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FORUM REVIEW ARTICLE

Selenium and the Selenoprotein Thioredoxin Reductase in the Prevention, Treatment and Diagnostics of Cancer

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Abstract

Selenium is an essential element that is specifically incorporated as selenocystein into selenoproteins. It is a potent modulator of eukaryotic cell growth with strictly concentration-dependant effects. Lower concentrations are necessary for cell survival and growth, whereas higher concentrations inhibit growth and induce cell death. It is well established that selenium has cancer preventive effects, and several studies also have shown that it has strong anticancer effects with a selective cytotoxicity on malignant drug-resistant cells while only exerting marginal effects on normal and benign cells. This cancer-specific cytotoxicity is likely explained by high affinity selenium uptake dependent on proteins connected to multidrug resistance. One of the most studied selenoproteins in cancer is thioredoxin reductase (TrxR) that has important functions in neoplastic growth and is an important component of the resistant phenotype. Several reports have shown that TrxR is induced in tumor cells and pre-neoplastic cells, and several commonly used drugs interact with the protein. In this review, we summarize the current knowledge of selenium as a potent preventive and tumor selective anticancer drug, and we also discuss the potential of using the expression and modulation of the selenoprotein TrxR in the diagnostics and treatment of cancer. *Antioxid. Redox Signal.* 12, 867–880.

Selenium Metabolism

The metabolism of selenium compounds follows three main pathways (Fig. 1), where the first is common for primarily redox active selenium compounds, the second for selenoamino acids, and the third for compounds that are direct precursors of monomethylselenol. Metabolism of redox active selenium compounds was clarified >60 years ago, and since then a number of classical works, still in all parts true and of great value, have been published from the 1950s to the 1990s. These works that form a basis for our understanding of cancer prevention and cancer treatment mechanisms are summarized below. They are fused with current knowledge of methylation and demethylation reactions and the formation of excretory products, including multimethylated forms and selenosugar.

The most redox active selenium compounds selenite and selenodiglutathione (GS-Se-SG) are very strong oxidizing agents and have a high reactivity with thiols. In 1941, Painter (98) described the classical reaction where selenious acid reacts with thiols to form disulfides and mixed so-called selenium trisulfides:

$$4\ RSH + H_2SeO_3 \rightarrow RSSR + RS\text{-}Se\text{-}SR + 3\ H_2O$$

In another classical work, selenite was shown to be a better oxidant of reduced glutathione (GSH) than copper and that catalytic amounts of selenite could oxidize a large quantity of GSH in the presence of oxygen (140). GS-Se-SG was also shown to be the active intermediate. This and other selenotrisulfide derivatives are only moderately stable and may be synthesized *in vitro* using exact stoichiometry 1:4/selenite:GSH (18). Selenite may also form selenotrisulfide bridges in protein thiols, causing cross linking, multimerization, and loss of function (38, 109). Selenite is directly and readily reduced nonenzymatically by cysteine (93) and enzymatically by the thioredoxin system, mammalian thioredoxin reductase, or by the glutaredoxin system (68). Under anaerobic conditions, the reaction stops after consumption of three molecules of NADPH consistent with complete 6-electron reduction to selenide:

$$SeO_3^{2-} + 3\ NADPH + H^+ \rightarrow Se^{2-} + 3\ NADP^+ + 3\ H_2O$$

After admission of oxygen, a linear reaction starts with a slight lag-phase showing redox cycling of selenide, oxygen, and the thioredoxin system under the consumption of NADPH. The reaction is very efficient since the concentration of selenite giving half-maximal velocity is 20 μM for mammalian TrxR. GS-Se-SG is also a very good substrate to mammalian TrxR and

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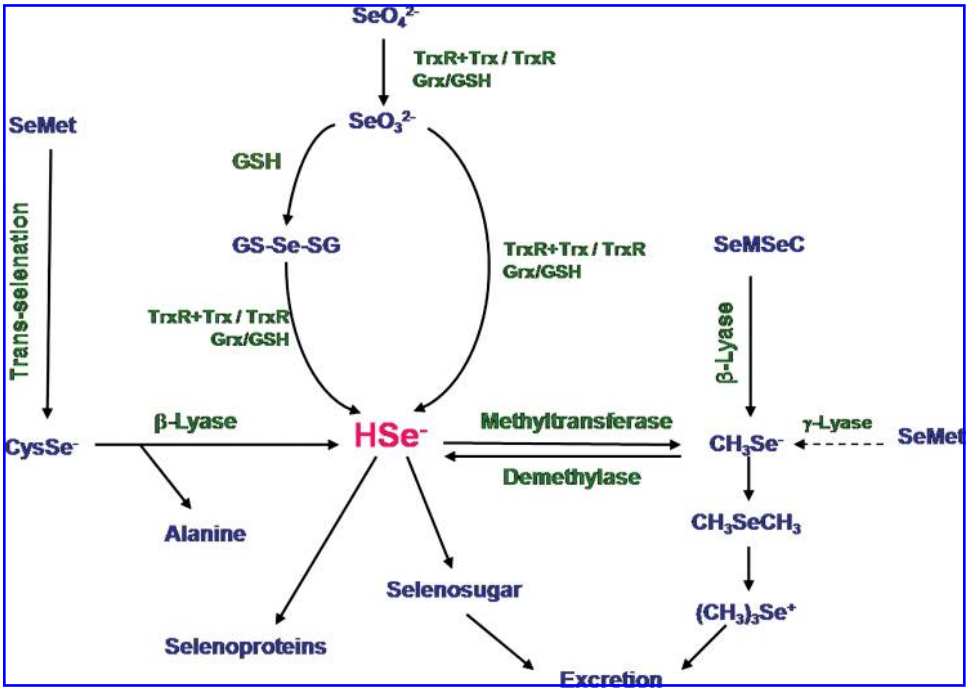

