ANTIOXIDANTS & REDOX SIGNALING
Volume 10, Number 6, 2008

DOI: 10.1089/ars.2007.1985

Forum Review

Thioredoxin in Vascular Biology: Role in Hypertension

TALIN EBRAHIMIAN[1] and RHIAN M. TOUYZ[2]

ABSTRACT

The thioredoxin (TRX) system consists of TRX, TRX reductase, and NAD(P)H, and is able to reduce reactive oxygen species (ROS) through interactions with the redox-active center of TRX, which in turn can be reduced by TRX reductase in the presence of NAD(P)H. Among the TRX superfamily is peroxiredoxin (PRX), a family of non-heme peroxidases that catalyzes the reduction of hydroperoxides into water and alcohol. The TRX system is active in the vessel wall and functions either as an important endogenous antioxidant or interacts directly with signaling molecules to influence cell growth, apoptosis, and inflammation. Recent evidence implicates TRX in cardiovascular disease associated with oxidative stress, such as cardiac failure, arrhythmia, ischemia reperfusion injury, and hypertension. Thioredoxin activity is influenced by many mechanisms, including transcription, protein–protein interaction, and post-translational modification. Regulation of TRX in hypertensive models seems to be related to oxidative stress and is tissue- and cell-specific. Depending on the models of hypertension, TRX system could be upregulated or downregulated. The present review focuses on the role of TRX in vascular biology, describing its redox activities and biological properties in the media and endothelium of the vessel wall. In addition, the pathopysiological role of TRX in hypertension and other cardiovascular diseases is addressed. *Antioxid. Redox Signal.* 10, 1127–1136.

INTRODUCTION

In physiological conditions, intra- and extracellular reactive oxygen species (ROS) influence metabolic, signaling, and transcriptional processes in cells, whereas in pathological conditions, increased bioavailability of ROS (termed oxidative stress) in cardiovascular cells contributes to cardiovascular disease (6, 35, 52). Cellular redox homeostasis is regulated by the coordinate activity of various antioxidant mechanisms, including glutathione (GSH) and thioredoxin (TRX) (Fig. 1). These two thiol-reducing systems are critically involved in defense against excessive generation of ROS, as well as in redox regulation of signaling events involved in cell growth, apoptosis, and inflammation (13). These molecules maintain the intracellular milieu in a reduced state. Glutathione is used by glutathione peroxidase (GPXs) to reduce peroxides, producing glutathione disulfide or oxidized glutathione (GSSG). Glutathione reductase reduces GSSG to GSH. Thioredoxins are a class of small 12-kDa redox proteins present in all eukaryotic and prokaryotic organisms and are essential for cell viability.

Thioredoxin knockout mice are lethal, indicating the importance of TRX for cell viability (45). To date, three distinct variants of human TRX, encoded by separate genes, have been characterized. TRX1, the prototype and best characterized, is located in the cytosol; TRX2 is the mitochondrial form; and SpTRX is highly expressed in spermatozoa. TRX1 is normally present in the cytosol but translocates to the nucleus under stress conditions. The TRX system also includes thioredoxin peroxidase (peroxiredoxin). Thioredoxin reduces peroxiredoxin, which then catalyzes H_2O_2 to produce H_2O. Thioredoxin is characterized by a highly conserved active site that contains two cysteine residues. The TRX system reduces cysteine groups

[1]Lady Davis Institute for Medical Research, Sir Mortimer B. Davis–Jewish General Hospital, Hypertension and Vascular Research Unit, McGill University, Montreal, Quebec, Canada.
[2]Kidney Research Centre, Ottawa Health Research Institute, University of Ottawa, Ontario, Canada.

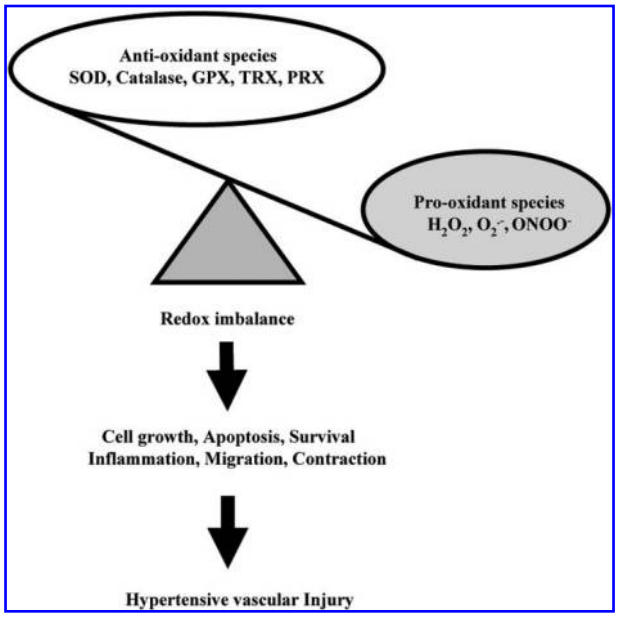

FIG. 1. Cellular redox imbalance. The cellular redox state is determined by the balance between pro-oxidant and antioxidant species. Redox imbalance leads to oxidative stress that influences cellular function and oxidative damage that are important mediators in cardiovascular diseases such as hypertension, atherosclerosis, and diabetes. GPX, glutathione peroxidase; H_2O_2, hydrogen peroxide; $O_2.^-$, superoxide anion; $ONOO^-$, peroxynitrite; PRX, peroxiredoxin; SOD, superoxide dismutase.

on proteins through an interaction with its redox-active center, by forming a disulfide bond which in turn can be reduced by the flavoenzyme TRX reductase (TRXR) and NAD(P)H. TRX, TRXR, and NAD(P)H constitute the TRX system. (37, 47). Mammalian TRX has a variety of biological activities as a hydrogen donor for various intracellular molecules and exerts its antioxidant function in its reduced form by scavenging H_2O_2 through TRX peroxidase (Fig. 2).

Independent of its antioxidant function, TRX can act as a signaling molecule. Thioredoxin activates some important transcription factors involved in cell growth/apoptosis and inflammation, such as nuclear factor-κB (NF-κB), activator protein-1 (AP-1), and redox factor-1 (Ref-1), by its TRX activity (59). As such, TRX has been shown to stimulate cell growth and inhibit apoptosis. Thioredoxin is also secreted and has cytokine- and chemokine-like activity. The function of TRX is regulated by an endogenous inhibitor, vitamin D_3–upregulated protein (VDUP)-1 (also called TRX-binding protein, TRXBP) with which it interacts (18). The redox-active site of TRX mediates this association, which leads to a reduction in TRX activity, suggesting that the TRX–VDUP-1 interaction may be an important regulatory mechanism of cellular redox state.

Since increased generation of ROS plays an important pathophysiological role in the development of hypertension, antioxidants including glutathione, catalase, thioredoxin, and peroxiredoxin may be involved in counter-regulatory actions and hence have protective actions. Dysregulation of pro-oxidant and antioxidant coupling mechanisms leads to oxidative damage and cardiovascular disease. The present review focuses on the TRX system in vascular biology, particularly with respect to its antioxidant actions and signal transduction regulatory properties, and discusses the potential importance of this system in vascular disease.

PEROXIREDOXIN (TRX PEROXIDASE)

Among the TRX superfamily, peroxiredoxins (PRXs) constitute a recently discovered family of non-heme peroxidases present in all organisms that catalyze the reduction of various hydroperoxides into the corresponding alcohol and water (39). Thioredoxin scavenges H_2O_2 through PRX. Mammalian cells express six PRX isoforms (PRX I-VI), PRX I, II, and VI are localized in the cytosol, PRX III and V in mitochondria, and PRX IV in the extracellular space (3). All isoforms contain a conserved cysteine residue that is the primary site of oxidation by H_2O_2. (Fig. 3). Two mechanisms may be responsible for the temporary inactivation of PRX: its phosphorylation by cyclin B-dependent kinase/Akt and the hyperoxidation of its active site (63) (Fig. 4).

