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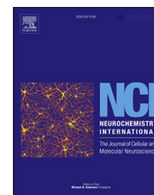
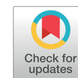


Peroxiredoxin-3 attenuates traumatic neuronal injury through preservation of mitochondrial function



Wei Hu [a, c], Xing-Bo Dang [c], Gang Wang [c], Shuai Li [c], Yue-Lin Zhang [a, b, *]

[a] Xi'an Jiaotong University Health Science Center, 76 Yanta West Road, Xi'an, Shannxi 710061, China
[b] Department of Neurosurgery, Shaanxi Provincial People's Hospital, The Third Affiliated Hospital, School of Medicine, Xi'an Jiaotong University, Xi'an, Shaanxi 710068, China
[c] Department of Emergency, Shaanxi Provincial People's Hospital, The Third Affiliated Hospital, School of Medicine, Xi'an Jiaotong University, Xi'an, Shaanxi 710068, China

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ABSTRACT

Peroxiredoxins (PRDXs) are a highly conserved family of thiol peroxidases that scavenge peroxides in cells. PRDX3 is one member of PRDXs localized in the mitochondria, and has been shown to be involved in antioxidant defense and redox signaling. In this study, we investigated the role of PRDX3 in neuronal trauma using a traumatic neuronal injury (TNI) model in primary cultured cortical neurons. We found that TNI significantly decreased the expression of PRDX3 at both mRNA and protein levels. Overexpression of PRDX3 by lentivirus (LV-PRDX3) transfection attenuated lactate dehydrogenase (LDH) release and neuronal apoptosis after TNI. The results of immunostaining showed that LV-PRDX3 transfection markedly reduced TNI-induced intracellular ROS production, protein radical formation and lipid peroxidation. In addition, overexpression of PRDX3 preserved mitochondrial membrane potential (MMP) levels and ATP generation, and inhibited mitochondrial cytochrome *c* release in TNI-injured neurons. The results of polymerase chain reaction (PCR) showed that PRDX3 overexpression also increased mitochondrial DNA (mtDNA) content and upregulated the expression of mitochondrial biogenesis-related factors. Taken together, our data demonstrate that PRDX3 protects against TNI insult by preserving mitochondrial function and mitochondrial biogenesis, and may have potential therapeutic value for traumatic brain injury (TBI).

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1. Introduction

Traumatic brain injury (TBI) is a major cause of death and disability, affecting approximate 10 million people worldwide annually, many of which require specialist care (Stocchetti et al., 2017). Morbidity and mortality after TBI are associated with the initial injury, and are also affected by the secondary intracranial processes, including intracranial hypertension, cerebral edema and neuronal inflammation (Abboud et al., 2016; Chen et al., 2017). As one of the most important organelle in cells, mitochondrion plays key roles in the secondary damage processes after TBI, and targeting mitochondrial dysfunction has been shown to be an ideal therapeutic strategy (Hiebert et al., 2015).

The peroxiredoxins (PRDXs) are a family of thiol peroxidases that provide antioxidant defense by scavenging peroxides in cells (Rhee, 2016). Six mammalian PRDX homologs have been identified, named PRDX1-6, which have conserved reactive cysteine residues in the active sites. Among these PRDXs members, PRDX3 and PRDX5 are localized in the mitochondria, PRDX1, PRDX2 and PRDX6 in the cytosol, and PRDX4 found in the endoplasmic reticulum (Cox et al., 2009). As a mitochondrial antioxidant protein, PRDX3 can scavenge not only hydrogen peroxide (H_2O_2), but also peroxynitrite ($ONOO^-$), and thus also known as antioxidant protein-1 (AOP-1) (Mitsumoto et al., 2001). It is the most abundant and efficient H_2O_2-eliminating enzyme in mitochondria of most cell types. PRDX3 has been shown to reduce lipopolysaccharide-induced lung injury in mice (Li et al., 2007), and protect against ibotenic acid-induced hippocampal injury in rats (Hattori et al., 2003). However, the role of PRDX3 in TBI has not been determined. In this study, a mechanical trauma injury model was performed in primary cultured cortical neurons to mimic TBI in vitro and investigate the

* Corresponding author. Department of Neurosurgery, Shaanxi Provincial People's Hospital, The Third Affiliated Hospital, School of Medicine, Xi'an Jiaotong University, Xi'an, Shaanxi 710068, China.
E-mail address: zhangyuelin_xjtu@163.com (Y.-L. Zhang).

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effect of PRDX3. We also investigated the possible related mechanisms with focus on mitochondrial dysfunction and mitochondrial biogenesis.

