Effects of Mammalian Thioredoxin Reductase Inhibitors

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Contents

Abstract

The mammalian thioredoxin system is driven by NADPH through the activities of isoforms of the selenoprotein thioredoxin reductase (TXNRD, TrxR), which in turn help to keep thioredoxins (TXN, Trx) and further downstream targets reduced. Due to a wide range of functions in antioxidant defense, cell proliferation, and redox signaling, strong cellular aberrations are seen upon the targeting of TrxR enzymes by inhibitors. However, such inhibition can nonetheless have rather unexpected consequences. Accumulating data suggest that inhibition of TrxR in normal cells typically yields a paradoxical effect of increased antioxidant defense, with metabolic pathway reprogramming, increased cellular proliferation,

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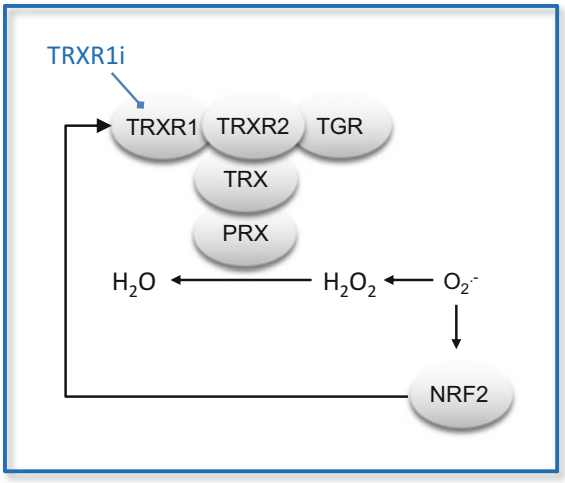
and altered cellular differentiation patterns. Conversely, inhibition of TrxR in cancer cells can yield excessive levels of reactive oxygen species (ROS) resulting in cell death and thus anticancer efficacy. The observed increases in antioxidant capacity upon inhibition of TrxR in normal cells are in part dependent upon activation of the Nrf2 transcription factor, while exaggerated ROS levels in cancer cells can be explained by a non-oncogene addiction of cancer cells to TrxR1 due to their increased endogenous production of ROS. These separate consequences of TrxR inhibition can be utilized therapeutically. Importantly, however, a thorough knowledge of the molecular mechanisms underlying effects triggered by TrxR inhibition is crucial for the understanding of therapy outcomes after use of such inhibitors.

Graphical Abstract



The mammalian thioredoxin system is driven by thioredoxin reductases (TXNRD, TrxR), which keeps thioredoxins (TXN, Trx) and further downstream targets reduced. In normal cells, inhibition of TrxR yields a paradoxical effect of increased antioxidant defense upon activation of the Nrf2 transcription factor. In cancer cells, however, inhibition of TrxR yields excessive reactive oxygen species (ROS) levels resulting in cell death and thus anticancer efficacy, which can be explained by a non-oncogene addiction of cancer cells to TrxR1 due to their increased endogenous production of ROS. These separate consequences of TrxR inhibition can be utilized therapeutically.

Keywords

Reactive oxygen species · Redox signaling · Selenoprotein · Thioredoxin reductase

1 Thioredoxin Reductases and Redox Biology

Redox biology is fundamental to all aspects of life, and altered redox processes are related to several diseases, including aspects of excessive levels of ROS, hypoxia, ischemia-reperfusion injury, and disturbed compartmentalized formation of reactive oxygen species (Forbes et al. 2008; Ryter et al. 2007; Ye et al. 2015). Enzymatically regulated formation of reactive oxygen species, especially H_2O_2, is also essential in several physiologically normal intracellular signaling pathways (Finkel 2000, 2011; Holmstrom and Finkel 2014; Rhee 2006). Different therapies that target the enzymatic systems of redox biology may thereby affect normal physiological events as well as pathways distorted in disease. Most if not all therapies that perturb redox states of cells will be likely to involve, or at least affect, the thioredoxin (Trx) and glutathione (GSH) systems, which are the main mammalian enzyme systems for control of reductive pathways in cells (Arnér 2009; Nordberg and Arnér 2001; Rundlöf and Arnér 2004). Direct drug targeting with inhibition of both of these two redox pathways can have therapeutic effects in cancer treatment (Harris et al. 2015), but simultaneous targeting of both pathways can also result in major unwanted toxicity and severe side effects. Several lines of observations suggest that targeting of Trx reductases (TrxRs) alone may however yield therapeutic efficacy in disease with less severe toxicity to normal cells. This will be discussed here, but first the selenoprotein nature of TrxRs shall be introduced.

