GeroScience (2018) 40:453–468
https://doi.org/10.1007/s11357-018-0039-6

ORIGINAL ARTICLE

Thioredoxin overexpression in both the cytosol and mitochondria accelerates age-related disease and shortens lifespan in male C57BL/6 mice

Geneva M. Cunningham · Lisa C. Flores · Madeline G. Roman · Christie Cheng · Sara Dube · Colton Allen · Joseph M. Valentine · Gene B. Hubbard · Yidong Bai · Thomas L. Saunders · Yuji Ikeno

Received: 24 May 2018 / Accepted: 9 August 2018 / Published online: 18 August 2018

Abstract To investigate the role of increased levels of thioredoxin (Trx) in both the cytosol (Trx1) and mitochondria (Trx2) on aging, we have conducted a study to examine survival and age-related diseases using male mice overexpressing Trx1 and Trx2 (TXNTg × TXN2Tg). Our study demonstrated that the upregulation of Trx in both the cytosol and mitochondria in male TXNTg × TXN2Tg C57BL/6 mice resulted in a significantly shorter lifespan compared to wild-type (WT) mice. Cross-sectional pathology data showed a slightly higher incidence of neoplastic diseases in TXNTg × TXN2Tg mice than WT mice. The incidence of lymphoma, a major neoplastic disease in C57BL/6 mice, was slightly higher in TXNTg × TXN2Tg mice than in WT mice, and more importantly, the severity of lymphoma was significantly higher in TXNTg × TXN2Tg mice compared to WT mice. Furthermore, the total number of histopathological changes in the whole body (disease burden) was significantly higher in TXNTg × TXN2Tg mice compared to WT mice. Therefore, our study suggests that overexpression of Trx in both the cytosol and mitochondria resulted in deleterious effects on aging and accelerated the development of age-related diseases, especially cancer, in male C57BL/6 mice.

Keywords Thioredoxin · Transgenic mouse · Oxidative stress · Cancer · Aging

G. M. Cunningham · L. C. Flores · M. G. Roman · C. Cheng · S. Dube · C. Allen · J. M. Valentine · G. B. Hubbard · Y. Ikeno
Barshop Institute for Longevity and Aging Studies, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78245, USA

G. B. Hubbard · Y. Ikeno
Department of Pathology, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, USA

Y. Bai
Department of Cell Systems and Anatomy, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, USA

T. L. Saunders
Transgenic Animal Model Core, University of Michigan, Ann Arbor, MI 48109, USA

Y. Ikeno
Geriatric Research and Education Clinical Center, Audie L. Murphy VA Hospital, South Texas Veterans Health Care System, San Antonio, TX 78229, USA

Y. Ikeno (✉)
Barshop Institute for Longevity and Aging Studies, The University of Texas Health Science Center at San Antonio, 15355 Lambda Drive, San Antonio, TX 78245-3207, USA
e-mail: ikeno@uthscsa.edu

Introduction

Thioredoxin (Trx) is a small protein (12 kDa) with two redox-active cysteine residues in the active center (Cys-Gly-Pro-Cys) (Arnér and Holmgren 2000), and two Trxs have been identified in humans, one cytosolic (thioredoxin 1, Trx1) (Tagaya et al. 1989) and one

mitochondrial (thioredoxin 2, Trx2) (Spyrou et al. 1997). Trx was first recognized in the early 1960s as the reductant for a variety of enzymes (Arnér and Holmgren 2000; Brot et al. 1981; Brot and Weissbach 2000; Chae et al. 1999a, b; Kim et al. 1988; Levine et al. 1999). Trx also plays an important role in maintaining a reduced environment in cells through thiol-disulfide exchange reactions (Arnér and Holmgren 2000). Because the thiol-disulfide exchange reactions are rapid and readily reversible, this reaction is ideally suited to control protein function via the redox state of structural or catalytic SH groups. Therefore, Trx could play important roles in aging either through its ability to attenuate the level of oxidative stress/damage or through alterations in redox-sensitive signaling, which has diverse effects on pathophysiology. To test the pathophysiological roles of Trx, mice overexpressing Trx1 were generated using a transgene containing the human thioredoxin cDNA fused to the β-actin promoter [Tg (act-*TXN*)$^{+/0}$ mice] (Takagi et al. 1999). The studies using Tg (act-*TXN*)$^{+/0}$ mice demonstrated that Trx1 overexpression resulted in resistance to ischemic injury and lower levels of protein oxidation in brain tissue (Takagi et al. 1999), and more importantly, Tg (act-*TXN*)$^{+/0}$ mice had an increased lifespan compared to their wild-type (WT) littermates (Mitsui et al. 2002; Nakamura et al. 2002; Takagi et al. 1999). Although these observations are very exciting, the study was conducted under conventional housing conditions, and the lifespan of WT C57BL/6 mice in their colony was shorter than WT C57BL/6 mice in aging colonies under optimal conditions. Because of this, our laboratory conducted an aging study with the same line of Tg (act-*TXN*)$^{+/0}$ mice to examine the effects of increased levels of Trx1 on oxidative stress and aging under optimal housing conditions. In our study with Tg (act-*TXN*)$^{+/0}$ mice, Trx1 overexpression showed a significant increase in the survival of male Tg (act-*TXN*)$^{+/0}$ mice compared to WT mice only during the first half of their lifespan; however, no increase in maximum lifespan was observed. This result was confirmed by another survival study using both male and female mice (Pérez et al. 2011). Tg (act-*TXN*)$^{+/0}$ mice showed that the levels of overexpression significantly decreased with age possibly due to the β-actin promoter driving expression of the transgene, which could cause an age-related decrease in expression of the transgene. Therefore, we subsequently generated new transgenic mice with clones of the human *TXN* gene containing endogenous promoters [Tg (*TXN*)$^{+/0}$] to ensure that the transgene is overexpressed throughout the lifespan, and examined the effects of continuous Trx1 overexpression on aging. Our findings with Tg (*TXN*)$^{+/0}$ mice suggest that increased levels of Trx1 over the lifespan showed some beneficial effects in the earlier part of life but had no significant effects on median or maximum lifespans. Tg (*TXN*)$^{+/0}$ mice also showed that Trx1 overexpression accelerates cancer development in old mice, which is consistent with the pathology results in Tg (act-*TXN*)$^{+/0}$ mice.

Thus, the results from two lines of Trx1 transgenic mice showed that overexpression of Trx1 alone does not have beneficial effects on the later part of life, and also may indicate that Trx overexpression in mitochondria is required for maximum lifespan extension. Because the study by Schriner et al. (2005) demonstrated that altering the antioxidant defense system of mitochondria plays important roles in aging. In their study, transgenic mice overexpressing catalase in mitochondria showed significantly increased lifespans and a reduction of some types of cancers; however, overexpressing catalase in the nucleus or peroxisome did not change lifespan (Schriner et al. 2005). To examine the effects of Trx overexpression in mitochondria on aging, we also conducted a survival study using transgenic mice generated with a clone of the human *TXN2* gene containing the endogenous promoter (TXN2Tg). Our study demonstrated that overexpression of Trx in mitochondria showed a slight extension of lifespan in the early part of life, but no significant extension was observed in the later part of life in male mice. Therefore, overexpression of Trx in either cytosol or mitochondria alone is beneficial only in the early part of survival in mice.

These results led us to question whether it is necessary to have increased expression of Trx in both the cytosol and mitochondria to have maximum impact on aging, i.e., extend both the earlier and later part of lifespan and attenuate cancer development. Thus, the purpose of this study is to test the effects of Trx overexpression in both the cytosol and mitochondria on aging and age-related diseases using male TXNTg × TXN2Tg mice.

We report that upregulating Trx in both the cytosol and mitochondria in TXNTg × TXN2Tg male mice unexpectedly resulted in a significantly shorter lifespan. TXNTg × TXN2Tg male mice also showed a significant increase in both the severity of lymphoma and the total number of histopathological changes in the whole body

(disease burden) compared to WT mice. Our results suggest that the overexpression of both Trx1 and Trx2 has deleterious effects on aging and accelerates the development of age-related diseases, especially cancer, in male C57BL/6 mice.

Methods

Animals and animal husbandry

The Trx1 transgenic mice in this study were generated using a fragment of the human genome containing the *TXN* gene [a BAC clone (RP11-427L11), Children's Hospital Oakland Research Institute (CHORI)'s BACPAC Resources Center (BPRC), Oakland, CA] and 8.3 kb and 12.3 kb of the 5′- and 3′-flanking sequences, respectively. We also generated the Tg $(TXN2)^{+/0}$ mice using the human thioredoxin 2 gene [a PAC clone (RP5-1119A7), CHORI's BPRC, Oakland, CA], which contained the *TXN2* gene and 8.4 kb and 5.1 kb of the 5′- and 3′-flanking sequences, respectively. These transgenic mice were produced by pronuclear microinjection of zygotes obtained from the mating of (C57BL/6J X SJL/J)F1 females with (C57BL/6J X SJL/J)F1 males (Jackson Laboratory; stock no. 100012) and were backcrossed to C57BL/6 mice ten times. Male hemizygous Trx1 mice were crossed to hemizygous Trx2 females to generate hemizygous TXNTg × TXN2Tg and WT control mice.

