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Forum Review

From Oxygen Sensing to Heart Failure: Role of Thioredoxin

YUMA HOSHINO,[1] KEISUKE SHIOJI,[2] HAJIME NAKAMURA,[1]
HIROSHI MASUTANI,[3] and JUNJI YODOI[3]

ABSTRACT

Oxidative stress has been widely recognized to be involved in the pathogenesis of cardiopulmonary disorders. In ischemic heart diseases, it is involved not only in the development of atherosclerosis but also in ongoing ischemic injury, especially in the reperfusion process. Cardiomyopathy is another cardiac disorder in which oxidative stress is involved. In diabetic cardiomyopathy, homocysteine, a well-known source of oxidative stress, is believed to play major roles in its development. Thioredoxin (TRX) is a redox-acting protein ubiquitously present in the human body. It also is inducible by a wide variety of oxidative stresses. TRX is a multifunctional protein and has anti-inflammatory and antiapoptotic effects, as well as antioxidative effects. It is therefore feasible to think that TRX is a potential therapy for cardiac disease. Moreover, serum TRX is a well-recognized biomarker of various diseases involving oxidative stress, and this is also the case for cardiac disorders. Here we discuss how TRX is useful as a biomarker of and therapeutic agent for cardiopulmonary disorders, especially focusing on ischemic heart disease, myocarditis and oxygen sensing, and acute respiratory distress syndrome. *Antioxid. Redox Signal.* 9, 689–699.

Thioredoxin-1 (TRX) was originally cloned from *Escherichia coli* as a cofactor of ribonucleotide reductase (43). TRX is a redox-acting small protein, well-conserved across species from plants to mammals (25). Human TRX was originally reported as a T-cell leukemia–derived factor in transformed cells with human T-cell leukemia virus-1, whose primary function is the induction of the beta chain of interleukin-2 receptor (83, 95). Human TRX is a 12-kDa small protein consisting of 105 amino acids. It has a conserved CXXC construct in its active site, which exchanges disulfide to dithiol to maintain the reducing status of various molecules. As shown in Fig. 1, the reducing activity of TRX is maintained by NADPH and thioredoxin reductase (43). TRX is ubiquitously present in the human body and also is induced by a wide variety of stress conditions, such as UV or x-ray irradiation, viral infection, ischemia/reperfusion, and drugs such as antineoplastic agents (58, 59). TRX itself has the ability to scavenge singlet oxygen or hydroxyl radical (9). It also works as a radical scavenger in cooperation with peroxiredoxin, as does the glutathione system (88). In addition to its antioxidative effects, TRX is known to have diverse properties such as anti-inflammation and antiapoptosis (57, 76). These properties are, in some part, related to its regulation of intracellular signal transduction. TRX is known to regulate various intracellular signaling pathways. TRX inhibits ASK-1 signals to suppress apoptosis (75). p38 MAPK also is suppressed by TRX (17). DNA binding of transcription factors such as NF-κB, AP-1, and p53 are known to be regulated by TRX (22, 68, 102). The activity of TRX is regulated by a molecule called thioredoxin-binding protein-2 (TBP-2), also called thioredoxin-interacting protein or vitamin-D_3–upregulated protein-1 (VUDP-1) (64).

In contrast to intracellular TRX, extracellular TRX is predominantly in an oxidized form. Truncated TRX (TRX80, 10 kDa) is another form of extracellular TRX, which lacks protein disulfide reductase activity but has a potent chemoattractant activity for monocytes (73). TRX is secreted from cells in response to oxidative stress, although it has no secretory

[1]Department of Experimental Therapeutics, Translational Research Center, Kyoto University Hospital, Kyoto; [2]Department of Cardiovascular Medicine, Kishiwada City Hospital; and [3]Department of Biological Responses, Institute for Virus Research, Kyoto University, Kyoto, Japan.

