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Interacting with Thioredoxin-1—Disease or No Disease?

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Abstract

Significance: Many cardiovascular disorders are accompanied by a deregulated cellular redox balance resulting in elevated levels of intracellular reactive oxygen species (ROS). One major antioxidative cellular molecule is thioredoxin-1 (Trx-1). Its indispensability is demonstrated by the embryonic lethality of Trx-1 deficient mice. Trx-1 is ubiquitously expressed in cells and has numerous, diverse functions. It not only reduces oxidized proteins or, together with peroxiredoxins, detoxifies H_2O_2, but also binds to several proteins and thereby regulates their functions. The interaction partners of Trx-1 differ depending on its localization in the cytosol or in the nucleus. ***Recent Advances/Critical Issues:*** Over the past decade it has become clear that Trx-1 is not only critical for tumor functions, which has resulted in therapeutic approaches targeting this protein, but also essential for proper functions of the vasculature and the heart. Changes in post-translational modifications of Trx-1 or in its interactions with other proteins can lead to a switch from a physiologic state of cells and organs to diverse pathologies. This review provides insights into the role of Trx-1 in different physiological situations and cardiac hypertrophy, ischemia reperfusion injury, heart failure, atherosclerosis, and diabetes mellitus type 2, underscoring the central role of Trx-1 in cardiovascular health and disease. ***Future Directions:*** Thus, the manipulation of Trx-1 activity in the heart and/or vasculature, for example, by small molecules, seems to be a promising therapeutic option in cardiovascular diseases, as general anti-oxidant treatments would not take into account interactions of Trx-1 with other proteins and also eliminate vital ROS. *Antioxid. Redox Signal.* 18, 1053–1062.

Introduction

Cardiovascular diseases are the leading cause of death in the world today. There are many factors known increasing the risk for cardiovascular diseases. The major modifiable risk factors include for example, tobacco use and alcohol abuse, unhealthy diet, obesity, physical inactivity, and stress. Cardiovascular complications, such as hypertension, atherosclerosis, pathological hypertrophy, ischemic heart disease, and myocardial infarction, result in enormous direct and indirect annual costs (28). Thus, treatment of these complications has a tremendous impact. Therefore, an overall understanding of the underlying causes and mechanism is required to find new possible treatments and enhance those already established.

It is widely accepted that the imbalance of prooxidative and antioxidative systems results in deregulated redox signaling and contributes to the abnormal cellular changes observed in diverse cardiovascular diseases. Redox regulation describes reduction and oxidation events that are responsible for keeping a proper cellular environment. These are essential physiologic processes that include reversible post-translational protein modifications changing the functional properties of the affected molecules, for example, oxidative inactivation of phosphatases or reductive activation of transcription factors (30, 57). They can be found in almost all cells including endothelial cells (EC) and cardiomyocytes. If prooxidative systems or situations take the upper hand and antioxidative systems cannot compensate those signals, the cell faces oxidative stress, which can lead to apoptosis. In cardiovascular cells the induction of apoptosis by oxidative stress is predominantly triggered by activation of caspases (6).

In cardiovascular cells in particular there are two major antioxidative defense systems, the glutathione (GSH) and the thioredoxin (Trx) systems. The tripeptide GSH can neutralize reactive oxygen species (ROS) and reduce oxidized molecules. The reactive GSH produced in these processes forms glutathione disulfide, which is regenerated to GSH by glutathione reductase and NADPH. The Trx system consists of Trx and the corresponding thioredoxin reductase (TR). TR utilizes the

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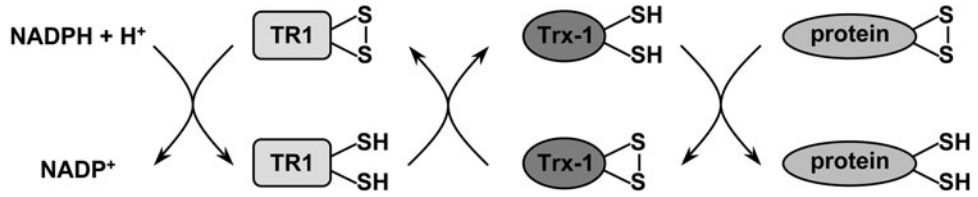

FIG. 1. The Trx-1 system. One important function of Trx-1 is to catalyze redox reactions. Upon reduction of a target protein, Trx-1 itself is oxidized. Regeneration of oxidized Trx-1 is sustained through TR1, which itself is restored in an NADPH-dependent manner. TR, thioredoxin reductase; Trx-1, thioredoxin-1.

electron donor NADPH to reduce and regenerate the dithiol active site in oxidized Trx [for review see ref. (22) and Fig. 1]. Along with Trx-1, which can be found in the cytosol and nucleus, another Trx, Trx-2, exists, which is localized in the mitochondria (47). Besides those two ubiquitously expressed Trx systems, a testis-specific Trx system has been described (32). This review, however, will focus on the role of Trx-1 in cardiovascular diseases.