The importance of peroxiredoxins is increasingly being recognized as an important regulator of H_2O_2 homeostasis (3). When overexpresssed or partially depleted using RNAi methodology in various cells, platelet-derived growth factor and tumor necrosis factor-α-stimulated H_2O_2 levels were reduced or increased respectively (4). In addition to reducing H_2O_2, PRX, via peroxynitrite reductase, reduces peroxynitrite levels (55).

THIOREDOXIN IN VASCULAR BIOLOGY

The TRX system is functionally active in the vasculature and is present in endothelial cells and vascular smooth muscle cells (VSMC). Thioredoxin is unique among antioxidants in that it interacts directly with various intracellular-signaling molecules as well as transcription factors, thereby directly influencing vascular cell growth and survival. Proteins that interact with TRX-1 include apoptosis signal-regulated kinase-1 (ASK-1), vitamin D3-upregulated protein 1 (VDUP1), phosphatase and tensin homolog (PTEN), the DNA repair enzyme, Ref-1, and transcription factors, including NF-κB and AP-1.

Thioredoxin is abundantly expressed in endothelial cells and is upregulated by oxidative stress, possibly as a counter-regulatory response to increased ROS formation. Thioredoxin has multiple actions in endothelial cells: it plays a protective role against H_2O_2-induced endothelial cytotoxicity (34), it regulates heme oxygenase (HO)-1, a stress response protein, in bovine aortic endothelial cells (53), and it induces mitochondrial manganese superoxide dismutase (SOD) in human lung microvascular endothelial cells (5).

Thioredoxin can also be protective against nitrosative stress. Endothelial cells are continually exposed to both exogenous and endogenous sources of nitric oxide (NO) and NO-derived species. HUVECs have a unique protective mechanism that allows the maintenance of balance in intracellular redox status via TRXR induction as an adaptive response to nitrooxidative stress. Overexpression of TRX prevents NO-induced reduction

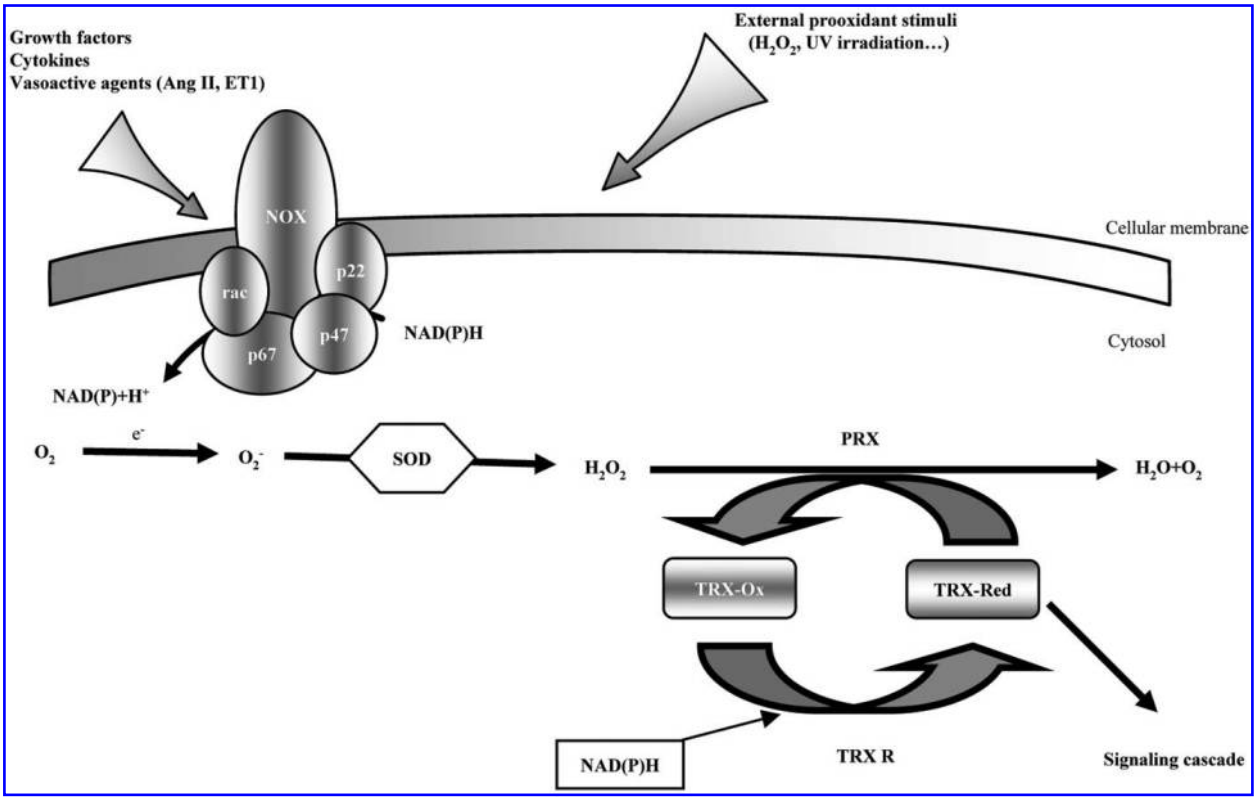

FIG. 2. Mechanism of action of thioredoxin (TRX). Peroxidase-dependent antioxidant effect of thioredoxin in the presence of oxidative conditions. The reduced thioredoxin (TRX-Red) scavenges hydrogen peroxide (H_2O_2) through peroxiredoxin (PRX). The oxidized Trx (TRX-Ox) in turn is reduced by Trx reductase in the presence of nicotinamide adenine dinucleotide phosphate (NAD(P)H). TRX-Red can interact with signaling molecules. Ang II, angiotensin II; ET1, endothelin-1; NADPH oxidase subunits (NOX, p67, p47, p22); O_2^-, superoxide anion; SOD, superoxide dismutase.

of eNOS activity in lung endothelial cells (65). In addition, exposure of endothelial cells to NO donors results in increased levels of TRX protein. Thioredoxin may participate in the regulation of NOS activity and be involved in NO functions via multiple mechanisms (44) (Fig. 5).

Thioredoxin and apoptosis

Through protein–protein interactions, TRX alters the enzymatic activity or the subcellular localization of its partner proteins, thereby influencing cellular functions. One of the best defined antioxidant-independent functions of TRX is its effect on apoptosis, through its interaction with ASK-1, a mitogen-activated protein kinase kinase kinase (MAPKKK). ASK-1 is activated by many stress- and cytokine-related stimuli, and in turn activates other downstream MAP kinases. Thioredoxin and ASK-1 physically interact in a redox-dependent manner. The reduced form of TRX1 inactivates ASK-1 (40), an upstream kinase of mitogen-activated protein (MAP) kinases, p38 MAP kinase, and JNK, thereby inhibiting apoptosis (Fig. 6). Pro-apoptotic stimuli such as tumor necrosis factor and ROS activate ASK1 in part by oxidizing TRX (forming intramolecular disulfide) to release TRX from ASK (24). Another mechanism whereby TRX influences apoptosis, is through its effects on the Akt/PI3K pathway, that induces phosphorylation and inactivation of Bad, a pro-apoptotic protein, and procaspase-9. In addition, TRX influences apoptosis by enhancing the DNA binding activity of NF-κB.

Dimmeler *et al*. demonstrated that the anti-apoptotic function of physiological concentrations of ROS is dependent on TRX expression in HUVECs (11). Overexpression of TRX inhibited oxidative stress-induced apoptosis. This seems to occur only when cysteine 69 is *S*-nitrosylated (10). Thioredoxin has also been implicated in angiogenesis. Pharmacological inhibition of endothelial cell TRX was associated with increased vascular endothelial growth factor (VEGF) receptor expression, enhanced cell migration, proliferation, and angiogenesis (48).

