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Forum Review

Selenium and Cancer Chemoprevention: Hypotheses Integrating the Actions of Selenoproteins and Selenium Metabolites in Epithelial and Non-Epithelial Target Cells

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ABSTRACT

The trace element nutrient selenium (Se) discharges its well-known nutritional antioxidant activity through the Se-dependent glutathione peroxidases. It also regulates nuclear factor activities by redox mechanisms through the selenoprotein thioredoxin reductases. Converging data from epidemiological, ecological, and clinical studies have shown that Se can decrease the risk for some types of human cancers, especially those of the prostate, lung, and colon. Mechanistic studies have indicated that the methylselenol metabolite pool has many desirable attributes of chemoprevention, targeting both cancer cells and vascular endothelial cells, whereas the hydrogen selenide pool in excess of selenoprotein synthesis can lead to DNA single strand breaks, which may be mediated by some reactive oxygen species. We propose a new paradigm based on a consideration of the post-initiation biology of avascular early lesion expansion microenvironment, physiochemistry of Se delivery, and the obligatory need for angiogenesis to sustain lesion progression. Our model integrates the roles of selenoproteins and specific Se metabolites to account for cancer risk reduction or enhancement. For future studies, speciation (profiling) methods for Se metabolites and for Se forms in foods and supplements are much needed for hypothesis testing and for the development of mechanism-based Se status markers for cancer prevention. Randomized cancer prevention trials are necessary to test the efficacy of methyl selenium compounds. *Antioxid. Redox Signal.* 7, 1715–1727.

INTRODUCTION

CANCER IS A LEADING CAUSE OF DEATH for people in developed countries. Cancer chemoprevention refers to the use of naturally occurring and/or synthetic chemicals to block, inhibit, or reverse the development of cancer. Chemoprevention holds the exciting promise to decrease the morbidity and mortality of cancer and is essential for winning the war on cancer.

Converging data from epidemiological, ecological, and preclinical studies have implicated selenium (Se) as a risk modifier for some, but not all, cancers (7, 27, 69). The results of the landmark cancer prevention trial by the late Larry Clark, Gerald Combs, and co-workers (5, 6, 8, 13–15, 57) in the United States have strengthened the plausible proposal that Se, as a nutritional supplement, is a safe and effective preventive agent against the genesis of solid cancers in multiple organ sites, particularly in the prostate, colon, and lung.

The trial by Clark *et al.* (5) was a prospective, double-blinded, randomized, placebo-controlled trial involving 1,312 patients (three-fourths of them were males) who were recruited initially because of a history of non-melanoma skin cancers (NMSC) including basal cell and squamous cell carcinomas. Participating clinics were from the Southeastern states of the United States, where soil Se levels tend to be lower than the rest of the country. Subjects in the treatment arm were given 200 μg of Se (approximately four times the recommended daily value of 55 μg/day) as selenized brewer's

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yeast (predominantly selenomethionine) per day for a mean of 4.5 years, and the subjects were followed for a mean of 6.5 years. Subjects who received the Se-yeast supplement showed significantly lower incidences of cancers of the prostate [relative risk (RR) = 0.37, $p = 0.002$], colon (RR = 0.42, $p = 0.03$), and lung (RR = 0.54, $p = 0.04$) than the placebo group. Total cancer-related mortality was also significantly decreased by 41%. However, there was an Se-yeast-associated increase in the risk of secondary NMSC (RR = 1.10), which were the primary endpoints of the trial. The protective effects of Se supplementation for prostate cancer (RR = 0.51, $p = 0.009$) and colon cancer (RR = 0.46, $p = 0.055$) persisted upon longer follow-up for a mean of 7.8 years (5, 13).

A series of follow-up papers have recently been published documenting critical interactions between the baseline plasma Se level and the risk reduction by Se supplement on cancers of the prostate (6, 14) (Fig. 1A) and lung (57) (Fig. 1B) and total cancer excluding NMSC (13) (Fig. 1C) using the complete data sets that included several additional years of follow-up. It is particularly important to note that the subjects with baseline plasma Se in the lowest tertile (less than ~105 ng/ml, or 1.33 μ*M*) showed the most reduction of prostate and lung cancer and total non-NMSC cancers upon Se-yeast intervention. Subjects entering the trial with higher baseline Se did not show a reduction of risk for these cancers with Se-yeast supplement. As in the original report (5), a small yet statistically significant increase in the occurrence of secondary NMSC due to Se-yeast supplement persisted with longer follow-up (RR = 1.27, $p = 0.001$) (15). It is noteworthy that the increase in secondary NMSC incidence was observed in subjects entering the trial within the 2nd (105–123 ng/ml) and 3rd tertile (123 ng/ml, or 1.58 μ*M*) of baseline Se concentration (Fig. 1D). These results clearly indicate organ site specificities of Se-yeast at the dose of 200 μg/day for the prevention of several solid cancers and for Se-associated increase in NMSC risk in this male-dominated and NMSC-patient cohort. The significant associations of lower baseline plasma Se with a greater sensitivity to the preventive effects of prostate, colon, and lung cancers and of higher baseline Se with greater increases in secondary NMSC, if confirmed, not only have practical implications for the application of Se-yeast for prevention of certain cancers in the target populations based on their Se status, but also challenge the current state of understanding of the mechanisms of cancer preventive activity.