All mice were fed a commercial chow (Teklad Diet LM485; Madison, WI) and acidified (pH = 2.6–2.7) filtered reverse osmosis water ad libitum. To measure the amount of food consumption, the amount of chow removed from the cage hopper and the spillage (the chow on the bottom of the cage) were weighed monthly. Actual food consumed was calculated by subtracting the spillage from the chow removed from the hopper. All of the mice were weighed monthly and maintained pathogen-free in microisolater units on Tek FRESH® ultra laboratory bedding. Sentinel mice housed in the same room and exposed weekly to bedding collected from the cages of experimental mice were sacrificed on receipt and every 6 months thereafter for monitoring of viral antibodies (Mouse Level II Complete Antibody Profile CARB, Ectro, EDIM, GDVII, LCM, M. Ad-FL, M. Ad-K87, MCMV, MHV, *M. pul.*, MPV, MVM, Polyoma, PVM, Reo, Sendai; BioReliance, Rockville, MD). All tests were negative.

Determination of Trx1 expression

Cytosolic fractions obtained from tissues homogenized as previously described (Pérez et al. 2008; Pérez et al. 2011) were used to determine Trx1 levels in several tissues from TXNTg × TXN2Tg and WT mice by Western blot analysis using goat anti-human Trx1 polyclonal antibodies (Catalog No. 705; American Diagnostica, Inc., Greenwich, CT). These antibodies recognize total Trx1 (both oxidized and reduced forms). After incubation with the primary antibody, membranes were incubated with the peroxidase-linked secondary antibody (Catalog No. P0449; Dako, Carpinteria, CA). Chemiluminescence was detected with an ECL Western blot detection kit (Amersham Biosciences Corp., Piscataway, NJ).

Thioredoxin 2 levels

Trx2 levels were measured using mitochondria obtained from the liver of TXNTg × TXN2Tg and WT mice as previously described (Pérez et al. 2008; Pérez et al. 2011). Western blot analysis was performed using rabbit anti-Trx2 polyclonal antibody (Catalog No. LF-PA0012; LabFrontier, Seoul, Korea). After incubation with the primary antibodies, membranes were incubated with the respective peroxidase-linked secondary antibodies (Catalog Nos. P0449 and P0217; Dako, Carpinteria, CA). Chemiluminescence was detected using the ECL Western blot detection kit (Amersham Biosciences Corp., Piscataway, NJ).

Glutaredoxin and total glutathione levels

Glutaredoxin (Grx) levels were measured using total homogenate fractions obtained from the liver of TXNTg × TXN2Tg and WT mice as previously described (Pérez et al. 2008; Pérez et al. 2011). Western blot analysis was performed using goat anti-human glutaredoxin polyclonal antibody (Catalog No. 710; American Diagnostica, Inc., Greenwich, CT). After incubation with the primary antibodies, membranes were incubated with the respective peroxidase-linked secondary antibodies (Catalog Nos. P0449 and P0217; Dako, Carpinteria, CA). Chemiluminescence was detected using the ECL Western blot detection kit (Amersham Biosciences Corp., Piscataway, NJ). The levels of total glutathione were determined using the Bioxytech GSH-

420 kit (Catalog No. 21023; Oxis International, Inc., Foster City, CA).

Determination of major antioxidant enzyme activities: Cu/ZnSOD, MnSOD, glutathione peroxidase, and catalase

The activities of major antioxidant enzymes (Cu/ZnSOD, MnSOD, glutathione peroxidase (GPx), and catalase) were measured in tissue homogenates from TXNTg × TXN2Tg and WT mice. The supernatants were used for the antioxidant defense enzymatic activity assay. GPx activity in tissue homogenates was measured as described by Sun et al. (1988). Catalase activity was determined by measuring the decomposition of hydrogen peroxide at 520 nm using the Catalase-520™ assay kit (*Oxis*Research™, Portland, OR). MnSOD and Cu/ZnSOD levels were measured by activity gels as previously described (Beauchamp and Fridovich 1971; Williams et al. 1998). Gel images were analyzed using ImageQuant software.

Determination of ASK1 signaling pathway activity

The levels of ASK1 were measured by Western blot using an ASK1 antibody (Santa Cruz, CA). The activation of ASK1 was also measured by Western blot with a phospho-ASK1 antibody [Thr845] (Cell Signaling Technology, Inc., MA). The tissues were homogenized in lysis buffer (20 mM Tris-HCl [pH 7.5], 150 mM NaCl, 1 mM EDTA, 10% glycerol, 1% NP-40, and 1× protease inhibitor cocktail I [Calbiochem]) and incubated on ice for 30 min. After centrifugation, the supernatant was separated, and the protein concentration was determined by the Bradford assay. Proteins (100 μg) were separated on polyacrylamide gel electrophoresis (PAGE) and transferred onto nitrocellulose membranes electrophoretically. Specific proteins on the membranes were detected by standard Western blotting procedures using secondary antibodies conjugated to horseradish peroxidase (HRP). Signals were detected with an ECL Western blot detection kit (Amersham Biosciences Corp., Piscataway, NJ).

Measurement of the mitochondrial apoptosis pathway

The mitochondrial apoptosis pathway was assessed by measuring caspase activity and cytochrome c release from the mitochondria. The caspase activity and cytochrome c release were determined by Western blot assay. The caspase-3 activity in liver tissue was visualized by the cleavage forms of caspase-3 (p20) as an indicator of caspase activity using anti-caspase-3 antibody (Cell Signaling Technology, Inc., Danvers, MA). The intensities of cleavage bands corresponding to p20 for caspase-3 were quantified by densitometry using ImageQuant v5.0, and β-actin was used as a loading control. The cytochrome c release from mitochondria was measured as levels of cytochrome c in the cytosolic fractions from the liver. Equal amounts of protein were separated on a 4–20% SDS-polyacrylamide gel, transferred to nitrocellulose membranes, and subjected to Western blotting with an anti-cytochrome c antibody (Santa Cruz Biotechnology, Santa Cruz, CA). The data were normalized and expressed as relative units respective to β-actin loading controls.

Tricarboxylic acid (TCA) cycle activity measurement

As a part of the metabolic pathway analyses, the (TCA) cycle activity was compared in the liver of young TXNTg × TNX2Tg and WT mice. The levels of citrate, alpha-ketoglutarate, fumarate, malate, and succinate were measured using gas chromatography/mass spectrometry as previously described (Lawton et al. 2008; Patel et al. 2017).

Measurement of the NFκB pathway

The amounts of NFκB and IκB were measured using Western blot with antibodies for the NFκB (p50, p65; Cell Signaling Technology, Inc., Danvers, MA) and IκB (Cell Signaling Technology, Inc., Danvers, MA) families. The tissues were homogenized in lysis buffer (20 mM Tris-HCl [pH 7.5], 150 mM NaCl, 1 mM EDTA, 10% glycerol, 1% NP-40, and 1× protease inhibitor cocktail I [Calbiochem]) and incubated on ice for 30 min. After centrifugation, the supernatant was separated and protein concentration was determined by the Bradford assay. Proteins (100 μg) were separated by PAGE and electrophoretically transferred onto nitrocellulose membranes. Specific proteins on the membranes were detected by standard Western blotting procedures using secondary antibodies conjugated to HRP. Signals were detected with the ECL Western blot detection kit (Amersham Biosciences Corp., Piscataway, NJ). The amounts of NFκB, IκB, and β-actin were quantified by a densitometer, and the data were expressed as the

relative amount of NFκB and IκB in lysates using β-actin as an internal standard.

Determination of mTOR signaling pathway activity

Levels of p70S6K1 (phosphorylated and non-phosphorylated forms) were measured in the total cell lysates from the liver of TXNTg × TXN2Tg and WT mice by Western blot analysis using mouse p70S6K1 and phospho-p70S6K1 antibodies (Cell Signaling Technology, Inc., Danvers, MA).

Determination of HIF-1α levels

Total cell lysates from various tissues of the mice were prepared, and detection of HIF-1α was performed using Western blots. The amount of HIF-1α was quantified by a densitometer, and the data were expressed as the relative amount of protein in lysates using β-actin as an internal standard.

Survival study

Mice in the survival groups were allowed to live out their lives, and the lifespan for individual mice was determined by recording the age of spontaneous death. A survival study consisting of 22 male TXNTg × TXN2Tg and 33 WT mice was conducted. The survival curves were compared statistically using the log-rank and Wilcoxon tests (Andersen et al. 1993; Custudio and Maria 2007; Philonenko and Postovalov 2015). The median, mean, and 10th percentile (when 90% of the mice have died) survivals were calculated for each group. The mean survivals for each experimental group were compared to the respective WT group by performing Student's *t* test upon log-transformed survival times. The median and 10th percentile survivals for each group were compared to the WT group using a score test adapted from Wang et al. (2004).

Cross-sectional pathological assessment

After the gross pathological examinations, the following organs and tissues were excised and preserved in 10% buffered formalin: brain, pituitary gland, heart, lung, trachea, thymus, aorta, esophagus, stomach, small intestine, colon, liver, pancreas, spleen, kidneys, urinary bladder, reproductive system (prostate, testes, epididymis, and seminal vesicles), thyroid gland, adrenal glands, parathyroid glands, psoas muscle, knee joint, sternum, and vertebrae. Any other tissues with gross lesions were also excised. The fixed tissues were processed conventionally, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin-eosin. The diagnosis of each histopathological change was made with histological classifications in aging mice as previously described (Bronson and Lipman 1991; Ikeno et al. 2005). A list of pathological lesions was constructed for each mouse that included both neoplastic and non-neoplastic diseases. Based on these histopathological data, the tumor burden, disease burden, and severity of each lesion in each mouse were assessed as previously described (Ikeno et al. 2003; Ikeno et al. 2005; Ikeno et al. 2009; Pérez et al. 2011).

