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Thioredoxin and Ventricular Remodeling

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Abstract

Increasing bodies of evidence indicate that reactive oxygen species (ROS) produced by mitochondria and other sources play an essential role in mediating ventricular remodeling after myocardial infarction and the development of heart failure. Antioxidants scavenge ROS, thereby maintaining the reduced environment of cells and inhibiting ventricular remodeling in the heart. Thioredoxin not only functions as a major antioxidant in the heart but also interacts with important signaling molecules and transcription factors, thereby modulating various cellular functions. The activity of thioredoxin is regulated by a variety of mechanisms, such as transcription, localization, protein-protein interaction, and post-translational modification. In this review, we will summarize the cardiac effects of thioredoxin and the mechanisms by which thioredoxin mediates inhibition of ventricular remodeling.

Keywords

thioredoxin; ventricular remodeling; cardiac hypertrophy; heart failure

1. Thioredoxin

Thioredoxin (Trx) is a major antioxidant in cells along with the tripeptide glutathione (GSH, Glu-Cys-Gly) [1–3]. These molecules maintain the intracellular space to be in reduced states [1,4]. Trx contains dithiol within its conserved active site (-Cys-Gly-Pro-Cys-; Cys at 32 and at 35 in Trx1) and reduces thiol-groups in various redox-sensitive proteins, while Trx itself is oxidized to form disulfide [1,5]. To work properly, Trx requires thioredoxin reductase (TrxR) and NADPH: TrxR reduces the active site of oxidized Trx using NADPH, thereby regenerating reduced Trx [2]. Therefore, the expression and activity of TrxR and the abundance of NADPH may affect Trx function. “The Trx system”, comprised of these three molecules, is conserved from *E.coli* to mammals, and is ubiquitously present in various tissues [6,7], which implicates the importance of Trx in cellular functions. In mammals, there are at least 3 members in the Trx family [3]: Trx1, Trx2, and Sp-trx (sperm trx, also designated as $p32^{TrxL}$) [6,8,9]. In the broad sense, the Trx system may include thioredoxin peroxidase (peroxiredoxin): Trx reduces peroxiredoxin, which then catalyzes H_2O_2 to produce water [3].

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1.1. Trx1

Among the three Trx family proteins, Trx1 has been most intensively examined thus far. Mammalian Trx1 is a small 12kDa protein, which was originally identified as adult T cell leukemia-derived factor produced by human T cell lymphotrophic virus-I-transformed T cells [10]. Trx1 activity is regulated by its expression level, localization (nucleus, cytosol, or extracellular space), interaction with other molecules, and post-translational modification (Figures 1 and 2).

1.1.1. Regulation of Trx1 expression—The promoter region of the *trx1* gene contains a series of stress-responsive elements [11]. Trx1 transcript is induced by various stimuli and/or stresses, such as TNF-α, H_2O_2, UV, and heat shock [1,12–14]. In addition, estrogen, prostaglandins E1, and cAMP are reported to induce mRNA, protein, and secretion of Trx1 [11,15]. Geranylgeranylacetone, a natural plant constituent and an anti-ulcer drug, is a molecule known to induce Trx1 expression [11,12,16]. In *in vivo* mouse models, pressure-overload and ischemia and reperfusion (I/R) induces Trx1 in the heart [17,18]. Among transcription factors, heat shock factor 2 (HSF2) is reported to induce Trx1 expression [19].

1.1.2. Localization of Trx1—Trx1 is normally present in the cytosol, but translocates into the nucleus under stress conditions [13], despite having no known nuclear localization sequence. A basic region, including Lys-81, 82, and 85, may interact with importin, a critical component of the nuclear transport system, thereby participating in nuclear localization of Trx1 [20]. Alternatively, the nuclear localization of Trx1 may be mediated by Thioredoxin1-interacting protein (TXNIP), which interacts with both Trx1 and importin α_1 [21,22] (**see section 6.2 and** Figure 1). In the nucleus, Trx1 regulates the activity of various transcription factors, such as nuclear factor κB (NFκB), activator protein-1 (AP-1), and p53, in a redox-sensitive manner [23,24], although the activation pattern of transcription factors by Trx1 may be cell type-dependent (**see section 6.4 and** Figure 1). Furthermore, Trx1 can be secreted to the extracellular space under stress conditions [11], and shows cytoprotective [23], anti-apoptotic [24], anti-inflammatory activity [25], as well as a growth-promoting effect as an autocrine/paracrine factor [26] (Figure 1). However, it is not well understood as to how Trx1 is secreted, and how extracellular Trx1 transmits its signal into the intracellular space. The extracellular function of Trx1 may be unique among various antioxidants, allowing Trx1 to alert neighboring cells and possibly prevent the expansion of the disaster [27].

1.1.3. Regulated activity of Trx1 by its interacting protein—The activity of Trx1 is negatively regulated by TXNIP at least at cellular levels [28,29] (**see section 6.2 and** Figure 1). Although TXNIP directly binds to a reduced form of Trx1, presumably at its catalytic center [28,29], the exact molecular mechanism by which TXNIP inhibits the protein–disulfide oxidoreductase activity of Trx1 remains to be elucidated. TXNIP existing in the cytoplasm also suppresses nuclear translocation of Trx1, thereby inhibiting nuclear effects of Trx1 in some cell types [30], although this observation contradicts the view that TXNIP exists primarily in the nucleus [21].

