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Forum Mini Review

The Role of Thioredoxin in the Aging Process: Involvement of Oxidative Stress

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ABSTRACT

Reactive oxygen species are produced by various stressors derived from internal and external sources, including endogenous metabolic activities. Glucose metabolism is one of the most primitive sources for energy production for most cells; however, it may at the same time yield hazardous oxidative stress via simultaneous oxidant production. The protective mechanism against oxidative stress is thus an indispensable biological function. Recently, genetic mutation loci affecting life span were isolated from experimental model organisms, and several locus products were found to be closely linked with machinery either producing or defending oxidative stress. Thioredoxin (TRX) is a small protein having strong antioxiradical quenching capabilities and other multiple functions depending on the cellular redox state. In this review, we focus on the role of TRX in the aging process (senescence) as a redox-regulating molecule against oxidative stress. We also discuss the possibility of the TRX system serving as an index marker for cellular proliferation and senescence. *Antioxid. Redox Signal.* 5, 563–570.

THIOREDOXIN (TRX) AS AN ANTIOXIDANT DEPENDING ON CELLULAR REDOX STATE

GLUCOSE METABOLISM is one of the major biological systems for producing necessary ATP molecules in conjunction with mitochondrial aerobic respiration. However, this energy production system at the same time yields hazardous materials such as reactive oxygen species (ROS) and other free radical species such as nitric oxide. Active aerobic respiration may increase the chance of electrons leaking from the mitochondrial electron transfer system, which causes severe cellular damage. A protective mechanism against oxidative stress is thus an indispensable biological function.

There are many proteins and low-molecular-weight antioxidants in the typical antioxidant system. One kind is radical quenching or scavenging proteins that include superoxide dismutase (SOD), catalase, glutathione peroxidase, glutathione reductase, TRX, peroxiredoxin, and TRX reductase. Another kind includes low-molecular-weight antioxidants such as glutathione, vitamin C, vitamin E, uric acid, and bilirubin. A small thiol protein, TRX, has recently attracted much attention due to its strong antioxiradical quenching capabilities and other important biological functions related to the regulations of cellular redox state.

TRX has been cloned as an adult T-cell leukemia-derived factor, produced by human T-cell leukemia virus-I-transformed T-cells (58, 69), or as an interleukin-1-like autocrine growth factor from Epstein–Barr virus-transformed cells (65). TRX is a 12-kDa thiol-mediated protein with a redox-active disulfide/dithiol group within the conserved active-site sequence Cys-Gly-Pro-Cys. Reduced TRX catalyzes the reduction of disulfide bonds in many proteins, and oxidized TRX is re-

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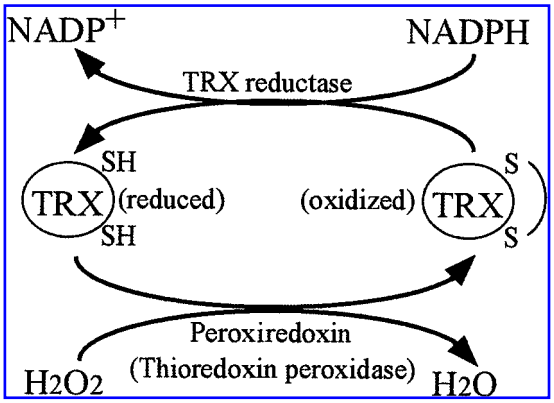

FIG. 1. TRX reducing cycle. TRX exists in either a reduced form containing two thiol groups or an oxidized form containing a disulfide bond. Oxidized TRX is reduced by TRX reductase and NADPH. The reduced form of TRX can reduce disulfide bonds of target proteins.

versibly reduced by the action of TRX reductase and NADPH (18) (Fig. 1). The TRX system (TRX, TRX reductase, and NAPDH) is widely conserved from fungus to higher eukaryotes. Several TRX-related molecules have been identified and are actively involved in the cellular antioxidant system and redox regulation with other TRX family proteins. Table 1 summarizes the TRX superfamily, which holds similar active site sequences regardless of the localization they express.