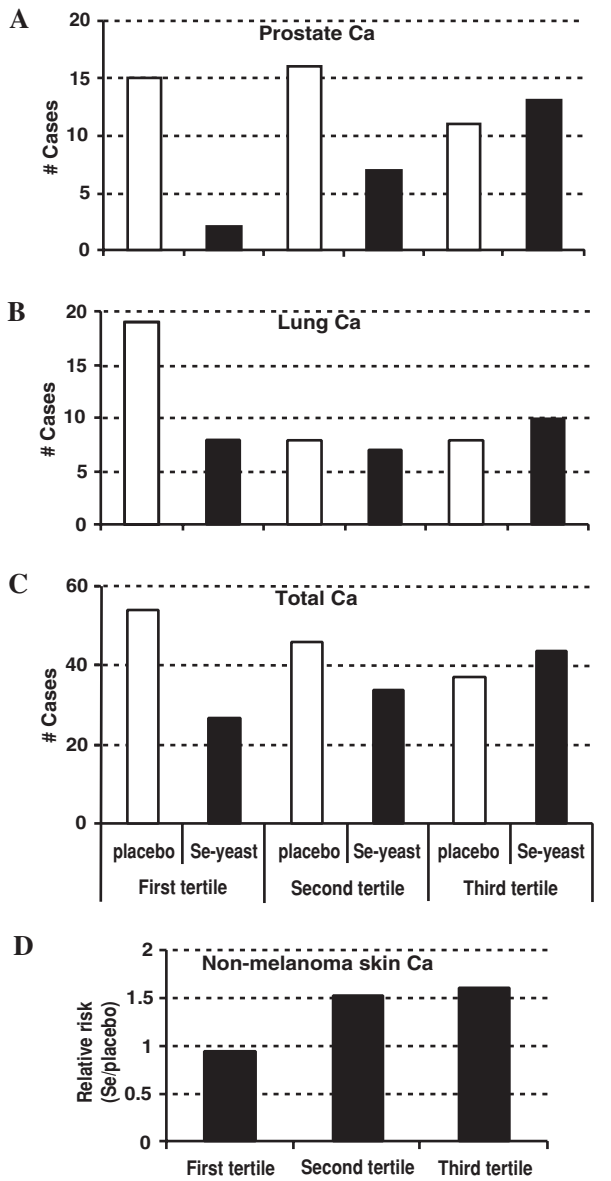

FIG. 1. The impact of baseline Se by tertile on the preventive effect of Se-yeast on (Ca) prostate cancer (A), lung Ca (B), total Ca (excluding NMSC) (C) and NMSC (D). Graphs were plotted based on data that have been reported in the literature (13–15, 57). Se intervention was given for an average of 4.5 years, and the subjects were followed up for an average of 7.8 years. The baseline Se tertile distribution was as follows: low, <105.3 ng/ml; middle, 105.3–121.6 ng/ml; high, >121.6 ng/ml.

NEW CANCER PREVENTION TRIALS WITH Se

In spite of the striking cancer risk reduction observed in the prostate, colon, and lung, the above findings were secondary endpoints of the trial. Because NMSC are rarely life-threatening, the potential benefit of preventing lethal solid cancers of the prostate and the lung has stimulated great research interests that culminated into two large clinical prevention trials in the United States and Canada to validate the preventive efficacy of Se for prostate cancer and lung cancer. The Selenium and Vitamin E Cancer Prevention Trial (SELECT) (44) is a randomized, prospective, double-blind study designed to determine whether Se as selenomethionine, which is a principal Se component of Se-yeast (2, 34), and vitamin E alone and in combination can reduce the risk of prostate cancer among healthy men. Preclinical, epidemiological, and Phase III data from randomized, placebo-controlled clinical trials suggest that both Se and vitamin E have potential efficacy in prostate cancer prevention. Coordinated by the Southwest Oncology Group (SWOG), SELECT is a 2 × 2

factorial study with an accrual goal of 32,400 men aged 55 years or older (age 50 or older for the African-American men) with nonsuspicious digital rectal examination and serum prostate-specific antigen (PSA) of 4 ng/ml or lower. Enrollment began in 2001 with final results anticipated in 2013. During the 12-year study period approximately 1,500–2,000 cases of prostate cancer, 800 lung cancers, and 500 colon cancers are estimated to be diagnosed.

A Phase III randomized chemoprevention study of Se in participants with previously resected stage I non-small cell lung cancer has been initiated by the Eastern Cooperative Oncology Group (protocol ECOG-5597) with several oncology groups participating (see http://www.cancer.gov/clinicaltrials/). Eligibility criteria include at least 18 years old, at least 6 weeks but no more than 3 years since surgery to remove lung tumors, no evidence of lung cancer after surgery, and no previous chemotherapy or radiation therapy for lung cancer. Patients will be randomly assigned to one of two groups. Patients in Group 1 will receive Se in the form of Se-yeast (200 μg of Se) by mouth once a day. Treatment will continue for up to 4 years. Patients in Group 2 will receive a placebo by mouth once a day. All patients will be evaluated once a year.

