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Research Report

Peroxiredoxin 6 delivery attenuates TNF-α-and glutamate-induced retinal ganglion cell death by limiting ROS levels and maintaining Ca^{2+} homeostasis

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ABSTRACT

Higher expression of reactive oxygen species (ROS) is implicated in neurological disorders. A major event in glaucoma, the death of retinal ganglion cells (RGCs), has been associated with elevated levels of glutamate and TNF-α in the RGCs' local microenvironment. Herein we show that the transduction of Peroxiredoxin 6 (PRDX6) attenuates TNF-α- and glutamate-induced RGC death, by limiting ROS and maintaining Ca^{2+} homeostasis. Immunohistochemical staining of rat retina disclosed the presence of PRDX6 in RGCs, and Western and real-time PCR analysis revealed an abundance of PRDX6 protein and mRNA. RGCs treated with glutamate and/or TNF-α displayed elevated levels of ROS and reduced expression of PRDX6, and underwent apoptosis. A supply of PRDX6 protected RGCs from glutamate and TNF-α induced cytotoxicity by reducing ROS level and NF-κB activation, and limiting increased intracellular Ca^{2+} influx. Results provide a rationale for use of PRDX6 for blocking ROS-mediated pathophysiology in glaucoma and other neuronal disorders.

1. Introduction

Reactive oxygen species (ROS) are produced intracellularly as byproducts of a variety of physiological processes in the microenvironment of cells, and can also be generated as a result of external environmental stresses. ROS-driven oxidative stress and inadequacy of antioxidant defense have important roles in initiation of diseases (Maier and Chan, 2002; Granot and Kohen, 2004). ROS-mediated insult to neuronal cells has been shown to be a cause of the development and progression of various neurodegenerative disorders, and there has been a correlation between antioxidant levels and neuronal function (Romero, 1996; Beal, 1998; Nunomura et al., 2001; Perry et al., 2003). Several lines of evidence indicate that oxidative damage and antioxidant responses lead to clinical and pathological manifestations in glaucoma (Kurysheva et al., 1996; McKinnon, 1997; Roth, 1997; Rose et al., 1998; Levin, 1999; Lieven et al., 2006).

Glaucoma, one of the leading causes of blindness in the world, is associated with selective death of retinal ganglion cells (RGCs). The disease is characterized by an elevation in intraocular pressure (IOP), which leads to increased glutamate

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and TNF-α levels (Tezel and Wax, 2000; Nucci et al., 2005; Okuno et al., 2006), both of which are producers of ROS (Moreno et al., 2004). The progressive loss of RGCs in glaucomatous patients has been suggested to result from long-term oxidative damage induced by ROS which were generated by higher levels of glutamate and TNF-α within the cellular microenvironment (Okuno et al., 2006). Although the underlying cause for this cell death remains unknown, the primary risk factor associated with glaucoma is an increase in IOP. Moreover, RGCs in animal studies and from patients with elevated IOP have been reported to contain high levels of metabolic products of lipid peroxidation, suggesting that oxidative stress plays a pivotal role in the pathophysiology of this disease (Muller et al., 1997; Bonne et al., 1998; Okuno et al., 2006; Tezel, 2006; Izzotti et al., 2006). Recently, ROS have gained attention because of their role in cellular signaling for various biomolecules such as glutamate (Aoun et al., 2003), cytokines such as TNF-α, and growth factors, such as PDGF and TGFβ, (Kim et al., 2006). TNF-α has been shown to cause RGC death following ischemia (Pahl and Baeuerle, 1994; Madigan et al., 1996; Fontaine et al., 2002; Tezel et al., 2004; Tezel and Yang, 2005). Moreover, recent evidence reveals that a high level of glutamate is toxic to and results in apoptosis of RGCs both *in vivo* if injected in vitreous, and *in vitro* when used as a treatment of cultured retinal neuronal cells (Aoun et al., 2003; Zhang et al., 2004; Guo et al., 2005, 2006). Glutamate is present in RGCs in very high concentrations and activates several types of cell receptors, including NMDA receptors, that can enhance intracellular levels of ROS and calcium concentrations (Chen and Diamond, 2002; Ullian et al., 2004) that may lead to inappropriate activation of the apoptotic program. The generation of ROS by these molecules has been associated with the activation and deactivation of several survival factors (Rhee, 1993). Studies in a variety of experimental systems have demonstrated that RelA containing NF-κB complex has an antiapoptotic effect (Barkett and Gilmore, 1999). In glaucoma, NF-κB is highly activated in RGCs and has been suggested to be proapoptotic (Kasibhatla et al., 1988; Schreck and Baeuerle, 1994; Pahl and Baeuerle, 1994; Kucharczak et al., 2003). Moreover, the levels of ROS are controlled by both their rate of production and their metabolism. To cope with deleterious factors which enhance levels of ROS, cells initiate expression and activation of various antioxidant enzymes including peroxiredoxins (Spector et al., 2001; Reddy et al., 2004; Fatma et al., 2005; Ma et al., 2006).

