Free Radical Research, 2002 Vol. 36 (9), pp. 949–956

Taylor & Francis healthsciences

Thioredoxin, an Anti-oxidant Protein, Protects Mouse Embryos from Oxidative Stress-induced Developmental Anomalies

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Accepted by Professor N. Taniguchi

(Received 11 March 2002; In revised form 19 March 2002)

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During the early postimplantation period, rodent embryos survive in a relatively anaerobic environment *in utero* and are vulnerable to a high oxygen pressure. They become resistant to oxygen stress when they are exposed to a higher oxygen pressure after the uteroplacental circulation is established. However, it is unknown how embryos acquire such resistance against oxidative stress. This study was undertaken to examine whether an antioxidant protein thioredoxin (TRX) plays a significant role in the embryonic acquisition of the tolerance to oxidative stress. E7.5 embryos of C57BL/6 wild-type (WT) and human TRX (hTRX) inserted-transgenic (Tg) embryos were cultured under 10 or 25% O_2 and their growth and morphological differentiation were evaluated. The TRX expression and the products of oxidative stress (8-hydroxy-2′-deoxy-guanosine and carbonylated proteins) in their tissues were also examined. When WT embryos were cultivated *in vitro* under 25% O_2, their growth was significantly disturbed and various developmental abnormalities were induced, which did not occur in embryos grown under 10% O_2. However, such embryotoxic effects of oxygen were significantly attenuated in the hTRX Tg embryos that continuously express hTRX. Accumulation of the products of oxidative stress was significantly reduced in hTRX Tg embryos as compared with that in WT embryos. The TRX transgene appears to provide the embryo with the resistance against oxidative stress and may play a crucial role in the redox regulation in embryos.

Keywords: Thioredoxin; Oxidative stress; Mouse embryo; hTRX transgenic mouse; Whole embryo culture; Dysmorphogenesis

INTRODUCTION

In vitro culture of whole rodent embryos is widely used to investigate the mechanisms of normal and abnormal development as well as the embryotoxic effects of environmental agents.[1] When rodent embryos at the headfold and neurulation stages (embryonic day (E) 7.5–9.5 in mice and E9–11 in rats) are cultivated *in vitro*, they are empirically grown under a relatively low oxygen concentration (5–10% O_2) because their growth and differentiation are significantly perturbed and various developmental anomalies are induced if they are grown under higher oxygen concentrations,[2,3] which means that early postimplantation rodent embryos are vulnerable to oxygen stress. A number of previous papers reported on the teratogenicity of hyperoxia *in vivo*. Ferm[4] exposed pregnant hamsters to 3–4 atmospheres of oxygen on the 6th, 7th or 8th day of gestation and produced umbilical hernia, excencephaly, spina bifida and limb defects in the offspring. Cardiac malformations such as interventricular septal defects and transposition of great vessels were frequently produced in rat pups from dams exposed to air or oxygen at three atmospheres on E8 or E13.[5] Hyperbaric oxygen was also found to increase resorptions[6] and to reduce fetal weights.[7]

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ISSN 1071-5762 print/ISSN 1029-2470 online © 2002 Taylor & Francis Ltd
DOI: 10.1080/1071576021000006626

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Although rodent embryos up to the neurulation stage are highly susceptible to oxidative stress, they become less sensitive to oxygen toxicity as development progresses and their oxygen requirement increases.[8] In fact, the oxygen requirement of explanted embryos increases to 95% by E10 in mice and E11 in rats.[8,9] Chen *et al.*[10] showed that rat embryos treated with 45% O_2 from E9 exhibited severe abnormalities, most prominently neural tube closure defects, while those treated on E10–11 were morphologically normal. The developmental stage at which the embryonic oxygen requirement remarkably changes seems to correspond to the stage when the definitive allantoic placenta is formed and replaces the yolk sac placenta. The yolk sac placenta supports the respiration and nutrition of the embryo during the early postimplantation period and takes oxygen and nutrients from blood circulation by diffusion through the chorion and visceral yolk sac. Around E9.5 in mice and E11.0 in rats, the yolk sac placenta regresses and the definitive allantoic placenta takes over an increasing share of respiratory and nutrient exchanges. Coincidentally with such a transition in the uteroplacental circulation, embryos should be acutely exposed to a higher oxygen pressure, which benefits rapid growth and differentiation, and therefore need to acquire a defense mechanism against oxidative stress.