The outcomes of these two trials can be expected in about a decade. These trials are billed as the definitive tests for validating the preventive efficacy of Se (in the forms of Se-yeast or selenomethionine) for prostate and lung cancers. Several small-scale clinical trials and pilot studies concerning prostate cancer prevention have also been either completed or initiated in the United States (18, 64, 65) and other countries including the Prevention of Cancer by Intervention by Selenium (PRECISE) Trial pilot studies in the United Kingdom and Denmark (46). The protective effects of an Se supplement on cancer risk in different organ sites were also seen in a few other studies (reviewed in 1), including a protective effect against liver cancer (69–71).

A better understanding of the mechanisms will provide novel insights for interpreting the results of these new Se trials and for guiding the design of future trials to fully exploit the beneficial effects of Se for cancer prevention. Here we review recent advances in mechanistic studies that may help account for the observed effect in the trial by Clark and coworkers and to suggest directions for future research. For a more thorough treatment of the literature, the reader is referred to recent comprehensive reviews (7, 27, 49, 60, 69).

"KNOWN" MECHANISMS

Proposed mechanisms include an antioxidant protection against peroxide- and reactive oxygen-driven initiation and promotion events, an enhanced carcinogen detoxification, an enhanced immune surveillance, an inhibition of proliferation, and an induction of death by apoptosis of transformed epithelial cells, to name a few (7, 27, 69). More recently, we have shown that an inhibition of angiogenesis by Se may also contribute to the preventive activity (37, 38, 49). In addition, the critical Se metabolite(s) hypothesis has steadily gained both *in vivo* and *in vitro* support during the last decade (7, 27, 48, 49, 60). Mechanisms should address key elements such as the organ site specificity, the selectivity against transformed phenotype, the forms and effective doses of Se, and the responsive carcinogenesis stages and molecular targets. Much remains debatable as to what mechanisms account for the cancer preventive activity of Se. It is likely that a combination of these mechanisms will operate depending on the host Se status as has been documented in the trial by Clark and coworkers (Fig. 1), the form and dose of Se supplement, and the organ site specificities of cancer etiology as well as the capacity for Se metabolism. Figure 2 schematically illustrates the nutritional and chemopreventive ranges of Se intake and associated selenoprotein and non-protein Se parameters.

Selenoproteins, anti-peroxidation, and redox regulation

The best-studied function of Se in the nutritional range of intake is as an integral part of a cellular antioxidant enzyme family, the Se-dependent glutathione peroxidases (SeGpx) (1, 3). Hypotheses based on antioxidant protection against reactive oxygen-driven cancer initiation and promotion are intuitive and rationally sound for human cancer prevention, especially in individuals with marginal and deficient Se status (Fig. 3). Mutagenic oxidative stress is generally thought to be a major factor in the initiation of human carcinogenesis, as the electron-rich DNA bases are susceptible to electrophilic attack by reactive oxygen species (ROS), resulting in genetic damages, mutant oncogenes and tumor suppressor genes or epigenetic changes that altered their expressions, and the expression of malignant phenotypes. Endogenously produced ROS include superoxide radical, hydrogen peroxide (H_2O_2), singlet oxygen, and hydroxyl radical, as well as electrophilic metabolites of xenobiotics and other reactive intermediary metabolites. Accordingly, there has been considerable interest in the possibility of whether the cancer chemopreventive activity of Se is mediated through the SeGpx.

The putative connections for other selenoproteins such as thioredoxin reductases (23, 66) and thyroxine deiodinases

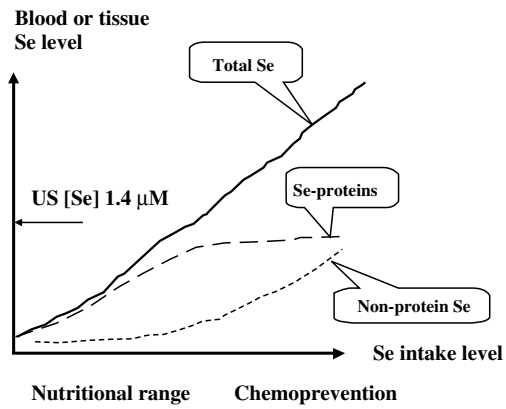

FIG. 2. A stylized representation of blood and tissue Se and selenoprotein status as functions of Se intake. The range of Se intake is divided into nutritional and chemoprevention based on the saturation status of selenoproteins and the appearance of non-selenoprotein Se. The value of 1.4 μ*M* plasma Se for subjects in the placebo group [based on data from Clark *et al.* (5)] is given for reference.

