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Peroxiredoxin 3 (PDRX3) is highly expressed in the primate retina especially in blue cones

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Abstract

Oxidative stress and loss of mitochondrial function have been implicated in age-related ocular diseases and thus studying enzymes involved in these processes may be of particular importance in these diseases. Peroxiredoxin III (PRDX3) is one of a family of six known peroxiredoxins which are known to protect cells against oxidative damage. PRDX3 is localized to the mitochondria and has been reported to be induced by hydrogen peroxide in aortic endothelial and lens epithelial cells. Using a highly specific commercially available antibody, PRDX3 was readily detected by immunoblot in monkey neural retina. Immunohistochemical analysis of human and monkey retina localized PRDX3 mainly to the photoreceptor inner segments, the outer and inner plexiform layers and the ganglion cells. These are areas of known high mitochondrial content. In the monkey retina some of the cone inner segments were much more strongly labeled than others. Dual-labeling experiments using specific anti-cone opsin antibodies determined that the high expressing cones were of the blue subtype. By contrast, in the human retina all of the cone inner segments were immunoreactive. This difference may be due to a postmortem induction of PRDX3 in the human sample. These results suggest that PRDX3 may be important in protecting photoreceptor mitochondria especially in blue cones.

Keywords

blue cones; photoreceptors; mitochondria

Oxidative stress is suspected of playing a major role in numerous age-related diseases (Jones, 2006) and neurodegenerative disorders (Reynolds et al., 2007), and in the eye, several diseases including age-related cataract (Truscott, 2005), glaucoma (Lundmark et al. 2007), and age-related macular degeneration (Beatty et al. 2000) are associated with endogenous and exogenous oxidative stress. A key characteristic of oxidative stress damage is loss of mitochondrial function. Thus, identifying mitochondrial specific antioxidant systems is likely to increase our understanding of these diseases. In the present report, we detected and localized for the first time mitochondrial Peroxiredoxin III (PRDX3) in the primate retina and determined that blue cones have higher expression than other cones types.

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PRDXs have been shown to be involved in the degradation of hydrogen peroxide, organic hydroperoxides and peroxynitrite with electrons donated by the presence of a thioredoxin enzyme (Rhee et al., 2005). Of the six known PRDXs, PRDX3 is specifically targeted to the mitochondria [Wood et al., 2003a]. PRDX3 is a 2-Cys Typical PRDX which refers to the two reactive cysteines involved in its peroxidase activity [Wood et al., 2003, Watabe et al., 1997]. The redox-active peroxidatic cysteine oxidizes to a cysteine sulfenic acid (Cys-SOH) upon peroxide damage which is then attacked by the resolving cysteine of a neighboring PRDX3 to form an intersubunit disulfide bond. Thioredoxin reduces this disulfide bond [Wood et al., 2003] regenerating the oxidized cysteine and completing the catalytic cycle. In addition to its peroxidase activity, other functions of PRDX3 have been previously reported including acting as a free radical scavenger [Gourlay et al., 2003] and participating in redox-related signaling transduction pathways [Chang et al., 2004, Rhee et al., 2005, Wood et al., 2003b].

To investigate the presence of PRDX3 we performed immunoblot and immunohistochemical analyses in human and monkey retina samples using a highly specific commercially available antibody to PRDX3 (Abcam, Cambridge, MA). Immunoblot analyses of protein extracts from monkey neural retina (MNR) and retinal pigment epithelium-choroid (RPE/CH) fractions detected PRDX3 expression mainly in the MNR (Fig. 1). Monkey liver (ML) protein extract was used as a positive control (Fig. 1). The immunoblot revealed a 27 kDa immunoreactive band in the retina correlating with the expected size for PRDX3. PRDX3 was observed as a doublet band in the RPE/choroid and monkey liver suggesting the possibility of multiple isoforms as previously reported [Wood et al., 2003 and Gourlay et al., 2003].

The monkey tissue was collected from young (6–7 yr) female rhesus macaques (*macaca mulatta*) by the Pathology Department of the Division of Veterinary Resources after completion of approved institute protocols at the National Institutes of Health (NIH). All work was conducted according to the NIH's guidelines for Care and Use of Laboratory Animals.

In order to determine the localization of PRDX3 in the retina, vibrotome sections (100 μ m thick) were prepared and immunofluorescence confocal microscopy was performed using the same antibody as above (Fig. 1) as previously described [Lee et al., 2006; Tserentsoodol et al., 2006]. PRDX3 localized throughout the monkey retina, including the RPE, photoreceptor inner segments, inner and outer plexiform layer, and ganglion cells and nerve fiber layer following general mitochondrial-specific pattern (Fig. 2A). The rod photoreceptor inner segments were homogeneously labeled but only a few scattered cones were immunoreactive (Fig. 2A). This suggested the possibility that PRDX3 expressed preferentially in certain cone subtypes. To determine the cone subtype that highly expressed PRDX3, dual labeling immunohistochemistry was performed using anti-cone opsin specific antibodies. Dual-labeling with anti-red/green opsin labeled the cone outer segments of those cones which have low or no expression of PRDX3 (Fig. 2B). Dual-labeling with the anti-blue cone opsin labeled the cones that were PRDX3 immunoreactive (Fig. 2C). This indicated that blue cones preferentially or constitutively express PRDX3.

