cell line, and examine their cytoprotective functions in these two lines.
METHODS

Cell culture
MCF-7 cells were cultured in ATCC-formulated Eagle's minimum essential medium containing bovine insulin (0.01 mg/mL) and 10% fetal bovine serum. MCF-10A cells were cultured in MEBM medium, supplemented with BPE (13 mg/mL), hydrocortisone (0.5 mg/mL), hEGF (10 µg/mL), and insulin (5 mg/mL). Both cell types were cultured at 37°C in a 5% CO₂ atmosphere.

Hydrogen peroxide treatment
The source of hydrogen peroxide for cell treatments was a 30% stock solution (Sigma), which was diluted into media and added immediately to cells at the desired final concentrations.

Suppression of Prdx1 and Prdx6 by siRNA
MCF-7 and MCF-10A cells were seeded in a 96-well plate at a density of approximately 15% in 100 µL media volume. Twenty-four hours after plating, cells were transfected with 33 nmol of siRNA using the Lipofectamine 2000 reagent (Invitrogen), according to manufacturer’s recommendations for siRNA transfections. Briefly, 33 nmol of siRNA were mixed with 0.5 µL Lipofectamine 2000 reagent and 22.5 µL Opti-MEM I serum-free media (Invitrogen), allowed to precipitate at room temperature for 25 min, and subsequently added to wells. Validated Silencer Select siRNAs (Applied Biosystems) were used for all siRNA experiments, and included human Prdx1 siRNA ID# s10007, human Prdx6 siRNA ID# s18429, and Negative Control siRNA #1 as a nonspecific siRNA control. A vehicle control containing no siRNA was also used in every experiment. After transfection, cells were cultured under normal growth conditions for 48 hr, determined to be the time of optimal suppression. All treatments were performed in triplicate wells, and experiments were repeated at least twice. For protein analysis, cells were seeded in T-25 flasks at a density of approximately 60%, and transfected with 33 nM siRNA using 0.5 mL Lipofectamine 2000 reagent and 0.5 mL Opti-MEM media, according to manufacturer’s suggestions.

Real-time PCR
To measure mRNA levels, transfected cells were washed in cold PBS and lysed in the wells using the TaqMan Gene Expression Cells-to-C<sub>T</sub> Kit (Applied Biosystems), according to manufacturer’s recommendations. Lysates were immediately used for reverse transcription reactions using the TaqMan Gene Expression Cells-to-C<sub>T</sub> Kit. Twenty-microliter real-time PCR reactions were performed in triplicate for each reverse transcription reaction with each target gene using the TaqMan Gene Expression Cells-to-C<sub>T</sub> Kit reagents and protocol. Reactions included 1 µL of the appropriate human TaqMan assay: Prdx1 (Hs00602020_mH), Prdx6 (Hs00705355_s1), or β-actin (ACTHs00242273_ml). To ensure that PCR amplification was not due to genomic DNA contamination, mock reverse transcription reactions containing no reverse transcriptase were conducted for each experiment and used in parallel PCR reactions with each target primer set. In all cases, reactions lacking reverse transcriptase amplified no product, or resulted in cycle threshold (Ct) values beyond 35 and at least seven Cts higher than the positive reactions, representing negligible genomic DNA contamination. Relative expression was calculated using the ΔΔC<sub>T</sub> method (39), with each target gene normalized against β-actin levels for the same sample. For each experiment, an appropriate sample was selected as calibrator for relative quantification.

Western blotting
MCF-7 and MCF-10A cells seeded in T25 flasks were used for protein analysis. At the time of analysis, cells were rinsed with 1 mL PBS and lysed in MPER (Mammalian Protein Extraction Reagent; Thermo Scientific), according to product suggestions. Protein was quantified using the Coomassie Blue Protein Assay Reagent (BioRad), and lysates were separated on a 12% Tris-HCl gel (BioRad) and electrophoretically transferred onto a PVDF membrane. Blots were blocked, incubated with primary antibodies for Prdx1 (ab59538, Abcam), Prdx6 (ab16947, Abcam), or β-actin (A5441, Sigma), and subsequently processed with the appropriate secondary antibody and chemiluminescent CDP-Star Reagent (GE Healthcare). Blots were imaged with X-OMAT film (Kodak) and the net band intensity was quantified using Kodak Gel Imaging software. Relative Prdx1 and Prdx6 protein expression was normalized against β-actin levels.

