ANTIOXIDANTS & REDOX SIGNALING
Volume 11, Number 11, 2009

DOI: 10.1089/ars.2009.2683

FORUM REVIEW ARTICLE

Redox Regulation of Cell Survival by the Thioredoxin Superfamily: An Implication of Redox Gene Therapy in the Heart

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Abstract

Reactive oxygen species (ROS) are the key mediators of pathogenesis in cardiovascular diseases. Members of the thioredoxin superfamily take an active part in scavenging reactive oxygen species, thus playing an essential role in maintaining the intracellular redox status. The alteration in the expression levels of thioredoxin family members and related molecules constitute effective biomarkers in various diseases, including cardiovascular complications that involve oxidative stress. Thioredoxin, glutaredoxin, peroxiredoxin, and glutathione peroxidase, along with their isoforms, are involved in interaction with the members of metabolic and signaling pathways, thus making them attractive targets for clinical intervention. Studies with cells and transgenic animals have supported this notion and raised the hope for possible gene therapy as modern genetic medicine. Of all the molecules, thioredoxins, glutaredoxins, and peroxiredoxins are emphasized, because a growing body of evidence reveals their essential and regulatory role in several steps of redox regulation. In this review, we discuss some pertinent observations regarding their distribution, structure, functions, and interactions with the several survival- and death-signaling pathways, especially in the myocardium. *Antioxid. Redox Signal.* 11, 2741–2758.

THE REDUCTION and oxidation process responsible for the cyclic maintenance of the redox state in a cell is commonly known as redox regulation. Redox regulation is an essential physiologic process in the cell survival of virtually all types of cells, including cardiomyocytes. Imbalance in redox regulation leads to development of oxidative stress in the cells, resulting in an impairment of cellular function, lipid peroxidation, degradation of proteins, and even breakage of the nucleic acids that are the major mediators of cardiovascular diseases. To neutralize the oxidative stress, myocardial cells are equipped with two major antioxidant systems: thioredoxin (TRX) and glutaredoxin (GRX), which are involved in redox regulation to protect the cells from oxidative stress and to stop apoptosis, thereby converting the death signals to survival signals. The TRX system consists of TRX, NADPH, and TRX reductase (TrxR), whereas the GRX system consists of GRX, NADPH, glutathione (GSH), and glutathione reductase (GR) (Fig. 1). Apart from these two antioxidant systems, another two potent antioxidant subsystems also exist: TRX-dependent TRX peroxidase, peroxiredoxin (PRX), and GRX-dependent glutathione peroxidase (GPX) (15, 19, 20).

Historical Perspective of the Thioredoxin Superfamily

In 1964, the small protein TRX was identified by Peter Reichard and his group (67, 97) as a hydrogen donor to ribonucleotide reductase (RNR), which is an essential enzyme for DNA synthesis in *Escherichia coli*. In 1974 Yodoi *et al.* (199) found a new disease in Japan, adult T-cell leukemia (ATL), which is caused by a human T-cell leukemia virus type-I (HTLV-I) infection. Overexpression of interleukin-2 (IL-2) receptor α-chain (CD25) is a characteristic feature of ATL cells. In 1987, ATL-derived factor (ADF) was reported as a cytokine-like factor, which is involved in induction of CD25 in HTLV-I–transformed ATL-2 cells (197, 198). Two years later in 1989, this ADF was cloned as a human TRX, which is present in the cytosolic compartment of the cells (hereafter we call it Trx-1) (170). Trx-1 is a small (12 kDa) multifunctional ubiquitous redox-active protein, consisting of 105 amino acids, although the Trx-1 largely present in the human body consists of 104 amino acids (67, 68). During its translational process, the first N-terminal methionine is mostly removed by methionine excision (132). Trx-1 has two redox-active cysteine residues in

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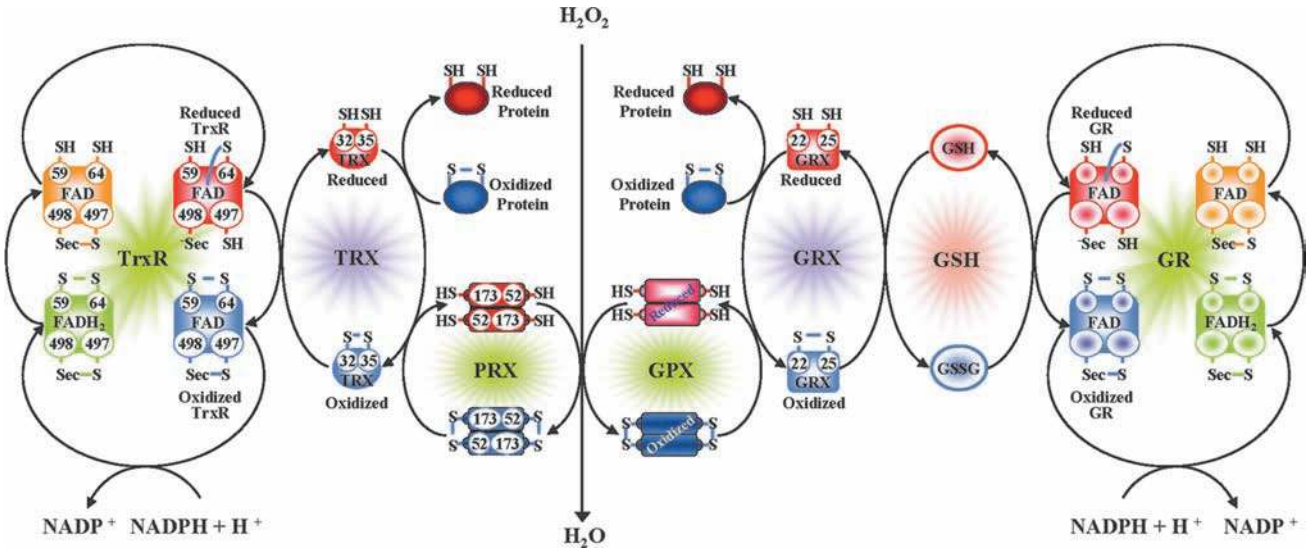

FIG. 1. Thioredoxin and glutaredoxin systems. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

its conserved active-site sequence:-Cys32-Gly-Pro-Cys35-. The active site of Trx-1 was discovered in 1968, and Trx-1 was shown to be a general protein disulfide reductase together with NADPH and TRX reductase 1 (TrxR1), which are present in all living cells (67). In addition to these two cysteine residues, human Trx-1 has three additional cysteine residues, Cys-62, Cys-69, and Cys-73, which are absent in *Escherichia coli* and also are absent in the mammalian mitochondrial thioredoxin, thioredoxin-2 (Trx-2). These extra cysteine residues constitute disulfide forms of Trx-1, but rarely form dimers and multimers, depending on the grade of oxidation of the protein (45, 53, 84, 188).

Conversely, GRX was first discovered in 1976 as a glutathione-dependent electron donor for RNR, which restored the growth of a Trx-1 mutant *Escherichia coli* (63–65). Functionally, TRXs and GRXs share a number of common features; but compared with TRXs, GRXs are more versatile with respect of the choice of substrate and reaction mechanism. Similar to TRXs, a -Cys-Pro-Tyr-Cys- active-site sequence also is present in the dithiol GRXs; however, in monothiol GRXs, the C-terminal cysteine residue is replaced by a serine, making -Cys-Gly-Phe-Ser- the active-site motif. This became the basis for classification of GRXs (Fig. 2) (103). GRXs use the reducing power of glutathione to catalyze the reduction of protein disulfides by a dithiol mechanism, or the reduction of mixed GS-S protein disulfides through a monothiol mechanism (36, 103). Unlike TRXs, GRXs have a stronger affinity toward the mixed disulfides. To reduce a disulfide, both active-site cysteines are required (the dithiol mechanism), and for the monothiol mechanism involving GSH-mixed disulfides, only the N-terminal cysteine is necessary.

