Iranian Journal of Basic Medical Sciences
Vol. 14, No. 3, May-June 2011, 191-204
Received: Oct 23, 2010; Accepted: Feb 4, 2011

IJBMS

Review article

The Human Thioredoxin System: Modifications and Clinical Applications

*Seyed Isaac Hashemy

Abstract

The thioredoxin system, comprising thioredoxin (Trx), thioredoxin reductase (TrxR) and NADPH, is one of the major cellular antioxidant systems, implicated in a large and growing number of biological functions. Trx acts as an oxidoreductase via a highly conserved dithiol/disulfide motif located in the active site (-Trp-Cys-Gly-Pro-Cys-Lys-). Different factors are involved in the regulation of Trx activity, including its expression level, localization, protein-protein interactions, post-translational modifications and some chemical inhibitors. Mammalian TrxRs are selenoproteins which have a –Cys-Val-Asn-Val-Gly-Cys- N-terminal active site, as well as a C-terminal selenium-containing active site. Besides two Cys-residues in the redox-regulatory domain of cytosolic Trx (Trx1), human Trx1 has three additional Cys-residues. Post-translational modifications of human Trx1 which are involved in the regulation of its activity can happen via modification of Cys-residues including thiol oxidation, glutathionylation and S-nitrosylation or via modification of other amino acid residues such as nitration of Tyr-49. Because of the numerous functions of the thioredoxin system, its inhibition (mainly happens via the targeting TrxR) can result in major cellular consequences, which are potentially pro-oxidant in nature, leading to cell death via necrosis or apoptosis if overexpression of Trx and other antioxidative enzymes can not recuperate cell response. Considering this feature, several anticancer drugs have been used which can inhibit TrxR. Elevated levels of Trx and/or TrxR have been reported in many different human malignancies, positively correlated with aggressive tumor growth and poor prognosis. Moreover, anti-oxidative and anti-apoptotic effects of Trx are reasons to study its clinical application as a drug.

Keywords: Post-translational Modification, Thioredoxin, Thioredoxin Reductase

Department of Biochemistry, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
*Corresponding author: Tel: +98-511-8828573; Fax: +98-511-8828574; email: hashemyi@mums.ac.ir

Introduction

The thioredoxin system, comprising thioredoxin (Trx), thioredoxin reductase (TrxR) and NADPH, is one of the major antioxidant systems applied by cells to provide and maintain reduced states in intracellular environment (1) as well as defending against oxidative and nitrosative stress (2, 3).

The thioredoxin system is a major cellular protein disulfide reductase for which Trx provides electrons for protein disulfides reduction (1). After a non-covalent binding between a hydrophobic surface area around the active site of Trx and the target protein-disulfide, the target disulfide undergoes a nucleophilic attack by the surface-exposed N-terminal thiolate group of Trx's active site. This reaction leads to the transient formation of a mixed disulfide intermediate which is subsequently reduced by the C-terminal thiolate group, causing the reduction of the target protein-disulfide accompanied by the oxidation of the active site in Trx (Reaction 1). Thereafter, oxidized Trx is reduced by NADPH via a reaction catalyzed with TrxR (Reaction 2) (4). This thiol-disulfide exchange reaction is reversible and efficient for electron transport.

$$\text{Trx-}(SH)_2 + \text{Protein-}S_2 \longrightarrow \text{Trx-}S_2 + \text{Protein-}(SH)_2 \quad (1)$$

$$\text{Trx-}S_2 + NADPH + H^+ \xrightarrow{\text{TrxR}} \text{Trx-}(SH)_2 + NADP^+ \quad (2)$$

In addition, the Trx system is implicated in a large and growing number of other biological functions such as DNA synthesis and cell proliferation (5, 6), angiogenesis (7), control and regulation of numerous transcription factors activities (8-9), protecting cells against apoptosis (10, 11), as well as involvement in cell signaling pathways through the interaction with other proteins (12, 13).

Thioredoxin is a small protein, ubiquitously present in various species and tissues (14, 15); however, the active site is highly conserved (-Trp-Cys-Gly-Pro-Cys-Lys-) among species from bacteria to humans (16). In mammals, three different forms of Trx have been described which are Trx1, Trx2, and SpTrx. Trx1 is localized mainly in cytosol; however, it can be translocated to the nucleus with an unknown mechanism (17). This translocation has been reported to happen after treatment of cells with the cancer drug cisplatin (18), UV irradiation (19), H_2O_2 (20), and hypoxia (21). It is also detected in nucleus of normal cells (22) as well as tumors (23). Human Trx2 is a mitochondrial protein which has the conserved catalytic active site of Trx, but lacks the structural cysteine residues. SpTrxs are tissue-specific members of this family which do not have thiol-disulfide oxidoreductase activity despite their Trx domain (24).

Trx1 can be secreted from a variety of normal cells such as hepatocytes, fibroblasts, and activated monocytes and lymphocytes as well as transformed and cancer cells. For example, adult T cell leukemia-derived factor (ADF) which had been first identified as a growth factor secreted by human T lymphotropic virus I-transformed leukemic cell lines was later shown to be identical to human Trx (25). The precise mechanism of Trx1 secretion is unknown, but it mainly occurs under oxidizing conditions and inflammations (26). For example, elevated level of Trx in plasma has been reported during cardiovascular diseases such as heart failure and cardiomyopathy (27), abdominal aortic aneurysms (28), airway-related disorders like asthma (29), patients with human immunodeficiency virus (HIV) infection (30), rheumatoid arthritis (31), Sjogren's syndrome (32), type II diabetes (33), and sepsis (34). In a very recent study on first-episode schizophrenic patients, increased serum Trx was observed which was positively correlated with positive symptoms of schizophrenia (35). The elevated level of Trx1 has also been reported in patients with tumors, such as hepatocellular carcinoma (36) and pancreatic ductal carcinoma (23). The measurement of serum/plasma levels of Trx1 to evaluate the oxidative stress and prognosis in a number of diseases has been studied. For example, it is shown that the plasma level of Trx1 in HIV infection is negatively correlated with the levels of intracellular glutathione in lymphocytes and prognosis (37). The serum levels of Trx can

also be utilized to estimate the oxidative stress in chronic liver diseases such as nonalcoholic steatohepatitis (38) and hepatitis C virus infection (39), as well as cardiovascular disorders like acute ischemic heart disease (40) and chronic heart failure (41). When Trx1 is secreted, it is involved in a variety of physiologic and pathophysiologic functions. For example, secretory Trx1 acts as a co-cytokine and chemokine for immune cells, stimulating the growth of lymphocytes and in this way it is involved in immunomodulation (2, 42). Secreted Trx1 has also been suggested to be an autocrine growth factor for normal fibroblasts and several tumor cell lines (43). In addition, a truncated form of Trx (Trx80) which has the first 80 or 84 N-terminal amino acids is identified which has been found to be secreted or located to the surface of monocytic cell lines (44). Trx80 is present in human plasma, enhancing eosinophilic cytotoxic activity compared to wild type Trx1 (44, 45). It also has the chemotactic activities for monocytes and polymorphonuclear neutrophiles as Trx1 possesses (46). Trx80 lacks the oxidoreductase activity of Trx1 even though it has the conserved active site, and it is not a substrate for TrxR. However, Trx80 can be reduced by Trx1 (47).

Different factors are involved in the regulation of Trx activity, including its expression level, localization, protein-protein interaction, post-translational modifications, and the inhibition by chemical substances. It is also shown that Trx activity can be affected by a mechanical force. Wiita *et al* showed the inhibition of Trx activity and diminished antioxidant properties of the enzyme following the application of the increased mechanical forces (48). Regarding the expression of Trx1, there are different regulatory elements which affect the promoters of TXN1 gene and induce the transcription. For instance, antioxidant responsive elements can induce the expression of Trx1 upon oxidative stress (49). The promoter region of TXN1 gene also contains other stress-responsive elements such as oxidative response (50) and heat shock responsive elements (51). The promoter region of the human TXN1 gene has regulatory binding motifs compatible with both constitutive or inducible expression (52). The expression of Trx is reported to increase because of different stress stimuli in cells such as O_2 (53), H_2O_2 (54), photochemical oxidative stress, hypoxia (55), viral infections, lipopolysaccharide (56), X-radiation and UV irradiation (57). The expression of Trx is also up-regulated by TNF-α, estrogen, and prostaglandin E1, and following the treatment with certain drugs such as adriamycin and a histone deacetylase inhinitor-suberoylanilide hydroxamic acid (SAHA) (58), and during some diseases like heart failure, myocarditis and in atherosclerotic plaques (59). On the other hand, the expression of Trx is reported to be down-regulated by cathepsin D (60), SAHA (61) and hypertension (62). In fact under some certain conditions, the expression of Trx is either negatively or positively regulated, which can be explained by differences regarding cell types, cell conditions, or strength of stimulation. Post-translational modifications of Trx1 as well as the inhibitory effects of some chemicals will be discussed in details later.