Thioredoxin and inflammation

The TRX system has also been implicated in inflammatory processes, in large part, through its modulatory effects on pro-inflammatory transcription factors, NF-κB and AP-1. Thioredoxin increases DNA binding of NF-κB by reducing cysteine 62 of the NF-κB P50 subunit (27). TRXR1 acts as a positive regulator of NF-κB and inflammation in endothelial cells (41). Activation of the AP-1 transcription factor is mediated by TRX nuclear translocation and its physical interaction with Ref-1 (57). The TRX-Ref -1 complex increases the DNA-binding activity of AP-1 (Fig. 7).

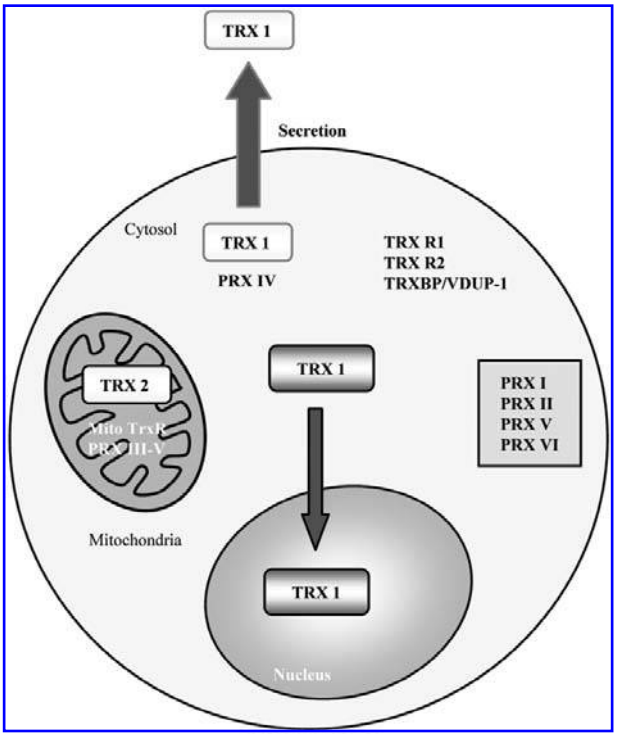

FIG. 3. Cellular localization of thioredoxin (TRX) and peroxiredoxin (PRX) isoforms. TRX and PRX isoforms are located in the cytosol and in the mitochondria. They can be translocated to the nucleus or can be secreted. TRXBP (called also VDUP-1) is the endogenous inhibitor of TRX. Mito Trx R, mitchondrial thioredoxin reductase; TRX R, thioredoxin reductase; TRXBP, thioredoxin interacting protein; VDUP-1, vitamin D3–upregulated protein.

Berk *et al*. (62) demonstrated that fluid shear stress inhibits vascular inflammation by decreasing VDUP-1 in endothelial cells. VDUP-1 and TRX are key components of biomechanical signal transduction and are potentially novel regulators of TNF signaling and inflammation in endothelial cells (62). Another target of TRX that has recently been identified is Peroxisome Proliferator-Activated Receptor-α (PPARα). Activation of PPARα leads to increased TRX expression as well as its translocation from cytoplasm to nucleus. Overexpression of TRX in the nucleus dramatically inhibits PPARα activation (22).

Thioredoxin is also abundantly expressed in human and rodent vascular smooth muscle cells (VSMCs) from mesenteric arteries and aorta (38). In the cytosol of VSMCs, TRX maintains the reduced thiol–disulfide status and is considered to regulate many cell functions, including differentiation, gene transcription, cell growth, and apoptosis. In VSMCs, TRX seems to plays a role in proliferation and cell hypertrophy, similar to what has been demonstrated in the heart. Adenoviral gene transfer of TRX in human aortic VSMCs, enhanced TRX enzyme activity and significantly increased DNA synthesis (42). In this paradigm, redox-dependent VSMC proliferation is regulated by the TRX endogenous inhibitor, VDUP-1, through interaction with TRX, and unlike in endothelial cells, treatment of VSMC with H_2O_2 did not modify TRX expression (64). Nitric oxide increases TRX activity in VSMCs by suppressing VDUP-1 expression (43). Similar to endothelial cells, TRX also has antiapoptotic properties, induced in part through induction of HO-1 (58). Depending on the redox state and cell type, TRX has the potential to both promote cell growth and to induce apoptosis. Low dose ROS seems to induce TRX expression, whereas a high dose of ROS inactivates TRX. Hence the response of TRX against different levels of ROS may differentially influence vascular responses of cell growth *versus* apoptosis.

Recent findings indicate that protein disulfide isomerase (PDI) regulates the TRX superfamily in VSMCs. PDI is a dithiol/disulfide oxidoreductase chaperone of the thioredoxin superfamily that closely associates with NAD(P)H oxidase to influence the subunit traffic/assembling (15).

PEROXIREDOXIN IN VASCULAR BIOLOGY

Peroxiredoxin modulates intracellular signaling pathways, influences cellular redox status, and regulates transcription factor activity (17, 20). Different PRX isoforms seem to modulate different cellular responses. Transfection of rat pulmonary artery VSMCs with a PRXI expression plasmid significantly

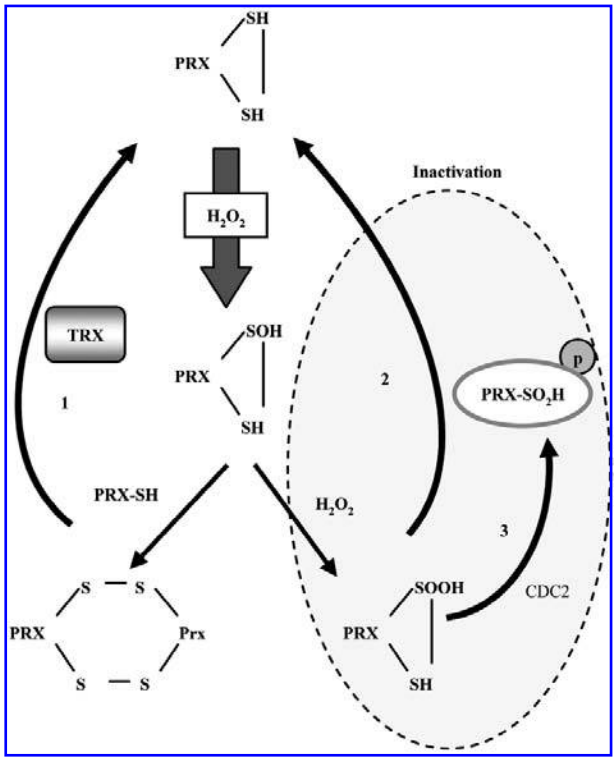

FIG. 4. The catalytic cycle of 2- Cys peroxiredoxin (PRX). The conserved cysteine residue of Prx is oxidized by hydrogen peroxide (H_2O_2), leading to formation of an intermolecular disulfide that is reduced specifically by thioredoxin (TRX) (1). Prx can be temporarily inactivated either by hyperoxidation of its active site (2) or by phosphorylation by cyclin B-dependent kinase (CDC2) (3).

