Molecular Targets for Selenium in Cancer Prevention

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Abstract: Mounting evidence reveals that selenium is a dietary constituent with anticarcinogenic and antitumorigenic properties. Various forms of selenium appear to be effective in bringing about these effects, although preclinical studies suggest that differences may arise as the quantity provided is reduced. The literature also documents the greater sensitivity of neoplastic cells to selenium than their nonneoplastic counterparts. Unfortunately, the minimal amount needed to bring about a positive effect in humans remains elusive. If there is a positive response to exaggerated intakes, it will likely be dependent on many factors, including the consumption of other dietary constituents, as well as variation in a host of genetic pathways involved with cancer. Although the biological basis of the reduction in cancer risk ascribed to selenium remains to be established, its consistency in retarding various experimentally induced tumors and suppressing the growth of various types of neoplasms in vitro and in vivo suggests that several mechanisms are involved. Depressed carcinogen bioactivation, reduced cell proliferation, and increased apoptosis raise the possibility that selenium works at a number of specific molecular targets involved with the cancer process. This review will focus on molecular targets involved with cell proliferation and apoptosis as possible mechanisms by which selenium might alter the cancer process.

Introduction

Selenium is an essential nutrient and, thus, has fundamental importance in maintaining health. For decades it was recognized for its ability to serve in conjunction with vitamin E to retard vascular and muscular dystrophy in animals (1). Early studies also identified its critical role in sperm formation and, thus, overall reproductive capacity (2). Its antioxidant properties stem from its key regulatory function within several selenoproteins (3). Today, ~20 eukaryotic and 15 prokaryotic selenoproteins containing selenocysteine have been identified, partially characterized, and/or cloned. Although considerable attention has been given to the potential health benefits of increasing selenium intakes, its importance as a modifier of cardiovascular disease risk remains equivocal (4). Nevertheless, considerable evidence points to the importance of an adequate supply of selenium for maintaining immunocompetence (5). Likewise, it has been reported to have a fundamental role in determining the virulence of some viruses (6). As mentioned below, substantial evidence indicates that selenium may alter cancer at several sites and by multiple mechanisms.

Because carcinogen metabolism and immunocompetence are discussed elsewhere in this special issue, this review focuses on how selenium might influence molecular targets associated with cell proliferation and apoptosis. Some of the possible molecular targets that selenium may alter include nuclear factor-κB (NF-κB), activator protein-1 (AP-1), cdk2, cyclooxygenase, and/or lipoxygenase.

Anticancer Effects

Almost 30 years ago, evidence surfaced that selenium might be a physiologically important deterrent to cancer (7,8). Since that time, several epidemiological and preclinical studies have added to the belief that higher intakes of selenium might retard the incidence and biological behavior of a variety of tumors. The more recent study by Clark and associates (9) provides some of the most compelling data that selenium might truly be a deterrent to cancer. In their study, a 200-µg selenium supplement per day for a mean of 4.5 yr, as selenized yeast, depressed cancer-related mortality by ~40%. Additional clinical intervention studies are needed to confirm these observations.

The ability of selenium to retard chemically induced cancers, including those induced in mammary tissue, prostate, lung, colon, pancreas, and liver, suggests that a common metabolic change, rather than a tissue-specific reaction, may account for its physiological actions (10–12). However, changes in carcinogen metabolism cannot totally explain the anticancer effects of selenium, especially those associated with altered rates of neoplastic proliferation (13–18).

The impact of the form of selenium on the efficacy of cancer prevention has not been extensively examined. Nevertheless, several selenium-containing compounds with diverse chemical structures have been found to inhibit cell
proliferation. Although limited evidence exists, organic selenium compounds such as selenomethionine and selenocysteine may be slightly less effective on a molar basis than selenite (13). The form of selenium was also important in a chemically induced lung tumor model when synthetic 1,4-phenylenebis(methylene)selenocyanate (p-XSC) was found to be superior to selenite (19). In a 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary model, p-XSC was again shown to be more effective than selenite in inhibiting tumors (20). In vitro studies by Thompson et al. (21) compared the effects of p-XSC and selenite on the induction of apoptosis in a mouse mammary carcinoma cell line. They found that p-XSC was far more effective than selenite. Exposure of mammary tumor cells to p-XSC has been found to suppress DNA, RNA, and protein synthesis, as well as inhibit mitochondrial transmembrane potential (22). Likewise, p-XSC has been shown to inhibit protein kinase C and protein kinase A activities in fibroblast cells (23). Although comparisons of selenite and p-XSC are interesting, variations in the biological response to other forms of selenium are less well documented. The use of exaggerated quantities of selenium in dietary and tissue culture studies makes it difficult to detect significant differences among forms.

