Review

Thioredoxin system inhibitors as mediators of apoptosis for cancer therapy

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The thioredoxin (Trx) system is a major antioxidant system integral to maintaining the intracellular redox state. It contains Trx, a redox active protein, which regulates the activity of various enzymes including those that function to counteract oxidative stress within the cell. Trx can also scavenge reactive oxygen species (ROS) and directly inhibits proapoptotic proteins such as apoptosis signal-regulating kinase 1 (ASK1). The oxidized form of Trx is reduced by thioredoxin reductase (TrxR). The cytoplasm and mitochondria contain equivalent Trx systems and inhibition of either system can lead to activation of apoptotic signaling pathways. There are a number of inhibitors with chemotherapy applications that target either Trx or TrxR to induce apoptosis in cancer cells. Suberoylanilide hydroxamic acid (SAHA) is effective against many cancer cells and functions by up-regulating an endogenous inhibitor of Trx. Other compounds target the selenocysteine-containing active site of TrxR. These include gold compounds, platinum compounds, arsenic trioxide, motexafin gadolinium, nitrous compounds, and various flavonoids. Inhibition of TrxR leads to an accumulation of oxidized Trx resulting in cellular conditions that promote apoptosis. In addition, some compounds also convert TrxR to a ROS generating enzyme. The role of Trx system inhibitors in cancer therapy is discussed in this review.

Keywords: Apoptosis / Cancer / Redox control / Thioredoxin

Received: November 29, 2007; revised: May 29, 2008; accepted: June 21, 2008

1 Introduction

Redox control processes are now well established as a major regulatory component of many cell signaling pathways in addition to their primary role of maintaining the intracellular redox state. As a major source of reactive oxygen species (ROS) during respiration the mitochondria both influence and are responsive to this intracellular redox state. While ROS have a physiological role through mediating several signal transduction pathways, high levels of ROS can cause oxidative stress, which can subsequently result in cell death by influencing apoptotic pathways. The redox control systems function by counteracting the ROS and their damage and also have direct involvement with apoptotic pathways [1].

Apoptosis is a highly regulated form of cell death and is necessary to maintain a constant cell number and to rid the body of damaged cells [2]. A series of biochemical pathways can be activated by either intracellular or extracellular events and lead to the commitment of the cell to undergo apoptosis. There are two major pathways, the extrinsic pathway, which is a receptor-mediated apoptotic pathway and the intrinsic pathway, which is a mitochondria-mediated apoptotic pathway. There is some crosstalk between the two pathways and both require the activation of caspases, which are a family of cysteine proteases that function as the executioners of apoptosis [3]. The intrinsic pathway can be activated by stress, UV radiation, or by administration of cytotoxic drugs that perturb the redox balance within a cell. This review will examine the influence of cellular redox state on the mitochondrial apoptotic pathways and

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Abbreviations: ASK1, apoptosis signal-regulating kinase 1; **ATO,** arsenic trioxide; **DNCB,** 1-chloro-2,4-dinitrobenzene; **FAD,** flavin adenine dinucleotide; **IAP,** inhibitor of apoptosis; **JNK,** Jun N-terminal kinase; **MAPK,** mitogen-activated protein kinase; **MGd,** motexafin gadolinium; **NADPH,** α-nicotinamide adenine dinucleotide phosphate; **PX-12,** 1-methyl-propyl-2-imidazolyl disulfide; **ROS,** reactive oxygen species; **SAHA,** suberoylanilide hydroxamic acid; **TBP-2,** thioredoxin binding protein 2; **TRAF,** TNF receptor-associated factor; **TrxR,** thioredoxin reductase; **VEGF,** vascular endothelial growth factor

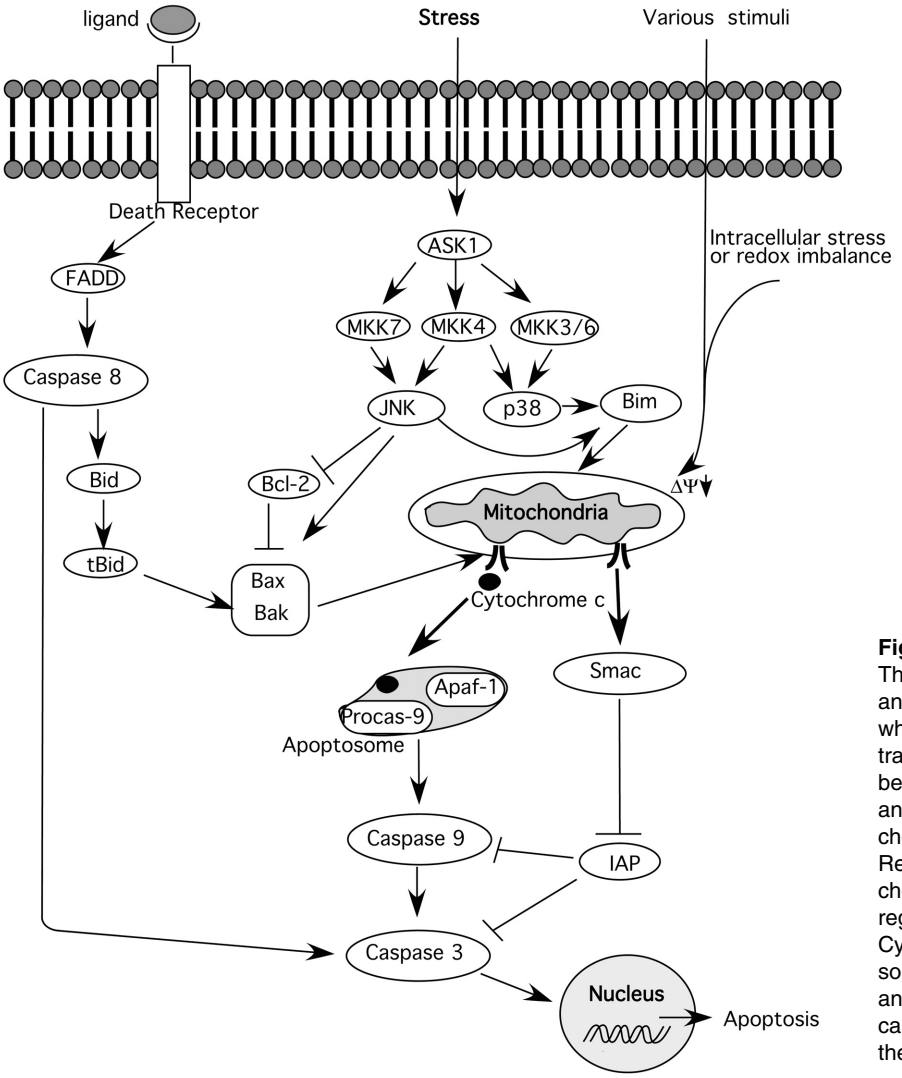

Figure 1. Apoptotic signaling pathways. The extrinsic (death receptor activated) and the intrinsic apoptotic pathways, which involve the mitochondria are illustrated, together with the crosstalk between the pathways. Various stresses and apoptotic stimuli decrease the mitochondrial membrane potential ($\Delta\psi$). Release of cytochrome *c* from the mitochondria through membrane pores is regulated by the Bcl-2 family of proteins. Cytochrome *c* forms part of the apoptosome, which then activates the caspases and leads to apoptosis. Stress can also cause apoptosis by activating ASK1 and the JNK and p38 signaling pathways.

then discuss the cancer therapeutic molecules that target the redox control systems with the goal of promoting apoptosis.