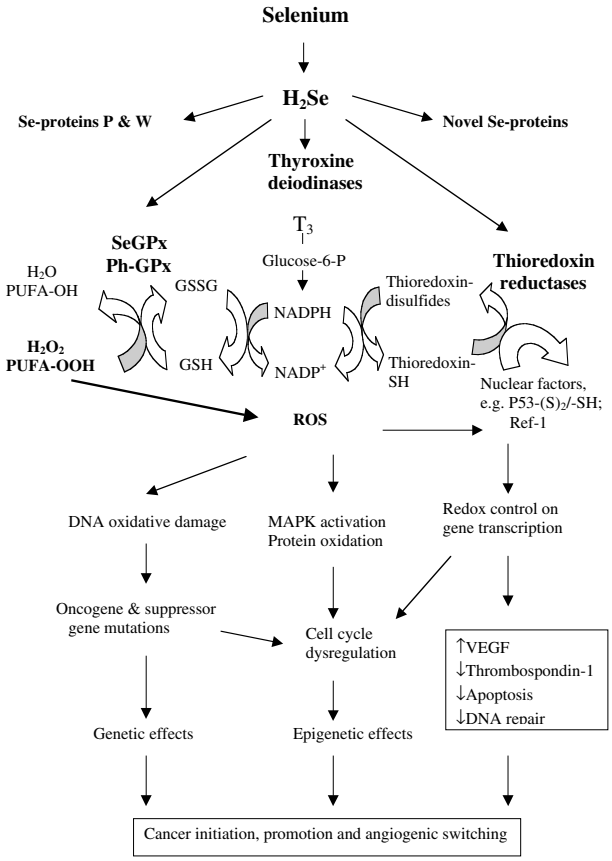

FIG. 3. Proposed roles of known selenoenzymes against ROS-driven cancer initiation, promotion, and angiogenic switch through genetic and epigenetic pathways in a nutritional context. Selenium in the form of hydrogen selenide is incorporated into selenoproteins through co-translational incorporation using the SeCys codon (Seryl-tRNA^{UGA}). Glucose-6-P, glucose-6-phosphate; GSH, reduced glutathione; GSSG, oxidized glutathione; MAPK, mitogen-activated protein kinase; Ph-Gpx, phospholipid glutathione peroxidase (Gpx4); PUFA, polyunsaturated fatty acid; T_3, triiodothyronine.

(45, 47) to regulate ROS and cellular redox are also depicted schematically in Fig. 3. In particular, Se has been shown to modulate p53 activity through redox modification of Cys^{275} and Cys^{277} mediated by Ref-1 (58), resulting in an enhanced repair of drug- or radiation-induced DNA damages (59). Because p53 is known to suppress the expression of the angiogenic factor vascular endothelial growth factor (VEGF) (72) and to induce the expression of the angiogenesis suppressor thrombospondin-1 (9), a selenoprotein-mediated increase of p53 activity could play a pivotal role in turning off the angiogenic switch in the early lesions. In this context, the selenoproteins could be very important mediators for decreasing cancer risk in populations with frank or marginal Se deficiency status.

The Se status of individuals residing in the United States has been considered "nutritionally" adequate judging by intake and the plasma Se content. According to the Third National Health and Nutrition Examination Survey (NHANES III), the mean intake of all ages was 103 μg (19), nearly twice the National Research Council's recommended daily allowance of 55 μg (20). From the 18,597 persons for whom serum Se values were available in NHANES III (21, 55), the mean concentration was 1.58 μ*M*, and the median concentration was 1.56 μ*M*. This is much higher than the 1 μ*M*, or 80 ng/ml, that was found to be the upper limit for SeGpx responses to supplemental Se in healthy adults (54). This was also borne out in the trial of Clark and co-workers in which the placebo group had a baseline plasma Se level of 114 ng/ml (1.4 μ*M*) (5, 13). Only two subjects (1.5%) had Se levels below 80 ng/ml (13). Because of this fact, most animal models and *in vitro* cell culture studies since the mid-1980s have dealt with chemopreventive levels of Se intake and have focused on the cancerous epithelial cells as the targets of its anti-cancer effects. Most animal models have shown cancer chemopreventive activity of Se intake that is 20–50 × greater than the nutritional requirement (27). Based on a large body of data from these studies, it has been articulated that cancer chemoprevention by Se is independent of the antioxidant activity of plasma or tissue SeGpx (7, 27). This paradigm was based on the observation that the dietary level of Se (2 ppm or greater) needed to achieve a significant cancer preventive activity in animal models far exceeded that required (*i.e.,* 0.1 ppm) to support maximal SeGpx in the blood (now known as Gpx3) or the target tissues from which experimental cancers arise (27). This view has prevailed since the mid-1980s and has been extended to the other selenoproteins identified subsequently in the last decade, including phospholipid glutathione peroxidase (also known as Gpx4), selenoprotein P, selenoprotein W, thyroxine deiodinases, and thioredoxin reductases (27).

Non-selenoprotein Se metabolites

Regarding non-selenoprotein Se metabolites, Fig. 4 shows schematically possible metabolic pathways for Se from inorganic salts and from selenoanimo acids. Hydrogen selenide is co-translationally incorporated into selenocysteine (Se Cys)-containing proteins. Excess Se beyond the need for selenoprotein synthesis is methylated into methylselenol. Milner (53) has shown that selenodiglutathione (GSSeSG), an intermediate of reductive metabolism of selenite, was significantly more efficacious than the parent compound selenite using a leukemia ascites model. However, the transient nature of GSSeSG under physiological pH *in vivo* and the fact that food-derived selenoanimo acids, which do not produce GSSeSG through *in vivo* metabolism (7, 69), exert anti-cancer activity support the role of more downstream Se metabolites as the likely critical anti-cancer Se species.