Thioredoxin reductases are the only enzymes which are able to reduce the active site of Trxs. Similar to Trxs, they are ubiquitously present in all living cells. Mammalian TrxRs are dimeric selenoproteins which have a conserved –Cys-Val-Asn-Val-Gly-Cys- N-terminal active site (63). Another prominent feature of mammalian TrxRs is their C-terminal selenium-containing active site which is not present in TrxRs of lower organisms (64). The penultimate carboxyl-terminal –Gly-Cys-Sec-Gly motif is essential for the redox activity of mammalian TrxRs (63), particularly regarding the Sec residue. Three isoenzymes for mammalian TrxRs have been introduced: TrxR1 which is mainly targeted to cytosol (65), TrxR2 which is in mitochondria (66), and the testis specific thioredoxin glutathione reductase (TGR) (67). The expression and intracellular concentration of TrxR1 can increase under prolonged oxidizing conditions, such as exposure of cells to H_2O_2 (68).

Modifications of Thioredoxin System

The activity, structure, and/or localization of proteins can be modulated through the post-

translational modifications of their amino acid residues. These modifications can be reversible or irreversible; however the former is the mechanism of choice for cellular regulation and signal transduction. There are a variety of proteins which are subjected to different kinds of post-translational modifications, and some can be regulated by more than one type of modifications. Human Trx1 is an example that can be modulated via different modifications, which are suggested to be involved in signaling pathways.

Besides two Cys residues in the redox-regulatory domain of Trx1 which are common in all kingdoms of life, mammalian cytosolic Trxs have additional structural cysteines which are first reported to be involved in protein aggregation and inactivation via their oxidation (69). Human Trx1 has three additional Cys residues at positions 62, 69 and 73 which have unknown biological functions (Fig. 1); however, it is suggested that they can be modified leading to the regulation of Trx1 activity. Among these structural cysteine residues, Cys 73 is the closest one to the active site in the three-dimensional structure of Trx, protruding from the surface.

Post-translational modifications of human Trx1 which are involved in the regulation of its activity can happen via modification of Cys residues including thiol oxidation, glutathionylation and S-nitrosylation or it may occur via modification of other amino acid residues such as nitration of Tyr 49.

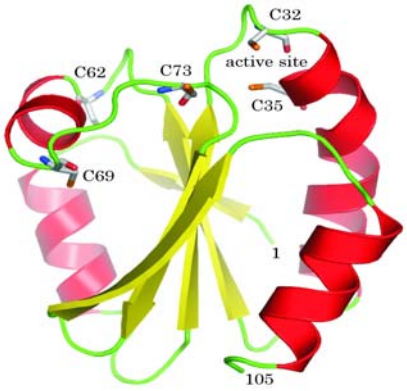

Figure 1. Human cytosolic Trx1 (PDB code: 1ERT)

An intermolecular disulfide bond has been identified via Cys 73 residues of two Trx1 molecules, suggested to be involved in homodimerization (70); though it is not essential for dimerization (71). Homodimerization of human Trx1 in solution occurs especially under strong oxidizing conditions or when it is stored at high concentrations. After homodimerization of Trx1 via a disulfide bond between two Cys 73 residues, the active site is inaccessible to TrxR, and Trx activity is therefore inhibited (71, 72). The physiological importance of Trx1 dimerization is not known. Interestingly, the homodimerization of Trx in the oxidizing extracellular environment limits the growth-stimulating effects of this protein (73). Besides Cys 73, other structural cysteines are also involved in the formation of intermolecular disulfides since under strong oxidizing conditions, the formation of oligomers has also been reported (74).

Under relatively mild oxidizing conditions, a second intramolecular disulfide bond may form between Cys 62 and Cys 69; which is not a substrate for TrxR and prohibits the reduction of active site disulfide by TrxR (75, 76). This inhibitory effect is probably mediated via attenuation the accessibility of TrxR to Trx1. The disulfide between Cys 62 and Cys 69 can be reduced by the active site of Trx1 (74). The formation of this intramolecular disulfide bond between two structural cysteines by diamide has been identified by mass scpectrometry, suggested to be involved in providing a redox mechanism for control of Trx1 function as well as providing more time for redox-dependent signaling processes (74, 76).

S-glutathionylation, another kind of oxidative post-translational modification of proteins, is a reversible process that is suggested to be involved in the buffering of oxidative stress as well as the protection of proteins against irreversible oxidation and regulation of protein activity (77). S-glutathionylation of Trx1 is shown to occur at Cys 73 both *in vitro* as shown by mass spectrometry and *in vivo* after treatment of cells with diamide which is a strong oxidant generating oxidized glutathione (GSSG) (78). It happens during oxidative stress via formation of a mixed disulfide between the protein and reduced glutathione (GSH) (78).

Even though glutathionylation may occur under physiological conditions, Trx1 could only become glutathionylated under oxidative stress. The enzymatic oxidoreductase activity of Trx1 was shown to be inhibited because of this modification; however, it is reversible via a process of auto-activation with sigmoidal kinetics. Glutathionylation of Trx has also been demonstrated in plants under conditions of oxidative stress (79).

Trx1 can also be modified by reactive nitrogen species, leading to S-nitrosylation of the protein. For the first time, *S*-nitrosylation of Trx1 was reported by Haendeler *et al* in 2002. They reported that human cytosolic Trx is nitrosylated on Cys 69 under basal conditions, a modification which was necessary for its anti-apoptotic function, scavenging reactive oxygen species as well as the redox regulatory activity via enzymatic activation (80). Thereafter, this modification has been extensively studied, leading to controversial results, showing the complexity of this area of research. In another study which was performed by Mitchell and Marletta, the S-nitrosylation of Trx1 was studied by mass spectrometry, immunological methods and site-directed mutagenesis. They reported that after treating His-tagged Trx1 with 50 molar equivalents of nitrosoglutathione (GSNO), a nitrosylating agent, no modification of Cys 73 could be detected; Cys 69 was however fully nitrosylated and a disulfide bridge was formed between two cysteine residues in the active site (81). In this study, they also showed a transnitrosation process between caspase-3 and Trx1, which was proved later to be required for S-nitrosation of procaspase-3 and the inhibition of apoptosis in Jurkat cells (82). In addition to caspase-3, Trx is involved in the denitrosylation of several other proteins such as metallothionein, albumin and peroxiredoxin 1 (83, 85) as well as low molecular weight compounds containing nitrosothiols such as GSNO (86), S-nitroso-$_L$-cysteine (CysSNO), S-nitroso-$_L$-homocysteine (HCysSNO), and S-nitroso-N-acetylpenicillamine (SNAP) without any significant substrate selectivity (87). Sengupta *et al* showed that all HepG2 cell-derived S-nitrosoproteins with a molecular mass of 23-30 kDa are substrates for the Trx system.

The first crystal structural study of nitrosylated Trx1 was done by Weichsel *et al* (88, 89). In this study, GSNO-treated human Trx1 did not exhibit any modification of Cys 73. However, a disulfide bond was formed between Cys 32 and Cys 35, and Cys 62 and Cys 69 were nitrosylated. This nitrosylation was relatively stable, even in the presence of 1 mM glutathione, and showed a pH-dependent pattern, i.e. at pH 7.0, 1 mol of S-NO/mol of Trx was found corresponding to the nitrosylation of Cys 62. While both Cys 62 and Cys 69 became nitrosylated at pH 9. At pH 5.6, no nitrosylation was detected (88).