Thioredoxin (TRX) is a small ubiquitous protein (12 kDa) and is known to function as an antioxidant protein, catalyzing thiol-disulfide oxidoreductions.[11,12] Its active consensus sequence Cys–Gly–Pro–Cys is conserved across species and the two cysteine residues can be reversibly oxidized to participate in reversible oxidation–reduction reactions.[13] TRX is induced in various cells and tissues when they are exposed to oxidative stress[14] and is involved in various biological reactions including cell proliferation, hormone secretion, and cellular signaling.[12,15] It has shown that TRX regulates the DNA binding activity of several transcriptional factors such as activator protein 1 (AP-1) (c-Fos/c-Jun complex),[16] nuclear factor (NF)-κB[17] and p53.[18,19] TRX has been reported to induce AP-1 through *de novo* transcription of c-Fos and c-Jun.[20]

We previously examined immunohistochemically the ontogenesis of two antioxidant proteins, TRX and glutaredoxin (GRX), in mouse embryos and fetuses, and found that they begin to be expressed in the embryonic heart at E8.5 and in many other tissues after E11.[21] We assume that these antioxidant proteins may contribute in providing the embryo with resistance against oxidative stress and help it adapt to a new aerobic environment. In the present study, in order to examine the roles of TRX in the acquisition of tolerance of mouse embryos against oxidative stress, we cultivated embryos of the TRX-transgenic (Tg) mouse, which constantly overexpress human TRX (hTRX)[22] and examined their susceptibility to oxygen toxicity. The hTRX Tg embryos were found to be significantly more resistant to oxygen toxicity than wild-type (WT) embryos and the products of oxidative stress were significantly reduced in the tissues of Tg embryos. Our findings provide an insight into the redox regulation in the mouse embryo and its possible roles in development.

MATERIALS AND METHODS

Animals

C57BL/6 strain mice were purchased from Japan SLC Co. Ltd., (Hamamatsu, Japan) and reared in our laboratory for using as WT controls. TRX Tg mice were kindly provided by Ajinomoto, Co. Inc. Basic Research Laboratory (Kawasaki, Japan).[22] For producing the Tg mouse, hTRX cDNA was inserted between the β-actin promoter and the β-actin terminator and the transgene was microinjected in the pronuclei of fertilized eggs from C57BL/6 strain mice.[22] Embryos were obtained by mating the transgenic female and male at noon of the day on which a vaginal plug was found which was designated as embryonic day 0.5 (E0.5). The presence of TRX transgene was confirmed by PCR.

Whole Embryo Culture

At E7.5, pregnant females were killed by overdosage of ether, and their uterus was carefully opened. The decidua and Reichert's membrane of the conceptuses were removed and the embryos and their yolk sacs were prepared for culture. They were explanted in the culture medium consisting of 75% rat serum and 25% Tyrode buffer and cultivated in rotating bottles for 48 h.[1] Before starting the culture, each bottle was flushed for two minutes with a gas mixture containing a given concentration of O_2, 5% CO_2 and N_2 balance, and then sealed air-tight. Embryos were cultured under 10 or 25% O_2 and their growth and differentiation were compared between the groups. We used 10% O_2 because early postimplantation rodent embryos are empilically cultured under 5–10% O_2[8] and 25% O_2 was used as a hyperoxic condition. Cultured embryos were harvested after 48 h and evaluated for growth and morphological differentiation under a dissection microscope according to the criteria proposed by Klug *et al.*[23]

Histological Examination

After observation, the embryos were fixed in 70% ethanol, overnight at 4°C. The samples were dehydrated in graded concentrations of ethanol, embedded in paraffin, and sectioned at 4 μm