Statistical analysis

Unless otherwise specified, all experiments were done at least in triplicate. Data were expressed as means ± SEM and were analyzed by the non-parametric test ANOVA. All pair-wise contrasts were computed using the Tukey error protection at 95% confidence interval (CI) unless otherwise indicated. Differences were considered statistically significant at $p < 0.05$.

Results

Overexpression of Trx1 and Trx2 in tissues from TXNTg × TXN2Tg mice

The levels of Trx1 and Trx2 in tissues from young (4–6 months old) TXNTg × TXN2Tg and WT mice were measured using Western blot analysis. The Trx1 protein levels were significantly higher (2- to 7-fold increase in the liver, kidney, and heart; 14-fold increase in the brain) in all of the four tissues examined in the young TXNTg × TXN2Tg mice compared to their WT littermates (Fig. 1a), which were similar to the Tg $(TXN)^{+/0}$ mice; however, the levels of Trx1 overexpression were higher than those in Tg (act-$TXN)^{+/0}$ mice. The Trx2 protein levels were also significantly higher (1.8- to 2.3-fold) in all of the four tissues examined in the young TXNTg × TXN2Tg mice compared to their WT littermates (Fig. 1b), which were similar to the TXN2Tg mice.

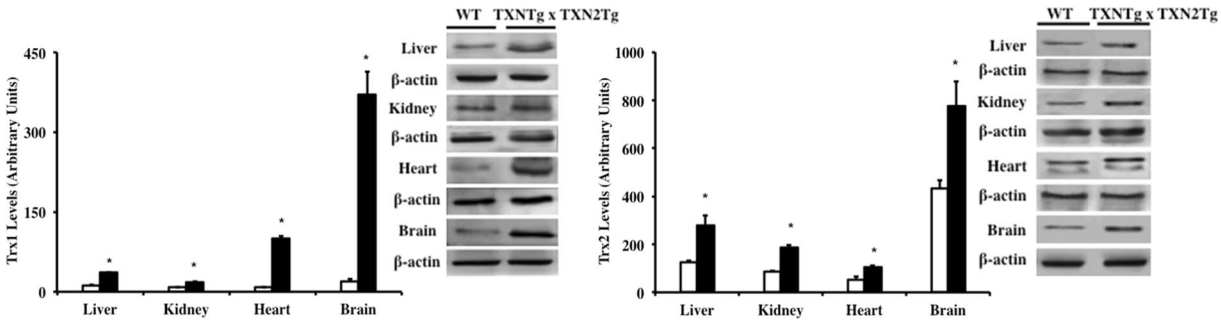


Fig. 1 Overexpression of Trx1 and Trx2 in TXNTg × TXN2Tg and WT mice. The levels of Trx1 and Trx2 were determined by Western blot in four tissues of young (4–6 months old) TXNTg × TXN2Tg (closed bar) and WT (open bar) mice. Trx1 levels were significantly higher in the tissues of TXNTg × TXN2Tg mice compared to WT mice ($*p < 0.05$). Trx2 levels were also significantly higher in four tissues of the TXNTg × TXN2Tg mice compared to WT mice ($*p < 0.05$). The data are the mean ± SEM from five mice

Levels of glutaredoxin and glutathione in tissues from TXNTg × TXN2Tg mice

We determined whether the levels of glutaredoxin and glutathione were altered in response to increased Trx levels because of similar biological functions. No significant changes in glutaredoxin (Fig. 2a) levels were observed in the liver of young (4–6 months old) TXNTg × TXN2Tg mice compared to WT control mice. Total glutathione levels in the liver were similar in the young (4–6 months old) TXNTg × TXN2Tg and WT control mice (Fig. 2b). Therefore, the data in Fig. 2 show that the overexpression of Trx1 and Trx2 in TXNTg × TXN2Tg mice was not associated with downregulation or upregulation of glutaredoxin and total glutathione levels.

Major antioxidant enzyme activities in tissues from TXNTg × TXN2Tg mice

The activities of other major antioxidant enzymes were also measured in TXNTg × TXN2Tg and WT control mice because it is possible that an increase in Trx activity could initiate a compensatory reduction in the activities of other components of the antioxidant system. The data in Fig. 3 show that the activities of Cu/ZnSOD, MnSOD, GPx, and catalase were similar in the tissues from the young (4–6 months old) TXNTg × TXN2Tg and WT control mice. Thus, the data in Fig. 3 show that Trx1 overexpression in the tissues of TXNTg × TXN2Tg mice did not downregulate the major antioxidant defense system.

Body weight

The body and organ weights of young (4–6 months old) TXNTg × TXN2Tg and WT control mice are shown in Table 1. The body and organ weights were similar between TXNTg × TXN2Tg and WT control mice. Based on the body and organ weights data, there was no evidence that overexpression of Trx in both the cytosol and mitochondria led to changes in the growth and development of male C57BL/6 mice.

Survival curves

To test whether overexpression of Trx in both the cytosol and mitochondria affects aging, we conducted a survival study to examine the effects of overexpression of both Trx1 and Trx2 on lifespan using male TXNTg × TXN2Tg and WT control mice. Our study showed that the survival curves were significantly different between male TXNTg × TXN2Tg and WT control mice (Fig. 4; log-rank: $p = 0.048$; Wilcoxon: $p = 0.04$). The mean, median, and 10th percentile survival for WT mice were 831, 833 (95% confidence interval 760–896), and 1087 (95% confidence interval 1010–1227) days, respectively. The mean, median, and 10th percentile survival for TXNTg × TXN2Tg mice were 710, 697 (95% confidence interval 644–833), and 1011 (95% confidence interval 935–1170) days, respectively. TXNTg × TXN2Tg mice had shorter mean (14.6%), median (16.3%), and 10th percentile (7%) lifespans compared to WT mice, although these differences did not reach statistical significance ($p > 0.05$).

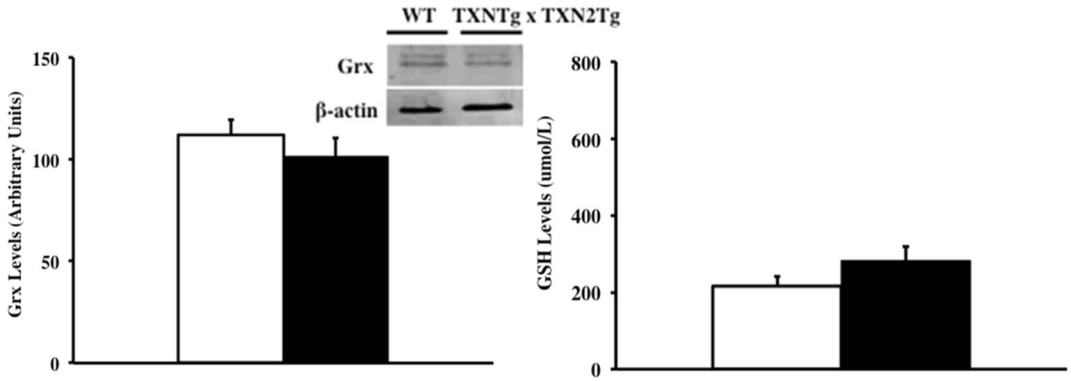


Fig. 2 Levels of glutaredoxin and total glutathione in TXNTg × TXN2Tg and WT mice. The levels of glutaredoxin and total glutathione were measured in the liver from young (4–6 months old) TXNTg × TXN2Tg (closed bar) and WT (open bar) mice. No significant differences were observed in glutaredoxin and total glutathione levels of TXNTg × TXN2Tg mice compared to WT mice. The data in **a** and **b** are the mean ± SEM from five mice

Cross-sectional pathology

Cross-sectional pathology data were collected from male TXNTg × TXN2Tg and WT mice at 20–22 months of age. Approximately 61% of WT mice and 72% of TXNTg × TXN2Tg mice had neoplastic diseases, the major disease being lymphoma. The incidence of lymphoma in male TXNTg × TXN2Tg mice was slightly higher (72%) than WT mice (61%), which was not statistically significant. However, the severity of lymphoma was significantly higher in TXNTg × TXN2Tg mice compared to WT mice