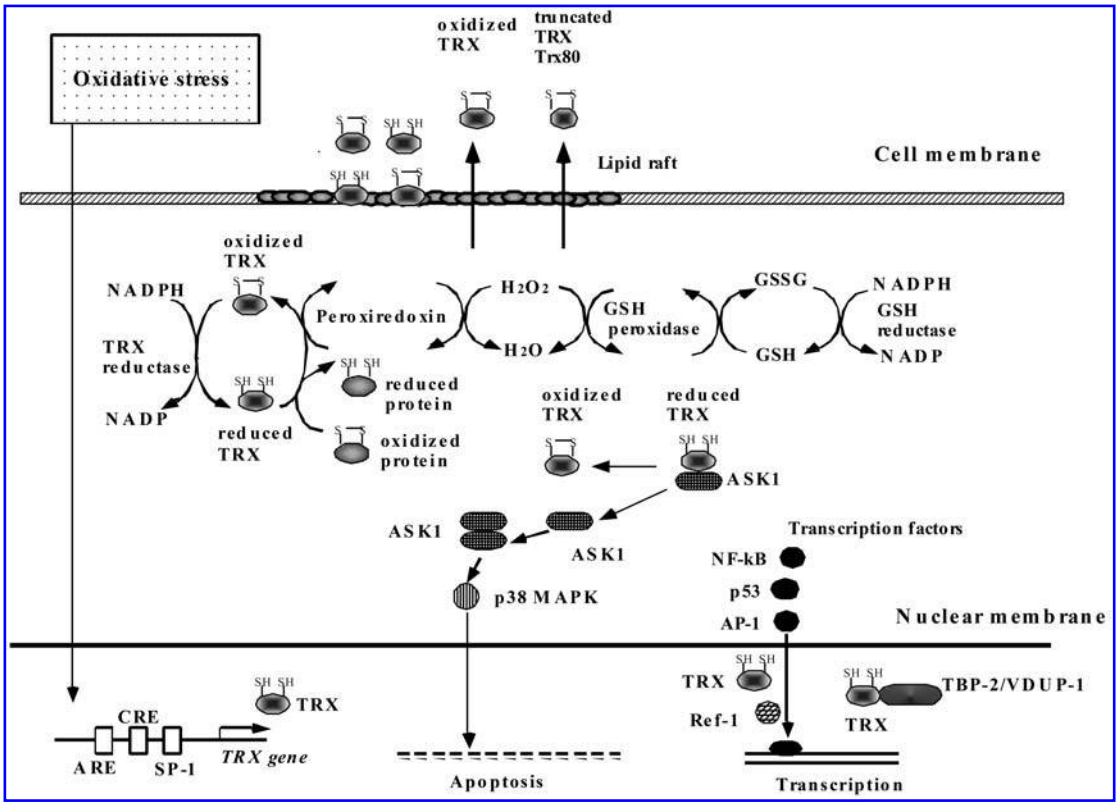

FIG. 1. Current understanding of biologic functions of thioredoxin. Thioredoxin (TRX) is a redox-acting protein that exchanges disulfide to dithiol to maintain the reducing status of various molecules. In the cytoplasm, TRX acts as a radical scavenger, either by itself or in cooperation with peroxiredoxin. TRX also has antiapoptotic and anti-inflammatory effects, some of which are attributed to the regulation of intracellular signal transduction, such as ASK-1, p38 MAPK, and DNA binding of NF-κB, AP-1, and p53. TRX is ubiquitously present in the human body and is also inducible by a wide variety of stress conditions via the modulation of transcriptional factor binding to its promoter region. The roles of extracellular TRX are discussed in Fig. 4.

signal sequence, as well as macrophage migration inhibitory factor (MIF), which is a classic inflammatory cytokine and another member of TRX family (42).

It is thus assumed that TRX is a good therapeutic candidate for various diseases in which oxidative stress, inflammation, and apoptosis are involved. Its effectiveness has been reported in many animal models either by using TRX transgenic mice (36, 60, 65, 70, 78, 85, 91, 97) or by injection of recombinant TRX (18, 47, 65, 101). Based on the findings observed in animal studies, we are now preparing a clinical trial evaluating the effectiveness of an intravenous injection of TRX against acute respiratory distress syndrome. Another therapeutic approach is to use TRX-inducing agents. Several agents are capable of inducing TRX and are suggested to be effective against various *in vivo* disease models or have cytoprotective effects *in vitro* (23, 90, 111). As shown in Fig. 1, the mechanisms of TRX induction are suggested to be regulated by binding transcription factors to the SP-1 site, antioxidant responsive element (ARE) or cyclic AMP-responsive element (CRE) in its 5' flanking sequence (33, 37, 89).

In addition to its therapeutic potential, TRX also is a useful biomarker for a wide variety of diseases. In the human body, TRX concentration is much lower in blood (10–100 ng/ml) compared with intracellular levels (1,000–10,000 ng/ml). Blood TRX level is elevated in various stress conditions up to 100–200 ng/ml, and therefore, it is suggested to be a good noninvasive marker of oxidative stress in clinical settings. So far, it has been reported that serum/plasma TRX is a useful marker not only of systemic diseases such as human immunodeficiency virus (HIV) infection (56), rheumatoid arthritis (30), type II diabetes (35), and acute lung injury (6), but also for local diseases such as nonalcoholic fatty liver disease (81) and asthma attacks (107).

Here we discuss the current topics of TRX in relation to cardiopulmonary disorders, focusing on ischemic heart disease, myocarditis, oxygen sensing, and acute lung injury.

OXYGEN SENSING AND THIOREDOXIN

Oxygen is essential to maintain homeostasis in the human body, but excessive oxygen is rather harmful, generating oxygen radicals, which enhance tissue injury. It is therefore necessary to measure oxygen concentration properly and to maintain its equilibrium. In the human body, several systems of oxygen sensing are found (104), one of which is the carotid body at the carotid artery bifurcation, which increases action-potential frequency in the carotid sinus nerve in response to hypoxia, thus stimulating respiration. Small-resistance pulmonary and fetoplacental arteries are other sensing systems that contract in response to hypoxia to optimize oxygen transfer in

the lung and placenta. Neuroepithelial bodies in the lungs and adrenomedullary cells in the fetus are also known to sense oxygen. Conversely, the ductus arteriosus exhibits an opposite response to contraction when oxygen levels increase, redirecting blood through the newly expanded lungs of the newborn.

In the clinical setting, the oxygen-sensing system plays critical roles in pulmonary hypertension, high-altitude pulmonary edema, persistent patent ductus arteriosus, and sudden infant death syndrome due to carotid-body chemoreceptors (15, 55, 96, 105). It is also suggested that the development of cardiac disorders by intermittent hypoxia in sleep apnea is associated with a failure of this oxygen sensing (82).