Peroxiredoxin (PRX) is a relatively newer candidate in the redox field. The absence of catalase in the mitochondria may render the organelle most vulnerable to oxidative stress, raising the possibility of having another important and potent enzyme in cellular oxidative stress reduction. PRX was initially named a thiol-specific antioxidant, in which thiol is necessary as an electron donor (85, 151). Soon the name was modified to thioredoxin peroxidase for its dependence on TRXs (19). Later, it was identified as an electron receiver from GRXs (152, 184). Hence, it finally was renamed as peroxiredoxin (PRX), in which the key component for the antioxidant defense mechanism involves a reactive cysteine residue in the conserved active-site sequence. Reversible oxidation of a reactive cysteine residue to a sulfinic acid (Cys-SOH) derivative with a high affinity for H_2O_2 is the pioneer step that would be followed by disulfide formation after reaction with a thiol (148, 149).

Thioredoxin-Glutaredoxin Family Proteins, Their Related Molecules, and Their Distribution in Cells

Thioredoxin-glutaredoxin family proteins in cells

In the three-dimensional structure of *Escherichia coli*, a fold of TRX was discovered in 1975, defined as a TRX fold (69). Several proteins share this fold of TRX and have structural similarity; they are called the TRX superfamily or TRX family proteins (Fig. 3). Human Trx-1 is a 12-kDa cytosolic protein present in all living cells and a classic member of the TRX family proteins, probably the most intensively studied in mammals. In addition to redox regulation, they play several important roles in signal transduction, cell growth, and apoptosis (134). After certain stimuli, Trx-1 is translocated in the nucleus (11, 52, 79). In contrast, Trx-2 is an 12.2-kDa (immature protein, 18.2 kDa) mitochondria-specific TRX family protein, and plays a crucial role in the regulation of programmed cell death by inhibiting cytochrome *c* release from the mitochondria and by increasing membrane potential in the mitochondria (27, 167, 173). Trx-2 is also an essential protein in mammals, like Trx-1, and together they cause embryonic lethality if they are deficient (114, 138). They are 2 kDa larger than Trx-1; another cytosolic TRX was found, known as TRX-related protein of 14 kDa (TRP14), which reactivates PTEN (78, 99). A 32-kDa cytosolic protein conserved the N-terminal TRX active-site sequence and has 99% homology with Trx-1, the TRX-related protein 32 (TRP32)/TRX-like protein 1 (Txl-1) (98). TRX-like protein 2 (Txl-2) is a unique member of TRX-family proteins, associated with microtubules of lung airway

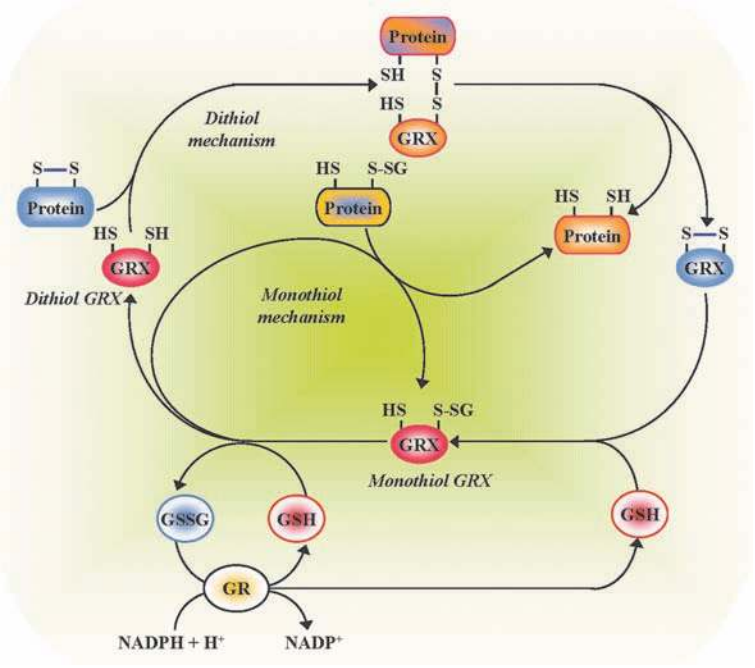

FIG. 2. Monothiol-dithiol redox mechanism of glutaredoxin systems. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

epithelial cilia and the manchette and axoneme of spermatids (155). Posttranslational modification was reported in the case of Trx-1, and this modification developed C-terminal truncated 10-kDa thioredoxin (TRX80). TRX80 consists of N-terminal 80/84 amino acids, which are secreted extracellularly and stimulate proliferation of monocytes in peripheral blood mononuclear cells (PBMCs) (144). In response to oxidative stress, Trx-1 becomes oxidized and then released or secreted extracellularly, although it has no secretory signal peptide like macrophage migration inhibitory factor (MIF). MIF, which is a 12-kDa protein, acts as an inflammatory cytokine and also is a member of TRX-family proteins (87, 90). Another member of TRX-family proteins is nucleoredoxin (NRX), a 48-kDa molecular-mass protein located in the nucleus, which regulates transcription factors (59, 93). In the endoplasmic reticulum, a large number of TRX-family members are present, including protein disulfide isomerase (PDI), calcium-binding protein 1 (CaBP1), ERp72, ERdj5/JPDI, and TRX-related transmembrane protein (TMX), which are involved in the maintenance of protein structure as molecular chaperones (25, 71, 91, 115, 146, 164). NRX also regulates the redox state of the nuclear proteins, such as transcription factors NF-κB, activation protein-1 (AP-1), and cyclic-AMP response element–binding (CREB) protein (59, 93, 96).

Glutaredoxin (GRX) is a glutathione-dependent TRX-family protein, which has four isoforms in humans, Grx-1, Grx-2, Grx-3 (PICOT), and Grx-5 (55, 103). The Grx-1 is 12-kDa cytosolic protein, which is most well characterized in the mammalian GRXs. Although it is present mainly in the cytosol, under certain stimuli, it can be translocated into the nucleus (Fig. 3) (8). Recently, Pai *et al.* (140) documented the existence of Grx-1 in the mitochondrial intermembrane space (140). Grx-1 also was detected in the plasma and sputum of healthy donors as well as in patients with cardiovascular and respiratory diseases (109, 135, 145). Mammalian mitochondrial Grx-2 was discovered in 2001 (48, 110). The molecular mass of the Grx-2 is 16 kDa, and it has a -Cys-Ser-Tyr-Cys- active-site motif. Only 34% sequence homology exists between Grx-1 and Grx-2. In an early study, two splice variants were reported in the Grx-2: Grx-2a, containing a mitochondrial signal peptide on the first exon (exon-I), which is located in the mitochondria; and Grx-2b, with an alternative exon-I, which is located in the perinuclear region, although a nuclear localization also was proposed (48, 110). Another recent study showed a third existing splicing variant of Grx-2: Grx-2c, in addition to Grx-2a and Grx-2b (107). Grx-2a is ubiquitously expressed in cells, and Grx-2b and Grx-2c are located in both the nucleus and the cytosol. In the scanned tissues, Grx-2b and Grx-2c were found exclusively in testis and some cancer cell lines (Fig. 3). More recently, another transcript variant, Grx-2d, was identified in mice; it appears to be mouse specific (72). The mammalian Grx-3, also named PICOT (protein kinase C–interacting cousin of thioredoxin), was discovered in 2000 with a yeast two-hybrid screening system as a binding partner of PKCθ (191). Grx-3 was described as a 37.5-kDa protein existing in the cytosol. Grx-3 possesses an N-terminal TRX homologue domain followed by two tandem GRX domains with a -Cys-Gly-Phe-Ser- active-site motif; this evolutionarily conserved domain is called the PICOT homology domain (75, 191). The N-terminal TRX homologue domain lacks the conserved -Cys-Gly-Pro-Cys- active-site motif. Instead, it contains an -Ala-Pro-Gln-Cys- motif; this domain is responsible for the PKCθ binding (55, 103, 191). The Grx-5 is a single-domain monothiol GRX homolog of yeast-Grx-5, possessing a -Cys-Gly-Phe-Ser- active-site motif. Human Grx-5 contains a mitochondrial targeting sequence, and it is located in the mitochondria (Fig. 3). A mouse homologue of Grx-5 was found in the mitochondria (124).