EXTENDED LIFE SPAN MUTANTS AND RESISTANCE TO OXIDATIVE STRESS

Caloric restriction (CR) has been identified as the only unequivocally accepted scheme to extend life span significantly for most organisms. Recently, the molecular basis of CR has been partially revealed as a reduced load of growth hormone signal transduction and the insulin pathway. Insulin is a primitive mitogenic factor that promotes glucose uptake by cells when the extracellular glucose concentration is high. Glucose metabolism is one of the most primitive sources for energy production for most cells, and is critical to support general biological activities. There is accumulating evidence that defective growth hormone components and insulin pathways frequently affect life span (Table 2). Since the first extended life span mutant *age-1,* a homologue of phosphatidylinositol 3-kinase (PI3K) in mammals, was reported in *C. elegans* (25), several similar mutations have been identified as extending life span significantly, including *daf-2* (insulin/IGF-I receptor homologue), *daf-16* (forkhead transcription factor) in *C. elegans* (29, 34), and *chico* (IRS, insulin receptor substrate), *InR* (insulin receptor) in *D. melanogaster* (8, 61). In mammals, $prop1^{df/df}$ (Ames dwarf) and $pit1^{dw/dw}$ (Snell dwarf) mice both display impaired pituitary gland development and lower levels of growth hormone (3, 14), and newly reported knockout mice of insulin receptor (2) and IGF-1 receptor (19) were all found to have significantly extended life span.

Many studies have indicated that CR treatment leads to strong resistance against various oxidative stresses in many animals (1, 13). It should be also noted that extended life span mutants caused by growth hormone defects and the insulin pathway simultaneously demonstrated strong resistance to various stresses, including free radical species. *Age-1* and *daf-2* showed strong resistance against oxidative stress (21, 33, 55) and increased mitochondrial Mn-SOD activities (20). Moreover, many extended and reduced life span mutation genes in *C. elegans* have been found to be components of the mitochondrial electron transport system such as *clk-1* (CoQ enzyme), *isp-1* (complex III), *gas-I* (complex I), and *mev-1* (complex II) (12, 23, 28, 32). Methuselah (*mth*) was first isolated as an extended life span mutant in *D. melanogaster* and found to show strong resistance to paraquat-induced oxidative stress (35). Several transgenic fruit flies introducing SOD and catalase (49), msra (52), and human SOD-1 (50) showed significant extension of life span. It is therefore possible that lowering the efficiency of the insulin pathway may affect energy production in mitochondria. When efficient energy production drops due to insufficient amounts of glucose,

TABLE 1. LIST OF TRX SUPERFAMILY GENES

TRX family gene	*kDa*	*Localization*	*Active-site sequence*
Thioredoxin (TRX)	12	Cytosol	-Cys-Gly-Pro-Cys-
Thioredoxin-2 (TRX-2)	12	Mt	-Cys-Gly-Pro-Cys-
TRX-related protein (TRP32)	32	Cytosol	-Cys-Gly-Pro-Cys-
Transmembrane TRX-related protein (TMX)	30	ER	-Cys-Pro-Ala-Cys-
Sperm-specific TRX	53	Cytosol	-Cys-Gly-Pro-Cys-
Glutaredoxin (GRX)	12	Cytosol	-Cys-Gly-Tyr-Cys-
Glutaredoxin-2 (GRX-2)	18	Nucleus, Mt	-Cys-Ser-Tyr-Cys-
Nucleoredoxin	48	Nucleus	-Cys-Pro-Pro-Cys-
Protein disulfide isomerase (PDI)	55	ER	-[Cys-Gly-His-Cys]$_2$-
Ca binding protein 1 (CaBP1)	49	ER	-[Cys-Gly-His-Cys]$_2$-
Ca binding protein 1 (Erp72)	72	ER	-[Cys-Gly-His-Cys]$_3$-
Phospholipase Cγ (PLCγ)	61	ER	-[Cys-Gly-His-Cys]$_2$-

ER, endoplasmic reticulum; Mt, mitochondria.