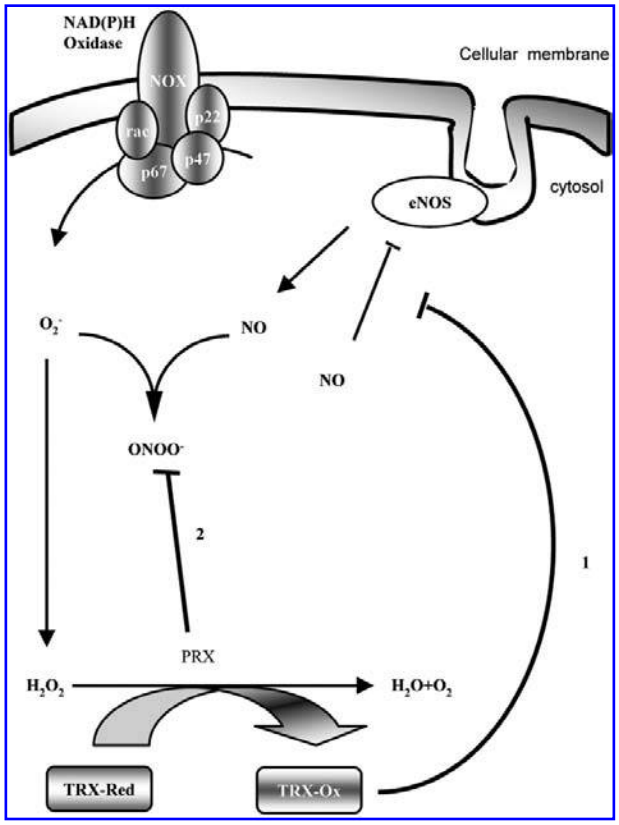

FIG. 5. Thioredoxin (TRX) and the nitric oxide (NO) pathway. Nitric oxide produced by endothelial nitric oxide synthase (eNOS), and superoxide anion (O_2^-) produced by NAD(P)H oxidase forms peroxynitrites ($ONOO^-$). Thioredoxin (TRX) inhibits the inhibitory effect of NO on eNOS (1). Peroxiredoxin (PRX) has a peroxynitrite reductase effect (2).

increased their growth (14). PRXII downregulation resulted in increased activation of platelet-derived growth factor receptor signaling and associated increased cell proliferation and migration (19). Overexpression of PRXV prevented p53-dependent generation of ROS and p53-induced apoptosis (66).

In contrast to TRX knockout mice, which are lethal, PRX knockout mouse are viable with a specific cardiac phenotype. Nagy *et al*. reported that hearts from PRXVI knockout mice are more susceptible to ischemia–reperfusion-induced injury, as evidenced by reduced postischemic recovery, increased infarct size, and apoptotic cell death, compared with those in hearts obtained from their wild-type littermates (33), suggesting a protective effect of PRXVI. Conversely, PrdxVI-null mice were shown to be hypersensitive to hyperoxia, providing evidence that PRXVI is an important antioxidant enzyme in *in vivo* conditions. Moreover, overexpression of mitochondrial PRXIII prevents left ventricular remodeling and cardiac failure post myocardial infarction in mice (26).

THIOREDOXIN IN CARDIAC AND VASCULAR DISEASES

Changes in TRX status, in part related to changes in ROS levels, have been implicated in various cardiovascular diseases, including atherosclerosis, ischemia reperfusion injury, myocarditis, cardiac hypertrophy, heart failure, myocardial infarction, and hypertension.

Hypertension and thioredoxin

Increased oxidative stress in hypertension has been attributed, in part, to decreased antioxidant capacity. Although alterations in the glutathione system and changes in levels of antioxidant vitamins have been studied in hypertension and atherosclerosis, little is known about the status of the TRX system in these conditions. Mansego *et al*. (25) recently demonstrated that in untreated hypertensive patients plasma and monocyte levels of GSH were reduced, GSSG levels were increased, and GSSG/GSH was elevated, compared with normotensive individuals. This was associated with reduced mRNA expression of *GSS, GSR, GCLC, GCLM, GPX1, GPX4*, and *GPX6* genes in the hypertensive group, but there was no relationship with blood pressure levels. On the other hand, mRNA levels of the thioredoxins *TRX1* and *TRX2* and the thioredoxin reductases,

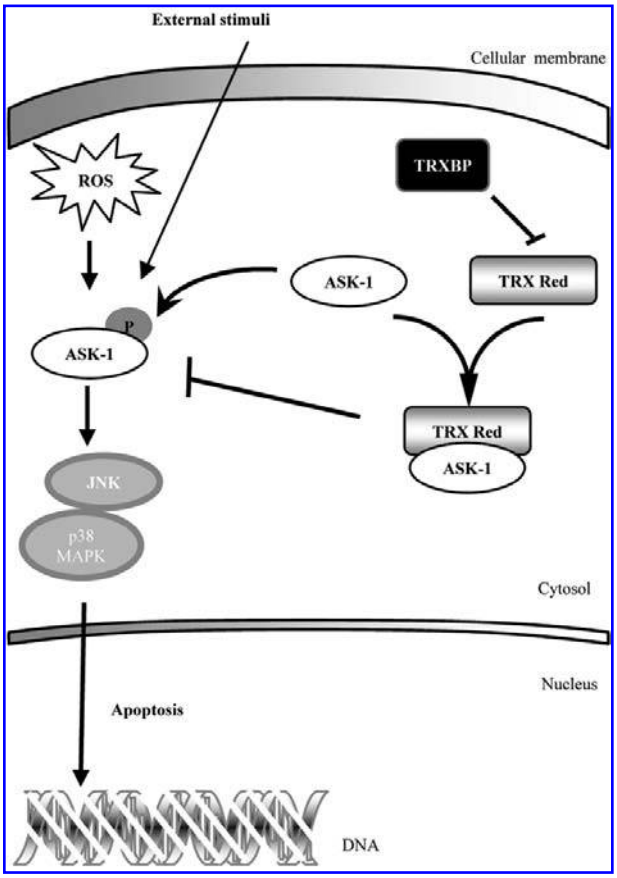

FIG. 6. Thioredoxin (TRX) and apoptosis. Apoptosis signal-regulating kinase 1 (ASK1) activation is mediated by reactive oxygen species (ROS). The reduced thioredoxin (TRX-Red) interacts with ASK-1 and maintains it in an inactivated state. Upon dissociation of TRX from ASK-1 induced by ROS, ASK-1 is activated by phosphorylation and induces apoptosis through activation of the MAP kinases pathway. JNK, c-Jun N-terminal kinase; TRXBP, thioredoxin binding protein.

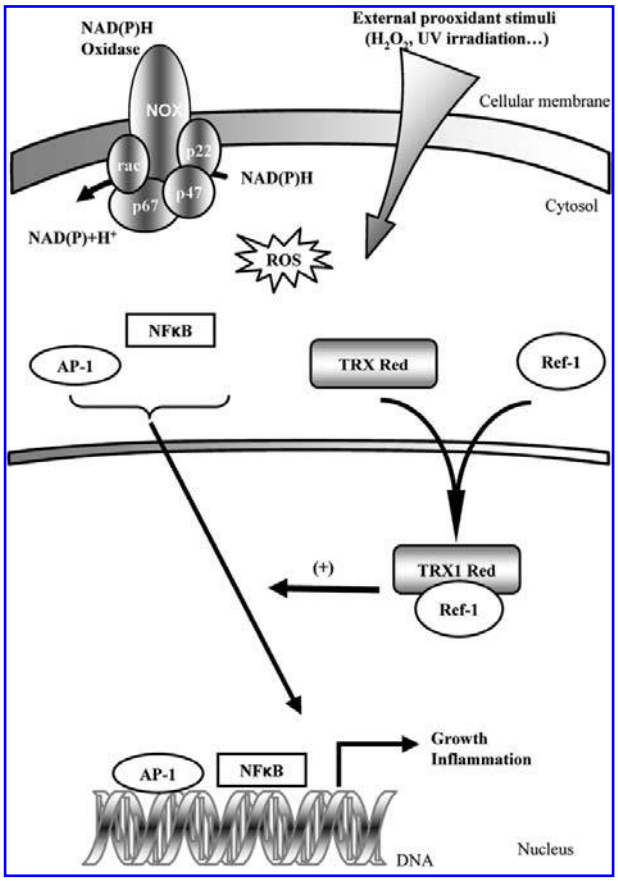

FIG. 7. Thioredoxin (TRX) and cellular signaling. Thioredoxin (TRX) exerts its actions via various biochemical mechanisms. Under oxidative conditions, Trx as well as Redox Factor-1 (Ref-1) are translocated from the cytosol into the nucleus, where they interact physically. Indeed, TRX-Ref-1 complex increases the DNA binding activity of some transcription factors such as nuclear factor kappa B (NF-κB) and activator protein-1 (AP-1) that are involved in cellular growth and inflammation.