1.1.4. Post-translational modification of Trx1—Redox-based modifications are reported to regulate Trx1 activity (Figure 2). Besides the two cysteine residues in the catalytic site, Trx1 harbors three other cysteines, Cys-62, Cys-69, and Cys-73, which can be modified by ROS and nitric oxide (NO). It is suggested that Cys-62 and Cys-69 form another disulfide bond in the presence of oxidative stress, which may attenuate the accessibility of TrxR1 to Trx1, thereby suppressing Trx1 activity [31]. In addition, under oxidized conditions, Cys-73 reacts with glutathione to form a mixed disulfide, *i.e.* glutathionylation (S-SG), resulting in inhibition of the enzymatic activity [32]. In contrast, Cys-69 can be nitrosylated by NO, which may be required for scavenging ROS and for preserving its redox regulatory activity [33]

(Figure 2). For example, in hearts subjected to ischemia/reperfusion, pretreatment of human Trx1 with s-nitrosoglutathione, which leads to nitro sylation of Trx1 at Cys-69, enhances the cardioprotective effect of Trx1 applied through intraperitoneal injection [24]. In contrast, *E. coli* Trx, which lacks Cys-69, does not possess this cardioprotective role [24]. Very recently, it has been reported that Trx1 undergoes irreversible nitrosative modification (Tyr-NO_2) by peroxynitrite at Tyr-49 in the region critical for protein folding. In contrast to thiol-nitrosylation at Cys-69, the tyrosine nitration inhibits the activity of Trx1 [34]. Thus, a balance of ROS and NO in cells may determine the activity of Trx1.

1.2. Trx2

Trx2 is targeted to the mitochondria by its N-terminal mitochondria localization sequence, which is cleaved upon mitochondrial import. Trx2 contains the catalytic active site, but lacks the other modifiable cysteines found in Trx1 [3,35]. The mitochondrial Trx2 system, comprising Trx2, TrxR2, NADPH and peroxiredoxin-3 [36], is ubiquitously expressed, but very highly expressed in the metabolically active tissues, such as the heart, the brain and the liver. Mitochondria convert 1–2% of the oxygen molecules consumed into superoxide anion [37]. The superoxide production occurs in the electron transport chain, especially at complex I (NADH dehydrogenase) and complex III (ubiquinone-cytochrome *c* reductase). In addition, mitochondria are very vulnerable to ROS. Since the heart is one of the oxygen-consuming organs and its energy is absolutely dependent on ATP made in mitochondria [38], Trx2 appears to play an important role in protecting mitochondria from damages by ROS in the heart along with other mitochondrially localized antioxidant proteins, such as mitochondrial superoxide dismutase (SOD) [39], glutaredoxin-2 [40], and catalase [41].

1.3. KO mice in the thioredoxin system

1.3.1. Trx1 and TrxR1—While heterozygous deletion of Trx1 in mice causes no obvious phenotype, the homozygous mutants die shortly after implantation [42]. Trx1 expression seems to be essential for early differentiation and morphogenesis of the mouse embryo [42]. Although cardiac specific deletion of Trx1 has yet to be reported, mice with cardiac specific overexpression of mutant Trx1 that suppresses endogenous Trx1 activity display hypertrophy [17] (**see section 3.1**).

Ubiquitous inactivation of TrxR1 causes early embryonic lethality between 9.5 and 10.5 days in mice [43]. In contrast, mice with heart-specific inactivation of TrxR1 develop normally and appear healthy, suggesting that TrxR1 is dispensable at least for cardiac development [43]. By extension, if TrxR1 is only the reductase responsible for Trx1, Trx1 may be dispensable for development of the heart.

1.3.2. Trx2 and TrxR2—Cells deficient in Trx2 display increased cellular ROS and apoptosis [44]. In addition, The *Trx2*$^{-/-}$ homozygous mutant embryos display increased apoptosis at 10.5 days and are not present by 12.5 days [45]. The timing of the embryonic lethality is consistent with the maturation of the mitochondria, suggesting that Trx2 is needed for mitochondrial function.

TrxR2 knockout mice display embryonic death at embryonic day 13 [46]. Cardiac specific ablation of TrxR2 results in fatal dilated cardiomyopathy and death shortly after birth, suggesting that removal of toxic ROS species from mitochondria by TrxR2 (and also Trx2) is critical for cardiac function, which is absolutely dependent upon the maintenance of mitochondrial integrity [46].

2. "Ventricular remodeling" and ROS

Postnatal growth of the heart is primarily due to non-proliferative cardiac myocyte enlargement, termed hypertrophy, in response to mechanical or hormonal factors. Cardiac hypertrophy is also induced by many pathological insults, and is often accompanied by functional deterioration as well as histopathological changes, including apoptosis and fibrosis, a step called "ventricular remodeling" [47]. Besides structural remodeling, an altered utilization of the metabolic fuels from fatty acid to glucose, termed "metabolic remodeling", occurs during hypertrophy and heart failure [48,49] (**see section 4**). Although ventricular remodeling may be initially an adaptive and beneficial physiological event for the maintenance of cardiac function, sustained remodeling processes often lead to pathological outcomes, *i.e.* heart failure [50].

