PRDX6 Protects ARPE-19 Cells from Oxidative Damage via PI3K/AKT Signaling

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ARPE-19 • PRDX6 • Oxidative stress • Apoptosis • PI3K/AKT signaling pathway

Abstract
Background/Aims: Oxidative stress that damages cells of the retinal pigment epithelium (RPE) can cause the development of hereditary retinal disease (HRD). PRDX6, which is a member of the PRDX family, is essential for removing metabolic free radicals from the body. However, the effect of PRDX6 on oxidative stress in HRD remains unknown. In this study, we sought to investigate the role of PRDX6 in oxidative stress-induced HRD in ARPE-19 cells and the molecular mechanism involved. Methods: ARPE-19 cells were used in the current study. Intracellular ROS levels were determined by flow cytometry. Lipid peroxidation was measured using a commercial MDA assay kit. Cellular variability was determined by MTT assay. Apoptosis was determined using an Annexin V-FITC Apoptosis Detection Kit. mRNA and protein expression levels were detected by real-time PCR and western blot analysis, respectively. Results: We found that \( \text{H}_2\text{O}_2 \) and blue light could induce significant oxidative stress damage and cell death in ARPE-19 cells. Furthermore, we found that PRDX6 levels significantly decreased after \( \text{H}_2\text{O}_2 \) treatment. PRDX6 overexpression protected ARPE-19 cells from \( \text{H}_2\text{O}_2 \) - and blue light-induced oxidative damage, while PRDX6 knockdown enhanced oxidative damage in these cells. Mechanistically, we found that PRDX6 prevented oxidative damage and promoted ARPE-19 cell survival through the PI3K/AKT signaling pathway. Conclusions: Collectively, these results suggest that PRDX6 protects ARPE-19 cells from \( \text{H}_2\text{O}_2 \)-induced oxidative stress and apoptosis and that this protection is mediated at least partially through the PI3K/AKT pathway.
Introduction

Hereditary retinal disease (HRD) is primary retinal degeneration that can lead to severe visual impairments and refractory blindness [1]. The average incidence of HRD is approximately 1/3000 in developed countries, although its average incidence in China is much higher (1/1000) [2, 3]. Among blindness-causing eye diseases, HRD has the highest incidence in the global working-age population. Therefore, HRD has received international attention in ophthalmology.

The retinal pigment epithelium (RPE), which is in the outermost layer of the retina, plays an extremely important physiological role in maintaining photoreceptor function and structure [4]. Unfortunately, RPE cells are susceptible to oxidative stress induced by their own metabolic products. Growing evidence has shown that oxidative damage causes HRD [5, 6]. In vitro, oxidative stress has been reported to cause damage to RPE cells, leading to photoreceptor cell apoptosis, which subsequently contributes to the pathogenesis of HRD [2, 7]. In vivo, HRD mice were found to be more vulnerable to short-wavelength radiation due to higher reactive oxygen species (ROS) production compared to WT mice [8].

Peroxiredoxin 6 (PRDX6) is a protein found in mammals [9-12] that belongs to PRDX, a newly identified antioxidant family. PRDX6 was first isolated from bovine ciliary proteins by Shichi et al. and named non-selenium-dependent glutathione peroxidase [13]. Functionally, PRDX6 removes metabolically produced free radicals in the body by neutralizing peroxides, peroxynitrite, and phospholipid hydroperoxides [14]. PRDX6 is involved in many different diseases, such as diabetes, cancer, and lung damage [15, 16]. Additionally, PRDX6 plays a role in oxidative damage in a lung epithelial cell line. For instance, PRDX6 overexpression significantly decreased oxidative stress-induced lipid peroxidation and cell membrane damage [15]. In contrast, RNA silencing of PRDX6 in rat lung epithelial cells markedly increased lipid peroxidation, cell membrane damage and apoptosis upon oxidative stress stimulation [17]. However, whether PRDX6 plays a role in oxidative damage to the RPE and whether its dysfunction contributes to HRD progression remain undetermined.

