FEBS Letters 583 (2009) 1511–1515

journal homepage: www.FEBSLetters.org

TNF-related apoptosis-inducing ligand suppresses PRDX4 expression

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ARTICLE INFO

Article history:
Received 17 March 2009
Accepted 4 April 2009
Available online 11 April 2009

Edited by Barry Halliwell

Keywords:
PRDX4
Transcription
TRAIL

ABSTRACT

TNF-related apoptosis-inducing ligand (TRAIL) is currently considered a promising target for developing anti-cancer therapies. Accumulating evidences have now shown that oxidative stress is involved in the TRAIL-mediated cell death. The peroxiredoxins (PRDXs) are a ubiquitous family of proteins involved in protection against oxidative stress through the detoxification of cellular peroxides. Here we demonstrated that endogenous expression of PRDX4 was significantly decreased by TRAIL at the transcriptional level. In addition, overexpression of PRDX4 dramatically suppressed TRAIL-induced apoptosis. Taken together, these data for the first time suggested that TRAIL suppressed the PRDX4 gene at the transcriptional level and that downregulation of PRDX4 might facilitate cell death induced by TRAIL.

1. Introduction

TNF-related apoptosis-inducing ligand (TRAIL) is now considered to be a promising anticancer reagent, since its selective cytotoxicity in transformed tumor cells but not in normal cells [1,2]. TRAIL engages the extrinsic apoptotic pathway by binding to its membrane-bound death receptors (DR4 and DR5), an event followed by recruitment of intracellular adaptor molecule FADD and apoptosis initiator procaspase-8, forming the death-inducing signaling complex (DISC). When caspase-8 is cleaved, active fragment of caspase-8 is released into the cytosol where executioner of apoptosis, caspase-3 is activated for apoptosis [3]. The activation of caspase-8 by TRAIL induces the translocation of other cytosolic proapoptotic proteins to the mitochondria, causing a dissipation of the mitochondrial membrane potential [4]. Consequently, mitochondria release reactive oxygen species (ROS) and pro-apoptotic proteins into the cytoplasm thus inducing cellular and DNA damage [5]. The activation of pro-caspase-8 is believed to be dependent solely on proximity to other pro-caspase-8 units during recruitment to the DISC [6]. However, accumulating evidences suggested that whereas proximity is required for activation of pro-caspase-8, ROS could modulate the initiation of apoptosis signaling [7–10].

Peroxiredoxins (PRDXs) comprise an extended family of small antioxidant proteins which conserve a thioredoxin-dependent catalytic function that can contribute to cell protection from ROS [11,12]. PRDXs perform their protective antioxidant role in cells through their peroxidase activity whereby hydrogen peroxide, peroxynitrite and a wide range of organic hydroperoxides are reduced and detoxified [12]. At least six PRDXs were identified in mammalian cells [11,12]. In human, PRDX1–4, the typical 2-Cys subgroup, share two conserved motifs centered on Cys residues, which contain an additional 'resolving' cysteine near the C-terminus. PRDX5 differs because its C-terminal cysteine is not in the conserved position [13,14]. PRDX6 conserves only the Cys nearer the NH2-terminus, which is the catalytic site. PRDX are ubiquitously and abundantly expressed in the various tissues of the human body [15] and were reported to act in a mutually non-redundant and sometimes stress-specific fashion to protect human cells from oxidant injury [16].

ROS generation is one of the critical events in TRAIL-induced cancer cell death, but the role of PRDXs during TRAIL treatment has not been explored. We found that TRAIL specifically suppressed PRDX4 expression at the transcriptional level. In some cancer cells TRAIL did not cause cell death in spite of PRDX4 decrease. However, our data indicated that subtraction of PRDX4 during apoptosis, although not sufficient for initiation of apoptosis, is probably relevant in promoting cell death, since overexpression of PRDX4 dramatically suppressed TRAIL-induced apoptosis. Our data suggested that although PRDX4 suppression per se was not sufficient to induce cell death, its downregulation might facilitate cell death induced by TRAIL.