TABLE 2. SUMMARY TABLE FOR EXTENDED LIFE SPAN MUTANTS IN EXPERIMENTAL MODEL ORGANISMS

Loci	*Species*	*Life span*	*Putative protein function*	*Reference*
[*sir-2*]	*S. cerevisiae*	+40%	Histone deacetylase	22
mth	*D. melanogaster*	+35%*	G protein coupling receptor	35
indy	*D. melanogaster*	+50%***	Sodium dicarboxylate cotransporter	51
[*sod*]	*D. melanogaster*	+48%	Superoxide dismutase	56
[*sod/cat*]	*D. melanogaster*	+30%	Superoxide dismutase/catalase	49
[*msra*]	*D. melanogaster*	+70%	Methionine sulfoxide reductase	52
age-1	*C. elegans*	+110%***	PI3K p110 subunit	25
daf-2	*C. elegans*	+100%*	Insulin-like/IGF-I receptor	29
daf-16	*C. elegans*	ND	Forkhead transcription factor	34
clk-1	*C. elegans*	+175%*	Coenzyme Q10 synthesis	32
isp-1	*C. elegans*	+62%	Electron transport complex III	12
inr	*D. melanogaster*	+85%	Insulin-like receptor	61
chico	*D. melanogaster*	+48%**	Insulin receptor substrate	8
prop-1	Mouse	+64%*	Pituitary function	3
pit-1	Mouse	+40%*	Pituitary function	14
p66shc	Mouse	+30%*	Tyrosine receptor adaptor	39
ghr	Mouse	+55%*	Growth hormone receptor	71
inr	Mouse	+18%*	Insulin receptor	2
igf1r	Mouse	+33%*	IGF-1 receptor	19
[*trx*]	Mouse	+35%**	Thioredoxin	41

When life span varies among sex, longest life spans are adopted. The listed life spans are average (*), median (**), or maximum (***) depending on the source, and no symbol means not described in the source. Life spans of hetrozygous mutants are indicated in case lethal phenotypes were observed in homozygous mutants. [ ], transgenic strain or augmented expressions of the loci; ND, not described in detail or ineffective effect by locus alone.

it may reduce ROS, potential by-products of aerobic respiration. Reduction of harmful intermediates may explain why CR treatment can extend life span for most species.

LIFE SPAN EXTENSION BY AUGMENTED EXPRESSION OF TRX IN THE MODEL ANIMAL

In mammals, at least two studies of knockout mice suggested evidence for a direct connection between oxidative stress and life span. One study showed that the $p66^{shc-/-}$ mouse had a significantly extended life span and enhanced resistance to paraquat-induced oxidative stress (39). The ablation of $p66^{shc-/-}$ also enhanced cellular resistance to plasma low-density lipoprotein oxidation, arterial oxidation epitopes, and early antherogenic lesions (46). Another study showed that the methionine sulfoxide reductase A (*msra*) −/− mouse had a shorter life span and high sensitivity toward oxidative stress (42). As for the biological function of msra, it catalyzes the reduction of oxidized methionine in protein by converting methionine sulfoxide to methionine. The catalytic enzyme reaction is completely dependent on the TRX redox system. TRX can serve as an electron donor in order to reduce oxidized form of msra, therefore, the effective reaction of msra is closely associated with TRX, TRX reductase, and NADPH. Meanwhile, Ruan *et al.* recently reported that msra transgenic animals showed significant resistance to paraquat-induced oxidative stress and extended life span (52). It will be then intriguing to study further possible interactions between msra and TRX on the role of msra-mediated life span extension.

As TRX is a strong antioxidant protein that has significant quenching capability against induced oxidative stress, it serves to reduce cellular oxidative stress significantly (40, 44). Although age-associated changes of the TRX system have not been studied extensively, several reports demonstrate decreased activity along with age (54). It should be also noted that CR treatment can prevent age-associated reduction of TRX as well as TRX reductase in cytoplasm (7). Therefore, the TRX system can be effective in controlling an animal's life span. We have recently produced human TRX transgenic mice driven under the control of the human β-actin promoter to elucidate the direct influence of the TRX redox system on an animal's life span. The TRX transgenic mice showed not only strong resistance to oxiradical stress in a variety of ischemic tissues and organs, but also significant extension of the maximum life span (22%) and median life span (35%) (41, 59) (Fig. 2). These findings demonstrate that antioxidant functions of the cellular redox system, including TRX, effectively contribute to lengthening an animal's life span, even in mammals.

TRX SUPERFAMILY INVOLVING CELLULAR RESPONSES AGAINST OXIDATIVE STRESS

If food availability is limited and cells are under harsh conditions, organisms cannot repair and support their biological activities. As a result, the cellular proliferation is completely blocked at the G_1 cell cycle, and the typical phenotypes of cells will show virtually similar multiple aspects of cellular aging or senescence. Indeed, several genetic mutations have