FIG. 1. Schematic illustration of the selenium metabolic pathway. Three main pathways are used to metabolize selenium compounds. The first one is common for redox active selenium compounds, the second one for selenoamino acids, and the third one for compounds that are precursors of monomethylselenol. Abbreviations: CH_3Se^-, monomethylselenol; $CysSe^-$, selenocysteine; GS-Se-SG, selenodiglutathione; HSe^-, selenide; MeSeCys, selenomethylselenocysteine; SeMet, selenomethionine; SeO_3^{2-}, selenite; SeO_4^{2-}, selenate.

a superior oxidant of reduced Trx (17). Under anaerobic conditions, the reaction stops after consumption of a stoichiometric amount of NADPH showing complete reduction to selenide and GSH. As in the case of selenite, the reaction starts after the admission of air with a slight lag phase. GS-Se-SG is also reduced by glutathione reductase, but the reaction with TrxR is superior as measured by NADPH consumed (17, 37). In selenate, selenium is present in its highest natural oxidation state and this molecule is far less reactive compared to selenite. However, in the presence of GSH, selenate is slowly reduced to selenite by the thioredoxin or glutaredoxin systems [(16) and unpublished data from our laboratory]. The thioredoxin family proteins and especially TrxR are thus very important for the formation of selenide to general selenoprotein synthesis (Fig. 1). Selenoprotein biosynthesis is not covered by this review where the focus is selenium and cancer. For a comprehensive overview of the specific processes involved, see the review by Papp *et al.* (101) and also "Threading the Needle: Getting Selenocysteine into Proteins" in this issue.

The natural occurring selenium analogues of the sulfur-containing amino acids cysteine and methionine are treated like the sulfur amino acids (*i.e.*, they are randomly incorporated in proteins that could influence the function). Furthermore, the trapping of selenomethionine in proteins will delay physiological effects. However, selenomethionine has been shown to be converted to selenocysteine by trans-selenation (7). Selenomethionine may also be directly converted to monomethylselenol by the γ-lyase reaction although this reaction is slow in mammals and the physiological importance of this pathway is not known (136). Selenocysteine and methylated species including selenomethylselenocysteine, selenohomolanthionine, and γ-glutamyl-Se-methylselenocysteine are cleaved by β-lyase to R-SeH species that eventually will be demethylated to selenide or undergo methylation to dimethylselenol or trimethylselenonium that will be excreted in the urine. Selenide formed from either pathways will be utilized in selenoprotein synthesis, undergo methylation or be converted to selenosugar [*i.e.*, 1-glutathionylseleno-*N*-acetyl-D-galactosamine and 1-methylseleno-*N*-acetyl-D-galactosamine (92, 136, 141)]. In Fig. 1, the metabolism of naturally occurring selenium compounds are schematically shown.

The Selenium Paradox in Cancer—From Prevention to Growth Inhibition

Selenium is a potent modulator of eukaryotic cell growth. More than 30 years ago, selenium was already shown to be one of the essential factors for cell culture under serum-free conditions, and that serum is in fact a source for selenium required for cell growth under normal culture conditions (83). However, the growth modulating effects are strictly concentration dependent since low concentrations are an absolute requirement for cell survival and growth but slightly higher concentrations potently inhibit growth (Fig. 2). The interval representing the "euselenic state" for normal cells is very narrow, but is even narrower for neoplastic cells and thus a "therapeutic window" is obvious, offering great potential for the use of selenium in cancer treatment. Mechanisms of cancer prevention and direct effects on manifest tumors differ greatly (Fig. 3) and are often mixed up in the literature. Selenium is best known as an antioxidant, and this effect is probably an important mechanism in cancer prevention. However, in tumor cells, the "selenium paradox" is evident and the antioxidant to normal cells is acting as a prooxidant reacting with several factors characterizing the resistant phenotype expressed by preneoplastic and neoplastic cells, for example, increased levels of intracellular thiols (12, 14).

Selenium-mediated cancer prevention

In the early 1970s, people living in selenium-rich areas of the United States were shown to have lower mortality in

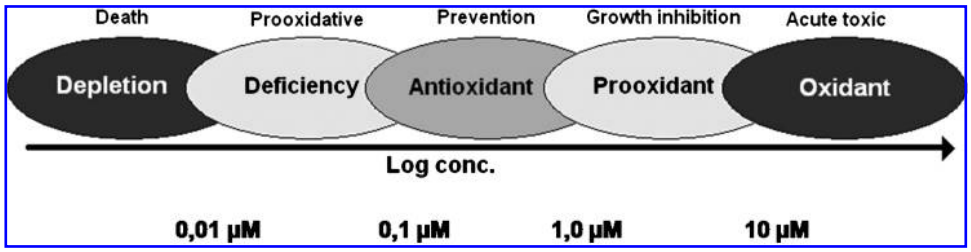

FIG. 2. The effects of selenium are strictly concentration dependent. The *arrow* indicates a logarithmic scale of the selenium concentration. The cellular effect results in cell death both at very low and high selenium concentrations. At moderate concentration selenium possesses antioxidant properties with cancer preventive effect, but is converted to a strong oxidant at higher levels with resulting growth inhibition.

cancers compared to people from selenium poor areas (122). These findings were supported by the results from additional studies in the 1970s and 1980s showing a correlation between selenium intake, plasma selenium levels, and cancer mortality (118, 150). Since then, numerous studies have indicated cancer preventive effects of selenium and the field was revitalized in 1996 by the well-performed double-blind placebo controlled intervention study by Clark and co-workers, showing a dramatic decrease in the incidences of several common cancers along with an overall decrease in cancer mortality (26). In this and other positive trials, selenized yeast was used and in this form of selenium supplementation a mixture of species are present where the dominant part is selenomethionine; but there is also a fraction of selenomethylselenocysteine and other methylated species (70). Selenomethylselenocysteine is a dominating source of the highly efficient monomethylselenol and is thus a considerably better precursor for cancer preventive selenium metabolites than selenomethionine (141). Selenomethylselenocysteine is also a safe form of selenium with a No Observable Adverse Effect Level (NOAEL) of <0.5 mg/kg/day in rats and 0.15 mg/kg/day in dogs (57). Several studies reveal clear chemopreventive effects of second-generation selenium compounds (*i.e.*, monomethylselenol generating species) (143). The role of selenomethionine is less clear, but it is known that free and protein-bound selenomethionine may scavenge ROS in the presence of GSH (3, 71). The physiological relevance of these findings is however still not known. The tRNA for methionine does not discriminate between methionine and the selenium analogue and thus selenomethionine supplementation leads to the trapping of selenium in proteins (22) with the consequent delayed release of active selenium species. Yeast preparations may thus be considered combination drugs with immediate and prolonged release of active selenium species. Therefore, this mechanism may be one possible explanation to the disappointing result from the SELECT trial (selenomethionine) where the positive results from the Clark study (selenized yeast) were not reproduced. Instead, there were no effects of selenomethionine or a combination of selenomethionine and vitamin E (77). The cancer preventive effects are complicated and there seems to be strict dose dependence. Prostate cancer is extensively studied and current data suggest preventive roles of selenium mainly in populations with low selenium intake and plasma levels (29, 107).