Other tissue-specific TRX-family proteins are SpTrx-1 (53 kDa), SpTrx-2 (67 kDa), and SpTrx-3 (15 kDa), which are present in the spermatocytes/spermatid and play a crucial role in the regulation of spermatogenesis process in the testis (80, 118, 154). A member protein of TRX named PC-TRP is present in plasma cells (193). The mitochondrial SP-22 (22 kDa) is a TRX-dependent peroxide reductase and another member of the TRX super family (57, 187).

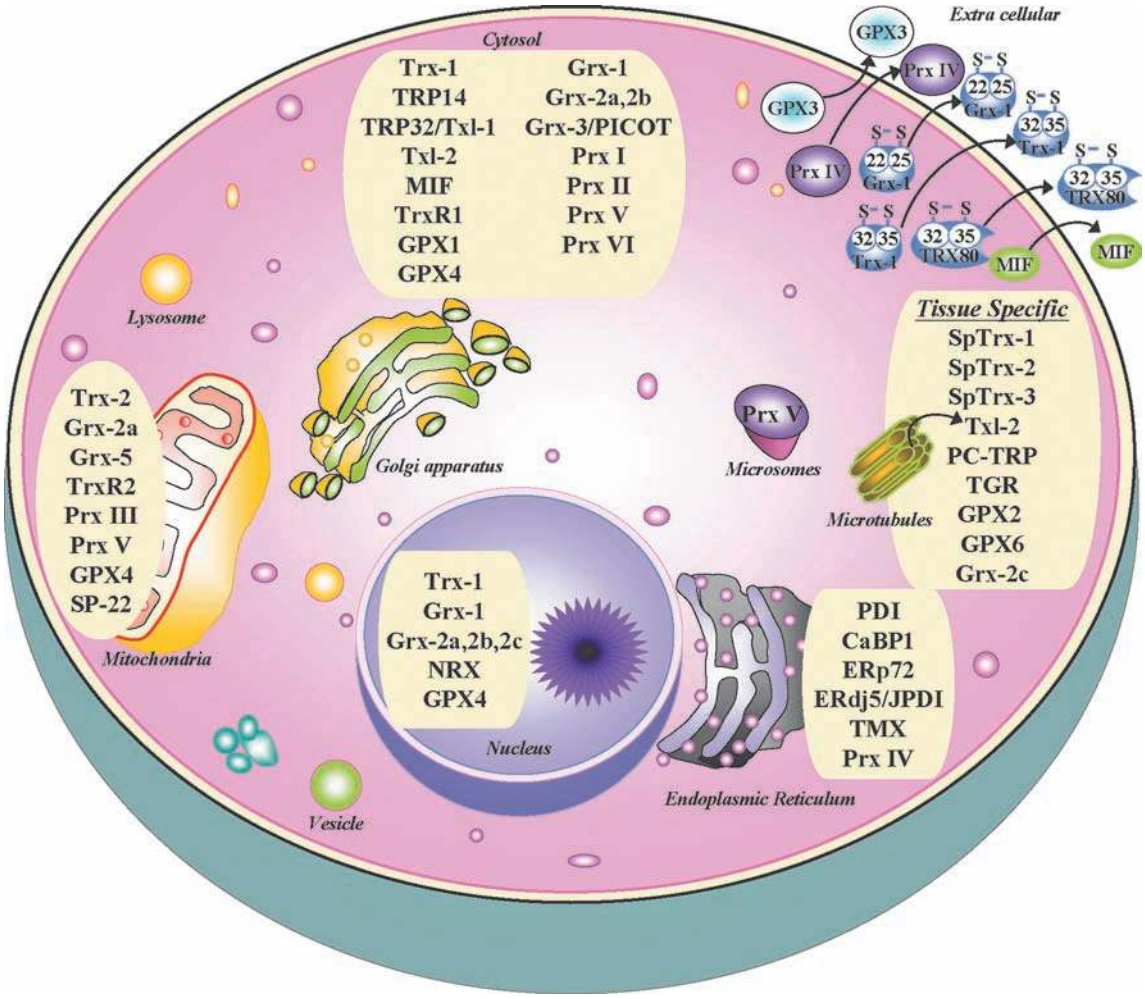

FIG. 3. Cellular distribution of thioredoxin-glutaredoxin family proteins. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

Thioredoxin-glutaredoxin–related molecules in cells

Thioredoxin reductases (TrxRs) are the homodimer form of flavoenzymes, which have three isoforms: cytosolic TrxR1, mitochondria-specific TrxR2, and the testis-specific TGR (Fig. 3) (119, 153, 206). The cytosolic human TrxR1 and rat TrxR1 are highly homologous to glutathione reductase (GR) (44, 207). TrxRs have two active-site sequence motifs; the N-terminal one is -Cys^{59}-Val-Asn-Val-Gly-Cys^{64}-, which is identical to the redox-active disulfide of glutathione reductase (GR). The C-terminal motif is -Gly-Cys^{497}-SeCys^{498}-Gly-, which contains an essential selenocyteine (Sec) residue. The Sec residue catalyzes the function of TrxRs, and deficiency of selenium from the Sec leads to a major loss of redox activity of TrxRs (10, 43, 206, 207). TrxRs are involved in the reduction of oxidized TRXs, and GR is involved in the reduction of GRXs, as well as glutathione disulfide (GSSG) (Fig. 1). Mammalian TrxRs have several splicing variants and react not only with TRXs but also with a wide range of substrates, including selenite, lipid hydroperoxides, H_2O_2, lipoamide, and alloxan (28, 92, 168, 169, 208).

Peroxiredoxins (PRXs) are heterogeneous and TRX-dependent peroxidases. They have six isoforms. According to their number of active cysteines and positional variation, PRXs are divided into three main groups: (a) typical 2-Cys PRXs, (b) atypical 2-Cys PRXs, and (c) 1-Cys PRXs (149, 150, 160). Prx I, II, III, and IV are typical 2-Cys PRXs, containing conserved Cys residues at both N-terminal and C-terminal, and requiring both of them for their catalytic function. Prx I and Prx II are present in the cytosol, whereas Prx III is present in the mitochondria (Fig. 3). Prx IV is a cytosolic PRX and is secreted from the cells. Prx V is an atypical 2-Cys member and has only the N-terminal Cys, but it needs one additional nonconserved Cys residue for catalytic activities. Prx V also exists in mitochondria and microsomes. In contrast, Prx VI is a sole member of 1-Cys PRX and contains only the N-terminal cysteine that uses glutathione as its electron donor instead of thioredoxin (37, 112). In addition to glutathione peroxidases (GPXs), PRXs scavenge hydrogen peroxide (H_2O_2) (Fig. 1). GPXs have also a selenium (Se) and, depending on the selenium, GPXs have five isoforms in the human (15). GPX1 is a cytosolic GPX and major scavenger of H_2O_2 as well as lipid hydroperoxides. GPX2 is a gastrointestinal tract epithelial cell–specific GPX, whereas GPX3 is secreted from the cell and is present in plasma. Interestingly, GPX3 can take electrons from TRXs, GRXs, as well as directly from GSH (179). GPX4 is present in cytosol and mitochondria as well as in the nucleus by its alternative splicing variants and prevents membrane phospholipid oxi-

dation. GPX6 is a newly found GPX, and it is also tissue specific to olfactory mucosa and embryonic tissue (15, 141).