Measurement of cell viability
Cell viability was determined using an MTT assay. Cells were seeded at a density of 35%–50% in 96-well plates and treated in replicates of four. After treatment incubation, cells were rinsed with phenol red-free medium and incubated with freshly prepared 0.5 mg/mL MTT (diluted in phenol red-free medium) for 1 hr at 37°C. This solution was replaced with 50 µL acidic isopropanol and then cells were incubated for 30 min at 37°C. The absorbance of the solubilized product was measured at 590 nm using the absorbance of cell-free wells for background subtraction.

Measurement of cytotoxicity
Cytotoxicity was determined by the indirect measurement of lactate dehydrogenase (LDH) activity using the CytoTox 96 assay (Promega). In order to assay for enzymatic activity, cells were seeded at a density of 35%–50% in 96-well plates and were either untransfected or transfected with 33 nM of siRNA as described above and cultured for 48 hr. Each siRNA treatment was conducted in replicates of six to eight for cytotoxicity measurements. Cells subsequently were treated for 24 hr with either 0, 0.05, or 0.5 mM H₂O₂. To measure released LDH, cell medium was removed for analysis. Intact adherent cells were lysed in situ with Promega CytoTox 96 lysis buffer (1 × in medium) for 45 min at 37°C and 5% CO₂, and 30–40 µL of lysate and media were assayed (separately), using an equal volume of substrate mix, and processed according to manufacturer’s recommendations. Absorbance was measured at 490 nm using a BioRad Benchmark microplate reader (BioRad). The absorbance of media blanks (with no
cells) was subtracted from each value, and relative cytotoxicity was calculated as released LDH/cellular LDH.

Measurement of apoptosis
The Caspase-Glo 3/7 Assay (Promega) was used to measure apoptosis in MCF-10A cell lysates, according to product recommendations. Briefly, 10–20 μL of lysate generated for cytotoxicity experiments (above) was aliquoted into a white-bottom 96-well plate and mixed with an equal volume of caspase 3/7 substrate, followed by incubation in the dark for 30 min. Luminescence levels were read in an Infinite F200 plate reader (Tecan). To account for well:well variability, these values were normalized against cellular LDH levels determined as described above) in each sample.

Statistical analysis
The means of individual treatment groups in each quantitative experiment were statistically compared using a two-tailed student’s t-test, assuming equal variances. Throughout the manuscript, the following designations are used: *p < .05; **p < .01; ***p < .005; ****p < .001.

RESULTS
Previous studies have reported increased peroxide-induced toxicity in MCF-10A cells, as compared with MCF-7 cells (19). To determine the susceptibility of these cells to hydrogen peroxide in our system, we treated cells with four different concentrations of hydrogen peroxide and measured cell viability after 4, 8, and 24 hr using an MTT assay. The results are shown in Figures 1(a) and (b). As shown in Figure 1(a), the concentrations used produced a dose-dependent and time-dependent decrease in viable cell number in MCF-7 cells. A modest statistically significant decrease was observed after 8 and 24 hr of treatment with the highest dose (0.5 mM), resulting in an approximately 20% reduction as compared with untreated cells. In contrast, the response to hydrogen peroxide was more dramatic in MCF-10A cells [Figure 1(b)]. Treatment with 0.25 mM H2O2 for 24 hr reduced cell viability by approximately 25%, while treatment with 0.5 mM H2O2 for 24 hr led to a 50% reduction in cell viability. These data suggest that MCF-10A cells exhibit an increased susceptibility to peroxide-induced toxicity in our system, and validate the use of this concentration range for our studies.

To confirm the MTT results, we also measured the ratio of released LDH (lactate dehydrogenase) to cellular LDH in each cell line 24 hr after treatment. LDH is released from cells upon cell death and thus can be compared with cellular LDH levels as an indirect measure of cytotoxicity. Since the overall LDH levels may vary between the cell types, the peroxide-induced cytotoxicity was calculated as a relative value compared with that observed in untreated cells for each line. As shown in Figure 1(c), relative cytotoxicity increased by less than 1.5 fold in treated MCF-7 cells, but approximately 3.5 fold in treated MCF-10A cells. This data confirm the MTT results, demonstrating a decreased resistance to peroxide-induced toxicity in the normal cells.