The finding of PRDX3 at high levels in the photoreceptor inner segments than in other cells of the retina is consistent with a mitochondrial function for PRDX3. It has been reported that 60–65% of retinal mitochondria are located in the photoreceptor inner segments [Hoang et al., 2002 and Perkins et al., 2004]. The high expression in blue cones is also consistent with the known function of these cells which absorb lower frequency light which is more ionizing to cells. PRDX3 may play a critical role in preserving and maintaining blue cone mitochondrial integrity, function, and production of ATP.

Immunolocalization in human retina showed an overall similar PRDX3 immunoreactivity pattern (data not shown) as for monkey retina (Fig. 3). The main difference between the human

and monkey retina was in the labeling of the cones. In the human all cones were immunoreactive for PRDX3 (Fig. 3) not just the blue cones. The human donor tissue was provided by the National Eye Institute image core facility and corresponded to an 85 year-old Caucasian male. The eye used in this study (right eye) had macular drusen while the contra lateral (not shown) had atrophic macular degeneration.

The reason(s) for the difference in the cone labeling between the human and the monkey are unclear. The most obvious reason may be a postmortem effect. The monkey eyes are harvested and fixed within minutes of euthanasia while human samples cannot be handled as expediently. The prolonged hypoxia in the postmortem human tissues often leads to highly variable expression of different genes. Another possibility is the age difference, the monkey was relatively young (7 yr) while the human was old (80 yr).

Although the function of PRDX3 in the retina is not known, PRDX3 has been shown to be induced by 2 μ M H₂O₂ in human lens epithelial cells [Lee et al., *In Press*] and by 500 μ M H₂O₂ in bovine aortic endothelial [Araki et al., 1999] cells. Targeted gene silencing of PRDX3 in bovine aortic endothelial cells [Araki et al., 1999] and HeLa cells [Chang et al., 2004] resulted in reactive oxygen species accumulation and loss of cell viability. In the macrophages of PRDX3 knockout mice, an increase in reactive oxygen species levels were detected [Li et al., 2007] suggesting that PRDX3 may defend and/or protect these cells against oxidative stress insult through its antioxidant functions. In addition to its direct antioxidant properties, PRDX3 has been proposed to participate in H₂O₂-mediated signaling pathways [Chang et al., 2004] and it is possible that PRDX3 could have a signaling function in the retina.

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Figure 1. Immunoblot of PRDX3 in monkey retina

Protein homogenates (25 μ g/lane) from monkey neural retina (MNR), retinal pigmented epithelium/choroid (MPEC) and liver (ML) were separated by SDS-PAGE. The immunoblot was probed with a rabbit anti-PRDX3 polyclonal antibody (Abcam, Cambridge, MA) at 1:20,000 and the blot was developed using an HRP-conjugated secondary antibody using Supersignal West Pico Chemiluminescent Substrate (Pierce, Rockford, IL) following manufacturer instructions. X-ray film was exposed for 10 seconds.

Figure 2. Immunofluorescent localization of PRDX3 and cone opsins in monkey retina

The immunolabeling for PDRX3 and cone opsins was developed with an anti-rabbit Alexa 633 (red) secondary antibody. Nuclei were stained with DAPI (blue). **A.** Low magnification of anti-PRDX3 (red) localization throughout the monkey retina. Arrows point to strongly labeled cone inner segments. **B.** Higher magnification of photoreceptor layer demonstrating the dual localization of anti-PDRX3 (red) and anti-red/green cone opsin (red). **C.** Higher magnification of photoreceptor layer demonstrating the dual localization of anti-PDRX3 (red) and anti-blue cone opsin (red). In both B and C autofluorescence (green) was incorporated to visualize the photoreceptor structures. The anti-cone opsins antibodies were purchased from Chemicon (Temecula, CA). CH, choriocapillaris; RPE, retinal pigmented epithelium; ROS, rod outer

segments; PIS, photoreceptor inner segments; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer.

Figure 3. Immunofluorescent localization of PRDX3 in human retina

Human retina sample was prepared as in Fig. 2. Nuclei were stained with DAPI (gray) PRDX3 immunoreactivity was visualized using anti-rabbit Alexa 633 (red) secondary antibody. Rod outer segments were visualized with mouse anti-rhodopsin (Chemicon) using an Alexa 568 anti-mouse secondary antibody. RPE autofluorescence is shown in green. The PRDX3-labeled cone and rod inner segments and the drusen particle are marked by arrows.