Selenium (Se) is an essential trace element for mammals, due to its role as the defining constituent of the 21st amino acid, selenocysteine (Sec), found in selenoproteins (Johansson et al. 2005). The human genome has 25 selenoprotein-encoding genes, mostly encoding enzymes with a single catalytic Sec residue in their active sites (Kryukov et al. 2003). The chemical features of Sec make this amino acid an ideal catalyst for redox reactions, with Sec being much more chemically reactive than its more common sulfur-containing Cys analog (Arnér 2010) and also more resistant to overoxidation (Reich and Hondal 2016). Sec can in many cases be regarded as a "super cysteine," which helps to explain the higher activities of selenoenzymes that are typically seen when compared to their corresponding Sec-to-Cys mutants (Johansson et al. 2005; Reich and Hondal 2016). Some of the mammalian selenoproteins are essential, as illustrated by the early embryonic lethality in mouse knockout models for cytosolic thioredoxin reductase (TrxR1, encoded by *Txnrd1*) (Bondareva et al. 2007), mitochondrial thioredoxin reductase (TrxR2, *Txnrd2*) (Conrad et al. 2004), and glutathione peroxidase 4 (GPx4, *Gpx4*) (Yant et al. 2003). It was also shown that GPx4 protects cells against ferroptosis in a strictly Sec-dependent manner, which may be one of the major functions explaining a need for Sec in this enzyme and for selenoprotein expression overall, at least in certain cell types (Ingold et al. 2017).

Interestingly, cellular TrxR1 status also effectively controls cellular phenotype and differentiation patterns, with its genetic deletion reprogramming metabolism in hepatocytes of mouse liver (Iverson et al. 2013), activating Nrf2 (Cebula et al. 2015) and promoting fibroblasts in culture to undergo adipogenesis (Peng et al. 2016). Such effects of TrxR1 on cellular differentiation relate, among other mechanisms, to modulation of PTP1B signaling linked to tyrosine receptor stimulation (Dagnell et al. 2013b, 2017) and to the direct modulation of redox-sensitive transcription factors such as Nrf2, HIF, and NFκB (Johansson et al. 2017; Kipp et al. 2017). It is clear that TrxR1 can modulate cellular signaling pathways on many different, yet interlinked, levels in cells (Dagnell et al. 2018). A better understanding of those pathways will be important in order to understand and predict the possible outcomes of drug-mediated TrxR inhibition.

1.1 TrxR Genes and Proteins

The mammalian Trx system is an important reductive enzyme system in cells that acts together or in parallel with the glutathione (GSH) system (Arner and Holmgren 2000; Becker et al. 2000; Gromer et al. 2004; Nordberg and Arnér 2001). The Trx system encompasses Trx1 (encoded in human by *TXN*) and several additional Trx-fold enzymes, being kept reduced and thus redox active by the actions of thioredoxin reductases (TrxRs) using NADPH. The Trx-fold proteins can subsequently act to support reductive pathways or modulate redox regulatory systems in a multitude of cellular functions. The human genome encodes three specific TrxR isoenzymes, namely, cytosolic TrxR1 (encoded by *TXNRD1*), mitochondrial TrxR2 (encoded by *TXNRD2*), and testis-specific TGR (encoded by *TXNRD3*), with all three enzymes being selenoproteins (Arner and Holmgren 2000; Arnér 2009; Gromer et al. 2004; Martin 1995; Miranda-Vizuete et al. 2004; Nordberg and Arnér 2001). The differences between these isoforms are discussed further in Sect. 1.2.