Morphological changes in the cells after 24 hr of 0.5 mM H2O2 treatment are also shown. Both untreated and treated MCF-7 cells [Figures 1(d) and (e)] exhibit the typical rounded, clumped morphology of this line. In contrast, the MCF-10A cells are elongated and well attached under normal growth conditions [Figure 1(f)], but become more rounded and retracted after 24 hr of peroxide treatment [Figure 1(g)]. Also apparent in the treated culture are some round detached cells, and an overall decrease in cell number after treatment.

Since Prdx1 and Prdx6 have been reported as overexpressed in various breast cancer samples, we next sought to compare the levels of Prdx1 and Prdx6 expression between the two cell lines. Using real-time PCR, we determined that Prdx1 mRNA is approximately 2.5 fold higher in MCF-7 cells, as compared with MCF-10A cells [Figure 2(a)]. Using western blotting, we demonstrated a similar increase in Prdx1 protein expression in MCF-7 cells [Figure 2(c)], with an average of approximately three times higher Prdx1 protein levels found in MCF-7 cells as compared with MCF-10A cells [Figure 2(d)]. In contrast, Prdx6 mRNA levels were only slightly higher in MCF-7 cells [Figure 2(b)], and no significant difference was observed at the protein level [Figure 2(c/d)]. Together, these data suggest that MCF-7 cells express elevated levels of Prdx1 and similar levels of Prdx6 as compared with MCF-10A cells.

To begin to examine peroxiredoxin function, we used transient transfection of siRNA to specifically suppress Prdx1 and/or Prdx6 in each cell line. Cells were transfected with 33 nM of either a gene-specific siRNA or a nonspecific negative control siRNA (“neg”). After 48 hr, gene expression was measured by real-time PCR and western blotting. Figures 3(a) and (b) represent the mRNA expression of Prdx1 and Prdx6 in each of the transfection conditions, relative to expression levels in untransfected cells. As shown in Figure 3(a), Prdx1 mRNA levels were significantly suppressed in both lines using Prdx1 siRNA alone, or in conjunction with Prdx6 siRNA. Expression of Prdx1 in transfected cells was approximately 90% lower than the levels in untransfected cells. Similar results were obtained for Prdx6 expression, with nearly 90% suppression achieved in both lines using Prdx6 siRNA alone, or in conjunction with Prdx1 siRNA [Figure 3(b)]. Interestingly, MCF-10A cells displayed a slight but significant increase in the expression of one Prdx gene when the other was suppressed: Prdx1 expression increased approximately by 30% in MCF-10A cells transfected with Prdx6 siRNA [Figure 3(a)], and Prdx6 expression increased approximately by 30% when Prdx1 was suppressed [Figure 3(b)]. However, this result was not observed in the cancer line, which showed no significant change in the expression of one Prdx upon suppression of the other. These data confirm effective knockdown of the target mRNAs in both lines and suggest a possible compensatory upregulation only in MCF-10A cells.

Western blotting was used to determine the resultant protein suppression in transfected cells. As shown in Figure 3(c) and quantified in Figure 3(d), Prdx1 levels were decreased with Prdx1 siRNA in both lines, but to a greater extent in MCF-10A cells. This was observed in cells transfected with Prdx1 siRNA alone, or in conjunction with Prdx6 siRNA. As
Figure 1. Susceptibility to H₂O₂-induced cytotoxicity in MCF-7 and MCF-10A cells. (a,b) Cells were cultured and treated with indicated concentrations of H₂O₂ for 4, 8, or 24 hr and cell viability was determined by measuring solubilized MTT. Average MTT for (a) MCF-7 cells and (b) MCF-10A cells relative to untreated levels was calculated for 3–4 samples in each treatment, and is shown (±SD). (c) Cells were treated with or without 0.5 mM H₂O₂ and released and cellular LDH levels were measured 24 hr later using the CytoTox 96 reagent. Average cytotoxicity (released LDH/cellular LDH) relative to untreated levels was calculated for 7–8 samples in each treatment (±SD). (d–g) Cells were plated and treated in 96-well plates and photographed with phase contrast microscopy at 100× magnification 24 hr after treatment with or without 0.5 mM H₂O₂. (d) Untreated MCF-7 cells, (e) treated MCF-7 cells, (f) untreated MCF-10A cells, and (g) treated MCF-10A cells. *p < .05; **p < .01; ***p < .005; ****p < .001.
shown in Figures 3(c) and (e), transfection of both lines with Prdx6 alone or in conjunction with Prdx1 siRNA resulted in marked protein suppression. Despite the increase observed in Prdx1 and Prdx6 mRNA in MCF-10A cells when the opposite gene was suppressed [Figures 3(a) and (b)], no corresponding increases at the protein level were observed [Figures 3(c)–(e)]. Together, these data demonstrate that the expression of the Prdx1 and Prdx6 proteins are reduced in both lines after 48 hr of siRNA transfection.

