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Forum Mini Review

Redox Regulation by Thioredoxin in Cardiovascular Diseases

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ABSTRACT

Increasing evidence has indicated that the modulation of intracellular redox states has important aspects to cellular events, such as cellular proliferation, activation, growth inhibition, or death via the regulation of intracellular signal transduction and gene expression. Thioredoxin (TRX) is a multifunctional stress-inducible protein, which protects cells from various types of stresses. TRX has not only a scavenging activity of reactive oxygen species, but also a regulating activity of various intracellular molecules including transcription factors. We demonstrated that the serum TRX levels are correlated with the severity of heart failure, and are negatively correlated with left ventricular ejection fractions of patients with heart failure. The expression of TRX is enhanced in endothelial cells and macrophages in human atherosclerotic plaques, in balloon-injured rat arteries, and in damaged cardiomyocytes of rats with acute myocarditis. Overexpression of TRX in transgenic mice attenuates adriamycin-induced cardiotoxicity by reducing oxidative stresses. These findings suggest that TRX and the redox system modulated by TRX have an important role in cellular defense against oxidative stress in cardiovascular diseases. *Antioxid. Redox Signal.* 5, 795–802.

INTRODUCTION

HUMAN THIOREDOXIN (TRX) was cloned as adult T-cell leukemia–derived factor (ADF), an inducer of interleukin-2 receptor α-chain produced by human T-cell leukemia virus type-I–transformed cells (44, 51, 56). TRX was originally reported as a hydrogen donor for ribonucleotide reductase, an essential enzyme for DNA synthesis in *Escherichia coli.* TRX is a 12-kDa protein that has disulfide-reducing activity. Two cysteine residues of conserved active site sequence, -Cys-Gly-Pro-Cys- (Cys32 and Cys35), serve for its reducing activity. Reduced TRX has dithiols, and oxidized TRX has a disulfide bond in this active site. Oxidized TRX is reduced by NADPH and TRX reductase (16, 28). Recently, Haendeler *et al.* (8) reported that *S*-nitrosylation at Cys69 is required for scavenging reactive oxygen species (ROS), for preserving the redox regulatory activity, and for the anti-apoptotic function of TRX. The TRX system, composed of TRX reductase, TRX, and peroxiredoxin, is important in regulating the redox balance. Increasing evidence suggests that redox (reduction and oxidation) regulation by the TRX system in addition to the glutathione system plays important roles in biological responses against oxidative stresses.

TRX is a stress-inducible ubiquitous protein, which protects cells from various types of stresses, *e.g.,* viral infection, exposure to ultraviolet light, X-ray irradiation, and hydrogen peroxide (26, 28) (Fig. 1). Recent studies showed that ROS generated by a variety of oxidative stresses are not only cytotoxic to the cells but also important in signal transductions of cellular activation and cell death. It is well accepted that relatively low levels of oxidative stress promote cellular proliferation rather than cause degeneration or cell death. The intracellular redox balance regulated by reducing factors, including TRX, has an important role in cellular apoptosis or death (52).

TRX negatively regulates activation of p38 mitogen-activated protein (MAP) kinase (10) and apoptosis signal-regulating kinase-1 (ASK-1) (37). Reduced TRX binds to ASK-1 and inhibits its activation. When TRX is oxidized by ROS, the binding between TRX and ASK-1 is dissociated, and ASK-1 is activated to transduce the signal of apoptosis (Fig. 1). TRX was