Although ROS at low concentrations could mediate physiological cellular functions, high concentrations of ROS are detrimental. ROS may be involved in both steps of ventricular remodeling, *i.e.* developments of hypertrophy and transition from compensated hypertrophy to heart failure [51–55]. ROS are generated by many hypertrophic stimuli, such as Gαq/Gα11-coupled receptor agonists (e.g., phenylephrine, angiotensin II) [56,57] and mechanical forces [58]. These ROS are generated by mitochondrial electron transport leakage [59,60], NAD(P)H oxidases [61–63], xanthine oxidase [64], and uncoupled nitric oxide synthase (NOS) [65, 66].

3. Roles of Trx1 in ventricular remodeling

Since increased production of ROS plays an important role in development of ventricular remodeling, antioxidants, such as glutathione, thioredoxin, SOD [67], catalase, glutaredoxin, and peroxiredoxin [36], are expected to play counteracting and thus protective roles. Interestingly, although a low dose of ROS induces Trx1 expression [14], a high dose of ROS inactivates Trx1 through post-translational modifications caused by imbalance between ROS and NO (see **Section 1.1.4**). Thus, the reactivity of Trx1 against different levels of ROS may differentially affect cellular responses of cardiac myocytes, *i.e.* hypertrophy or apoptosis.

3.1. Cardiac hypertrophy and Trx1

Since a low dose of ROS promotes cell growth [2,38,62], antioxidants, such as Trx1, may attenuate cell growth. However, Trx1 has the potential to promote cell growth in some cell types, such as cancer cells [1,12,68]. Thus, these conflicting effects of Trx1 raise the following question, "What is the net effect of Trx1 on cardiac hypertrophy?"

In order to address this question, we made transgenic mice with cardiac specific overexpression of Trx1 (Tg-Trx1) and catalytically defective Trx1 harboring the double substitution of Cys32/35Ser (Tg-Trx1-DN) [17]. Interestingly, suppression of endogenous Trx1 by overexpression of Trx1-DN induces cardiac hypertrophy at baseline and exacerbates hypertrophic response induced by thoracic aortic constriction (TAC). On the other hand, baseline cardiac hypertrophy was never observed in Tg-Trx1 mice, while cardiac hypertrophy induced by TAC is significantly suppressed in Tg-Trx1 mice [17]. These findings indicate that Trx1 suppresses pathological cardiac hypertrophy. It is not clear why cardiac myocyte apoptosis is not prominent in Tg-Trx1-DN mice, even though ROS are increased in the mouse hearts. The level of ROS produced by suppression of Trx1 may not be sufficient to induce apoptosis. Alternatively, ROS may be produced in a subcellular compartment which couples to hypertrophy but not apoptosis. A possible mechanism underlying the Trx1-mediated anti-hypertrophic effect could be inhibition of the Ras-MAPK signaling pathway [17]. Consistent with our findings, Kuster et al have shown that Trx1 inhibits Ras activation induced by α-adrenergic receptor stimulation, thereby suppressing hypertrophy in adult rat ventricular

myocytes [69]. Ras has redox-sensitive cysteine residues, and during α-adrenergic receptor-stimulated hypertrophy, ROS, probably produced by NAD(P)H oxidase or mitochondria, oxidize the thiols in the cysteines, leading to enhanced activation of Ras [63]. By contrast, Trx1 directly reduces the thiols in Ras, thereby inhibiting Ras [69] (Figure 1). It should be noted, however, that this may not be the sole mechanism by which Trx1 inhibits cardiac hypertrophy, because Trx1 modulates various protein-protein interactions and signaling pathways.

3.2. Ischemia/reperfusion injury, preconditioning and Trx1

Preconditioning is the phenomenon that sublethal stress increases the tolerance of myocardium against a subsequent prolonged ischemic insult [70,71]. Preconditioning is one of the most powerful mechanisms of protection of heart cells from injury and death induced by I/R. Both ROS and Trx1 may play indispensable roles in mediating preconditioning. Molecules released from cells subjected to I/R, including adenosine, angiotensin II, and nitric oxide [70–72], often activate protein kinase C, a major mediator of preconditioning, which is involved in ROS production during ischemic preconditioning [73–76]. On the other hand, cardioprotective effects of preconditioning are abolished when the heart is pre-perfused with N-acetyl cysteine, a scavenger of ROS [77]. Thus, ROS production is required for initiation of preconditioning. Turoczi et al reported, using ex vivo working rat hearts, that Trx1 was upregulated by ischemic preconditioning [18]. In addition, overexpression of Trx1 improved post-ischemic ventricular recovery and reduced myocardial infarct size in mouse hearts, implicating a protective role of Trx1 in ischemic myocardium [18]. Furthermore, Malik et al showed that inhibition of Trx1 by RNA interference attenuates the cardioprotective effect of Trx1 during preconditioning, where nuclear translocation of Trx1 and subsequent activation of NFκB may be critical for cardioprotection [78] (Figure 1). Das and colleagues propose that Trx1 plays a critical role in mediating the preconditioning of myocardial ischemia [72,77].

Plasma Trx1 levels are elevated in patients with acute myocardial infarction [79]. The elevated levels of Trx1 may be protective under ischemic conditions, because exogenously applied human Trx1 was incorporated into myocardial tissue and reduced both infarct size induced by I/R [24] and occurrence of arrhythmia [80].