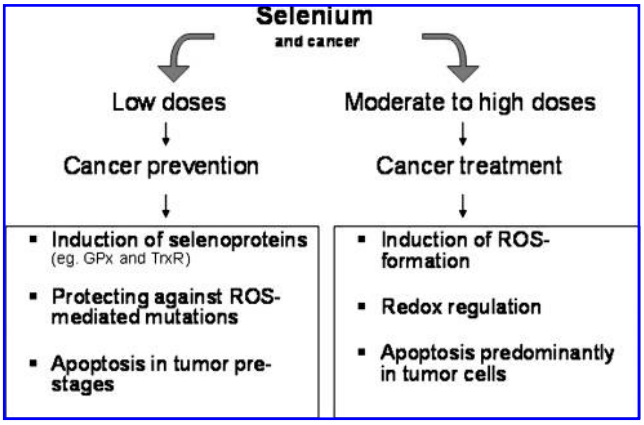

FIG. 3. Selenium and cancer. Selenium at low doses is known to have cancer preventive effects that are believed to be at least in part dependent on increased levels of selenoproteins with antioxidant capacity. Selenium at moderate to high doses has been shown to have a selective cytotoxic effect and induces apoptosis in tumor cells. The cytotoxic effect is explained by increased ROS formation and redox regulation.

The exact mechanisms explaining the cancer preventive effects are not known. Numerous studies have focused on the antioxidant effects of selenium and especially the role for glutathione peroxidases and TrxR. The basic hypothesis is that GPx:s prevent mutations through decrease in the concentrations of reactive oxygen species (ROS). TrxR may, in addition to direct reduction of hydroperoxides (15), exert antioxidant activity through regeneration of low molecular weight antioxidants (*e.g.*, ubiquinol) (152). However, effects on ROS scavenging are probably not the major factor since tumor prevention extends beyond selenium levels leading to saturation of selenoproteins. One indication that other mechanisms apart from the antioxidant effects are important is the fact that antioxidant supplementation has shown no effect or even an increase in cancer incidences (10). In fact, selenium was the only antioxidant where all included studies showed significant decrease in the incidence of gastrointestinal malignancies in a meta-analysis. For other antioxidants (*e.g.*, carotene and vitamin E) some studies showed no effect or opposing results with increase in cancer mortality (10). Furthermore, in the SELECT trial, vitamin E led to an increase (nonsignificant but still regarded as an alarming observation by the study group) in the incidence of prostate cancer, again showing that antioxidant effects may not fully explain any cancer preventive effects of selenium.

Several studies suggest that selenium exerts cancer preventive effects by action in the very first step in the carcinogenic process through inducing apoptosis in initiated cells (27, 149). Furthermore, selenium supplementation during the

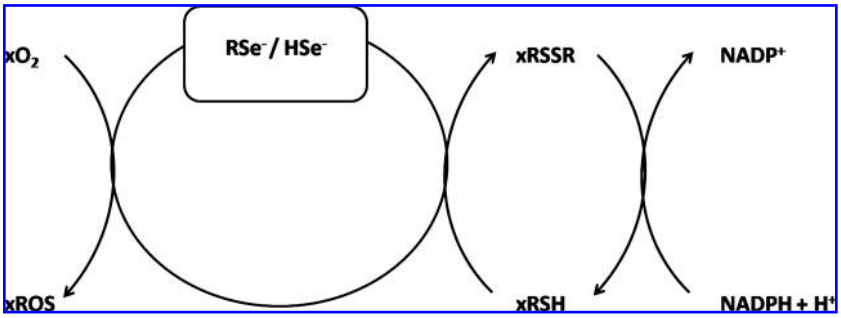

FIG. 4. Redox cycling and ROS formation with selenide and monomethylselenol. Selenide and monomethylselenol react with thiols and create redox cycles leading to thiol oxidation and the formation of ROS.

promotion and progression phases of chemical carcinogenesis results in a marked prevention of the carcinogenic process (12). Suggested targets of selenium include transcription factors and enzymes. Selenium increases the activity of p53 and the resulting induction of DNA repair (121). Furthermore, selenium interferes with transcription factors of importance for proliferation and growth, including NF-kB (63) and AP-1 (131). Also, selenium may limit the growth of transformed cells by interfering with the supply of deoxyribonucleotides through inhibition of the thioredoxin–glutaredoxin systems and ribonucleotide reductase (109, 132). Other suggested targets for selenium-mediated prevention include inhibition of carcinogenic biactivation (149), the immune system (67), and cytosine methyltransferase (34). The complex patterns of mechanisms behind selenium-mediated cancer prevention are further demonstrated by gene expression analysis of human subjects after selenium supplementation. Two human global gene expression studies have revealed that selenium influences the expression of a great number of genes, including ribosomal protein, transcription factors, and phase 2 enzymes (97, 106).