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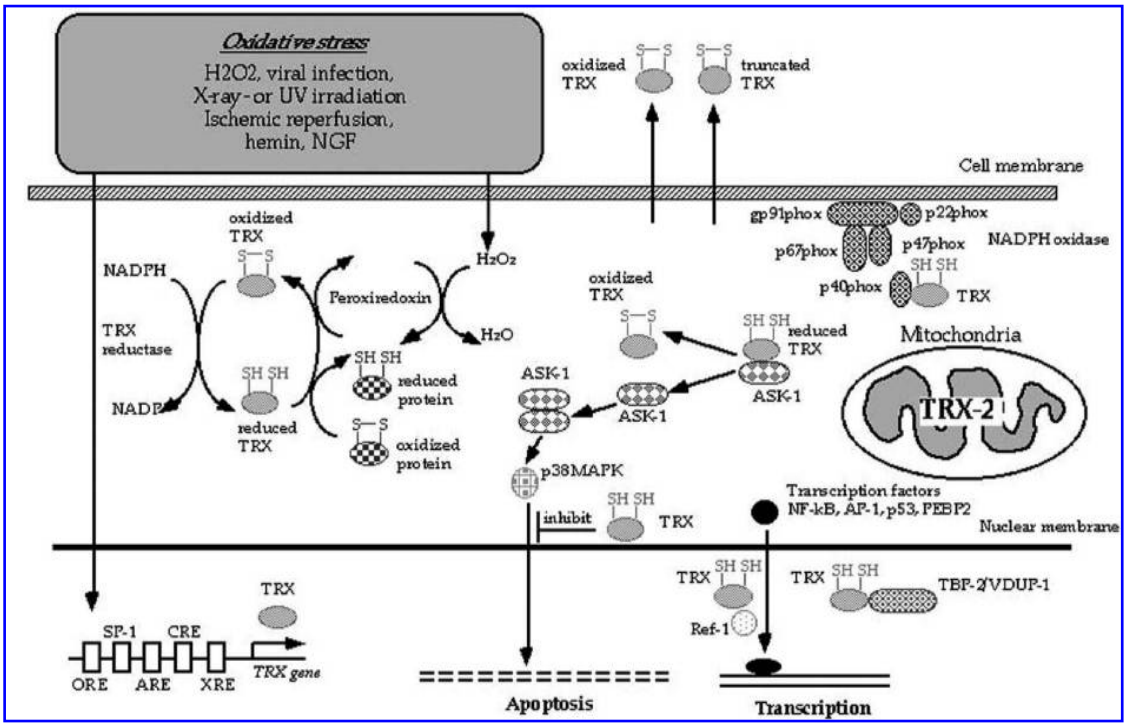

FIG. 1. Schema of biological functions of TRX. The TRX system (TRX, TRX reductase, and NADPH) reduces peroxiredoxin or oxidized proteins. Peroxiredoxin catalyzes the reduction of hydrogen peroxide. TRX has interactions with ASK-1, the p38 MAP kinase (MAPK) pathway, or p40phox (TBP-1) in cytosol. Oxidative stresses induce TRX expression and nuclear translocation of TRX. In the nucleus, TRX has interactions with transcription factors or TBP-2/VDUP-1. Oxidized TRX or truncated TRX is considered to be extracellularly secreted.

reported to induce ASK-1 ubiquitination and degradation to inhibit ASK-1-induced apoptosis (22).

TRX promotes DNA binding of transcription factors such as activator protein-1 (AP-1) (13), nuclear factor-κB (NF-κB) (9, 14), p53 (53), and phosphatidylethanolamine-binding protein-2 (PEBP-2) (2). DNA binding of AP-1 is modified by redox factor-1 (Ref-1), the activity of which is regulated by TRX (13). TRX reduces the cysteine 62 residue of NF-κB, which is important for the binding of NF-κB to DNA. Overexpression of TRX in the cytoplasm suppresses NF-κB activation, whereas overexpression of TRX in the nucleus enhances DNA binding of NF-κB (14). Overexpression of Ref-1 inhibits hypoxia or tumor necrosis factor-induced endothelial cell apoptosis through NF-κB-independent and -dependent pathways (9). The TRX–Ref-1 cascade interacts with p53, a gatekeeper against DNA damage and an inducer of G1 arrest, to afford cells time to repair damaged cells, and up-regulates p53-dependent p21 expression in response to oxidative stress (53). Accordingly, the redox status balanced by generated ROS and endogenous antioxidants plays a crucial role in the regulation of signal transduction in biological responses.

The gene encoding human TRX was reported to be mapped to 9q31 on chromosome 9 (11).The promoter region of the human TRX gene has the SP-1 site, the cyclic AMP responsive element (CRE), and the oxidative responsive element (ORE) in the 5′ flanking sequence (19, 48). TRX is induced by hemin through the binding of a transcription factor, nuclear factor-erythroid 2-related factor 2 (Nrf2), to the antioxidant response element (ARE) (20). Nerve growth factor (NGF) activates the TRX gene through a regulatory region positioned from −263 to −217 bp, containing the CRE. Insertion of a mutation in the CRE in this region abolishes the response to NGF. NGF also induces binding of CRE binding protein to the CRE of the TRX promoter (4). The promoter sequences of the TRX gene also contain SP-1 and the xenobiotics responsive element (XRE) (Y.W. Kwon *et al.*, manuscript submitted).