In the present study, we used \( \text{H}_2\text{O}_2 \) and blue light to induce oxidative damage in ARPE-19 cells and demonstrated for the first time a protective role for PRDX6 against oxidative damage in ARPE-19 cells. Mechanistically, we found that PRDX6 protected cells from oxidative damage through the PI3K/AKT pathway. These findings not only defined the role of PRDX6 in oxidative damage to the RPE but also provided a potential therapeutic target for future HRD treatment.

Material and Methods

Reagents

The human RPE cell line ARPE-19 was obtained from American Type Culture Collection (ATCC, USA). Assay kits for MTT, MDA and ROS were purchased from Beyotime (Nantong, China). The antibodies for p-AKT, AKT and β-actin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The PI3K/AKT signaling pathway inhibitor LY294002 was purchased from Selleck Company. Dulbecco’s modified Eagle’s medium and fetal bovine serum were obtained from Gibco BRL (Grand Island, NY). An annexin V-fluorescein isothiocyanate (FITC)-propidium iodide (PI) apoptosis kit was purchased from Becton Dickinson (Mountain View, CA).

Cell culture

ARPE-19 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum, 100 \( \mu \text{g/ml} \) streptomycin, and 100 U/ml penicillin. The cells were incubated at 37°C in a humidified 5% CO\(_2\) atmosphere, and the medium was changed every other day. The cells were grown to an appropriate density and used at passages 10–15.
**MTT assay**

Cell viability was measured by quantitative colorimetric MTT assay (Beyotime, Nantong, China). Briefly, ARPE-19 cells were seeded in 96-well plates (6 × 10^3 cells/well) and maintained in growth media for 24 h with 5% CO_{2} at 37°C. When the cells reached 60% confluence, they were treated with 0–1 mmol/l H_{2}O_{2} and blue light (470 ±20 nm) for 24 h. Next, 10 µl of MTT solution (5 mg/ml) was added to each well, and the cells were incubated for another 4 h at 37°C. After formazan crystals formed, the MTT medium was aspirated and replaced with 150 µl of dimethyl sulfoxide (DMSO) (Beyotime, Nantong, China) to solubilize the crystals. Then, the plates were shaken for 5 min. The absorbance of each well was recorded using a microplate spectrophotometer at 570 nm. Relative cellular growth was determined by the ratio of the average absorbance of treated cells versus the average absorbance of control cells. Cell viability was calculated as the ratio of optical densities.

**Detection of intracellular ROS levels**

To measure ROS generation within H_{2}O_{2}-treated cells, FACS analysis was performed. Briefly, cells were exposed to H_{2}O_{2} for the indicated times and then stained with 5 µg/ml DCF-DA (Beyotime, Nantong, China) for 30 min. Next, the cells were subjected to flow cytometry using a Partec CyFlow flow cytometer (Partec, Germany) and analyzed using FloMax 2.82 software.

**Determination of cellular lipid peroxidation via MDA assay**

Malondialdehyde (MDA), an end product of lipid peroxidation, was measured to estimate the levels of lipid peroxidation in ARPE-19 cells using a commercial MDA assay kit (Beyotime). The results are expressed as nM/mg protein.

**8-Hydroxy-2’-deoxyguanosine (8-OHdG) ELISA**

The 8-OHdG content in lysis solution was detected by ELISA. A human 8-OHdG ELISA kit was purchased from Cusabio Biotech Company (Wuhan, China) and used according to the manufacturer’s instructions.

**Analysis of apoptosis**

Apoptosis was quantified using an Annexin V-FITC Apoptosis Detection Kit (Beyotime, Nantong, China). Briefly, ARPE-19 cells were seeded in 6-well plates at a density of 4 × 10^6 cells/ml. After the cells were treated, they were harvested by trypsinization. Then, the cells were centrifuged and washed twice with cold PBS. Between 1 × 10^5 and 1 × 10^6 cells were resuspended in 300 µl of 1× binding buffer and then transferred to a sterile glass flow cytometry tube. In total, 5 µl of annexin V-FITC was added to the suspension, and then the suspension was incubated in the dark for 30 min at room temperature. Next, the cells were incubated in the dark with 5 µl of PI and analyzed using a flow cytometer (FACS Partec, Germany).