TRXR1, TRXR2, and *TRXR3* were higher in hypertensive patients than controls and decreased during antihypertensive treatment. These differences between the GSH and TRX systems may be attributed to the basal state of oxidative stress. It was proposed that the TRX system is activated by chronic increased oxidative stress, whereas the GSH system response is not. In hypertension, despite activation of the TRX system, this may be insufficient for maintaining a normal redox state, especially in the presence of an impaired GSH response. Increased serum levels of TRX have also been demonstrated in patients with major risk factors for cardiovascular disease, including hypertension, smoking, and hypercholesterolemia.

In experimental models of hypertension, the TRX system has been shown to be downregulated. In aorta, heart, and kidney from SHR and SHRSP, which exhibit increased oxidative stress, expression of TRX, estimated by immunohistochemistry and Western blot analysis, and expression of TRX gene estimated by real-time RT-PCR were markedly suppressed, compared with Wistar Kyoto (WKY) (50). Moreover, induction of TRX was impaired after Ang II stimulation in peripheral blood mononuclear cells isolated from SHR and SHRSP, compared with those from WKY (50). Yamagata et al. (60) reported similar data for cultured cortical neurons isolated from SHRSPs after ischemia/reperfusion. Taken together, these studies suggest that decreased TRX in tissues of SHRs and SHRSPs may contribute to hypertension. Zhang *et al*. (65) demonstrated regulation of endothelial function by a mitochondria-specific TRX, which reduces oxidative stress and increases NO bioavailability, thereby preserving vascular endothelial cell function and preventing vascular injury in hypertension and atherosclerosis.

Unlike studies in genetically hypertensive rats, and similar to what has been reported in human hypertension, we recently demonstrated upregulation of the TRX system in Ang II-infused C57B6 male mice. In particular, cardiac TRX expression was increased and activity was enhanced in Ang II-infused mice, compared with controls (7). This was associated with cardiac hypertrophy and increased oxidative stress, suggesting that oxidative stress induced by Ang II could be responsible for upregulation of the TRX antioxidant system, possibly as a counter-regulatory adaptive process. Interestingly, the upregulation of the TRX system by Ang II was only observed in male and ovariectomized female mice and not in intact female mice where basal TRX levels were already high (7). These results suggest that normally estrogens may exert beneficial cardiovascular actions by augmenting the TRX system (Fig. 8). Others have also shown that Ang II activates NAD(P)H oxidase-mediated ROS production and that oxidative stress induces expression/activity of TRX (8). Taken together, the existing data indicate that the TRX system is both increased and de-

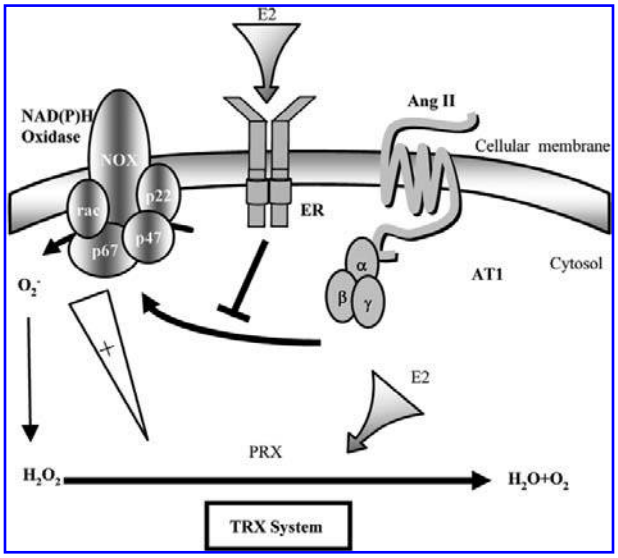

FIG. 8. Thioredoxin (TRX) and hypertension. Putative regulation of the thioredoxin (TRX) system in Ang II-induced hypertension. Angiotensin II activates NAD(P)H oxidase, leading to increased production of superoxide anions (O_2^-) and hydrogen peroxide (H_2O_2), which in turn activate the Trx system as a counter regulatory mechanism. In female mice, estrogen (E2) seems to inhibit effects of Ang II on NAD(P)H oxidase and Trx. AT1R, Angiotensin receptor type 1; ER, estrogen receptor; NAD(P)H oxidase subunits, NOX, rac, p67, p47, p22; PRX, peroxiredoxin.

creased in different models of hypertension. It may be possible that in some circumstances TRX is acting primarily as a signaling molecule influencing cardiovascular remodeling, whereas in other situations it is acting as an antioxidant, which may be blunted in hypertension. Hence, the exact role of the TRX system in hypertension still awaits clarification.

Atherosclerosis and thioredoxin

Miyamoto *et al*. demonstrated increased plasma levels of TRX in patients with atherosclerosis, diabetes, coronary artery disease and acute coronary syndrome (12, 30, 32, 46). Changes in TRX status have also been reported at the cellular level in atherosclerosis. In human atherosclerotic neointimal plaques (38) and in balloon-injured rat arteries (49), endothelial and macrophage TRX expression is increased. TRXR is also upregulated in human atherosclerotic plaques, possibly due to macrophage-derived low density lipoproteins in monocyte-derived macrophages (9). Other studies reported that extracellular TRX inhibits lipopolysaccharide-induced interleukin-1beta expression in human monocyte-derived macrophages (2). Whether increased TRX and TRXR are reflective of increased oxidative stress in atherosclerosis or whether they are acting as signaling molecules involved in atherogenesis is still unclear.

Ischemia–reperfusion and thioredoxin

Ischemia–reperfusion injury in mice is associated with reduced cardiac TRX expression (54). Overexpression of cardiac TRX was associated with improved postischemic ventricular recovery and decreased myocardial infarct size *versus* hearts from wild-type mice (54), whereas downregulation of cardiac TRX, (by overexpressing cardiac-specific dominant negative TRX) was associated with increased cardiac injury and oxidative stress, implicating a protective role of TRX in ischemia-reperfusion injury. In support of this, studies of *in vivo* models of ischemia–reperfusion in the heart showed that increasing the concentration of TRX, either by inducing endogenous TRX or intravenous administration of human recombinant TRX, reduces markers of tissue damage (5). Exogenously applied TRX reduces myocardial ischemia/reperfusion injury and decreases oxidative damage *in vivo* (51). Possible mechanisms for this protective effect against oxidative-induced injury include activation of transcription factors that regulate pro-survival and antioxidant genes and modulation of kinases, such as ASK-1, which coordinate cell growth and apoptosis. Protection against reperfusion-induced arrhythmias by human TRX has also been reported (1).

Cardiac hypertrophy and thioredoxin

Thioredoxin, possibly due to its cell growth regulating properties and antioxidant properties, has also been implicated in the pathogenesis of cardiac hypertrophy. In *in vivo* studies downregulating TRX, by dominant negative TRX overexpression or by antisense approaches, was associated with decreased oxidative stress and attenuated cardiac hypertrophy in response to pressure overload (61). Yoshioka *et al*. (64) demonstrated that VDUP-1, an endogenous inhibitor of TRX, controls cardiac hypertrophy by modulating TRX activity. Thioredoxin overexpression in rat ventricular myocytes prevents alpha adrenergic-stimulated hypertrophic signaling by inhibiting Ras activation (21).

Myocarditis and thioredoxin

Increased oxidative stress has been implicated in autoimmune myocarditis, due in part to alterations in the TRX system. Liu *et al*. (23) demonstrated a decrease in the development of myocarditis and reduced inflammation in mice treated with TRX. Other studies demonstrated that TRX is involved in the development of age-related cardiac changes (29).

Clinical implications

Thioredoxin may be a biomarker of oxidative stress, as well as a potentially important therapeutic agent in cardiovascular disease. Recent clinical studies reported an increase in circulating levels of TRX in patients with chronic cardiac failure where TRX elevation correlated significantly with increased oxidative stress (16). Plasma levels of TRX are also increased in patients with unstable angina compared to those with stable angina (31) and in post-angioplasty in patients with peripheral vascular disease plasma (56). Upregulation of the cardiac TRX system has also been demonstrated in patients with myocarditis and cardiomyopathies, as evidenced by increased TRX expression in endomyocardial biopsy samples (36). Variations of TRX concentrations have been suggested as an indicator of the severity of some cardiac pathologies, and in some studies TRX levels have been used as biomarkers of cardiovascular disease (28).