PLASMA/SERUM TRX LEVEL AS AN OXIDATIVE STRESS MARKER IN CARDIOVASCULAR DISEASES

TRX is secreted from cells by a leaderless pathway. Since TRX was cloned as ADF, a cytokine-like factor, there is accumulating evidence that TRX shows cytokine-like functions. Exogenous TRX enhances the cell growth by itself and shows comitogenicity with other cytokines. TRX also shows chemokine-like functions (5). Elevations of serum TRX levels are observed in patients suffering from oxidative stress, for example, in patients with HIV (27, 30), rheumatoid arthritis (24, 58), severe burn injury (1), or hepatitis C virus infection (43). It is suggested that TRX secretion into plasma may be a kind of host defense response against oxidative stress. Truncated TRX (1–80), which

was isolated as eosinophil cytotoxicity-enhancingfactor, was reported to act as an inducer of cytokine expression (Fig. 1) (35).

We previously documented that the level of plasma oxidized TRX is increased during reperfusion of the postcardioplegic heart because of systematic oxidative stress (29). We investigated the clinical significance of the serum TRX levels of patients with heart failure (21). The serum TRX level in patients with III and IV functional classes of New York Heart Association (NYHA) was significantly higher than in the control subjects (Fig. 2A). In addition, the serum TRX levels are negatively correlated with left ventricular ejection fractions of the patients (Fig. 2B). The serum TRX levels are elevated in patients with acute coronary syndrome or dilated cardiomyopathy compared with the control.

Recently, we reported that the serum level of TRX is high during the acute phase, and slowly decreases during the chronic phase in a patient with fulminant myocarditis (41). Circulating TRX inhibits the neutrophil recruitment into the inflammatory site in the mouse air pouch model (31). Therefore, it is possible that the elevation of serum TRX in patients with myocarditis is associated with not only the host defense response against oxidative stress, but also the inhibition of the neutrophil recruitment into the myocarditis lesion.

In conclusion, these results suggest the possible association between the elevated level of TRX and the severity of heart failure. The measurement of plasma/serum TRX levels is one of the useful tools to check how much the host is suffering from oxidative stress in patients with heart failure.

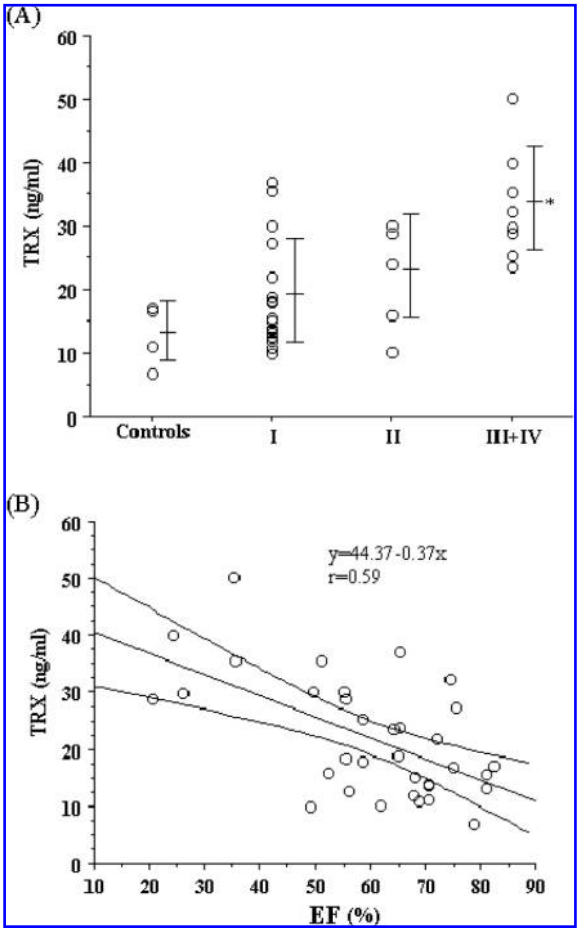

FIG. 2. Serum TRX levels with heart failure. (A) Comparison of serum TRX levels among patients with NYHA functional class I (n = 17, 19.1 ± 8.5 ng/ml), II (n = 5, 21.9 ± 8.5 ng/ml), and III plus IV (n = 8, 33.3 ± 8.6 ng/ml) and control subjects (n = 4, 13.0 ± 4.9 ng/ml). Significant differences were found between patients with NYHA III plus IV and control subjects, but no significant differences were found between patients with NYHA I or II and control subjects. **(B)** The relation between serum TRX levels and left ventricular ejection fractions (EF). The serum TRX levels were inversely correlated with EF ($r = 0.59$, $p < 0.001$) in all subjects. Bold lines indicate ±95% reliability zone.