Regarding to this modification, I and my colleague showed that the fully reduced protein was nitrosylated by GSNO on both Cys 69 and Cys 73 *in vitro*; Cys 32 and Cys 35 which are in the active site of Trx1 were not nitrosylated by GSNO, but became oxidized to a disulfide bond. In addition, Pro 34 was involved in protection of the active site against S-nitrosylation since the *E. coli* P34H Trx1 was sensitive to S-nitrosylation (74, 90).

Interestingly, human cytosolic glutaredoxin (Grx1) which has a high homology to Trx1 with three structural cysteines besides its two Cys residues in the active site does not show a same pattern of modification in response to nitrosylating agents such as GSNO (91). Under anaerobic conditions, all three structural cysteines of human Grx1 can be S-nitrosylated, suggesting that S-nitrosylation of human Trx1 on only two of three structural cysteines should be a specific rather than a random modification.

The physiological and pathophysiological significance of S-nitrosylation of mammalian Trxs as well as its kinetics remain to be fully explored. Nitrosylation of Trx1 may provide a protective mechanism against irreversible oxidation of thiol groups or inactivation of the protein via disulfide formation between structural cysteines during oxidative and nitrosative stress. Nitrosylation of hTrx1 might be a protective effect against nitrative stress by removing NO, leading to the inhibition of $ONOO^-$ formation.

Moreover, Trx1 is reported to be affected by peroxynitrite via nitration of Tyr 49. Mammalian Trx1 has only one tyrosine which is located within a region that is essential for its folding. Nitration of this tyrosine residue leads to an irreversible inhibition of Trx1 redox regulatory activity through a conformational change. Nitrated Trx1 loses its antiapoptotic and cardioprotective effects as well (92), specially under hyperglycemic condition (93). The nitration of Trx1 also results in its dissociation from ASK-1, leading to ASK-1 activation and apoptosis (92). The decrease of Trx activity in the aging heart was reported which was due to the post-translational nitrative modification and resulted in apoptotic cardiomyocyte death. According to this study, the expression of Trx is increased in the aging heart; and therefore, it does not contribute to the reduction of Trx activity (94). Peroxynitrite is also shown to inhibit the activity of TrxR *in vivo* via a reaction with the selenocysteine residue, a reaction which is irreversible (95).

In a very recent study, glycation of Trx was reported for the first time as a novel post-translational modification, inhibiting the oxidoreductase activity of Trx. But aminoacids which are involved in this modification are still unknown (96).

The importance of other amino acids in the structure of Trxs and their effects on the activity and/or structure of these proteins has not been investigated as much as the Cys residues. However, there are several studies in this field which are mainly based on the mutagenesis of Trxs to investigate the effects on protein characteristics. For example, an extensive study has shown that two amino acids which are located in the active site between two redox active cysteines are crucial for the redox properties of Trx (97).

Inhibitors of thioredoxin system and clinical applications

Because of the numerous functions of the thioredoxin system, the inhibition of this oxidoreductase system which mainly happens via the targeting TrxR can result in major cellular consequences, e.g. less total antioxidant capacity, impairment of the whole reductive capacity of cells, increased levels of reactive oxygen species (ROS) and the subsequent oxidative stress, decreased GSH levels and increased GSSG and glutathionylated proteins, increased expression and secretion of Trx, increased expression of TrxR, and increased secretion of NF-κB- dependent proteins (98). Because of these effects which are potentially pro-oxidant in nature, inhibition of the Trx system results in cell death via necrosis or apoptosis if overexpression of Trx and other antioxidative enzymes can not recuperate the cell response (99).

On the other hand, elevated levels of Trx and/or TrxR have been reported in many different human malignancies such as lung, hepatic, colorectal, pancreatic, thyroid, prostate and cervical cancer (100-102). The level of Trx and TrxR is positively correlated with aggressive tumor growth, poor prognosis and decreased patient survival (103-104). In a recent study, a positive correlation between Trx expression and the relapse of acute myeloid leukemia was reported (105). However, a relationship between Trx catalytic activity and tumor growth and stage is less clear. Yoo *et al* showed a direct role in carcinogenesis for TrxR1 *in vivo*, implicating that the enzyme is essential for the growth of a murine tumor (106).

Moreover, several studies suggest that Trx may confer resistance to the cytotoxic effects of anti-cancer drugs. In one study with several human bladder and prostatic cancer cell lines resistant to cis-diamminedichloroplatinum(II) (cisplatin), all drug-resistant cell lines had much higher levels of thioredoxin than drug-sensitive cells. By introducing thioredoxin antisense expression plasmids into drug-resistant cell lines, increased sensitivity to cisplatin and also to other superoxide-generating agents, i.e., doxorubicin, mitomycin C, etoposide, and hydrogen peroxide, as well as to UV irradiation was observed (107). Tumor sensitivity to cisplatin is also negatively correlated with mRNA levels of Trx in hepatocellular carcinoma (108) and human cervical carcinoma cell lines (109). Resistance to adriamycin is also reported in various T-cell leukemia cell lines including adult T-cell leukemia (ATL) cell lines with high

expression of thioredoxin (110). In another study, the positive correlation between thioredoxin expression and resistance to docetaxel therapy in breast cancer patients was reported (111). This effect may be due to the role of Trx in cell survival and its anti-apoptotic functions, or may be derived from the scavenging of ROS which are increased during chemotherapy or radiation therapy, leading to the apoptosis.

The Trx system provides tumor cells with a survival advantage through different mechanisms, such as its anti-apoptotic effects, role as a growth factor, stimulating angiogenesis, etc. Hence, this enzymatic system is an appealing target for anticancer therapy (112). Since thioredoxin reductase has a crucial role in the thioredoxin system and it has a highly reactive C-terminal active site which can be targeted by different electrophilic compounds, TrxR has been particularly considered as a target of anticancer agents (113). Another feature of TrxR which makes it a good target for antitumor therapy is its inducible NADPH-oxidase activity which leads to formation of ROS. DNCB (1-chloro-2,4-dinitrobenzene) is an electrophilic compound which irreversibly inhibits the activity of mammalian TrxRs with second order kinetics (114). This effect is due to the alkylation of both Cys and Sec residues in the C-terminal active site of NADPH-reduced TrxR (115). But upon alkylation, DNCB induces the NADPH oxidase activity of TrxR about 30-fold higher than the enzyme. This effect is dependent on the presence of oxygen, leading to an aerobic redox cycling and the formation of ROS (114). It has also been shown that certain anticancer drugs such as cisplatin as well as the chemopreventive agent curcumin form selenium-compromised thioredoxin reductase with an enhanced NADPH oxidase activity, leading to the production of more ROS and causing oxidative stress (109). In fact, these compounds switch TrxR from an antioxidant to a prooxidant. The irreversible inhibition of TrxR by curcumin is dose- and time-dependent and the curcumin-modified enzyme shows a strongly induced NADPH oxidase activity producing ROS (116). Motexafin gadolinium (MGd) is another cytotoxic drug which was initially introduced as a radiation sensitizer, selectively accumulates in tumor cells (117) and binds to TrxR, leading to the induction of its NADPH-oxidase activity as well as the inhibition of the protein-disulfide reductase activity of the enzyme (118).

There are several anticancer compounds which are in clinical use and can inhibit TrxR, like retinoic acid (119), nitrosoureas such as carmustine (bis-chloroethyl-nitrosurea, BCNU) and fotemustine (120), platinum compounds such as cisplatin (109), and quinones (121).

The inhibition of TrxR by BCNU and other nitrosourea drugs is irreversible and happens through carbamoylation of a cysteine in the catalytic site of reduced TrxR; hence oxidized enzyme is not a target for these compounds (122). Similar to nitrosourea drugs, cisplatin irreversibly inhibits the NADPH-reduced (but not oxidized) TrxR; however via covalent modification of the reduced selenocysteine residue. Recently, new platinum compounds have been synthesized which inhibit TrxR irreversibly with nanomolar concentrations directed to the C-terminal active site and micromolar affinities for the N-terminal active site (112). Phosphole complexes containing Au- and Pt-phospholes are new drug candidates which inhibit TrxR both *in vitro* and in cell culture (123). TrxR is also a target for arsenic trioxide which is an effective anticancer drug for acute promyelocytic leukemia with potential therapeutic applications in a wide range of solid tumors (124).