The effects of selenium on the growth of tumor cells

The leading mechanisms in selenium-mediated growth inhibition are redox effects. Redox active selenium compounds including selenite, selenocysteine, selenodiglutathione (GS-Se-SG), and precursor compounds to monomethylselenol [*e.g.*, selenomethylselenocysteine and to some extent selenomethionine (16, 17, 89, 112, 130)] inhibit the growth of malignant cells. Selenide and monomethylselenol react readily with thiols, creating redox cycles leading to thiol oxidation and the formation of ROS (Fig. 4). The effect is general, and reported in several tumor forms, including prostate, lung, and bone (25, 31, 73,158). The involvement of redox cycles and the resulting formation of ROS as a critical step in selenium-mediated cytotoxicity is demonstrated by the prevention of cytotoxic effects and sustained viability after inclusion of antioxidant compounds, such as Pycnogenol®, to cells exposed to selenite with the resulting decrease in ROS (36, 50, 53, 54, 62, 65, 89, 112, 127, 154). Apart from addition of antioxidant compounds, overexpression of antioxidant proteins after treatment with selenite have also been proven to protect the cells from the cytotoxic effects of selenite (80). In addition to the ROS production caused by selenium compounds, it leads to a shift in the glutathione ratio, with the resulting increase in the amount of GSSG (124, 125).

Selenite, selenodiglutathione, and selenocysteine are directly toxic to cells in culture, but selenomethionine and selenomethylselenocysteine require activation by methionase or lyases in order to form monomethylselenol, and the ß-lyase-mediated activation of selenocysteine derivatives may occur already in the intestine by gastrointestinal bacterial species (119). Selenide and monomethylselenol may react indiscriminately with thiols and flavoenzymes. Redox cycles involving the thioredoxin system is however not contributing to selenium-mediated cytotoxicity since overexpression of TrxR on the contrary protect cells upon selenite exposure (80). The sensitivity of a tumor cell to selenite is however not only dependent on the protein level and activity of TrxR, but more importantly, on the ability of the cell to induce the activity of this enzyme in response to selenite (58, 120). Moreover, cotreatment of selenium and auranofin, specifically inhibiting TrxR at nontoxic doses, increases the sensitivity to selenium (110, 120). Selenium treatment has been shown to result in an upregulation of the total TrxR1-mRNA expression in different cell lines as well as of a majority of the splice variants of TrxR1, although activity measurements have shown that selenium at low levels induce the activity whereas higher concentrations lead to a pronounced decrease in enzyme activity (120). These observations may reflect that the Trx system primarily resuscitates critical cellular thiols lost as a result of redox cycles involving selenide, oxygen, and thiols.

Selenide and monomethylselenol induce apoptosis but there is however a clear difference in how primary selenide forming selenium compounds and selenium species generating monomethylselenol induce apoptosis (69, 86, 134). Monomethylselenol induce apoptosis by caspase-dependent mechanisms whereas selenide may induce primarily caspase independent apoptosis along with DNA strand breaks and phosphorylation of p53 (56, 58, 64, 73). Furthermore, selenide leads to accumulation of Bax, increased phosphorylation of p53, increased p21, activation of the p38 pathway, and upregulation of protein kinases (50, 56, 88, 112, 158). Monomethylselenol upregulates p21/CIP1 and p16/INK4a (144) and downregulates Bcl-2 (72).

Apart from apoptosis, the two major redox active selenium metabolites affect a number of cellular events and signal pathways differently. Selenide will cause S-phase arrest whereas monomethylselenol will cause arrest in G1 (56, 132). Selenide may inhibit protein function by forming intra- and intermolecular disulfides or mixed disulfides/selenides. By this mechanism, human thioredoxin is inhibited due to structural changes (109). Furthermore, oxidation of structural cysteine residues will lead to decreased DNA binding of p53 and AP-1 (88, 131). The thiol oxidation causes a direct inhibition of several proteins including signaling enzymes. This is, for instance, the case for caspases (90, 102) and JNK (103). Selenide inhibits the functions of iron enzymes by interference with active site irons, thereby preventing ferrous/ferric redox cycles (19).

TrxR as a Diagnostic Marker and Drug Target in Cancer

The need for improvement of cancer diagnostics and treatment regimens is imperative. One possible strategy for achieving improvement within this area is the development of more efficient biomarkers. The basis for functional biomarkers in cancer diagnostics is distinguishable differences between normal and malignant cells. An efficient marker should furthermore be able to discriminate between degrees of differentiation and proliferation. The propensity of cancer cells to overexpress the selenoprotein TrxR make it an apparent target in research directed at improved diagnostics and effectual drug targets. Malignant cells often display upregulation of cellular antioxidant systems as a consequence of abnormal levels of oxidative stress. Previous data indicate that proteins belonging to the Trx family are particularly relevant in highly malignant and resistant tumors. The Trx system is linked to diverse cellular pathways and is crucial for cellular adaptation since it is essential for maintaining redox homeostasis in normal cells and supports progression and growth upon malignant transformation (1, 91, 105). TrxR1 has been shown to enhance tumor proliferation and chemotherapy resistance, and to be coupled to prosurvival signaling and effects (87). Specific examples include the association of TrxR expression to tumor grading and subsequent prognosis in lung cancer (33) and brain tumors (45, 55).

TrxR in cancer diagnostics

TrxR1 is widely expressed in tissues and several reports have shown that TrxR1 is induced in tumor cells and preneoplastic cells (8, 41, 76). Upregulated levels of TrxR1 have been observed in lung malignancies such as non-small cell carcinomas (129) and malignant mesothelioma (59, 89, 135). TrxR1 has also been found in high levels in hepatocellular carcinoma (28), astrocytic brain tumors (45), and in tumor nodules in a model for hepatocellular carcinogenesis (13). The upregulation of TrxR1 in tumors could reflect the need of this protein for DNA synthesis in cancer cells as well as the increased level of oxidative stress in tumor cells, but might also be linked to the regulatory response related to p53. It has been shown that TrxR sustains a functional state of p53 via Ref-1 and furthermore, cells exposed to electrophilic prostaglandins, which inhibit TrxR, have been shown to accumulate p53 with impaired function (85). Moreover, TrxR1 deficiency has been shown to reverse the morphology and tumorigenicity of lung carcinoma cells (156) and using TrxR1 knock down cells, it was also suggested that TrxR1 is essential for tumor growth in mice. A correlation between level of TrxR1 and degree of differentiation has also been observed in mesothelioma cell lines with various phenotype with increased levels in cells of epithelioid morphology compared to cells of sarcomatoid phenotype (115). Publications establishing the potential of TrxR as a useable biomarker in the development of improved diagnostics are summarized in Table 1.