UP-REGULATION OF TRX IN ATHEROSCLEROSIS

We reported that the expressions of TRX protein and TRX mRNA are enhanced in endothelial cells and macrophages in human atherosclerotic plaques, but not in nonatherosclerotic lesions (45). In atherosclerotic lesions of autopsy samples of human coronary arteries, Okuda *et al.* (34) reported that infiltrating macrophages highly express TRX in addition to glutaredoxin (GRX), which catalyzes protein disulfide reductions coupled with glutathione, glutathione reductase, and NADPH. The expressions of TRX protein and TRX mRNA are also increased after injury in the neointimal regenerating endothelial cells of balloon-injured rat arteries (45).

Accordingly, the development of atherosclerosis, neointimal hyperplasia after vascular injury, may be, at least in part, regulated by the cellular redox states via thiol-disulfide oxidoreductases such as TRX and GRX.

UP-REGULATION OF TRX IN INFLAMMATORY MYOCARDITIS

Excessive production of ROS at the inflammatory site contributes to the inflammatory process by induction of the expression of adhesion molecules, proinflammatory cytokines, and chemoattractants. An ROS scavenger such as superoxide dismutase has a therapeutic potential for myocarditis (12).

We demonstrated that the expression of TRX protein is up-regulated in association with an oxidative stress marker, 8-hydroxy-2′-deoxyguanosine, in infiltrating cells and damaged myocytes in rats with giant cell myocarditis during the acute stage (39). Since the expression of NF-κB is also up-regulated in damaged myocytes, TRX may have a protective role against the progressive myocardial damage in cardiomyocytes during acute inflammatory myocarditis through the activation of NF-κB. We also reported that the expression of TRX protein is increased in inflammatory cells and cardiomyocytes of left ventricular biopsy samples in a patient with fulminant myocarditis (41). Accordingly, acute inflammatory myocardi-

tis may be, at least partly, regulated by the cellular redox state via TRX.

CARDIOVASCULAR DRUGS HAVE CYTOPROTECTIVE EFFECTS IN CARDIOMYOCYTES THROUGH THE UP-REGULATION OF TRX

We found that geranylgeranylacetone (GGA), which is widely used as an anti-ulcer drug, can induce TRX and that GGA suppresses ethanol-induced cytotoxicity in cultured hepatocytes via the induction of TRX and the activation of transcription factors such as NF-κB and AP-1 (15).

We recently reported that treatment with temocapril, a nonsulfhydryl-containingangiotensin-convertingenzyme, enhances the protein expression of TRX, but not TRX2, copper/zinc-superoxide dismutase or manganese-superoxide dismutase in the myocardium of rats. Treatment with temocapril ameliorates the severity of the disease in rats with experimental autoimmune myocarditis with the reduction of protein oxidation by inducing TRX up-regulation in a preconditioning manner. Thus, treatment with temocapril ameliorates autoimmune myocarditis partially because of enhanced cardiomyocyte TRX expression (59). The supposed mechanism of temocapril against acute myocarditis via the up-regulation of TRX is described in Fig. 3. It is suggested that many drugs have cytoprotective effects via the modulation of redox state, including the up-regulation of TRX.

TREATMENT WITH RECOMBINANT TRX OR OVEREXPRESSION OF TRX IN TRANSGENIC MICE REDUCES ROS-INDUCED CARDIOTOXICITY

Heterozygotes carrying a targeted disruption of the mouse TRX gene are viable and fertile and appear normal in mice. In contrast, homozygous mutants die shortly after implantation (23). These results suggest that TRX is essential for early differentiation and morphogenesis of the mouse embryo.

Overexpression of human TRX by the β-actin promoter in mice (TRX transgenic [TRX-TG] mice) prolongs life span (25). TRX-TG mice are more resistant to oxidative stress such as postischemic reperfusion injury in the brain (46), retinal photic injury (50), and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced hematotoxicity (57). Moreover, specific overexpression of human TRX by the insulin promoter in pancreatic islet β-cells in mice prevents autoimmune or streptozotocin-induced diabetes *in vivo* (17).

Treatment with recombinant human TRX reduces hypoxia–reoxygenation injury in murine endothelial cells *in vitro*. In an in *vivo* study, treatment with recombinant human TRX also protects against retinal photic injury in mice (49), against reperfusion injury in canine lung transplantation (55), and against reperfusion-induced arrhythmias in an isolated rat heart model (3).