In this review, we focus mainly on TRXs (Trx-1 and Trx-2), GRXs (Grx-1 and Grx-2), and PRXs, concentrating in the areas related to myocardial cell survival and cardiovascular diseases and their diagnosis, including possible treatment plans.

Thioredoxin Superfamily in the Heart

Thioredoxins (TRXs) have been implicated in a large number of cardiovascular diseases, including ischemic heart disease, cardiac hypertrophy, diabetes, obesity, atherosclerosis, and hypertension. Plasma TRX levels are either elevated or depressed in the pathologic heart. A brief description of the role of TRXs in the diseased heart is furnished later.

Ischemic heart diseases

The TRX system regulates the postischemic redox imbalance elicited by oxidative stress, contributing to the pathogenic remodeling of the K^+ channel, which underlies arrhythmogenesis and contractile dysfunction in the falling heart (101). The chronic intermittent hypoxia reduces ischemia/reperfusion–induced myocardial injury by enhancing the expression level of Trx-1. However, the short-term intermittent hypoxia enhances myocardial injury, whereas the expression level of Trx-1 is minimal (142). The same study shows that ischemia/reperfusion–induced myocardial injury is enhanced by the inhibition TrxR1, suggesting that postischemic injury is regulated by alteration of Trx-1 levels (142). Plasma levels of Trx-1 of the patients undergoing open-heart surgery are significantly reduced (135). *Euryale ferox*, a ROS scavenger, exerts its postischemic cardioprotective effect by enhancing the expression level of Trx-1 and TRP32 (29). The mitochondrial protein SP-22 exerts its cardioprotective effect in rat heart as an antioxidant molecule (5). However, the higher expression level of serum or plasma Trx-1 is indicative of a risk factor for the patients with acute myocardial infarction (AMI), in which Trx-1 could enhance platelet aggregability and reduce the left ventricular ejection fraction (123, 165).

Cardiac hypertrophy

Higher levels of ROS and ROS-induced cardiomyocyte apoptosis play an important role in developing cardiac hypertrophy. Trx-1 attenuates cardiac hypertrophy not only by scavenging ROS but also by regulating the protein expression in the signal-transduction pathway (3). Trx-1 attenuates cardiac hypertrophy by upregulating DnaJb5 and heat-shock protein 40 and by forming multiple protein complexes with DnaJb5 and class II histone deacetylases (HDACs) (2). Apoptosis signal-regulating kinase 1 (ASK1) is involved in cardiac apoptosis, leading to cardiac remodeling and hypertrophy. Both cardiac hypertrophy and fibrosis are associated with the downregulation of Trx-1 and the upregulation of ASK1/caspase signaling in the menopausal mouse model (33). Trx-1 and Trx-2 directly bind with the N-terminal regulatory domain of ASK1 and inhibit the ASK1-induced cardiac apoptosis as well as cardiac hypertrophy (137). The antioxidative properties of estrogen prevent congestive heart failure by inhibiting ASK1 through upregulation of Trx-1 and TrxR1 (158). Trx-1 inhibits cardiac hypertrophy by enhancing mitochondrial functions through the upregulation of mitochondrial proteins PGC-1α and nuclear respiratory factors (NRFs) (4). PICOT/Grx-3 attenuates cardiac hypertrophy by inhibiting hypertrophic signal transduction and by disrupting calcineurin-NFAT signaling by dissociating muscle LIM protein (MLP)–calcineurin interaction (76, 77, 157). α-Adrenergic receptor–stimulated myocardial hypertrophy in the adult rat is mediated through Trx-1–sensitive posttranslational oxidative modification of thiols on the Ras-Raf-MEK1/2-ERK1/2 signaling pathway (94).

Diabetes

Diabetes is associated with increased oxidative stress and inflammation. Chronic diabetic hyperglycemia can cause excessive oxidative stress, leading to cardiac complications, including hypertension, left ventricular hypertrophy, dilated cardiomyopathy, and myocardial infarction. Diabetic cardiomyopathy causes hyperglycemia-induced left ventricular dysfunction, leading to myocardial apoptosis, cardiac hypertrophy, and fibrosis, mediated by hyperactivity of ASK1. The 14-3-3 protein regulates ASK1 signaling and protects the heart from diabetic cardiomyopathy by upregulating the activity of TrxR1 (177). Resveratrol exerts its potent cardioprotective effect on streptozotocin-induced diabetic cardiomyopathy through upregulation of Trx-1, HO-1, and NO (178). The activities of the antioxidant system TRX and GRX are downregulated in streptozotocin-induced diabetic rat heart (102), indicating that TRX superfamily members are critically involved in diabetes-induced myocardial complications.

Atherosclerosis and hypertension

Diabetes-induced oxidative stress and oxidative stress–mediated formation of oxidized LDL and the activation of monocytes and macrophages are closely involved in the pathogenesis of atherosclerosis, leading to narrowing of vessel structure that finally develops hypertension. Hyperhomocysteinemia impairs Trx-1 function, leading to increased ROS production and secretion of MCP-1 from macrophages in the $ApoE^{-/-}$ mice and accelerates the atherogenesis process (26). Uptake of oxidized LDL by macrophages upregulates the expression of the TRX and GRX systems, indicating that the cellular defense mechanism against oxidized LDL is mediated through the TRXs and GRXs systems (49, 139).

Thioredoxins in the Vascular Smooth Muscle and Endothelial Cells

TRX superfamily in smooth muscle cells

Redox regulation by Trx-1 is critically important for the prevention of mechanical stress–induced cardiac hypertrophy or cardiac smooth muscle cells (CSMCs) growth. Overexpression of Trx-1 increases DNA synthesis in the CSMCs, suggesting the growth-promoting role of Trx-1 (159). The mechanical pressure overload–induced upregulation of Trx-1 enhances the proliferation of CSMCs in the myocardium and cardiac hypertrophy. An endogenous negative regulator of Trx-1, Trx-1–binding protein-2 (TBP-2), also known as Trx-1–interacting protein (TXNIP) or vitamin D_3 upregulated protein 1 (VDUP1), prevented smooth muscle cell growth by inhibition of functional activity of Trx-1, indicating critical regulation of biomechanical signaling of Trx-1 by TBP-2 (201). The endogenous Trx-1 is an essential component for the myocardial antioxidant system and plays a vital role in regulation of oxidative-stress homeostasis in the myocardium. A