The redox system is deeply involved in oxygen sensing. Intracellular redox signaling is a major downstream mediator of oxygen sensing, regulating vascular tone. The precise mechanism is as follows: reactive oxygen species (ROS) from the mitochondria, NADPH oxidase, or redox couples may control potassium-channel gating and membrane potential and thus calcium entry. The same redox signaling may control calcium release from the sarcoplasmic reticulum. Calcium stores in the sarcoplasmic reticulum, in turn, are replaced by calcium entry through store-operated channels. Rho kinase augments the response of actin-myosin at any level of cytosolic calcium.

It has not been reported that TRX itself is involved in oxygen sensing; however, hypoxia-inducible factor (HIF), one of the major mediators of oxygen sensing, is known to be regulated by TRX by enhancing the stability of the HIF gene (27). It is therefore possible that TRX is involved in the oxygen-sensing system. Conversely, it also is of note that the "thioredoxin-like fold" structure of protein disulfide isomerase and other chaperone proteins is associated with its oxygen-sensing ability (8). So far, TRX itself is rather involved in the processes after the failure of oxygen sensing, especially in the embryonic period. During the early postimplantation period, rodent embryos encounter a transition from a relatively anaerobic environment to a higher oxygen pressure after uteroplacental circulation is established. They have to lessen this oxidative stress, and TRX is likely to be involved in this process. Kobayashi *et al.* (41) demonstrated that TRX transgenic mice are more resistant to this oxidative stress compared with wild-type C57 mice. After exposure to a hyperoxic condition, mouse embryos overexpressing TRX had better growth and fewer developmental abnormalities than did wild-type embryos. The mechanism is suggested to be scavenging intracellular ROS produced by the transient increase of oxygen pressure. This might explain why TRX knockout mice are embryonic lethal (48).

It is thus concluded that TRX has a protective effect against transient hyperoxia in the fetus, a consequence of abnormal oxygen sensing. The possible roles of TRX in adaptation to hypoxia and hyperoxia are summarized in Fig. 2, apoptosis signal-regulating kinase-1.

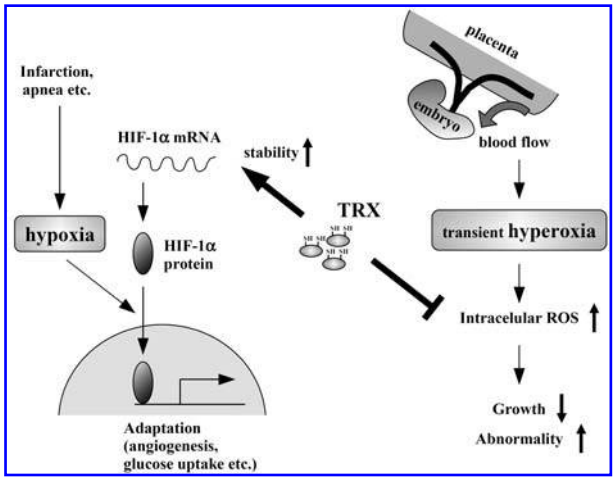

FIG. 2. Roles of thioredoxin in adaptation to hypoxia and hyperoxia. Thioreodxin enhances the stability of HIF-1α mRNA, which might facilitate the adaptation to hypoxic conditions such as organ infarction or apnea. Conversely, TRX is protective against transient hyperoxia induced by the establishment of uteroplacental circulation.

ISCHEMIC HEART DISEASE AND THIOREDOXIN

Thioredoxin as a biomarker of ischemic heart disease

Coronary artery disease is a leading cause of death in the United States (49). It is recognized that atherosclerosis and vasospasm are the major pathogeneses preceding ischemic heart disease.

It is well known that oxidative stress is involved in various cardiac disorders (7, 44, 87). As mentioned earlier, serum/plasma TRX is a useful marker of oxidative stress, and that is also the case with ischemic heart disease. First, TRX is a marker distinguishing acute coronary syndrome. Plasma TRX levels were elevated in patients with acute myocardial infarction (AMI) and unstable angina compared with stable exertional angina and chest-pain syndrome (24, 80). They also suggested that TRX may be helpful for predicting adverse cardiac events. In patients with AMI, higher levels of plasma TRX on admission were a risk factor for failure in emergency reperfusion therapy. In unstable angina patients, high plasma TRX is also associated with the risk of recurrent myocardial ischemia. The results indicate that blood TRX is a useful noninvasive index for diagnosis and for evaluating the response to therapeutic intervention and predicting adverse cardiac events.

It also is suggested that the serum TRX level is negatively associated with cardiac function. As in the mentioned reports, Kishimoto *et al.* (39) demonstrated that serum TRX levels were increased in acute coronary syndrome, whereas in stable angina patients, serum TRX is not elevated, suggesting that serum TRX reflects ongoing tissue injury by cardiac ischemia. In this study, serum TRX levels in patients with severe heart failure (New York Heart Association functional classes III and IV) were significantly higher than in control subjects. Moreover, serum TRX was negatively correlated with left ventricle ejection fraction.

Elevated TRX in the plasma of patients with myocardial infarction is associated with platelet hyperaggregability, which is also believed to be a pathogenesis of ischemic heart disease (54). They showed that plasma TRX levels and platelet aggregability increased concomitantly in patients with AMI, and increased plasma TRX was associated with platelet

hyperaggregability and lower left ventricular ejection fraction. The degree of oxidative stress was negatively correlated with fibrinolytic activity, a defense mechanism against thrombus formation, which augments narrowing of the constricted coronary artery (53). Serum plasminogen activator inhibitor levels and serum TRX levels were both elevated in patients with vasospastic angina, and treatment with vitamin E, an antioxidant, decreased both indices. It is therefore speculated that TRX is a good marker for the evaluation of therapeutic response.