The reducing activity of the Trx system can also be inhibited by an organotellurium analog of vitamin E which is able to inhibit the growth of tumor cells (125). Azelaic acid is another example of a suggested TrxR inhibitor with growth inhibitory effect on skin cells. Azelaic acid was proposed to competitively inhibit the electron transfer from the enzyme's active site (126). 1, 2-[bis(1,2-benzisoselenazolone-3(2H)-ketone)]ethane (BBSKE) is a novel organoselenium compound which was shown to be another thioredoxin reductase inhibitor with the inhibitory effect on the growth of a variety of human cancer cells (127). Wang *et al* showed the inhibition of TrxR *in vivo* as a molecular mechanism for ifosfamide, an oxazaphosphorine alkylating agent with a broad spectrum of

antineoplastic activity in both adults and children (128). In this study, a transient inactivation of TrxR with an unknown mechanism was detected which was followed by a dramatic deceleration in tumor progression. This inhibitory effect was relatively specific to TrxR, since other antioxidants such as catalase, superoxide dismutase and glutathione S-transferase were not affected. The *in vivo* inhibition of TrxR has also been shown to be involved in antitumor effects of cyclophosphamide (129).

Mammalian TrxRs can also be inhibited by a variety of low molecular weight electrophilic compounds such as thiol-alkylating compounds including 4- vinyl pyridine (115) and iodo-acetic acid (115), arsenicals (130), nitroaromatic compounds such as tetryl (131), quinone compounds (132), polyphenols (133), flavonoids (134), gold compounds (135), and dinitrohalobenzenes, e.g., DNCB (115). Gold is well-known for its affinity to thiols. Two gold-containing antirheumatic drugs, auranofin and aurothioglucose, can inactivate the NADPH-reduced form of TrxR at nanomolar concentrations via binding to the Sec residue (136). As mentioned above, DNCB is an irreversible inhibitor of human TrxR which modifies the selenocysteine residue and is uniquely capable of inducing the NADPH oxidase activity of TrxR simultaneously, leading to the formation of superoxide (114). The fluoride analoge to DNCB, 1-fluoro-2, 4-dinitrobenzene (DNFB), is also an inhibitor of TrxR and induces the NADPH oxidase activity as well. Recently, Wataha *et al* showed the potent inhibition of TrxR1 activity by Hg (II) in both cell-free and intracellular assays using monocytes. This inhibition was dose-dependent and assumed to be mediated via thiol or selenol dependent mechanism. This mercurial compound is also shown to result in a transient decrease in Trx1 level which is suggested to happen because of Trx1 secretion (137). In 2008, I and my colleague analyzed the effects of mercuric chloride ($HgCl_2$) and monomethylmercury (MeHg) on the Trx system both *in vitro* and *in vivo*, showing that the inhibition of recombinant rat TrxR as well as the binding between fully reduced human Trx1 and mercury led to its oxidation and loss of the activity (138). Later, the inhibitory effect of methylmercury on mice thioredoxin reductase was also shown (139). The inhibitory effect of mercury on TrxR activity is reversible by selenite (140).

Since the Trx system has crucial roles in cell survival, the inhibition of this system leads to different side effects. In order to partially overcome this problem, the inhibition of TrxR can be directed specifically to one of different isoenzymes. For example, mitochondrial TrxR2 can be selectively inhibited by organogold(III) complexes, leading to Ca^{2+}-dependent mitochondrial membrane permeability and cytochrome C release (141).

On the other hand, several compounds have been reported to affect the Trx system via the modification of Trx. PX-12 is such a drug, a substituted 2-imidazolyl disulfide which is in phase I clinical trial in patients with advanced metastatic cancer. PX-12 affects the activity of Trx via thioalkylation of Cys 73, and the effect is irreversible (142). Pleurotin is another inhibitor of Trx which decreases angiogenesis in cancer cells via the inhibition of hypoxia-induced factor-1α (HIF-1α) and vascular endothelial growth factor (VEGF) formation (142).

The anti-oxidative and anti-apoptotic effects of Trx have been reasons for some investigators to study the clinical application of Trx as a drug. This approach seems especially appealing because of the small size and stable structure of Trx, which can function both intracellularly and extracellularly. This goal can be achieved by means of exogenous Trx which can enter into cells or via the induction of Trx1 expression. The administration of recombinant human Trx and its therapeutic advantages have been studied for acute lung injury (143), and cerebral ischemia (144). The protective effect of exogenous Trx in cardiovascular diseases such as autoimmune myocarditis (145), reperfusion-induced arrhythmias (146), myocardial apoptosis and infarct size (147), and age-induced cardiac hypertrophy and fibrosis (148) has also been shown. The application of Trx1 gene therapy for the regulation of angiogenic signaling and ventricular remodeling in infarcted myocardium of diabetic rats was also recently reported (149).

References

1. Holmgren A. Thioredoxin. Annu Rev Biochem 1985; 54:237-271.
2. Nakamura H, Nakamura K, Yodoi J. Redox regulation of cellular activation. Annu Rev Immunol 1997; 15:351-369.
3. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature 2000 9; 408:239-247.
4. Holmgren A. Thioredoxin structure and mechanism: conformational changes on oxidation of the active-site sulfhydryls to a disulfide. Structure 1995; 3:239-243.
5. Laurent TC, Moore EC, Reichard P. Enzymatic synthesis of deoxyribonucleotides. Iv. Isolation and characterization of thioredoxin, the Hydrogen donor from escherichia Coli B. J Biol Chem 1964 ; 239:3436-3444.
6. Luthman M, Eriksson S, Holmgren A, Thelander L. Glutathione-dependent hydrogen donor system for calf thymus ribonucleoside-diphosphate reductase. Proc Natl Acad Sci U S A 1979; 76:2158-2162.
7. Welsh SJ, Bellamy WT, Briehl MM, Powis G. The redox protein thioredoxin-1 (Trx-1) increases hypoxia-inducible factor 1alpha protein expression: Trx-1 overexpression results in increased vascular endothelial growth factor production and enhanced tumor angiogenesis. Cancer Res 2002; 62:5089-5095.
8. Matthews JR, Wakasugi N, Virelizier JL, Yodoi J, Hay RT. Thioredoxin regulates the DNA binding activity of NF-kappa B by reduction of a disulphide bond involving cysteine 62. Nucleic Acids Res 1992; 20:3821-3830.
9. Abate C, Patel L, Rauscher FJ, 3rd, Curran T. Redox regulation of fos and jun DNA-binding activity in vitro. Science. 1990; 249:1157-1161.
10. Powis G, Kirkpatrick DL, Angulo M, Baker A. Thioredoxin redox control of cell growth and death and the effects of inhibitors. Chem Biol Interact 1998; 111-112:23-34.
11. Powis G, Montfort WR. Properties and biological activities of thioredoxins. Annu Rev Pharmacol Toxicol. 2001;41:261-95.
12. Saitoh M, Nishitoh H, Fujii M, Takeda K, Tobiume K, Sawada Y, *et al*. Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. Embo J 1998; 17:2596-2606.
13. Junn E, Han SH, Im JY, Yang Y, Cho EW, Um HD, *et al*. Vitamin D3 up-regulated protein 1 mediates oxidative stress via suppressing the thioredoxin function. J Immunol 2000; 164:6287-6295.
14. Holmgren A. Thioredoxin. VI. The amino acid sequence of the protein from *Escherichia coli* B. Eur J Biochem 1968; 6:475-484.
15. Holmgren A. Thioredoxin and glutaredoxin systems. J Biol Chem 1989; 264:13963-13966.
16. Eklund H, Gleason FK, Holmgren A. Structural and functional relations among thioredoxins of different species. Proteins 1991; 11:13-28.
17. Imamoto N, Kamei Y, Yoneda Y. Nuclear transport factors: function, behavior and interaction. Eur J Histochem 1998; 42:9-20.
18. Ueno M, Masutani H, Arai RJ, Yamauchi A, Hirota K, Sakai T, *et al*. Thioredoxin-dependent redox regulation of p53-mediated p21 activation. J Biol Chem 1999; 274:35809-35815.
19. Masutani H, Hirota K, Sasada T, Ueda-Taniguchi Y, Taniguchi Y, Sono H, *et al.* Transactivation of an inducible anti-oxidative stress protein, human thioredoxin by HTLV-I Tax. Immunol Lett 1996 ; 54:67-71.
20. Makino Y, Yoshikawa N, Okamoto K, Hirota K, Yodoi J, Makino I, *et al*. Direct association with thioredoxin allows redox regulation of glucocorticoid receptor function. J Biol Chem 1999 29; 274:3182-3188.
21. Ema M, Hirota K, Mimura J, Abe H, Yodoi J, Sogawa K, *et al*. Molecular mechanisms of transcription activation by HLF and HIF1alpha in response to hypoxia: their stabilization and redox signal-induced interaction with CBP/p300. Embo J 1999; 18:1905-1914.
22. Maruyama T, Sachi Y, Furuke K, Kitaoka Y, Kanzaki H, Yoshimura Y, *et al.* Induction of thioredoxin, a redox-active protein, by ovarian steroid hormones during growth and differentiation of endometrial stromal cells *in vitro*. Endocrinology 1999; 140:365-3672.
23. Nakamura H, Bai J, Nishinaka Y, Ueda S, Sasada T, Ohshio G, *et al*. Expression of thioredoxin and glutaredoxin, redox-regulating proteins, in pancreatic cancer. Cancer Detect Prev 2000; 24:53-60.
24. Miranda-Vizuete A, Sadek CM, Jimenez A, Krause WJ, Sutovsky P, Oko R. The mammalian testis-specific thioredoxin system. Antioxid Redox Signal 2004; 6:25-40.
25. Tagaya Y, Maeda Y, Mitsui A, Kondo N, Matsui H, Hamuro J, *et al*. ATL-derived factor (ADF), an IL-2 receptor/Tac inducer homologous to thioredoxin; possible involvement of dithiol-reduction in the IL-2 receptor induction. Embo J 1989; 8:757-764.
26. Rubartelli A, Bajetto A, Allavena G, Wollman E, Sitia R. Secretion of thioredoxin by normal and neoplastic cells through a leaderless secretory pathway. J Biol Chem 1992; 267:24161-24164.
27. Kishimoto C, Shioji K, Nakamura H, Nakayama Y, Yodoi J, Sasayama S. Serum thioredoxin (TRX) levels in patients with heart failure. Jpn Circ J 2001; 65:491-494.
28. Martinez-Pinna R, Lindholt JS, Blanco-Colio LM, Dejouvencel T, Madrigal-Matute J, Ramos-Mozo P, *et al*. Increased levels of thioredoxin in patients with abdominal aortic aneurysms (AAAs). A potential link of oxidative stress with AAA evolution. Atherosclerosis 2010; 212:333-338.