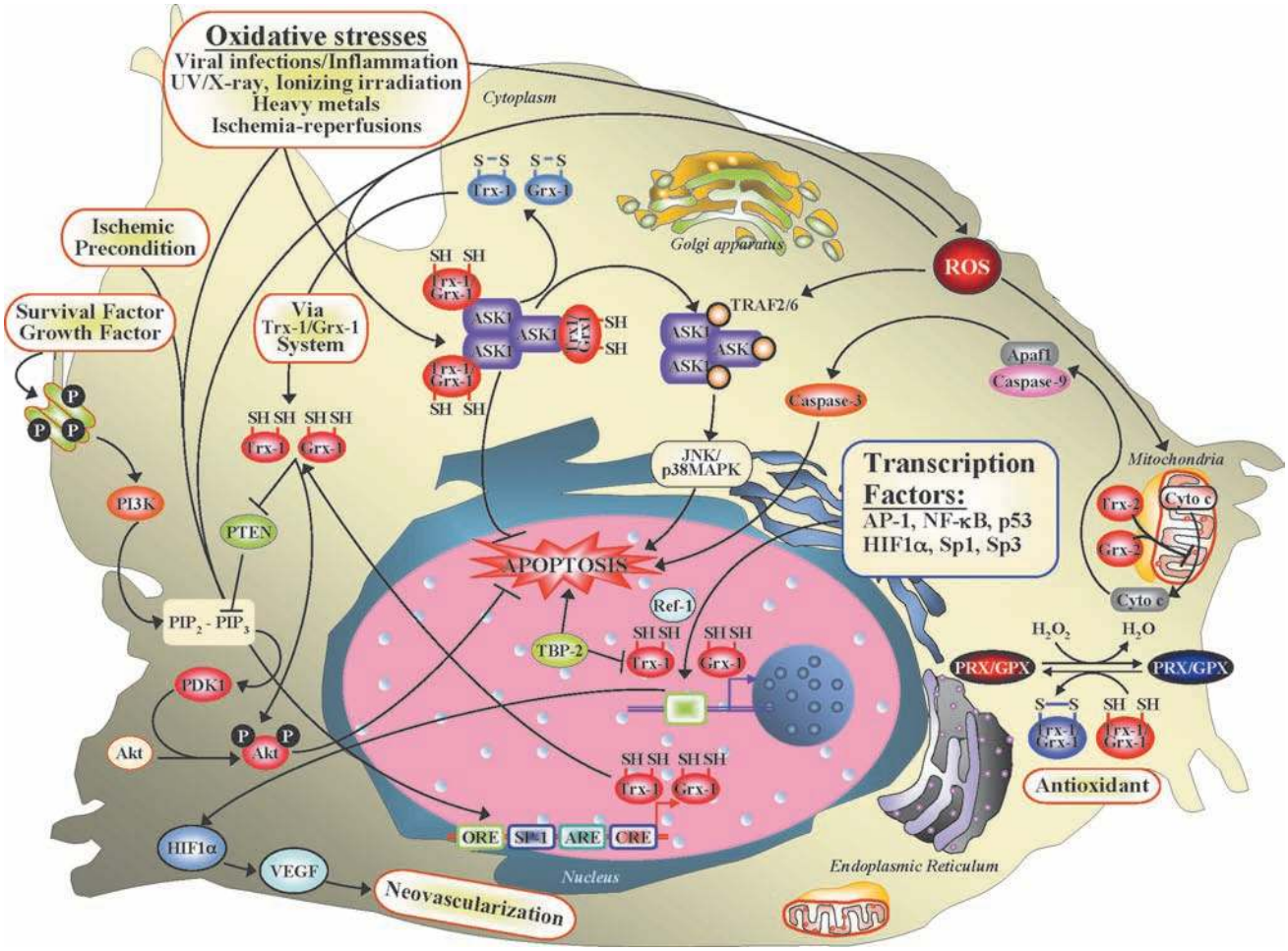

FIG. 4. Redox regulation by thioredoxin-glutaredoxin family proteins in myocardial cells. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

transgene of the dominant-negative double mutant (C32S/C35S) of Trx-1 enhances accumulation of markers of oxidative stress and causes simultaneous hypertrophy in the basal condition or pressure overload or both through the redox-sensitive mechanisms (194).

TRX superfamily in vascular endothelial cells

Vascular endothelial–specific overexpression of a member of TRX superfamily, Trx-2, plays a crucial role in preserving the proper function of endothelium to protect atherosclerotic lesions by reducing oxidative stress and by increasing nitric oxide (NO) (203). The study showed that Trx-2 improved endothelial cell function and reduced atherosclerotic lesions in the apolipoprotein-E–deficient mouse. In another study, Trx-1 stimulated endothelial cell growth and vascular smooth muscle cell migration through the activation of ERK1/2 (192). A significant number of articles exist in the literature showing that mitochondrial ROS significantly contribute to endothelial cell dysfunction and the progression of atherosclerosis, and mitochondrial Trx-2 plays a role in this process (7, 143). A cysteine-modified mutant of recombinant human Trx-1 (rhTrx-1-C35S) was found to bind to human umbilical vein endothelial cells (HUVECs) and to enter these cells *via* lipid rafts (51). The same study showed that endogenous Trx-1 was expressed on the surface of HUVECs, including lipid rafts (51). In another related study, overexpression of Trx-1 prevented NO-induced reduction of NO synthase activity in lung endothelial cells (204).

Redox Regulation by Thioredoxin-Glutaredoxin and Related Molecules in Myocardial Cell Survival

Thioredoxin and related molecules as biomarkers of cardiovascular diseases

Trx-1 is one of the major redox-sensing proteins, which is ubiquitously expressed in all organs including those of the cardiovascular system. The promoter region of the *Trx-1* gene showed several responsive elements, such as oxidative responsive element (ORE), SP-1, antioxidant-responsive element (ARE), and cyclic AMP–responsive element (CRE), induced by various types of oxidative stresses including reactive oxygen species (ROS), as well as ischemia/reperfusion and preconditioning (Fig. 4) (28, 113, 134). Both types of TRXs are dominantly present in the myocardial cells: Trx-1 is localized in both cytosol and nucleus, and Trx-2, in the mitochondria, both of them taking part in the redox reaction. Reduced TRXs form dithiol (-SH, -SH), and oxidized TRXs form disulfide (S-S) bonds in the active site. In the cytoplasm, reduced Trx-1 gives electrons to the disulfide bond of the oxidized target protein to reduced it, whereas Trx-1 itself becomes

oxidized. Oxidized Trx-1 reversibly changes to the reduced form by TrxR1 and NADPH (13, 62, 66). Trx-1–dependent peroxiredoxin (PRX) scavenges hydrogen peroxide (Fig. 1). Thus, cytoplasmic Trx-1 also possesses an antioxidant effect together with NADPH, TrxR1, and PRX (Prx I and Prx II). Similarly, mitochondrial Trx-2 also shows an antioxidant effect together with NADPH, TrxR2, and Prx III, and it is strongly expressed in heart tissue (39, 173). Trx-1 and Trx-2 show redox-regulatory functions in signal transduction of myocardial cell survival and prevent apoptosis. Apoptosis signal–regulating kinase 1 (ASK1) is a mitogen-activated protein kinase kinase kinase (MAPKKK), which is associated with the reduced form of Trx-1 in cytosol and Trx-2 in mitochondria in the normal state of a cell to prevent cellular apoptosis (156). During oxidative stress–induced apoptosis in the cells, TRXs become oxidized, followed by the dissociation of ASK1 from TRXs, and then ASK1 interacts with TRAF2/6, inducing the phosphorylation of JNK and p38 MAPK to transduce the apoptosis signal (Fig. 4) (38, 205). TRXs also interfere with the Ras/Raf/ERK pathway to inhibit apoptosis (94, 95). Reduced Trx-1 also inhibits apoptosis by regulating or preserving phosphorylation status of Akt, or both, by inhibiting PTEN, which is known as a PI3K-Akt pathway inhibitor or by upregulation of survivin expression (83, 99, 172). Trx-1 is known to be involved in redox regulation of a numbers of transcriptional factors, including Ref-1, AP-1, NF-κB, HIF-1α, Sp1, and Sp3. During redox regulation, the promoter of Trx-1 binds the Sp1 and Sp3 to enhance the expression of Trx-1 (14). The DNA binding of AP-1, NF-κB, p53, and HIF1α is regulated by Trx-1– or Ref-1–dependent reduction of their key cysteine residues, or both (34, 58, 60). DNA binding of NF-κB regulates several gene expressions in response to inflammatory cytokines, infections, ROS, carcinogenesis, cellular stress, and apoptosis inducers (47, 189). Transactivation of p53 causes upregulation of p21 expression in the cells transiently transfected with Trx-1 (181). The glucocorticoid receptor is a transcription factor, the activity of which also is regulated by Trx-1 (117). The mitochondrial Trx-2 (12.2 kDa) plays a crucial role in the regulation of programmed cell death by inhibition of cytochrome *c* release from the mitochondria and by increasing mitochondrial membrane potential, leading to the inhibition of the caspase-mediated apoptosis pathway (Fig. 4) (27, 173). Trx-1 enhances cardioprotection by postmyocardial neovascularization through the Trx-1–HO-1–VEGF pathway (82).