Most studies with regard to effects of inhibitors have been performed on TrxR1 or TrxR2, while TGR has been much less studied. However, several pathogenic parasites rely on TGR orthologs, which may be inhibited through drug therapy as a novel form of antiparasitic therapy. This includes targeting of the TGR enzyme in *Schistosoma mansoni* (Kuntz et al. 2007; Lea et al. 2008; Rai et al. 2009; Silvestri et al. 2018; Simeonov et al. 2008), *Schistosoma japonicum* (Huang et al. 2015; Song et al. 2012), *Fasciola gigantica*, *Fasciola hepatica*, and other helminth parasites (Maggioli et al. 2011; Shukla et al. 2018; Williams et al. 2013), the tapeworm *Mesocestoides vogae* (Pasquet et al. 2015), *Taenia crassiceps cysticerci* (Martinez-Gonzalez et al. 2015), *Echinococcus granulosus* (Saiz et al. 2014), and additional cestode and trematode flatworms (Otero et al. 2010; Ross et al. 2012). The rest of this chapter shall however discuss drug targeting of the human forms of TrxR.

1.2 Isoforms and Expression Patterns of Human TrxRs

The human *TXNRD1* gene encodes predominantly cytosolic TrxR1, which is ubiquitously expressed and has Trx1 as its major substrate (Arner and Holmgren 2000; Rundlof and Arner 2004; Rundlof et al. 2004; Sun et al. 2001b). Mitochondrial TrxR2 encoded by *TXNRD2* reduces mitochondrial Trx2 as its main substrate (Lee et al. 1999; Miranda-Vizuete et al. 1999; Rigobello et al. 1998). The *TXNRD3* gene, finally, encodes TGR (thioredoxin glutathione reductase) that has a glutaredoxin (Grx) domain at the N-terminal part of the protein, in addition to its major TrxR module that otherwise is similar in domain structure to that found in TrxR1 and TrxR2. TGR is involved in maturation of sperm cells and mainly expressed in early spermatids (Su et al. 2005; Sun et al. 2001a, 2005).

The *TXNRD1* gene on chromosome 12 (12q23-q24.1) has a complex organization, with numerous transcripts displaying extensive splicing at their 5′-ends, thus producing several different protein isoforms of TrxR1 (Osborne and Tonissen 2001; Rundlof et al. 2000, 2004; Su and Gladyshev 2004; Sun et al. 2001b). One isoform, TXNRD1_v3 ("v3"), has three additional exons encoding a Grx domain, which is expressed in N-terminal fusion to the classical TrxR1 module. This is similar to TGR but v3 has a dithiol active site in contrast to the monothiol site found in TGR (Dammeyer et al. 2008; Rundlof et al. 2004, 2007; Su and Gladyshev 2004). Humans, chimpanzees, and dogs express v3, but mice or rats do not (Su and Gladyshev 2004). The v3 enzyme can be myristoylated and palmitoylated, being targeted to cell membranes where it seems to associate with lipid rafts and trigger formation of filopodia (Cebula et al. 2013; Damdimopoulou et al. 2009; Dammeyer et al. 2008). It is not clear if v3 is also targeted by drugs inhibiting TrxR, but this possibility should not be disregarded. Other major splice variants of TrxR1 are TXNRD1_v1 that is the "classical" form of the enzyme and TXNRD1_v2 (also called TrxR1b) that can be channeled to the nucleus and there interact with transcription factors including the estrogen receptor (Arnér 2009; Damdimopoulos et al. 2004).

The human *TXNRD2* gene is found on chromosome 22 (22q11.21) and mouse *Txnrd2* on chromosome 16. Similarly to *TXNRD1* there is evidence for extensive alternative splicing at the 5′-end of the corresponding transcripts, encoding protein variants with different N-terminal domains (Sun et al. 2001c). Thus, also in the case of TrxR2 there is a chance that drug inhibition of the enzyme also targets several isoforms within the same cells, or in different organs. It should here be noted that not all TrxR isoenzymes are expected to be targeted with the same efficiency upon use of inhibitors, with the final effects both depending upon different affinities for the specific enzymes and upon possible compartmentalization effects. In a side-by-side comparison, it was indeed shown that TrxR1 and TrxR2 differ in their sensitivities to different inhibitors (Rackham et al. 2011) and certain compounds, such as auranofin or isothiocyanates, were shown to target mainly mitochondrial TrxR2 before they inhibit TrxR1 within the cellular context (Brown et al. 2008; Cox et al. 2008).