Because of its function as an antioxidant and as a negative regulator of hypertrophy and cardiac failure, TRX is potentially an interesting therapeutic target. Moreover TRX is a small molecule, it is stable, and is functionally active in the intracellular and extracellular compartments, making it especially attractive as a therapeutic possibility.

CONCLUSIONS

Thioredoxin influences many cellular responses, both as an antioxidant and as a modulator of signaling pathways via direct interaction with signaling kinases and transcription factors. Regulation of TRX activity is complex and involves many processes, including transcription, cellular localization, protein–protein interaction, and post-translational modification. Thioredoxin-interacting proteins important in cardiovascular biology include ASK-1, TXNIP/VDUP/TBP-2, PTEN/PI3K/Akt, and transcriptional factors such as NF-κB and AP-1. Activation of the TRX system has been shown to be both increased and decreased in experimental and human hypertension, whereas in atherosclerosis and cardiac disease, TRX appears to be primarily upregulated. The (patho)physiological significance of these TRX differences in cardiovascular disease has yet to be determined. It is still unclear whether elevated TRX in pathological conditions is an index of increased oxidative stress, whether it is upregulated to counterbalance increased bioavailability of ROS, or whether increased TRX is responsible for aberrant cellular signaling. Further investigations are needed to

understand the mechanisms of regulation, downstream targets, and the role of TRX in oxidative balance in the cardiovascular system, and the pathophysiological significance of alterations of TRX in cardiovascular disease.

ACKNOWLEDGMENTS

RMT holds a Canada Research Chair and is funded through grants from the Canadian Institutes of Health Research, Heart and Stroke Foundation of Canada, and the Canadian Foundation for Innovation.

ABBREVIATIONS

AP1, activator protein-1; ASK-1, apoptosis signal-regulated kinase- 1; CDC2, cyclin B-dependent kinase; ET1, endothelin-1; GPX, glutathione peroxidase; GSH, glutathione; GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide; HO-1, heme oxygenase-1; JNK, c-Jun N-terminal kinase; MAPKKK, mitogen-activated protein kinase kinase kinase; NF-κB, nuclear factor-κB; $O_2.^-$, superoxide anion; $ONOO^-$, peroxynitrite; PDI, protein disulfide isomerase; PPARα, peroxisome proliferator-activated receptor-alpha; PRX, peroxiredoxin; PTEN, phosphatase and tensin homolog; Ref-1, redox factor-1; SOD, superoxide dismutase; TRX, thioredoxin; TRXBP, thioredoxin binding protein; TRXR, thioredoxin reductase; VDUP-1, vitamin D3-upregulated protein; VEGF, vascular endothelial growth factor; WKY, Wistar Kyoto.

REFERENCES

1. Aota M, Matsuda K, Isowa N, Wada H, Yodoi J, and Ban T. Protection against reperfusion-induced arrhythmias by human thioredoxin. *J Cardiovasc Pharmacol* 27: 727–732, 1996.
2. Billiet L, Furman C, Larigauderie G, Copin C, Brand K, Fruchart JC, and Rouis M. Extracellular human thioredoxin-1 inhibits lipopolysaccharide-induced interleukin-1beta expression in human monocyte-derived macrophages. *J Biol Chem* 280: 40310–40318, 2005.
3. Chae HZ, Kim HJ, Kang SW, and Rhee SG. Characterization of three isoforms of mammalian peroxiredoxin that reduce peroxides in the presence of thioredoxin. *Diabetes Res Clin Pract* 45: 101–112, 1999.
4. Choi MH, Lee IK, Kim GW, Kim BU, Han YH, Yu DY, Park HS, Kim KY, Lee JS, Choi C, Bae YS, Lee BI, Rhee SG, and Kang SW. Regulation of PDGF signalling and vascular remodelling by peroxiredoxin II. *Nature* 435: 347–353, 2005.
5. Das KC, Lewis–Molock Y, and White CW. Elevation of manganese superoxide dismutase gene expression by thioredoxin. *Am J Respir Cell Mol Biol* 17: 713–726, 1997.
6. De Champlain J, Wu R, Girouard H, Karas M, EL Midaoui A, Laplante MA, and Wu L. Oxidative stress in hypertension. *Clin Exp Hypertens* 26: 593–601, 2004.
7. Ebrahimian T, He Y, Schiffrin EL, and Touyz RM. Differential regulation of thioredoxin and NAD(P)H oxidase by angiotensin II in male and female mice. *J Hypertens* 25: 1263–1271, 2007.
8. Ejima K, Layne MD, Carvajal IM, Nanri H, Ith B, Yet SF, and Perrella MA. Modulation of the thioredoxin system during inflammatory responses and its effect on heme oxygenase-1 expression. *Antioxid Redox Signal* 4: 569–575, 2002.
9. Furman C, Rundlof AK, Larigauderie G, Jaye M, Bricca G, Copin C, Kandoussi AM, Fruchart JC, Arner ES, and Rouis M. Thioredoxin reductase 1 is upregulated in atherosclerotic plaques: specific induction of the promoter in human macrophages by oxidized low-density lipoproteins. *Free Radic Biol Med* 37: 71–85, 2004.
10. Haendeler J, Hoffmann J, Tischler V, Berk BC, Zeiher AM, and Dimmeler S. Redox regulatory and anti-apoptotic functions of thioredoxin depend on S-nitrosylation at cysteine 69. *Nat Cell Biol* 10: 743–749, 2002.
11. Haendeler J, Tischler V, Hoffmann J, Zeiher AM, and Dimmeler S. Low doses of reactive oxygen species protects endothelial cells from apoptosis by increasing thioredoxin-1 expression. *FEBS Lett* 577: 427–433, 2004.
12. Hokamaki J, Kawano H, Soejima H, Miyamoto S, Kajiwara I, Kojima S, Sakamoto T, Sugiyama S, Yoshimura M, Nakamura H, Yodoi J, and Ogawa H. Plasma thioredoxin levels in patients with unstable angina. *Int J Cardiol* 99: 225–231, 2005.
13. Holmgren A. Antioxidant function of thioredoxin and glutaredoxin systems. *Antioxid Redox Signal* 2: 811-820, 2000.
14. Ihida–Stansbury K, McKean DM, Gebb SA, Martin JF, Stevens T, Nemenoff R, Vaughn J, Lane K, Loyd J, Wheeler L, Morrell NW, Ivy D, and Jones PL. Regulation and functions of the paired-related homeobox gene PRX1 in pulmonary vascular development and disease. *Chest* 128: 591S, 2005.
15. Janiszewski M, Lopes LR, Carmo AO, Pedro MA, Brandes RP, Santos CX, and Laurindo FR. Regulation of NAD(P)H oxidase by associated protein disulfide isomerase in vascular smooth muscle cells. *J Biol Chem* 280: 40813–40819, 2005.
16. Jekell A, Hossain A, Alehagen U, Dahlstrom U, and Rosen A. Elevated circulating levels of thioredoxin and stress in chronic heart failure. *Eur J Heart Fail* 6: 883–890, 2004.
17. Jin DY, Chae HZ, Rhee SG, and Jeang KT. Regulatory role for a novel human thioredoxin peroxidase in NF-kappaB activation. *J Biol Chem* 272: 30952–30961, 1997.
18. Junn E, Han SH, Im JY, Yang Y, Cho EW, Um HD, Kim DK, Lee KW, Han PL, Rhee SG, and Choi I. Vitamin D3 up-regulated protein 1 mediates oxidative stress via suppressing the thioredoxin function. *J Immunol* 164: 6287–6295, 2000.
19. Kang SW, Rhee SG, Chang TS, Jeong W, Choi MH. 2-Cys peroxiredoxin function in intracellular signal transduction: therapeutic implications. *Trends Mol Med* 11: 571–578, 2005.
20. Kim H, Lee TH, Park ES, Suh JM, Park SJ, Chung HK, Kwon OY, Kim YK, Ro HK, and Shong M. Role of peroxiredoxins in regulating intracellular hydrogen peroxide and hydrogen peroxide-induced apoptosis in thyroid cells. *J Biol Chem* 275: 18266–18270, 2000.
21. Kuster GM, Pimentel DR, Adachi T, Ido Y, Brenner DA, Cohen RA, Liao R, Siwik DA, and Colucci WS. Alpha-adrenergic receptor-stimulated hypertrophy in adult rat ventricular myocytes is mediated via thioredoxin-1-sensitive oxidative modification of thiols on Ras. *Circulation* 111: 1192–1198, 2005.
22. Liu GH, Qu J, and Shen X. Thioredoxin-mediated negative autoregulation of peroxisome proliferator-activated receptor alpha transcriptional activity. *Mol Biol Cell* 17: 1822–1833, 2006.
23. Liu W, Nakamura H, Shioji K, Tanito M, Oka S, Ahsan MK, Son A, Ishii Y, Kishimoto C, and Yodoi J. Thioredoxin-1 ameliorates myosin-induced autoimmune myocarditis by suppressing chemokine expressions and leukocyte chemotaxis in mice. *Circulation* 110: 1276–1283, 2004.
24. Liu Y and Min W. Thioredoxin promotes ASK1 ubiquitination and degradation to inhibit ASK1-mediated apoptosis in a redox activity-independent manner. *Circ Res* 90: 1259–1266, 2002.
25. Mansego ML, Blesa S, Gonzalez–Albert V, Tormos MC, Saez G, Redon J, and Chaves FJ. Discordant response of glutathione and thioredoxin systems in human hypertension? *Antioxid Redox Signal* 9: 507–514, 2007.
26. Matsushima S, Ide T, Yamato M, Matsusaka H, Hattori F, Ikeuchi M, Kubota T, Sunagawa K, Hasegawa Y, Kurihara T, Oikawa S, Kinugawa S, and Tsutsui H. Overexpression of mitochondrial peroxiredoxin-3 prevents left ventricular remodeling and failure after myocardial infarction in mice. *Circulation* 113: 1779–1786, 2006.