The 12 kDa small protein Trx-1 was first discovered in 1964 by Peter Reichard and his group as a hydrogen donor for the ribonucleotide reductase, an essential enzyme involved in DNA synthesis in *Escherichia coli* (27). Shortly thereafter, the amino acid sequence of *E. coli* Trx-1 was determined and the dithiol active site (–Cys–Gly–Pro–Cys–), conserved from bacteria to mammals and essential for the redox-regulatory function, described (21). A few years later the three-dimensional structure of *E. coli* Trx-1 revealed the so-called Trx fold, a structural element of proteins of this class (23). Trx-1 is ubiquitously expressed in mammalian cells and has numerous, diverse functions. Its essential role was demonstrated by genetic targeting leading to severe developmental disorders in the early mouse embryo resulting in embryonic lethality (31). Together with the peroxiredoxins (Prxs) Trx-1 is involved in reducing peroxides, for example, hydrogen peroxide, by regenerating oxidized Prxs through reduction of their redox-active cysteines (39) (Fig. 2). Interestingly, Day *et al.* recently demonstrated in fission yeast that the inactivation of the only 2-Cys Prx Tpx1 by hydrogen peroxide is required for Trx-1 mediated reduction of oxidized proteins and thus cell survival (9). However, it is not clear whether the same mechanism applies in cardiovascular cells, since they express more than one 2-Cys Prx.

Besides this indirect scavenging of peroxides, Trx-1 interacts with several proteins *via* disulfide bridges thereby modulating protein functions (11). For example, Trx-1 binds to the apoptosis signal-regulating kinase 1 (ASK-1) and prevents apoptosis or to the thioredoxin-interacting protein (Txnip, also called vitamin D3-upregulated protein 1 or thioredoxin-binding protein 2) inhibiting its functions (41, 59). Another study recently showed that in response to physiological amounts of ROS or tumor necrosis factor α (TNFα) the Trx-1/Txnip complex translocates to the plasma membrane and promotes a cell survival signal through VEGFR2 in EC (56) (Fig. 2). Upon translocation into the nucleus Trx-1 enhances DNA binding of several transcription factors in concert with APEX nuclease (multifunctional DNA-repair enzyme) 1, including activator protein 1 and nuclear factor κB (NFκB) (17, 18, 44) (Fig. 2). Interestingly, cytosolic Trx-1 has an opposite effect on NFκB activation by preventing the degradation of the NFκB inhibitor IκB and thus the translocation of NFκB into the nucleus (18, 43). Most of these examples demonstrate that Trx-1 facilitates the reduction of proteins in the cytosol and in the nucleus by cysteine thiol-disulfide exchange reaction.

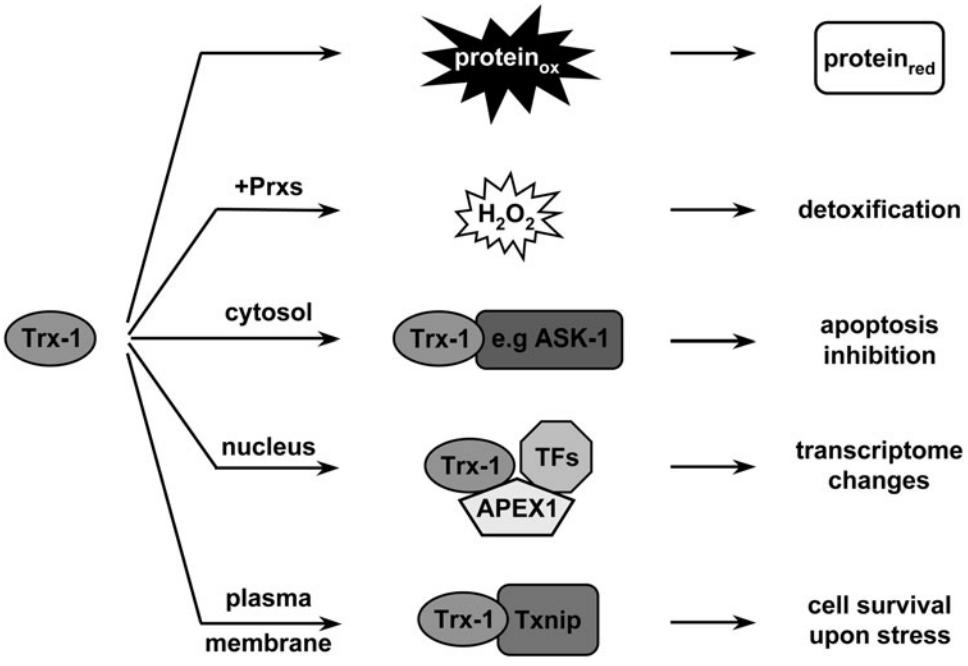

FIG. 2. The multiple functions of Trx-1. Trx-1 is capable of reducing proteins and thus restoring their functions. Together with the Prx system Trx-1 detoxifies peroxides. Further, binding of Trx-1 to cytosolic proteins, for example ASK-1, protects against apoptosis and reductive activation of transcription factors in the nucleus together with APEX1 results in transcriptome changes. Upon stress, translocation to the plasma membrane together with Txnip leads to a cell survival signal. APEX1, APEX nuclease (multifunctional DNA-repair enzyme) 1; ASK-1, apoptosis signal-regulating kinase 1; Prx, peroxiredoxin; TF, transcription factor; Txnip, thioredoxin-interacting protein.