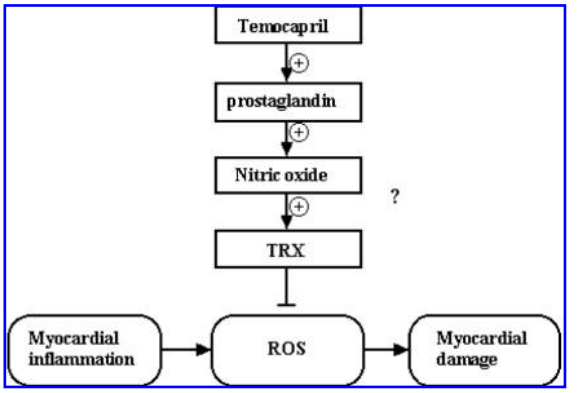

FIG. 3. The supposed mechanism of temocapril against acute myocarditis via the up-regulation of TRX. Long-term angiotensin-converting enzyme inhibition may increase prostaglandin production and stimulate the release of nitric oxide. Nitric oxide and peroxynitrite donors induce TRX protein and mRNA (45). Excessive production of ROS at the inflammatory site contributes to myocardial damage. TRX induced by treatment with temocapril scavenges ROS, leading to the suppression of myocardial damage.

To investigate the protective role of TRX in cardiomyocytes, we subjected wild-type (WT) and TRX-TG mice to adriamycin (ADR), which induces cardiotoxicity due, at least in part, to free ROS-mediated cellular damage. The formation of hydroxyl radicals in ADR-treated heart homogenates of TRX-TG mice was decreased compared with those of WT mice. Ultrastructural morphology was better maintained in ADR-treated TRX-TG mice than in ADR-treated WT mice. For the survival study, all WT mice treated with 24 mg/kg ADR died within 6 weeks, but five of six TRX-TG mice treated with ADR survived much longer. We also showed that treatment with high-dose, but not low-dose, of recombinant human TRX1 reduced ADR-induced injury in neonatal rat cardiomyocytes *in vitro*. Accordingly, the up-regulated expression of TRX by ADR is not enough to protect the heart against ADR-induced cardiotoxicity in WT mice. However, TRX-TG mice whose TRX expressions in the hearts were 50-fold greater than those of WT mice showed attenuated ADR-induced cardiotoxicity *in vivo,* and TRX-TG mice survived longer than WT mice (Fig. 4). Accordingly, TRX has a protective role against ADR-induced cardiotoxicity by reducing oxidative stresses (40). These findings suggest that TRX and the redox system modulated by TRX have important roles in the cellular defense against oxidative stress in cardiomyocytes.

TRX2 AND CARDIOVASCULAR DISEASES

Mammalian cells were suggested to contain only one form of TRX located in the cytosol that could be translocated to the nucleus under certain conditions. Recently, mammalian TRX localized in the mitochondria (TRX2) was cloned (42). TRX2 is more resistant to oxidation than TRX, because TRX2 lacks structural cysteine that can be oxidized to form a dimer, which

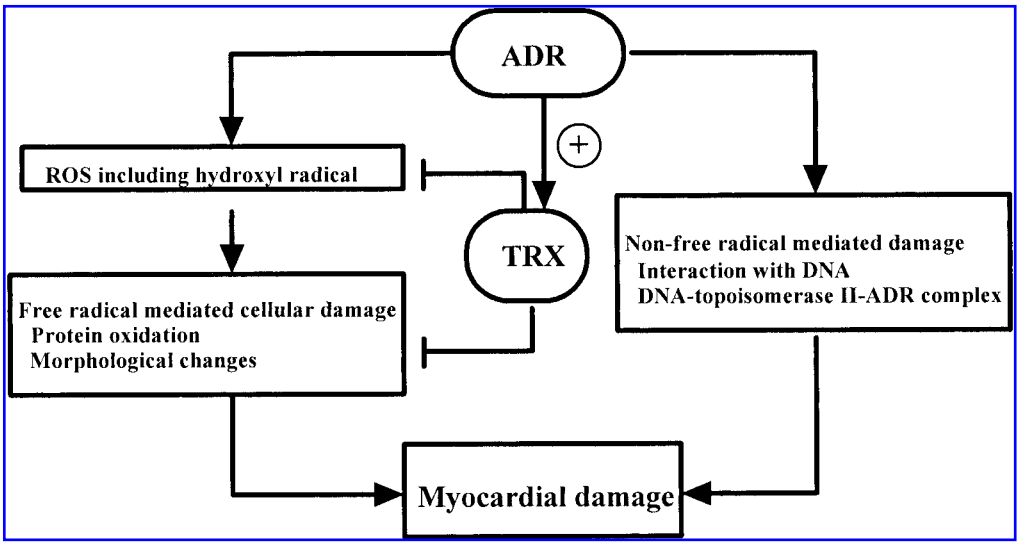

FIG. 4. The effects of TRX on ADR-induced cardiotoxicity. ADR has non–free radical mediated anti-tumor activity and causes free radical-mediated damage. ROS induced by ADR induce cellular damage in the heart. TRX is induced by treatment with ADR in the heart. TRX scavenges hydroxyl radical and prevents protein oxidation, which leads to prevention of ADR-induced myocardial damage in TRX-TG mice.