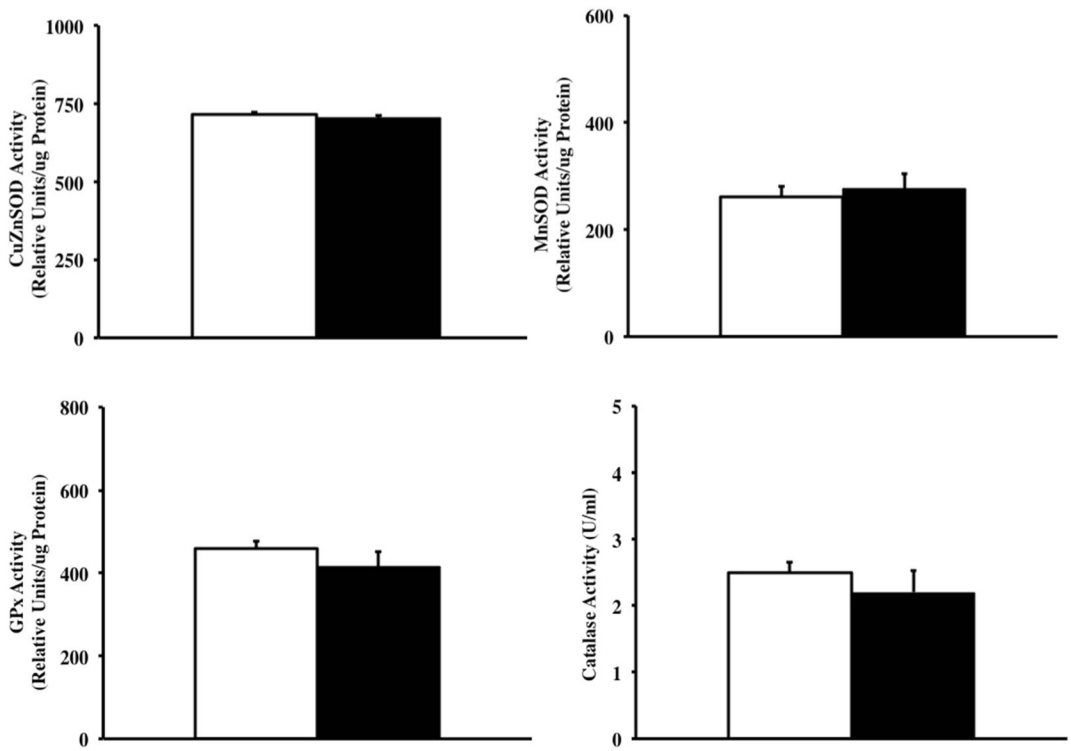


Fig. 3 Cu/ZnSOD, MnSOD, GPx, and catalase activities in TXNTg × TXN2Tg and WT mice. The activities of Cu/ZnSOD, MnSOD, GPx, and catalase were measured in tissue extracts from young (4–6 months old) mice. The activities of Cu/ZnSOD, MnSOD, GPx, and catalase were similar between TXNTg × TXN2Tg and WT mice. The data are the mean ± SEM from five mice

Table 1 Body and organ weights of the TXNTg × TXN2Tg mice

	WT ($n = 6$)	TXNTg × TXN2Tg ($n = 5$)
Body weight (g)	30.6 ± 1.96	29.1 ± 2.61
Liver (g)	1.47 ± 0.088	1.38 ± 0.183
Spleen (g)	0.085 ± 0.004	0.077 ± 0.005
Pancreas (g)	0.169 ± 0.016	0.170 ± 0.016
Heart (g)	0.158 ± 0.011	0.161 ± 0.021
Lung (g)	0.198 ± 0.007	0.214 ± 0.017
Left kidney (g)	0.217 ± 0.008	0.228 ± 0.023
Right kidney (g)	0.228 ± 0.013	0.232 ± 0.022
Left testicle (g)	0.123 ± 0.005	0.126 ± 0.007
Right testicle (g)	0.127 ± 0.007	0.132 ± 0.006
Brain (g)	0.432 ± 0.002	0.449 ± 0.010

(Fig. 5a; $p = 0.015$). The tumor burden of male TXNTg × TXN2Tg mice was slightly higher (0.778) than WT mice (0.611), which was not statistically significant (Fig. 5b). The total number of histopathological changes in the whole body (disease burden) was significantly higher in TXNTg × TXN2Tg mice compared to WT mice (Fig. 5c; $p = 0.039$). Therefore, the lifespan data in Fig. 4 and the pathology data in Fig. 5a, c convincingly show that overexpression of Trx in both the cytosol and mitochondria has deleterious effects on aging, i.e., a significant reduction in lifespan, an accelerated lymphoma development, and an increase in age-related pathology in the male TXNTg × TXN2Tg mice compared to their WT littermates.

ASK1 levels

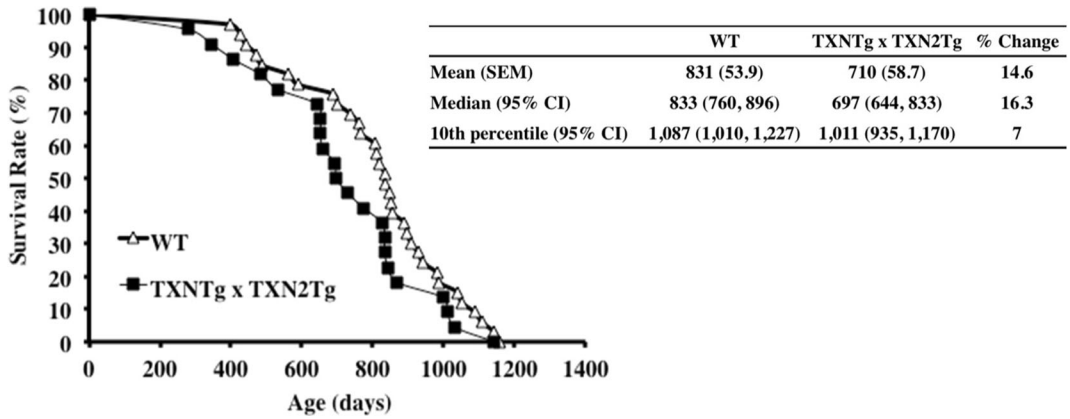
As we have previously shown that Tg (act-*TXN*)$^{+/0}$ mice had higher levels of the ASK1/Trx1 complex, which could result in reduced ASK1 phosphorylation (Pérez et al. 2011), we measured the levels of ASK1 and phosphorylated ASK1. Figure 6a shows levels of ASK1 were similar between male TXNTg × TXN2Tg and WT mice. However, phosphorylated ASK1 levels were significantly lower in young (6–7 months old) male TXNTg × TXN2Tg mice compared to WT mice (Fig. 6b).

Measurement of mitochondrial apoptosis pathway

The mitochondrial apoptosis pathway was assessed for caspase activity and cytochrome c release from the mitochondria because Trx1 overexpression alters the ASK1 pathway. Cytochrome c release and cleaved caspase-3 levels were similar between male TXNTg × TXN2Tg and WT mice (Fig. 7a, b).

TCA cycle activity

When young male TXNTg × TXN2Tg mice were compared to young WT mice, decreases were observed in citrate, alpha-ketoglutarate, fumarate, and malate (data not shown). Succinate levels, meanwhile, were significantly elevated in young male TXNTg × TXN2Tg compared to WT mice (Fig. 8).



	WT	TXNTg x TXN2Tg	% Change
Mean (SEM)	831 (53.9)	710 (58.7)	14.6
Median (95% CI)	833 (760, 896)	697 (644, 833)	16.3
10th percentile (95% CI)	1,087 (1,010, 1,227)	1,011 (935, 1,170)	7

Fig. 4 Survival curves of TXNTg × TXN2Tg and WT mice. The survival curves, mean, median, and 10th percentile lifespans (days) and the percent differences of TXNTg × TXN2Tg and WT mice are presented. The survival study was conducted with 22 TXNTg × TXN2Tg and 33 WT male mice. The survival curves were significantly different between TXNTg × TXN2Tg and WT mice (log-rank: $p = 0.048$; Wilcoxon: $p = 0.04$). TXNTg × TXN2Tg mice had shorter mean (14.6%), median (16.3%), and 10th percentile (7%) lifespans compared to WT mice, which were not statistically significant ($p > 0.05$)

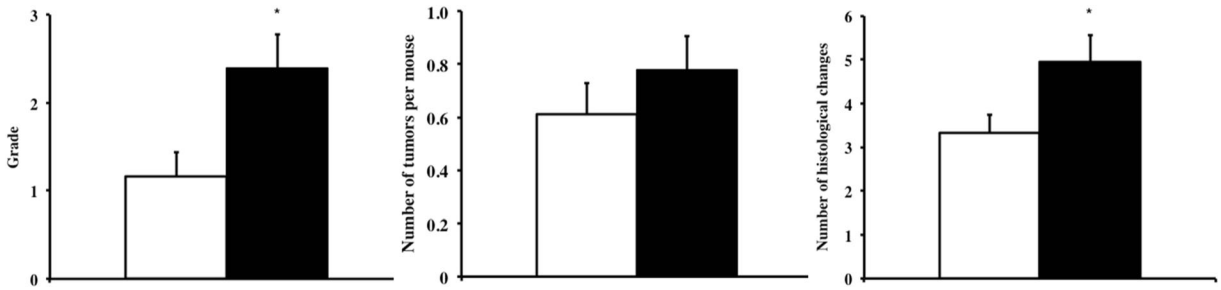


Fig. 5 Severity of lymphoma, tumor burden, and disease burden in TXNTg × TXN2Tg and WT mice. The average severity of lymphoma in TXNTg × TXN2Tg (closed bar) mice was significantly higher than that in WT (open bar; $p = 0.015$) mice. The tumor burden of male TXNTg × TXN2Tg mice was slightly higher (0.778) than WT mice (0.611) but was not statistically significant. The disease burden per mouse is the total number of pathological changes of any type found in individual mice from each group. The disease burden in TXNTg × TXN2Tg mice (closed bar) was significantly higher than that WT mice (open bar; $p = 0.039$)

Determination of mTOR signaling pathway activity

Since substantial evidence showed that mTOR activity is one of the key pathways for cancer development and lifespan, levels of p70S6K1 (phosphorylated and non-phosphorylated forms) were measured in the total cell lysates from the liver of TXNTg × TXN2Tg and WT mice by Western blot analysis using mouse p70S6K1 and phospho-p70S6K1 antibodies. The data in Fig. 9 show that TXNTg × TXN2Tg mice had similar levels of p70S6K1 (Fig. 9a) and phospho-p70S6K1 (Fig. 9b) compared to WT littermates.

