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Redox Regulation by Thioredoxin Superfamily; Protection Against Oxidative Stress and Aging

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Thioredoxin (TRX) is a 12 kD protein with redox-active dithiol in the active site; -Cys-Gly-Pro-Cys-. We originally cloned human TRX as adult T cell leukemia derived factor (ADF) produced by HTLV-I transformed cells. TRX and related molecules maintain a cellular reducing enviroment, working in concert with the glutathione system. Physiologically, TRX has cytoprotective effects against oxidative stress. TRX promotes DNA binding of transcription factors such as NF-kB, AP-1, p53, and PEBP-2. The TRX superfamily, including thioredoxin-2 (mitochondrial thioredoxin) and glutaredoxin, are involved in biologically important phenomena via the redox-regulating system. Thioredoxin-binding protein-2, which we recently identified by a yeast two-hybrid system, is a type of endogenous modulator of TRX activity. TRX is secreted from the cells and exhibits cytokine-like and chemokine-like activities. Redox regulation by TRX plays a crucial role in biological responses against oxidative stress.

Keywords: Thioredoxin, Thioredoxin superfamily, Thioredoxin-2, Glutaredoxin, oxidative stress, Thioredoxin-binding protein

INTRODUCTION

Cells are exposed to various stresses, among which oxidative stress has been studied intensively. Reactive oxygen species (ROS) generated by a variety of oxidative stresses are harmful to cells. However, recent research has provided evidence that ROS plays important roles in signal transductions of cellular activation and cell death. Multiple mechanisms against oxidative stress exist to protect cells.

Thioredoxin is a small protein of 12kDa involved in many different functions each of which is dependent on thiol-disulfide interchange catalyzed by the conserved active site WCGPC. TRX operates together with NADPH and TRX reductase as an efficient reducing system of exposed protein disulfides [1]. Several transcription factors including AP-1, NF-kB, p53, and PEBP-2, and nuclear receptors like glucocorticoid receptors or estrogen receptors have been shown to be activated by TRX. Moreover, TRX is secreted from the cells and regulates the redox balance extracellularly, showing cytokine- and chemokine-like functions. Recently, several proteins sharing the similar active sites and similar three-dimensional structures with TRX have

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been cloned. These proteins are known as members of the TRX superfamily (Table I), and are involved in biologically important phenomena via the redox-regulating system.

EXTRACELLULAR FUNCTIONS OF TRX

Previous reports have shown that TRX is secreted from the cells through a unique leaderless pathway like IL-1 beta [2]. Since TRX was cloned as ADF [3], a cytokine-like factor, accumulating evidence has shown that TRX possesses cytokine-like functions. Exogenous TRX enhances the cell growth in 3B6 by itself [4] and shows comitogenicity with other cytokines. Moreover, TRX shows chemokine-like functions. Eosinophil cytotoxicity enhancing factor was revealed to be a truncated form of TRX comprising N-terminal 1–80 or 1–84 amino acids [5]. TRX was previously reported to have a chemotactic effect on eosinophils [6] and recently to be chemoattractive for neutrophils, monocytes and lymphocytes [7]. More recently, oxidized TRX has been shown to be more chemoattractive for polymorphonuclear cells than reduced TRX. Intravenous injection of TRX suppresses extravasation of leukocytes induced by lipopolysaccharides in the mouse air pouch model. Several reports have shown that exogenous administration of TRX protects the cells or tissues from damage caused by anti-Fas antibody [8], hydrogen peroxide [9], and ischemia-reperfusion [10]. TRX also prevents EBV-transformed cells from proceeding into the lytic phase, and regulates the cohabitation of EBV and its host cells.

TRX SUPERFAMILY

Thioredoxin-2 (TRX2)

Thioredoxin-2 (TRX2) was cloned as a mitochondria TRX by Spyrou et al [11]. TRX2 is a 18.2-kDa protein possessed of the conserved TRX – active site, Cys-Gly-Pro-Cys, and localized in the mitochondria. TRX2 differs from TRX by the presence of a 60-aminoacid extension at the N terminus. This extention has properties characteristic of a mitochondrial translocation signal. Futhermore, TRX2 lacks structural cysteine. All previously described mammalian TRX have 2 or 3 additional cysteine residues to the 2 cysteines located in the active site. These structural or noncatalytic cysteine residues can be oxidized to form a dimer which leads to inactivation. In contrast, TRX2 has resistance to oxdation. However, the function of TRX2 is unknown.