3.3. Heart failure and Trx1

Chronic heart failure is a complex neuro-hormonal and inflammatory syndrome [81]. Oxidative stress is enhanced during chronic heart failure [62], where production of ROS is enhanced or the activity of antioxidants is suppressed. ROS not only induce apoptosis of cardiac myocytes [82] but also stimulate fibrosis [23]. ROS also elicit reduced cardiac pump function [83] through suppressed activity of ion channels, such as L-type calcium channels on the sarcolemma [84], and of the sarcoplasmic reticulum Ca^{2+} ATPase, SERCA2 [85]. During hypertrophy and heart failure, ROS are produced primarily by mitochondria [59,62], NAD(P)H oxidase [61,62] and uncoupled eNOS [66]. High amounts of ROS further damage mitochondria, through ROS-induced ROS production, thereby forming a vicious cycle of ROS and mitochondrial dysfunction [86].

Although the exact tissue level of Trx1 during heart failure in humans has yet to be elucidated, serum concentrations of Trx1 are known to be increased in patients with heart failure and correlate with the severity of the disease [87,88]. The upregulation of Trx1 is assumed to counteract increased oxidative stress in cardiac myocytes and myocardium. Circulating Trx1 suppresses inflammation by blocking chemotaxis of neutrophils [25,89] and death of cardiac myocytes [24] (Figure 1). However, there has been no direct evidence so far to show that Trx1 prevents the occurrence and progression of heart failure. We have recently found, using a mouse model of dilated cardiomyopathy in which mammalian sterile 20 like kinase 1 (Mst1) is

overexpressed specifically in the heart [90], that overexpression of Trx1 prevents progression of heart failure in the Mst1 mice (Ago and Sadoshima, unpublished data).

3.3.1. Apoptosis and Trx1—Apoptosis is one of the important components of heart failure. Trx1 exerts an anti-apoptotic effect through its antioxidant effect [33] and interaction with critical proteins that regulate apoptosis, such as apoptosis signal-regulating kinase 1 (ASK-1) [91–94] and Akt [95,96]. The reduced form of Trx1 inactivates ASK-1, an upstream kinase that leads to activation of p38 and JNK, critical mediators of apoptosis in cardiac myocytes, thereby inhibiting apoptosis [91]. Trx1 appears to activate Akt [95], which directly phosphorylates and inactivates Bad, a pro-apoptotic protein of the Bcl-2 family [97], and procaspase-9, a critical component of apoptosomes [98]. Trx1 enhances the DNA binding activity of NFκB, which is anti-apoptotic under some conditions, such as I/R, by inducing anti-apoptotic genes [99]. The anti-apoptotic mechanisms involving these molecules induced by Trx1 will also be discussed in **section 6** (Figure 1).

3.3.2. Cardiac fibrosis and Trx1—The extracellular matrix, produced by cardiac fibroblasts, normally maintains the myocardial architecture. However, increased synthesis of collagen causes fibrosis. Matrix metalloproteinase (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs) mainly regulate collagen deposition and degradation. Dysregulation of the extracellular matrix by these molecules contributes to abnormal cardiac muscle fibrosis, leading to progression of heart failure. It is reported that ROS activate expression of MMPs, such as MMP-2 and MMP-9 [100], whereas ROS decrease TIMP expression and fibrillar collagen synthesis in cardiac fibroblasts [101,102]. Furthermore, ROS directly activate MMPs post-translationally [102]. In contrast, inhibition of ROS derived from NAD(P)H oxidase [103] and mitochondria [36] leads to reduced myocardial remodeling in animal models [102]. Trx1 prevents pro-inflammatory cytokine- or bleomycin-induced lung fibrosis, which is mediated by ROS [104]. Trx1 suppresses progression of cardiac fibrosis in a mouse model of heart failure, although the underlying molecular mechanism has yet to be elucidated (our unpublished data).

3.4. Aging heart and Trx1

One of the major hypotheses regarding the molecular mechanism of aging is that oxidative stress underlies aging processes [4]. Thus, protective mechanisms against oxidative stress may be important in counteracting the aging process [4,105]. Systemic overexpression of Trx1 in transgenic mice resulted in extended median and maximum life spans compared with wild-type mice [106,107]. Interestingly, although enhanced telomerase activity was found in the Trx1 transgenic mice, proliferative changes, especially cancer, do not frequently occur [106]. In the heart, Trx1 is expected to attenuate structural and functional changes with aging. Indeed, Trx1 appears to attenuate age-induced cardiac hypertrophy in mice (Ago and Sadoshima, unpublished data). It has been shown that expression of TrxR2 is severely attenuated by aging, a condition which is normalized by caloric restriction, a factor for longevity [108].

Trx1 also serves as an electron donor for methionine sulfoxide reductases (Msr) as well as peroxiredoxins [109,110]. Msr, expressed in all human tissues, including the heart, catalyzes the thioredoxin-dependent reduction of methionine sulfoxide residues back to methionine residues [111]. The importance of Msr is highlighted by the fact that aging is associated with a loss of Msr activity in a number of animal tissues, and that C57BL/J6 mice lacking Msr display a decreased maximum life span [112], whereas overexpression of Msr leads to a dramatic increase in the maximum life span [113]. Thus, a possible mechanism by which Trx1 antagonizes aging may be due to keeping Msr in an active state.