Over the past decade it has become clear that Trx-1 is not only critical for tumor functions where Trx-1 is often upregulated, which has resulted in therapeutic approaches targeting this protein [for review see ref. (37)], but is also essential for proper functions of the vasculature and the heart. Therefore, this review will focus on the role of Trx-1 in different physiological situations and pathophysiological changes within the cardiovascular system.

Cardiac Hypertrophy

Cardiac hypertrophy is characterized by a significant increase in the size of cardiomyocytes together with increased ventricular chamber size and a thickening of the ventricular wall. Healthy cardiac hypertrophy results from normal physiological stimuli such as athletic training or pregnancy and is the normal adaptive response to the enhancement in working load. This increase in heart muscle mass and pumping ability is not accompanied by a long-term disease pattern. Pathological hypertrophy, in contrast, is characterized as the response of the heart to stress such as chronic hypertension or myocardial infarction, both of which are associated with pressure or volume overload leading to contractile dysfunction and heart failure. Many of those pathological changes are associated with elevated ROS levels, which induce damage to proteins, lipids, and DNA, whereas in physiological hypertrophy ROS act as second messengers and influence central signaling pathways without inducing damage [for review see ref. (48)].

To study the role of Trx-1 in cardiac hypertrophy, mice were generated overexpressing wild-type or a dominant negative Trx-1 mutant, in which the cysteines of the catalytic center Cys 32 and Cys 35 are exchanged to serines, in the heart (58). Animals with cardiac-specific overexpression of wild-type Trx-1 were protected against lipid peroxidation, did not exhibit hypertrophy at baseline, and showed reduced hypertrophy after aortic banding. In contrast, expression of the dominant negative mutant leads to cardiac hypertrophy under baseline conditions and in response to pressure overload. These mice were also characterized by increased lipid peroxidation, DNA damage, oxidized GSH, extracellular signal-regulated kinase 1/2, Ras and Raf-1 activation. An involvement of oxidative stress in baseline cardiac hypertrophy was demonstrated by administration of an antioxidant (58).

Other studies showed that not only detoxification of ROS is involved in protection against baseline hypertrophy, but also the interaction of Trx-1 with other proteins. One prominent interaction partner of Trx-1 is Txnip (45, 60). However, studies in Txnip deficient mice revealed a transient attenuation of pressure overload induced hypertrophy not accompanied by changes in Trx-1 expression and activity, suggesting a Trx-1-independent protective mechanism for Txnip in cardiac hypertrophy (62).

In contrast, Ras induced hypertrophy is Trx-1 dependent. Upon oxidative stress exerted through hypertrophic stimuli, Trx-1 seems to keep cysteine 118 of Ras in a reduced state thereby preventing a Ras-mediated hypertrophic response in cardiac myocytes (36) (Fig. 3). This was shown by overexpression of Trx-1 in these cells, which prevented alpha adrenergic receptor induced, Ras-mediated hypertrophy. In this situation, ROS levels were not decreased, however, Ras activation was reduced. Involvement of a Trx-1-dependent redox regulatory process was substantiated by TR1 inhibition with azelaic acid, which potentiated protein synthesis leading to hypertrophy (26).

Another known interaction partner of Trx-1 is ASK-1. Reduced Trx-1 binds to ASK-1 *via* cysteines 32 and 35 in its catalytic center and thus inactivates the enzyme (41). Free ASK-1 itself is well described to play a major role in cardiac hypertrophy and also in promoting apoptosis of cardiomyocytes (19, 25). ROS may disrupt the Trx-1/ASK-1 interaction through oxidative modifications of the Trx-1 catalytic center, which in turn releases ASK-1 subsequently leading to the aforementioned changes (Fig. 3).

Mammalian class II histone deacetylases (HDACs) have been reported to play a crucial role in the regulation of cardiac