27. Matthews JR, Wakasugi N, Virelizier JL, Yodoi J, and Hay RT. Thioredoxin regulates the DNA binding activity of NF-kappa B by reduction of a disulphide bond involving cysteine 62. *Nucleic Acids Res* 20: 3821–3830, 1992.
28. Miwa K, Kishimoto C, Nakamura H, Makita T, Ishii K, Okuda N, Yodoi J, and Sasayama S. Serum thioredoxin and alpha-tocopherol concentrations in patients with major risk factors. *Circ J* 69: 291–294, 2005.
29. Miyamoto M, Kishimoto C, Nimata M, Nakamura H, and Yodoi J. Thioredoxin, a redox-regulating protein, is expressed in spontaneous myocarditis in inbred strains of mice. *Int J Cardiol* 95: 315–319, 2004.
30. Miyamoto S, Kawano H, Hokamaki J, Soejima H, Kojima S, Kudoh T, Nagayoshi Y, Sugiyama S, Sakamoto T, Yoshimura M, Nakamura H, Yodoi J, and Ogawa H. Increased plasma levels of thioredoxin in patients with glucose intolerance. *Intern Med* 44: 1127–1132, 2005.
31. Miyamoto S, Kawano H, Sakamoto T, Soejima H, Kajiwara I, Hokamaki J, Hirai N, Sugiyama S, Yoshimura M, Yasue H, Nakamura H, Yodoi J, and Ogawa H. Increased plasma levels of thioredoxin in patients with coronary spastic angina. *Antioxid Redox Signal* 6: 75–80, 2004.
32. Miyamoto S, Sakamoto T, Soejima H, Shimomura H, Kajiwara I, Kojima S, Hokamaki J, Sugiyama S, Yoshimura M, Ozaki Y, Nakamura H, Yodoi J, and Ogawa H. Plasma thioredoxin levels and platelet aggregability in patients with acute myocardial infarction. *Am Heart J* 146: 465–471, 2003.
33. Nagy N, Malik G, Fisher AB, and Das DK. Targeted disruption of peroxiredoxin 6 gene renders the heart vulnerable to ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol* 291: H2636–H2640, 2006.
34. Nakamura H, Matsuda M, Furuke K, Kitaoka Y, Iwata S, Toda K, Inamoto T, Yamaoka Y, Ozawa K, and Yodoi J. Adult T cell leukemia-derived factor/human thioredoxin protects endothelial F-2 cell injury caused by activated neutrophils or hydrogen peroxide. *Immunol Lett* 42: 75–80, 1994.
35. Nakazono K, Watanabe N, Matsuno K, Sasaki J, Sato T, and Inoue M. Does superoxide underlie the pathogenesis of hypertension? *Proc Natl Acad Sci USA* 88: 10045–10048, 1991.
36. Nimata M, Kishimoto C, Shioji K, Ishizaki K, Kitaguchi S, Hashimoto T, Nagata N, and Kawai C. Upregulation of redox-regulating protein, thioredoxin, in endomyocardial biopsy samples of patients with myocarditis and cardiomyopathies. *Mol Cell Biochem* 248: 193–196, 2003.
37. Nordberg J and Arner ES. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free Radic Biol Med* 31: 1287–1312, 2001.
38. Okuda M, Inoue N, Azumi H, Seno T, Sumi Y, Hirata Ki, Kawashima S, Hayashi Y, Itoh H, Yodoi J, and Yokoyama M. Expression of glutaredoxin in human coronary arteries: its potential role in antioxidant protection against atherosclerosis. *Arterioscler Thromb Vasc Biol* 21: 1483–1487, 2001.
39. Rhee SG, Chae HZ, and Kim K. Peroxiredoxins: a historical overview and speculative preview of novel mechanisms and emerging concepts in cell signaling. *Free Radic Biol Med* 38: 1543–1552, 2005.
40. Saitoh M, Nishitoh H, Fujii M, Takeda K, Tobiume K, Sawada Y, Kawabata M, Miyazono K, and Ichijo H. Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. *EMBO J* 17: 2596–2606, 1998.
41. Sakurai A, Yuasa K, Shoji Y, Himeno S, Tsujimoto M, Kunimoto M, Imura N, and Hara S. Overexpression of thioredoxin reductase 1 regulates NF-kappa B activation. *J Cell Physiol* 198: 22–30, 2004.
42. Schulze PC, De Keulenaer GW, Yoshioka J, Kassik KA, and Lee RT. Vitamin D3-upregulated protein-1 (VDUP-1) regulates redox-dependent vascular smooth muscle cell proliferation through interaction with thioredoxin. *Circ Res* 9: 689–695, 2002.
43. Schulze PC, Liu H, Choe E, Yoshioka J, Shalev A, Bloch KD, and Lee RT. Nitric oxide-dependent suppression of thioredoxin-interacting protein expression enhances thioredoxin activity. *Arterioscler Thromb Vasc Biol* 26: 2666–2672, 2006.
44. Shao LE, Tanaka T, Gribi R, and Yu J. Thioredoxin-related regulation of NO/NOS activities. *Ann NY Acad Sci* 962: 140–150, 2002.
45. Slaby I and Holmgren A. Thioredoxin reductase-dependent insulin disulfide reduction by phage T7 DNA polymerase reflects dissociation of the enzyme into subunits. *J Biol Chem* 264: 16502–16506, 1989.
46. Soejima H, Suefuji H, Miyamoto S, Kajiwaram I, Kojima S, Hokamaki J, Sakamoto T, Yoshimura M, Nakamura H, Yodoi J, and Ogawa H. Increased plasma thioredoxin in patients with acute myocardial infarction. *Clin Cardiol* 26: 583–587, 2003.
47. Spyrou G, Enmark E, Miranda–Vizuete A, and Gustafsson J. Cloning and expression of a novel mammalian thioredoxin. *J Biol Chem* 272: 2936–2941, 1997.
48. Streicher KL, Sylte MJ, Johnson SE, and Sordillo LM. Thioredoxin reductase regulates angiogenesis by increasing endothelial cell-derived vascular endothelial growth factor. *Nutr Cancer* 50: 221–231, 2004.
49. Takagi Y, Gon Y, Todaka T, Nozaki K, Nishiyama A, Sono H, Hashimoto N, Kikuchi H, and Yodoi J. Expression of thioredoxin is enhanced in atherosclerotic plaques and during neointima formation in rat arteries. *Lab Invest* 78: 957–966, 1998.
50. Tanito M, Nakamura H, Kwon YW, Teratani A, Masutani H, Shioji K, Kishimoto C, Ohira A, Horie R, and Yodoi J. Enhanced oxidative stress and impaired thioredoxin expression in spontaneously hypertensive rats. *Antioxid Redox Signal* 6: 89–97, 2004.
51. Tao L, Gao E, Hu A, Coletti C, Wang Y, Christopher TA, Lopez BL, Koch W, and Ma XL. Thioredoxin reduces post-ischemic myocardial apoptosis by reducing oxidative/nitrative stress. *Br J Pharmacol* 149: 311–318, 2006.
52. Touyz RM. Oxidative stress and vascular damage in hypertension. *Curr Hypertens Rep* 2: 98–105, 2000.
53. Trigona WL, Mullarky IK, Cao Y, and Sordillo LM. Thioredoxin reductase regulates the induction of haem oxygenase-1 expression in aortic endothelial cells. *Biochem J* 394: 207–216, 2006.
54. Turoczi T, Chang VW, Engelman RM, Maulik N, Ho YS, and Das DK. Thioredoxin redox signaling in the ischemic heart: an insight with transgenic mice overexpressing TRX1. J *Mol Cell Cardiol* 35: 695–704, 2003.
55. Uwayama J, Hirayama A, Yanagawa T, Warabi E, Sugimoto R, Itoh K, Yamamoto M, Yoshida H, Koyama A, and Ishii T. Tissue Prx I in the protection against Fe-NTA and the reduction of nitroxyl radicals. *Biochem Biophys Res Commun* 339: 226–231, 2006.
56. Wahlgren CM and Pekkari K. Elevated thioredoxin after angioplasty in peripheral arterial disease. *Eur J Vasc Endovasc Surg* 29: 281–286, 2005.
57. Wei SJ, Botero A, Hirota K, Bradbury CM, Markovina S, Laszlo A, Spitz DR, Goswami PC, Yodoi J, and Gius D. Thioredoxin nuclear translocation and interaction with redox factor-1 activates the activator protein-1 transcription factor in response to ionizing radiation. *Cancer Res* 60: 6688–6695, 2000.
58. Wiesel P, Foster LC, Pellacani A, Layne MD, Hsieh CM, Huggins GS, Strauss P, Yet SF, and Perrella MA. Thioredoxin facilitates the induction of heme oxygenase-1 in response to inflammatory mediators. *J Biol Chem* 275: 24840–24846, 2000.
59. World CJ, Yamawaki H, and Berk BC. Thioredoxin in the cardiovascular system. *J Mol Med*. 84: 997–1003, 2006.
60. Yamagata K, Tagami M, Ikeda K, Yamori Y, and Nara Y. Altered gene expressions during hypoxia and reoxygenation in cortical neurons isolated from stroke-prone spontaneously hypertensive rats. *Neurosci Lett* 284: 131–134, 2000.
61. Yamamoto M, Yang G, Hong C, Liu J, Holle E, Yu X, Wagner T, Vatner SF, and Sadoshima J. Inhibition of endogenous thioredoxin in the heart increases oxidative stress and cardiac hypertrophy. *J Clin Invest* 112: 1395–1406, 2003.
62. Yamawaki H, Pan S, Lee RT, and Berk BC. Fluid shear stress inhibits vascular inflammation by decreasing thioredoxin-interacting protein in endothelial cells. *J Clin Invest* 115: 733–738, 2005.
63. Yang KS, Kang SW, Woo HA, Hwang SC, Chae HZ, Kim K, and Rhee SG. Inactivation of human peroxiredoxin I during catalysis as the result of the oxidation of the catalytic site cys-