4. Metabolic remodeling during hypertrophy and heart failure

Cardiac muscle needs a high turnover of ATP made by mitochondria in order to contract efficiently. Under aerobic conditions, fatty acid oxidation accounts for up to 90% of the total acetyl-CoA, the substrate of the TCA cycle [114]. However, during cardiac hypertrophy and heart failure, the metabolic process to supply acetyl-CoA in the heart shifts from fatty acid oxidation to glucose-mediated metabolism, termed metabolic remodeling [48], which is accompanied by a degree of cellular oxidative stress [115]. Alteration of gene expression responsible for metabolic remodeling during cardiac hypertrophy and heart failure is in part mediated by the fatty acid-activated peroxisome proliferator-activated receptors (PPARs) and PPARγ coactivator-1α (PGC-1α), major regulators of mitochondrial biogenesis and respiration [116,117]. Expression of these molecules is extensively suppressed during hypertrophy and heart failure [49,117–119]. Cardiac specific deletion of either PPARs [120–122] or PGC-1α [123] leads to decreased fatty acid oxidation and subsequent cardiac dysfunction, suggesting that PPARs and PGC-1α primarily regulate cardiac function and prevent the progression of heart failure [124]. We have recently found, using cDNA microarray analyses, that expression of PGC-1α and the activity of mitochondrial enzymes are significantly upregulated by Trx1 overexpression in the heart [125]. This finding suggests that Trx1 may play a protective role against hypertrophy and heart failure by attenuating metabolic remodeling through an enhancement of PGC-1α activity (Figure 1).

5. Channel remodeling during hypertrophy and heart failure

Along with structural and metabolic remodeling, “channel remodeling” also occurs during hypertrophy and heart failure, and leads to contractile dysfunction and arrhythmias characterized by downregulation of K^+ channels that control action potential morphology [126,127]. Li et al suggested that Trx1 upregulates expression of cardiac ion channels responsible for transient outward current, and reverses channel remodeling through its redox activity [128]. Thus, Trx1 may also work protectively against “channel remodeling”.

6. Trx1-interacting proteins involved in cardiac remodeling

Trx1 not only reduces redox-sensitive molecules but also interacts with various proteins, such as intracellular signaling molecules and transcription factors (Figure 1). The interaction is often dependent on the redox state and localization of Trx1. Through protein-protein interaction, Trx1 alters the enzymatic activity or the subcellular localization of the partner protein, thereby affecting various cellular functions.

6.1. ASK-1

ASK-1, a MAPKKK [129], is one of the most prominent molecules that are regulated by Trx1. Increasing lines of evidence suggest that ASK-1 plays an important role in mediating both cardiac hypertrophy and apoptosis [94]. For example, G-protein–coupled receptor (GPCR) agonists such as angiotensin II, endothelin-1 and phenylephrine generate ROS and activate ASK-1, thereby leading to cardiac myocyte hypertrophy [130] (Figure 1). In ASK-1 knockout mice, angiotensin II-induced cardiac hypertrophy and remodeling, as well as p38 and JNK activation, are significantly attenuated [131]. Trx1 and ASK-1 physically interact with one another and this interaction is redox-dependent: oxidized Trx1 is unable to interact with ASK-1 [91]. Through this direct interaction, Trx1 suppresses the kinase activity of ASK-1, thereby inhibiting the apoptotic as well as hypertrophic function of ASK-1 [91,129] (Figure 1). In addition, Trx1 may promote ubiquitination and degradation of ASK-1, a function for which a single cysteine in the catalytic site of Trx1 is sufficient [92]. Thus, both anti-hypertrophic and anti-apoptotic actions of Trx1 may be mediated at least in part by the interaction with ASK-1. ASK-1 associates with troponin T and phosphorylates it, thereby reducing contractility in

cardiac myocytes [132], which may also be attenuated by Trx1. Recently, it has been shown that Trx2 also interacts with ASK-1 in the mitochondria, thereby inhibiting ASK1-induced apoptosis [93].

6.2. TXNIP/ VDUP1/TBP-2

TXNIP was originally identified as an upregulated protein in HL-60, human promyelocytic leukemia cells, treated with 1α, 25 dihydroxyvitamin D_3, and was thus designated vitamin D_3-upregulated protein 1 (VDUP1) [133]. Using a yeast two hybrid system, Nishiyama et al identified this same protein as a Trx1-binding protein, and originally named it TBP-2 [28]. TXNIP binds to reduced but not oxidized Trx1, suggesting that the catalytic center of Trx1 is important for this interaction [28]. Although it is thought that TXNIP attenuates Trx1 activity, at least as measured by insulin-reducing activity at in vitro and cellular levels [28,29], Trx1 activity is not enhanced in TXNIP knockout mice [134]. Expression of TXNIP in cardiac myocytes is rapidly suppressed by hypertrophic stimuli, such as mechanical strain, hydrogen peroxide, and pressure-overload [135,136]. In contrast, ectopic overexpression of TXNIP suppresses cellular proliferation in tumor cells [137] and, in cardiac myocytes, attenuates cardiac hypertrophy in response to mechanical strain, phenylephrine, or angiotensin II [136], and causes apoptotic cell death [135]. Thus, TXNIP serves as both a negative regulator of cardiac hypertrophy and a positive mediator of cell death. If TXNIP functions only to attenuate Trx1 activity, these findings may not be consistent with the reported Trx1 functions in the heart [17]. Rather, Trx1 and TXNIP may function in a cooperative fashion to suppress hypertrophy. Indeed, HSF, a transcription factor, upregulates both Trx1 [19] and TXNIP [138], and TXNIP may mediate the translocation of Trx1 through interaction with importin α_1 into the nucleus, where Trx1 plays a critical role in regulating transcription [21] (Figure 1). However, it should be noted that TXNIP may inhibit nuclear translocation of Trx1 in some cell types [30]. It remains to be elucidated how Trx1 and TXNIP regulate their activities of one another during different disease states in the heart.

