ANTIOXIDANTS & REDOX SIGNALING
Volume 4, Number 4, 2002

Rapid Letter

Overexpression of Human Thioredoxin in Transgenic Mice Controls Oxidative Stress and Life Span

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ABSTRACT

Transgenic (Tg) mice overexpressing human thioredoxin (TRX), a small redox-active protein, were produced to investigate the role of the protein in a variety of stresses. Bone marrow cells from TRX-Tg mice were more resistant to ultraviolet C-induced cytocide compared with those from wild type (WT) C57BL/6 mice. TRX-Tg mice exhibited extended median and maximum life spans compared with WT mice. Telomerase activity in spleen tissues in TRX-Tg mice was higher than that in WT mice. These results suggest that overexpression of TRX results in resistance against oxidative stress and a possible extension of life span without apparent abnormality in mammals. *Antioxid. Redox Signal.* 4, 693–696.

INTRODUCTION

THIOREDOXIN (TRX) is a small, ubiquitous multifunctional protein with a redox-active disulfide/dithiol within the conserved active-site sequence: -Cys-Gly-Pro-Cys- (2). TRX regulates via thiol redox control various intracellular molecules, including transcription factors such as nuclear factor-κB and activator protein 1 (7). TRX gene promoter contains a variety of stress responsive elements (3), and TRX is induced by various stresses, including viral infection, ischemia–reperfusion, and hydrogen peroxide (7). Furthermore, administration of recombinant TRX protein shows a cytoprotective effect against oxidative stress-induced cell damage (6). These data suggest that TRX plays a number of important biological roles in both intra- and extracellular compartments.

We produced TRX-overexpressing transgenic (Tg) mice and investigated the role of TRX in stress response, as well as longevity. Previous study demonstrated that brain focal ischemic injury is attenuated in TRX-Tg mice and that generation of oxidized protein is suppressed in TRX-Tg mice after the ischemic insult (13). In the present study, we examined ultraviolet (UV)-induced cytocide of hemopoietic progenitor cells in TRX-Tg mice and control wild-type (WT) C57BL/6 mice, and also determined telomerase activities, as well as their life spans.

MATERIALS AND METHODS

TRX-Tg mice

TRX-overexpressing Tg mouse lines were established as described previously (13). In brief, plasmid vector, pADF-βAP/T, was constructed by inserting human β-actin promoter from pHβAPr-3-neo, human TRX cDNA (12), and human β-actin terminator from pHβAPr-3-neo in pUC18. To generate Tg mice, 5.5-kb Xba I-Vsp I fragment of the recombinant

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plasmid was microinjected into the pronuclei of the C57BL/6. Mouse line (βAc-ADF-2) containing four copies of the human TRX cDNA per genome was used for further studies. TRX-Tg mice were selected by polymerase chain reaction (PCR) with mouse genomic DNA as a template and synthetic oligonucleotides as primers: forward primer, 5′-CAGATCGACAAGAC-3′; reverse primer, 5′-CAGGAA-ACAGCTATGAC-3′. Animals were maintained on normal diet under the specific pathogen-free conditions.

TRX assay

TRX activities in the organ homogenate were determined by NADPH/TRX reductase-dependent insulin reducing assay (5). In brief, the assay mixture (120 μl) contained 0.1 *M* Tris-Cl, pH 7.5, 2 m*M* EDTA, 0.2 m*M* NADPH, 140 μ*M* insulin, 0.4 units/ml human placenta TRX reductase, and organ homogenate. The reaction was determined from the oxidation of NADPH at 340 nm at 25°C. The activity was expressed as ΔA_{340}/min.

Survival of CFU-GM

Bone marrow cells from the TRX-Tg mice were exposed to a graded dose of UVC (248 nm) in each Petri dish, followed by semisolid *in vitro* culture for colony formation of hemopoietic progenitor cells (CFU-GM) as described elsewhere (8). In brief, marrow cells (2×10^4/ml) in minimum essential medium Eagle (α modification) containing 0.8% methyl cellulose, 30% (vol/vol) fetal bovine serum, 1% deionized bovine serum albumin (fraction V; Sigma), 100 μ*M* 2-mercaptoethanol, and 10 ng/ml recombinant murine granulocyte-macrophage growth factor (R&D Systems, Minneapolis, MN, U.S.A.) were plated into 35-mm Petri dishes. The cultures were incubated at 37°C, 5% CO_2 for 6 days, and colonies were scored under a dissecting microscope.

Survival analysis of mice

TRX-Tg mice and normal littermates were maintained under identical conditions with free access to food and water. Identification of either TRX-Tg or normal animals was done by PCR described above immediately after the death. Survival curves of each group were plotted according to the Kaplan–Meier method, and the survival data were analyzed by the generalized Wilcoxon test. The median and maximum life spans of the animals were calculated from the time (in months) at which mortality reached 50% and 100% of the starting population of each group.