The human *TXNRD3* gene encoding TGR is located at chromosome 3 (3q21.3), while mouse *Txnrd3* is at chromosome 6. These are yet the least characterized TrxR-encoding genes and also the least characterized TrxR isoenzymes. It should nonetheless be noted that TGR has the same Sec-containing active site motif as the other TrxRs, and it is thus both possible and plausible that also TGR may be targeted upon the use of drugs inhibiting TrxR isoenzymes.

1.3 Catalytic Mechanisms and Propensity for Drug Inhibition of TrxR

All human TrxRs share the same C-terminal -Gly-Cys-Sec-Gly-COOH motif being the proper active site reducing Trx (Arscott et al. 1997; Gladyshev et al. 1996; Lee et al. 2000; Tamura and Stadtman 1996; Zhong et al. 1998, 2000; Zhong and Holmgren 2000). Several crystal structures of Sec-to-Cys substituted mutant enzymes revealed the general domain structure and catalytic mechanism of mammalian TrxRs (Biterova et al. 2005; Eckenroth et al. 2006, 2007a, b; Fritz-Wolf et al. 2007; Sandalova et al. 2001), with a crystal structure of Sec-containing TrxR1 subsequently confirming the proposed formation of a selenenylsulfide at the C-terminus of the oxidized protein (Cheng et al. 2009). Importantly, in the NADPH-reduced enzyme, the selenenylsulfide becomes reduced to a selenolthiol motif, with its highly reactive and nucleophilic Sec residue being fully exposed to solvent and thus serving as a prime target for inhibition by electrophilic compounds (Cheng et al. 2009).

The first part of the reductive half-reaction of TrxR1 utilizes NADPH to reduce an enzyme-bound FAD in one subunit of the dimeric enzyme. The reduced FAD subsequently reduces a disulfide in a -CVNVGC- active site motif present in the same subunit, thus producing a dithiol. This part of the catalytic cycle is similar to that seen in glutathione reductase and other enzymes of the pyridine nucleotide disulfide oxidoreductase family (Williams 1992). However, instead of next reducing a substrate in solution, as with GSSG reduction by glutathione reductase, the C-terminal selenenylsulfide motif in the opposing subunit of TrxR1 is reduced, which may finally reduce substrates of TrxR1 including Trx1 (Cheng et al. 2009). Mammalian TrxRs also reduce several other substrates in addition to Trxs. Two additional and potentially important direct protein substrates of mammalian TrxR1 are glutaredoxin 2 (Johansson et al. 2004) and TRP14 (also called TXNDC17) having several redox signaling roles in cells (Espinosa and Arner 2019; Jeong et al. 2004; Pader et al. 2014; Woo et al. 2004). Another protein substrate of TrxR of potential importance is protein disulfide isomerase (PDI) that, like other ER proteins including CaBP1 and CaBP2 (Erp57), carries Trx domains with active sites that can be reduced by TrxR (Lundström-Ljung et al. 1995). It is interesting that cytosolic TrxR1 somehow reduces ER-resident proteins, which indeed can explain phenomena such as the reductive activation of immunotoxins through PDI being reduced by TrxR (Bellisola et al. 2004) or reduction of the disulfides in misfolded ER proteins being dependent upon TrxR1 (Poet et al. 2017). TrxR1 was

also shown to directly reduce the active site of another protein of the Trx family, Trx-like-1 (TXL-1, TXNL-1 or TRP32) (Jimenez et al. 2006), that is a cytosolic protein (Lee et al. 1998) involved in glucose metabolism (Jimenez et al. 2006) and endocytosis (Felberbaum-Corti et al. 2007). Additional protein disulfide substrates for TrxR include Trx isoforms in male germ cells (Jimenez et al. 2002, 2004; Miranda-Vizuete et al. 2004) and the antibacterial peptide NK-lysin (Andersson et al. 1996). Furthermore, TrxR activities are important in controlling the persulfidation states of proteins, including key signaling proteins (Doka et al. 2016, 2020). Again, all of these enzymatic functions may be considered to be inhibited or affected upon the use of TrxR inhibitors.