It is reported that the phenotype of the mutant mouse strain, HcB-19/Dem, which shows characteristics of familial combined hyperlipidemia, may be caused by a nonsense mutation in TXNIP [139]. Consistently, TXNIP knockout mice display impaired fatty acid oxidation and utilization by the TCA cycle, with resultant increased levels of fatty acid and reduced levels of glucose in plasma [134]. These findings suggest that TXNIP may regulate fatty acid metabolism and metabolic remodeling in the heart as well. As we mentioned, microarray analyses of Tg-Trx1 mice show that Trx1 increases PGC-1α expression [125], which may enhance fatty acid oxidation. Thus, it is possible that Trx1 and TXNIP may function cooperatively to maintain the normal metabolic state in the heart (Figure 1).

6.3. PTEN/PI3K/Akt pathway

Trx1 has been shown to interact with Phosphatase and Tensin homolog (PTEN), a protein phosphatase that attenuates PI3K/Akt activity [95]. PTEN has a critical cysteine at residue 212 in the C2 lipid-binding domain, which mediates interaction with Cys-32 in the catalytic site of Trx1 to form a disulfide bond. The interaction leads to steric hindrance of the catalytic site of PTEN [95]. Thus, the Trx1-PTEN interaction attenuates the phosphatase activity of PTEN, thereby activating PI3K and Akt (Figure 1). Since a signaling cascade that leads to Akt activation promotes growth and survival of many cell types, including cardiac myocytes, stimulation of Akt via suppression of PTEN may explain the anti-apoptotic effect of Trx1. There are some reports showing that ROS enhance Akt activity by directly inhibiting PTEN via oxidation [140]. Consistently, in ischemic preconditioning, oxidative stress, rather than Trx1 induction, may induce PTEN inactivation and further activation of Akt [141]. In these studies, however, a possibility remains that ROS-induced upregulation of Trx1 may mediate Akt activation.

Growing bodies of evidence suggest that the IGF-PI3K-Akt pathway mediates physiological rather than pathological hypertrophy [142]. Interestingly, *akt1*$^{-/-}$ mice develop an exacerbated form of cardiac hypertrophy in response to pressure-overload, suggesting an antagonizing effect of Akt against pathological hypertrophy, although physiological hypertrophy induced by swimming was significantly suppressed in these mice [143]. Thus, there is a possibility that Trx1-mediated activation of PI3K/Akt through PTEN inhibition may participate in physiological cardiac hypertrophy, while attenuating pathological hypertrophy as observed in Tg-Trx1 mice [17]. Alternatively, the anti-hypertrophic effect of Trx1 may be totally independent of the PTEN/PI3K/Akt pathway.

6.4. Transcriptional factors and their regulators

The activity of several transcription factors is altered by redox modification of specific cysteine residues. The DNA-binding activity of NFκB is markedly augmented by direct interaction with Trx1 *in vitro* [144]. However, Trx1 also inhibits NFκB activation: in contrast to the event in the nucleus, Trx1 blocks degradation of IκB and prevents nuclear translocation of NFκB [145] (Figure 1). The DNA-binding activity of AP-1 is modified by a DNA repair enzyme, redox factor 1 (Ref-1). Ref-1 activity is regulated by interaction with Trx1 [146]. Trx1 associates directly with Ref-1 in the nucleus only in its reduced form, which then potentiates AP-1 activity through direct interaction [147] (Figure 1). Because NFκB and AP-1 are well known transcription factors related to the development of cardiac hypertrophy [148,149], it is expected that Trx1 is involved in hypertrophic responses in the heart through these transcription factors. However, we have recently shown, using cDNA microarray analyses, that the activity of these transcription factors is not enhanced in the heart of Tg-Trx1 mice at baseline [125]. Thus, the functional outcome of transcription factor regulation by Trx1 may be different among various cell types and cell conditions (*i.e.* normal vs diseased states, such as I/R and heart failure). Using microarray analyses in conjunction with transcription factor binding site analyses, we have found that the transcriptional activity of CREB and nuclear respiratory factor 1 (NRF1) is enhanced in Tg-Trx1 mouse hearts [125] (Figure 1). Although it has been reported that Trx1 reduces a cysteine residue of NRF2, thereby enhancing the DNA binding activity of NRF2 [150], it remains unknown whether CREB and NRF1 also have similar redox sensitive cysteines regulated by Trx1.

7. Therapeutic implications

Trx1 may function as a negative regulator of hypertrophy and heart failure. Modulating the negative regulators of hypertrophy may become one of the important strategies for combating heart failure in the future [151]. Although some negative regulators of hypertrophy induce apoptosis [151,152], Trx1 suppresses both hypertrophy and apoptosis, which would be ideal when considered as a therapeutic agent for many heart diseases. In addition, Trx1 has advantages for use in clinical therapy: it is small-sized and structurally stable, and can function both intracellularly and extracellularly, which would allow administration of Trx1 directly into either the myocardium or coronary circulation. Indeed, in animal models, direct administration of Trx1 exhibited salutary effects on some heart disease states [12,24]. However, higher doses of Trx1 may be needed so far to reduce oxidative stress and inflammation [12]. In addition to direct infusion of Trx1, the administration of reagents that induce Trx1 expression is also worthy of attention. Geranylgeranylacetone is one such drug, which is already used as an anti-ulcer drug. Interestingly, resveratrol, a major component of red grapes, is known to induce Trx1 [153]. Finding the molecules that elicit Trx1 expression effectively and in a highly selective fashion may expand our choice for cardiac therapy. In summary, we believe that Trx1 may be one of the most promising molecules to be used for therapy for ischemic heart disease and congestive heart failure.