The cytoprotective function of specific peroxiredoxins is often examined in individual cell lines by either siRNA suppression or transient overexpression. To measure this activity in this experiment, we used the CytoTox 96 Cytotoxicity Assay (Promega) to determine the ratio of released LDH (indicative of cell death) to cellular LDH (indicative of viable cells) under each transfection condition. Figure 4(a) shows the cytotoxicity results in the absence of exogenous hydrogen peroxide treatment. As shown, there was no effect of Prdx suppression in the MCF-7 cancer cells. In contrast, Prdx suppression led to a statistically significant increase in MCF-10A cytotoxicity with either Prdx6 siRNA alone or in conjunction with Prdx1 siRNA [Figure 4(a)]. The largest effect was observed when both genes were suppressed, resulting in a 70% increase in cytotoxicity relative to cells transfected with the negative control siRNA. These data demonstrate a clear effect of Prdx suppression in MCF-10A cells.

To determine the added effect of oxidative stress on peroxiredoxin-suppressed cells, we also examined cytotoxicity in transfected cells treated with low or high doses of hydrogen peroxide. To eliminate the contribution of peroxiredoxin suppression to basal cytotoxicity, data for each transfection condition were calculated relative to the untreated condition. As shown in Figure 4(b), 0.05 mM hydrogen peroxide had no added effect on any siRNA-transfected group. In contrast, 0.5 mM hydrogen peroxide produced a small but consistent increase in cytotoxicity in cells transfected with Prdx1 and/or Prdx6. This increase, of approximately 50%, was similar between all three transfection conditions, and was comparable to the increase seen in untransfected cells [Figure 1(c)]. While MCF-10A cells, similar to MCF-7 cells, showed no significant increase in cytotoxicity after 0.05 mM hydrogen peroxide treatment, the cytotoxicity of cells transfected with both Prdx1 and Prdx6 increased greatly (approximately fivefold) after high-dose
Figure 3. Suppression of Prdx1 and Prdx6 by siRNA in MCF-7 and MCF-10A cells. Cells were cultured and transfected with 33 nM of the negative control siRNA, Prdx1 siRNA, Prdx6 siRNA, or both Prdx1 siRNA and Prdx6 siRNA using the Lipofectamine 2000 reagent and Opti-MEM media. After 48 hr, Prdx1 and Prdx6 mRNA and protein were measured as previously described. Average RQ of mRNA (± SD) is shown for (a) Prdx1 and (b) Prdx6, with *p < .05; **p < .01; ***p < .005; ****p < .001. (c) A representative western blot is shown. Net band intensities for Prdx1 and Prdx6 on replicate blots were normalized against β-actin levels, and averages for each gene were calculated relative to levels in the negative control-transfected cells. Data are shown for (d) Prdx1 and (e) Prdx6. (Since each transfection experiment achieves different levels of suppression, the data were not analyzed statistically.)

hydrogen peroxide treatment [Figure 4(c)]. Together, these data demonstrate a synergistic effect of Prdx1/Prdx6 suppression in MCF-10A cells in the absence or presence of oxidative stress.