Other reports suggest TRX as a good marker for evaluating therapeutic responses. The administration of edaravone, a free radical scavenger, before reperfusion of the heart with MI, decreased serum TRX level along with a decreased infarct size, reperfusion arrhythmia, and increased cumulative event-free rate (98).

Serum TRX also is associated with nitrate tolerance of patients with coronary spastic angina, which is an obstacle of long-term management with nitrate. Patients treated with nitroglycerin have a higher plasma TRX level and less arterial dilatation in response to additional nitroglycerin than do patients treated with nitroglycerin in combination with angiotensin II–receptor blocker (19).

Conversely, a recent report suggested that serum TRX is elevated even at the preclinical stage of cardiovascular disease. Serum TRX levels were elevated in subjects with coronary risk factors such as hypertension, hypercholesterolemia, and cigarette smoking (50). In contrast, serum levels of α-tocopherol, another antioxidant, decreased in patients having multiple risk factors. This suggests that prolonged oxidative stress in patients with coronary risk factors wastes the serum antioxidant pool such as vitamin C, and serum TRX is recruited for compensation.

Thioredoxin as a therapeutic for IHD

Pathologic changes in acute coronary syndrome, such as AMI and unstable angina, are suggested to be a disruption of atherosclerotic plaque and subsequent hemagglutination (12, 46).

Oxidative stress is deeply involved in the processes; oxidized low-density lipoprotein (LDL) is phagocytosed by subintimal macrophages, which subsequently release numerous inflammatory mediators and enhance atherosclerosis. Inflammation and injury of the vascular endothelium is also important for the progression of atherosclerosis. In the early pathogenesis of atherosclerosis, intimal injury enhances the expression of adhesion molecules and promotes the recruitment of monocytes and macrophages. These recruited mononuclear cells are transformed into foamy cells. Prolonged intimal inflammation spreads to arterial media and enhances the proliferation of arterial smooth muscle cells. Leukocytes such as T lymphocytes are recruited, and platelets are agglutinated, making atherosclerotic plaque unstable (73). It is thus assumed that antioxidant TRX is a useful oxidative marker and is also an effective therapeutic for IHD.

Several reports have suggested that TRX is effective against ongoing cardiac ischemia. TRX administration diminished MI in mice by inhibiting myocardial apoptosis and p38MAPK signals (92). *S*-nitrosation of C69 in TRX molecules has been suggested to be crucial for its antiapoptotic effect (14); however, in this animal model, *S*-nitrosation was not a crucial but an augmenting step for its antiapoptotic effect. In addition, exogenously administered TRX decreased peroxynitrite and increased SOD in the ischemic myocardium to inhibit apoptosis and ameliorate infarction (93). In their latest work, it was demonstrated that even the oxidized form of TRX exerted a cardioprotective effect against ischemia (94). It is also of note that protein nitration, which is observed in MI, inactivated the antiapoptotic effect of TRX by inhibiting ASK-1/TRX binding.

Essential roles of inducible endogenous TRX for protection against cardiac ischemia were also suggested. Mild and repeated ischemic preconditioning induced adaptation to subsequent cardiac ischemia (99). TRX depletion abolished this cardioprotection, as evidenced by a reduction of postischemic ventricular recovery, increase in myocardial infarct size, cardiomyocyte apoptosis, and a decreased amount of ROS. The cardioprotective role of TRX was also confirmed with TRX transgenic mice. A TRX-inducing agent might also be protective against ischemic heart disease. Geranylgeranylacetone, an antiulcer drug known to have the ability to induce TRX and heat-shock protein 72 (23), is protective against cardiac ischemia in rats, although the authors did not mention the involvement of TRX (71).

Recently, another role of TRX as a therapeutic for ischemic heart disease was suggested. Treatment with resveratrol, a naturally occurring polyphenol, improved neovascularization in the infarcted myocardium, a crucial step in the healing process of ischemia, and the effect was mediated by the induction of TRX, which led to hemeoxygenase-1 induction, followed by the upregulation of vascular endothelial growth factor (32).

It is suggested that TRX is not only an effective therapeutic of ongoing myocardial ischemia but also preventive against atherosclerosis underlying IHD. In human specimens, TRX expression was enhanced in endothelial cells and macrophages in atherosclerotic plaques (84). The same enhancement was observed in the neointima of balloon-injured arteries in rats. Because TRX expression ameliorated peroxynitrite-induced cytotoxicity, they suggested that TRX and the cellular redox state modified by TRX play a crucial role in arterial neointima formation in atherosclerosis. Thioredoxin family glutaredoxin also plays protective roles against atherosclerosis formation (69). Hagg *et al.* (16) reported that the uptake of oxidized LDL induces the upregulation of glutathione and thioredoxin systems and suggested that these systems may participate in cellular defense against oxidized LDL and possibly modulate the development of atherosclerosis (16). TRX also attenuated the inflammatory reaction of human monocyte-derived macrophages, cells that are suggested to be involved in atherosclerosis formation (4). Although no evidence directly shows that TRX inhibits atherosclerosis, these findings suggest the potential preventive role of TRX against atherosclerosis.