Alternative splicing of TrxR

Alternative splicing has emerged as an important mechanism in regulation of cellular functions, and it has been established that human cancers often involve significant changes in expression profiles of alternative transcripts (20, 40, 47, 52, 60, 99, 133, 139, 146–148, 153). These changes may contribute functionally to the maintenance of the transformed state of the cell and reflect changes coupled with tumorigenesis, and could thereby be used as diagnostic markers or targets in cancer therapy. In addition to its tumorigenic overexpression, TrxR displays an intricate pattern of alternative splicing, further highlighting the potential for clinical use. The mainly cytosolic TrxR1 protein is encoded by the TXNRD1 gene located on chromosome 12 and has been mapped to 12q23-q24.1 (39). This gene contains several alternative exons in the 5′-region apart from 15 core exons encoding the major part of the TrxR1 protein (Fig. 5). These extra exons give rise to several alternative splice forms, some of which encode different protein variants (96, 114). Adding to the complexity of TrxR1 expression and regulation is the fact that additional intra-intronic genes can be found within the TXNRD1 gene (4, 114). Although the gene has alternative transcription promoters, a core promoter guides most of its transcripts. This promoter has the characteristics of a housekeeping gene, lacking TATA or CCAAT boxes, and interacts with transcription factors Oct-1 and Sp1/Sp3 (113). Transcriptional regulation is also affected by an Nrf2-regulated antioxidant responsive element (48, 49, 117). The number of alternative mRNA splice variants surpasses 20, however, several of the differentially spliced transcripts differ in untranslated regions, and therefore potentially encodes five different protein isoforms that have previously been designated TrxR1v.1–5 (114). The function of different splice variants is far from clarified but could possibly be coupled to tissue and cell-specific regulation of expression at the mRNA level. Several of the different mRNA forms of TrxR1 have been shown to be expressed in human tumor cell lines originating from mesothelioma and lung cancer (115, 120). The expression of Trx1, TrxR1, and the isoforms of TrxR1v.2,3,5 has further been examined in tissue from non-small cell lung cancer patients (Fig. 6). This revealed that both Trx1 and TrxR1v.2,3,5 proteins showed a significant correlation to the degree of differentiation in adenocarcinoma and squamous carcinoma (33).

TrxR as a drug target

The potential of the thioredoxin system as a drug target in cancer therapy has been extensively studied, (reviewed in Refs. 5, 9, 44, 104, 142) often with special focus on TrxR. The importance of TrxR expression for tumor cell development is exemplified by inhibition experiments in lung cancer (157) and hepatocellular carcinoma (35). TrxR is also strongly connected to apoptotic processes through Trx regulation of apoptosis signal-regulating kinase 1 (ASK1) (116). TrxR1 is an important component of the resistant phenotype and has important functions in neoplastic growth. Thus, TrxR1 has a great potential in diagnostics and treatment of resistant tumor diseases. As many tumors that overexpress TrxR are drug resistant, inhibiting TrxR could possibly contribute to prevent or reverse the resistance mechanisms. TrxR is known to be inhibited by several electrophilic compounds via reactions with the redox-active residues and has been proposed as a target for anticancer therapy (87, 142, 151). These include several clinically used drugs such as platinum-containing compounds (2, 6), arsenicals (74, 75, 79), nitrosoureas (43), quinones (82), motexafin gadolinium (46), and diverse gold compounds (42, 78, 81, 111). Several of these gold-based TrxR inhibitors target the highly reactive active site Sec residue and

Table 1. Publications Investigating the Potential of TrxR as a Diagnostic Biomarker

TrxR Expression	*Tissue/cell type*	*Author*
Significant correlation between differentiation and TrxR expression	Non small cell lung carcinoma tissue	Fernandes *et al.* 2009 (33)
Significantly elevated expression level of TrxR in tumor tissue compared to internal control	Hepatocellular carcinoma biopsies	Cunnea *et al.* 2007 (28)
Significantly higher TrxR protein levels in epithelioid cells compared to sarcomatoid cells	Malignant mesothelioma cell lines	Nilsonne *et al.* 2006 (89)
Increased expression of TrxR in liver nodules compared to surrounding tissue	Chemically induced hepatocarcinogenesis in a rat model	Björkhem-Bergman *et al.* 2005 (12)
Significant association between TrxR expression and malignancy grade in astrocytomas	Astrocytic brain tumor tissue	Haapasalo *et al.* 2003 (45)
Overexpression of TrxR in a majority of samples as compared to corresponding patient matched samples	Tissue samples from breast cancer, thyroid, prostate and colorectal carcinoma, and malignant melanoma	Lincoln *et al.* 2003 (76)
23 of 26 biopsies showed positive immunohistochemical staining compared to six cases of histologically healthy pleural mesothelium which showed no immunoreactivity	Malignant mesothelioma biopsies	Kahlos *et al.* 2001 (59)
TrxR expression correlating to tumor grade with a 2-fold decrease between grade I/II and III	Non small cell lung carcinoma tissue	Soini *et al.* 2001 (129)
2-fold higher TrxR activity in cells with epithelioid differentiation compared to cells with a sarcomatoid growth pattern associated with worse prognosis	Malignant mesothelioma cell lines	Sun *et al.* 2000 (135)
~1,5-3-fold induction of TrxR in malignancies relative to controls	Liver tumors in a transgenic mouse model and prostate cancer cell lines	Gladyshev *et al.* 1998 (41)
2-fold increase of TrxR protein and activity in colorectal tumors compared to normal mucosa	Human primary colorectal tumors	Berggren *et al.* 1996 (8)

display highly effective inhibition at low concentrations (95). Gold has an inherent strong affinity for thiols, which makes the nucleophilic selenolate of reduced TrxR a principal target for gold-containing drugs. Moreover, the oxidation state of the gold atom is not decisive for the inhibition capacity which potentially could result in retained effect after intracellular metabolism. More specific observations regarding TrxR inhibition and cancer includes induction of apoptosis in cisplatin-resistant ovarian cancer cells by the gold compound auranofin (81) and inhibition of both TrxR and selenium incorporation in the process of general selenoprotein synthesis by the same compound (137). In conclusion, a growing body of experimental results suggests a strong potential for TrxR as both a marker in cancer diagnostics and efficient drug target.