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TABLE I Development of C57BL/6 embryos cultured under different oxygen concentrations

Group	Number of embryos examined	Number of somites (mean ± SD)	Crown-rump length (mm) (mean ± SD)	Score* (mean ± SD)	No. of abnormal embryos (%)		
					Open neural tube	Microcephaly	Not rotated
10% O_2							
Wild-type	19	21.6 ± 2.1	2.0 ± 0.4	31.3 ± 4.0	2 (16.7)	0 (0.0)	3 (22.2)
TRX transgenic	13	22.8 ± 3.6	2.2 ± 0.3	33.8 ± 4.3	1 (7.7)	1 (7.7)	1 (7.7)
25% O_2							
Wild-type	19	17.0 ± 2.1†	1.7 ± 0.5†	20.8 ± 5.0†	19 (100.0)†	9 (47.4)†	18 (94.7)†
TRX transgenic	17	23.3 ± 2.5‡	2.0 ± 0.3‡	33.1 ± 3.0‡	6 (35.3)‡	1 (5.9)‡	0 (0.0)‡

*According to Ref. [23]. †Significantly different from the 10% wild-type group ($P < 0.05$). ‡Significantly different from the wild-type group ($P < 0.05$).

thickness for histological and immunohistochemical studies.

Immunohistochemical Localization of TRX and AP-1

For immunohistochemical detection of TRX and AP-1 proteins, the paraffin sections were incubated with 0.3% hydrogen peroxide for 30 min to remove the endogenous peroxidase activity and immersed in a blocking solution to reduce non-specific background staining. Then the sections were immunoreacted with respective antibodies. The antibodies used were a rabbit anti-mouse TRX polyclonal antibody,[24,25] a mouse anti-human TRX monoclonal antibody (ADF-11 mAb),[26] a rabbit anti-c-Fos polyclonal antibody (K-25) (Santa Cruz Biotechnology, Inc., Santa Cruz) and rabbit anti-c-Jun polyclonal antibody (H-79) (Santa Cruz Biotechnology, Inc., Santa Cruz). We confirmed that endogenous mouse IgG was not detected in these embryonic tissues before using the mouse anti-hTRX monoclonal antibody (ADF-11 mAb). The immunoproducts were detected by the ABC method and the bound peroxidase was visualized with diaminobenzidine (DAB). Immunohistochemical staining was performed on more than four embryos from each experimental group.

Western Blot Analysis

To detect TRX proteins in embryonic tissues, the hearts of 15–20 embryos were collected and lysed in a solubilizing buffer (0.5% Nonidet P-40/10 mM Tris–HCl pH 7.2/150 mM NaCl/1 mM PMSF) and protein was quantitated by the Micro BCA protein assay method (Pierce Ltd., Co., Rockford).

After boiling for 10 min in the sample buffer, 10 μg protein per lane was separated in a 15% polyacrylamide gel and transferred to a polyvinylidene defluoride membrane (Millipore, MA) by electroelution. After blocking in 5% skim milk, the membrane was incubated with a rabbit anti-mTRX polyclonal antibody.[24,25] Horseradish peroxidase-conjugated anti-rabbit IgG (Funakoshi, Tokyo) was used as the secondary antibody. The bands reactive with the antibody were visualized by the horseradish peroxidase-ECL method. Each test was repeated three times.

Immunohistochemical Detection of 8-OHdG

The 8-hydroxy-2′-deoxy-guanosine (8-OHdG) is a DNA base-modified product induced by oxidative stress and is used as a maker of oxidative stress.[27] To visualize 8-OHdG, paraffin sections of embryos were pretreated by boiling 500 mM citrate acid buffer (pH 6.0) for 5 min and immunoreacted with an anti-8-OHdG monoclonal antibody (kindly gifted by Dr S. Toyokuni, Kyoto University). More than four

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embryos from each group were subjected to the immunohistochemical examination for 8-OHdG.