Given the previously reported role of peroxiredoxins in preventing hydrogen-peroxide-induced apoptosis, we sought to determine whether hydrogen-peroxide-induced cell death in MCF-10A cells was due to apoptosis. To do this, we measured caspase 3/7 activity in cell lysates from transfected cells treated for 24 hr with 0.5 mM hydrogen peroxide. As shown in Figure 5, we observed a significant increase in caspase activity in untreated cells when one or both genes were suppressed. This increase was almost twofold with Prdx1 siRNA, and almost threefold with Prdx6 siRNA or both siRNAs (as compared with cells transfected with the control siRNA). For cells transfected with Prdx1 or Prdx6 alone, peroxide treatment caused no additional increase in caspase activity. In contrast, cells in which both genes were suppressed showed an additional twofold increase in caspase activity. These data suggest that peroxiredoxin suppression in untreated cells increases apoptosis, and that loss of both genes further increases apoptosis in response to oxidative stress.
DISCUSSION

The present studies were conducted to examine the function of two important yet distinct peroxiredoxins, Prdx1 and Prdx6, in noncancerous and cancerous breast epithelial cells. Both genes have been reported to be overexpressed in breast cancer, yet no studies to date have examined the effect of their silencing in normal and cancerous breast cell lines. The present study reports several new findings: (a) MCF-7 and MCF-10A cells exhibit differences in time-/dose-dependent peroxide-induced toxicity; (b) MCF-7 and MCF-10A cells express comparable levels of Prdx6, while Prdx1 is significantly elevated in MCF-7 cells; (c) transient transfection of siRNA into both lines leads to comparable suppression of Prdx6 protein in both lines, and greater suppression of Prdx1 protein in MCF-10A cells; (d) levels of Prdx1/Prdx6 suppression achieved in MCF-7 cells had no effect on cytotoxicity; (e) suppression of Prdx6 alone or in conjunction with Prdx1 significantly increased cytotoxicity in MCF-10A cells; and (f) combined suppression of both genes in MCF-10A cells resulted in a marked increase in peroxide-induced apoptotic death. Overall, these data confirm the cytoprotective role of peroxiredoxins in normal cells and reveal a nonredundant synergistic effect of Prdx1 and Prdx6 in MCF-10A cells.
Overexpression of Prdx1 and Prdx6 is associated with breast cancer in both patient samples and established cell lines (9, 11, 14, 18–20). While these studies have been useful in establishing this link, a comparison of normal and cancerous cell lines can provide a closer examination of the precise role of these genes in breast cancer. The MCF-7 estrogen-receptor-positive mammary adenocarcinoma cell line has been extensively used for studies on the molecular and cellular properties of breast cancer. By comparing the properties of this line with those of the nontumorigenic MCF-10A cell line, a valuable model can be established for investigating the mechanisms associated with carcinogenesis in breast tissue. Our data show an increased susceptibility to peroxide-induced toxicity in MCF-10A cells, as compared with MCF-7 cells. Other studies have reported a similar difference (19), although higher concentrations were used and viability was determined using the less reliable trypan blue method. Our data use two alternative methods (MTT and LDH release) to confirm this difference, and establish the method of peroxide treatment to be used in subsequent siRNA experiments. While relatively high concentrations (0.01–0.5 mM) of hydrogen peroxide were used in our study, the extent of cell death in both lines is less than other studies, which have reported other normal and cancer cells, although our results are comparable to that seen by Bae et al. for these cell lines (19). This may be attributed to the relative instability of aqueous hydrogen peroxide in our studies, and it is likely that the actual cytosolic concentrations achieved in our cultures may be much lower.

Our demonstration that Prdx1 is significantly overexpressed at the mRNA and protein levels in MCF-7 cells is also consistent with a previous study reporting overexpression of Prdx1 and Prdx2 in these cells (19), although this study did not compare Prdx6 expression, nor did it examine the effects of peroxiredoxin suppression on toxicity. Given that many cancers exhibit elevated Prdx expression, it is likely that overexpression represents a cellular adaptation to the cancerous state, although the mechanism for this upregulation is still unknown. Our mRNA data do suggest, however, that Prdx1 expression is elevated by a transcriptional mechanism. In contrast, we demonstrated that Prdx6 is not overexpressed in the cancer line. Despite this observation, differences in peroxiredoxin activity besides levels of expression may also exist between normal and cancer cells, including peroxiredoxin phosphorylation state, oxidation state, or oligomerization, all of which are likely to modify the corresponding enzyme activity. Since these modifications may lead to differences in both basal and suppressed peroxiredoxin activity between the lines, an investigation of these endpoints in these cells, as well as other cancer cell models, will allow us to more completely characterize the cell-specific role for these proteins.