**Measurement of mitochondrial membrane potential (ΔΨm)**

ΔΨm was analyzed by JC-1 assay (Beyotime, Nantong, China). JC-1 is a cationic dye that indicates ΔΨm by reversibly shifting its fluorescence emission between green and red. Briefly, a staining mixture of 300 nM JC-1 was prepared according to the manufacturer’s instructions. Cells were incubated with the staining mixture for 30 min at 37°C. Next, the cells were washed twice with medium and finally resuspended in fresh medium. ΔΨm was monitored using a fluorescence microscope.

**Plasmid construction**

The PRDX6-pcDNA3.1 vector was generated from the human PRDX6 sequence obtained from GenBank (PRDX6; GenBank accession no. NM_004905.2). PRDX6 was cut with the restriction enzymes EcoRI and Xhol, and then PRDX6 was inserted into a pcDNA3.1 vector (Invitrogen, USA) for sequencing. STK33 siRNA oligos were inserted into the pSilencer 4.1-CMV plasmid using BamHI and HindIII enzyme sites. All transfection experiments were repeated in independent triplicates.

**Cell transfection**

Cells were transfected with the indicated PRDX6 plasmids or siRNAs using Lipofectamine 2000 (Invitrogen, USA) in serum-free medium according to the manufacturer’s instructions. The cells were collected after 24 h of transient transfection for further experiments.
Real-time qPCR

Total RNA from ARPE-19 cells was isolated using TRizol Reagent (TaKaRa, Japan) according to the manufacturer’s protocol. The RNA concentration was quantitated by measuring the absorbance at 260 nm, and the RNA purity was determined by the A260/A280 ratio. The total RNA (1 µg) from each tissue sample was reverse-transcribed to cDNA using a Prime Script RT Reagent Kit (TaKaRa) in the following reaction system: 8 µl of 5× Prime Script Buffer (for real-time), 2 µl of Prime Script RT Enzyme Mix, 0.1 nmol oligo(dT) primer, 0.2 nM random hexamers, 2 µg of total RNA, and RNase-free deionized water to a final volume of 40 µl. The reverse transcription program was as follows: 15 min at 37°C and 5 sec at 85°C. The specific primers were designed using Primer Premier 5.0. All primers were synthesized by Sangon Biotechnology. An ABI 7300 Real-Time PCR System (ABI, USA) was used for RT-PCR amplification and detection. RT-PCR reactions were prepared in triplicates in 20-µl reaction volumes as follows: 10 µl of 2× SYBR Premix Ex Taq II, 0.4 µM forward and reverse primers, 2 µl of cDNA (50 ng) template, and 6.4 µl of RNase-free water. Master Mix without cDNA template was used as a negative control. RT-PCR cycling conditions were used as suggested in the SYBR Premix Ex Taq II Kit (TaKaRa, Japan) instructions. Melting curves were checked to ensure the specificity of the PCR products in the SYBR Green reactions. Relative mRNA levels of the target genes were normalized to β-actin mRNA. The PRDX6 primer sequences used were F: GGAGCAAGGATATCAATGCTTACA and R: CCAAAAACAAACACCACACCGA.

Western blot analysis

Cells were blotted twice in ice-cold PBS and resuspended in protein lysis buffer (Beyotime, China) containing a protease inhibitor cocktail. The supernatants were centrifuged at 10,000 × g for 15 min at 4°C to obtain the protein supernatants. Protein concentrations were determined using Bio-Rad protein assay kits (Bio-Rad, USA). Then, the samples were separated by SDS-PAGE on 10% polyacrylamide gels and electro-transferred to Hybond polyvinylidene fluoride (PVDF) membranes. The PVDF membranes were soaked for 2 h at room temperature with 5% nonfat milk in TBST buffer composed of 50 mM Tris (pH 7.6), 150 mM NaCl and 0.1% Tween-20 and then incubated with primary antibody overnight at 4°C. After the membranes were washed three times with TBST buffer for 5 min each, they were incubated at room temperature for 2 h in TBST containing horseradish peroxidase (HRP)-conjugated goat anti-mouse or goat anti-rabbit IgG antibody (Santa Cruz, Inc., USA). Next, the membranes were washed three times with TBST buffer for 10 min each. Then, the membranes were incubated in ECL reagent (Pierce, Thermo Co., Ltd, USA) for HRP detection and exposed to autoradiography film (Bio-Rad, Co., Ltd, USA) for band visualization. ß-Actin was used as a loading control. The relative amounts of various proteins were analyzed, and the results were quantified using ImageJ software.