2. Materials and methods

2.1. Cell culture

The panel of cancer cells were maintained in DMEM (Sigma–Aldrich, Saint Louis, MO) supplemented with 10% FBS (Sigma–Aldrich).

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doi:10.1016/j.febslet.2009.04.009

2.2. Reagents and antibodies

Human recombinant TRAIL and actinomycin D were purchased from Calbiochem (San Diego, CA) and Sigma–Aldrich (Saint Louis, MO), respectively. Western immunoblotting was performed using primary antibodies against PRDX1 (Novus Biologicals Inc., Littleton, CO), PRDX2 (GeneTex Inc., San Antonio, TX), PRDX3 (Novus Biologicals Inc.), PRDX4 (Abcam, Cambridge, MA), PRDX5 (Abnova, Walnut, CA), PRDX6 (Abcam), cytochrome c (BD Bioscience, San Jose, CA), poly ADP-ribose polymerase (PARP) (Cell Signaling Technology, Danvers, MA) or γ-tubulin (Sigma, Saint Louis, MO).

2.3. RNA isolation and real-time reverse transcription-polymerase chain reaction (RT-PCR)

RNA isolation and real-time RT-PCR were performed as previously reported [17]. 5′-CACAGCTGTTATGCCAGATG-3′ and 5′-ACT GAAAGCAATGATCTCCG-3′, 5′-AGATCATCGCGTTCAGCAAC-3′ and 5′-ATCCTCAGACAAGCGTCTGG-3′, 5′-GTCGCAGTCTCAGTGGATTC-3′ and 5′-AACAGCACACCGTAGTCTCG-3′, 5′-AACAGCTGTGATCGA TGGAG-3′ and 5′-TCAAGTCTGTCGCCAAAAGC-3′, 5′-CAAGAAGGG TGTGCTGTTTG-3′ and 5′-TAACACTCAGACAGGCCACC-3′, 5′-ATGCC TGTGACAGCTCGTGTG-3′ and 5′-TCTTCTTCAGGGATGGTTGG-3′ primer pairs were used to amplify PRDX1–6, respectively. For β-actin, the forward primer was 5′-GAGACCTTCAACACCCCAGCC-3′ and the reverse was 5′-GGATCTTCATGAGGTAGTCAG-3′. Results were normalized against those of β-actin.

2.4. Isolation of cytosolic and mitochondrial fractions

Cytosolic and mitochondrial fractions from HeLa cells were prepared as previously described [18].

2.5. Western blot analysis

Cells were lysed in lysis buffer (20 mM Tris–HCl, 150 mM NaCl, 2 mM EDTA, 1% Triton-X100 and protease inhibitor cocktail (Sigma–Aldrich). Cell extract protein amounts were quantified using the BCA protein assay kit. Equivalent amounts of protein (25 μg) were separated using 12% SDS–PAGE and transferred to PVDF membrane (Millipore Corporation, Billerica, MA).

2.6. Generation of PRDX4 promoter luciferase constructs

The 5′-flanking region of human PRDX4 genomic DNA between −979 and +24 (+1 represents the translation start site, and the upstream nucleotide adjacent to the transcription start site is defined here as −1) was amplified by PCR from HeLa genomic DNA and subcloned into the reporter plasmid pGL4 (Promega, Madison, WI).

2.7. Luciferase assay

The luciferase activity was determined using the Dual-Luciferase® Reporter Assay System (Promega), according to the manufacturer's instructions. All transfection experiments were repeated for three times in triplicate. Firefly (*Photinus pyralis*) luciferase activities normalized by *Renilla* (*Renilla reniformis*) activities are presented as fold induction relative to the normalized firefly luciferase activity in cells transfected with the pGL4 empty vector only, which was taken as 1.0.