The possible roles of TRX in ischemic heart diseases are summarized in Fig. 3. In short, TRX is a useful biomarker of IHD for its early detection and as a marker of disease activity and therapeutic response. TRX also has potential for its

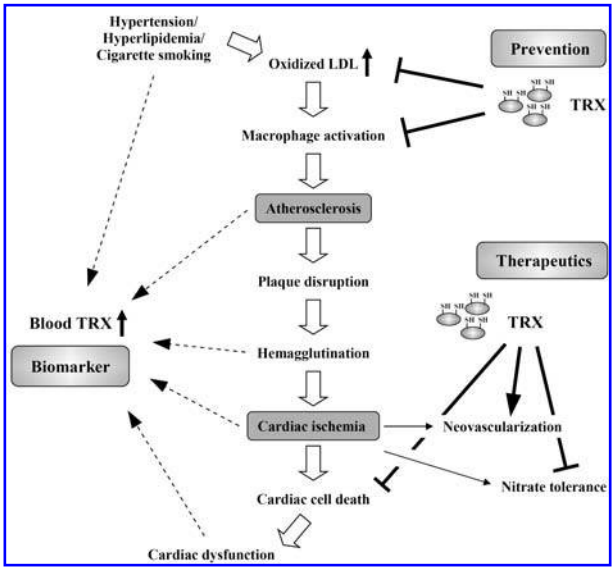

FIG. 3. Proposed roles of thioredoxin in ischemic heart disease. TRX is a useful biomarker of IHD for its early detection, as a marker of disease activity and therapeutic response. TRX also has a potential for its prevention and in the therapy for acute coronary syndrome by scavenging ROS and facilitating neovascularization.

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MYOCARDITIS/CARDIOMYOPATHY AND THIOREDOXIN

Oxidative stress in myocarditis/cardiomyopathy

Myocarditis is an inflammatory injury of the myocardium and is usually caused by viral infection such as coxsackievirus, adenovirus, and echovirus. The pathology of myocarditis is characterized by inflammatory cell infiltration and necrosis of myocardial cells. This pathologic condition is caused primarily by viral infection itself, but the involvement of postinfectious autoimmune reaction also is suggested. Persistent myocardial injury causes tissue remodeling and occasionally leads to severe myocardial dysfunction—in other words, cardiomyopathy (11, 72). Cardiomyopathy is a cardiac dysfunction caused by myocardial injury. Most are idiopathic, but some are secondary to a wide variety of disorders such as hypertension, diabetes, and autoimmune disease.

Oxidative stress is thought to be involved in the pathogenesis of myocarditis because antioxidants have a beneficial effect against viral myocarditis (20, 21). The fact that oxidative stress predisposes subjects to viral infection also indicates the association of oxidative stress with the development of myocarditis (3). Another report suggested that oxidative stress also affects cardiac remodeling in myocarditis (86).

The involvement of oxidative stress in myocarditis and cardiomyopathy is further suggested in relation to TRX. TRX was upregulated in cardiac tissue obtained from patients with myocarditis or cardiomyopathy in the active necrotic stage (61). This upregulation of cardiac TRX is associated with DNA damage and may reflect oxidative stress overload in a hemodynamically uncontrolled state.

The involvement of TRX is discussed in a more specific subset, such as diabetic cardiomyopathy. Cardiomyopathy is one of the critical complications of diabetes mellitus that significantly affects its prognosis. Li *et al*. (45) examined changes in the potassium channel and TRX system in an experimental diabetes model in rats and found that the potassium channel and TRX reductase were significantly suppressed in cardiomyocytes (45). Disturbed TRX systems in diabetes might lead to impairment of the potassium channel and increase the incidence of cardiac arrhythmia and sudden death.

Another mechanism of diabetic cardiomyopathy is suggested by Tyagi *et al.* (100). In diabetes, homocysteine levels are increased because homocysteine metabolism by glucose and insulin, and its renal clearance, are impaired. This homocysteine suppresses peroxisome proliferator–activated receptor-γ (PPAR-γ) activity and enhances oxygen radical formation. These changes result in a decrease of cellular antioxidative defense including TRX, peroxiredoxin. Subsequently, metalloprotease activity is enhanced and promotes dissociation of the endothelium from myocytes. The dissociating space is filled with stiff oxidized collagen, and, as a result of this tissue remodeling, cardiac dilatation is impaired.

Thioredoxin as a biomarker of myocarditis

In clinical settings, serum/plasma TRX is a useful biomarker of myocarditis and cardiomyopathy. Kishimoto *et al*. (39) reported that serum TRX levels are elevated in patients with dilated cardiomyopathy and suggested that the serum TRX level correlated with the severity of heart failure and negatively correlated with left ventricle ejection fraction (39).

Serum TRX is useful for evaluating therapeutic response in cardiomyopathy. Kishimoto *et al.* (38) reported the effectiveness of intravenous immunoglobulin administration for acute inflammatory cardiomyopathy in humans. They found that the improvement of left ventricle ejection fraction by intervention is associated with the suppression of inflammatory cytokines and the reduction of oxidative stress, as evaluated by serum TRX.

In animal models of myocarditis, TRX is a well-established index of oxidative stress and reflects the response to interventions. In a rat model of immune-mediated myocarditis induced by immunization with myosin, TRX was upregulated in the acute stage along with NF-κB activation, but not in the chronic stage (79). TRX expression was correlated with the severity of disease. It is therefore suggested that TRX may be induced by acute inflammatory stimuli and served as a regulator of the immune response.