Fluorescence microscopy and indirect immunofluorescence staining

Cells were grown in 6-well glass-bottomed dishes. After the cells were treated, they were fixed in 4% paraformaldehyde for 30 min and then permeabilized with 0.2% Triton X-100 in PBS. Nonspecific binding sites were blocked with normal goat serum (Sigma-Aldrich, USA) diluted in 0.1% Triton-X-100 in PBS for 2 h. Then, the cells were incubated overnight at 4°C with primary antibodies at a 1:200 dilution in blocking buffer. Anti-p-AKT antibody was purchased from Proteintech Technology Company. The next day, the cells were incubated with appropriate fluorophore-conjugated secondary antibodies. DAPI was used to stain nuclei before acquiring images. The images were acquired using a fluorescence microscope.

Results

\(\text{H}_2\text{O}_2\) and blue light can induce cell death and oxidative stress in ARPE-19 cells

The redox reaction is a basic biochemical reaction in human bodies that is indispensable for cellular survival. However, when the cells in our bodies suffer from harmful factors that disrupt this balance, they can be injured and eventually undergo apoptosis [18, 19]. In this study, we exposed ARPE-19 cells to \(\text{H}_2\text{O}_2\) together with blue light to generate an oxidative stress injury model. ARPE-19 is a human RPE cell line with an epithelial morphology and a rapid proliferation rate. To confirm that \(\text{H}_2\text{O}_2\) together with blue light (subsequently referred to simply as “\(\text{H}_2\text{O}_2\)”) could affect cell survival and oxidative stress, we first treated ARPE-19...
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Cells with different concentrations of H$_2$O$_2$ (0, 0.5, and 1.0 mM) under blue light for 24 h and then detected cell proliferation by MTT assay. We found that H$_2$O$_2$ could inhibit cell growth in a dose-dependent manner, with 50% of cell growth inhibited at 1 mM H$_2$O$_2$ (Fig. 1A). These results indicated that H$_2$O$_2$ and blue light might induce apoptosis in ARPE-19 cells. Next, using annexin V/PI double staining, we detected the apoptotic effect of H$_2$O$_2$ on ARPE-19 cells. Using flow cytometry, we found that H$_2$O$_2$ significantly increased both the apoptotic (Fig. 1B) and necrotic (Fig. 1C) populations of ARPE-19 cells at 24 h. These findings indicated that H$_2$O$_2$ induced apoptosis and necrosis in ARPE-19 cells. In addition, we examined the effect of H$_2$O$_2$ on the ΔΨm. We observed that H$_2$O$_2$-treated cells exhibited significantly more green JC-1 fluorescence compared to control cells under a fluorescence microscope, which suggested a reduction in the ΔΨm in H$_2$O$_2$-treated ARPE-19 cells (Fig. 1D).

In subsequent experiments using MDA and 8-OHdG assays, we found that the oxidative stress end products MDA and 8-OHdG increased in a dose-dependent manner in H$_2$O$_2$-treated cells compared to control cells (Fig. 1E, F). To further determine the effect of H$_2$O$_2$ on ROS induction, we measured intracellular ROS production using DCF-DA fluorescence dye. As expected, we found that H$_2$O$_2$ significantly enhanced intracellular ROS production in a dose-dependent manner (Fig. 1G). Taken together, these data suggested that H$_2$O$_2$ could induce oxidative damage in ARPE-19 cells.