2 Apoptotic pathways and the mitochondria

Mitochondria play a pivotal role in the intrinsic pathway of apoptosis [4] (Fig. 1). The key event for activating the intrinsic pathway is the permeabilization of the mitochondrial outer membrane, which occurs in response to various stimuli and is regulated by many cytoplasmic proteins including members of the Bcl-2 family. Some members of this family promote apoptosis and include Bax, Bak, Bim, and Bid while other members such as Bcl-2 and Bcl-xL act to prevent their action and are antiapoptotic [5]. The exact mechanisms by which the various members of the Bcl-2 family affect the mitochondria are largely unknown but they can either form pores in conjunction with mitochondrial membrane proteins or they can open other existing channels [6, 7].

Once permeabilization occurs there is a release of cytochrome *c* and other proteins including second mitochondrial-derived activator of caspases (Smac) from the mitochondria into the cytoplasm [1]. Cytochrome *c* is anchored in the mitochondria by cardiolipin, an anionic phospholipid and once it is released it recruits other proteins including apoptotic protease activating factor-1 (Apaf-1) and procaspase 9 to form the apoptosome [1, 8]. The apoptosome cleaves procaspase 9, which in turn activates caspase 3, which acts on the nucleus to induce apoptotic events including DNA fragmentation [9]. A further negative regulatory event occurs through the action of IAP (inhibitor of apoptosis), which inhibits the action of caspases 9 and 3 [10]. Smac, a protein released from the mitochondria along with cytochrome *c*, acts to inhibit the action of IAP and is thus a proapoptotic protein [11].

Various stresses including tumor necrosis factor (TNF), UV light, and oxidative stress can lead to apoptosis through activation of the mitogen-activated protein kinase (MAPK) pathway [12], which also exerts effects on the mitochondria (Fig. 1). Apoptosis signal-regulating kinase 1 (ASK1) is a MAPK kinase kinase (MAPKKK) and it activates several MAPK kinases such as MKK3, 4, 6, and 7, which then activate the c-Jun N-terminal kinase (JNK) and the p38 MAPK pathways [13]. These kinases phosphorylate and activate specific proapoptotic targets including members of the Bcl-2 family like Bim [14, 15] and Bax [16]. They also phosphorylate and activate the transcription factor p53 [17], which can play an important role in apoptosis, particularly in response to damaged DNA, by up-regulating the transcription of proapoptotic genes including Bax [18].

The extrinsic apoptotic pathway is activated by ligands binding to the death receptor, which then cleaves procaspase 8 [19]. Upon its activation caspase 8 can either act on Bid to exert apoptotic effects through the mitochondria or caspase 8 can directly activate caspase 3 without involving the mitochondria [19] (Fig. 1). Thus there is some crosstalk between the extrinsic and intrinsic pathways that appears to be dependent on cell type and signal.

All of these pathways have specific components that can be regulated by the intracellular redox state of the cell. External stresses impact on the intracellular redox state by stimulating the production of ROS, which can lead to apoptosis through the pathways described above. The mitochondria often play an integral role in determining if apoptosis will progress but also have a major influence on the redox state of a cell due to their own production of ROS.

3 ROS and the mitochondria

ROS are chemically reactive molecules that are derived from oxygen. They include the superoxide anion (O_2^-), the hydroxyl radical ($HO^\bullet$), singlet oxygen ($^1O_2$), peroxyl radicals ($ROO^\bullet$), peroxynitrate ($ONOO^-$), nitric oxide ($NO^\bullet$), and hydrogen peroxide (H_2O_2) [20]. Each of these can exert different effects on a cell due to differences in reactivity. All aerobic cells generate ROS as by-products during routine metabolic reactions and also in response to certain stimuli. Mitochondria are a major source of ROS as they are formed during the respiratory chain but ROS are also produced by electron transport chains present in the ER and nuclear membranes [21].

Within the mitochondria the primary site of ROS production occurs during respiration and the electron transport chain [1, 21]. Approximately 1–2% of the molecular oxygen utilized by the cell is converted into superoxide radicals, which being charged cannot readily diffuse across the mitochondrial membranes [22]. However, the superoxide anion can pass into the cytosol by activating the voltage-dependent anion channel (VDAC). Moreover the O_2^- anion can be converted to hydrogen peroxide by enzymes within the mitochondria, namely superoxide dismutases (SOD), which can then easily move across the membrane and into the cytoplasm [22]. Thus the formation and metabolism of ROS in and by the mitochondria can make an impact on the cytosolic redox state.

An excess of ROS can occur in the mitochondria if the cell is subjected to external stresses such as chemical poisoning, UV light or a dysfunctional complex within the electron transport chain. Oxidative stress is the result of excess ROS, which can cause damage to several mitochondrial components including proteins, lipids, and DNA. ROS can affect the protein components of membranes where they cause protein fragmentation, formation of protein carbonyls by oxidation of certain amino acids and crosslinking of cysteine residues resulting in protein aggregation. Lipid peroxidation is primarily caused by OH– radicals while damage to DNA can occur through oxidative modification of the bases, deoxyribose backbone and by direct breakage of the chain (reviewed in detail by [1]).

There is now evidence that ROS also play fundamental roles in cell death signaling pathways. Addition of ROS or intracellular accumulation of ROS can lead to cell death [1, 21, 23]. While ROS undoubtedly can cause much general damage to the mitochondrial components if left unchecked, they may also have some specific targets in the apoptotic pathway. Mounting evidence suggests that ROS facilitates the detachment of cytochrome *c* from cardiolipin before its release into the cytoplasm through the pores created by proapoptotic Bcl-2 family members [24]. Recently cytochrome *c* was shown to catalyze a hydrogen peroxide dependent lipid peroxidation reaction of cardiolipin that facilitates the release of cytochrome *c* from the mitochondrial membranes [25]. While the exact mechanisms are not yet known it raises the possibility that ROS may play important roles in the permeabilization of the membrane. Other roles for ROS have also been suggested and have recently been reviewed by Orrenius *et al.* [1].

Another major effect of increased ROS is to cause an imbalance in the intracellular redox state [21]. This redox imbalance can also occur by disrupting the antioxidant defense mechanisms that usually function to control ROS levels within the cell. Redox control systems are essential to enable a cell to get rid of ROS and protect cells from oxidative stress and include both cytoplasmic and mitochondrial enzyme and nonenzyme systems. The diet contributes some antioxidants in the forms of vitamins, but it is the endogenous systems that exert the greatest effects.

If these redox systems are affected it can lead to either an inability to get rid of ROS or to a redox imbalance, which are conditions that favor apoptosis. In addition, these redox control systems have direct interactions with components of the apoptotic signaling pathways. The two most important systems are the thioredoxin (Trx) and glutathione systems. While the glutathione system is also extremely impor-