Ip (27) has spear-headed the effort to identify the putative *in vivo* Se metabolite pool using a mammary chemical carcinogenesis model. In a typical rodent cancer chemoprevention study, the chemical carcinogen-exposed animals were given Se in diets or drinking water for 6 months or longer. In the rat, the dietary Se level to achieve a significant inhibition (50%) of mammary carcinogenesis was ~3 ppm (3 μg/g) in the form of sodium selenite in comparison with a control group consuming 0.1 ppm Se. Dietary level of Se as selenite above 5 ppm usually caused a depression of body weight gain, indicative of systemic toxicity. A chemopreventive level

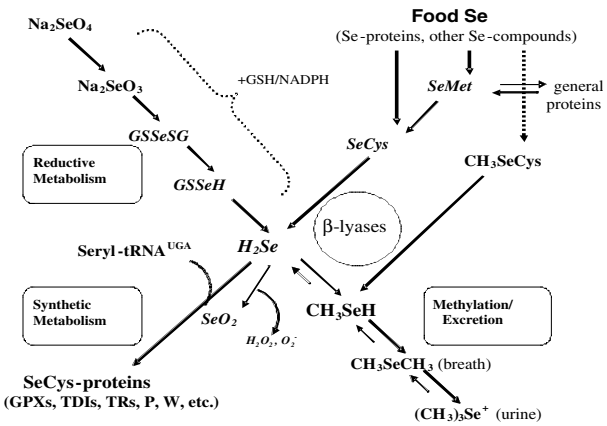

FIG. 4. Possible metabolic pathways for inorganic Se and selenoanimo acids in a chemoprevention context. For selenoamino acids, tissue cysteine β-lyases release hydrogen selenide and methylselenol from SeCys and methylselenocysteine, respectively. The methylselenol pool may be selectively enriched by using methylselenol precursors either as purified compounds or as functional foods such as Se-garlic, bypassing the hydrogen selenide pool. TDIs, thyroxine deiodinases; TRs, thioredoxin reductases.

of Se intake results in a plasma Se level of 600–800 ng/ml (7.6–10.1 μ*M* Se). The plasma Se level for animals consuming the "requirement" level of 0.1 ppm Se is approximately 300–400 ng/ml (3.8–5 μ*M* Se). The plasma or target tissue SeGpx enzyme activities are usually not appreciably increased by the chemopreventive level of Se intake. This is not surprising because the "nutritional requirement" for Se is defined as the minimal dietary Se level needed to support maximal blood SeGpx and tissue SeGpx activities. Even though it is often true that the cancer preventive activity is proportional to the tissue Se accumulation for many Se forms when studied individually, the total tissue Se content did not predict cancer preventive efficacy when different Se forms were compared (29). These observations indicate that the chemical nature of non-protein Se might be a critical determinant of the cancer chemopreventive efficacy.

Ip (27) proposed that the active anti-cancer Se metabolite was likely a monomethylated Se species (presumably methylselenol) and that the chemopreventive efficacy of a given Se compound might depend on the rate of its metabolic conversion to that active Se form. Strong supporting evidence was obtained by comparing the cancer preventive efficacy of different forms of Se that fed into different Se metabolite pools, with precursors of methylselenol displaying greater preventive efficacy than those for hydrogen selenide or dimethylselenide in the chemically induced rodent mammary carcinogenesis model (28, 30). In addition, arsenic was used as an inhibitor of the Se methylation steps, and the data showed that blocking the conversion of hydrogen selenide to methylselenol decreased the anti-cancer activity, whereas inhibiting the further methylation of methylselenol increased the efficacy. Extending on the methylselenol structure–activity theme, subsequent work had shown that the alkylselenol and allyl-selenol precursor compounds were more active against mammary carcinogenesis than methylselenol precursors on a molar basis of dietary Se intake (32, 33). However, these structure–activity studies have not been extended beyond the mammary carcinogenesis model for assessing the general applicability of the methylselenol hypothesis in other organ sites.

Consistent with the concept of non-selenoprotein Se metabolites for chemoprevention, el-Bayoumy and coworkers undertook to develop new organoselenium cancer chemopreventive agents with less toxicity than some of the classic Se compounds, such as sodium selenite (16, 17, 31, 71–73). A series of organoselenium compounds have been synthesized and evaluated for their chemopreventive efficacy *in vivo*. Parallel to these studies, short-term *in vitro* and *in vivo* assays were employed to understand the mechanisms of action. They have demonstrated that one of the most effective of these organoselenium compounds is 1,4-phenylenebis (methylene)selenocyanate. This agent is capable of inhibiting tumors in the mammary glands, colon, and lung of laboratory animals (16, 17). The structural feature of X-CH_2SeCN (where X represent aromatic phenyl groups of agents in this series) for generating X-CH_2SeH may in part explain their many similarities with methylselenol precursors. While these agents are better tolerated in rodent models than inorganic selenite and selenoamino acids (60), their applicability for human cancer chemoprevention requires more investigation.