Several lines of evidence indicate that Trx-1 reduces myocardial injury by reducing both oxidative and nitrative stress. For example, treatment of heat cells with Trx-1 significantly reduced myocardial apoptosis and upregulated MnSOD (176). In this study, Trx-1 also reduced peroxynitrite donor SIN-1 (3-morpholinosydnonimine)-induced cardiomyocyte apoptosis. In another study, high glucose was found to sensitize cardiomyocytes to ischemia/reperfusion injury due to nitrative inactivation of Trx-1 (108). The treatment of cardiomyocytes with *rh*Trx-1 exerts its cardioprotective effect by suppressing the ischemia/reperfusion-induced nitrative stress (176).

Although the function of extracellular TRXs or recombinant human Trx-1 (*rhTrx-1*) is distinct from that of redox-regulating function of TRXs and largely unclarified, it may play an important role in the protection of myocardial cells through its antiinflammatory function, which is mediated by its combined effects: (a) antichemotaxis effect (131, 136); (b) MIF-inhibitory effect (90,171); (c) antiadhesion effect (51, 89); and (d) anti-transmigration effect through suppression of complement activation (74), illustrated in Fig. 5.

Redox regulation by glutaredoxin in myocardial cell survival

Similar to TRXs, Oxidized GRXs are reduced by GSH, glutathione reductase (GR), and NADPH (Figs. 1 and 2). In normal conditions, glutaredoxin catalyzes the reduction of

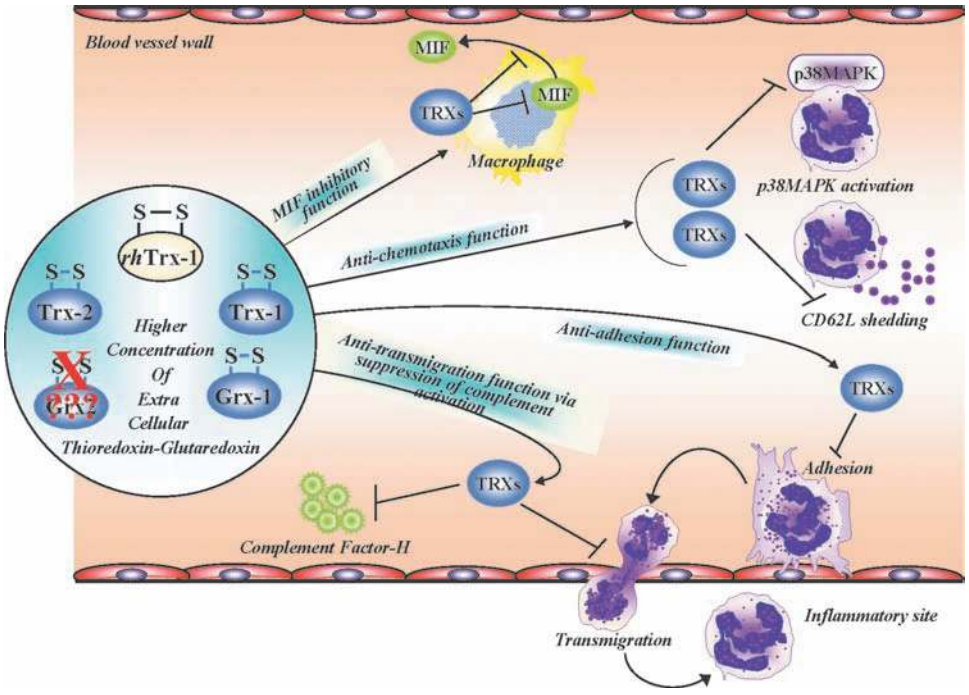

FIG. 5. Antiinflammatory function of recombinant or circulatory human thioredoxin-glutaredoxin family proteins. Those are maximally oxidized proteins lacking antioxidant function. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

some protein disulfides and GSH-mixed disulfides, but in certain conditions, also can catalyze the reverse reaction, leading to GS-S-protein mixed disulfide formation (23, 41, 161).

Like Trx-1, Grx-1 also was shown to bind and negatively to regulate the activation of ASK1, and the overexpression of Grx1 represses the ASK1/SEK1/JNK1 pathway and prevents cytotoxicity induced by metabolic oxidative stress in a glutathione-dependent manner (Fig. 4) (166). Overexpression of Grx1 maintains the redox status of Akt, facilitates cell survival, and suppresses apoptosis (127). 17/β Estrogen (E2) induces Grx1 and protects H9c2 from apoptosis by the same Akt pathway (182). Conversely, Grx1 can regulate the Ras/Raf/Erk pathway as a response to hypertrophic stimuli. The *S*-glutathionylation as a response to hypertrophic stimuli of Ras causes activation of the Raf/Mek/Erk pathway, leading to hypertrophy. Overexpression of Grx-1 inhibited the *S*-glutathionylation of the Ras, repressed the activation of the Erk, and suppressed the increased protein synthesis induced by strain stimulation (147). Grx-1 also suppresses the activation of p38 induced by angiotensin II (1).

Grx-2 plays a central role in the maintenance of the mitochondrial redox environment. Depending on the ratio of the GSH to GSSG, Grx-2 can catalyze glutathionylation and deglutathionylation of the protein. Unlike Grx-1, Grx-2 is a substrate for mitochondrial TrxR2. During oxidative stress, when the GSH/GSSG ratio is decreased, this alternative reducing mechanism becomes operative, suggesting an important role for Grx-2 (81). Grx-2 regulates the activity of mitochondrial proteins like complex I by reversible *S*-glutathionylation (9). Human Grx-2 is the first member of TRX superfamily characterized as an iron–sulfur protein. Grx-2 forms a (2Fe-2S) bridged dimer, which is enzymatically inactive, but the (2Fe 2S) cluster serves as a redox sensor for Grx-2 (12, 103, 104). Overexpression of Grx-2 protects the cells against apoptosis induced by 2-deoxy-glucose and doxorubicin, by inhibiting the release of cytochrome *c* as well the activation of caspase 3, and the loss of cardiolipin (Fig. 4) (35, 105). Furthermore, overexpression of Grx-2 promotes the activation of survival protein Akt, redox-sensitive transcription factor NF-κB, and antiapoptotic Bcl2 (129).

Witte *et al.* (191) reported that the overexpression of Grx-3 in T cells inhibited the activation of AP-1 and NF-κB in a dose-dependent manner (191). In response to oxidative stress, Grx-3/PICOT is translocated into the nucleus (6). Cardiac-specific overexpression of PICOT diminished the hypertrophic response of the hearts after pressure overload. PICOT was found to enhance the sensitivity of the myofilaments toward Ca^{2+}, and increased the Ca^{2+} reuptake by SR. Excessive PICOT transfection repressed the activation of PKCα, PKCε, and PKCζ after hypertrophic insults, whereas the activation ERK and JNK was decreased (76). Direct interaction between the PICOT GRX domain (PICOD-HD) and muscle LIM protein (MLP) has been identified. PICOT competes with calcineurin for binding to MLP in a dose-dependent manner. Overexpressed PICOT extruded the calcineurin from binding with MLP, and this inhibited the PE-induced increased phosphatase activity of calcineurin, as well the binding, dephosphorylation, and nuclear translocation of NFAT with calcineurin (18, 76, 77). Grx-5 deficiency leads to inappropriate Fe-S cluster synthesis and further deflects the heme synthesis. Grx-5 deficiency in humans causes anemia with iron overload (17, 190). All of these studies suggested that redox regulation by GRXs plays an essential role in myocardial cell survival.