2. Materials and methods

2.1. Primary culture of cortical neurons

Cortical neurons were cultured from Sprague-Dawley rats using a modified method (Chen et al., 2012). Briefly, cerebral cortices were removed from embryos at 16–18 days, and the tissues were dissociated by 0.25% trypsin digestion for 15 min at 37 °C. After gentle trituration, neurons were resuspended in neurobasal medium containing 2% B27 supplement and 0.5 mM L-Glutamine. Then the neurons were plated on the culture vessels, which were pre-coated with poly-L-Lysine (PLL, 50 μg/ml). Neurons were maintained at 37 °C in a humidified 5% CO_2 incubator and half of the culture medium was changed every other day.

2.2. Traumatic neuronal injury (TNI) model

TNI was performed according to the Faden's method with modifications (Faden et al., 2001). In brief, traumatic injury was performed on cultured cortical neurons by using a punch device that consisted of stainless steel blades joined together. As a result, the injury produced parallel cuts at 2-mm internals that were uniformly distributed through the cell layer. These cuts caused immediate neuronal death under the blade and secondary neuronal injury at a distance from the cuts (Fig. 1A).

2.3. Immunocytochemistry (ICC)

The neurons were fixed with 4% paraformaldehyde, and incubated with 1% H_2O_2 for 10 min. Following two PBS rinses, the cells were incubated with blocking solution for 20 min and incubated with the primary PRDX3 or DMPO antibody at 4 °C overnight. The neurons were then rinsed with PBS and incubated with fluorescein isothiocyanate (FITC) labelled secondary antibodies for 1 h at room temperature. Coverslips were mounted in mounting medium and visualized using a fluorescence microscope.

2.4. Lactate dehydrogenase (LDH) release assay

Neuronal toxicity was determined by measuring the release of LDH with a diagnostic kit according to the manufacturer's instructions. Briefly, 50 μl of supernatant from each group was collected, and the samples were incubated with a reduced form of nicotinamide-adenine dinucleotide (NADH) and pyruvate for 15 min at 37 °C. The activity of LDH was calculated from the absorbance at 440 nm, and the results are presented as fold of the control.

2.5. TUNEL staining

Neurons were fixed by 4% methanol-free formaldehyde for 20 min at room temperature and permeabilized with 0.2% Triton X-100 for 5 min. Neurons were labelled with fluorescein TUNEL reagent mixture for 60 min at 37 °C according to the manufacturer's suggested protocol. Then, the neurons were examined by fluorescence microscopy, and the TUNEL-positive (apoptotic) cells were counted. The apoptotic rate was determined as the percentage of total number of neurons (stained by DAPI).

2.6. Measurement of ROS generation

Intracellular ROS generation was evaluated by the probe 2′,7′-dichlorofluorescin diacetate (DCFH-DA, Sigma). Neurons were incubated with 50 μg/ml DCFH-DA for 1 h at 37 °C in dark, and then washed with PBS. ROS levels were determined by the fluorescence intensity of 2,7-dichlorodihydrofluorescein diacetate (H2DCF-DA) with a fluorescence microscope.

2.7. Measurement of mitochondrial membrane potential (MMP)

MMP was determined by rhodamine 123 (RH123) staining. Briefly, RH123 was added to neurons to achieve a final concentration of 10 mM for 30 min at 37 °C after the cells were washed with PBS. The fluorescence was observed using a microscope with the appropriate fluorescence filters (excitation wavelength of 480 nm and emission wavelength of 530 nm).

2.8. Measurement of ATP synthesis

Neurons were subjected to fission and centrifuged at 12 000 g for 5 min. In 24-well plates, 100 μl of each supernatant was mixed with 100 μl ATP working dilution. Luminance was measured using a monochromator microplate reader. The ATP release levels were expressed as a fold of the luminescence levels in the treated control cells.

2.9. Real-time reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA was prepared from cortical neurons as previously described (Chen et al., 2013). The expression levels of D-loop, ATP8, PGC-1, NRF-1, TFAM and PRDX3 mRNA were determined by RT-PCR. The condition of amplification was: 5 min at 94 °C; 35 cycles of 45 s at 94 °C, 1 min at 56 °C, 1 min at 72 °C; followed by 10 min at 72 °C. The relative expression value was normalized to the expression value of GAPDH.

2.10. Western blot analysis

Total protein isolated from each sample was loaded and separated by 12% SDS-PAGE gels, and transferred to polyvinylidene difluoride (PVDF) membranes. Membranes were blocked with 5% skimmed milk solution in TBST for 1 h, and incubated overnight at 4 °C with the primary antibody dilutions. After that the membranes were washed and incubated with secondary antibodies for 1 h at room temperature. Immunoreactivity was detected with Super Signal West Pico Chemiluminescent Substrate (Thermo Scientific, Rockford, IL, USA). Image J software was used to quantify the optical density of each band.

2.11. Statistical analysis

Statistical evaluation was carried out with GraphPad Prism software by one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparisons. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Expression of PRDX3 after traumatic injury in cortical neurons

As shown in Fig. 1A, neurons in TNI group showed damaged neurites and shrunken cell bodies. The morphological staining showed that PRDX3 expression was significantly reduced in

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