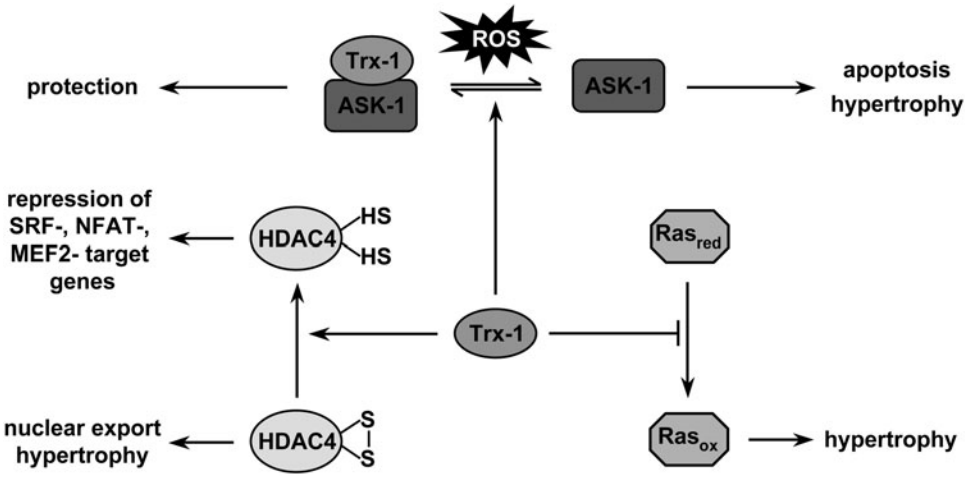

FIG. 3. Multiple roles of Trx-1 in cardiac hypertrophy. Trx-1 exerts anti-hypertrophic actions through interaction with and reduction of different proteins. Hypertrophic stimuli such as ROS result in oxidation and nuclear export of HDAC4. In a multi-protein complex Trx-1 reduces oxidized HDAC4, which then re-enters the nucleus and represses SRF-, NFAT-, and MEF2-target genes involved in hypertrophy. Protective binding of Trx-1 to ASK-1 is disrupted through ROS; free ASK-1 then promotes apoptosis and hypertrophy in cardiovascular cells. Moreover, Trx-1 prevents a Ras-mediated hypertrophic response through keeping Ras in a reduced state. HDAC, histone deacetylase; MEF2, myocyte enhancer factor 2; NFAT, nuclear factor of activated T-cells; ROS, reactive oxygen species; SRF, serum response factor.

hypertrophy through repression of target genes of several transcription factors, such as serum response factor, nuclear factor of activated T cells, and myocyte enhancer factor 2 (7, 8, 15). Translocation of class II HDACs from the nucleus to the cytoplasm occurs in response to G protein-coupled receptor signaling and subsequent phosphorylation by Ca^{2+} calmodulin-dependent kinases, for example, CaMKIIδ, and other kinases like protein kinase C (PKC) δ [for review see ref. (35)]. Besides this known shuttle of class II HDACs, a novel phosphorylation-independent and redox-sensitive nuclear export of HDAC4 has been proposed. Upon hypertrophic stimuli HDAC4 is oxidized under formation of a disulfide bond between Cys-667 and Cys-669 and exported from the nucleus possibly through unmasking a nuclear export signal within its C-terminal region. In the cytosol Trx-1 partially exerts its anti-hypertrophic capacity by reducing oxidized HDAC4 in a multiprotein complex with Txnip and DnaJb5, thereby allowing HDAC4 to re-enter the nucleus. At first, Trx-1 reduces an intramolecular disulfide bond between cysteines 274 and 276 in DnaJb5 to allow binding to HDAC4, which is then reduced by Trx-1 after its regeneration by TR1 (Fig. 4) (1). Even more, CaM kinases, like for example, CaMKII may also be activated through oxidation of methionine residues in response to angiotensin II, possibly leading to phosphorylation of HDACs. Trx-1 is capable of reducing methionine sulfoxide reductases, which in turn can inactivate CaMKII. Thus, it is possible that Trx-1 may negatively regulate CaMKII, which could contribute to the protective effect of Trx-1 in cardiac hypertrophy.

In summary, Trx-1 has a general protective role in cardiac hypertrophy not only through antioxidative mechanisms, but also *via* direct interactions with several proteins. A direct interaction and thereby a reduction of the target proteins by Trx-1 has been shown for Ras and HDAC4 preventing pro-hypertrophic responses. In the case of ASK-1 Trx-1 functions as a scavenger molecule precluding the pro-apoptotic activity of the kinase.

Ischemia/Reperfusion

Ischemia is a period of restricted or even no blood supply to an organ, for example, after rupture of an atherosclerotic plaque and subsequent arterial occlusion leading to an infarcted heart. Once the blood flow is re-established the affected part of the organ is subjected to reperfusion. Paradoxically, this reperfusion phase generally results in lethal tissue damage, for example, due to inflammation and/or oxidative stress rather than physiological recovery. This so-called lethal reperfusion injury can be alleviated by a process called preconditioning. Herein, repeated short-term non-lethal ischemic and reperfusion periods ultimately protect the myocardium from a consecutive potential lethal ischemia (33).

In a model of working isolated rat hearts it was shown that in response to ischemia/reperfusion Trx-1 is slightly downregulated whereas preconditioning increases Trx-1 expression, decreases infarct size, cardiomyocyte apoptosis, and oxidative stress. These cardioprotective effects were abrogated in response to cisplatin, which has been described as a non-specific inhibitor of Trx-1 and therefore should be regarded with caution due to possible side effects. Further, in the same study, it was demonstrated that transgenic mouse hearts overexpressing Trx-1 exhibited significantly improved post-ischemic ventricular recovery and reduced myocardial infarct size in comparison to wild-type hearts after ischemia/reperfusion (53). Along the same line, infusion of recombinant human Trx-1 (rhTrx-1) shortly before reperfusion of the ischemic myocardium significantly reduced apoptosis and myocardial infarct size. Immunohistochemical analysis confirmed the uptake of rhTrx-1 throughout the ischemic/reperfused myocardium. However, it is not clear from this study whether rhTrx-1 is taken up in its reduced or oxidized form. S-nitros(yl)ation of rhTrx-1 prior to administration potentiated those protective effects, whereas a bacterial isoform isolated from *E. coli* (eTrx) lacking cysteine 69 showed similar effects as non-modified rhTrx-1, indicating an important role