Detection of Oxidized Proteins

Proteins was extracted from 5–13 embryos for each group and derivatized to 2,4-dinitrophenylhydraxone (DNP-hydrazone) by a reaction with 2,4-dinitrophenylhydrazine (DNPH) using an oxidized protein detection kit (S7150) (Intergen, New York) to detect carbonylated proteins, a marker of cellular protein oxidation. Ten micrograms of the DNP-derivatized protein sample per lane was separated by 12% polyacrylamide gel electrophoresis and transferred to a polyvinylidene defluoride membrane (Millipore, MA) by electroelution followed by Western blotting. The amount of oxidized proteins was also compared between groups by dot blot analysis. Western blot and dot blot analyses were repeated three times.

Statistical Analysis

Student's *t*-test was applied for comparing means and chi-square test for comparing frequencies.

RESULTS

When E7.5 WT C57BL/6 embryos were cultivated for 48 h under 10% O_2, rotation of the body axis and/or neural tube closure were not completed in 3 of the 19 embryos (22.2%), suggesting that this condition could be slightly toxic to embryos. Only one of the Tg embryos (7.7%) was abnormal, but the frequency of abnormal embryos was not significantly different from that of the WT embryos. The growth and morphological differentiation of hTRX Tg embryos, as judged by the somite number, body length and developmental scores,[23] were comparable to those of WT C57BL/6 embryos. When WT embryos were cultivated under 25% O_2, their growth was significantly retarded as compared with those grown under 10% O_2, and developmental abnormalities were frequently induced (Table I). Malformations induced in the 25% O_2 group included microcephaly (47.4%), open neural tube (100%) and malrotation of the body axis (94.7%) (Fig. 1). When hTRX Tg embryos were cultivated under 10 or 25% O_2, the prevalence of developmental abnormalities was decreased as compared with the corresponding values for WT controls, the difference being significant between the 25% O_2 groups (Table I). When the expression of TRX proteins in E7.5 embryos was examined immunohistochemically, mTRX was not detected in the tissues of WT and TRX Tg embryos (Fig. 2A and B) as was shown previously in ICR embryos[21]. The mTRX began to be detected at E8.5 in WT C57BL/6 embryos (data not shown) similarly as in ICR embryos. While hTRX protein was not detected in the embryonic proper tissue of E7.5 WT embryos, it was intensely expressed in tissues of E7.5 hTRX Tg embryos (Fig. 2C and D). When WT embryos were cultured under 10% O_2, mTRX was clearly expressed in embryonic tissues at the end of the culture period, whereas its immunoreactivity was weaker in WT embryos exposed to 25% O_2 (Fig. 3A and B). The mTRX expression in hTRX Tg embryos was not altered by the elevated oxygen concentration (Fig. 3C and D). An immunoblot analysis confirmed the expression of mTRX in the heart of WT embryos grown under 10% O_2 but it was barely detectable in WT embryos exposed to 25% O_2 (Fig. 3G). We used the heart of cultured embryos for immunoblot analysis because the protein content of the whole body was significantly decreased in the 25% O_2 group because of growth retardation but the protein amount was relatively well maintained in the heart.

To evaluate the magnitude of oxidative stress in explanted embryos, the expression of 8-OHdG and the generation of carbonylated proteins, which are biochemical markers of cellular oxidative stress, were examined in cultured whole embryos (Fig. 4). In WT embryos, 8-OHdG induction increased remarkably in various tissues when they were