HIF-1α levels

HIF-1α, one of the signaling molecules shown to be redox-sensitive and affected by Trx, could play important roles in age-related cancer development. Thus, the levels of HIF-1α were measured using Western blots. The data in Fig. 10 show that TXNTg × TXN2Tg mice had significantly increased levels of HIF-1α compared to WT littermates. These data suggest that increased levels of HIF-1α could also play an important role in cancer growth and development.

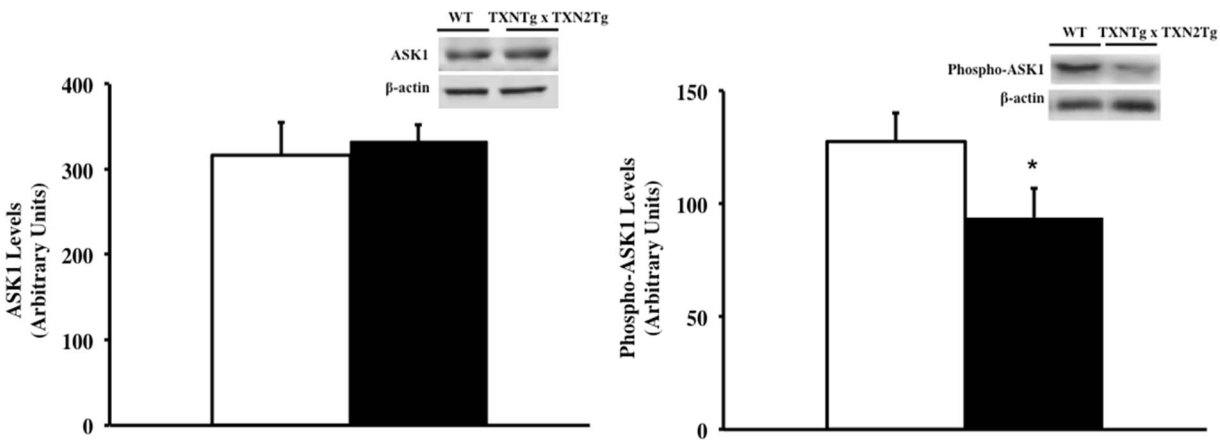


Fig. 6 Levels of ASK1 and phosphorylated ASK1 in TXNTg × TXN2Tg and WT mice. The levels of ASK1 were measured using Western blot in the liver of TXNTg × TXN2Tg (closed bar) mice compared to WT (open bar) mice at 6–7 months of age. The levels of ASK1 were similar between TXNTg × TXN2Tg and WT mice. Levels of phosphorylated ASK1 were measured by Western blot analysis in the cytosolic liver fractions from 6- to 7-month-old mice. The levels of phosphorylated ASK1 were significantly lower in TXNTg × TXN2Tg mice (closed bar) compared to WT (open bar; $p < 0.05$) mice. The data are the mean ± SEM from five mice

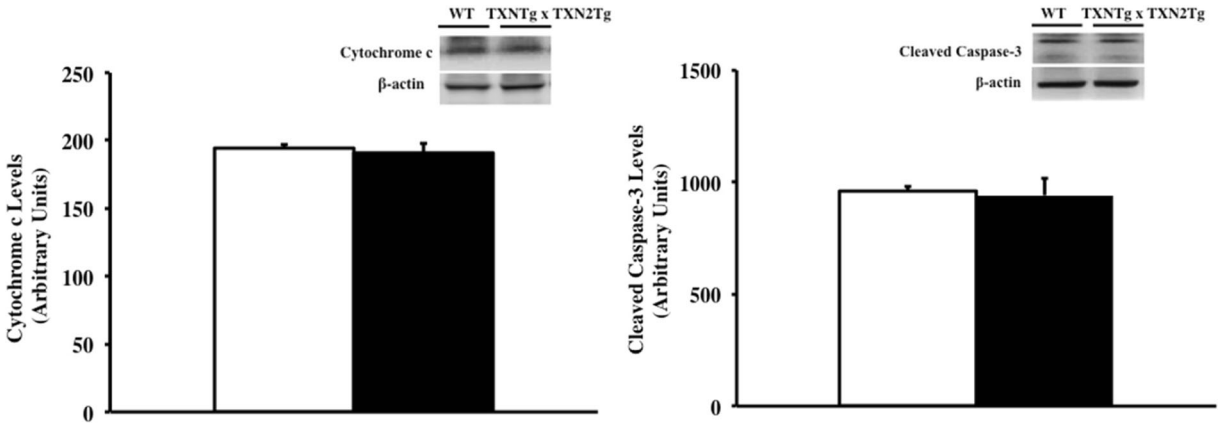


Fig. 7 Levels of cytochrome c and cleaved caspase-3 in TXNTg × TXN2Tg and WT mice. The levels of cytochrome c and cleaved caspase-3 were measured in the liver of TXNTg × TXN2Tg (closed bar) and WT (open bar) mice. No significant differences were observed in cytochrome c and cleaved caspase-3 levels in TXNTg × TXN2Tg mice compared to WT mice. The data in the graphs are the mean ± SEM from five mice

Measurement of the NFκB pathway

NFκB is one of the redox-sensitive transcription factors and contains cysteine residues. Therefore, Trx directly and indirectly affects NFκB activities. Since NFκB plays important roles in oxidative stress, inflammation, apoptosis, and cancer, the levels of NFκB (p65 and p50) and IκB were measured using Western blot. The data in Fig. 11 show that TXNTg × TXN2Tg mice had significantly increased levels of NFκB p65 compared to WT littermates (Fig. 11a), while levels of NFκB p50 and IκB were similar between TXNTg × TXN2Tg mice and their WT littermates (Fig. 11b, c, respectively).

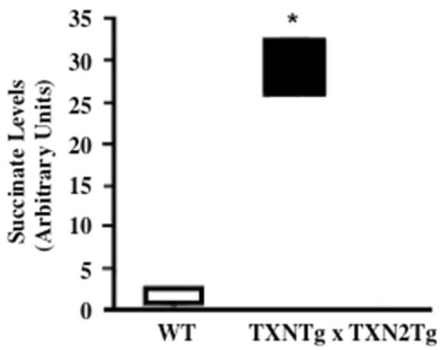
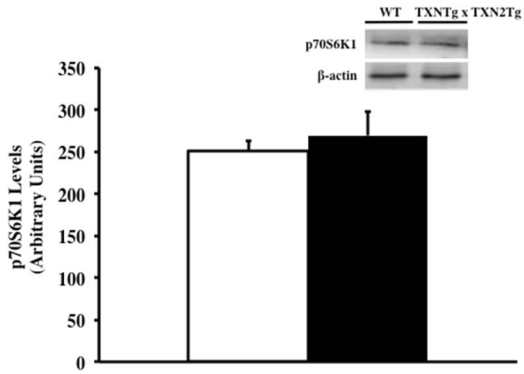


Fig. 8 Levels of succinate in TXNTg × TXN2Tg and WT mice. The levels of succinate were measured in the liver of young (4–6 months old) TXNTg × TXN2Tg (closed bar) and WT (open bar) mice. The levels of succinate in TXNTg × TXN2Tg mice were significantly elevated compared to WT mice (*$p < 0.05$). The data are the mean ± SEM from five mice

Discussion

Thioredoxin (Trx) was first recognized in the early 1960s as the major reductant for a variety of enzymes (Arnér and Holmgren 2000). A major role of Trx is to donate a hydrogen atom to enzymes involved in reductive reactions [e.g., ribonucleotide reductase, which reduces ribonucleotides to deoxyribonucleotides for DNA synthesis; peroxiredoxin (Prx), which reduces peroxides (Chae et al. 1999a, b; Kim et al. 1988); and methionine sulfoxide (MetO) reductase, which reduces MetO in proteins and provides protection against oxidative stress (Brot et al. 1981; Brot and Weissbach 2000; Levine et al. 1999)]. By maintaining a reduced environment in cells through thiol-disulfide exchange reactions, Trx protects cells and tissues from oxidative stress and also plays critical roles for the normal function of proteins that contain cysteine residues (Abate et al. 1990; Galter et al. 1994; Takagi et al. 1999; Toledano and Leonard 1991). For example, Trx could (1) directly regulate DNA binding activity of cysteine-containing transcription factors and (2) control redox-sensitive signaling pathways. Due to these unique features, Trx could have more diverse effects on the pathophysiological changes during aging compared to other antioxidant enzymes (e.g., superoxide dismutases, catalases), which play a role in reducing oxidative damage.