TABLE I Members of the Thioredoxin superfamily

member	*kD*	*location*	*Active site*
Thioredoxin	12	cytoplasm	-Cys-Gly-Pro-Cys-
Thioredoxin 2	12 (18)	mitochondria	-Cys-Gly-Pro-Cys-
Thioredoxin- related protein 32	32	cytoplasm	-Cys-Gly-Pro-Cys-
Glutaredoxin	12	cytoplasm	-Cys-Pro-Tyr-Cys-
Nucleoredoxin	48	nucleus	-Cys-Pro-Pro-Cys-
Protein disulfide isomerase	55	endoplasmic reticulum	-[Cys-Gly-His-Cys]$_2$-
Ca binding protein 1	49	endoplasmic reticulum	-[Cys-Gly-His-Cys]$_2$-
Ca binding protein 2	72	endoplasmic reticulum	-[Cys-Gly-His-Cys]$_3$-
Phosphlipase C gamma	61	endoplasmic reticulum	-[Cys-Gly-His-Cys]$_2$-

To investigate the biological mechanism of TRX2 action, we disrupted the TRX2 gene in DT40, chicken B cell line, by homologus recombination and created DT40 clones heterozygous for the TRX2 gene (TRX2 +/-).

TRX2 expression in TRX2 +/- mutant clone was lower than that in wild-type clone (Fig. 1). The specific mitochondrial respiratory chain inhibitor antimycin A induced cell death in TRX2 +/- mutant clone more than in wild-type cells (Fig. 2). This suggests that heterozygous mutant clones carrying a targeted disruption of the TRX 2 gene in chicken DT-40 cells, are more sensitive to oxidative stress.

Further investigate TRX2 function, we constructed TRX2- null clones TRX2-/- cells expressing a tetracyclin (tet)-responsible chicken TRX2 transgene.

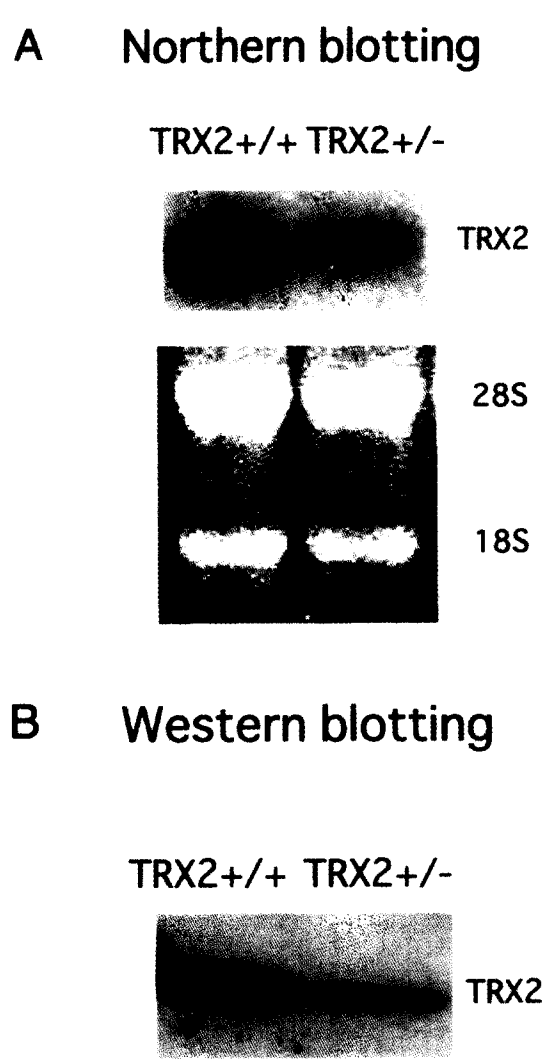

FIGURE 1 TRX2 expression in DT40cells or TRX2+/- mutant cells A: Northern blotting B: Western blotting

Glutaredoxin

Glutaredoxin (GRX) is a 12kD redox-active protein catalyzing GSH-dependent disulfide reduction. There is accumulating evidence that GRX as well as TRX plays an important role in redox regulation of signal transduction. GRX regulates the activation of transcription factors such as nuclear factor I [12], OxyR [13] and PEBP-2 [14]. We detected differential expressions of GRX and TRX in the differentiation of macrophage [15] and mouse embryos. It is also reported that GRX is detected within the HIV-1 virus and regulates the activity of glutathionylated HIV-1 protease [16].