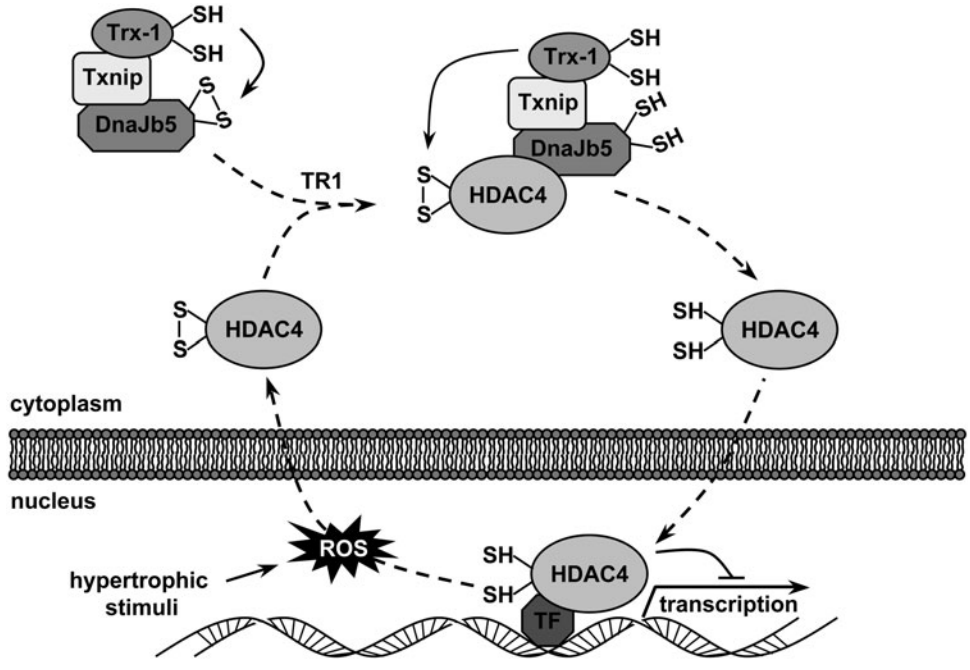

FIG. 4. Protective interaction of Trx-1 with HDAC4 in the hypertrophic response. In the nucleus reduced HDAC4 plays a crucial role in prevention of cardiac hypertrophy through repression of transcription. Hypertrophic stimuli lead to ROS formation and oxidation of HDAC4, which then is exported out of the nucleus. In the cytosol Trx-1 exerts in part its anti-hypertrophic effect through reducing HDAC4 in a multi-protein complex with Txnip and the heat shock protein DnaJb5. To form this reducing complex Trx-1 first reduces DnaJb5 allowing HDAC4 to bind. After regeneration by TR, Trx-1 is capable of reducing HDAC4, which can then re-enter the nucleus.

of this post-translational modification in cardioprotection without being a prerequisite. Mechanistically, this anti-apoptotic impact to some extent appears to be carried out through diminished p38-mitogen-activated protein kinase (MAPK) activation, a known downstream target of ASK-1 (50) (Fig. 5). This observation is in accordance with data describing an increase in the anti-apoptotic function of Trx-1 if S-nitros(yl)ated on cysteine 69 in EC (12). It is known that there is a significant increase in the content of nitrated proteins under various pathological conditions. This is in agreement with data presented by Yin *et al.* (61) who demonstrated that Trx-1 is nitrated in ischemia/reperfusion subsequently leading to its inactivation. Administration of nitrated Trx-1 in working mouse hearts prior to reperfusion did not result in a significant reduction in infarct size (Fig. 5) (61). In agreement with this observation is the decrease in ischemia/reperfusion induced caspase-3 activation in a similar experimental setting by administration of unmodified Trx-1 or a non-nitratable mutant Trx-1 (Y49F) in contrast to nitrated Trx-1. Of note, nitration of Trx-1 in the ischemic/reperfused cardiac tissue diminished the binding of Trx-1 to ASK-1 and enhanced p38-MAPK activation (51, 63) (Fig. 5). Trx-1 may also carry out its protective influence through antagonizing ion channel remodeling in the post myocardial infarcted heart leading to arrhythmia and contractile dysfunction. Especially, the expression of ventricular K^+ channels seems to be negatively regulated by the impaired cardiac Trx-1 system after ischemia/reperfusion through intensified ASK-1-Jun-N-terminal kinase (JNK)-p38-MAPK signaling (49). Finally, there is evidence that overexpression of Trx-1 induces genes coding for parts of the oxidative phosphorylation machinery and the citric acid cycle in mitochondria (2). The downregulation of Trx-1 in ischemia/reperfusion might thus contribute to the well-described damage of these organelles under these conditions.

Overall, during ischemia/reperfusion the protective effects of Trx-1 not only rely on its binding partners, including transcription factors, which change gene expression programs, but also on different post-translational modifications of Trx-1 itself, which can result in opposing activation states.

Heart Failure

The inability of the heart to supply sufficient blood flow to the whole body is generally defined as heart failure. Myocardial infarction, ischemic heart diseases, and cardiomyopathies can often lead to heart failure. As described above, Trx-1 is protective against ischemia/reperfusion and cardiac hypertrophy. Thus, these beneficial effects may also prevent the progression of heart failure. In a therapeutic setting this would require long-term treatment with Trx-1. Therefore, the development of small molecules that can increase Trx-1 activity in the heart or mimic its action would provide an excellent long-term treatment for heart failure.