Therefore, our laboratory has been conducting the first detailed studies on the role of Trx in the cytosol (Trx1) and in the mitochondria (Trx2) on aging and age-related diseases using unique mouse models either



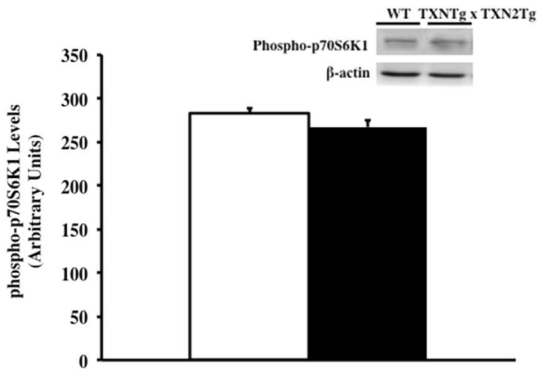


Fig. 9 Levels of p70S6K1 and phospho-p70S6K1 in TXNTg × TXN2Tg and WT mice. The levels of p70S6K1 and phospho-p70S6K1 were measured in the liver of TXNTg × TXN2Tg (closed bar) and WT (open bar) mice. No significant differences were observed in p70S6K1 and phospho-p70S6K1 levels in TXNTg × TXN2Tg mice compared to WT mice. The data in **a** and **b** are the mean ± SEM from five mice

overexpressing or downregulating Trx1 or Trx2. Our previous work with Tg (act-*TXN*)$^{+/0}$ mice demonstrated that overexpressing Trx1 showed a significant increase in the survival of male Tg (act-*TXN*)$^{+/0}$ mice compared to WT mice only during the first half of their lifespan. This result was confirmed by another survival study using both male and female mice from another cohort (Pérez et al. 2011). Our pathology data from Tg (act-*TXN*)$^{+/0}$ mice showed a significantly reduced incidence of lung inflammation in young mice compared to WT mice, but a slightly higher incidence of total fatal tumors and fatal lymphomas in old mice compared to WT mice (Pérez et al. 2011). Since Tg (act-*TXN*)$^{+/0}$ mice showed that the levels of overexpression significantly decreased with age, possibly due to the β-actin promoter driving expression of the transgene, we subsequently examined the effects of continuous Trx1 on aging using another line of transgenic mice [Tg (*TXN*)$^{+/0}$]. Our findings with Tg (*TXN*)$^{+/0}$ mice regarding longevity and age-related pathology were consistent with the results of Tg (act-*TXN*)$^{+/0}$ mice: (1) Trx1 overexpression showed some beneficial effects in the earlier part of life but had no significant effects on median or maximum lifespans and (2) Trx1 overexpression accelerates cancer development in old mice.

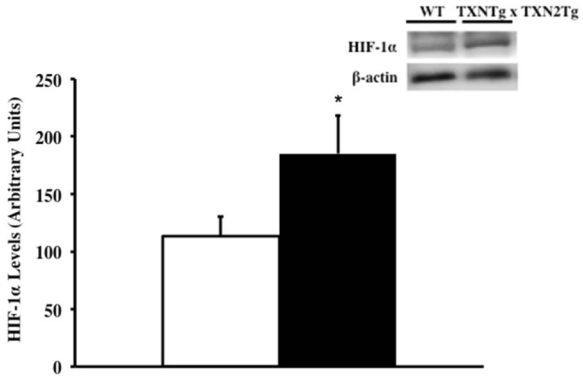


Fig. 10 Levels of HIF-1α in TXNTg × TXN2Tg and WT mice. The levels of HIF-1α were measured in the liver of TXNTg × TXN2Tg (closed bar) and WT (open bar) mice. HIF-1α levels were significantly increased in TXNTg × TXN2Tg mice compared to WT mice (*$p < 0.05$). The data are the mean ± SEM from five mice

Thus, the results from our studies with Tg (act-*TXN*)$^{+/0}$ and Tg (*TXN*)$^{+/0}$ mice showed that overexpression of Trx1 alone does not have beneficial effects on the later part of life and led us to question whether increased expression of Trx in both the cytosol and mitochondria is required to have maximum impact on aging, i.e., to extend both the earlier and later parts of lifespan and attenuate cancer development. Because the important role of antioxidant overexpression in mitochondria in aging and age-related disease was strongly suggested by the study of Schriner et al. (2005) with transgenic mice overexpressing catalase, this study demonstrated that overexpressing catalase in the mitochondria significantly extended lifespan and suppressed specific cancers compared to their WT littermates; however, overexpressing catalase in the nucleus or peroxisome did not have beneficial effects. Thus, the purpose of our study was to test the effects of Trx overexpression in both the cytosol and mitochondria on aging and age-related diseases using male TXNTg × TXN2Tg C57BL/6 mice.

We found that levels of Trx1 and Trx2 were significantly higher in all the tissues examined in young male

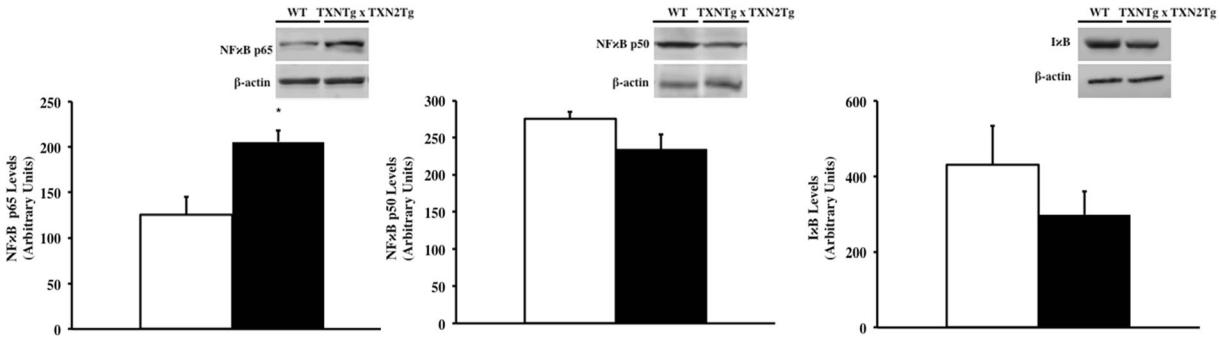


Fig. 11 Levels of NFκB (p65 and p50) and IκB in TXNTg × TXN2Tg and WT mice. The levels of NFκB p65, NFκB p50, and IκB (**c**, right) were measured in the liver of TXNTg × TXN2Tg (closed bar) and WT (open bar) mice. NFκB p65 levels were significantly higher in TXNTg × TXN2Tg mice compared to WT mice; however, no significant differences were observed in the NFκB p50 and IκB levels (*$p < 0.05$.) The data in are the mean ± SEM from five mice

TXNTg × TXN2Tg mice compared to the WT control mice. The levels of Trx1 and Trx2 were 2- to 14-fold and 1.8- to 2.3-fold higher in all of the tissues examined, respectively. The increased levels of Trx1 and Trx2 in male TXNTg × TXN2Tg mice did not affect glutaredoxin and total glutathione levels or major antioxidant enzyme activities.

Our survival study showed that the survival curve of male TXNTg × TXN2Tg mice was significantly different from WT control mice. The mean, median, and 10th percentile lifespans of male TXNTg × TXN2Tg mice were approximately 14.6%, 16.3%, and 7% shorter than the WT control mice, respectively. The cross-sectional pathology demonstrated that approximately 61% of WT mice and 72% of TXNTg × TXN2Tg mice had neoplastic diseases. The total number of tumors (tumor burden) was also slightly higher in TXNTg × TXN2Tg mice (0.778) compared to WT mice (0.611). The incidence of lymphoma, a major neoplastic disease, was slightly higher in TXNTg × TXN2Tg mice compared to WT mice. Although the slight increase in the incidence of lymphoma in TXNTg × TXN2Tg mice was not statistically significant, the severity of lymphoma was significantly higher in the TXNTg × TXN2Tg mice compared to WT mice. This indicates that overexpression of both Trx1 and Trx2 plays a more important role in the development and growth of lymphoma. Furthermore, the total number of histopathological changes in the whole body (disease burden) was significantly higher in TXNTg × TXN2Tg mice compared to WT mice. Therefore, the pathology data further support the results of the survival study, and the survival and pathological data indicate that overexpression of both Trx1 and Trx2 caused deleterious effects on aging.

These results were unexpected and paradoxical because (1) our previous work with Tg (act-*TXN*)$^{+/0}$ mice showed beneficial effects of Trx1 overexpression on the first half of lifespan (Pérez et al. 2011) and (2) catalase overexpression in mitochondria has been previously shown to extend lifespan and reduce some cancers (Schriner et al. 2005). However, our results strongly indicate that the role of Trx in pathophysiology, including aging and age-related diseases, is more complex than we initially expected. Supporting our observation with TXNTg × TXN2Tg mice, a series of studies have demonstrated the complex roles of thioredoxin in pathophysiology. For example, a study with transgenic mice overexpressing Trx1 in the nucleus (NLS-Trx1Tg) showed an increased mortality by influenza H1N1, which was associated with increased inflammatory signaling (Go et al. 2011), while studies using mice that overexpress Trx in mitochondria and the cytosol showed that Trx protects against various stresses (Nakamura et al. 2002; Zhang et al. 2007). Another series of studies also demonstrated that the Trx and thioredoxin-interacting protein (Txnip) complex plays very important roles in physiology by changing intracellular and/or extracellular redox signaling, and its dysregulation could underlie various disease processes (Yoshihara et al. 2013). In addition, our previous study with Tg (act-*TXN*)$^{+/0}$ mice further supports the complex role of thioredoxin in pathophysiology. The Tg (act-*TXN*)$^{+/0}$ mice showed a significantly reduced incidence of lung inflammation in young mice compared to WT mice, while old Tg (act-*TXN*)$^{+/0}$ mice had a slightly higher incidence of total fatal tumors and fatal lymphomas compared to WT mice (Pérez et al. 2011).