Experimentally, the anticancer properties of selenium appear to arise at intakes that are substantially greater than those associated with maximal expression of most selenium-containing enzymes. Thus, although it is possible that one or more of the selenoenzymes might account for some of the anticancer protection, much of the evidence points to selenium metabolites as instrumental in the overall biological response (10,12).

Intracellularly Generated Selenocompounds

Historically, research has revealed the propensity of selenium to interact with thiols, especially protein thiols. Selenate is slowly reduced to selenite by glutathione and other sulfhydryl compounds. Selenite is further reduced to relatively stable compounds such as selenenitrusulfide and selenopersulfide. Both may produce free radicals and ultimately lead to DNA damage or alter a host of thiols and, thus, alter several metabolic events. Kuchan and Milner (24) provided rather compelling evidence that intracellular concentrations of glutathione were instrumental in determining the ability of selenite to alter cellular proliferation. Thus it is conceivable that the various forms of selenium will not be equal in their efficacy but will be highly dependent on not only the quantity provided but how it is metabolized.

Selenite and selenodiglutathione (GSSeSG) are recognized as efficient oxidants of reduced thioredoxin and reduced thioredoxin reductase (TR) (25). Selenite and other redox-active selenocompounds are recognized to modify a host of cellular proteins, including the tumor promoter protein kinase C (26). By using phorbol ester-promoted JB6 epidermal cell transformation assay, Gopalakrishna et al. (26) found that selenite-, selenocystine-, and selenodiglutathione-inactivated protein kinase C was reversed by treatment with thiol agents. Spallholz (27) indicates that several forms of selenium are capable of forming Se-S bonds with thiol-containing amino acids and/or proteins.

The formation of selenenitrusulfide or selenenylsulfide bonds may be important in the anticancer properties associated with selenium. Several studies suggest that selenite may increase intracellular concentrations of the selenenitrusulfide GSSeSG. Exposure of cells to GSSeSG has been found to markedly inhibit the growth of tumor cells, possibly by arresting protein biosynthesis (28–30). Although this exaggerated effect may relate to the delivery of selenium to a target site in the cell, it may also be one of the active forms of selenium that brings about a depression in tumor proliferation.

Monomethylated forms of selenium may also be involved in the chemopreventive effects of selenium (12,31). Selenite and selenomethionine exposures are known to increase the formation of methylated metabolites, including methylsel-enol, dimethylselenide, and trimethylselenonium. In vitro experiments reveal that methylseleninic acid is more potent than Se-methylselenocysteine in retarding cell proliferation and promoting apoptosis whether wild-type or nonfunctional p53 was present in hyperplastic mammary epithelial cells. Furthermore, the change in proliferation or apoptosis could not be attributed to DNA damage. In general, methylseleninic acid was ~10 times more effective than Se-methylselenocysteine in cells in culture (32). It is possible that cells vary in their ability to generate a monomethylated selenium species from Se-methylselenocysteine because of variation in the activity of β-lyase. The overall significance of this variation is unclear, since in vivo studies suggest that methylseleninic acid and Se-methylselenocysteine are comparable in retarding tumors resulting from methylnitrosourea or DMBA (32).

Selenium Inactivates NF-κB and AP-1

Although the mechanism by which selenium inhibited experimentally induced cancer remains to be determined, initial studies focused on selenoproteins such as cytoplasmic glutathione peroxidase (cGPx). In most preclinical studies, the protection provided by selenium occurred when it was provided at concentrations beyond that required to optimize the activity of cGPx and possibly other selenium-containing enzymes. Nevertheless, it is possible that a correction of cellular selenoenzyme deficiencies might account for some anticarcinogenic actions. Support for the involvement of several selenium-containing enzymes continues to emerge because of their involvement in determining the cell’s redox status.