8. Concluding remarks

Trx1 has a wide variety of cellular functions, not only as an antioxidant but also as a regulator of diverse signaling pathways through direct interaction with intracellular signaling molecules and transcription factors. Through these functions, Trx1 prevents the development of ventricular remodeling and congestive heart failure. However, the molecular mechanisms by which Trx1 confers advantages to the heart are still not completely understood. Further investigations are needed in order for us to apply our knowledge regarding the potentially salutary effects of Trx1 to treatment for cardiovascular patients.

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List of Abbreviations

AP-1

activator protein 1

ASK-1

apoptosis signal-regulating kinase 1

DN
dominant-negative

GPCR
G-protein–coupled receptor

HSF
heat shock factor

I/R
ischemia/reperfusion

MMP
matrix metalloproteinase

Msr
methionine sulfoxide reductases

NFκB
nuclear factor κB

NO
nitric oxide

NOS
nitric oxide synthase

PGC-1α
PPARγ coactivator-1α, PPAR, peroxisome proliferator-activated receptor

PTEN
Phosphatase and Tensin homolog

Ref-1
redox factor 1

ROS
reactive oxygen species

SOD
superoxide dismutase

TAC
thoracic aortic constriction

Tg-Trx1
transgenic mice with cardiac specific thioredoxin-overexpression

TIMP
tissue inhibitors of matrix metalloproteinase

Trx
thioredoxin

TrxR
thioredoxin reductase

TXNIP

thioredoxin-interacting protein