Redox regulation by peroxiredoxin in myocardial cell survival

Peroxiredoxin (PRX) is a potent antioxidant that scavenges H_2O_2 and produces by-product H_2O with the help of reduced TRX (Fig. 4). Prx I is present in both cytosol and nuclear compartments of a cell. Cytosolic Prx I inhibits NF-κB p50 activation and prevents nuclear translocation. However, nuclear Prx I does not affect the nuclear translocation but promotes the activity of NF-κB receptor (50). Decreased Prx I is associated with increased ROS production, leading to p53 activation, which regulates apoptosis through the activation of the caspase-mediated pathway (185). Overexpression of Prx I also is associated with increased cell proliferation, indicating that overexpression of Prx I prevents apoptosis by scavenging ROS (73, 125). PRX also scavenges/reduces peroxynitrite, acting as a peroxynitrite reductase (183). Prx II regulates inflammatory responses through the NF-κB and MAP kinase pathways, and thereby controls the macrophage response to pro-inflammatory stimuli, like LPS and cytokines (195). Prx III is the mitochondrial isoform of the PRX family, which serves as the first line of defense against ROS generation and subsequent myocardial cell damage (21). *In vivo* transfer of the Prx III gene prevents cell death induced by oxidative stress (54). Depletion of Prx III results in increased intracellular H_2O_2, which makes the cells prone to apoptosis by TNF-α or staurosporine (21). Prx IV regulates the expression of the TP-β receptor and prevents oxidative stress (46). Prx V also prevents p53-dependent generation of ROS and p53-induced apoptosis (209). These two mechanisms are involved in inactivation of all PRXs, phosphorylation of threonine-90 residue by the cyclin-dependant kinase cdc2, and hyperoxidation of cysteine residues of the active-site sequence (21, 196). These studies suggest that redox regulation by PRXs is an essential factor for myocardial cell survival against ROS and ROS-induced apoptosis.

Thioredoxin-Glutaredoxin and Related Molecules as Biomarkers of Cardiovascular Diseases

Thioredoxin and related molecules as biomarkers of cardiovascular diseases

Cardiovascular diseases are among the major causes of death all over the world and involve oxidative stress. Oxidative stress is deeply involved in atherosclerotic plaque formation and uptake of oxidized LDL (oxLDL) by macrophages and leads to induction of Trx-1 (139). Higher oxLDL levels in serum are associated with higher serum levels of Trx1, indicating its absence as a risk factor of coronary atherosclerotic diseases (Ahsan M.K. *et al.*, unpublished data). TrxR1 was also induced in atherosclerotic plaques (40). Plasma Trx-1 is elevated during reperfusion of the post-cardioplegic heart because of systemic oxidative stress (133). Serum Trx-1 levels are elevated in the patients with acute coronary syndrome and dilated cardiomyopathy but not in stable angina patients (86, 163). The serum levels of Trx-1 are highly elevated in the acute phase but not in the chronic phase of patients with fulminant myocarditis, Trx-1 is expressed in

inflammatory cells as well as in cardiomyocytes in the biopsy samples of the left ventricle in patients with fulminant myocarditis (162). Plasma Trx-1 levels are elevated in patients with unstable angina, spastic angina, and acute myocardial infraction compared with those in stable exertional angina and chest-pain syndrome (61, 120, 165). Elevated Trx-1 levels in acute myocardial infraction are associated with hyperaggregation of platelet, which indicates further risk as well as pathogenesis of ischemic heart diseases (123). Highly elevated plasma or serum Trx-1 levels are reported in the patients with diabetes mellitus (DM), especially in DM type II as well as in patients with glucose intolerance (IGT) and patients with hypertension, hypercholesterolemia, and atherosclerosis, all of these being the major risk factors for cardiovascular diseases (121, 122, 139). Conversely, in spontaneous hypertensive rats, the Trx-1 expression is decreased in the aorta or in the aortic arch (174). In the heart, preconditioning-induced hormesis is involved in cGMP-dependent induction of redox protein Trx-1 and Trx-2, including MnSOD and heat-shock protein 70 (HSP70), leading to cardioprotection (22). Therefore, the postischemic heart disease–induced induction of Trx-1 and Trx-2 in serum might show a good adaptive response for the heart, although we do not have any direct evidence. Therefore, plasma or serum TRXs levels may be good markers to identify the risk factor or pathologic state or postadaptive signs of cardiovascular diseases.

Glutaredoxin and related molecules as biomarkers of cardiovascular diseases

Only a handful number of studies investigated the detection of extracellular levels of Grx-1. Nakamura *et al.* (135), through ELISA assays, found reduced plasma levels of Grx-1 in preoperative cardiac patients. Lundberg and colleagues (109) also used sandwich ELISA to determinate the cellular and plasma levels of Grx-1 and Grx-2; the Grx-2 level was not detectable in the plasma of healthy donors (109). The study revealed that the Grx-1 level was increased during cardiopulmonary bypass surgery, probably because of the increased hemolysis. This study also reported that peripheral blood mononuclear cells secrete Grx-1 without stimulation. A related study reported that the plasma levels of Grx-1 of the control patients (angiography group) were noted to be higher compared with those of the angiography group. However, this study could not find a correlation between the plasma level of Grx-1 before and after angioplasty (186). It was also reported that blood glutathione reductase (GR) was decreased in patients with myocardial infarction (32). Therefore, plasma or serum levels of GRXs and related molecules may also to be good markers to identify the risk factor or pathologic state or postadaptive response of cardiovascular diseases. Further studies must be conducted to investigate the extracellular level and the role and the source of GRXs and related molecules in diverse cardiovascular disease conditions compared with normal conditions to confirm that GRXs and the related molecules can be used as biomarkers of cardiovascular diseases.

Peroxiredoxin as a biomarker of cardiovascular diseases

A recent study shows that the plasma levels of oxidized Prx II and VI are increased in Alzheimer disease patient (200). Although oxidized Prx II and VI already are established as biomarkers of Alzheimer disease, in cardiovascular diseases, the role PRXs as biomarkers is yet to be confirmed. We may detect PRXs levels in plasma by using the assay kits in several cardiovascular disease states to establish PRXs as biomarkers of cardiovascular diseases. This hypothesis was further strengthened by Brixius *et al.* (16); they showed a selective downregulation of some PRX isoforms (Prx III to VI) in patients with dilated cardiomyopathy (16). Further studies might establish whether PRXs could be used as biomarkers of cardiovascular diseases.

Implication of Thioredoxin, Glutaredoxin, and Peroxiredoxin Gene Therapy in Cardiovascular Diseases