Miyamoto *et al.* (51, 52) reported strain-dependent predisposition to viral myocarditis and spontaneous myocarditis in mice. Coxsackievirus B3 infection induced severe myocarditis in DBA/2 mice, moderate myocarditis in BALB/c mice, and mild myocarditis in C57BL/6 mice. TRX was upregulated in cardiac tissue, and its expression was correlated with the severity of disease. In a spontaneous myocarditis model,

they also found similar strain dependency. In addition, treatment with *N*-acetylcysteine, an antioxidant, suppressed the development of spontaneous myocarditis, suggesting that oxidative stress is involved in the pathogenesis.

Moreover, TRX reflects the therapeutic response in myocarditis. Kishimoto *et al*. (40) reported that, in a mouse model of viral myocarditis, the upregulation of myocardial TRX with DNA damage induced by inflammatory stimuli by the virus was suppressed by coenzyme Q10 treatment, which may reflect the antioxidant effects of coenzyme Q10.

Thioredoxin as a therapy for myocarditis

Accumulating evidence implicates the therapeutic application of TRX for myocarditis and cardiomyopathy.

The protective effects of TRX against doxorubicin (Adriamycin)-induced cardiotoxicity were reported by using animal models of myocarditis. TRX-overexpressing transgenic mice treated with doxorubicin had less cellular damage, less oxidative stress, and better survival than wild-type mice treated with doxorubicin, a cardiotoxic agent (78).

Another report suggested cardioprotection by inducible endogenous TRX. Temocapril, an inhibitor of angiotensin-converting enzyme, was shown to have the ability to induce TRX in myocytes *in vitro* and *in vivo* (111). In a rat model of experimental autoimmune myocarditis, temocapril treatment suppressed oxidative stress and ameliorated myocarditis.

Exogenously administered TRX is also effective against myocarditis. Liu *et al*. (47) reported that TRX administration ameliorated myosin-induced autoimmune myocarditis in mice. The mechanism is suggested to suppress leukocyte chemotaxis by TRX with the inhibition of chemokines rather than by its direct antioxidative effect (Fig. 4).

Based on these findings, it is concluded that TRX is a useful biomarker of myocarditis/cardiomyopathy for the evaluation of disease severity and therapeutic response. TRX and its inducers might be good candidates for therapy for myocarditis/cardiomyopathy with antioxidative and antichemotactic properties.

ACUTE LUNG INJURY AND THIOREDOXIN

Cardiac failure frequently accompanies pulmonary edema, the manifestation of which resembles acute respiratory distress syndrome/acute lung injury (ARDS/ALI), a noncardiogenic pulmonary edema. ARDS is a disease entity with severe respiratory failure caused primarily by severe infection, aspiration, lung disease, drugs, and so on. The pathogenesis is increased alveolar permeability, leading to an accumulation of exudates and inflammatory cells in the alveolar space, causing severe impairment of gas exchange. The fact that oxidative stress and inflammation of predominantly neutrophils play critical roles in the pathogenesis of ARDS (10) implies the potential of TRX as a biomarker for the disease as well as a therapy.

It has been reported that TRX is upregulated in airway epithelial cells treated with bleomycin, an antineoplastic agent, and is also known to cause acute lung injury (13) in bronchial epithelial cells in bleomycin-treated mouse lung (13), and in lung tissue obtained from patients with ARDS, especially localized in alveolar macrophages and alveolar type II epithelial cells (6). As a biomarker for ARDS, TRX levels were elevated in bronchoalveolar lavage fluid (BALF) and plasma obtained from patients with ARDS. It is of note that BALF TRX levels correlated with BALF IL-8 levels. Moreover, these BALF markers were higher in patients with ARDS of lung origin compared with ARDS caused by diseases outside the lung.

The therapeutic potential of TRX against acute lung injury has been suggested in several *in vitro* and *in vivo* models, and the potential effects of both TRX enhancement and inhibition in ARDS have been discussed elsewhere (2). First, the

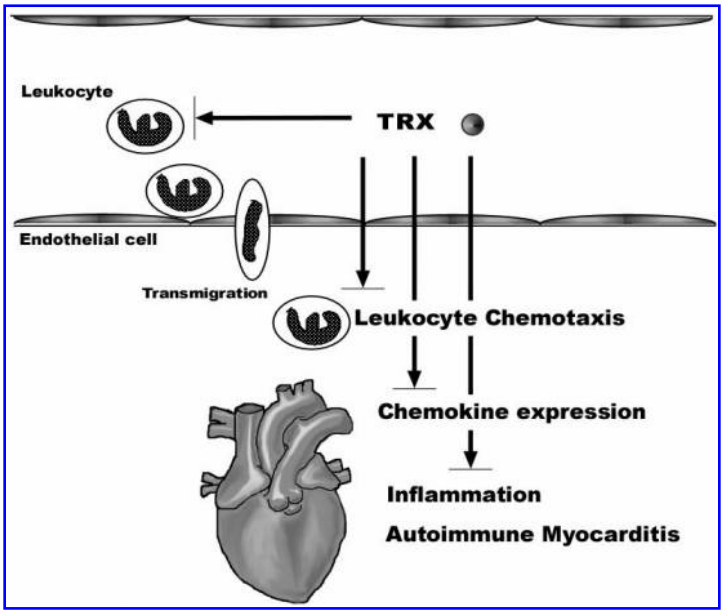

FIG. 4. Thioredoxin ameliorates autoimmune myocarditis through suppressing inflammation. Administration of recombinant thioredoxin ameliorated myosin-induced autoimmune myocarditis in mice. The mechanism is thought to be the suppression of leukocyte chemotaxis by the inhibition of chemokines rather than its direct antioxidative effect.