Selenium as a Specific Cytotoxic Agent—Mechanism for Tumor-Specific Selenium Uptake

There is a high degree of selectivity of selenium for tumor cells where selenium induces apoptosis at concentrations that do not influence the growth and viability of normal, benign primary cells (54, 89). Several studies have also shown that drug-resistant cells are more sensitive to selenite compared to drug-sensitive cells (11, 58, 89, 93). Furthermore, *in vitro* studies have shown that selenium compounds efficiently inhibit the development of drug resistance and also inhibit the growth of resistant cells (23). The specificity of selenium to generate cell damage predominantly in tumor cells is however not fully elucidated. As apoptotic pathways and antioxidant defense systems have been rigorously studied, the specific cytotoxicity observed in malignant cells is still largely unexplained.

Findings from *in vivo* experiments in human subjects from the 1960s using selenite as a tumor localizing agent clearly demonstrated a high degree of tumor specificity for selenium. Cavalieri *et al.* demonstrated, through intravenous administration of radioactive selenite, a high affinity selenium uptake by malignancies in brain and thorax compared to normal tissue (24). In the context of current knowledge, these findings may suggest that malignant cells rather than being more sensitive to selenium *per se,* are intracellularly exposed to higher levels compared to normal cells, due to a tumor-specific uptake.

Furthermore, the connection between glutathione levels and selenite sensitivity has been investigated (123). An interesting unanticipated finding in this study was that supplementation of GSH extracellularly together with selenite significantly increased cytotoxicity. Similar findings have been demonstrated in yeast where millimolar tolerance to

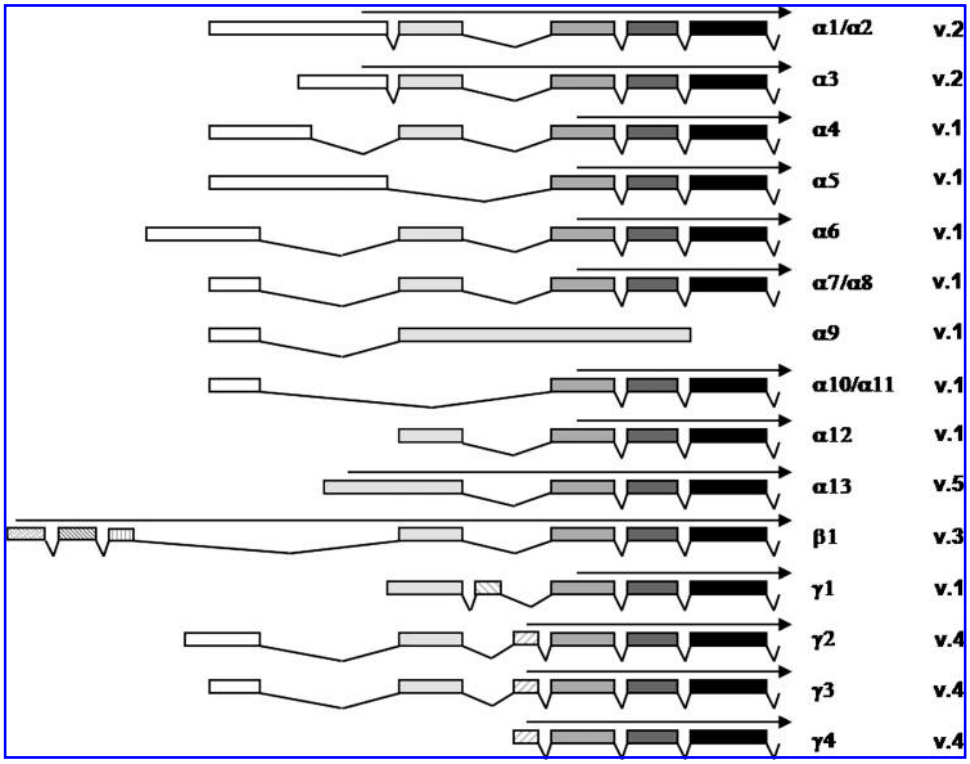

FIG. 5. Schematic illustration of variant mRNA forms of TrxR1 with different exon combinations. Exons are shown by *boxes*, with α1/2 representing the classical TrxR1 transcript variant (for nomenclature, see Ref. 114). The translated open reading frames for the different variants are indicated with *horizontal arrows* and five different N-terminal domain variants of TrxR1 are possible. Corresponding protein isoforms to the different exon combinations are shown in the *right hand side* of the figure.

selenite could be reduced to the micromolar range by addition of glutathione to the growth medium (138).

Extracellular reduction of selenite to a reduced form, possibly selenide, was recently demonstrated to lead to a high affinity uptake of selenium in lung cancer cells (93). The innate variability in secretion of thiols between different cell lines, correlated positively to the uptake and cytotoxicity of selenite, selenodiglutathione, and selenocystine. Furthermore, the extracellular thiols consisted mainly of cysteine and were dependent on cystine uptake through the xc- cystine glutamate antiporter and cysteine secretion through multidrug resistance proteins (MRPs) (Fig. 7), both regulated by the antioxidant response element (ARE) (126). The demonstrated dependence on the xc- antiporter and multidrug resistance proteins, both frequently expressed in cancer cells, may explain the cancer-specific low dose cytotoxicity of redox active selenium compounds, especially in drug-resistant malignancies. The accumulation of selenide in cancer cells will cause redox cycling with an extensive oxidation of thiols accompanied by extensive ROS formation. Selenium is thus not only more efficiently taken up due to induced resistance factors, but selenide also cause more damage due to the induced levels of thiols in resistant cells compared to normal cells (14, 30). There is indeed a rationale for the selenium-specific cytotoxicity since many parts of the resistant phenotype are targets for selenium. Selenium is therefore a very interesting therapeutic alternative in multidrug resistant tumor diseases.