**PRDX6 mRNA levels increase after H2O2 and blue light stimulation in ARPE-19 cells**

PRDX6 serves as an antioxidant and plays an important role in the maintenance of redox balance. To detect whether PRDX6 was involved in H$_2$O$_2$-induced cellular injury, we first quantified PRDX6 mRNA expression by real-time PCR. As shown in Fig. 2A, we observed
that PRDX6 mRNA expression significantly increased after H$_2$O$_2$ treatment (24 h) in a dose-dependent manner (approximately 5-fold increase at 1 mM H$_2$O$_2$). To investigate the role of PRDX6 further, we generated a PRDX6 overexpressing plasmid (PRDX6-pcDNA3.1) and a silencing plasmid (PRDX6-pSilencer 4.1). The transfection efficiencies of PRDX6-pcDNA3.1 and PRDX6-pSilencer 4.1 were confirmed by both qPCR and western blot assays (Fig. 2B, C). Both plasmids achieved the highest transfection efficiencies (more than 50%) at 48 h post-transfection (Fig. 2B, C).

**PRDX6 can protect ARPE-19 cells from cell death and oxidative stress induced by H$_2$O$_2$ and blue light**

To determine whether PRDX6 played a role in H$_2$O$_2$-induced cell death and oxidative injury in ARPE-19 cells, we first transfected these cells with PRDX6-pcDNA3.1 or PRDX6-pSilencer 4.1 and then exposed them to H$_2$O$_2$ (0.5 or 1.0 mM) and blue light treatment for 24 h. Flow cytometric assay indicated that PRDX6 overexpression decreased the population of apoptotic (Fig. 3A) or necrotic (Fig. 3B) cells. In addition, using JC-1 immunostaining, PRDX6 overexpression increased the ΔΨm induced by H$_2$O$_2$ (Fig. 3C). In contrast, PRDX6 knockdown

![Fig. 2. PRDX6 expression significantly increased after stimulation with H$_2$O$_2$ and blue light. ARPE-19 cells were treated with H$_2$O$_2$ for 24 h under blue light. (A) Relative gene expression of PRDX6 was detected by real-time PCR in ARPE-19 cells with and without varying doses of H$_2$O$_2$ stimulation and blue light. Stimulations were performed using 0, 0.5 and 1.0 mM H$_2$O$_2$ for 24 h (n = 3). The results are presented as the average fold change in PRDX6 gene expression (normalized to β-actin) after 24 h of 0, 0.5, and 1.0 mM H$_2$O$_2$ and blue light exposure. (B) qPCR analysis was performed using isolated total RNA to quantify PRDX6 mRNA levels after PRDX6-pcDNA3.1 and PRDX6 siRNA transfection. (C) The expression of PRDX6 protein was detected by western blot after PRDX6-pcDNA3.1 and PRDX6 siRNA transfection. The data are representative of three experiments with similar results (Student's t-test, *p < 0.05). β-Actin was used as the loading control.](image-url)
increased the population of dead cells and decreased H$_2$O$_2$-induced changes to the ΔΨm (Fig. 3A-C). Moreover, we found that MDA and 8-OHdG decreased following PRDX6 overexpression (Fig. 3D, E). Similarly, intracellular ROS production was further confirmed by flow cytometry, followed by probing with DCF-DA at 488 nm excitation. Compared to the H$_2$O$_2$-treated group, we found that knocking down PRDX6 with PRDX6-pSilencer 4.1 significantly increased intracellular ROS production, while overexpressing PRDX6 with PRDX6-pcDNA3.1 decreased ROS production (Fig. 3F). Together, these observations demonstrated that PRDX6 might be a protective gene in the H$_2$O$_2$-induced-ARPE-19 cell injury model.