overexpression of TRX was suggested to be effective against acute lung injury. An *in vitro* study demonstrated that TRX-transfected L929 cells were more resistant to bleomycin-induced cytotoxicity (13). TRX-transgenic mice are resistant to cytokine-induced (IL-2 and IL-18) lung injury and bleomycin-induced lung injury in mice by suppressing inflammatory cell infiltration and lung cell death (26). TRX transgenic mice are also resistant to acute lung injury caused by diesel exhaust particles (DEPs) (34). The mechanism of the protective effect is suggested to be that TRX recovers Akt-mediated antiapoptotic signals suppressed by DEP. Figure 5 summarizes the possible roles of TRX in acute lung injury.

Conversely, the administration of exogenous TRX has been suggested to be effective against acute lung injury. TRX administration ameliorated cytokine-induced lung injury and bleomycin-induced lung injury in mice (26). When considering the clinical setting, however, it is necessary to refine the method of TRX administration. We thus developed two models of acute lung injury in rats and established an administration method. In a lipopolysaccharide-induced acute lung injury model in rats, it was observed that continuous intravenous infusion of TRX is necessary to attenuate BALF neutrophilia instead of bolus injection (101). We also established a lung-injury model with more-sustained inflammation by using low-dose administration of bleomycin to rats. In this model, continuous intravenous administration of TRX on days 4–7 from onset was found to be effective against neutrophil infiltration to the lungs. It is therefore speculated that TRX is effective even if administration is delayed after onset. Based on these preclinical observations, we are now preparing a clinical trial of intravenous TRX injection against ARDS/ALI.

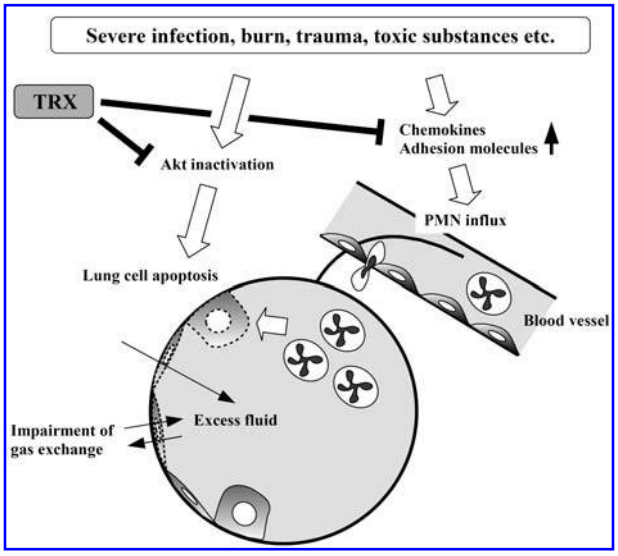

FIG. 5. Role of thioredoxin in acute lung injury. In acute lung injury, lung cell death and excessive fluid in alveolar space lead to an impairment of gas exchange. Thioredoxin protects lung cells by suppressing Akt inactivation. It also inhibits neutrophil influx by suppressing chemotactic signals such as chemokines and adhesion molecules.

The anti-inflammatory effect of TRX in the lungs also was reported in a mouse model of bronchial asthma (29). By using an OVA-sensitized mouse model, it was shown that recombinant TRX inhibited the asthmatic response after sensitization but not sensitization itself. It was also demonstrated that its redox regulation is necessary for the effect because mutant TRX having weak redox activity did not show an effect.

It is thus speculated that TRX is not only a promising therapy for ARDS but it could also be applied as therapy for a wide variety of oxidative lung diseases such as interstitial pneumonia, bronchial asthma, and chronic obstructive pulmonary disease.

THIOREDOXIN BINDING PROTEIN-2 IN CARDIAC DISORDERS

The activity of TRX is regulated by a molecule called thioredoxin-binding protein-2 (TBP-2), also called thioredoxin-interacting protein or vitamin-D_3–upregulated protein-1. TBP2 is a negative regulator of TRX (64) and has multiple regulatory functions in cellular redox, growth, differentiation, apoptosis, and aging (1, 31, 62–64, 109). TBP-2 knockout mice have characteristics that resemble Reye syndrome in humans (66). It is suggested that Krebs cycle–mediated fatty acid utilization is impaired in knockouts. A TBP-2 homologue, TBP-2–like inducible membrane protein, is known to regulate PPAR, suggesting its potential involvement in lipid metabolism (67).