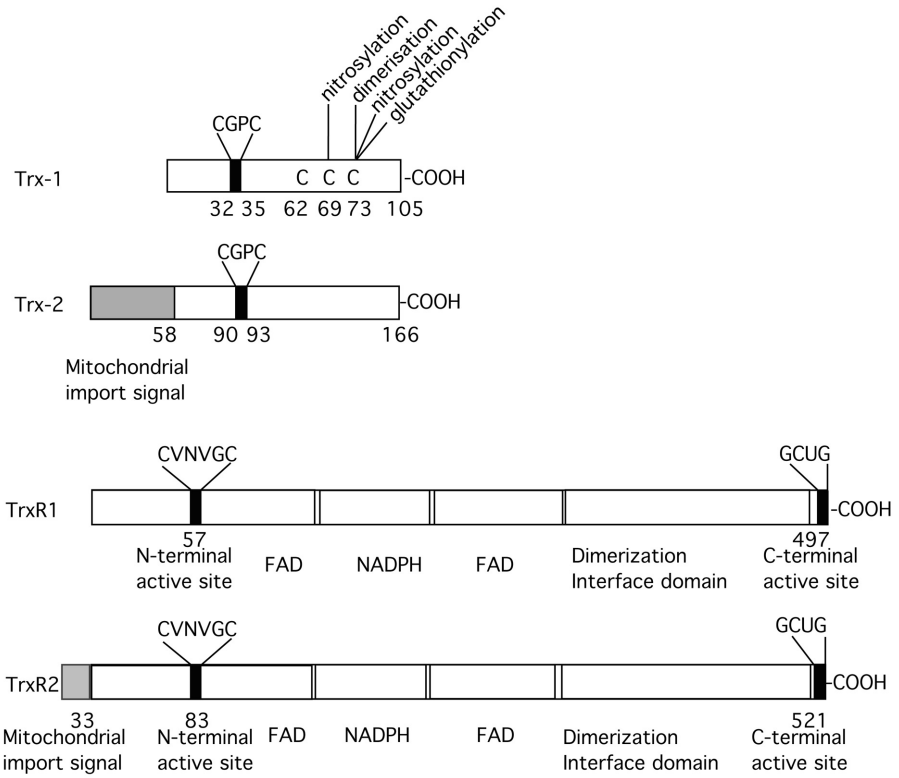

Figure 2. Structure of Trx and TrxR proteins. Trx-1 is the cytoplasmic form of Trx and Trx-2 is the mitochondrial form, which is initially synthesized with a predicted 58 amino acid mitochondrial import sequence [28]. The active site cysteines are indicated for each protein with corresponding amino acid positions. Trx-1 contains an additional three-cysteine residues that have been implicated in various functions [32]. TrxR1 is the cytoplasmic form of TrxR and the hatched patterns represent binding regions that have been mapped to 3-D domains. Structural studies revealed that TrxR1 contains a single FAD binding domain, one NADPH binding domain and a dimerization interaction domain [35]. There are two active site regions with the C-terminal active site containing a selenocysteine (U) residue [34]. TrxR2 is the mitochondrial form that is similar in structure to TrxR1 except that it contains an additional mitochondrial import sequence [29].

tant to the cell with respect to maintaining redox state, this review will focus on the Trx system due to the large number of therapeutic reagents that have been designed to treat cancer by targeting the Trx system. However, there is some interplay between the systems and therefore some reagents can exert effects through both systems.

4 The Trx systems

The Trx system is a major redox control system in the cytoplasm of cells and there is a corresponding system in the mitochondria. The cytoplasmic system consists of a 12 kDa redox active protein called thioredoxin (Trx-1), a homodimeric seleno-protein called thioredoxin reductase (TrxR1), and α-nicotinamide adenine dinucleotide phosphate (NADPH; Fig. 2) [26]. The active site of Trx contains two cysteine residues at positions 32 and 35 that undergo reversible oxidation to form a disulfide bond during the transfer of reducing equivalents to a disulfide substrate. The oxidized Trx protein is then recycled to a reduced state through the action of TrxR1 and NADPH [26]. Through this reversible redox reaction Trx can regulate the activity of several protein substrates in numerous pathways, including members of the peroxiredoxin family that target the degradation of hydrogen peroxide [27].

The mitochondrial Trx system has been far less studied but is known to consist of the 12 kDa thioredoxin-2 protein (Trx-2) [28] and the selenoprotein thioredoxin reductase-2 (TrxR2) [29] and NADPH. Trx-2 is synthesized with an additional N-terminal extension that targets the protein to the mitochondria and is then cleaved to the 12 kDa form (Fig. 2). The mitochondrial system utilizes a similar mechanism of action to that of the cytoplasmic system with TrxR2 able to reduce the oxidized Trx2. Two mitochondrial peroxidases have been identified to date with one of them Prdx3 [30] exclusively detected in the mitochondria leading to the suggestion that they may function to provide a defense system against the hydrogen peroxide formed during respiration in the mitochondria. However, the relative importance

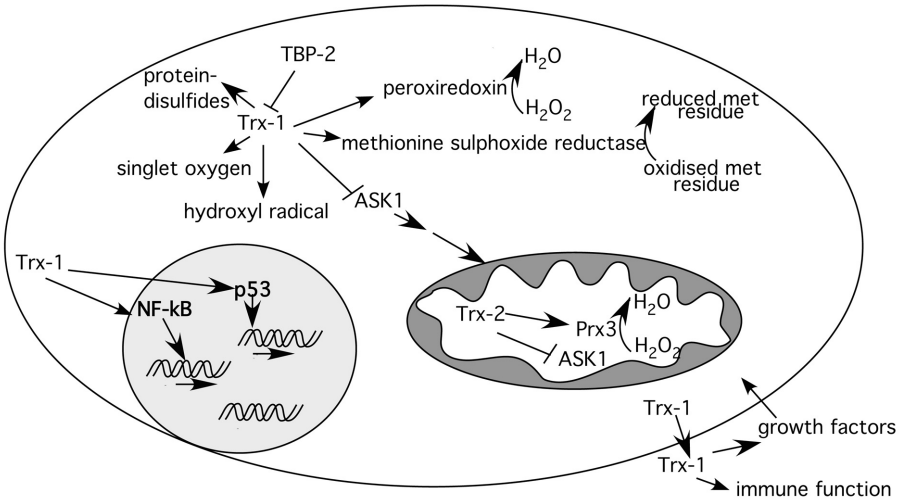

Figure 3. Trx cellular functions. Some of the most important pathways and substrates regulated by Trx are shown together with their subcellular localization. Inhibitory interactions are also indicated.

of the Trx system to peroxide detoxification compared to the glutathione system within the mitochondria is not known [31].

The cytoplasmic and mitochondrial Trx both contain a conserved active site sequence of cys-gly-pro-cys, but Trx-1 also contains three additional cysteine residues at positions 62, 69, and 73 (Fig. 2). These cysteines are often referred to as the structural cysteines and post-translational modifications of these three residues including glutathionylation, oxidation, and *S*-nitrosylation contribute to the regulation of Trx function [32]. Trx-1 also forms disulfide linked homodimers through the cysteine-73 residue [33].

Both TrxR1 and TrxR2 are flavoproteins and consist of homodimers with each subunit containing two active site regions (Fig. 2). TrxR1 has an active site region positioned at the N-terminus from residues 59 to 64 containing the following motif -cys-val-asn-gly-cys-. The second region is located at the C-terminus of the protein and contains a selenocysteine -gly-cys-sec-gly- that is essential for activity [34]. The first active site receives electrons from NADPH *via* the flavin adenine dinucleotide (FAD) molecule and transfers electrons to the selenocysteine redox active site present in the other subunit. The selenolate formed in the C-terminal active site region can then act to reduce the required substrate [35]. The mitochondrial TrxR2 has a similar structural domain organization to TrxR1 including the conserved C-terminal selenocysteine active site region [36, 37]. This region is not only important for the function of TrxR but also represents a target for many therapeutic reagents to specifically inhibit TrxR.

A third form of TrxR, called TrxR3, is expressed in mammals from a separate gene and is able to reduce glutathione disulfides as well as Trx. It is therefore also referred to as thioredoxin glutathione reductase (TGR) [38] but appears to be expressed in a tissue specific manner, predominantly in the testis. For the purpose of this review TrxR will be used to refer collectively to all of the TrxR forms while TrxR1 will be used to define the cytoplasmic form and TrxR2 the mitochondrial form.

5 Thioredoxin functions – association with apoptosis

Trx was originally described as a hydrogen donor for ribonucleotide reductase [39] but has now been shown to function in numerous pathways within the cell. Trx regulates the DNA binding ability of certain transcription factors, acts as a cellular growth factor and has roles in regulating immune function (Fig. 3) [40 – 43]. However, one of its main functions is to counteract oxidative stress by scavenging ROS and by regulating other enzymes that help to reduce oxidative stress.