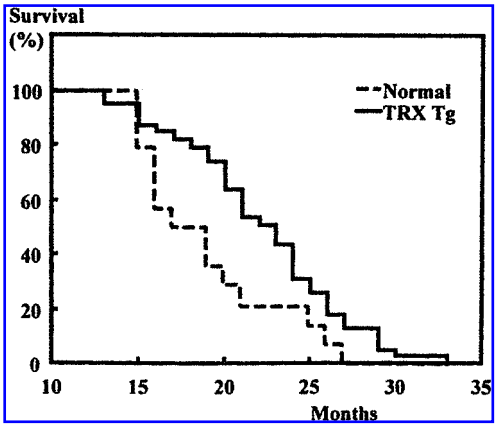

FIG. 2. Life span of the TRX transgenic mice. TRX transgenic mice had significantly extended life span as shown by the survival curves of TRX transgenic (Tg) mice (solid line) and wild-type control (dashed line). Survival curves were plotted according to the Kaplan–Meier method (41).

been identified to show shorter life spans if continuous cellular proliferation is inhibited by DNA damage (11), DNA repair defects (10, 64, 70), enhanced apoptosis (62), or decreased telomere length (17, 66) (Table 3). Those reduced life span mutants are frequently associated with conspicuous growth retardation some time after birth. If the cellular damages exceed a certain point, the cellular antioxidant system, including TRX, is also no longer effective at regulating the redox state and life span. In such cellular conditions, there is accumulating evidence that the expression of TRX is decreasing and the cellular apoptosis pathway is activated. TRX was found to bind and inhibit apoptosis signal regulating kinase (ASK-1), which is an important factor for initiating the p38 mitogen-activated protein (MAP) kinase-mediated apoptosis signal pathway (53). ROS and cytotoxic cytokines including tumor necrosis factor activate ASK-1 through oxidation of TRX to dissociate from ASK-1 (36). In addition, overexpression of TRX negatively regulates p38 MAP kinase signal transduction and p38 MAP kinase-mediated cytokine production, indicating that TRX has an important role in p38 MAP kinase activation (16).

On the other hand, the expression of TRX tends to be enhanced in tumor cell lines and tissues (27, 38, 45), and transfection of human TRX enhances cellular proliferation (15). At the same time, TRX-2 is uniquely expressed in mitochondria, where it regulates the mitochondrial redox state and plays an important role in cell proliferation (9, 60). TRX-2 was found to form a complex with cytochrome *c* localized in the mitochondrial matrix, and the releasing of cytochrome *c* from the mitochondria is significantly enhanced when the expression of TRX-2 is inhibited (48, 60). Overexpression of TRX-2 demonstrates resistance to oxidant-induced apoptosis in human ostosarcoma cells, indicating the critical role in the protection against apoptosis via TRX-2 in mitochondria (6) (Fig. 3). As both TRX and TRX-2 are known as regulators of the manifestation of apoptosis under redox-sensitive caspase (63), potential coordinated actions between TRX and TRX-2 may be possible. However, the function of TRX-1 and TRX-2 does not seem to compensate completely because knockout mice of TRX-2 were found be embryonic lethal (48).

TRX binding protein-2 (TBP-2) identified by yeast two-hybrid screening binds to reduced TRX, but not oxidized TRX (47). TBP-2 was originally identified as a vitamin D upregulated protein 1 (VDUP 1) in HL-60 cells treated with 1α,25-dihydroxyvitamin D (5), and is thought to be a negative regulator of TRX (47). Several cysteine residues were identified, and some of them were assumed to bind the active site of TRX (67). Therefore, the overexpression of TBP-2 may block the action of TRX, eventually leading to the inhibition of cellular proliferation. TBP-2 was indeed isolated from a cDNA library enriched for mRNA species that immediately increase by administration of BrdU as a senescence-associated gene (57). Enhanced expression of TBP-2/VDUP 1 was also reported to be sensitive to paraquat-induced oxidative stress (24). On the other hand, a significant reduction in TBP-2 expression was frequently observed in several tumor cell lines and tumor tis-

TABLE 3. SUMMARY TABLE FOR REDUCED LIFE SPAN MUTANTS IN EXPERIMENTAL MODEL ORGANISMS

Loci	*Species*	*Life span*	*Putative protein function*	*Reference*
sir-2	*S. cerevisiae*	−50%*	NAD-dependent deacetylase	26
gas-1	*C. elegans*	−25%	Electron transport complex I	28
mev-1	*C. elegans*	−30%*	Electron transport complex II	23
*top3*β	Mouse	−35%*	DNA topoisomeraseβ	31
ttd	Mouse	−50%*	Nucleotide excision repair	10
[*p53*]	Mouse	−65%***	Tumor suppressor	62
msra	Mouse	−40%*	Methionine sulfoxide reductase	42
ku86	Mouse	−61%*	Double-strand break DNA repair	64
terc	Mouse	ND	Telomerase	17
klotho	Mouse	−92%*	Calcium regulation?	30
wrn	Mouse	(−83%)	DNA helicase	37
lmna	Mouse	−95%	A-type lamins	43