These unexpected and paradoxical results led us to a question: why did the combined overexpression of Trx1 and Trx2 shorten lifespan? One of the striking observations of this study is that Trx overexpression in the cytosol and mitochondria increased the severity of lymphoma, which could be one of the major contributing factors to shorten lifespan. The tumor-promoting effects of Trx are not surprising, because (1) our previous studies showed that overexpression of Trx1 had a slightly higher incidence of total fatal tumors and fatal lymphomas compared to WT mice (Pérez et al. 2011) and (2) Trx has been shown to stimulate cell growth and have increased levels in various human cancers (Powis et al. 2000) and the experimental glioma model (Mahlke et al. 2011).

To seek the possible underlying mechanisms of why overexpression of Trx in both the cytosol and mitochondria accelerates cancer development and shortens lifespan, we measured ASK1 and mitochondrial apoptosis pathways, several redox-sensitive signaling pathways (i.e., mTOR, HIF-1α, NFκB), and TCA cycle activity in mitochondria, which could play important roles in aging and cancer.

As we have previously shown that Tg (act-*TXN*)$^{+/0}$ mice had reduced ASK1 phosphorylation (Pérez et al. 2011), we measured the levels of ASK1 and mitochondrial apoptosis pathways in TXNTg × TXN2Tg and WT mice. Levels of ASK1 were similar between TXNTg × TXN2Tg compared to WT mice; however, phosphorylated ASK1 levels were significantly lower in young (6–7 months old) TXNTg × TXN2Tg compared to WT mice (Fig. 6a, b). The mitochondrial apoptosis pathway was also assessed for caspase-3 activity and cytochrome c release from the mitochondria. Cytochrome c release and caspase-3 activity were similar between TXNTg × TXN2Tg and WT mice (Fig. 7a, b). Since reduced apoptosis could play important roles in carcinogenesis, the inhibition of the ASK1 pathway by Trx overexpression and its anti-apoptotic effects (Hansen et al. 2006; Hsieh and Papaconstantinou 2006; Pérez et al. 2011; Saitoh et al. 1998; Surh et al. 2005; Wong 2011) could be one of the contributing factors for the onset/progression of cancer (Wong 2011). Therefore, the suppression of the ASK1 pathway may be promoting cancer development, which may have resulted in the accelerated mortality of TXNTg × TXN2Tg mice, although there were no changes in the mitochondrial apoptosis pathway.

Next, we examined several redox-sensitive signaling pathways (i.e., mTOR, HIF-1α, and NFκB) since substantial evidence shows that these pathways play important roles in cancer development and lifespan and can also be attenuated by Trx.

Although substantial evidence showed that mTOR activity is one of the key pathways for cancer development and lifespan, our data showed similar levels of p70S6K1 (Fig. 9a) and phospho-p70S6K1 (Fig. 9b) compared to WT littermates. This suggests that the mTOR pathway did not play roles in the shorter lifespan and accelerated cancer development in mice by the overexpression of Trx in both the cytosol and mitochondria.

Hypoxia-inducible factor 1 is a heterodimeric protein that consists of two proteins, one being HIF-1α. Levels of HIF-1α were significantly increased in TXNTg × TXN2Tg mice compared to WT littermates. These observations are consistent with a previous study showing that Trx1 increases HIF-1α protein expression, and Trx1 overexpression resulted in enhanced tumor angiogenesis (Welsh et al. 2002). In addition to the direct effects of Trx, increased levels of HIF-1α could also be due to changes in the TCA cycle activity in mitochondria. Our data showed that succinate levels were significantly higher in the young male TXNTg × TXN2Tg compared to WT mice. Recent studies show that succinate is a key modulator of the hypoxic response and an important player in tumorigenesis (Jiang and Yan 2017). More importantly, succinate stabilizes HIF-1α by preventing a reaction that would allow HIF-1α to be broken down. Since HIF-1α plays important roles in cancer development (Semenza 2003; Semenza 2009) by activating the transcription of various genes that are involved in carcinogenesis, including those regulating angiogenesis, cell proliferation, and metastasis (Rankin and Giaccia 2008; Semenza 2003; Semenza 2009), increased levels of HIF-1α could be another contributing factor for accelerated cancer development in TXNTg × TXN2Tg mice.

Another notable change in the redox-sensitive signaling pathway was NFκB. TXNTg × TXN2Tg mice had significantly increased levels of NFκB p65 with slightly reduced levels of NFκB p50 and IκB compared to their WT littermates. Increased NFκB activity promotes tumor cell proliferation, suppresses apoptosis, and attracts angiogenesis (Xia et al. 2014). Furthermore, it also induces epithelial-mesenchymal transition, which facilitates distant metastasis (Huber et al. 2004).

Therefore, increased NFκB activity may play important roles in accelerated cancer development in TXNTg × TXN2Tg mice along with increased levels of HIF-1α and reduced ASK1 apoptotic pathway.

Our data suggest that overexpression of Trx in both the cytosol and mitochondria (1) increased HIF-1α levels and NFκB activity, both of which could play an important role in cancer growth (Hoesel and Schmid 2013; Huber et al. 2004; Semenza 2003; Xia et al. 2014), and (2) reduced ASK1 activity, which could suppress apoptosis (Saitoh et al. 1998) and benefit cancer development. These results are unanticipated because a series of comprehensive studies with transgenic mice overexpressing various antioxidant enzymes demonstrated that antioxidant overexpression protects cells/tissues from oxidative stress and does not show any deleterious effects on aging or acceleration of age-related pathology development including cancers (Pérez et al. 2009). Thus, to our knowledge, this study is the first to report that overexpression of the molecule, which has antioxidant properties and protects cells/tissues from oxidative stress, shows deleterious effects on aging. This is most likely due to the unique biological characteristics of thioredoxin, which could change the redox-sensitive signaling pathway activities that have more diverse biological effects on pathophysiology than the protection against oxidative stress. The molecular changes in redox-sensitive signaling pathways described above could accelerate cancer development, which could be one of the major contributing factors that shortened the lifespan in TXNTg × TXN2Tg mice compared to WT littermates.

Although accelerated cancer growth could be one of the explanations for why TXNTg × TXN2Tg mice had a shorter lifespan compared to WT mice, further study is required to determine the specific pathways that shorten lifespan and increase disease burden (the total number of diseases per mouse) by overexpression of Trx in both the cytosol and mitochondria. In addition, the effect of Trx1 and Trx2 overexpression on aging in female mice remains to be examined, as a survival study has not been conducted. To further test the role of Trx in the cytosol and mitochondria, we are currently investigating whether the downregulation of Trx in both the cytosol and mitochondria shows anti-aging and/or anti-cancer effects.

The results of this study along with the ongoing study with mice downregulating Trx in both the cytosol and mitochondria could (1) provide a major advance in our understanding of aging, cancer, and the role of oxidative stress and redox state and (2) lead us to the potential use of pharmacological interventions (e.g., thioredoxin inhibitors) to prevent the occurrence and/or delay the development of age-related cancer and extend healthspan.

Acknowledgements We acknowledge the Pathology Core in the San Antonio Nathan Shock Center (P30-AG013319) for the pathological analyses.

Funding information This research was supported by the VA Merit Review grant from the Department of Veteran Affairs (Y.I.), the NIH grant AG13319 (Y.I.), the American Federation for Aging Research (AFAR) grant (Y.I.), and a grant from the Glenn Foundation (Y.I.).

References

Abate C, Patel L, Rauscher FJ 3rd, Curran T (1990) Redox regulation of Fos and Jun DNA-binding activity in vitro. Science 249:1157–1161

Andersen PK, Borgan O, Gill RD, Keiding N (1993) Statistical models based on counting processes. Springer, New York

Arnér ESJ, Holmgren A (2000) Physiological functions of thioredoxin and thioredoxin reductase. Eur J Biochem 267: 6102–6109

Beauchamp C, Fridovich I (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 44:276–287

Bronson RT, Lipman RD (1991) Reduction in rate of occurrence of age related lesions in dietary restricted laboratory mice. Growth Dev Aging 55:169–184

Brot N, Weissbach H (2000) Peptide methionine sulfoxide reductase: biochemistry and physiological role. Biopolymers 55: 288–296

Brot N, Weissbach L, Werth J, Weissbach H (1981) Enzymatic reduction of protein-bound methionine sulfoxide. Proc Natl Acad Sci U S A 78:2155–2158

Chae HZ, Kang SW, Rhee SG (1999a) Isoforms of mammalian peroxiredoxin that reduce peroxides in presence of thioredoxin. Methods Enzymol 300:219–226

Chae HZ, Kim HJ, Kang SW, Rhee SG (1999b) Characterization of three isoforms of mammalian peroxiredoxin that reduce peroxides in the presence of thioredoxin. Diabetes Res Clin Pract 45:101–112