Distinct Se metabolite actions in cell culture models

Studies by us and others using cell culture models have shown that the monomethyl Se pool induced numerous cellular, biochemical, and gene expression responses that were distinct from those induced by the forms of Se that enter the hydrogen selenide pool (4, 12, 39, 40, 42, 43, 50–52, 62, 67, 68). These major cellular and biochemical effects are schematically summarized in Fig. 5. Together, these findings support the presence of at least two different pools of Se metabolites that induce distinct types of biochemical and cel-

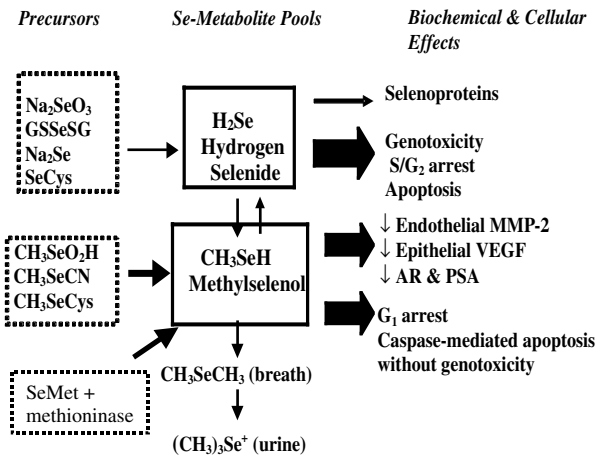

FIG. 5. Distinct biochemical and cellular effects of Se precursors feeding into the genotoxic hydrogen selenide pool or non-genotoxic methylselenol pool of metabolites in cell culture models. AR, androgen receptor.

lular responses and exert differential impacts on angiogenic processes.

The hydrogen selenide pool. Sodium selenite and sodium selenide, which feed into the hydrogen selenide pool first, rapidly (within hours of Se exposure) induced DNA single strand breaks (SSBs) (*i.e.,* genotoxic) and S phase/G_2 cell cycle arrest and led to subsequent cell death by apoptosis (40, 42, 48–52). Sodium selenide and SeCys recapitulated the DNA SSB induction and the apoptogenic effects of selenite (50). A superoxide dismutase (SOD)-mimetic compound, copper dipropylsalicylate, blocked DNA SSBs and apoptosis, indicating that selenite *per se* did not trigger these events (48). The role of superoxide generation by the hydrogen selenide pool has been confirmed with SOD or SOD-mimetics (40, 74). However, little is known about whether the hydrogen selenide pool could reach the pharmacological levels used in these *in vitro* studies to affect DNA integrity (genotoxicity).

The methylselenol pool. Our earlier work has shown that methylselenol precursors such as methylselenocyanate (MSeCN, CH_3SeCN) and Se-methylselenocysteine induced apoptosis of mammary tumor epithelial cells without the induction of DNA SSBs (42, 51, 52, 62). Furthermore, we and others have reported that methylselenium-induced cancer cell apoptosis is caspase-dependent, whereas selenite-induced cell death is independent of these death proteases in prostate cancer and leukemia cells (41, 43). The methylselenium group led to G_1 arrest (40, 42, 52, 67, 68). Specific inhibitory effects on cyclin-dependent kinases (61, 75) and protein kinase C (63) have been attributed to the methylselenium pool.

In addition to these cellular effects, methylselenol precursors exert a rapid inhibitory effect on the expression of key molecules involved in angiogenesis regulation. For example, we have shown that subapoptotic doses of methylseleninic acid (MSeA, CH_3SeO_2H) inhibit the expression and secretion of the angiogenic factor VEGF in several cancer cell lines (38). Methylselenium also inhibits the expression of matrix metalloproteinase (MMP)-2 in the vascular endothelial cells (38). These effects plus a potent inhibitory effect on the cell cycle progression of vascular endothelial cells (67, 68) indicate that methylselenol is a key inhibitor of angiogenic switch regulation in early lesions and in tumors (49). Furthermore, we and others have recently shown that methylselenol inhibits androgen receptor expression and its signaling to PSA (4, 12) as well as PSA stability (4). This action may offer an explanation for why the prostate is particularly sensitive to cancer prevention by Se in the trial by Clark and co-workers (5, 6, 14).

EFFECTS OF Se METABOLITES ON CANCER EPITHELIAL CELLS

Methylselenium-specific inhibitory effect of VEGF expression

The transformed epithelial cells contribute to angiogenic switching by up-regulating the expression and secretion of positive factors and/or down-regulating the expression of angiogenesis inhibitors. Because of the central role that VEGF plays in neo-angiogenesis, an inhibitory effect on VEGF expression by Se can be expected to repress the angiogenic switch for the early lesions. We have found that Se treatment, whether given in a chemoprevention setting or in an acute therapy setting, was associated with a significant inhibition of VEGF expression in some but not all mammary carcinomas induced by 1-methyl-1-nitroso urea in the rat (37). In cell culture, we have reported a methylselenium specificity of the inhibition of tumor epithelial VEGF expression (38). In human prostate (DU-145) and breast (MCF-7 and MDA-MB-468) carcinoma cell lines, exposure to MSeA led to a rapid and sustained decrease of the cellular and the secreted VEGF protein levels irrespective of the serum level (serum free vs. 10% fetal bovine serum) in which Se treatments were carried out. The concentration of MSeA required for suppressing VEGF expression was much lower than that needed for apoptosis induction. Selenite lacked any inhibitory activity in either acute or chronic exposure in these cells (38). Taken together, the data support the hypothesis that the monomethyl Se pool inhibits the expression of VEGF in the transformed epithelial cells. Efforts are underway to investigate how the methylselenium exerts this suppression activity. It is also important to integrate the impact of and the interaction of selenium metabolites and selenoproteins with hypoxia into the research questions (see model in Fig. 6). The impact of different pools of Se on the expression profile and activity of other angiogenic stimulators and inhibitors should be investigated to provide an overall picture of the angiogenic switch action.