Atherosclerosis

Atherosclerosis is a chronic inflammatory response in the vessel wall leading to the formation of atherosclerotic plaques that are characterized by accumulation of immune cells, lipids, and cellular debris covered by a fibrous cap (40). Upon rupture of the plaque sudden thrombotic occlusion of the artery can occur, which in the heart leads to myocardial infarction. One of the earliest steps in the development of atherosclerosis involves the loss of integrity of the endothelium, the innermost cellular layer in the vessel, which is in part due to oxidative stress and apoptosis (4, 54).

Trx-1 is one important anti-apoptotic protein in EC and mediates this effect through different mechanisms, which depend on post-translational modifications, subcellular localization, and different binding partners of Trx-1. The crucial role of Trx-1 in EC apoptosis has been shown by overexpression and downregulation leading to protection or an increased sensitivity toward programmed cell death, respectively (12, 14). The protective effects are mediated by different mechanisms; among them is translocation of Trx-1 into the nucleus under physiological concentrations of ROS. There, Trx-1 activates transcription factors binding to the antioxidant response element (ARE) culminating in an anti-oxidative gene expression program. Well-described examples are the upregulation of glutathione S-transferase P1, the promoter of

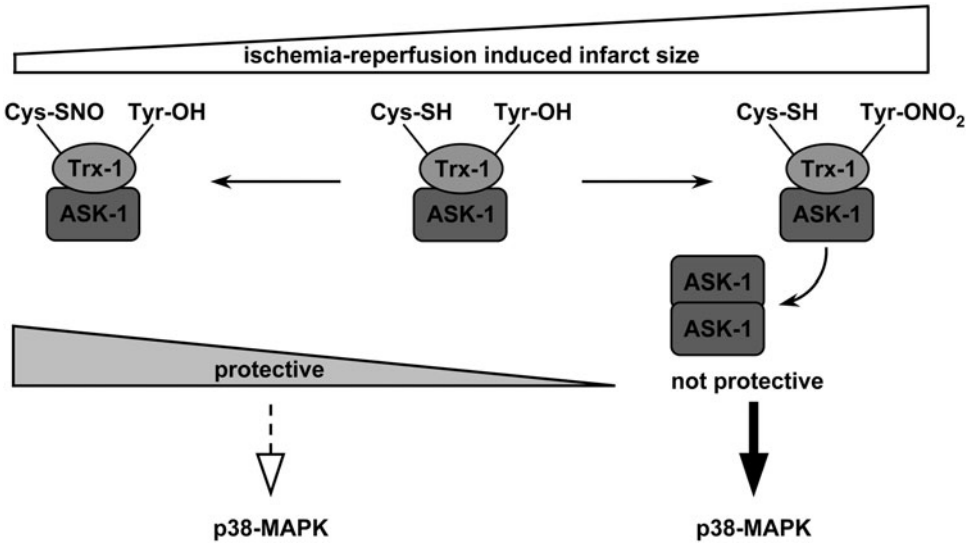

FIG. 5. Post-translational modifications of Trx-1 in ischemia/reperfusion injury. Sequestration of ASK-1 by Trx-1 is protective during ischemia/reperfusion in part through diminished p38-MAPK activation. S-nitros(yl)ation of cysteine 69 of Trx-1 potentiates the protective effect, whereas nitration of tyrosine 49 of Trx-1 leads to a dissociation of the Trx-1/ASK-1 complex resulting in enhanced p38-MAPK activation and thus loss of protection. MAPK, mitogen-activated protein kinase.

which contains several AREs (44), and the reduction of nuclear factor erythroid 2-related factor 2 (Nrf-2), enabling its binding to these regulatory elements (16). The latter is also evident in the Nrf-2 knockout mouse, in which one prominent phenotypical feature is the apoptosis of EC (38). A potent anti-apoptotic and therefore anti-atherosclerotic stimulus is the blood flow itself. It has been shown that in areas of human carotid atherosclerotic plaques with low or turbulent flow, apoptosis rates of EC are significantly higher than in those areas with normal, laminar blood flow (52). This laminar blood flow, also called shear stress, results in the downregulation of Txnip, releasing Trx-1 thus enhancing its binding to ASK-1. This interaction not only inhibits apoptosis through preventing homodimerization of ASK-1, a prerequisite for its activation, and making ASK-1 prone to ubiquitination and degradation (29), but also plays a role in preventing a pro-inflammatory response. Degradation of ASK-1 prevents activation of JNK and p38-MAPK thereby inhibiting TNFα-induced expression of vascular cell-adhesion molecule 1, a surface molecule important for the interaction of EC with T cells and monocytes in the early inflammatory response leading to atherosclerotic plaque formation (29, 60). Along the same lines Trx-1 downregulates monocyte chemoattractant protein-1 expression and secretion (5), thus, suppressing monocyte/macrophage recruitment and adhesion. For monocytes themselves it was shown that Trx-1 plays a role in preventing apoptosis (24). Interestingly, treatment of monocyte-derived macrophages with a synthetic peroxisome proliferator-activated receptor gamma agonist led to upregulation of Txnip and elevated apoptosis rates (3). Taken together, this indicates that the Trx-1/Txnip interaction may contribute to monocyte apoptosis regulation.