**PRDX6 protection against H$_2$O$_2$-induced cellular injury partially functions through PI3K/AKT signaling**

PI3K/AKT signaling plays an important role in cell growth and cellular oxidative stress [20]. Therefore, in this study we investigated the role of PI3K/AKT signaling in PRDX6
protection against oxidative damage. After \( \text{H}_2\text{O}_2 \) treatment, we found that the p-AKT level significantly decreased, suggesting the involvement of p-AKT (Fig. 4A) in this oxidative injury cellular model. Western blot analysis indicated that the decreased p-AKT level induced by \( \text{H}_2\text{O}_2 \) recovered significantly upon PRDX6 overexpression (Fig. 4A). The level of p-AKT was further confirmed by fluorescence microscopy (Fig. 4B). To confirm the effect of p-AKT, we incubated cells with 10 \( \mu \text{M} \) LY294002 for 1 h before \( \text{H}_2\text{O}_2 \) treatment. This synthetic inhibitor of the PI3K/AKT signaling pathway [21] clearly inhibited increased AKT phosphorylation by PRDX6 overexpression (Fig. 4A, B). To assess the extent to which cell growth and ROS production occurred downstream of the PI3K/AKT signaling pathway, we co-treated cells with PRDX6-pcDNA and LY294002 and tested their effects on cell growth and ROS production. We found that the alleviation of cell apoptosis and necrosis induced by PRDX6 overexpression significantly increased upon PI3K/AKT inhibitor treatment (Fig. 5A, B). Similar results were observed by JC-1 assay (Fig. 5C). Moreover, we found that the alleviation of ROS production by PRDX6 overexpression significantly increased upon PI3K/AKT inhibition (Fig. 5D). Together, these results indicated that PRDX6 protected against \( \text{H}_2\text{O}_2 \)-induced cell injury through the PI3K/AKT pathway.

**Discussion**

Previous ophthalmological studies have focused extensively on the crucial role of oxidative stress in the pathophysiology of age-related macular degeneration (AMD) [22]. Recently, some investigators hypothesized that oxidative stress could be linked to the occurrence of HRD [23]. In the present study, we used ARPE-19 cells challenged with oxidative stress (\( \text{H}_2\text{O}_2 \) and blue light) as an *in vitro* HRD model to study the underlying mechanism of oxidative damage in HRD. Considering the physiological function and location of RPE.
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...cells, which lead them to be constantly exposed to many ROS [24, 25], the protection of RPE cells from oxidative stress and damage might serve as an excellent target for treating HRD. Therefore, RPE cells seemed to be an ideal model for HRD study. Here, we used the human RPE cell line ARPE-19 and. We used H$_2$O$_2$ and blue light to induce oxidative stress in these cells. H$_2$O$_2$-induced oxidative stress is a classic model by which researchers have detected susceptibility to oxidative stress or antioxidant efficiency in RPE cells [26-28]. Additionally, light exposure has been linked to the occurrence and development of AMD possibly through oxidative stress-related pathology [29, 30]. While blue light illumination induced a variety of ROS in RPE cells and increased ROS production by mitochondria, light-induced oxidation has also been positively associated with age [31, 32]. We used the fluorescent dye DCF-DA to quantify ROS generation, which is not a direct assay of H$_2$O$_2$, NO, lipid peroxides, singlet O$_2$ or O$_2^-$ production. We observed that H$_2$O$_2$ and blue light irradiation together could induce robust oxidative stress (Fig. 1E-G). However, additional studies to specify the pool of hydrogen peroxide are warranted. Furthermore, apoptosis and necrosis significantly increased in the H$_2$O$_2$-treated group. These results demonstrated that H$_2$O$_2$ could induce oxidative stress in ARPE-19 cells and suggested that this treatment could be used to stimulate the disease model in our study. In this paper, we used both H$_2$O$_2$ and blue light to increase oxidative stress and to decrease antioxidant efficiency in ARPE-19 cells.