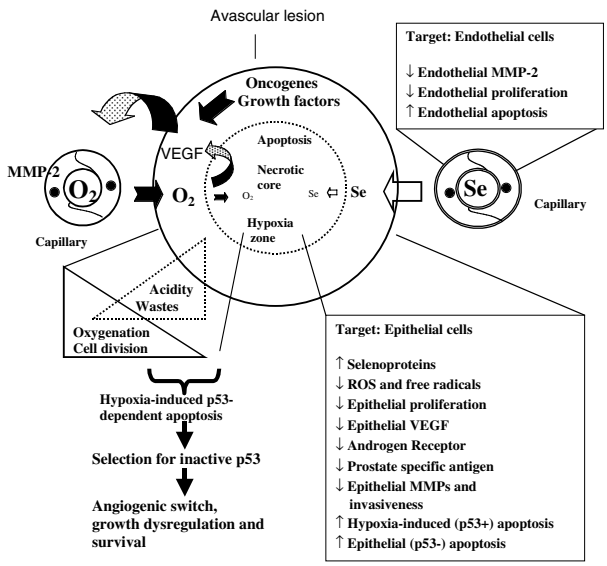

FIG. 6. A schematic representation of the avascular lesion expansion microenvironment and proposed modes of Se actions in a post-initiation model of cancer chemoprevention. We hypothesize that Se and selenoproteins follow a gradient distribution pattern like that of oxygen, creating a "conditional Se deficiency" in hypoxic cells in the interior of avascular clones even when the blood Se supply is considered adequate. Some known Se actions on cancer epithelial cells and vascular endothelial cells are outlined.

Methylselenium-specific inhibition of androgen receptor expression and signaling

PSA is widely used clinically for prostate cancer diagnostics and as an indicator of therapeutic efficacy and recurrence. PSA expression is strongly androgen-dependent in the prostate epithelial cells. In the androgen-responsive LNCaP prostate cancer cell model, we recently (4) have found that exposure to subapoptotic concentrations of MSeA or methylselenol inhibited PSA protein expression and secretion, whereas sodium selenite and selenomethionine lacked any inhibitory effect. The inhibition was detectable at 3 h of exposure and required a threshold level of MSeA to sustain. Turnover experiments showed that MSeA caused a rapid PSA degradation, which was partially blocked by lysosomal inhibitors, but not by a proteasomal inhibitor. Furthermore, MSeA treatment reduced the PSA mRNA level, downregulated the androgen receptor protein expression, and inhibited the androgen-stimulated PSA promoter transcription, confirming recent reports of two other groups (12, 73). These findings imply a unique mechanism to account for the prostate-specific cancer chemopreventive action of Se.

Methylselenium induction of caspase-mediated apoptosis

In terms of the pathways of apoptosis execution, caspases appear to be specifically activated by methylselenium exposure. We have reported recently that MSeA induced DU145 human prostate carcinoma cell apoptosis through caspase-dependent execution (39). Specifically, apoptosis induced by MSeA involved cell detachment, the activation of multiple caspases, mitochondrial release of cytochrome *c*, cleavage of poly(ADP-ribose) polymerase, and DNA nucleosomal fragmentation. The last three biochemical actions were shown to require the activation of caspases. Independently of and complementing to our work with MSeA, Se-methylselenocysteine has been shown to cause caspase-dependent apoptosis of HL-60 human leukemia cells (43), which grow in suspension culture and do not require cell attachment for survival and mitogenesis. Because DU145 and HL-60 cells do not contain functional p53, these results suggest that methylselenol induces apoptosis in a p53-independent manner.

Metabolite-specific effects on cancer cell cycle

In comparison to normal epithelial cells, transformed cells are characterized by a loss of growth control through oncogenic activation and/or a loss of tumor suppressor activities. Lesion size is governed by the rate of cell proliferation and the rate of cell death in the transformed epithelial cells. The rate of cell birth will be dependent on the ratio of the cycling cells to the non-cycling cells, which include the quiescent or the terminally differentiated cells. Prolonging the time required for each phase of the cell cycle of the cycling cells (cell cycle arrest) as well as diverting them into the non-cycling pool can impact the rate of overall growth of the lesion and may result in cell death.

We and other have shown that at dose levels lower than that required to induce cell death, a variety of tumor cells undergo cell cycle arrests, but in a Se metabolite-specific manner (42, 52, 62). It has been well documented that methylselenium induces a G_1 arrest, whereas selenite induces an S or S/G_2-M arrest. Whereas superoxide production and DNA SSBs may underlie the S or S/G_2-M phase arrest effect induced by selenite, very little is known about how methylselenium exerts the G_1 arrest. Methylselenium-induced inhibitions of specific protein kinase and cyclin-dependent kinases have been reported (61, 63, 75). Altered expressions of cell cycle regulatory gene networks have also been described using microarray profiling techniques (10, 11). Additional work is required to determine the precise pathways for methylselenium to exert its cell cycle arrest action.