29. Yamada Y, Nakamura H, Adachi T, Sannohe S, Oyamada H, Kayaba H, *et al*. Elevated serum levels of thioredoxin in patients with acute exacerbation of asthma. Immunol Lett 2003; 86:199-205.
30. Nakamura H, De Rosa S, Roederer M, Anderson MT, Dubs JG, Yodoi J, *et al*. Elevation of plasma thioredoxin levels in HIV-infected individuals. Int Immunol 1996; 8:603-611.
31. Yoshida S, Katoh T, Tetsuka T, Uno K, Matsui N, Okamoto T. Involvement of thioredoxin in rheumatoid arthritis: its costimulatory roles in the TNF-alpha-induced production of IL-6 and IL-8 from cultured synovial fibroblasts. J Immunol 1999; 163:351-358.
32. Kumagai S. [ADF/thioredoxin as an indicator of oxidative stress]. Rinsho Byori 1998 ; 46:574-580.
33. Kakisaka Y, Nakashima T, Sumida Y, Yoh T, Nakamura H, Yodoi J, *et al*. Elevation of serum thioredoxin levels in patients with type 2 diabetes. Horm Metab Res 2002; 34:160-164.
34. Leaver SK, MacCallum NS, Pingle V, Hacking MB, Quinlan GJ, Evans TW, *et al*. Increased plasma thioredoxin levels in patients with sepsis: positive association with macrophage migration inhibitory factor. Intensive Care Med 2010; 36:336-341.
35. Zhang XY, Chen da C, Xiu MH, Wang F, Qi LY, Sun HQ, *et al*. The novel oxidative stress marker thioredoxin is increased in first-episode schizophrenic patients. Schizophr Res 2009; 113:151-157.
36. Miyazaki K, Noda N, Okada S, Hagiwara Y, Miyata M, Sakurabayashi I, *et al.* Elevated serum level of thioredoxin in patients with hepatocellular carcinoma. Biotherapy 1998; 11:277-288.
37. Nakamura H, De Rosa SC, Yodoi J, Holmgren A, Ghezzi P, Herzenberg LA, *et al*. Chronic elevation of plasma thioredoxin: inhibition of chemotaxis and curtailment of life expectancy in AIDS. Proc Natl Acad Sci U S A 2001; 98:2688-2693.
38. Nakashima T, Sumida Y, Furutani M, Hirohama A, Okita M, Mitsuyoshi H, *et al*. Elevation of serum thioredoxin levels in patients with nonalcoholic steatohepatitis. Hepatol Res 2005; 33:135-137.
39. Sumida Y, Nakashima T, Yoh T, Nakajima Y, Ishikawa H, Mitsuyoshi H, *et al*. Serum thioredoxin levels as an indicator of oxidative stress in patients with hepatitis C virus infection. J Hepatol 2000; 33:616-622.
40. Miyamoto S, Kawano H, Sakamoto T, Soejima H, Kajiwara I, Hokamaki J, *et al*. Increased plasma levels of thioredoxin in patients with coronary spastic angina. Antioxid Redox Signal 2004; 6:75-80.
41. Shioji K, Nakamura H, Masutani H, Yodoi J. Redox regulation by thioredoxin in cardiovascular diseases. Antioxid Redox Signal 2003; 5:795-802.
42. Bertini R, Howard OM, Dong HF, Oppenheim JJ, Bizzarri C, Sergi R, *et al*. Thioredoxin, a redox enzyme released in infection and inflammation, is a unique chemoattractant for neutrophils, monocytes, and T cells. J Exp Med 1999; 189:1783-1289.
43. Gasdaska JR, Berggren M, Powis G. Cell growth stimulation by the redox protein thioredoxin occurs by a novel helper mechanism. Cell Growth Differ 1995; 6:1643-1650.
44. Pekkari K, Gurunath R, Arner ES, Holmgren A. Truncated thioredoxin is a mitogenic cytokine for resting human peripheral blood mononuclear cells and is present in human plasma. J Biol Chem 2000 ; 275:37474-37480.
45. Silberstein DS, Ali MH, Baker SL, David JR. Human eosinophil cytotoxicity-enhancing factor. Purification, physical characteristics, and partial amino acid sequence of an active polypeptide. J Immunol 1989; 143:979-983.
46. Bizzarri C, Holmgren A, Pekkari K, Chang G, Colotta F, Ghezzi P, *et al*. Requirements for the different cysteines in the chemotactic and desensitizing activity of human thioredoxin. Antioxid Redox Signal 2005; 7:1189-1194.
47. Pekkari K, Holmgren A. Truncated thioredoxin: physiological functions and mechanism. Antioxid Redox Signal 2004;6:53-61.
48. Wiita AP, Perez-Jimenez R, Walther KA, Grater F, Berne BJ, Holmgren A, *et al*. Probing the chemistry of thioredoxin catalysis with force. Nature 2007; 450:124-127.
49. Kim YC, Masutani H, Yamaguchi Y, Itoh K, Yamamoto M, Yodoi J. Hemin-induced activation of the thioredoxin gene by Nrf2. A differential regulation of the antioxidant responsive element by a switch of its binding factors. J Biol Chem 2001; 276:18399-18406.
50. Taniguchi Y, Taniguchi-Ueda Y, Mori K, Yodoi J. A novel promoter sequence is involved in the oxidative stress-induced expression of the adult T-cell leukemia-derived factor (ADF)/human thioredoxin (Trx) gene. Nucleic Acids Res 1996; 24:2746-2752.
51. Leppa S, Pirkkala L, Chow SC, Eriksson JE, Sistonen L. Thioredoxin is transcriptionally induced upon activation of heat shock factor 2. J Biol Chem 1997; 272:30400-30404.
52. Kaghad M, Dessarps F, Jacquemin-Sablon H, Caput D, Fradelizi D, Wollman EE. Genomic cloning of human thioredoxin-encoding gene: mapping of the transcription start point and analysis of the promoter. Gene 1994; 140:273-278.
53. Das KC, Guo XL, White CW. Induction of thioredoxin and thioredoxin reductase gene expression in lungs of newborn primates by oxygen. Am J Physiol 1999; 276:L530-539.
54. Higashikubo A, Tanaka N, Noda N, Maeda I, Yagi K, Mizoguchi T, *et al*. Increase in thioredoxin activity of intestinal epithelial cells mediated by oxidative stress. Biol Pharm Bull 1999; 22:900-903.