2.8. Construction of PRDX4 plasmid and generation of HeLa cells stably overexpressing PRDX4

A cDNA encoding human PRDX4 was generated by polymerase chain reaction (PCR) from human brain cDNA library (Invitrogen, Carlsbad, CA) and subcloned into the eukaryotic expression plasmid pcDNA3 (pcDNA3-PRDX4). HeLa cells were transfected with pcDNA3-PRDX4 or an empty vector (pcDNA3-Flag) using Lipofectamine 2000 according to the protocol of the manufacturer. 48 h later, the cells were incubated in growth medium containing G418 (800 μg/ml, Life Technologies) to select stable clones. Four stable clones were selected based on the overexpression of PRDX4, which was confirmed by Western blotting.

2.9. Detection of cell death

For cell death assays, cells were washed twice in phosphate-buffered saline and then stained with Annexin V-FITC (Biovision, Mountainview, CA) and propidium iodide (PI, Sigma–Aldrich) according to the manufacturer's instructions. After staining with annexin V-FITC and PI, samples were analyzed by fluorescence-activated cell scanner (FACScan) flow cytometer (Becton Dickinson, Franklin Lakes, NJ).

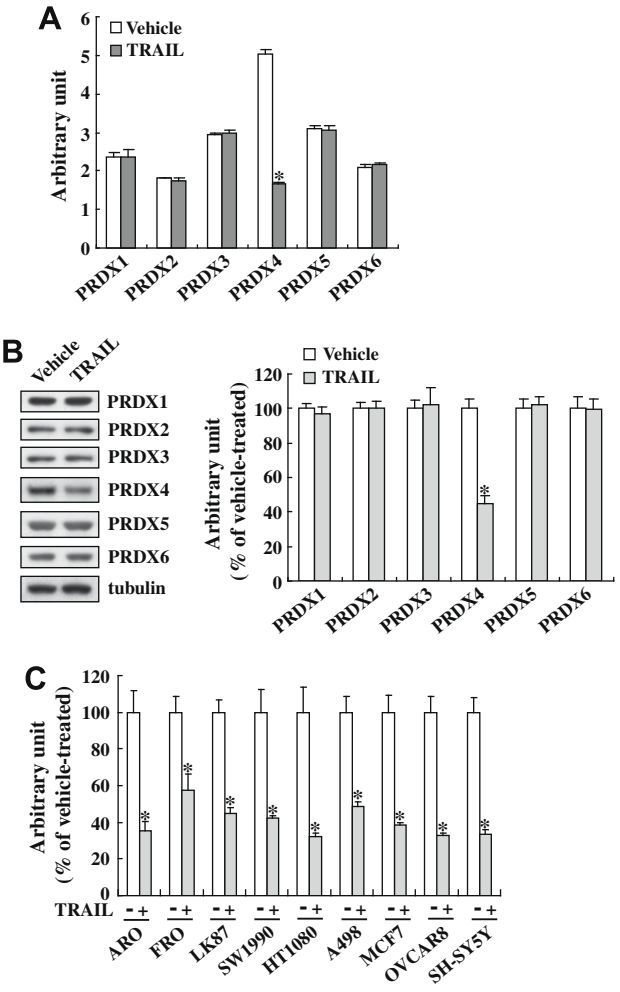

Fig. 1. The effect of TRAIL exposure on the PRDXs expression. (A) 8 h after cells had been treated with 1000 ng/ml TRAIL, total cellular RNA was extracted and real-time RT-PCR was performed. (B) HeLa cells were treated with 1000 ng/ml TRAIL for 8 h, total cellular proteins were isolated and Western blot was performed using indicated antibodies. A representative image was presented and the ratios *vs* that of vehicle-treated (normalized by tubulin) were graphed on the right of the image ($n = 3$). C, A panel of cancer cells was treated with 1000 ng/ml TRAIL for 8 h and PRDX4 expression was measured using real-time RT-PCR. All experiments were repeated three times, and each experimental condition was repeated in triplicate in each experiment. Data reported were average values ±S.D. of representative experiments. $^{*}P < 0.01$ as compared with vehicle-treated.