While there are other antioxidant systems within a cell, Trx has some specific protein substrates that give it some distinct functional roles for counteracting oxidative stress. Trx specifically reduces other enzymes that function to maintain the redox state of a cell, including members of the peroxiredoxin family (Fig. 3) [27]. These proteins regulate levels of hydrogen peroxide in the cell and thus are important contributors to maintaining redox balance. Trx also reduces glutathione peroxidase 3, thus providing a link between the two major antioxidant systems [44]. Another substrate for Trx is methionine sulfoxide reductase, which acts to reduce oxidized methionine residues [45]. Trx can also act directly on certain ROS through its action as a potent singlet oxygen quencher and hydroxyl radical scavenger, although interestingly its redox active site is not required for this function [46]. A functional Trx system is therefore essential to maintain the intracellular redox state

and to reduce oxidized proteins. Inhibiting the function of Trx can lead to an imbalance in the redox state that can ultimately cause apoptosis.

TrxR is the only enzyme in the cell known to reduce Trx. However, TrxR1 itself has a broad substrate specificity and in addition to protein disulfides it can also reduce low molecular weight disulfide compounds such as 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) [47], lipoic acid, and lipoamides [48]. It can also reduce nondisulfide substrates including vitamin K [49], hydrogen peroxide [34] and various quinones [50], and selenium containing compounds [51, 52]. This broad substrate specificity is due to the activity of the selenocysteine containing active site region at the C-terminus [34]. Therefore, TrxR1 contributes to maintaining the redox state of a cell both by regulating Trx and by its own direct action.

In addition to having a profound effect on maintaining redox state, Trx has a direct interaction with the apoptotic pathway through binding to ASK1, a member of the MAPKKK family. The reduced form of Trx binds to the N-terminal region of ASK1 and inhibits its kinase function, while under oxidizing conditions Trx is unable to bind [53]. If ROS are present Trx is converted to an oxidized form and it then dissociates from ASK1. In this case, ASK1 gains a kinase activity and forms part of a larger molecular weight complex by recruiting other factors including TNF receptor-associated factor 2 (TRAF2) and TRAF6 [54]. Apoptosis is then mediated *via* the JNK and p38 pathways (Fig. 1). Trx further regulates ASK1 by targeting it for ubiquitination and degradation [55]. Mitochondrial Trx has also been reported to interact with and inhibit ASK1 in the mitochondria [56] leading to an inhibition of apoptosis. In contrast to ASK1 signaling in the cytoplasm, ASK1 in the mitochondria mediates apoptosis through a JNK independent pathway that nonetheless still results in cytochrome *c* release and caspase-3 activation. Inhibition of Trx therefore results in activation of ASK1 and stimulation of apoptosis.

Recently, Trx has been shown to directly regulate apoptosis by interacting with procaspase 3 [57]. Trx catalyses a specific transnitrosylation reaction by transferring NO to the cysteine 163 residue of procaspase 3. The cysteine 73 residue of Trx was shown to be required for this nitrosothiol transfer reaction. The nitrosylation of procaspase-3 correlates with an inhibition of apoptosis, as has also been reported by other groups [58], thereby identifying yet another antiapoptotic role for Trx.

Trx regulates the activity of various transcription factors, including NF-κB, by reducing a key cysteine residue that is required for DNA binding [59]. NF-κB controls expression of a number of stress genes including some that are involved in blocking the apoptotic pathway. For example, NF-κB regulates expression of members of the Bcl-2 family (including Bcl-2 itself), the IAP family and the TRAF family, all of which have antiapoptotic roles [60, 61]. Therefore, Trx may also modulate the apoptotic pathways through regulation of NF-κB DNA binding activities. Inhibition of Trx will therefore not only lead to functional consequences but also transcriptional regulation within a cell will be altered such that expression of antiapoptotic proteins is reduced.

Another transcription factor regulated by Trx is p53, which controls expression of proapoptotic genes. Thus when p53 is required to induce apoptosis in response to specific stimuli, an intact Trx system is required [62]. This proapoptotic role is in direct contrast to the other roles of Trx and therefore for cancer cells to take advantage of the antiapoptotic functions of Trx the p53 gene is often inactivated [63, 64].

Trx is itself controlled by other proteins. A screen for interacting partners of Trx identified ASK1 and a protein called thioredoxin binding protein 2 (TBP-2), which was previously known as Vitamin D up-regulated protein 1 (VDUP-1) [65]. TBP-2 binds to and inhibits Trx function and therefore higher levels of TBP-2 can lead to the activation of apoptotic pathways due to repression of Trx functionality (Fig. 3).

The mitochondrial Trx system, while less studied, has also been implicated in the apoptotic signaling pathways. There have been suggestions that the mitochondrial Trx system plays a role in the redox control of the mitochondrial membrane permeability since some inhibitors of TrxR2 can stimulate an increase in membrane permeability and lead to the release of cytochrome *c* [66]. However, overexpression of TrxR2 does not result in any changes to permeability [31] indicating that expression levels alone do not have an effect on permeability and that the activity of TrxR2 may also play an important role. Other researchers have shown that oxidation of mitochondrial Trx-2 by exposure to hydrogen peroxide or diamide results in increased cell death [67]. The mitochondrial Trx-2 was oxidized more extensively and persistently than cytoplasmic Trx-1. This would suggest that Trx-2 is also an important sensor of oxidative stresses and that it too can regulate the onset and progression of apoptosis.

A delicate balance of the Trx system can thus dictate whether a cell undergoes apoptosis or is protected from cell death. This is particularly relevant in cancer where the cell uses the antiapoptotic functions of the Trx system to provide both a protective and functional advantage. Consequently therapeutic strategies can be designed that target the Trx system with the goal of promoting apoptosis of the cancerous cells.

6 The Trx system in cancer

High levels of Trx and TrxR are present in many different tumor types compared to levels observed in corresponding healthy cells from the same patient [68, 69]. These include both solid cancers and also lymphomas and leukemias [70]. Most transformed cell lines also express high levels of both

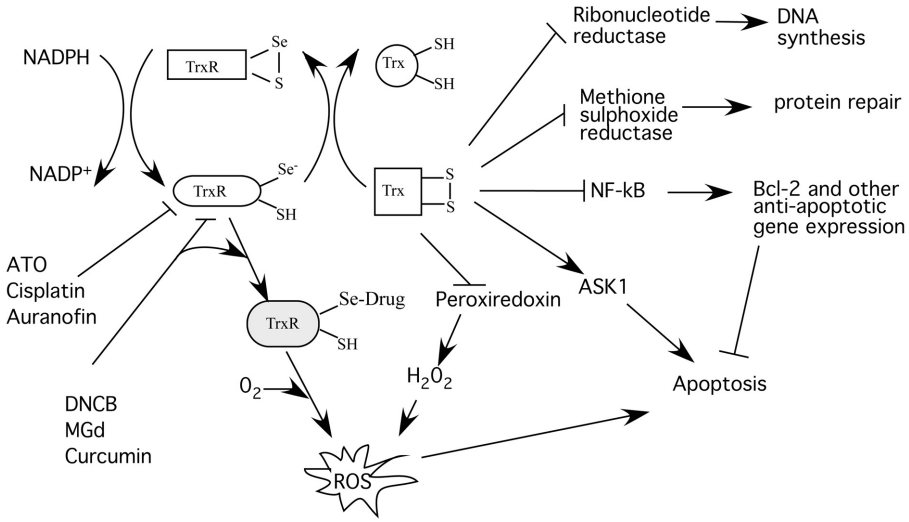

Figure 4. Consequences of TrxR inhibition. Inhibition of TrxR results in enhanced levels of oxidized Trx and a reduction of reduced Trx. Trx is therefore unable to activate the listed substrates, which results in inhibition of cell functions including a reduction in DNA synthesis, protein repair, transcription factor activity, and peroxidase function. An accumulation of hydrogen peroxide and free radicals subsequently occurs. This leads to oxidative stress conditions, which promote apoptosis. Another consequence is that oxidized Trx can no longer repress ASK1, allowing it to initiate apoptosis. In addition some TrxR inhibitors modify TrxR such that it acquires an oxidase function, which causes accumulation of ROS.