55. Berggren M, Gallegos A, Gasdaska JR, Gasdaska PY, Warneke J, Powis G. Thioredoxin and thioredoxin reductase gene expression in human tumors and cell lines, and the effects of serum stimulation and hypoxia. Anticancer Res 1996; 16:3459-3466.
56. Ejima K, Koji T, Nanri H, Kashimura M, Ikeda M. Expression of thioredoxin and thioredoxin reductase in placentae of pregnant mice exposed to lipopolysaccharide. Placenta 1999; 20:561-566.
57. Hoshi Y, Tanooka H, Miyazaki K, Wakasugi H. Induction of thioredoxin in human lymphocytes with low-dose ionizing radiation. Biochim Biophys Acta 1997; 1359:65-70.
58. Ungerstedt JS, Sowa Y, Xu WS, Shao Y, Dokmanovic M, Perez G, *et al*. Role of thioredoxin in the response of normal and transformed cells to histone deacetylase inhibitors. Proc Natl Acad Sci U S A 2005; 102:673-678.
59. Ago T, Sadoshima J. Thioredoxin1 as a negative regulator of cardiac hypertrophy. Antioxid Redox Signal 2007; 9:679-687.
60. Haendeler J, Popp R, Goy C, Tischler V, Zeiher AM, Dimmeler S. Cathepsin D and H_2O_2 stimulate degradation of thioredoxin-1: implication for endothelial cell apoptosis. J Biol Chem 2005; 280:42945-42951.
61. Butler LM, Zhou X, Xu WS, Scher HI, Rifkind RA, Marks PA, *et al*. The histone deacetylase inhibitor SAHA arrests cancer cell growth, up-regulates thioredoxin-binding protein-2, and down-regulates thioredoxin. Proc Natl Acad Sci U S A 2002; 99:11700-1175.
62. Tanito M, Nakamura H, Kwon YW, Teratani A, Masutani H, Shioji K, *et al.* Enhanced oxidative stress and impaired thioredoxin expression in spontaneously hypertensive rats. Antioxid Redox Signal 2004; 6:89-97.
63. Zhong L, Arner ES, Holmgren A. Structure and mechanism of mammalian thioredoxin reductase: the active site is a redox-active selenolthiol/selenenylsulfide formed from the conserved cysteine-selenocysteine sequence. Proc Natl Acad Sci U S A 2000; 97:5854-5859.
64. Zhong L, Arner ES, Ljung J, Aslund F, Holmgren A. Rat and calf thioredoxin reductase are homologous to glutathione reductase with a carboxyl-terminal elongation containing a conserved catalytically active penultimate selenocysteine residue. J Biol Chem 1998; 273:8581-8591.
65. Gasdaska PY, Gasdaska JR, Cochran S, Powis G. Cloning and sequencing of a human thioredoxin reductase. FEBS Lett 1995; 373:5-9.
66. Lee SR, Kim JR, Kwon KS, Yoon HW, Levine RL, Ginsburg A, *et al.* Molecular cloning and characterization of a mitochondrial selenocysteine-containing thioredoxin reductase from rat liver. J Biol Chem 1999; 274:4722-4734.
67. Sun QA, Kirnarsky L, Sherman S, Gladyshev VN. Selenoprotein oxidoreductase with specificity for thioredoxin and glutathione systems. Proc Natl Acad Sci U S A 2001; 98:3673-3678.
68. Sun QA, Wu Y, Zappacosta F, Jeang KT, Lee BJ, Hatfield DL, *et al.* Redox regulation of cell signaling by selenocysteine in mammalian thioredoxin reductases. J Biol Chem 1999; 274:24522-24530.
69. Engstrom NE, Holmgren A, Larsson A, Soderhall S. Isolation and characterization of calf liver thioredoxin. J Biol Chem 1974; 249:205-210.
70. Ren X, Björnstedt M, Shen B, Ericson ML, Holmgren A. Mutagenesis of structural half-cystine residues in human thioredoxin and effects on the regulation of activity by selenodiglutathione. Biochemistry 1993; 32:9701-9708.
71. Weichsel A, Gasdaska JR, Powis G, Montfort WR. Crystal structures of reduced, oxidized, and mutated human thioredoxins: evidence for a regulatory homodimer. Structure 1996; 4:735-751.
72. Holmgren A. Bovine thioredoxin system. Purification of thioredoxin reductase from calf liver and thymus and studies of its function in disulfide reduction. J Biol Chem 1977; 252:4600-4606.
73. Gasdaska JR, Kirkpatrick DL, Montfort W, Kuperus M, Hill SR, Berggren M, *et al.* Oxidative inactivation of thioredoxin as a cellular growth factor and protection by a Cys73-->Ser mutation. Biochem Pharmacol 1996; 52:1741-1747.
74. Hashemy SI, Holmgren A. Regulation of the catalytic activity and structure of human thioredoxin 1 via oxidation and S-nitrosylation of cysteine residues. J Biol Chem 2008; 283:21890-21898.
75. Luthman M, Holmgren A. Rat liver thioredoxin and thioredoxin reductase: purification and characterization. Biochemistry 1982; 21:6628-66233.
76. Watson WH, Pohl J, Montfort WR, Stuchlik O, Reed MS, Powis G, *et al*. Redox potential of human thioredoxin 1 and identification of a second dithiol/disulfide motif. J Biol Chem 2003; 278:33408-33415.
77. Cotgreave IA, Gerdes RG. Recent trends in glutathione biochemistry--glutathione-protein interactions: a molecular link between oxidative stress and cell proliferation? Biochem Biophys Res Commun 1998; 242:1-9.
78. Casagrande S, Bonetto V, Fratelli M, Gianazza E, Eberini I, Massignan T, *et al*. Glutathionylation of human thioredoxin: a possible crosstalk between the glutathione and thioredoxin systems. Proc Natl Acad Sci U S A 2002; 99:9745-9749.
79. Michelet L, Zaffagnini M, Marchand C, Collin V, Decottignies P, Tsan P, *et al*. Glutathionylation of chloroplast thioredoxin f is a redox signaling mechanism in plants. Proc Natl Acad Sci U S A 2005 ; 102:16478-16483.
80. Haendeler J, Hoffmann J, Tischler V, Berk BC, Zeiher AM, Dimmeler S. Redox regulatory and anti-apoptotic functions of thioredoxin depend on S-nitrosylation at cysteine 69. Nat Cell Biol 2002 ; 4:743-749.