Potential Clinical Use

Despite a great number of preclinical studies showing pronounced effects of selenium compounds on the growth and viability of tumor cells, the number of clinical trials with selenium on cancer patients are very few.

In 1956, Weisberger and Suhrland (145) published a case series where two patients with chronic myelogenous leukemia (CML), one patient with acute myelogenous leukemia (AML), and one patient with acute lymphoblastic leukemia (ALL) were treated with very high doses of selenocystine (an average dose of 100 mg/day, ranging from 50–200 mg/day and a duration from 10 to 57 days). The results on the leukemia were remarkable since there was a clear drop in LPK counts and in one patient a regained sensitivity to 6-mercaptopurine was noted. Unfortunately there were severe side effects due to the extreme dose, including severe persistent nausea, vomiting, and alopecia. However, the side effects were reversible and no overt organ changes, including liver and kidney functions, were observed. The great potential of selenium in the treatment of leukemia was recently further supported by an *ex vivo* study comparing the efficacy of selenite to the commonly clinically used cytostatic drugs in equipotent concentrations (94). Selenite in a low concentration (5 μM) induced massive apoptosis in blast cells from 39 patients with AML. In fact, selenite was superior to all tested cytostatic drugs and statistical analysis revealed that there was no cross resistance between selenium and any cytostatic drug. These results suggest that selenium is potentially an extremely interesting alternative in the treatment of leukemia and demonstrate the need for full scale clinical trials.

In two studies, selenium was given parenterally to cancer patients; Pakdaman and co-workers (100) performed an uncontrolled case series on patients with brain tumors and found decreased intracranial pressure in 76% of the study population along with improved blood parameters. No adverse effects of the relatively high dose (1 mg of sodium selenite/day during 4–8 weeks) were reported. In another placebo controlled study, selenium was given to patients undergoing

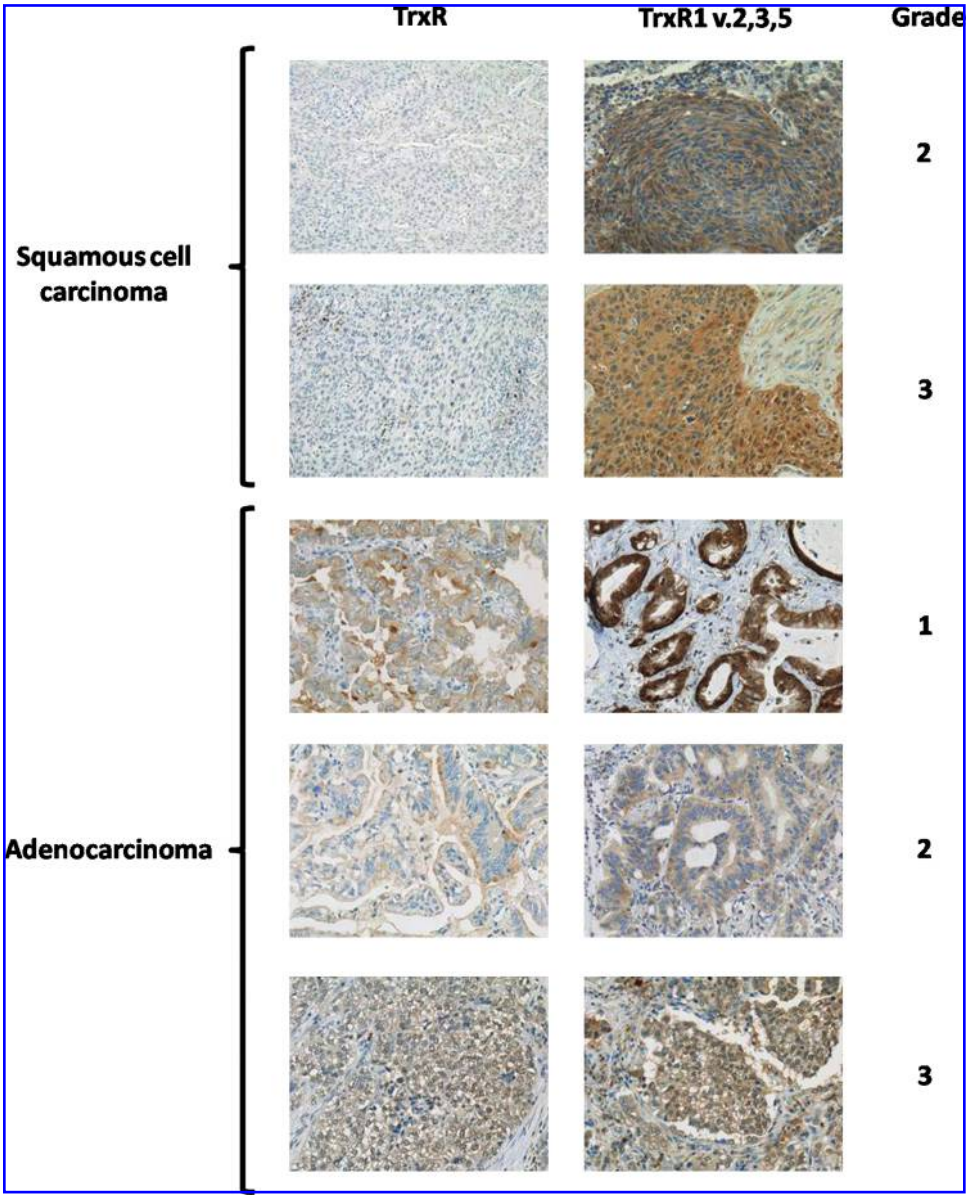

FIG. 6. Immunohistochemistry of human lung cancer sections stained against TrxR1 and TrxR1v.2,3,5. Lung cancer sections with adenocarcinoma, grades 1, 2, and 3, and squamous carcinoma grades 2 and 3. The expression levels are significantly elevated in the tumor area of the section and correlate to the degree of differentiation.

surgery for oral tumors and an inverse correlation was observed between selenium levels and edema (159).