Recent reports suggest that TBP-2 has the potential to play roles in the development of cardiovascular diseases. Because TBP-2 is closely associated with lipid metabolism, dysregulation of TBP-2 could facilitate atherosclerosis and predispose patients to coronary artery disease. A mutant mouse strain HcB-19/Dem shares a feature with familial combined hyperlipidemia (5). This mouse strain has a mutation in the genomic sequence coding the TBP-2 gene and mRNA expression of TBP-2 is constitutively at a low level. When fasting the mouse strain, insulin secretion was enhanced, and hypoglycemia and hypertriglyceridemia were observed, which were likely to be a consequence of abnormal cellular redox status caused by TBP-2 downregulation (28). Conversely, the same mouse strain has levels of TRX activity similar to those of wild-type mice, suggesting that abnormal lipid metabolism might be independent of redox regulation (77). In this context, TBP-2 is a negative regulator of atherosclerosis formation. A contradictory mechanism of TBP-2 is suggested by Yamawaki *et al.* (108). Disturbed blood flow upregulated TBP-2 in endothelial cells that decreased TRX activity and consequently enhanced TNF-related inflammation. It is therefore speculated that TBP-2 could facilitate atherosclerosis by enhancing endothelial inflammation.

The roles of TBP-2 in cardiac ischemia have been discussed. It is suggested that TBP-2 responds to stress and regulates the cell death of myocytes (103). Wang *et al.* (106) demonstrated that TBP-2 is downregulated in cardiomyocytes by oxidant exposure or mechanical stress that lead to an enhancement of TRX activity as a defense response. They also

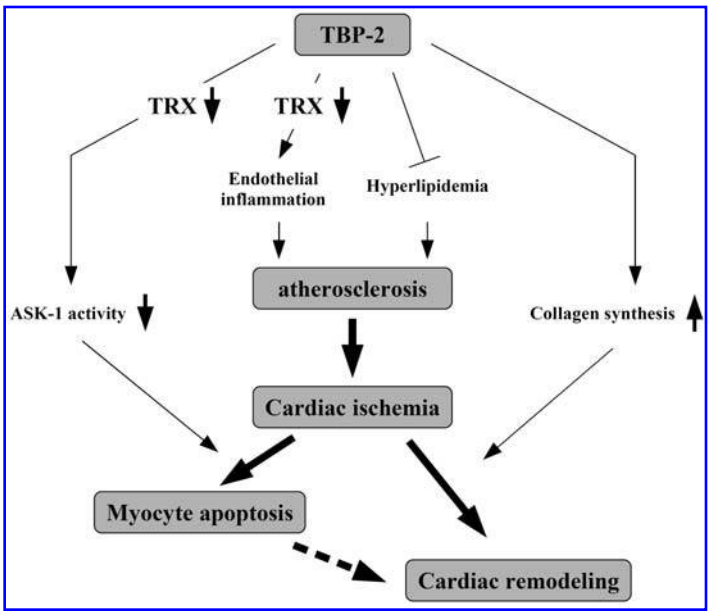

FIG. 6. Role of TBP-2 in ischemic heart disease. TBP-2 regulates lipid metabolism and endothelial inflammation and could therefore be involved in atherosclerosis formation. TBP-2 might facilitate myocyte apoptosis by regulating TRX-ASK-1 activity. Downregulation of TBP-2 suppresses collagen synthesis, leading to cardiac remodeling.

demonstrated that overexpression of TBP-2 made myocytes vulnerable to oxidant injury. In contrast, in an *in vivo* model of myocardial ischemia, TBP-2 was rather upregulated in myocytes (106). It was shown that suppression of TBP-2 attenuated myocyte apoptosis via the inhibition of ASK-1. Taken together, TBP-2 suppression seems beneficial against myocardial ischemia.

TBP-2 is also suggested to be involved in the remodeling process after cardiac ischemia. Downregulation of TBP-2 was associated with suppressed collagen synthesis and a reduction in myocardial scar formation (106).

Cardiac hypertrophy is a common disorder as an adaptive process against pressure overloads, and occasionally leads to lethal ventricular arrhythmias. The role of TBP-2 in cardiac hypertrophy was reported by Yoshioka *et al.* (110). In a rat model of aortic constriction, TBP-2 was downregulated, and TRX activity was enhanced in cardiomyocytes. Because TBP-2–overexpressed cells were less hypertrophic in this model, they suggested that TBP-2 overexpression might suppress cardiac hypertrophy; however, the involvement of TRX in this process remains to be discussed.

Thus, TBP-2 plays an important role in the regulation of lipid metabolism, endothelial function, myocardial ischemia, the remodeling process after cardiac ischemia, and cardiac hypertrophy (Fig. 6). The involvement of TRX in these processes seems various and should be further clarified. The modulation of TBP-2 and TRX expression might be considered a therapeutic measure for these conditions.

ABBREVIATIONS

ALI, acute lung injury; AMI, acute myocardial infarction; ARDS, acute respiratory distress syndrome; ARE, antioxidant responsive element; ASK-1, apoptosis signal-regulating kinase-1; BALF, bronchoalveolar lavage fluid; CRE, cyclic AMP–responsive element; DEP, diesel exhaust particles; HIF, hypoxia-inducible factor; IHD, ischemic heart disease; LDL, low-density lipoprotein; MIF, macrophage migration inhibitory factor; PPAR, peroxisome proliferator–activated receptor; ROS, reactive oxygen species; SOD, superoxide dismutase; TBP-2, thioredoxin-binding protein-2; TRX, thioredoxin; VUDP-1, vitamin-D_3–upregulated protein-1.

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Address reprint requests to:
Yuma Hoshino MD, PhD
54 Kawahara-cho, Shogoin, Sakyo-ku
Kyoto, 6068507 Japan

E-mail: yuma@kuhp.kyoto-u.ac.jp

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