81. Mitchell DA, Marletta MA. Thioredoxin catalyzes the S-nitrosation of the caspase-3 active site cysteine. Nat Chem Biol 2005; 1:154-158.
82. Mitchell DA, Morton SU, Fernhoff NB, Marletta MA. Thioredoxin is required for S-nitrosation of procaspase-3 and the inhibition of apoptosis in Jurkat cells. Proc Natl Acad Sci U S A 2007 ; 104:11609-11614.
83. Stoyanovsky DA, Tyurina YY, Tyurin VA, Anand D, Mandavia DN, Gius D, *et al*. Thioredoxin and lipoic acid catalyze the denitrosation of low molecular weight and protein S-nitrosothiols. J Am Chem Soc 2005; 127:15815-15823.
84. Wu C, Liu T, Chen W, Oka S, Fu C, Jain MR, *et al*. Redox regulatory mechanism of transnitrosylation by thioredoxin. Mol Cell Proteomics 2010; 9:2262-2275.
85. Benhar M, Thompson JW, Moseley MA, Stamler JS. Identification of S-Nitrosylated targets of thioredoxin using a quantitative proteomic approach. Biochemistry 2010; 49:6963-6969.
86. Nikitovic D, Holmgren A. S-nitrosoglutathione is cleaved by the thioredoxin system with liberation of glutathione and redox regulating nitric oxide. J Biol Chem 1996; 271:19180-19115.
87. Sengupta R, Ryter SW, Zuckerbraun BS, Tzeng E, Billiar TR, Stoyanovsky DA. Thioredoxin catalyzes the denitrosation of low-molecular mass and protein S-nitrosothiols. Biochemistry 2007 ; 46:8472-8483.
88. Weichsel A, Brailey JL, Montfort WR. Buried S-nitrosocysteine revealed in crystal structures of human thioredoxin. Biochemistry 2007; 46:1219-1227.
89. Weichsel A, Kem M, Montfort WR. Crystal structure of human thioredoxin revealing an unraveled helix and exposed S-nitrosation site. Protein Sci 2010; 19:1801-1806.
90. Hashemy SI, Holmgren A. Thioredoxin and glutaredoxin systems in cellular thiol redox homeostasis. Free Radic Res 2007; 41:S7-S.
91. Hashemy SI, Johansson C, Berndt C, Lillig CH, Holmgren A. Oxidation and S-nitrosylation of cysteines in human cytosolic and mitochondrial glutaredoxins: effects on structure and activity. J Biol Chem 2007; 282:14428-14436.
92. Tao L, Jiao X, Gao E, Lau WB, Yuan Y, Lopez B, *et al*. Nitrative inactivation of thioredoxin-1 and its role in postischemic myocardial apoptosis. Circulation 2006; 114:1395-13402.
93. Yin T, Hou R, Liu S, Lau WB, Wang H, Tao L. Nitrative inactivation of thioredoxin-1 increases vulnerability of diabetic hearts to ischemia/reperfusion injury. J Mol Cell Cardiol 2010; 49:354-361.
94. Zhang H,Tao L, Jiao X, Gao E, Lopez BL, Christopher TA, *et al*. Nitrative thioredoxin inactivation as a cause of enhanced myocardial ischemia/reperfusion injury in the aging heart. Free Radic Biol Med 2007; 43:39-47.
95. Park YS, Fujiwara N, Koh YH, Miyamoto Y, Suzuki K, Honke K, *et al.* Induction of thioredoxin reductase gene expression by peroxynitrite in human umbilical vein endothelial cells. Biol Chem 2002; 383:683-691.
96. Yuan Y, Jiao X, Lau WB, Wang Y, Christopher TA, Lopez BL, *et al*. Thioredoxin glycation: A novel post-translational modification that inhibits its antioxidant and organ protective actions. Free Radic Biol Med 2010; 49:332-338.
97. Mossner E, Huber-Wunderlich M, Glockshuber R. Characterization of *Escherichia coli* thioredoxin variants mimicking the active-sites of other thiol/disulfide oxidoreductases. Protein Sci 1998; 7:1233-1244.
98. Nordberg J, Arner ES. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free Radic Biol Med. 2001 Dec 1; 31:1287-312.
99. Tonissen KF, Di Trapani G. Thioredoxin system inhibitors as mediators of apoptosis for cancer therapy. Mol Nutr Food Res 2009; 53:87-103.
100. Lincoln DT, Ali Emadi EM, Tonissen KF, Clarke FM. The thioredoxin-thioredoxin reductase system: over-expression in human cancer. Anticancer Res 2003; 23:2425-2433.
101. Valdman A, Haggarth L, Cheng L, Lopez-Beltran A, Montironi R, Ekman P, *et al.* Expression of redox pathway enzymes in human prostatic tissue. Anal Quant Cytol Histol 2009; 31:367-374.
102. Lincoln DT, Al-Yatama F, Mohammed FM, Al-Banaw AG, Al-Bader M, Burge M, *et al*. Thioredoxin and thioredoxin reductase expression in thyroid cancer depends on tumour aggressiveness. Anticancer Res 2010; 30:767-775.
103. Kakolyris S, Giatromanolaki A, Koukourakis M, Powis G, Souglakos J, Sivridis E, *et al*. Thioredoxin expression is associated with lymph node status and prognosis in early operable non-small cell lung cancer. Clin Cancer Res 2001; 7:3087-3091.
104. Raffel J, Bhattacharyya AK, Gallegos A, Cui H, Einspahr JG, Alberts DS, *et al*. Increased expression of thioredoxin-1 in human colorectal cancer is associated with decreased patient survival. J Lab Clin Med 2003; 142:46-51.
105. Zhou FL, Zhang WG, Wei YC, Meng S, Bai GG, Wang BY, *et al*. Involvement of oxidative stress in the relapse of acute myeloid leukemia. J Biol Chem 2010; 285:15010-15015.
106. Yoo MH, Xu XM, Carlson BA, Gladyshev VN, Hatfield DL. Thioredoxin reductase 1 deficiency reverses tumor phenotype and tumorigenicity of lung carcinoma cells. J Biol Chem 2006; 281:13005-13008.
107. Yokomizo A, Ono M, Nanri H, Makino Y, Ohga T, Wada M, *et al*. Cellular levels of thioredoxin associated with drug sensitivity to cisplatin, mitomycin C, doxorubicin, and etoposide. Cancer Res 1995; 55:4293-4296.