Measurement of telomerase activity

A stretch PCR assay for telomerase activity was use as previously described (14). Spleen cells were prepared by Ficoll–Hypaque density centrifugation and lysed in lysis buffer on ice for 30 min. The protein concentration of lysates was measured using the Bio-Rad DC Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA, U.S.A.). Telomerase activities were analyzed by TeloChaser kit (Toyobo Co., Ltd., Osaka, Japan) in a 6-bp ladder when the PCR products were subjected to the electrophoresis on a 10% polyacrylamide gel and stained with ethidium bromide.

RESULTS

Generation of TRX-overexpressing Tg mice

TRX-Tg mice grew normally and were fertile. There were no observable phenotypic and behavioral differences between TRX-Tg and WT mice. Previous report demonstrated that human TRX protein content in TRX-Tg tissues was at least threefold higher than mouse endogenous TRX without exception (13). We also reconfirmed that the activity of TRX in brain and heart of TRX-Tg mice was roughly sixfold and twofold higher, respectively, compared with that of WT mice (Fig. 1).

Resistance against oxidative stress in TRX-Tg mice

Previous reports have shown that TRX overexpression introduces resistance to a variety of oxidative stresses *in vitro*. TRX-Tg mice show resistancy against oxidative stress, including cerebral ischemia (13). As UV exposure is known to produce oxidative stress, we examined here the resistancy of bone marrow cells from TRX-Tg and WT mice against UVC exposure. When exposed to UVC at 9.4 μW/cm², bone marrow cells from TRX-Tg mice were more resistant to UVC-induced cytocide than those from WT mice (Fig. 2).

Extended life span of TRX-Tg mice

Fifty-three TRX-Tg and WT mice were bred and maintained under the specific pathogen-free condition. Body weights of TRX-Tg and WT mice at 12 months after birth were not significantly different (data not shown), indicating that the Tg mice grew normally until at least this time. Survival curves of TRX-Tg and WT mice are shown in Fig. 3. In this experiment, the median survival of WT mice was relatively shorter than that of C57BL/6 mice previously reported as 21–23 months. However, under the same condition, the life spans of TRX-Tg and WT mice were significantly different ($p = 0.039$, determined by generalized Wilcoxon test), and the onset of median and 100% mortality (median and maxi-

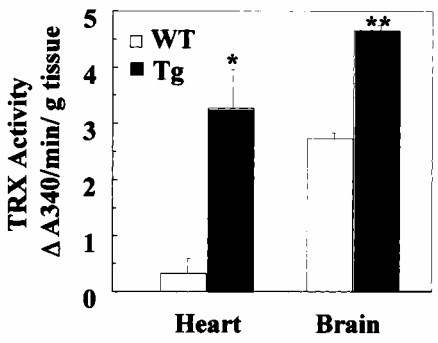

FIG. 1. TRX activities in the heart and the brain are elevated in TRX-Tg mice. TRX activity in tissue homogenate was measured by NADPH/TRX reductase-dependent insulin reducing assay. Open columns indicate TRX activity in WT organs ($n = 4$), and hatched columns represent TRX-Tg mice ($n = 3$). Data are expressed as means ± SEM (standard error of mean value). $*p < 0.05$; $**p < 0.001$, compared with WT mice.

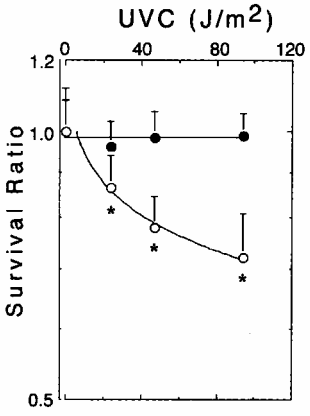

FIG. 2. Bone marrow cells from TRX-Tg mice are more resistant to exposure to UVC. CFU-GM survival after exposure to a graded dose of UV light was determined. Survival curves of CFU-GM of TRX-Tg (●) and WT (○) mice after exposure to UVC at 9.4 μW/cm^2 are shown.

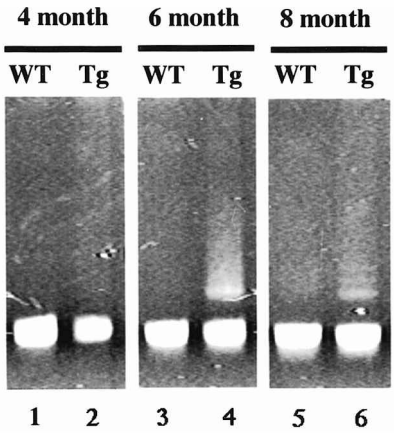

FIG. 4. Telomerase activity in TRX-Tg spleen tissue is higher than that in WT tissue. Telomerase activity in spleen tissues of 4-, 6-, and 8-month-old WT and TRX-Tg mice was examined as described in Materials and Methods. The intensity of 6-bp ladder over the lower band indicates telomerase activity.

mum life span) in TRX-Tg mice were delayed as compared with WT mice. Relative to the controls, the percent increase in TRX-Tg mice was 35% in the median life span and 22% in the maximum life span. To confirm this result, another independent experiment was performed. Fifty-one percent of TRX-Tg mice survived (42 alive/82 total) at 20 months after birth, whereas only 30% of WT mice survived (28 alive/94 total). The difference between TRX-Tg and WT mice was significant ($p = 0.004$, χ^2 test).