Thioredoxin-binding proteins

To investigate the molecular mechanism of TRX action, we used a yeast two-hybrid system to identify TRX-binding proteins. TRX-binding protein-1 (TBP-1) is p40 phox, a cytosolic component of phagocyte NADPH oxidase [17]. One of the candidates, designated as thioredoxin-binding protein-2 (TBP-2), was identical to vitamin D3 up-regulated protein 1 (VDUP1). TBP-2/VDUP1 was originally reported as an up-regulated gene in HL-60 cells stimulated by 1,25-dihydroxyvitamin D3 [18].

The function of VDUP1 remains unclear, although several homologous sequences from some mammals have been reported. In COS-7 and HEK293 cells transiently transfected with TBP-2 expression vector, a decrease in the insulin reducing activity of TRX and diminishment of

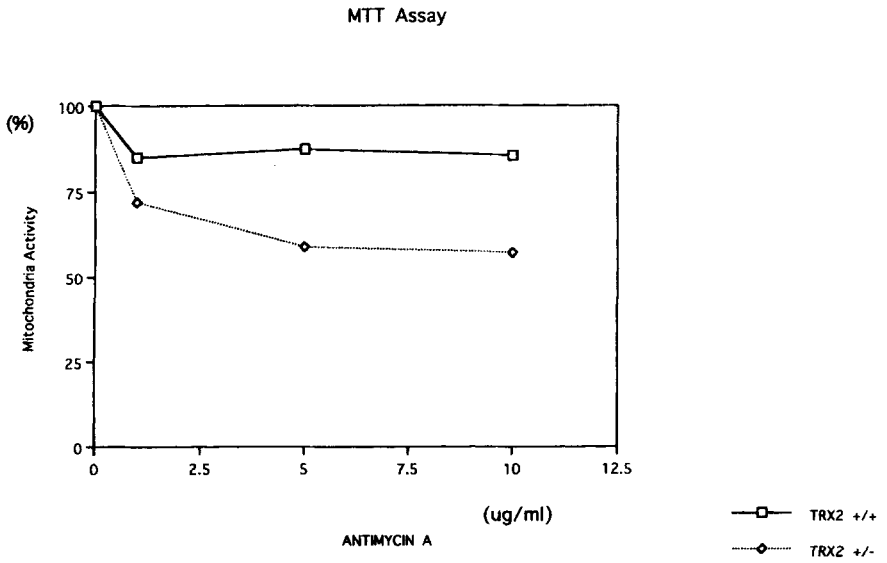

FIGURE 2 MTT assay

TRX expression was observed. Treatment of HL-60 cells with vitamin D3 Induced an increase in TBP-2 expression and down-regulation of the expression and reducing activity of TRX. Therefore, TBP-2 may serve as a negative regulator of the biological function and expression of TRX [17].

TRX KNOCKOUT AND TRANSGENIC MICE

In mice, heterozygotes carrying a targeted disruption of the mouse TRX gene are viable, fertile and appear normal. In contrast, homozygous mutants die shortly after implantation. This suggests that TRX is essential for the early differentiation and morphogenesis of the mouse embryo [19]. Overexpression of human TRX by beta-actin promoter in mice attenuates focal cerebral ischemia [20]. These TRX-transgenic mice are more resistant to oxidative stress such as paraquat (Hirabayashi et al. in preparation) and survive longer than control C57BL/6 mice (Mitsui et al. in preparation). Interestingly, specific-overexpression of human TRX by insulin promoter in pancreatic islet beta-cells in mice prevents autoimmune and streptozocin-induced diabetes [21].

CONCLUSION

Since ADF was proven to be human TRX, various biological functions of the redox-regulating system including TRX have been clarified. Recently members of molecules belonging to redox-regulating proteins including the TRX superfamily are expanding rapidly. There is accumulating evidence that redox-regulation is deeply involved in biologically important phenomena such as differentiation and apoptosis. Further analysis of redox-regulation in biological responses will contribute to new therapeutic approaches and to the development of new biotechnological tools.

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