Most reduced life span mutants in lower organisms were omitted. When life span varies among sex, longest life spans are adopted. The listed life spans are average (*), median (**), or maximum (***) depending on the source, and no symbol means not described in the source. Life span of *wrn* is relative to p53 null background. Life spans of hetrozygous mutants are indicated in case lethal phenotypes are observed in homozygous mutants. [ ], transgenic strain or augmented expressions of the loci; ND, not described in detail or ineffective effect by locus alone.

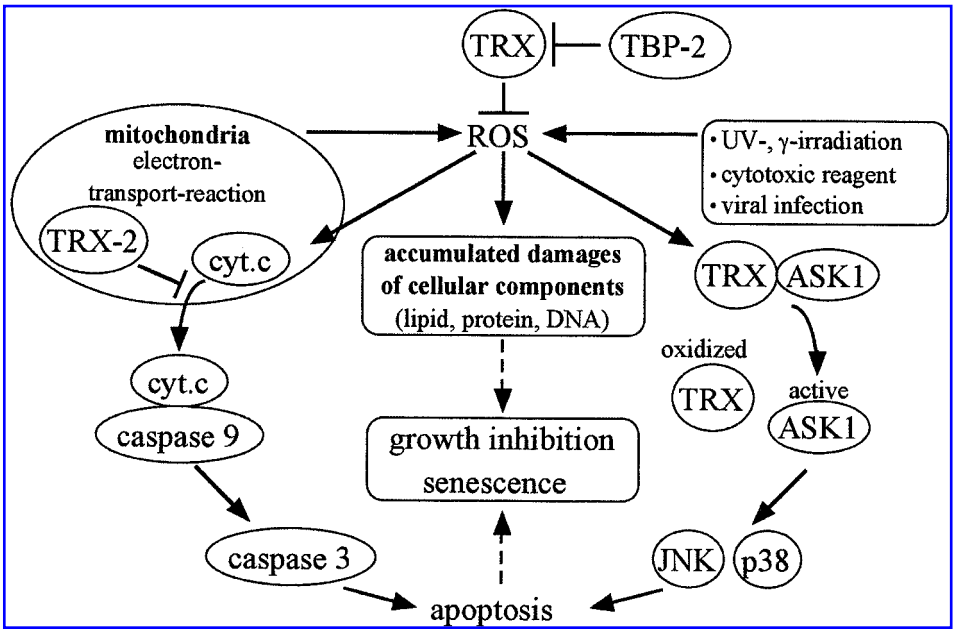

FIG. 3. Multiple functions of TRX and TRX-2 in oxidative stress. TRX and TRX-2 are involved in a variety of signal transductions including ROS-mediated cellular damage and apoptosis. The accumulated damages of cellular components eventually lead to growth inhibition and cellular senescence.

sues, including human primary breast and colon tumors (4, 68). Moreover, a strong antioncogenic histone deacetylase inhibitor, suberoylanilide hydroxamic acid, up-regulates the expression of TBP-2 accompanied by a significant reduction in TRX (4). These reports support the idea that the expression of TRX and TBP-2 are complementarily reflected in cellular proliferation and cellular senescence *in vivo* and *in vitro.*

CONCLUDING REMARKS

In conclusion, TRX is a strong redox-regulating antioxidant protein that has significant quenching capability against induced oxidative stress. Accumulating evidence demonstrates that TRX functions efficiently as an antioxidant against deterioration along with the aging process (senescence) via the cellular redox state, and can extend an animal's life span. The TRX system, including TBP-2, can also serve as an index marker for cellular proliferation and senescence based on their correlative expressions with cellular conditions.

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ABBREVIATIONS

ASK-1, apoptosis signal-regulating kinase; CR, caloric restriction; MAP, mitogen-activated protein; PI3K, phosphatidylinositol 3-kinase; ROS, reactive oxygen species; SOD, superoxide dismutase; TBP-2, thioredoxin binding protein-2; TRX, thioredoxin; VDUP 1, vitamin D up-regulated protein 1.

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