Trx and TrxR that are secreted into the medium [68, 71]. Results from various studies have suggested that Trx may have different functions in the cancer cell depending on the stage of cancer development. At early stages Trx may in fact be beneficial for preventing cancer due to its capability to counteract the oxidative stress caused by many carcinogens. Once a cell has initiated a cancer phenotype then high levels of Trx may assist cancer development due to its growth promoting [72, 73] and antiapoptotic functions [53]. At later stages of cancer progression Trx may have roles in angiogenesis and metastasis. Trx has been shown to regulate vascular endothelial growth factor (VEGF) expression and function [74] and also to modulate the activity of various members of the matrix metalloproteinase (MMP) family [75].

Increased levels of Trx have also been associated with resistance to chemotherapy drugs including cisplatin [76] and docetaxel [77]. Docetaxel is one of the most powerful anticancer drugs for breast cancer but a considerable number of patients do not respond to treatment. Microarray analysis showed that patients who do not respond to docetaxel have an increased expression of Trx compared to patients who do respond [78] and up-regulation of Trx protein was confirmed by immunocytochemistry [77]. When the MCF-7 breast cancer cell line, which is usually docetaxel sensitive, was transfected with the Trx gene it became tolerant to a 10 nmol/L dose of docetaxel [78]. Another important clinical finding was that breast cancer patients with increased Trx levels prior to treatment had a greater chance of developing resistance to docetaxel during the treatment phase [77]. This suggests the importance of quantifying Trx levels before treatment so that an optimal therapeutic regime can be selected. It could also identify patients that may benefit from an anti-Trx treatment prior to, or as an adjuvant therapy.

Due to the integral role that the Trx system plays in regulating apoptosis and its high expression levels in many cancer cells there is an interest in developing drugs that can target the Trx system. These drugs can either act alone or in combination with another chemotherapeutic drug and may target either Trx or TrxR.

7 Trx system inhibitors

The past decade has seen a large number of therapeutic reagents that target the Trx system being developed as possible cancer treatments or as adjuncts to existing therapy. Targeting the Trx system will result in modulation of the intracellular redox state, which can favor cells becoming apoptotic due to an accumulation of ROS and alteration of the intracellular redox state. Inhibiting Trx can also have other consequences such as activation of ASK1 [53], inhibition of procaspase 3 nitrosylation [57] and inhibition of NF-κB [59]. As described above all of these situations lead to stimulation of apoptosis within a cell.

Either Trx or TrxR can be targeted by a chemotherapeutic reagent since a nonfunctional TrxR will result in lower levels of reduced Trx in a cell, thus preventing the redox function of Trx. Targeting TrxR also has some additional affects since TrxR has its own substrates that it reduces directly without the need for Trx [47 – 52]. Some drugs are also capable of converting TrxR into a ROS generating system, in direct contrast to its usual role as a defensive enzyme. Figure 4 summarizes the effects of inhibition of TrxR and its possible physiological consequences.

The following sections describe some of the important inhibitors of the Trx system that are being promoted as cancer therapeutics. These compounds range widely in structure and mechanism but the goal in each case is to force the

Table 1. Trx system inhibitors

Drug	Class of drug	Target	Clinical studies	Trademark	References
PX-12	Imidazolyl disulfide	Trx1	In phase II clinical trials for pancreatic cancer		[79]
SAHA	HDAC inhibitors	Trx1	Approved by FDA (2006) for cutaneous T-cell lymphoma. In clinical trials for other cancers	Zolinza	[90, 92]
Cisplatin	Platinum compounds	TrxR	Approved for use by FDA in 1978 for cancer treatment. Often used in combination with other drugs (*e.g.* taxotere)	Platinol	[95, 96]
Auranofin	Gold containing compound	TrxR	Originally approved as an antiarthritic drug in 1985	Ridoura	[96, 104, 107, 108]
ATO	Arsenic containing compound	TrxR	Approved by FDA in 2000 for treatment of acute promyelocytic leukemia	Trisenox	[118, 122]
MGd	Texaphyrins	TrxR	Currently in clinical trials for multiple cancers	Xcytrin	[126, 129]
DNCB	Nitroaromatic compounds	TrxR	In trials as a sensitising agent (pretreatment) prior to chemotherapy of melanoma patients		[133, 136]
Curcumin, flavonoids	Polyphenols	TrxR	In clinical trials for various cancers		[134, 135]

cancer cell to die by affecting the intrinsic apoptotic pathway. Some of the more important inhibitors that have been considered for cancer therapy are shown in Table 1.

8 Trx inhibitors

8.1 1-Methyl-propyl-2-imidazolyl disulfide (PX-12)

Given the high expression of Trx observed in many cancers, it is not surprising that a number of different chemical inhibitors of Trx are being assessed for their effectiveness as antitumor agents. The most promising to date is 1-methyl-propyl-2-imidazolyl disulfide (IV-2), now known as PX-12, which is currently in phase II trials for pancreatic cancer [79].

PX-12 was originally discovered by screening numerous disulfide compounds for their ability to inhibit the growth of cancer cell lines *in vitro* [80]. PX-12 was also shown to inhibit, in a dose dependent manner, the growth of human MCF-7 breast cancer and HL-60 promyelocytic leukemia xenografts in *scid* mice. At the highest dose tested PX-12 displayed a 98% reduction against MCF-7 induced tumor formation, revealing its potential as a therapeutic agent [81].

PX-12 acts by binding to the cysteine 73 residue of Trx1, causing it to become irreversibly alkylated and this modified form of Trx1 is no longer able to act as a substrate for TrxR1 [82]. Reduced Trx1 is therefore unable to be regenerated, leading to inhibition of the Trx system, with downstream consequences on apoptosis and transcription factor activation. PX-12 was shown to be a potent inducer of apoptosis in HL-60 cells when compared to known apoptotic reagents such as dexamethasone [81]. In contrast, MCF-7 cells transfected with a construct that over-expresses Trx1 were resistant to apoptosis when treated with PX-12 [81], suggesting that PX-12 targeting of Trx1 may result in increased apoptosis of cancer cells, presumably by disrupting the ASK-1/Trx interaction.

Further investigation also revealed that cells and patients treated with PX-12 had decreased expression of VEGF [83], a necessary protein for angiogenesis and cancer metastasis. VEGF expression is regulated by the hypoxic inducible factor-1 (HIF-1) transcription factor, which is activated by Trx1. Overexpression of Trx1 leads to increased VEGF production and enhanced angiogenesis [84], while a decrease in Trx1 activity leads to lower levels of activated HIF-1 and reduced expression of VEGF. It is therefore likely that the anticancer effects of PX-12 are mediated through several cellular pathways.

While direct inhibition of Trx1 can lead to an anticancer response, a concern is whether the drug will be specific for cancer cells. A phase I clinical trial that administered PX-12 to patients with advanced solid tumors defined the dosage that could be tolerated by patients with minimal side effects [85]. A phase II clinical trial is now underway to test the efficacy of PX-12 treatment of advanced pancreatic cancer patients [79]. While other Trx inhibitory compounds have been described, they are yet to be assessed in clinical trials for efficacy or safety. An alternative therapeutic approach is to inhibit Trx through regulation of an endogenous inhibitor, and this indirect targeting of Trx is the mode by which suberoylanilide hydroxamic acid (SAHA) functions.