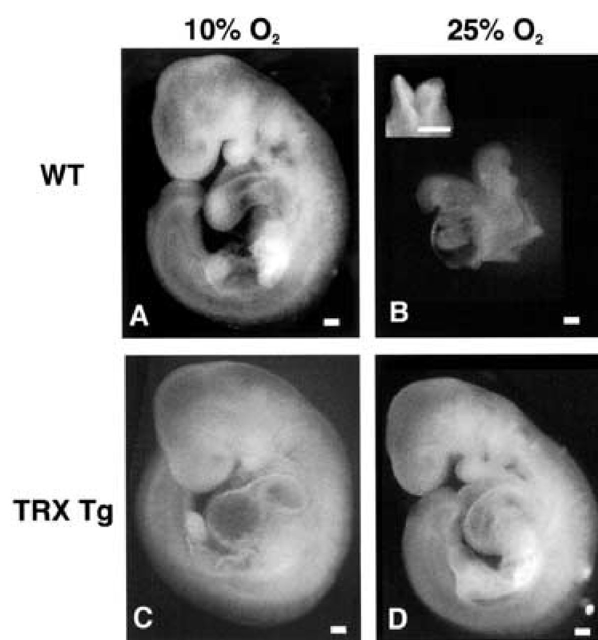

FIGURE 1 Mouse embryos cultured from E7.5 under different O_2 concentrations. WT (A,B) and hTRX Tg (C,D) embryos were cultured under 10% O_2 (A,C) or 25% O_2 (B,D) for 48 h. Both the WT and hTRX Tg embryos grew comparably when cultured under 10% O_2 (A,C). When exposed to 25% O_2, growth was retarded and malformations such as open neural tube (inserted in B), microcephaly, malrotation of the body axis were induced in WT embryos (B). The growth of hTRX Tg embryos (D) was significantly better than that of WT embryos (B). (Bars = 100 μm).

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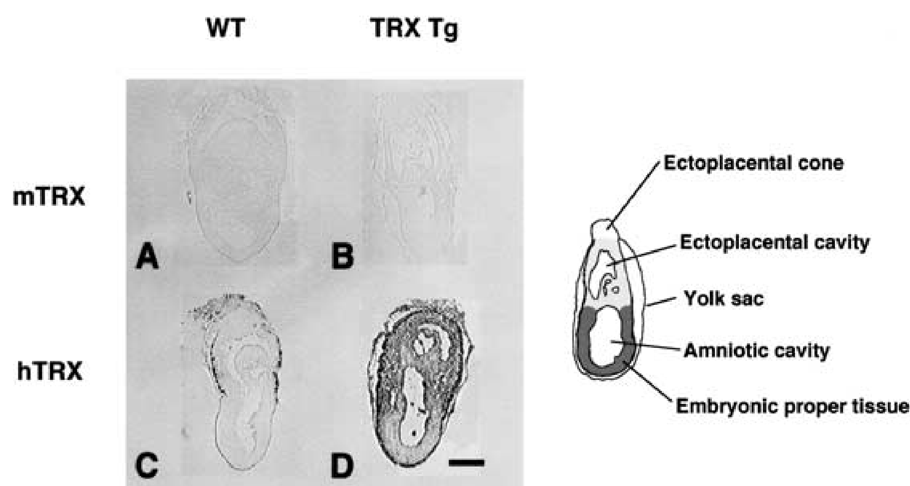

FIGURE 2 Expression of mTRX and hTRX in E7.5 embryos. mTRX was not detectable in E7.5 embryos (A,B). hTRX was not detected in E7.5 WT embryos (C) but was expressed intensely in TRX Tg embryos (D) (Bars = 100 μm).

exposed to 25% O_2 as compared with that in embryos cultured under 10% O_2 (Fig. 4A and B). In hTRX Tg embryos, however, the increase for 8-OHdG by the elevated oxygen pressure remained minimal and less than that in WT embryos (Fig. 4C And D). When the production of carbonylated proteins in WT embryos was detected by Western blotting, most bands were clearly more dense in the 25% O_2 group than in the 10% O_2 group (Fig. 4E). A dot blot analysis for comparing the relative density of carbonylated protein confirmed the difference between the two groups (Fig. 4F).

The expression of activator protein 1 (AP-1, c-Fos/c-Jun complex) in embryos was also examined immunohistochemically to clarify the relationship between TRX-dependent redox signaling and the attenuation of oxygen-induced developmental anomalies. Neither c-Fos nor c-Jun was detected at E7.5 and E8.5 both in WT and TRX Tg embryos (data not shown).