Custudio M, Maria RL (2007) Diagnostics for choosing between log-rank and Wilcoxon tests. Dissertation, Western Michigan University

Galter D, Mihm S, Droge W (1994) Distinct effects of glutathione disulphide on the nuclear transcripton factor kB and the activator protein-1. Eur J Biochem 221:639–648

Go Y-M, Kang S-M, Roede JR, Orr M, Jones DP (2011) Increased inflammatory signaling and lethality of influenza H1N1 by nuclear thioredoxin-1. PLoS One 1:e18918

Hansen JM, Go YM, Jones DP (2006) Nuclear and mitochondrial compartmentation of oxidative stress and redox signaling. Annu Rev Pharmacol Toxicol 46:215–234

Hoesel B, Schmid JA (2013) The complexity of NF-κB signaling in inflammation and cancer. Mol Cancer 12:86

Hsieh C-C, Papaconstantinou J (2006) Thioredoxin-ASK1 complex levels regulate ROS-mediated p38 MAPK pathway activity in livers of aged and long-lived Snell dwarf mice. FASEB J 20:259–268

Huber MA, Azoitei N, Baumann B, Grünert S, Sommer A, Pehamberger H, Kraut N, Beug H, Wirth T (2004) NF-kappaB is essential for epithelial-mesenchymal transition and metastasis in a model of breast cancer progression. J Clin Invest 114(4):569–581

Ikeno Y, Bronson RT, Hubbard GB, Lee S, Bartke A (2003) The delayed occurrence of fatal neoplastic diseases in Ames dwarf mice: correlation to the extended longevity. J Gerontol 58A:291–296

Ikeno Y, Hubbard GB, Lee S, Richardson A, Strong R, Fernandez E, Diaz V, Nelson JF (2005) Housing density does not influence the longevity effect of calorie restriction. J Gerontol 12:1510–1517

Ikeno Y, Hubbard GB, Lee S, Cortez LA, Lew CM, Rodriguez M, Bartke A (2009) Reduced incidence and delayed occurrence of fatal neoplastic diseases in growth hormone receptor/binding protein (GHR/BP) knockout mice. J Gerontol 64A: 522–529

Jiang S, Yan W (2017) Succinate in the cancer-immune cycle. Cancer Lett 390:45–47

Kim K, Kim IH, Lee KY, Rhee SG, Stadtman ER (1988) The isolation and purification of a specific "protector" protein which inhibits enzyme inactivation by a thiol/Fe (III)/O2 mixed-function oxidation system. J Biol Chem 263:4704–4711

Lawton KA, Berger A, Mitchell M, Milgram KE, Evans AM, Guo L, Hanson RW, Kalhan SC, Ryals JA, Milburn MV (2008) Analysis of the adult human plasma metabolome. Pharmacogenomics 9(4):383–397

Levine RL, Berlett BS, Moskovitz J, Mosoni L, Stadtman ER (1999) Methionine residues may protect proteins from critical oxidative damage. Mech Ageing Dev 107:323–332

Mahlke MA, Cortez LA, Ortiz MA, Rodriguez M, Uchida K, Shigenaga MK, Lee S, Zhang Y, Tominaga K, Hubbard GB, Ikeno Y (2011) The anti-tumor effects of CR are correlated with reduced oxidative stress in ENU-induced gliomas. Pathobiol Aging Age Relat Dis 1:7189

Mitsui A, Hamuro J, Nakamura H, Kondo N, Hirabayashi Y, Ishizaki-Koizumi S, Hirakawa T, Inoue T, Yodoi J (2002) Overexpression of human thioredoxin in transgenic mice controls oxidative stress and life span. Antioxid Redox Signal 4(4):693–696

Nakamura H, Tamura S, Watanabe I, Iwasaki T, Yodoi J (2002) Enhanced resistancy of thioredoxin-transgenic mice against influenza virus-induced pneumonia. Immunol Lett 1:165–170

Patel DP, Krausz KW, Xie C, Beyoğlu D, Gonzalez FJ, Idle JR (2017) Metabolic profiling by gas chromatography-mass spectrometry of energy metabolism in high-fat diet-fed obese mice. PLoS One 12(5):e0177953

Pérez VI, Lew CM, Cortez LA, Webb CR, Rodriguez M, Liu Y, Qi W, Li Y, Chaudhuri A, Van Remmen H, Richardson A, Ikeno Y (2008) Thioredoxin 2 haploinsufficiency in mice results in impaired mitochondrial function and increased oxidative stress. Free Radic Biol Med 44(5):882–892

Pérez VI, Van Remmen H, Bokov A, Epstein CJ, Vijg J, Richardson A (2009) The overexpression of major antioxidant enzymes does not extend the lifespan of mice. Aging Cell 8(1):73–75

Pérez VI, Cortez LA, Lew CM, Rodriguez M, Webb CR, Van Remmen H, Chaudhuri A, Qi W, Lee S, Bokov A, Fok W, Jones D, Richardson A, Yodoi J, Tominaga K, Hubbard GB, Ikeno Y (2011) Thioredoxin 1 overexpression extends mainly the earlier part of life span in mice. J Gerontol 66(12): 1286–1299

Philonenko P, Postovalov S (2015) A new two-sample test for choosing between log-rank and Wilcoxon tests with right-centered data. J Stat Comput Simul 85(14):2761–2770

Powis G, Mustacich D, Coon A (2000) The role of the redox protein thioredoxin in cell growth and cancer. Free Rad Biol Med 29:312–322

Rankin EB, Giaccia AJ (2008) The role of hypoxia-inducible factors in tumorigenesis. Cell Death Differ 15(4):678–685

Saitoh M, Nishitoh H, Fujii M, Takeda K, Tobiume K, Sawada Y, Kawabata M, Miyazono K, Ichijo H (1998) Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. EMBO J 17:2569–2606

Schriner SE, Linford NJ, Martin GM, Treuting P, Ogburn CE, Emond M, Coskun PE, Ladiges W, Wolf N, Van Remmen H, Wallace DC, Rabinovitch PS (2005) Extension of murine life span by overexpression of catalase targeted to mitochondria. Science 308:1909–1911

Semenza GL (2003) Targeting HIF-1 for cancer therapy. Nat Rev Cancer 3(10):721–732

Semenza GL (2009) Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. Oncogene 29(5):625–634

Spyrou G, Enmark E, Miranda-Vizuete A, Gustafsson J-A (1997) Cloning and expression of a novel mammalian thioredoxin. J Biol Chem 272:2936–2941

Sun Y, Elwell JH, Oberley LW (1988) A simultaneous visualization of the antioxidant enzymes glutathione peroxidase and catalase on polyacrylamide gels. Free Radic Res Commun 5: 67–75

Surh YJ, Kundu JK, Na HK, Lee JS (2005) Redox-sensitive transcription factors as prime targets for chemoprevention with anti-inflammatory and antioxidative phytochemicals. J Nutr 132:2993S–3001S

Tagaya Y, Maeda Y, Mitsui A, Kondo N, Matsui H, Hamuro J, Brown N, Arai K-I, Yokota T, Wakasugi H, Yodoi J (1989) ATL-derived factor (ADF), an IL-2 receptor/Tac inducer homologous to thioredoxin; possible involvement of dithiol-reduction in the IL-2 receptor induction. EMBO J 8: 757–764

Takagi Y, Mitsui A, Nishiyama A, Nozaki K, Sono H, Gon Y, Hashimoto N, Yodoi J (1999) Overexpression of thioredoxin in transgenic mice attenuates focal ischemic brain damage. Proc Natl Acad Sci U S A 96:4131–4136

Toledano MB, Leonard WJ (1991) Modulation of transcription factor NF-KB binding activity by oxidation-reduction in vitro. Proc Natl Acad Sci U S A 88:4328–4332

Wang C, Li Q, Redden DT, Weindruch R, Allison DB (2004) Statistical methods for testing effects on "maximum lifespan". Mech Ageing Dev 125:629–632

Welsh SJ, Bellamy WT, Briehl MM, Powis G (2002) The redox protein thioredoxin-1 (Trx-1) increases hypoxia-inducible factor 1α protein expression: Trx-1 overexpression results in increased vascular endothelial growth factor production and enhanced tumor angiogenesis. Cancer Res 62:5089–5095

Williams MD, Van Remmen H, Conrad CC, Huang TT, Epstein CJ, Richardson A (1998) Increased oxidative damage is correlated to altered mitochondrial function in heterozygous manganese superoxide dismutase knockout mice. J Biol Chem 273:28510–28515

Wong RSY (2011) Apoptosis in cancer: from pathogenesis to treatment. J Exp Clin Cancer Res 30:87

Xia Y, Shen S, Verma IM (2014) NF-κB, an active player in human cancers. Cancer Immunol Res 2(9):823–830

Yoshihara E, Masaki S, Matsuo Y, Chen Z, Tian H, Yodoi J (2013) Thioredoxin/Txnip: redoxisome, as a redox switch for the pathogenesis of diseases. Front Immunol 4:514

Zhang H, Luo Y, Zhang W, He Y, Dai S, Zhang R, Huang Y, Bernatchez P, Giordano FJ, Shadel G, Sessa WC, Min W (2007) Endothelial-specific expression of mitochondrial thioredoxin improves endothelial cell function and reduces atherosclerotic lesions. Am J Pathol 1:1108–1120