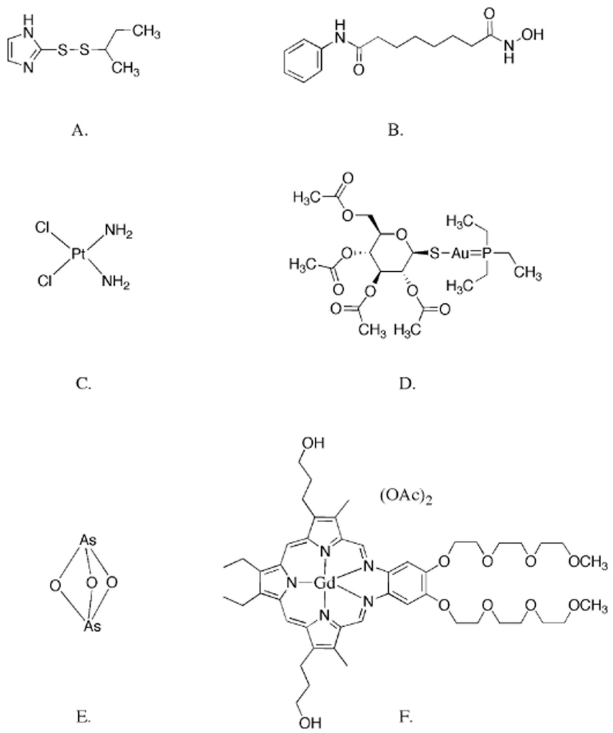

Figure 5. Chemical structures of Trx system inhibitors. (A) PX-12; (B) SAHA; (C) cisplatin; (D) auranofin; (E) ATO; (F) MGd.

8.2 SAHA

Histone deacetylase inhibitors (HDACi) are a relatively new class of cancer therapeutic agents that act by chromatin modification and thereby affecting gene expression. The acetylation and deacetylation of histones dictates transcriptional activity of the associated genes and is controlled by a number of histone acetylases and deacetylases [86]. Inhibiting the deacetylation process thus changes gene expression patterns. HDACi include several groupings of compounds (based on structure) with the most advanced for cancer treatment purposes being SAHA, which is a member of the hydroxamate group (Fig. 5B) [87]. SAHA binds to the catalytic site of class I and II HDAC enzymes and inhibits their activity.

SAHA inhibits the growth of a broad spectrum of solid tumors grown both *in vitro* and *in vivo* but does not inhibit the growth of normal cells [88]. *In vitro* studies showed that transformed cells were at least tenfold more sensitive to SAHA compared to normal cells [89]. Several different cell lines have been utilized and a recent study using prostate cancer cells showed that SAHA could completely inhibit cell growth of three different cell lines. Interestingly the selective effect of SAHA to target cancer cells is likely due to the differing responses of the cells to the drug. SAHA has been the subject of many clinical trials and is manufactured as the drug Zolinza by Merck. It was approved by the FDA in late 2006 for the treatment of cutaneous T-cell lymphoma [90] and is currently being assessed in clinical trials for its effectiveness in treating other cancers [91]. The action of SAHA was shown to be mediated by its effects on the Trx system [92].

Microarray analysis of prostate cancer cell lines treated with SAHA showed that several genes were specifically up-regulated, including TBP-2, the inhibitor of Trx [92]. They further reported that SAHA could up-regulate TBP-2 in other cancer cell types and that the promoter region for TBP-2 was directly responsive to SAHA [92]. As discussed previously, TBP-2 binds to Trx and inactivates its ability to function as a redox control protein [65], thereby leading to an accumulation of ROS. As expected, increased ROS is found in several different cell types treated with SAHA, but only in cancer cells [89]. Nontransformed cells appear to compensate by overexpressing Trx in response to the ROS, but cancer cells do not [89]. The mechanism by which this occurs has not yet been explained. The mechanism of SAHA-induced apoptosis appears to involve different targets in different cell types since both caspase dependent and independent pathways have been shown to play roles [93].

Inhibition of Trx can potentially lead to an activation of the ASK1 apoptotic pathway and it is therefore interesting that a recent study showed that ASK1 expression is also up-regulated by SAHA [94]. Whether this is a general mechanism applicable for all cells has yet to be established. The pre-existing levels of Trx in the cells may also determine the final response and also if resistance will occur during treatment.

This is an interesting mode of targeting the Trx system as the drug itself does not target Trx, but rather causes up-regulation of an endogenous inhibitor. In addition, it uses the protective effects of Trx to be of benefit to the normal cells surrounding the cancer during treatment. This aspect is likely to be a contributor to the low incidence of side effects observed when using SAHA as a cancer treatment [91].

9 TrxR inhibitors

9.1 Platinum compounds

Platinum compounds such as cisplatin (*cis*-diamminedichloridoplatinum (II); Fig. 5C), carboplatin, and oxaliplatin are a group of metal complexes with demonstrated ability to cause cell death in different cancer cell types, and have therefore been widely used in cancer therapy for the past 20 years [95, 96]. Once inside the cell they are able to bind to DNA, forming irreversible DNA – platinum adducts and are therefore generally recognized as DNA-damaging compounds [97]. Different signal transduction pathways will be activated or inactivated as a consequence of the DNA damage leading to cell cycle arrest, DNA repair, and/or cell death [97]. However, many studies have indicated that plati-

num compounds might also target other cellular molecules and pathways to account for their cytotoxic effects. The Trx system is one such target [96, 98, 99]. In particular, it has been shown that cisplatin can cause a dose and time dependent inhibition of human TrxR both *in vitro* and in HeLa cells cultured in the presence of cisplatin. A correlation was observed between the reduction in activity of the enzyme and the decrease in cell viability [99]. Further studies demonstrated that TrxR inhibition was irreversible and also highly specific since under the same conditions neither human or bacterial glutaredoxins were inhibited by cisplatin [98]. The glutathione adduct of cisplatin, on the other hand, was able to inhibit the glutaredoxins in addition to TrxR, implying that cisplatin affects both redox systems, the consequences of which are an altered intracellular redox state [98].

The effects of cisplatin and other platinum compounds on TrxR were also the focus of another study [100]. Cisplatin and its analog oxaliplatin showed similar reactivity with TrxR and a similar extent of inhibition, whereas another analog, carboplatin was not able to induce inhibition of the enzyme, suggesting a lower cytotoxicity of carboplatin compared to cisplatin at the same experimental conditions [100]. None of these compounds were able to inhibit the activity of glutathione reductase, a nonselenoprotein structurally related to TrxR but lacking the C-terminal selenocysteine [100, 101]. The highly reactive selenocysteine residue at the C-terminal domain of TrxR was therefore suggested to be the target of these platinum compounds [100].

The mechanism by which the platinum compounds inhibit TrxR appears to be an important trigger for apoptosis. Induction of apoptotic cell-death by cisplatin-derivatized TrxR1 was observed in a study conducted in human lung carcinoma A549 cells [102]. In this study, lipid-mediated delivery of different forms of TrxR1 that had the selenocysteine residue compromised through derivatization with cisplatin promoted apoptotic cell death in a similar manner to that seen when a truncated form of the enzyme (that did not contain the selenocysteine residue) was delivered to the cells. This result suggests an association between apoptosis and the unavailability of the C-terminal selenocysteine-containing redox active site of the protein [102]. In contrast, delivery of a full length TrxR1 with an intact selenocysteine did not promote cell death, confirming the selenol group of this redox enzyme as a potential target for successful anticancer therapy.