NF-κB is an inducible oncogenic nuclear transcription factor with a pivotal role in inducing genes involved in a number of physiological processes, including those associated with cytokines, growth factors, cell adhesion molecules, and immunoreceptors (33,34). The redox state is important in determining NF-κB activity. Support for this statement comes from the ability of chemically diverse anti-
oxidants such as N-acetyl-L-cysteine, α-lipoic acid, butylated hydroxyanisole, pyrrolidine dithiocarbamate, and α-tocopherol to block its activation in vivo (35–37).

Several factors are known to influence the redox status of the cell, including the concentration of reactive oxygen species arising from lipid and H₂O₂. The modulation of NF-κB by specific reactive oxygen species appears to be cell specific, rather than a general phenomenon (38). Selenium and selenium-containing enzymes, including cGPx, membrane glutathione peroxidase, and TR, may also be involved via alterations in H₂O₂ and lipid peroxide and/or thiol status (39–41). In human T47D cells, the overexpression of cGPx retards NF-κB activation, NF-κB nuclear translocation, and IκB degradation in response to tumor necrosis factor-α or H₂O₂ treatment. These phenomena were no longer observed when tetrameric cGPx activity was reduced by selenium depletion (40). Available evidence suggests that increased cGPx activity may interfere with the activation, but not the synthesis or stability, of NF-κB (40). Thus selenium intakes and variation in cGPx expression might be factors determining the rates of proliferation of some neoplasms.

Another glutathione peroxidase, phospholipid hydroperoxide glutathione peroxidase (PHGPx), is a monomeric, membrane-associated enzyme containing one atom of selenium per mole of protein. The involvement of this selenoenzyme in the interleukin-1 (IL-1)-induced NF-κB activation has been examined using the human umbilical endothelial cell line ECV 304 transfected with the PHGPx gene and the gene for selenophosphate synthase to foster selenoprotein biosynthesis (41). In these studies IL-1 induction of NF-κB was inhibited by supplementation with 50 nM selenite only in transfected cells. These results demonstrate that overexpression of PHGPx can inhibit NF-κB activation and suggests that NF-κB activation by IL-1 is mediated by a preferential substrate of PHGPx, such as a fatty acid hydroperoxide. Again, variation in selenium intake and PHGPx expression may account for some of the observed variation in rates of tumor proliferation.

The activity of TR, another selenoenzyme, has also been linked to NF-κB activation through its ability to regulate thioredoxin concentrations. Mammalian TR is homologous to glutathione reductase with a selenocysteine residue in the conserved COOH-terminal sequence (42). TR specifically reduces oxidized thioredoxin to its reduced form using NADPH (43). The reduced thioredoxin reduces disulfide bonds of several proteins, including NF-κB (44). Thioredoxin may associate with the NF-κB p50 subunit through its cysteine residues (45). In response to oxidative stress such as ultraviolet irradiation and tumor necrosis factor, human thioredoxin translocates from the cytoplasm into the nucleus, where it increases NF-κB transcriptional activity. The availability of selenium is a key factor that determines TR activity in cells in culture and in vivo (12,46). Providing supplemented selenium to HT-29 human colon cancer cells grown in serum-free medium markedly increased TR activity (47). Because feeding rats a high-selenium diet (1.0 ppm) has been reported to cause a transient increase in liver, kidney, and lung TR activity (46), the importance of this enzyme in explaining the antitumorigenic effects of selenium remains unclear.

AP-1 is another transcription factor involved in cell proliferation. Several forms of selenium are known to retard the binding of the AP-1 to DNA, presumably by altering redox control mechanisms. GSSEGš has been reported to be ~10 times more effective in inhibiting AP-1 DNA binding in nuclear extracts from 3B6 lymphocytes than is selenite (25). This nuclear transcription factor, as well as NF-κB, may be involved in the antitumorigenic effects of selenium.