leads to inactivation. TRX2 is distributed with the highest expression in metabolically active tissues such as heart, skeletal muscle, and adrenal gland (42).

TRX2 is an essential gene regulating mitochondria-dependent apoptosis (47), and overexpression of TRX2 in human embryo kidney-293 cells is more resistant to etoposide-induced apoptosis (7). TRX2 plays an important role in the regulation of the mitochondrial membrane potential (7). In the paraventricular hypothalamic nucleus and reticular thalamic nucleus, treatment with dexamethasone causes elevation of the TRX2 mRNA level (36). In the heart, the expression of TRX2 protein is not up-regulated in acute myocarditis (59) or ADR-induced cardiotoxicity (authors unpublished data).

TRX-BINDING PROTEINS AND CARDIOVASCULAR DISEASES

We identified several TRX-binding proteins (TBPs) by the yeast two-hybrid system. TBP-1 is p40phox, a cytosolic component of phagocyte NADPH oxidase (33). TBP-2 is identical to a protein reported previously as a vitamin D_3 up-regulated protein-1 (32). TBP-2 expression is induced in HL-60 cells treated with vitamin D_3, although TRX expression is suppressed. Transfection of TBP-2 suppresses the protein expression and insulin-reducing activity of TRX. TBP-2 can bind only to the reduced form of TRX, and the C32S/C35S mutant of TRX in its active site fails to bind with TBP-2 (32). Another study reported that overexpression of TBP-2 inhibits the TRX-dependent suppression of c-Jun N-terminal kinase activity and the interaction of TRX with ASK-1. In addition, overexpression of TBP-2 induces apoptotic cell death by treatment with tumor necrosis factor or hydrogen peroxide (18). Therefore, TBP-2 is a kind of endogenous negative modulator of TRX (Fig. 1).

Lee and co-workers reported that biochemical strain or hydrogen peroxide suppresses the expressions of TBP-2 protein and mRNA in rat primary cardiomyocytes. Overexpression of TBP-2 induces apoptosis of cardiomyocytes and sensitizes cells to oxidative stress-induced apoptosis, suggesting that TBP-2 acts a key molecule as a environmental stress-mediated regulator of cardiomyocyte viability (54). They also reported that overexpression of TBP-2 blocks platelet-derived growth factor–induced cell growth through the suppression of TRX activity in human aortic smooth muscle cells (38). It was recently reported that the mRNA expression of TBP-2 is decreased in a mutant mouse strain, HcB-19/Dem, which shares features with familial combined hyperlipidemia (6). Studies are in progress to clarify the involvement of TBP-2 in the progression of atherosclerosis and cardiovascular diseases.

CONCLUSIONS

These findings suggest that TRX and the redox system modulated by TRX1 have an important role in the cellular defense against oxidative stress in cardiovascular diseases. TRX and its family proteins have wide various effects in many biological functions. The analysis of redox regulation in biological responses will contribute to new therapeutic approaches towards cardiovascular diseases.

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ence and Technology, Japan, and the Research and Development Program for New Bio-industry Initiatives.

ABBREVIATIONS

ADF, adult T-cell leukemia–derived factor; ADR, adriamycin; AP-1, activator protein-1; ARE, antioxidant response element; ASK-1, apoptosis signal-regulating kinase-1; CRE, cyclic AMP responsive element; GGA, geranylgeranylacetone; GRX, glutaredoxin; MAP, mitogen-activated protein; NF-κB, nuclear factor-κB; NGF, nerve growth factor; Nrf2, nuclear factor-erythroid 2-related factor 2; NYHA, New York Heart Association; ORE, oxidative response element; Ref-1, redox factor-1; ROS, reactive oxygen species; TBP, TRX-binding protein; TRX, thioredoxin; TRX-TG, thioredoxin transgenic; WT, wild-type; XRE, xenobiotics responsive element.

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