In addition, laminar blood flow was demonstrated to enhance the activity of Trx-1 in EC through increasing the amount of S-nitros(yl)ated Trx-1 (20). Interestingly, apoptosis protection by Trx-1 involves not only activation of anti-oxidative gene programs and inhibition of pro-apoptotic signaling cascades, but also interactions with cytoskeletal components. We recently demonstrated that the binding of Trx-1 to γ-actin is a new, ASK-1-independent anti-apoptotic mechanism. Challenging the actin cytoskeleton with H_2O_2 leads to aberrant rearrangements and formation of actin stress fibers. This is accompanied by a reduction in Trx-1 protein levels possibly through Cathepsin D-mediated degradation and increased EC apoptosis (13, 64). Since overexpression of Trx-1 prevents stress fiber formation and inhibition of actin bundle formation blocks Trx-1 degradation, this interaction seems to mutually protect both proteins from oxidative stress (64) (Fig. 6).

Taken together, Trx-1 may exerts its protective role in atherosclerosis through preventing EC apoptosis at several levels, thereby ensuring the integrity of the vessel wall and preventing inflammatory processes.

Diabetes Mellitus

Diabetes mellitus is a metabolic disease, which is characterized by prevailing hyperglycemia in the blood. Type 1 diabetes mellitus, also known as juvenile diabetes, is considered an autoimmune disease in which the insulin producing beta-cells of the pancreas are destroyed resulting in very low levels of insulin and leading to high blood sugar levels. On the other hand, in type 2 diabetes mellitus, also called adult-onset diabetes, the responses of cells to insulin and the uptake of glucose are impaired resulting in high blood sugar levels. The prevalence of type 2 diabetes is ever increasing becoming the major form of diabetes diagnosed in patients and is considered to be a major risk factor for cardiovascular diseases, for example, atherosclerosis or myocardial infarction (10).

The diabetic disease state with hyperglycemic conditions has been associated with an increase in ROS. The elevated ROS levels seem to be generated in the mitochondria by a one electron transfer to oxygen. This mitochondrial superoxide overproduction may result in PKC and NFκB activation and an increase in advanced glycation end-products forming methylglyoxal, which in turn harms the endothelial monolayer (34). The intensified oxidative potential is challenging for the cellular antioxidative systems and may lead to deregulated protein interactions, as mentioned earlier. Indeed, in an animal model of streptozotocin-induced diabetes in rats, ROS were increased and Trx-1 activity was significantly decreased without a change in expression or protein levels in the diabetic animals in comparison to untreated littermates. The

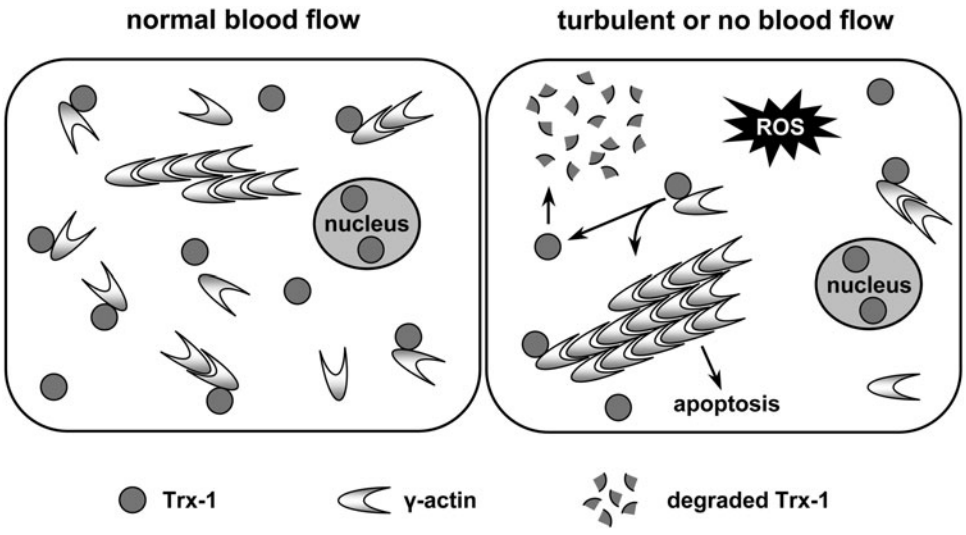

FIG. 6. The interaction of Trx-1 with γ-actin in EC. Under conditions of normal blood flow Trx-1 binds to γ-actin, which results in a mutual protection of both proteins against degradation and stress fiber formation, respectively and thus the EC itself. ROS occurring in areas of turbulent or no blood flow partly result in thick actin bundle formation leading to a dissociation of Trx-1 from actin and subsequently to Trx-1 degradation most likely exerted through Cathepsin D and stress fiber formation. Both events precede EC apoptosis and thus the onset of cardiovascular diseases like atherosclerosis. EC, endothelial cells.