108. Kawahara N, Tanaka T, Yokomizo A, Nanri H, Ono M, Wada M, *et al*. Enhanced coexpression of thioredoxin and high mobility group protein 1 genes in human hepatocellular carcinoma and the possible association with decreased sensitivity to cisplatin. Cancer Res 1996; 56:5330-5333.
109. Sasada T, Nakamura H, Ueda S, Sato N, Kitaoka Y, Gon Y, *et al.* Possible involvement of thioredoxin reductase as well as thioredoxin in cellular sensitivity to cis-diamminedichloroplatinum (II). Free Radic Biol Med 1999; 27:504-514.
110. Wang J, Kobayashi M, Sakurada K, Imamura M, Moriuchi T, Hosokawa M. Possible roles of an adult T-cell leukemia (ATL)-derived factor/thioredoxin in the drug resistance of ATL to adriamycin. Blood 1997; 89:2480-2487.
111. Kim SJ, Miyoshi Y, Taguchi T, Tamaki Y, Nakamura H, Yodoi J, *et al.* High thioredoxin expression is associated with resistance to docetaxel in primary breast cancer. Clin Cancer Res 2005 ; 11:8425-8430.
112. Urig S, Becker K. On the potential of thioredoxin reductase inhibitors for cancer therapy. Semin Cancer Biol 2006; 16:452-465
113. Nguyen P, Awwad RT, Smart DD, Spitz DR, Gius D. Thioredoxin reductase as a novel molecular target for cancer therapy. Cancer Lett 2006; 236:164-174.
114. Arner ES, Bjornstedt M, Holmgren A. 1-Chloro-2,4-dinitrobenzene is an irreversible inhibitor of human thioredoxin reductase. Loss of thioredoxin disulfide reductase activity is accompanied by a large increase in NADPH oxidase activity. J Biol Chem 1995; 270:3479-3482.
115. Nordberg J, Zhong L, Holmgren A, Arner ES. Mammalian thioredoxin reductase is irreversibly inhibited by dinitrohalobenzenes by alkylation of both the redox active selenocysteine and its neighboring cysteine residue. J Biol Chem 1998; 273:10835-10342.
116. Fang J, Lu J, Holmgren A. Thioredoxin reductase is irreversibly modified by curcumin: a novel molecular mechanism for its anticancer activity. J Biol Chem 2005; 280:25284-25290.
117. Young SW, Qing F, Harriman A, Sessler JL, Dow WC, Mody TD, *et al*. Gadolinium(III) texaphyrin: a tumor selective radiation sensitizer that is detectable by MRI. Proc Natl Acad Sci U S A 1996; 93:6610-6615.
118. Hashemy SI, Ungerstedt JS, Zahedi Avval F, Holmgren A. Motexafin gadolinium, a tumor-selective drug targeting thioredoxin reductase and ribonucleotide reductase. J Biol Chem 2006 ; 281:10691-10697.
119. Becker K, Gromer S, Schirmer RH, Muller S. Thioredoxin reductase as a pathophysiological factor and drug target. Eur J Biochem 2000; 267:6118-6125.
120. Schallreuter KU, Gleason FK, Wood JM. The mechanism of action of the nitrosourea anti-tumor drugs on thioredoxin reductase, glutathione reductase and ribonucleotide reductase. Biochim Biophys Acta 1990; 1054:14-20.
121. Mau BL, Powis G. Mechanism-based inhibition of thioredoxin reductase by antitumor quinoid compounds. Biochem Pharmacol 1992; 43:1613-1620.
122. Gromer S, Schirmer RH, Becker K. The 58 kDa mouse selenoprotein is a BCNU-sensitive thioredoxin reductase. FEBS Lett 1997; 412:318-320.
123. Urig S, Fritz-Wolf K, Reau R, Herold-Mende C, Toth K, Davioud-Charvet E, *et al*. Undressing of phosphine gold (I) complexes as irreversible inhibitors of human disulfide reductases. Angew Chem Int Ed Engl 2006; 45:1881-1886.
124. Lu J, Chew E-H, Holmgren A. Targeting thioredoxin reductase is a basis for cancer therapy by arsenic trioxide. Proc Natl Acad Sci 2007; 104:12288-12293.
125. Engman L, Al-Maharik N, McNaughton M, Birmingham A, Powis G. Thioredoxin reductase and cancer cell growth inhibition by organotellurium antioxidants. Anticancer Drugs? 2003; 14:153-161.
126. Schallreuter KU, Wood JM. Azelaic acid as a competitive inhibitor of thioredoxin reductase in human melanoma cells. Cancer Lett 1987; 36:297-305.
127. Shi C, Yu L, Yang F, Yan J, Zeng H. A novel organoselenium compound induces cell cycle arrest and apoptosis in prostate cancer cell lines. Biochem Biophys Res Commun 2003; 309:578-583.
128. Wang X, Zhang J, Xu T.Thioredoxin reductase inactivation as a pivotal mechanism of ifosfamide in cancer therapy. Eur J Pharmacol 2008;579:66-73.
129. Wang X, Zhang J, Xu T. Cyclophosphamide as a potent inhibitor of tumor thioredoxin reductase in vivo. Toxicol Appl Pharmacol . 2007; 218:88-95.
130. Lin S, Cullen WR, Thomas DJ. Methylarsenicals and arsinothiols are potent inhibitors of mouse liver thioredoxin reductase. Chem Res Toxicol 1999; 12:924-930.
131. Cenas N, Prast S, Nivinskas H, Sarlauskas J, Arner ES. Interactions of nitroaromatic compounds with the mammalian selenoprotein thioredoxin reductase and the relation to induction of apoptosis in human cancer cells. J Biol Chem 2006; 281:5593-5603.
132. Cenas N, Nivinskas H, Anusevicius Z, Sarlauskas J, Lederer F, Arner ES. Interactions of quinones with thioredoxin reductase: a challenge to the antioxidant role of the mammalian selenoprotein. J Biol Chem 2004; 279:2583-2592.

133. Du YT, Wu YF, Cao XL, Cui W, Zhang HH, Tian WX, *et al*. Inhibition of mammalian thioredoxin reductase by black tea and its constituents: Implications for anticancer actions. Biochimie 2009; 91:434-444.
134. Lu J, Papp LV, Fang J, Rodriguez-Nieto S, Zhivotovsky B, *et al*. Inhibition of Mammalian thioredoxin reductase by some flavonoids: implications for myricetin and quercetin anticancer activity. Cancer Res 2006; 66:4410-4418.
135. Bindoli A, Rigobello MP, Scutari G, Gabbiani C, Casini A, Messori L. Thioredoxin reductase: A target for gold compounds acting as potential anticancer drugs. Coord Chem Rev 2009; 253:1692-1707.
136. Gromer S, Arscott LD, Williams CH, Schirmer RH, Becker K. Human placenta thioredoxin reductase. Isolation of the selenoenzyme, steady state kinetics, and inhibition by therapeutic gold compounds. J Biol Chem 1998; 273:20096-20101.
137. Wataha JC, Lewis JB, McCloud VV, Shaw M, Omata Y, Lockwood PE, *et al.* Effect of mercury (II) on Nrf2, thioredoxin reductase-1 and thioredoxin-1 in human monocytes. Dent Mater 2008 ;24:765-72.
138. Carvalho CML, Chew EH, Hashemy SI, Lu J, Holmgren A. Inhibition of the human thioredoxin system-A molecular mechanism of mercury toxicity. J Biol Chem 2008; 283:11913-11923.
139. Wagner C, Sudati JH, Nogueira CW, Rocha JB. *In vivo* and *in vitro* inhibition of mice thioredoxin reductase by methylmercury. Biometals 2010.
140. Carvalho CM, Lu J, Zhang X, Arner ES, Holmgren A. Effects of selenite and chelating agents on mammalian thioredoxin reductase inhibited by mercury: implications for treatment of mercury poisoning. FASEB J 2011; 25:370-381.
141. Pia Rigobello M, Messori L, Marcon G, Agostina Cinellu M, Bragadin M, Folda A, *et al*. Gold complexes inhibit mitochondrial thioredoxin reductase: consequences on mitochondrial functions. J Inorg Biochem 2004; 98:1634-1641.
142. Welsh SJ, Williams RR, Birmingham A, Newman DJ, Kirkpatrick DL, Powis G. The thioredoxin redox inhibitors 1-methylpropyl 2-imidazolyl disulfide and pleurotin inhibit hypoxia-induced factor 1alpha and vascular endothelial growth factor formation. Mol Cancer Ther 2003; 2:235-243.
143. Hoshino T, Nakamura H, Okamoto M, Kato S, Araya S, Nomiyama K, *et al*. Redox-active protein thioredoxin prevents proinflammatory cytokine- or bleomycin-induced lung injury. Am J Respir Crit Care Med 2003; 168:1075-1083.
144. Hattori I, Takagi Y, Nakamura H, Nozaki K, Bai J, Kondo N, *et al*. Intravenous administration of thioredoxin decreases brain damage following transient focal cerebral ischemia in mice. Antioxid Redox Signal 2004; 6:81-87.
145. Liu W, Nakamura H, Shioji K, Tanito M, Oka S, Ahsan MK, *et al*. Thioredoxin-1 ameliorates myosin-induced autoimmune myocarditis by suppressing chemokine expressions and leukocyte chemotaxis in mice. Circulation 2004; 110:1276-1283.
146. Aota M, Matsuda K, Isowa N, Wada H, Yodoi J, Ban T. Protection against reperfusion-induced arrhythmias by human thioredoxin. J Cardiovasc Pharmacol 1996; 27:727-732.
147. Tao L, Gao E, Hu A, Coletti C, Wang Y, Christopher TA, *et al*. Thioredoxin reduces post-ischemic myocardial apoptosis by reducing oxidative/nitrative stress. Br J Pharmacol 2006; 149:311-318.
148. Ago T, Sadoshima J. Thioredoxin and ventricular remodeling. J Mol Cell Cardiol 2006; 41:762-773
149. Samuel SM, Thirunavukkarasu M, Penumathsa SV, Koneru S, Zhan L, Maulik G, *et al*. Thioredoxin-1 gene therapy enhances angiogenic signaling and reduces ventricular remodeling in infarcted myocardium of diabetic rats. Circulation 2010; 121:1244-1255.