Gene delivery of thioredoxin

Since recombinant human Trx-1 (*rh*Trx-1) was shown to possess antioxidant, antiinflammatory, and antiapoptotic functions that demonstrated its cytoprotective effect, Trx-1 has became one of the better therapeutic agents for the diseases related to oxidative stress, acute inflammation, and apoptosis/necrosis. Most of the cardiovascular diseases are related to oxidative stress, acute inflammation, and apoptosis/necrosis (56, 70, 88, 100). Moreover, resveratrol, a drug for myocardial infarction (MI), improves neovascularization in the infarct myocardium, and temocapril, a cardiovascular-protective drug, exerts cytoprotective effect through upregulation of Trx-1 and Trx-2 expression (82, 202). In addition, an animal model shows that endogenous Trx-1 has a protective role in the ischemic heart (180). The Trx-2–transgenic mice model shows reduced ROS, increased nitric oxide (NO) bioavailability, leading to reduced vasoconstriction and increased vasodilatation, indicating that Trx-2 may have a beneficiary effect in hypertension. Trx-2 also improves EC function and reduces atherosclerotic lesions in the apolipoprotein E–deficient mouse model (203). Conversely, cardiac cell–specific ablation of TrxR2 results in fatal dilated cardiomyopathy and death shortly after birth in a mice model, indicating that TrxR2 as well as Trx-2 may have also beneficial effects in dilated cardiomyopathy (24). *S*-nitrosylation of *rh*Trx-1 shows more potential cardioprotective effects against ischemic hearts in a mouse model (175, 176). *rh*Trx-1 ameliorates myosin-induced autoimmune myocarditis in mice (106). All these reports suggest that TRXs might be good therapeutic agents for clinical application against cardiovascular diseases. However, developing the drug-delivery technique and its bioavailability is one of the important steps to making a successful treatment plan. The half-life of *rh*Trx-1 in plasma is roughly 1 h in mice, 2 h in rats, and 8 h in monkeys (130). Moreover, our research experiment shows that a continuous flow of *rh*Trx-1 is needed for the maximal therapeutic efficacy of TRXs. To elongate the half-life of TRXs in plasma, a possible modification has been considered: conjugation with polyethylene glycol (PEG), which bridges with cysteine residues. This modification is not recommended for clinical trial, because these cysteine residues of TRXs play a crucial role in redox regulation. Therefore, one of the best techniques is gene therapy or gene delivery to make a successful treatment plan by using these redox proteins in cardiovascular diseases. This study is continuing in our Cardiovascular Research Center.

Gene delivery of glutaredoxin

Overexpression of both Grx-1 and Grx-2 renders the heart resistant to ischemia/reperfusion injury (42, 111, 129). Our recent studies showed that several natural food products, like resveratrol or broccoli, induce Grx-1 and Grx-2 and protect the heart from ischemia/reperfusion injury (31, 126). Doxorubicin, a known anticancer drug, exerts cardiac cell damage during chemotherapy. Overexpression of Grx-2 ameliorates doxorubicin-induced cardiac cell damage as well as ventricular remodeling of the heart (30). These results suggest Grx-2 as a possible therapeutic candidate for the cancer patient who is undergoing treatment with doxorubicin. The Grx-3 negatively regulates left ventricular hypertrophy, suggesting a possible therapeutic role for Grx-3 in left ventricular hypertrophy (18, 76, 77). Moreover, Grx-1 gene therapy can prevent ischemia/reperfusion injury in the early phase in the diabetic mouse model (Lekli I and Das DK *et al*., unpublished data). Based on all these studies, we may assume that GRX overexpression could have therapeutic value in different heart diseases. Although we observed all these data after the overexpression of GRXs, like a preventive medicine, further study is needed to address the therapeutic value of GRXs as a modern medicine after the disease condition develops. The overexpression GRXs could be done by direct gene delivery.

Gene delivery of peroxiredoxin

As mentioned earlier, ROS-induced apoptosis/necrosis and inflammation are involved in pathogenesis of a majority of cardiovascular diseases. All the isoforms of PRXs are involved in scavenging ROS and prevent ROS-induced apoptosis/necrosis, thus indicating the possible therapeutic values of PRXs in cardiovascular diseases. Deficiency of Prx VI makes the heart more susceptible to ischemia/reperfusion–induced injury and leads to an increase in infarct size and apoptotic cell death, suggesting a protective role of Prx VI on ischemia/reperfusion injury as well as myocardial infarction (MI) (128). Moreover, overexpression of mitochondrial Prx III in another mouse model shows resistance to MI and prevents ventricular remodeling and post-MI heart failure (116). Thus, PRXs should also be a potent therapeutic agent for cardiovascular diseases.

Concluding Remarks

The review is focused on the vital cellular antioxidant defense mechanism comprising TRXs, GRXs, and PRXs, along with their isoforms and their effects on the myocardium. Ischemia/reperfusion-induced injury generates a huge amount of ROS. Uncontrolled increment of ROS brings turbulence to the cellular microenvironment that ultimately triggers apoptosis. Given the fact that modulation of ROS is of immense importance for the maintenance of cardiovascular health. Overexpression of the few isoforms of TRXs, GRXs, and PRXs have been shown to be beneficial for the stressed myocardium, which works in redox chain to scavenge the ROS. Added to that, all these proteins seem to have a role in antiinflammatory activities. All our summarized information suggests myocardial cell survival as an effect of TRXs-, GRXs-, and PRXs-mediated redox regulation. It appears reasonable to speculate that improve cardiac health is possible by overexpressing these proteins by a successful and clinically applicable gene therapy.

Acknowledgments

We thank Dr. Monika Das, Dr. Narasimman Gurusamy, Subhendu Mukherjee. and Jocelyn Dudley (all in the Department of Surgery, UCONN Health Center) for their critical reviews of the manuscript. This study was supported by grants-in-aid from the National Institutes of Health: NIH HL 34360, HL 22559, and HL 33889.

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Date of first submission to ARS Central, May 28, 2009; date of final revised submission, July 1, 2009; date of acceptance, July 7, 2009.

Abbreviations Used

ADF = ATL-derived factor
AP-1 = activation protein-1
ARE = antioxidant responsive element
ASK1 = apoptosis signal regulating kinase 1
ATL = adult T-cell leukemia
CaBP1 = calcium-binding protein 1
CD25 = inteleukin-2 receptor α-chain
CRE = cyclic-AMP responsive element
CREB = cyclic-AMP response element binding protein
CSMCs = cardiac smooth muscle cells
DM = diabetes mellitus
GPX = glutathione peroxidase
GR = glutathione reductase
GRP 78 = 78-kDa glucose regulated protein
GRP 94 = 94-kDa glucose regulated protein
GRX = glutaredoxin
GSH = glutathione
HDACs = histone deacetylases
HDL = high density lipoprotein
HIF1α = hypoxia inducing factor 1 alpha
HO-1 = heme oxygenase-1
HSP70 = heat shock protein 70
HTLV-I = human T-cell leukemia virus type-I
HUVECs = human umbilical vein endothelial cells
IGT = glucose intolerance
IL-2 = interleukin-2
LAD = left anterior descendent coronary artery
LDL = low density lipoprotein
LPS = lipopolysaccharide
MAPKKK = mitogen-activated protein kinase kinase
MCP-1 = monocyte chemoattractant protein-1
MEF = mouse embryonic fibroblast
MI = myocardial infarction
MIF = macrophage migration inhibitory factor
MLP = muscle LIM protein
NF-κB = nuclear factor kappa B
NO = nitric oxide
NRFs = nuclear respiratory factors
NRX = nucleoredoxin
ORE = oxidative responsive element

Abbreviations Used (cont.)

PBMC = peripheral blood mononuclear cells
PDGF = platelet derived growth factor
PDI = protein disulfide isomerase
PGC-1α = peroxisome proliferators-activated receptor gamma co-activator-1 alpha
PICOT = protein kinase C-interacting cousin of thioredoxin
PICOT-HD = protein kinase C-interacting cousin of thioredoxin glutaredoxin domain
PRX = peroxiredoxin
*rh*Trx-1 = recombinant human thioredoxin-1
RNR = ribonucleotide reductase
ROS = reactive oxygen species
Sec = selenocysteine
SIN-1 = 3-morpholinosydnonimine
SOD = superoxide dismutase
TBP-2 = thioredoxin-1-binding protein-2
TMP = thioredoxin-related transmembrane protein
TMX = thioredoxin-related transmembrane protein
TNF-α = tumor necrosis factor alpha
TRP14 = thioredoxin-related protein of 14
TRP32 = thioredoxin-related protein 32
TRX = thioredoxin
TrxR = thioredoxin reductase
TRX80 = 10-kDa thioredoxin/truncated thioredoxin
Txl = thioredoxin-like protein
TXNIP = thioredoxin-1-interacting protein
UPR = unfolded protein response
VDUP1 = vitamin-D_3 up-regulated protein 1