TrxRs also have non-protein substrates that can play functional roles in a cellular context. This includes reduction of dehydroascorbate (May et al. 1997), lipoic acid (Arnér et al. 1996), cytochrome *c* (Nalvarte et al. 2004), toxoflavin (Gencheva et al. 2018), ubiquinone (Xia et al. 2003), and several other quinone compounds (Cenas et al. 2004). It is not clear if TrxR-mediated reduction of such substrates has a physiological importance, but also these activities will naturally be affected upon TrxR inhibition.

2 Inhibitors of Thioredoxin Reductases

TrxR1 is inhibited by a wide range of different compounds. The relative ease of inhibiting TrxR1 is mainly explained by its exceptionally reactive Sec residue that easily becomes covalently derivatized by many electrophilic inhibitors (Becker et al. 2000; Carvalho et al. 2008; Cebula et al. 2015; Krishnamurthy et al. 2008; Liu et al. 2008a; Prast-Nielsen et al. 2011; Witte et al. 2005). However, TrxR1 is a complex enzyme, and it should not be disregarded that inhibition of the enzyme can be achieved by reversible or irreversible interactions also of other motifs in TrxR1 than its Sec residue. For comprehensive discussions of different classes of TrxR1 inhibitors, see prior reviews on the topic (Arnér 2009; Cai et al. 2012; Cebula et al. 2015; Eriksson et al. 2009; Gromer et al. 2004; Liu et al. 2008a; Rackham et al. 2011; Urig and Becker 2006; Wipf et al. 2004; Zhang et al. 2016, 2018, 2019). Here the different classes of TrxR inhibitors shall not be repeated. Instead, we shall discuss the different cellular consequences of TrxR inhibition and their therapeutic potential.

3 Consequences of Thioredoxin Reductase Inhibition

A large number of compounds that inhibit TrxR1 have anticancer effects, and, moreover, several clinically used anticancer agents are known to inhibit TrxR1 (Arnér 2009; Arnér and Holmgren 2006; Cai et al. 2012; Casini et al. 2008; Chew et al. 2008; Eriksson et al. 2009; Fang et al. 2005; Gromer et al. 2004; Hashemy et al. 2006; Hedstrom et al. 2009; Lincoln et al. 2003; Liu et al. 2008a, b; Lu et al. 2007, 2006; Marzano et al. 2007; Peng et al. 2013; Prast-Nielsen et al. 2010; Prast-Nielsen

et al. 2011; Shi et al. 2014; Urig and Becker 2006; Wang et al. 2008; Wipf et al. 2004; Witte et al. 2005). It is not clear, however, whether an efficient anticancer therapy can be developed solely upon TrxR1 inhibition and/or if any specific consequences of TrxR1 targeting can form the basis for a successful anticancer therapy. Some inhibitors of TrxR1 however show clear antitumoral efficacy in mouse models (Stafford et al. 2018; Ye et al. 2017). It is furthermore possible, perhaps even plausible, that TrxR inhibition in normal non-cancerous cells may have therapeutic potentials for use in other diseases than cancer. This will be discussed next.

3.1 Paradoxically Increased Antioxidant Defense

The nuclear factor erythroid-2-related factor 2 (Nrf2) transcription factor activates transcription of several key enzymes supporting cellular antioxidant systems (Copple et al. 2008; Osburn and Kensler 2008; Tong et al. 2006; Zhang 2006). It has been suggested that antitumoral immune system functions require Nrf2 activation (Ghosh et al. 2015; Mougiakakos et al. 2012; Zhang 2006; Zhao et al. 2014) and, interestingly, many inhibitors of TrxR1 also activate Nrf2, indeed suggesting a direct functional link between TrxR1 and Nrf2 (Cebula et al. 2015). A question is whether Nrf2 activation in normal cells can be achieved by drug-mediated TrxR1 inhibition and whether this may have any therapeutic value. It would be possible that such therapy can be used to protect normal cells from damage by excessive ROS levels and indeed also perhaps strengthen the antitumoral immunity. Interestingly, it was, perhaps at first seemingly paradoxically so (Lei et al. 2016), found that TrxR1 inhibition in normal cells becomes highly protective against subsequent oxidative challenges, as a result of a strong Nrf2 activation (Iverson et al. 2013; Locy et al. 2012; Rollins et al. 2010). Such protective effects may help explain how the TrxR1-inhibiting compounds curcumin (Fang et al. 2005; Liu et al. 2008a) or isothiocyanates (Bacon et al. 2007; Brown et al. 2008; Hu et al. 2007; Jakubikova et al. 2006) have chemopreventive effects, provided that Nrf2 has the anticancer preventive capacity that has been proposed (Brigelius-Flohe 2008; Brigelius-Flohe and Banning 2006; Chew et al. 2010; Higgins and Hayes 2011; Hu et al. 2007; Lee et al. 2007; Lu et al. 2006; Poerschke et al. 2012; Surh et al. 2008; Zhang 2006).