DISCUSSION

Reactive oxygen species (ROS) including oxygen, superoxide and hydrogen peroxide, can react with various intracellular molecules and cause them damage. Fantel[28] extensively reviewed the roles of ROS in normal and abnormal development, and provided evidence that oxygen radicals underlie the embryotoxicity and teratogenicity of various exposures during the prenatal life. He suggested that some teratogens could induce transient utero-placental hypoperfusion and generate ROS, thereby inducing dysmorphogenesis. One of such malformations is a limb reduction defect caused by vascular disruptions, which can result from hypoxia, secondarily to hypoperfusion. The anticonvulsant drug phenytoin (dephenylhydantoin) is teratogenic in several animal models, as well as in humans, and produces various malformations including limb defects.[29,30] Winn and Wells[31] showed that maternal administration of polyethylene superoxide dismutase (SOD) can enhance phenytoin embryotoxicity and that its teratogenicity can be modulated by the antioxidant enzyme glycol-catalase, which supports the hypothesis that embryonic bioactivation and formation of ROS are associated with phenytoin teratogenesis. Similarly ROS have been

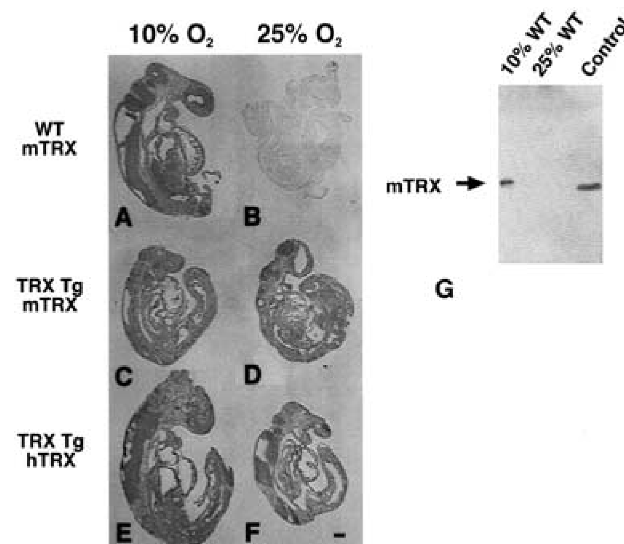

FIGURE 3 Expression of TRXs in mouse embryos cultured *in vitro*. (A–F) TRXs were detected immunohistochemically in embryos cultured from E7.5 for 48 h. The expression of mTRX in WT embryos was significantly suppressed when cultured at 25% O_2 (B) as compared with that in the 10% O_2 group (A). However, both mTRX (C,D) and hTRX (E,F) were expressed similarly in hTRX Tg embryos grown under 25% O_2 and those cultured under 10% O_2 (C–F). (Bars = 100 μm) (G) Western blot for mTRX with the heart of WT embryos cultured from E7.5 for 48 h. mTRX protein was detected in embryos grown under 10% O_2 (10% WT) but barely in those grown under 25% O_2 (25% WT). The lysate of limbs from E13.5 WT embryos was used as a positive control (Control).

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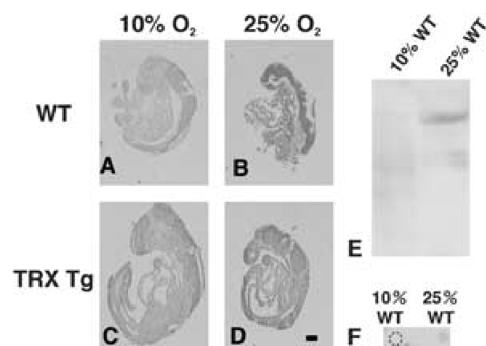

FIGURE 4 Products of oxidative stress (A–D) The accumulation 8-OHdG was immunohistochemically detected in WT and hTRX Tg embryos cultured from E7.5 under 10% O_2 (A,C) or 25% O_2 (B,D). The area of high staining intensity is shown red by density slicing by the NIH image software. The amount of 8-OHdG was clearly increased in WT embryos grown under 25% O_2 (B) when compared with those exposed to 10% O_2 (A). However, the 8-OHdG level increased only slightly in hTRX Tg embryos cultured under 25% O_2 (D) as compared with the 10% O_2 group (C). (Bar = 100 μm) (E) A quantitative analysis of carbonylated proteins by Western blotting. The bands show oxidized proteins, whose density is clearly more dense in the 25% O_2 group (25% WT) than in the 10% group (10% WT). (F) A dot blot analysis confirmed that carbonylated proteins increased in WT embryos exposed to 25% O_2 as compared with the 10% O_2 group.