Transient siRNA transfection in our studies resulted in a nearly 90% mRNA suppression, but only 30%–50% protein suppression in either line. The difference between mRNA and protein levels may represent differences in the transfection efficiency of the different formats used for RT-PCR analysis versus western blotting, or may indicate an increase in Prdx1/Prdx6 protein translation or protein stability in response to mRNA suppression. Regardless, we were able to achieve a reduction in protein expression for both genes in both cell types. However, the magnitude of Prdx1 suppression was significantly less in MCF-7 cells. This is interesting, and may represent a cell-specific resistance to Prdx1 loss by some unknown mechanism. Since this observation was reproducible and shown when Prdx1 siRNA was transfected alone or in conjunction with Prdx6, it appears to be gene specific.

We demonstrate a clear cytoprotective role for both Prdx1 and Prdx6 in MCF-10A cells. Our observation that Prdx6 suppression increased the cytotoxicity of these cells suggests that Prdx6 may play an important cytoprotective role in the absence of external oxidative stress. Although no effect was seen with Prdx1 suppression alone, a synergistic effect was observed in conjunction with Prdx6 suppression, suggesting nonredundant cytoprotective functions for these proteins in unstressed MCF-10A cells. The profound increase in peroxide-induced apoptotic death, which we observed in MCF-10A cells upon suppression of both peroxiredoxins, provides two additional insights into the putative mechanism of peroxiredoxin-mediated protection. First, the fact that the functional result of peroxiredoxin suppression is exacerbated by external oxidative stress suggests that peroxiredoxin-mediated cytoprotection in normal cells is likely attributable to its antioxidant activity and regulation of ROS levels. Second, the synergistic activities of these two proteins confer resistance to apoptosis. Together, these data demonstrate for the first time that Prdx6 is cytoprotective for MCF-10A cells, and has a synergistic effect with Prdx1 in the absence or presence of oxidative stress.

In contrast to our results with MCF-10A cells, we observed no effect of peroxiredoxin suppression in untreated MCF-7 cells. However, peroxiredoxin suppression did lead to a small (<50%) but significant increase in cytotoxicity in response to high-dose hydrogen peroxide treatment. The observation that this increase was consistent in all peroxiredoxin-suppressed cells suggests that the two proteins, and possibly other peroxiredoxins, may play more of a redundant role in MCF-7 cells. This relatively small effect is somewhat surprising, given the widely reported cytoprotective function of peroxiredoxins in other cancer cells. There are several possible explanations for this result. First, this may be a product of the less effective siRNA-mediated Prdx1 suppression achieved in MCF-7 cells. This level of suppression was confirmed in several experiments and with a different Prdx1 siRNA (data not shown), and thus may implicate the existence of a possible compensatory mechanism that acts to resist Prdx1 suppression in MCF-7 cells. However, marked Prdx6 suppression was successfully achieved in these cells, and still resulted in this low increase in cytotoxicity. Alternatively, this may represent a consequence of the cancerous state of these cells, in which numerous other alterations (genetic or epigenetic) are likely to exist that render these cells resistant to ROS-induced death, such as aberrant mitogenic or apoptotic signaling. Finally, other peroxiredoxins are likely to be overexpressed in these cells that may compensate for the reduction in any one peroxiredoxin. A comparison of the complete peroxiredoxin...
expression and activity profiles for these two lines will be required to fully understand the cell line differences in peroxiredoxin function.

The role of peroxiredoxins in cancer is likely to be complex, analogous to the complexity of ROS itself in carcinogenesis. On the one hand, peroxiredoxins clearly possess a tumor-supportive function, since suppression of peroxiredoxins in many cell lines induces apoptosis. On the other hand, abnormal levels of ROS are known to promote tumorigenesis, and peroxiredoxins can inhibit ROS and oxidative DNA damage associated with tumorigenesis (17). An ability to prevent oxidative DNA damage has been demonstrated for Prdx1 (26) and corresponding tumor-preventive activity has been shown in vivo with increased tumor incidence reported in Prdx1-/−mice (40). Our observation that can-}
tivity has been shown for Prdx1 (26) and corresponding tumor-preventive activity to prevent oxidative DNA damage has been demonstrated for Prdx1 (26) and corresponding tumor-preventive activity has been shown in vivo with increased tumor incidence reported in Prdx1-/−mice (40).

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DECLARATION OF INTEREST

The authors report no conflicts of interest.

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