Enhanced telomerase activity in TRX-Tg tissue

Previous reports have showed evidence of antioxidation and telomerase activity extending the life span (15). Recently, it was also reported that telomerase-deficient mice have impaired stress response and shorter life span than their WT counterparts (1, 10).

Thus, we examined the telomerase activity in spleen tissues of TRX-Tg and WT mice. Samples from spleen tissues of 4-, 6-, and 8-month-old TRX-Tg mice, but not WT mice, showed 6-bp ladder signals amplified by PCR with telomere sequence-specific primers (Fig. 4, lanes 2, 4, and 6). This indicates that telomerase activity in TRX-Tg spleen tissue was higher than that in WT tissue.

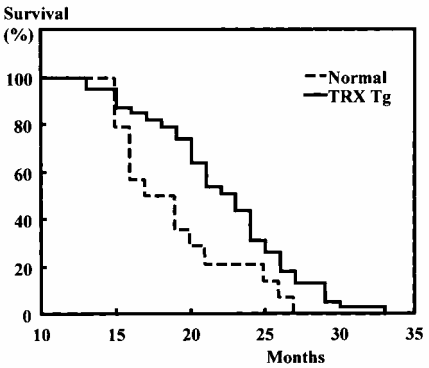

FIG. 3. TRX-Tg mice exhibit extended life span. Survival curves of TRX-Tg mice (solid line) and WT mice (dashed line) were plotted according to the Kaplan–Meier method.

DISCUSSION

We demonstrate here that overexpression of human TRX in Tg mice suppresses oxidative stress damage and elongates the life span. In our experiments, the mean body weight of the TRX-Tg mice was statistically not different from that of control WT mice. Although caloric restriction is one of the most effective ways of survival elongation (16–18) and further detailed study with well controlled food uptake is ideal, our study strongly suggests that the longer life span of TRX-Tg mice is not mainly due to nutrition factors. Considering the possible unknown influence of environmental factors on the survival data, a cooperative study in a different facility in the U.S.A. is now in progress.

Previous study demonstrated that focal ischemic injury of the brain was attenuated and the generation of oxidized protein after the ischemic insult was prevented in TRX-Tg mice (13). In the present study, TRX-Tg mice were shown to be resistant to oxidative tissue damage, such as UV-induced cytocide of hemopoietic progenitor cells. These data demonstrate that overexpressed TRX in murine tissue could protect the animals from reactive oxygen species-induced tissue injury.

Several lines of evidence have suggested that oxidative stress is closely related to the life span, and antioxidant enzymes are required for longevity of mammals. The maximum life span of mice, rats, guinea pigs, rabbits, pigs, and cows correlated positively with superoxide dismutase (SOD) and catalase activity in the brain, liver, and heart (11). The activity of mitochondrial SOD in the liver tissues of the senescence-accelerated mouse strain was about one-half that in the control strain (9).

Telomerase activity is also another determinant of longevity of various animals. We showed evidence of extension of life span of TRX-Tg mice along with higher expression of telomerase activities in spleen cells from 4-, 6-, and 8-month-old Tg mice compared with those in WT mice.

Recently, targeted mutation of the mouse $p66^{shc}$ gene has been demonstrated to prolong mouse life span by as much as 30% (4). This mutation enhanced resistance to hydrogen peroxide- or UV irradiation-induced cellular apoptosis, as well as *in vivo* resistance to paraquat, which generates superoxide anions upon cellular intake. These findings and our present study provide evidence that acquired stress resistance in mice is associated with extended life span.

It is to be noted that there was no significant incidence of tumor formation in TRX-Tg mice in our colony. Together with the increase of life span, TRX overexpression by itself may not increase the risk of oncogenesity, although a detailed study with local application of carcinogens is in progress.

In summary, TRX-overexpressing mice acquired resistance to various oxidative stresses, as well as extended life span. TRX-Tg mice provide a unique experimental system to study the mechanism of the aging process in mammals.

ACKNOWLEDGMENTS

We thank Drs. A. Richardson at THSC at San Antonio and Huber Warner at NIA, NIH for critical comments on the manuscript and ongoing cooperation for the further detailed study of longevity.

ABBREVIATIONS

PCR, polymerase chain reaction; SOD, superoxide dismutase; Tg, transgenic; TRX, thioredoxin; UV, ultraviolet, WT, wild-type.

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Received for publication April 8, 2002; accepted May 8, 2002.