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implicated in the teratogenicity of vasoreactive agents and some other disease states.[28] If the developmental transition of the embryonic internal environment from anaerobic to more aerobic states has some similarity to hypoxia/reperfusion, the oxidative damage in early embryos and the reperfusion injury in adult tissues may have some pathogenic mechanism in common.

It has also been suggested that embryonic dysmorphogenesis in diabetic environment or hyperglycemia may be induced by generation of ROS[32] and that ROS scavenging enzymes can protect diabetic embryos from glucose-induced malformations.[33] Trocino *et al.*[34] showed that anomalies induced in rat embryos grown under hyperglycemia conditions can be associated with GSH depletion and that GSH-esters can attenuate hyperglycemia-induced abnormalities. The teratogenic effect of diabetic serum could be prevented by SOD and *N*-acetylcysteine in rat whole embryo culture.[35] These results suggest that redox imbalance can be induced by some embryonic metabolic disorders and that the regulation of the proper redox status is essential for normal embryonic development.

Our present study demonstrated that the transgenic mouse embryos that overexpress hTRX were significantly more resistant to the teratogenic effects of hyperoxia than WT embryos. While the growth and differentiation of WT embryos were disturbed and developmental anomalies were frequently produced when they were cultured under 25% O_2, the development of hTRX Tg embryos was not significantly affected by the hyperoxic condition. Thus, TRX is most likely to provide early mouse embryos with attenuation to the embryopathic effects of oxygen stress.

Several studies with the TRX Tg mouse including ours, strongly imply that TRX and/or the redox status modified by TRX play a crucial role in biological protection against oxidative stress both in development and in various situations in the adult. Recently Takagi *et al.*[22] subjected TRX Tg mice to focal brain ischemia by means of middle cerebral artery occlusion and found that infarct areas were significantly smaller than in similarly treated WT mice, indicating that overexpression of the TRX gene suppresses ischemic neuronal injury. In addition, Mitsui *et al.* (submitted for publication) showed that bone marrow cells of hTRX Tg mice were more resistant to ultraviolet light-induced cytocide than those of WT embryos and that the life span of hTRX Tg mice was approximately 35% extended as compared with that of WT controls. Since hTRX Tg mice acquire the capacity to adapt their redox state to oxidative stress and become resistant against its toxic effects, TRX may possibly restrain aging by attenuating oxidative damage.

When organogenesis commences and the metabolic state of embryos becomes active, TRX protein begins to be expressed in embryonic tissues,[21] which should contribute to providing them with the resistance to oxygen toxicity and to adapt to more hyperoxic states. With this regard, it is interesting to note the fact that homozygous mutant mouse embryos which lack the TRX gene die shortly after implantation,[36] which corresponds to the stage when the TRX protein begins to be expressed in WT embryos.

Since growth and morphological differentiation were significantly inhibited in WT embryos cultivated from E7.5 under 25% O_2, the cells of these embryos may possibly be undifferentiated and too premature to express TRX. Actually mTRX was not detected in tissues of E7.5 mouse embryos (Fig. 2A and B) as shown previously.[21] On the other hand, hTRX was expressed in E7.5 hTRX Tg embryos. When examined at the end of the culture period, the level of oxidative stress, as measured by the production of 8-OHdG and carbonylated proteins, was found to be increased remarkably in the tissues of WT embryos exposed to 25% O_2, but the amount of 8-OHdG increased only minimally in hTRX Tg embryos exposed to 25% O_2. Since TRX protein can scavenge singlet oxygen and hydroxy radicals by its structural and catalytic cysteines,[37] hTRX protein expressed in the embryo from the start of culture was most likely to prevent TRX Tg embryos from oxidative modifications of DNA and proteins. With this regard, it is noteworthy that hydrogen peroxide is scavenged by recombinant hTRX[38] and by a TRX dependent peroxide reductase, peroxiredoxin.[39,40] Thus, it seems that

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inserted hTRX gene and/or overexpressed hTRX protein protected hTRX Tg embryos from teratogenesis by suppressing oxidative stress.