3.2 Affected Cell Differentiation Patterns

Mouse embryos lacking TrxR1 die prior to gastrulation and they display a lack of mesoderm formation (Bondareva et al. 2007). Conditionally knocked-out TrxR1 in hepatocytes of mouse liver triggers hyperproliferation, lack of signs of excessive ROS levels, metabolic aberrations, and very strong Nrf2 activation (Prigge et al. 2012a; Rollins et al. 2010; Iverson et al. 2013; Prigge et al. 2017; Suvorova et al. 2009), similar effects as those seen upon drug-mediated inhibition of TrxR1 (Locy et al. 2012), again suggesting that TrxR1 can be linked to control of Nrf2, with

activation of Nrf2 upon inhibition or loss of TrxR1 (Cebula et al. 2015; Schmidt 2015). *Txnrd1*-deficient mouse embryonic fibroblasts also display striking features in culture, with an increased cell differentiation, insulin responsiveness, and spontaneous adipogenesis (Peng et al. 2016). Notably, in such cells lacking TrxR1, its major substrate Trx1 is still reduced (Peng et al. 2016), likely through the action of GSH-dependent glutaredoxins (Du et al. 2013). This suggests that any effects of TrxR1 inhibition on cellular phenotypes must not necessarily be due to impaired Trx1 activities. TrxR1-lacking cells nonetheless show increased responses to PDGF in conjunction with exaggerated oxidative inhibition of PTP1B (Dagnell et al. 2013a), again illustrating strong effects of TrxR1 status on cellular signaling pathways. Similar effects may hence be triggered also upon use of TrxR1 inhibitors. In other words, it is possible that inhibition of TrxR1 increases the overall antioxidant capacity of normal cells due to Nrf2 activation, and it may also be possible that a number of immature cell types can become triggered to a propensity for increased differentiation.

3.3 Effects on the Immune System

Antitumoral efficacy of the immune system is an important feature for final eradication of cancer in any form of cancer therapy (Ruffell and Coussens 2015; Shahabi et al. 2015; Vinay et al. 2015). Important in this context is that the Trx system can modulate the effectiveness of the immune system against cancer at least by two different mechanisms. First, activation of Nrf2 seems to be important for antitumoral activities of the immune system (Ghosh et al. 2015; Manda et al. 2015; Mougiakakos et al. 2012; Ruffell and Coussens 2015; Vinay et al. 2015), which may hence be another potentially beneficial consequence of TrxR1 inhibition in cancer therapy. Second, if TrxR1 becomes inhibited in cancer cells, this might increase the secretion from these cells of Trx1 as well as its C-terminally truncated protein Trx80; both of those proteins when present in serum act as co-cytokines and chemokines that may be proposed to attract antitumoral immune cells toward the tumor (Arner and Holmgren 2000; Arnér and Holmgren 2006; Backman et al. 2007; Hori et al. 1993; Pekkari et al. 2005; Pekkari and Holmgren 2004). It should also be noted that auranofin, a classically used antirheumatic drug, is a very potent inhibitor of TrxR (Cox et al. 2008; Gromer et al. 2002; Marzano et al. 2007; Omata et al. 2006; Rigobello et al. 2005), and although it is not clear if or how TrxR inhibition is part of the antirheumatic efficacy of this gold compound, auranofin is now also being repurposed for use in therapy of cancer and other diseases where TrxR1 inhibition may be viewed as beneficial (Roder and Thomson 2015). It is also of significant interest that TrxR1 targeting yields prevention of STAT3 activation as a secondary downstream effect, which may also contribute to the anticancer efficacy of TrxR1 inhibitors (Busker et al. 2020).