Although highly effective and widely used in the treatment of several forms of cancer, including testicular, ovarian, cervical, head and neck, and nonsmall cell lung cancer, cisplatin presents some disadvantages due to some toxic side effects including nephrotoxicity, emetogenesis, and neurotoxicity and the occurrence of both inherent and acquired drug resistance in tumor cells [97]. In an attempt to overcome these problems, other platinum-containing compounds, platinum (IV) complexes, have been developed and are being considered as new therapeutic agents. The *in vitro* antitumor activity of some of these complexes have been tested against mouse fibrosarcoma L929 cells and human astrocytoma U251 cells and compared to cisplatin [103]. Results have shown that two Pt(IV) complexes with bidentate ethylenediamine-*N*,*N*′-di-3-propanoato esters displayed cytotoxic effects more rapidly than cisplatin. Also, it was observed that cisplatin was unable to generate a significant amount of ROS and caused mainly apoptotic cell death; whereas, the Pt(IV) complexes induced over production of ROS and mainly necrotic cell death indicating that the antitumor effects of these Pt(IV) complexes could be dependent on induction of ROS and mediated by a different cell death pathway [103]. Necrotic cell death linked to oxidative stress in the form of overproduction of ROS could provide an interesting alternative mechanism to apoptosis in promoting tumor regression [96].

9.2 Gold containing compounds

Gold containing compounds have been widely used as chemotherapeutic agents in the treatment of rheumatoid arthritis. Recently, due to their cytotoxic effects on a variety of human tumor cell lines, many gold (I) and gold (III) compounds have been the focus of several investigations as potential new anticancer agents [104 – 106]. Although the exact mechanism of action is still unknown, it is postulated that these compounds may exert their anticancer effects by direct interaction with DNA in a similar manner to that of platinum compounds or by interaction with other cellular targets such as proteins [104]. Gold is known for its high affinity to thiol groups and therefore it is possible that such groups are the prime targets of these compounds [96]. Several studies have indicated TrxR as a possible cellular target of gold compounds [96, 107 – 112].

Auranofin (*S*-triethylphosphinegold (I)-2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside; Fig. 5D) is a gold (I) compound well known as an antirheumatoid agent. *In vitro* studies have shown that this compound is able to inhibit the reduced form of TrxR (isolated from human placenta) at nanomolar levels (K_i = 4 nM) [107]. Much higher concentrations, at micromolar levels, were needed to inhibit glutathione reductase, an enzyme structurally similar to TrxR but lacking the C-terminal redox active site Cys-Se-Cys, leading to speculations that the selenol group could be the target of the compound [107]. In a study conducted to compare the cytotoxic effects of auranofin on human ovarian cancer cells that were either sensitive or resistant to cisplatin, similar results were obtained. TrxR activity was inhibited by auranofin in the nanomolar range whereas glutathione reductase activity was unaffected [108]. Furthermore, auranofin was able to promote cytotoxic effects in both cell lines and it was able to overcome cisplatin resistance to apoptosis [108], highlighting the potential of this drug in the treatment of cisplatin resistant tumors. Higher

activity of TrxR was observed in the cisplatin resistant cells, possibly indicating the importance of TrxR in the development of cisplatin resistance in these cells.

Upon auronofin treatment, increased production of hydrogen peroxide and release of cytochrome *c* was also observed [108]. It was suggested that inhibition of TrxR, by auranofin could be responsible for a dramatic change in the cellular redox state toward a more oxidized milieu, an increase in ROS production and ultimately apoptosis. Results from other studies add further support to this proposal. In isolated purified mitochondria, auronofin treatment is able to inhibit mitochondrial TrxR2 activity and to induce alterations of the mitochondrial membrane permeability with mitochondria swelling and loss of membrane potential ($\Delta\psi$) [112] and mitochondrial release of proapoptotic proteins such as cytochrome *c* [66]. In addition, a large accumulation of hydrogen peroxide occurs in the presence of auranofin and is independent of new formation of hydrogen peroxide by the respiratory chain. Instead, the high levels of hydrogen peroxide are caused by a decrease in the rate by which it is removed, possibly due to inhibition of the Trx system [113]. Taken together, these observations suggest a strong link between the anticancer effects of auranofin and alterations of the Trx system with mitochondrial malfunction caused by increase of membrane permeability, mitochondria swelling, release of cytochrome *c* with consequent induction of proapoptotic factors and rapid apoptotic cell death.

Selective inhibition of TrxR activity by auranofin has also been observed in experiments conducted in rat hearts subjected to an *in vitro* model of ischemia-reperfusion [114]. Under the experimental conditions used in those studies, the activity of another selenocysteine-containing enzyme, glutathione peroxidase, was not affected, indicating a very selective action of the gold compound.

Recently, a new gold (I) phosphine complex, [Au(d2pypp)$_2$]Cl, has been put forward as a new anticancer drug that targets the mitochondria and the Trx system [110]. Cell growth studies conducted with human MDA-MB-468 breast cancer cells and human HMEC mammary epithelial cells have shown that [Au(d2pypp)$_2$]Cl treatment selectively inhibited growth of the cancer cells but not of the normal cells. Furthermore, the mitochondria mediated apoptotic pathway was suggested as the mechanism of cell death in the breast cancer cells since activation of caspases 3 and 9 and depolarization of the mitochondrial membrane potential was observed. Both TrxR and Trx were indicated as potential cellular targets since [Au(d2pypp)$_2$]Cl was able to inhibit the activity of both enzymes to a greater extent in the MDA-MB-468 breast cancer cells than in the normal cells [110]. The higher enzymatic inhibition correlated with the selective toxicity to breast cancer cells. This gold (I) phosphine complex is quite interesting since it has been shown to accumulate preferentially in the mitochondria of cancer cells as a consequence of its lipophilic – cationic properties and the elevated mitochondrial membrane potential of the cancer cells [110]. The development of a drug that selectively targets the mitochondria in cancer cells and modulates the activity of the Trx system is a significant advancement in anticancer therapy.

Recently, TrxR1 has been shown to be the preferred target not only of gold(I) compounds such as auranofin but also of many other gold compounds (I and III) with different oxidation states and ligand configuration [109]. Results indicated that different gold compounds were able to inhibit TrxR1 activity at different potency and that the level of inhibition was affected by the ligand configuration and not so much by the initial oxidation state of the gold [109].

Studies conducted on the cellular effects of organogold (III) compounds containing the bipyridyl motif on the ovarian A2780 human cancer cell line have shown that these compounds are cytotoxic in the low micromolar range and able to promote apoptosis to a greater extent than cisplatin [115]. Rigobello *et al.* [111] have shown that these compounds are selective inhibitors of mitochondrial TrxR2. Considering that various gold (III) and organogold (III) compounds bind weakly *in vitro* to calf thymus DNA [116], the authors suggested that the capacity of these organogold (III) compounds to induce apoptosis could be linked to the activation of the mitochondria-mediated apoptotic pathway as a result of the inhibition of TrxR2 [115].

9.3 Arsenic trioxide (ATO)

ATO (Fig. 5E) is a very efficient and successful therapeutic agent recently used in the treatment of both newly diagnosed and relapsed acute promyelocytic leukemia [117, 118]. Induction of the mitochondrial apoptotic pathway has been suggested as one of the numerous effects ATO exerts on cells [119]. Although the exact mechanism of action of this compound is unknown as of yet, there are suggestions that proteins with accessible and closely spaced cysteine residues could be one of the cellular targets [120, 121]. *In vitro* irreversible inhibition of mammalian TrxR by ATO in the micromolar range (IC_{50} = 0.25 μM) supports this suggestion [122]. Results have shown that only reduced TrxR is inhibited by ATO indicating that the TrxR inhibition is dependent on the redox state of the protein and that the protein sulfhydryl groups are possibly the targets of the drug. MS analysis of trypsin-digested peptides of TrxR-ATO suggests that both the N-terminal redox active dithiol and the C-terminal selenothiol-active site may react with ATO, although the selenocysteine in the C-terminal active site seems to be critical for the inhibitory effect. Inhibition of TrxR by ATO was further seen in an investigation using human breast MCF-7 cancer cells. Growth inhibition of MCF-7 cancer cells was observed after 48 h treatment together with irreversible inhibition of TrxR and subsequent severe oxidation of Trx [122].