**Selenium Inhibits cdk2 and gadd45**

Various forms of selenium markedly retard the growth of neoplasms. Part of this effect may relate to the recognized ability of selenite to produce DNA breakage and cell death (17,29,30). The induction of apoptosis has been attributed to changes in genes such as cyclin-dependent kinase 2 (cdk2) and gadd45 (48,49). The cdk2 and DNA damage-inducible (gadd) genes are related to cell cycle arrest at G₁/S and G₂/M, respectively. Genomic stability in eukaryotes can be maintained by the checkpoints at G₁/S and G₂/M in response to DNA damage. In vitro, methylselenocysteine has been reported to arrest mouse mammary tumor epithelial cells (TM6) in the S phase, which coincided with a specific block of cdk2 kinase activity and an elevated expression of gadd34, gadd45, and gadd153 (48,50). Although the underlying mechanism accounting for these observations is not clear, the alterations in cdk2 and gadd45 suggest that the effect of selenium in these cells may be related to the p53-mediated apoptosis. The p53 protein is a factor that enhances transcription of several genes, including gadd45, p21⁵⁶⁴⁶,waf1,cip1, mdm2, cyclin G, bax, and insulin-like growth factor binding protein-3. Generally, the p53 protein is maintained at a low concentration, although it can be induced by physical or chemical DNA damage (51). p53 has been implicated in a G₁/M phase checkpoint, preventing premature entry into another S phase, possibly by altering gadd45 (52,53) and/or regulating the number of centrosomes in a cell (54).

**Selenium and Apoptosis**

Virtually all cells are endowed with the capacity for programmed cell death, i.e., apoptosis (55). This process typically involves activation of caspase-family cell death proteases. Apoptosis enhances the elimination of damaged and dysfunctional cells that may arise from oxidative stress, glycation, and DNA damage. Interestingly, apoptosis also can be triggered by selenium independent of DNA damage and in cells with a null p53 phenotype. p73 and p63 have homology to p53 in their respective transactivation, DNA-binding, and oligomerization domains. Both p73 and p63 transactivate p53-regulated promoters and induce apoptosis. Evidence suggests that p73 and p63 mediate apoptosis by mechanisms different from p53 (56). Although different forms of selenium
have different effects on apoptosis (57–59), it remains to be determined whether selenium alters these or other factors associated with non-p53-mediated apoptosis.

**Selenium Silences Lipoxygenases**

PHGPx is an enzyme recognized for its involvement in the removal of esterified lipid hydroperoxides. Additionally, PHGPx may be involved with the silencing of several lipoxygenases, including 5-, 12-, and 15-lipoxygenase (60–62). The importance of this regulation stems from the recognition that lipoxygenases generate metabolites that mediate signals for increasing cell growth and proliferation (63) and inhibiting apoptosis (64). Creation of selenium-deficient rat basophilic leukemia cells with <1% of normal cGPx activity and ~35% of normal PHGPx activity caused an approximately eightfold increase in release of lipoxygenase metabolites compared with controls. Addition of 0.25 µg of selenium per milliliter of medium to these cells reduced the amount of 5-lipoxygenase metabolites released to control values after 12 h and restored PHGPx. Injection of 500 µg of selenium as Na2SeO3 per kilogram to rats raised leukocyte PHGPx activity eightfold and significantly decreased lipoxygenase-generated metabolites within 114 h compared with controls (60). These results indicate that PHGPx, but not cGPx, is likely responsible for silencing 5-lipoxygenase activity by regulating the tone of membrane hydroperoxides (65). The significance of the interactions between dietary fats and selenium is exemplified by the general association of a high dietary fat intake with increased risk of some cancers, such as prostate, and the protection that may occur with dietary selenium supplementation (66). Because the selenoprotein PHGPx can silence lipoxygenases, this may partially explain the observed anticancerous effects of this trace element.

**Summary**

Overall compelling evidence exists that selenium is a deterrent to cancer cell proliferation in model systems. Nevertheless, the best dietary source, quantity, and biologically active form of selenium remain to be determined. Likewise, there remains a dearth of information about the impact of dietary selenium on the biological behavior of tumors occurring in humans. It is likely that not all individuals will respond identically because of differences in their absorption and metabolism of selenocompounds. Genetic differences may also contribute to variation in response to selenium. Only by having knowledge of molecular targets for selenium can individuals be adequately identified who might benefit most or be placed at risk by a dietary strategy to enhance selenium intakes for cancer prevention.

**Acknowledgments and Notes**

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