A few studies of different design (randomized controlled, nonrandomized, and randomized cross-over) with peroral treatment of cancer patients in combination with cytostatic drugs, radiation therapy and/or surgery have been reported (21, 51, 61, 84, 128). One study including patients with colorectal carcinoma, gastric carcinoma, or esophageal carcinoma, showed that nutritional supplementation of selenium reduced the side effects from the cytostatic treatment (32). This

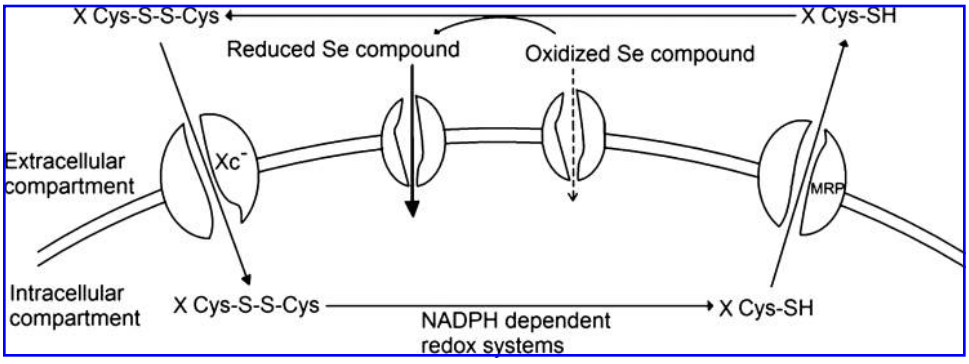

FIG. 7. Extracellular reduction of redox active selenium compounds. Reduction of selenite, selenodiglutathione, and selenocystine enhances toxicity through high affinity selenium uptake. Extracellular reduction is dependent on cystine uptake through the xc^- cystine antiporter and secretion of cysteine through MRPs.

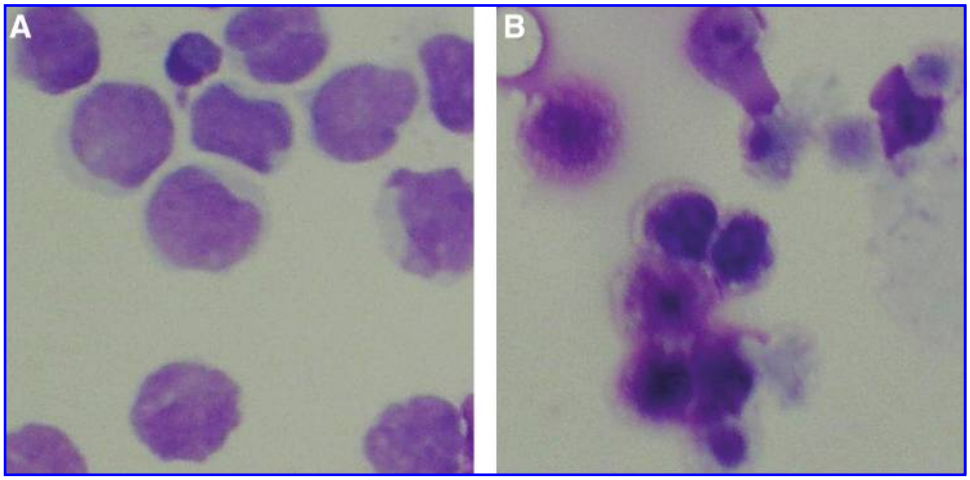

FIG. 8. Cytotoxic effect of selenite treatment on primary acute myeloid leukemic (AML) cells. Apoptotic morphological change showing pyknosis (shrunken cells with a condensed nucleus and chromatin clumping against a background of viable cells) of primary AML cells after treatment with 5 μM selenite for 4 days. (**A**) Control primary AML cells. (**B**) Primary AML cells treated with 5 μM selenite. Modified from Ref. 94.

finding was supported by other studies involving patients with ovarian cancer (128) and head and neck squamous carcinoma (66).

Selenium toxicity has been relatively well investigated and previous studies show that selenium concentrations toxic to tumor cells (up to 10 μM) are easily achievable with no or low toxicity in humans. It has been shown that minor symptoms of selenium toxicity may be observed at plasma concentrations corresponding to 13 μM (155) and another study indicates that oral intake up to 3200 μg/day over a long period of time can be tolerated (108). Treatment with too low concentration of selenite could possibly stimulate proliferation of tumor cells, indicating that the dose is critical and that the plasma levels should be carefully monitored.

Concluding Remarks

Selenium has a clear role in the regulation of normal and malignant cell growth. Despite the disappointing results from the SELECT trial, a great body of evidence suggests that selenium supplementation for a broad public could result in extraordinary health benefits. In fact, the results from the SELECT trial must not lead to the depreciation of all positive and interesting data generated over the past decades. The reported cancer preventive effects in several studies are extraordinary for selenium and there is a great need for further studies to precede worldwide recommendations. Selenium is unique in the sense that this element may be used both in cancer prevention and treatment. Selenium cytotoxicity is remarkably cancer specific, and the specificity is at least in part explained by drug resistance mechanisms since the more resistant a cancer cell is to conventional therapy the more sensitive that particular cell is to selenium. This property should clearly be used in cancer therapy and there is a demanding need for human trials. In the near future selenium may thus be used by the public as a cancer preventive dietary factor but also be used by the medical profession in the treatment of cancers.

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Abbreviations Used

ALL = acute lymphoblastic leukemia
AML = acute myelogenous leukemia
AP-1 = activator protein 1
ARE = antioxidant response element
ASK-1 = apoptosis signal-regulating kinase 1
Bax = Bcl-2-associated X protein
CML = chronic myelogenous leukemia
GPx = glutathione peroxidase
GSH = glutathione
GS-Se-SG = selenodiglutathione
GSSG = glutathione disulfide
JNK = c-Jun N-terminal kinase
MRPs = multidrug resistance proteins
NF$\kappa\beta$ = nuclear factor-kB
NOAEL = no observable adverse effect level
Nrf2 = NF-E2 related factor2
Oct-1 = organic cation transporter 1
Ref-1 = redox effector factor 1
Trx = thioredoxin
TrxR = thioredoxin reductase

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