The glutathione system together with the Trx system plays an important role in cellular redox control. Together

both systems are expected to modulate the proapoptotic effects of ATO. A dramatic increase in sensitivity of MCF-7 cells to ATO and reduction of TrxR activity was observed when the cells were pretreated with buthionine sulfoximine (BSO), an inhibitor of glutathione synthesis, suggesting that intracellular glutathione levels may be correlated to different sensitivity of cancer cell lines to ATO [123]. Glutathione has been identified as an important detoxifying agent of arsenicals [124] but no effect was observed on the activity of glutathione-related enzymes, glutathione reductase, glutathione peroxidases, and glutathione-*S*-transferase, indicating that these enzymes are probably not the targets in the arsenic toxicity mechanism [125]. The authors suggested that inhibition of both antioxidant systems, the Trx and GSH systems, could cause alterations in cellular events such as DNA synthesis and repair, protection against oxidative stress and consequently induction of apoptotic events [122].

9.4 Motexafin gadolinium (MGd)

MGd (Fig. 5F), is a member of the group of porphyrin-like molecules called texaphyrins, that is, coupled to the gadolinium (III) cation and utilized in cancer therapies [126]. The anticancer activity of this compound is linked to its capacity to undergo redox cycling and to generate superoxide and other ROS under aerobic conditions catalyzing the oxidation of intracellular reducing metabolites such as NADPH, GSH, ascorbic acid, and protein vicinal thiols [126, 127]. It has been shown to induce cytotoxicity in dexamethasone-sensitive and also in highly dexamethasone-resistant multiple myeloma cell lines by altering the cellular redox state due to an increased production of ROS [128]. ROS production and increased oxidative stress has been considered as a potential mechanism of action of MGd in the enhanced ionizing radiation sensitivity observed in cancer cell lines in the presence of MGd [127].

Recent studies have shown that the cytosolic TrxR1 is one of the target molecules of MGd [129]. Results show that MGd inhibits TrxR1 disulfide reductase activity and induces an NADPH oxidase activity in TrxR1, generating ROS such as superoxide and hydrogen peroxide. In the same study, it was also observed that MGd is a potent inhibitor of ribonucleotide reductase, which is an essential enzyme for DNA synthesis. The effects of MGd on these cellular systems could account for its anti cancer activity. Manipulation of the Trx redox system together with an increased production of ROS induces a prooxidant state in the cell, which in turn may trigger cellular injury and induction of apoptotic events in tumor cells. Inhibition of ribonucleotide reductase will block DNA synthesis and repair and cellular growth. The indirect action of MGd on oxidized Trx may induce apoptosis *via* ASK1 mediated cell death [129].

Other studies have indicated an involvement of MGd with alterations of zinc metabolism. Microarray analyses conducted in human cancer cell lines (A549 lung cancer cells and Ramos B-cell lymphoma line) have shown that MGd treatment causes up-regulation in the expression of genes controlling free zinc levels [130]. Increased intracellular free zinc levels may have detrimental effects on the redox active SH groups of enzymes such as TrxR or ribonucleotide reductase [129]. Inhibition of TrxR activity by zinc has been reported [130, 131] suggesting an additional indirect action of MGd on TrxR [126].

MGd is currently in clinical trials as a single drug or in combination with other chemotherapeutic agents or radiotherapy for the treatment of different type of cancers including nonsmall cell lung carcinoma, brain and central nervous system tumors, lymphoma, renal cell carcinoma, and pancreatic and biliary tumors [126, 132].

9.5 Nitroaromatic compounds (DNCB)

1-Chloro-2,4-dinitrobenzene (DNCB) is a nitroaromatic compound proven to be a potent inhibitor of TrxR activity by dinitrophenyl-derivatization of both the selenocysteine and its neighboring cysteine residue in the C-terminal domain [133]. This small electrophilic compound is not only capable of irreversibly inhibiting TrxR but also of inducing a superoxide generating NADPH oxidase activity of the protein [50, 133]. The molecular mechanism for this activity involves FAD catalyzed production of nitro anion radicals in the dinitrophenol groups of the modified enzyme, which in turn react with oxygen to generate superoxide [133]. DNCB is also able to induce apoptosis in human cancer cells [50]. Treatment of A549 lung cells and HeLa cells with DNCB has been shown to induce cytotoxic effects, to trigger mitochondrial caspase (3/7) activation and to cause inhibition of cellular TrxR [50]. In addition, treatment of HeLa cells over expressing Bcl-2 did not prevent the cytotoxic effect of the compound suggesting that the apoptotic events were not blocked by over expression of Bcl-2. The authors proposed that the modified TrxR formed upon interaction with the nitroaromatic compound might be a major trigger of the apoptotic events. This proposal is compatible with other findings previously described in this review [100, 102, 122].

9.6 Curcumin and flavonoids

Polyphenols such as curcumin and some flavonoids have been identified as potential anticancer agents with a mechanism of action that may be mediated by the Trx system [134, 135]. TrxR activity is irreversibly inhibited upon interaction of the C-terminal redox active site with curcumin and the modified enzyme is converted into a pro-oxidant that stimulates ROS production *via* an acquired NADPH oxidase activity [134]. Inhibition of TrxR is also observed when curcumin is added to cultured HeLa cells indicating that the enzyme is a major cellular target of the

polyphenol compound [134]. Curcumin is currently being assessed in clinical trials for its anticancer activity and the above results suggest that its mode of action may be through targeting TrxR.

A similar mechanism of action has been attributed to the flavonoids, myricetin, and quercetin. *In vitro* studies have shown that both of these flavonoids are able to irreversibly inhibit TrxR and that inhibition of the Trx system together with consequent ROS production might be responsible for the anticancer activity of these compounds [135].

10 Conclusions

Over the years, several groups of synthetic compounds and natural products have been explored as anticancer agents and observed to, directly or indirectly, modulate cellular antioxidant defense systems and to induce apoptosis in cancer cells. In this review, we have described some of the agents that specifically target components of the Trx system with particular emphasis given to compounds inhibiting the TrxR enzyme. Thiol groups and more specifically the selenothiol of TrxR is the selected target of these agents. The interaction of agents such as ATO, cisplatin and auranofin with TrxR causes inhibition of its activity with consequent alteration of the many cellular activities controlled by the enzyme. Agents like DNCB, MGd, and curcumin modify TrxR causing the induction of a pro-oxidant activity, which is responsible for an increased production of ROS. In both cases, the net result is an alteration of the cellular redox state and impairment of mitochondrial function, with consequent induction of apoptotic cell death (see Fig. 4).

Although many studies have provided information regarding the mechanisms by which these compounds may act on the Trx system and how they exert their downstream cellular effects, many questions still remain unanswered and many issues unresolved. These include the need for improved specificity and selectivity toward their targets and in particular toward cancer cells, increased effectiveness and reduction of side effects and how to overcome resistance against the cytotoxic effects of some drugs, with the ultimate aim of improving the overall anticancer treatment response. Much progress has already been made to improve the selectivity of certain agents toward cancer cells. A new anticancer agent, the gold (I) phosphine complex $[Au(d2pypp)_2]Cl$, which specifically targets the Trx system, selectively inhibits growth of cancer cells but not of normal cells as a consequence of preferential accumulation in the mitochondria of cancer cells. The higher affinity of this type of compounds toward vicinal protein thiols and selenols may explain their selectivity toward the Trx system.

Differences in sensitivity toward some of these anticancer agents between various types of cancer cells and/or between cancer and normal cells have been observed and associated with different expression levels and/or enzymatic activity of Trx and TrxR, suggesting a critical role of the Trx system in the development of cellular resistance against some anticancer agents. Elucidating the detailed and complete mechanisms by which the Trx system inhibitors exert their apoptotic effects will enable their full potential as potent chemotherapy agents to be realized.

We would like to thank Dr. Chris Brown for assistance with the Chemdraw program and Fig. 5.

The authors have declared no conflict of interest.

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