TRX acts as an antioxidant protein only after its reduction by TRX reductase. While we previously reported that TRX is expressed from E8.5 in mouse embryos,[21] it is unknown from which developmental stage TRX reductase is expressed in the embryo. In the present study, it was difficult to measure the TRX reductase activity in hTRX Tg embryos because the embryonic proper tissue was too small at the early post implantation stage. However, previous reports have shown that the TRX reductase activity in the fetal liver is much higher than in the neonatal liver[41] and that in newborn primates, gene expression of TRX reductase as well as TRX in newborn primates is enhanced by oxygen pressure.[42] Since the localization of TRX is similar to that of TRX reductase and since their distribution is compatible with function in thiol-disulfide interchange reaction,[43] the activity of TRX reductase may be enhanced in hTRX Tg mouse embryos when they are subjected to oxidative stress. Further studies are needed to investigate the ontogenesis of TRX reductase in rodent embryos and to elucidate its possible roles in prenatal development.

Cu/Zn-superoxide dismutase (SOD) and glutaredoxin (GRX) were detected immunohistochemically in WT embryos cultivated under 10 and 25% O_2 (data not shown). Since their expression was not different between the 10 and 25% oxygen conditions, TRX-dependent redox system may possibly function at this stage of development. Since the mice lacking Cu/Zn-SOD,[44] Mn-SOD,[45] extracellular (EC)-SOD[46] or glutathion peroxidase (GPX)-1[47,48] have been reported to be born normally, these enzymes may not be essential for embryonic development or other molecules may compensate their function.

It is also possible that oxidative stress-induced anomalies are caused by modulation of genes controlling cell proliferation, survival or death. Since TRX regulates the DNA binding activity of several transcriptional factors, it may attenuate the oxidative stress-induced modifications of these molecules. We failed to detect AP-1 (c-Jun/c-Fos) immunohistochemically both in WT and TRX Tg embryos at E7.5 and E8.5, suggesting that AP-1 complex may not be directly associated with the prevention of oxidative stress-induced anomalies in hTRX Tg embryos. Another possibility is that c-Fos and c-Jun proteins have not begun to function by E8.5. Further studies are underway in our laboratories to examine the expression patterns of TRX-dependent transcriptional factors such as AP-1 and NF-κB in early postimplantation embryos by using RT-PCR and *in situ* hybridization techniques.

TRX has also been shown to regulate apoptosis signaling. Saitoh *et al.*[49] showed that reduced TRX prevents apoptosis via inhibitory binding to apoptosis signal-regulating kinase 1 (ASK-1), which is a mitogen-activated protein (MAP) kinase[50] and that ASK-1 released from oxidized TRX can induce abnormal apoptosis. It remains to be elucidated whether TRX can induce abnormal apoptosis and result in embryonic abnormalities.

Unborn embryos are at risk of being damaged by excessive oxidative stress in utero especially during the early embryonic period. Antioxidant proteins including TRX are likely to play some crucial roles in their bioprotection which may be important in early development. Further studies would shed light on the redox regulation in mammalian embryos and reveal their unknown important functions in reproduction and development.

Acknowledgements

We thank Drs E. Bloom, S. Toyokuni, H. Masutani, Y. Miura and T. Miura for technical assistance and helpful comments. This work was supported by grants from the Japanese Ministry of Education, Culture, Sports, Science and Technology and the Japanese Ministry of Health, Labour and Welfare.

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