teine to cysteine-sulfinic acid. *J Biol Chem* 277: 38029–38036, 2002.
64. Yoshioka J, Schulze PC, Cupesi M, Sylvan JD, MacGillivray C, Gannon J, Huang H, and Lee RT. Thioredoxin-interacting protein controls cardiac hypertrophy through regulation of thioredoxin activity. *Circulation* 109: 2581–2586, 2004.
65. Zhang J, Li YD, Patel JM, and Block ER. Thioredoxin overexpression prevents NO-induced reduction of NO synthase activity in lung endothelial cells. *Am J Physiol* 275: L288–L293, 1998.
66. Zhou Y, Kok KH, Chun AC, Wong CM, Wu HW, Lin MC, Fung PC, Kung H, and Jin DY. Mouse peroxiredoxin V is a thioredoxin peroxidase that inhibits p53-induced apoptosis. *Biochem Biophys Res Commun* 268: 921–927, 2000.

Address reprint requests to:
Talin Ebrahimian, Ph.D.
Lady Davis Institute for Medical Research–SMBD Jewish General Hospital
McGill University
3999 Sainte Catherine Road
H3T 1E2, Montreal
Quebec, Canada

E-mail: tebrahimian@ldi.jgh.mcgill.ca

Date of first submission to ARS Central, November 26, 2007; date of acceptance, December 7, 2007.

This article has been cited by:

1. Paul J. Lijnen, Yvette Piccart, Tamara Coenen, John S. Prihadi. 2012. Angiotensin II-induced mitochondrial reactive oxygen species and peroxiredoxin-3 expression in cardiac fibroblasts. *Journal of Hypertension* **30**:10, 1986-1991. [CrossRef]
2. Qian Guo, Yan Wang, Qing Yun Li, Min Li, Huan Ying Wan. 2012. Levels of thioredoxin are related to the severity of obstructive sleep apnea: based on oxidative stress concept. *Sleep and Breathing* . [CrossRef]
3. Arthur I. Cederbaum, Lili Yang, Xiaodong Wang, Defeng Wu. 2012. CYP2E1 Sensitizes the Liver to LPS- and TNF #-Induced Toxicity via Elevated Oxidative and Nitrosative Stress and Activation of ASK-1 and JNK Mitogen-Activated Kinases. *International Journal of Hepatology* **2012**, 1-19. [CrossRef]
4. Lili Yang, Defeng Wu, Xiaodong Wang, Arthur I. Cederbaum. 2011. Depletion of cytosolic or mitochondrial thioredoxin increases CYP2E1-induced oxidative stress via an ASK-1–JNK1 pathway in HepG2 cells. *Free Radical Biology and Medicine* **51**:1, 185-196. [CrossRef]
5. Ramón Rodrigo, Jaime González, Fabio Paoletto. 2011. The role of oxidative stress in the pathophysiology of hypertension. *Hypertension Research* **34**:4, 431-440. [CrossRef]
6. Amadou K.S. Camara , Edward J. Lesnefsky , David F. Stowe . 2010. Potential Therapeutic Benefits of Strategies Directed to Mitochondria. *Antioxidants & Redox Signaling* **13**:3, 279-347. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
7. Ana M. Briones, Rhian M. Touyz. 2010. Oxidative Stress and Hypertension: Current Concepts. *Current Hypertension Reports* **12**:2, 135-142. [CrossRef]
8. Jungang Zhang, Guangrong Zhao, Jinling Liu, Xiangwu Ji. 2009. Protection of salvianolic acid B for human endothelial cells against hydrogen peroxide-induced oxidative damage. *Transactions of Tianjin University* **15**:6, 434-439. [CrossRef]
9. Yves Meyer, Bob B. Buchanan, Florence Vignols, Jean-Philippe Reichheld. 2009. Thioredoxins and Glutaredoxins: Unifying Elements in Redox Biology. *Annual Review of Genetics* **43**:1, 335-367. [CrossRef]