3.4 Anticancer Therapy

It is rather well established that cancer cells have increased endogenous ROS levels (Luo et al. 2009). The activities of their antioxidant systems are thereby also increased, which in turn makes tumor cells more vulnerable to treatments that further enhance their ROS levels (Gorrini et al. 2013; Wondrak 2009). Indeed, Nrf2 is typically highly activated in cancer cells as a means to support their own survival (Brigelius-Flohe and Flohe 2011; Ganan-Gomez et al. 2013; Mitsuishi et al. 2012; Osburn and Kensler 2008; Singh et al. 2008). The expression levels of TrxR1 in turn modulate the cytotoxic profiles of redox active anticancer drugs in cancer cells (Eriksson et al. 2009). It is thus not far-fetched to believe that TrxR1 targeting may be a plausible mechanism of action for anticancer drugs, and the notion that cancer cells have an inherently increased level of ROS that can be targeted for therapy is indeed gaining wide recognition (Galluzzi et al. 2013; Harris et al. 2015; Luo et al. 2009; Manda et al. 2015; Shi et al. 2014; Trachootham et al. 2006, 2009). This property of cancer cells also explains why they typically exhibit high endogenous Nrf2 activities, with increased levels of enzymes in the GSH and Trx systems, as a means of surviving (Brigelius-Flohe and Flohe 2011; Higgins and Hayes 2011; Mitsuishi et al. 2012; Singh et al. 2008; Zhang 2006). It should thus be a natural consequence that inhibition of TrxR in cancer cells should help triggering their cell death, while normal cells should typically survive the loss of TrxR activity (Arnér 2009; Arnér and Holmgren 2006; Chew et al. 2010; Harris et al. 2015; Shi et al. 2014; Trachootham et al. 2009). This may hence be a major principle by which TrxR inhibition can yield anticancer efficacy and reduction of tumor mass. This notion is further corroborated by findings showing that the lack of TrxR1 in cancer cells impairs their capacity to form tumors (Hatfield et al. 2009; Mandal et al. 2010; Yoo et al. 2006, 2007).

An additional effect of drug targeting of TrxR1 in cancer cells, which may contribute to tumor cell death, is the conversion of the enzyme to toxic pro-oxidant redox cycling forms of the protein, named SecTRAPs (*Se*lenium *c*ompromised *t*hioredoxin *r*eductase-derived *a*poptotic *p*roteins) that can further increase ROS levels and thus also help killing cancer cells (Anestål and Arnér 2003; Anestål et al. 2008; Cai et al. 2012; Cebula et al. 2015; Hashemy et al. 2006). These mechanisms of action are also compatible with the activities of novel TrxR1 inhibitors showing anticancer efficacy (Stafford et al. 2018).

As explained above, since compounds that target TrxR1 in cancer cells will likely also induce robust Nrf2 responses in normal cells that, paradoxically, *protect* normal cells from oxidative damage (Iverson et al. 2013; Lei et al. 2016; Locy et al. 2012; Prigge et al. 2012b), this opens the possibility that specific targeting of TrxR1 can have dual effects in anticancer therapy, namely, protection of normal cells with a boost of the immune system by Nrf2 activation on one hand and lethality to cancer cells due to excessive ROS levels on the other.

4 Conclusions

As discussed herein, the outcome of TrxR inhibition will depend upon the cellular context in which the enzyme is inhibited, as well as upon the nature of the isoenzyme (s) or isoform(s) of TrxRs that are being targeted. Notwithstanding the complexity of the profile of TrxR enzymes in cells, the picture emerges that inhibition of these enzymes in normal cells can trigger Nrf2 activation that protects these cells from excessive ROS levels, which in turn boosts the functions of the immune system. In contrast, cancer cells seem to be excessively sensitive to TrxR1 inhibition, and drugs inhibiting the enzyme can thereby have direct anticancer properties. In combination, these consequences of TrxR inhibition suggest that inhibitors of these enzymes are amenable to therapy development for treatment of a number of different diseases, mainly cancer but also diseases where normal cells suffer from injuries due to increased levels of ROS.

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