diminished activity of Trx-1 is thought to be due to an induction of Txnip expression through high glucose-induced activation of p38-MAPK signaling. Elevated levels of Txnip would result in more inhibitory binding to Trx-1 and explain the reduced Trx-1 activity. Of note, insulin treatment reduced high glucose-induced Txnip expression and rescued Trx-1 activity (46). One may speculate that this glucose-dependent upregulation of Txnip also disturbs the Trx-1/ASK-1 axis, since the enhanced inhibitory binding of Txnip to Trx-1 releases ASK-1, which then induces EC apoptosis. In terms of clinical approaches, there are recent studies that show a protective effect of Trx-1 after myocardial infarction in streptozotocin-induced animal models for diabetes. Adenoviral gene therapy with Trx-1 after myocardial infarction in diabetic rats reduced fibrosis, oxidative stress, and apoptosis and enhanced capillary and arteriolar density (42). Administration of rhTrx-1 in a comparable experimental setting in mice attenuated apoptosis, reduced infarct size, and improved cardiac function (61). This cumulative evidence suggests that Trx-1 may be a suitable therapeutic to decrease heart damage after myocardial infarction in diabetic patients.

Another mechanistic explanation for reduced Trx-1 activity could be glycative inhibition by methylglyoxal, a byproduct of metabolic pathways, which is elevated in diabetic patient plasma. In the setting of an ischemia/reperfusion model of H9c2 cardiomyoblasts, cells preincubated with methylglyoxal had lower Trx-1 activity accompanied by enhanced p38-MAPK activation and reduced binding of Trx-1 to ASK-1 (55).

In conclusion, diabetes is a disease closely correlated with cardiovascular morbidity and mortality, which is accompanied by excessive ROS formation and a modified Trx-1 system. Animal models suggest that functional improvement after cardiovascular insults may be obtained by Trx-1 delivery. One has to keep in mind that increased Trx-1 activity is present in nearly all tumors, such that therapeutic interventions of this kind bear an inherent danger. However, approaches targeting elevated mitochondrial ROS production or trying to increase mitochondrial Trx-2 activity to support classical diabetes therapies could be envisioned in the future.

Innovation

It has become clear that thioredoxin-1 (Trx-1) protects against cardiovascular diseases through multiple pathways in the cytoplasm and the nucleus. It can reduce many proteins to control their function or localization. In addition, it can interact with other proteins restraining them from harming the cell, for example, by inducing programmed cell death. On the other hand, these interactions can result in mutual protection of both partners against harmful influences. Thus, the manipulation of Trx-1 protein level and/or activity in the heart and vasculature, for example, by small molecules, seems to be a promising therapeutic option in cardiovascular diseases.

Conclusion and Future Directions

Trx-1 plays a central role in the physiology of the cardiovascular system. Inactivation or loss of Trx-1 has been demonstrated in multiple cardiovascular diseases. Therefore, Trx-1 has been used in animal models to protect against cardiovascular diseases. To understand the protective effects of Trx-1 in the cardiovascular system, several studies have investigated its antioxidative capacity, its interaction with several proteins and its potential in modifying gene expression programs. It has become clear that Trx-1 protects against cardiovascular diseases through multiple pathways. However, important unsolved problems are (i) how to increase the concentration of Trx-1 in the heart and vessels or (ii) how to administer Trx-1 to the heart. To increase intracellular Trx-1 levels, it could be envisioned to either inhibit its degradation or to upregulate its transcription. However, this alone might not be sufficient, because Trx-1, in order to execute its multiple functions, has to be kept in a reduced form, which requires TR1 and NADPH. Moreover, the threshold levels of Trx-1 necessary to exert its protective functions in the heart and vasculature are unknown and require further investigation. Another option would be to design small molecule drugs enhancing Trx-1 activity. An important issue when using systemically acting compounds is the expression of Trx-1 in tumors. This has been addressed in therapeutic approaches targeting this protein in cancer patients. However, all systemic approaches improving Trx-1 functions should be treated with caution, as they might foster tumor development and/or progression. Conversely, reciprocal considerations have to been taken into account, when trying to treat tumors by interfering with Trx-1 functions, because this might lead to severe cardiovascular dysfunction. Therefore, molecules, which could be targeted to specific tissues and cellular compartments would be the premier option.

Acknowledgments

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Abbreviations Used

APEX1 = APEX nuclease (multifunctional DNA-repair enzyme) 1
ARE = antioxidant response element
ASK-1 = apoptosis signal-regulating kinase 1
EC = endothelial cells
GSH = glutathione
HDAC = histone deacetylase
JNK = Jun-N-terminal kinase
MAPK = mitogen-activated protein kinase;
MEF2 = myocyte enhancer factor 2
NFAT = nuclear factor of activated T-cells
NFκB = nuclear factor κB
Nrf-2 = nuclear factor erythroid 2-related factor 2
Prx = peroxiredoxin
rhTrx-1 = recombinant human Trx-1
ROS = reactive oxygen species
SRF = serum response factor
TNFα = tumor necrosis factor α
TR = thioredoxin reductase
Trx-1 = thioredoxin-1
Txnip = thioredoxin-interacting protein