The peroxiredoxin (PRDX) family includes six known members (PRDX 1–6). Of particular interest is PRDX 6 cloned by our group from human lens epithelial cells cDNA library (Fatma et al., 2001). The PRDXs including PRDX6 use redox-active cysteine (Cys or C) to reduce peroxides (Kang et al., 1998a, b; Chae et al., 1994; Fatma et al., 2001, 2005). PRDX6, a "moonlighting" protein, can protect cells from membrane, DNA, and protein damage mediated by lipid peroxidation (Manevich et al., 2002). PRDX6 from human, rat, bovine, and mouse tissues show 94% homology with conserved redox-active Cys47 residue, and mutation of this residue abolishes its protective activity (Kim et al., 1988; Leyens et al., 2003; Fatma et al., 2005). Advances in the field of gene/protein delivery with identification of several protein transduction domains (PTDs) have made it possible to deliver proteins to targeted cells or organs (Green and Loewenstein, 1988; Frankel and Pabo, 1988). HIV-TAT domain has 11 amino acids (aa; YGRKKRRQRRR) and has been shown to hold 100% potential for intracellular delivery of proteins across the plasma membrane and the blood brain barrier, and has been found to be biologically active (Mann and Frankel, 1991; Rustani et al., 1997; Nagahara et al., 1998; Becker-Hapak et al., 2001). Taking

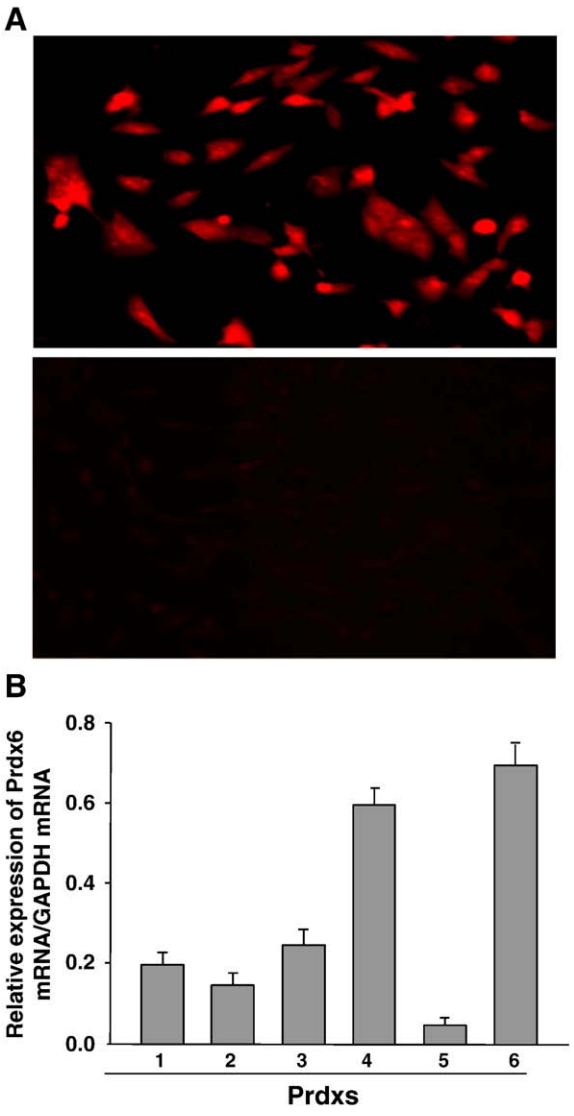

Fig. 1 – (A) Immunohistochemical analysis of RGC-5 using PRDX6 antibody. Positive imunostaining was observed in the cytoplasm of cells (A, upper panel). While in the control cells stained with neutralized PRDX6 antibody, staining was not visible (A, lower panel). (B) Quantitative real-time PCR showing differential expression of PRDX1-6 mRNA in normal RGCs. Total RNA was isolated and transcribed into cDNA. Real-time PCR was performed using specific primers (see Experimental procedures). mRNA expression of each PRDX was adjusted to the mRNA copies of GAPDH. Results indicate that mRNA expression level of PRDX6 was significantly high in comparison to other PRDXs. However, PRDX4 is also present at a significant level but could not provide protection against glutamate and/or TNF-α induced RGC death.