Fig. 5. The PRDX6 gene activated the PI3K/AKT signaling pathway to protect ARPE-19 cells against oxidative stress and cell death. Cells transfected with different PRDX6 plasmids were incubated with or without 10 μM LY294002 for 1 h and then exposed to 0.5 or 1 mM H$_2$O$_2$ and blue light for 24 h. (A and B) Apoptosis (A) and necrosis (B) were determined by flow cytometry after annexin V/PI double staining. (C) After treatment, the cells were immunostained using a JC-1 kit. The green fluorescence of JC-1 monomers and red fluorescence of J-aggregates clustered in groups. (D) Cells were stained with DCF-DA to detect intracellular ROS production. The values represent the mean ± S.D. of three independent experiments. Statistical significance was assessed using one-way ANOVA plus Tukey’s test using GraphPad Prism Version 5.0a software (*p < 0.05; **p < 0.01; ***p < 0.001 vs. control; †p < 0.05; ††p < 0.01; †††p < 0.001, vs. 1 mM H$_2$O$_2$ + blue light + PRDX6-pcDNA3.1 group).
When we explored the mechanism of H$_2$O$_2$-induced cellular oxidative stress, we hypothesized that PRDX6 could be involved. The mRNA expression levels of PRDX6 significantly increased after H$_2$O$_2$ treatment (Fig. 2A). Similar results were also found in other oxidative stress models. The mRNA and protein expression and enzymatic activity of PRDX6 markedly increase in the mouse when exposed to 100% oxygen [33]. Additionally, two-dimensional electrophoresis and mass spectrometry analyses of retinas of mice with HRD indicated higher PRDX6 expression [34]. In the present study, we demonstrated for the first time that PRDX6 could protect ARPE-19 cells against oxidative damage by H$_2$O$_2$. PRDX6 played an important role in resisting oxidative stress and in maintaining the balance of phospholipid metabolism. In addition, transiently overexpressing PRDX6 significantly decreased cell viability and increased apoptosis induced by H$_2$O$_2$ and blue light (Fig. 3A, B). These findings were consistent with previous observations in other disease models, where PRDX6 protein exerted essential protective effects against organ damage induced by a variety of stressors such as ischemia-reperfusion, oxidative stress, and lipopolysaccharide [35-37]. In one study, PRDX6 transgenic mice displayed significantly decreased oxidative damage induced by tobacco [38].

To explore factors downstream of PRDX6 in HRD, we assessed potential cell signaling pathways in cells after H$_2$O$_2$ treatment. We observed that p-AKT signaling, which is an important signaling pathway in ARPE-19 cells [39], significantly decreased in ARPE-19 cells when treated with H$_2$O$_2$ and blue light (24 h). PI3K/AKT signaling is a critical pathway that is involved in cell growth, survival, apoptosis, and other cellular functions [40]. However, previous studies have shown that AKT can be activated in RPE cells as early as 15 min after H$_2$O$_2$ stimulation [41]. This finding may be explained by several factors, including the differences in injury models (H$_2$O$_2$ vs. H$_2$O$_2$ and blue light), different treatment periods (15 min vs. 24 h), and different cells (primary cells vs. cell lines). Here, we further confirmed the role of AKT by introducing LY294002, a highly specific inhibitor of PI3K/AKT signaling. Upon LY294002 treatment, ROS production and apoptosis significantly increased in ARPE-19 cells under oxidative stress conditions (Fig. 5). In addition, after PRDX6 overexpression, H$_2$O$_2$-induced decreases in p-AKT levels were significantly ameliorated (Fig. 4A). These results indicated that AKT is a downstream factor of PRDX6 that drives redox balance during stress. However, we cannot exclude other factors also contributing to this signaling pathway, thus warranting further investigation of the underlying mechanism of this effect.

Taken together, our results showed that H$_2$O$_2$ and blue light could significantly induce oxidative stress and cell apoptosis in ARPE-19 cells. We further found that PRDX6 could protect ARPE-19 cells against oxidative damage induced by H$_2$O$_2$ and blue light. In addition, we revealed a link between PRDX6 and AKT in directly contributing to oxidative stress and cell survival after H$_2$O$_2$ stimulation. Collectively, these observations provided new insight into the role of PRDX6 in resisting oxidative stress and could indicate novel targets for clinical intervention.

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Disclosure Statement

The authors declare that no financial or commercial conflicts of interest exist.
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