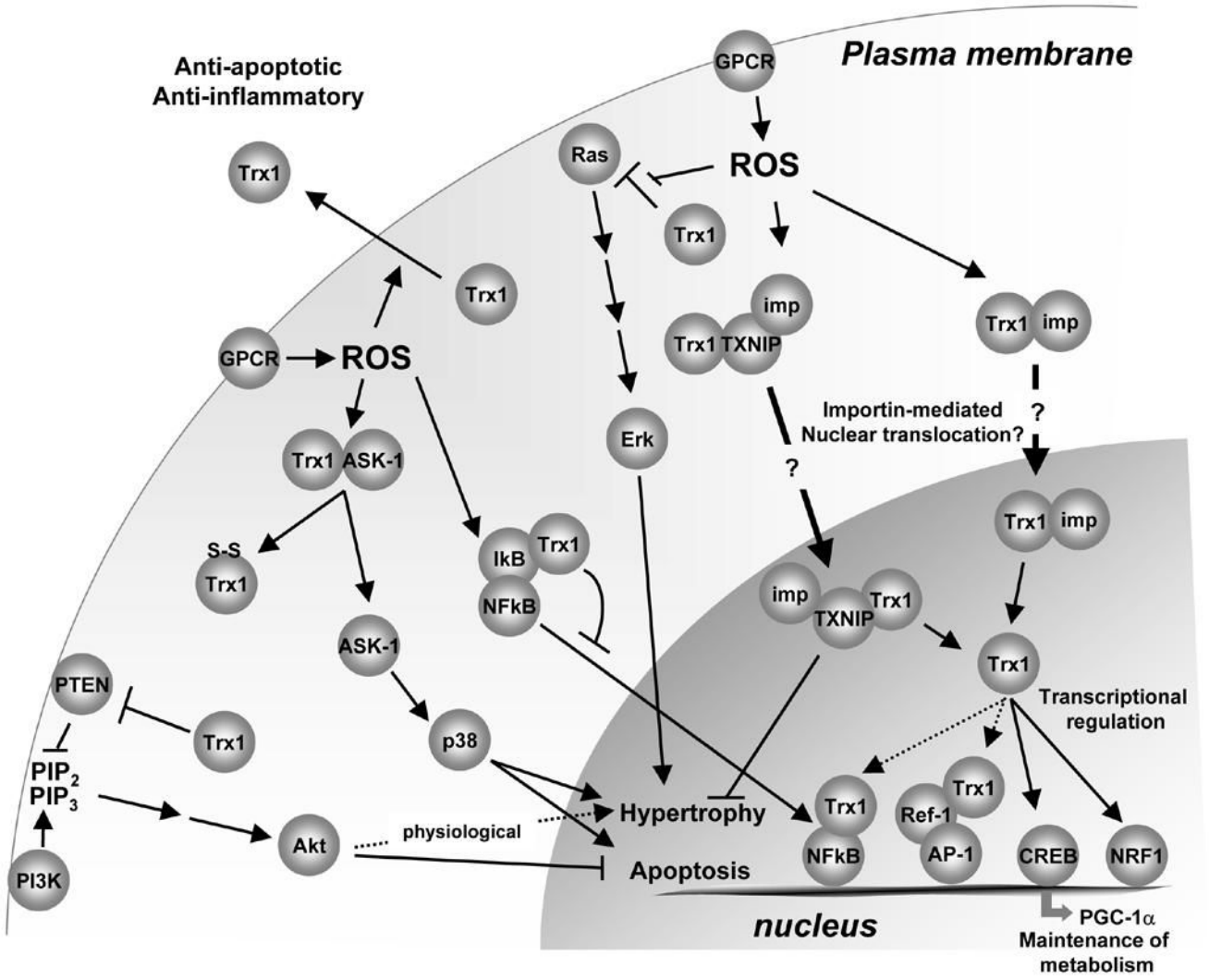

Figure 1. Anti-hypertrophic and anti-apoptotic effects of Trx1

Trx1 is normally localized in the cytosol, but translocates to the nucleus under stress conditions through direct interaction with importin (imp) or TXNIP. In the nucleus, Trx1 regulates the activity of various transcriptional factors. In the cytosol, Trx1 exerts anti-hypertrophic and anti-apoptotic effects by interacting with important signaling molecules, such as ASK-1, Ras, PTEN/Akt, and NFκB. Trx1 is also secreted to the extracellular space under stress conditions, thereby protecting neighboring cells.

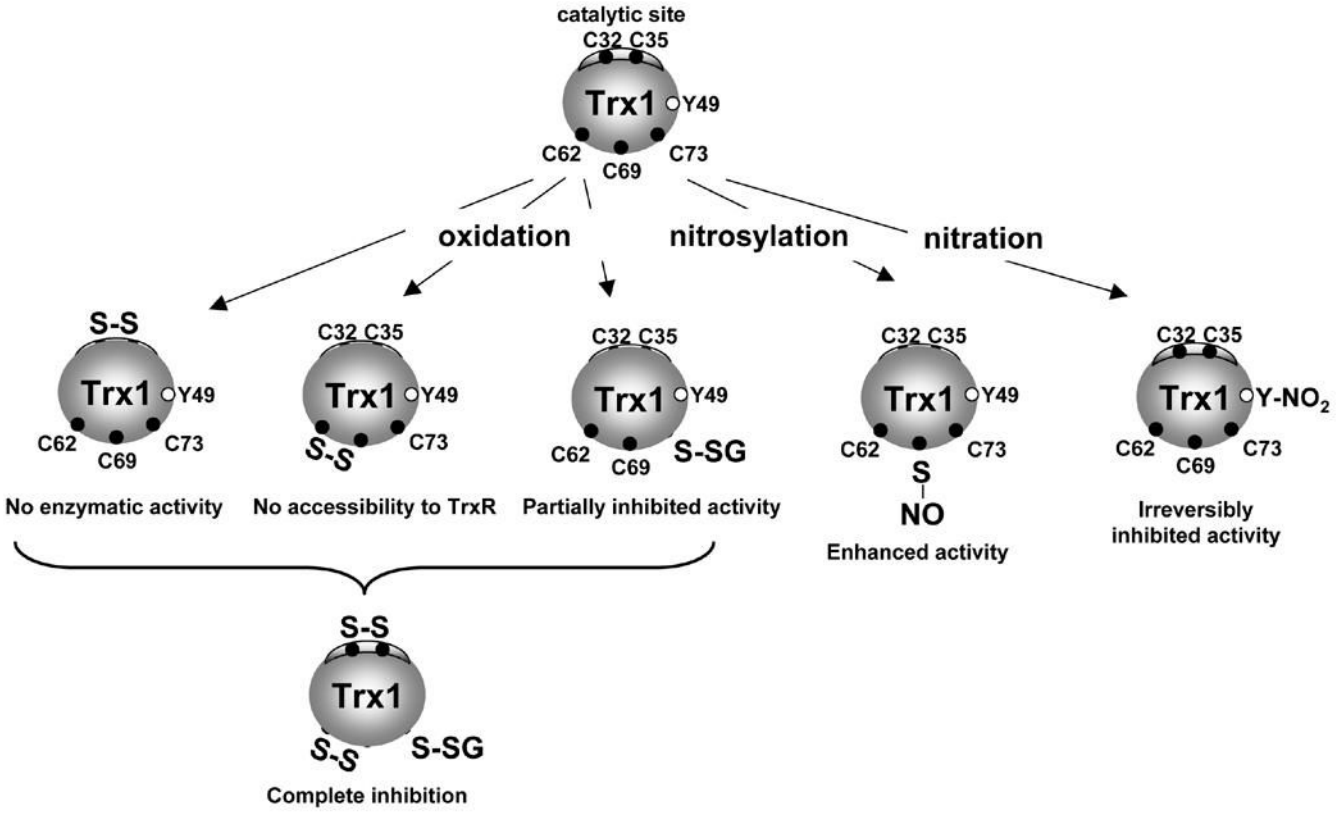

Figure 2. Post-translational modifications of Trx1

Trx1 has five modifiable cysteines (closed circles). Two of them, namely Cys-32 and Cys-35, in the catalytic site are involved in the enzymatic activity of Trx1. Under oxidative stresses, Trx1 forms disulfide bonds (S-S) between Cys-32 and Cys-35 and/or between Cys-62 and Cys-69, or a mixed disulfide bond with glutathione, called glutathionylation (S-SG). The activity of Trx1 or the accessibility of TrxR1 to Trx1 is inhibited by these oxidative modifications. On the other hand, Trx1 can be modified by NO and peroxynitrite. Trx1 is nitrosylated at Cys-69 (S-NO), which leads to the enhanced activity of Trx1. Trx1 has Tyr-49, which is nitrated by peroxynitrite. Nitration of Tyr-49 irreversibly suppresses the activity of Trx1.