EFFECTS OF METHYLSELENOL ON VASCULAR ENDOTHELIAL CELLS

The angiogenic microenvironment is likely conducive for a selective targeting of the stimulated endothelial cells by the blood-borne Se metabolites. Two physical and biological features of the vascular endothelial cells stand out: (a) As the lining of delivery channels, the endothelial cells are the first line of exposure to blood Se, and (b) the endothelial cells in normal tissues are essentially quiescent, whereas the angiogenic-stimulated endothelial cells are proliferative. Although the activity of selenoenzymes in blood and normal tissues do not increase once a threshold level of Se supply is reached (54), the total blood Se and therefore the non-protein Se in the blood and the tissues go up with an increased Se intake (Fig. 2). The elevated levels of non-protein Se metabolites, especially the methylselenol-related species, can be expected to directly affect the angiogenically stimulated endothelial cells to dampen their angiogenic responses through one or more of the following processes: extracellular matrix proteolytic activity, mitogenesis, and motility. Because endothelial cells are much less susceptible to mutagenesis in comparison with epithelial cells, the wild-type p53 status of the endothelial cells can ensure p53-dependent growth arrest and apoptosis, when the angiogenic and survival signals are interfered with by Se metabolites in the stimulated endothelial cells.

Methylselenium-specific inhibitory effect on MMP-2 expression

We have used a human umbilical vein endothelial cell model to examine the effects of Se exposure on the expression of MMP-2 and endothelial proliferation and survival to identify Se metabolite-specific activities on these critical components of an angiogenic response (37, 38, 67, 68). We have shown a methyl Se-specific inhibitory activity on MMP-2 expression (37, 38). This was supported by a clear contrast of the inhibitory effects of the methylselenol precursors MSeA and MSeCN with forms of Se that feed into the hydrogen selenide pool, *e.g.,* sodium selenite and sodium selenide. The MMP-2 inhibitory activity occurred at Se levels (IC_{50} of ~2 μ*M*) that were within human plasma Se and that occurred rapidly and required cellular activation. Expression, recruitment, and activation of MMP-2 and/or other MMPs by the stimulated endothelial cells are necessary to break down the adjacent extracellular matrix for the endothelial cells to

invade through during sprouting. A crucial role of MMP-2 or MMP-9 in setting the angiogenic switch has been shown in several models (26, 36). Many MMP inhibitors have been evaluated in animal models with promising efficacy for inhibiting cancer growth and metastasis, and some of these agents are in clinical trials. Taken together, these results support the methyl selenium pool for inhibiting the extracellular matrix degradation in vascular endothelial cells. It will be important to characterize the biochemical and molecular mechanisms for methyl selenium to inhibit MMP-2 expression in the endothelial cells and to determine whether such mechanisms are applicable to other MMPs and secretory proteins that are involved in angiogenesis and in tumor invasiveness, growth, and survival.

Methylselenium induces G_1 arrest through a phosphatidylinositol 3-kinase-related pathway

We have recently shown that MSeA inhibited the angiogenic factor-stimulated DNA synthesis (G_1 to S progression) with an IC_{50} of ~1 μ*M* and completely inhibited the stimulated DNA synthesis at 3 μ*M* (67). Methylselenium appeared to target a growth control mechanism in human umbilical vein endothelial cells during mid- to late-G_1 in a manner that can be recapitulated by phosphatidylinositol 3-kinase inhibitors (67). At higher levels of exposure that could be pharmacologically relevant, MSeA induced apoptosis through caspase pathways that were activated by p38 mitogen-activated protein kinase (41, 67). Furthermore, in an *in vitro* tube formation assay on Matrigel, prolonged treatment (>30 h) with either MSeA or MSeCN inhibited the retraction of human umbilical vein endothelial cell capillaries into spheroids due to endothelial apoptosis, whereas selenite at even higher exposure levels lacked this effect (38). These findings suggest that inhibiting the proliferation and survival of the activated endothelial cells by methylselenol or related Se metabolites may contribute to a reduced angiogenic response. Much remains to be done to delineate the signaling pathways and the target protein molecules involved in the metabolite-specific anti-mitogenic and pro-apoptotic effects in the endothelial cells, especially in the capillary microvascular endothelial cells that are the likely targets of angiogenic responses in the early lesions.

Possible chemical basis for Se metabolite effects

Ganther (22) had proposed a number of chemical reactions through which Se pools may directly modify the redox-sensitive enzymes or transcriptional factors to alter their functional activities: formation of selenotrisulfide bonds (-S-Se-S-); formation of selenylsulfide bonds (-S-Se-); catalysis of disulfide bond formation or its reversal (-SH ←Se→ -S-S-); and formation of diselenide bonds (-Se-Se-). The first three reactions would affect the activities of many enzymes with critical sulfhydryl groups, while the last reaction would specifically affect activities of SeCys proteins that have SeCys residues at these active centers. Identification of target proteins mediating these documented effects of Se metabolite pools remains a research priority before detailed studies of these proposed reactions are to be undertaken. An example is the redox regulation of protein kinase C isoforms by Se compounds (24, 25).

TRANSLATING THE MECHANISMS TO HUMAN APPLICATIONS

The prevention trial results of Clark and co-workers (5, 6, 13–15, 57) provide benchmarks to judge the likelihood of applicability of the preventive "mechanisms" observed from animal and cell culture models. Relevant to the mechanistic issues, the average plasma Se level in the supplemented group in the trial by Clark *et al.* (5) was 190 ng/ml (2.4 μ*M*) in comparison to that of the placebo group with 114 ng/ml (1.4 μ*M*). In spite of an increase of plasma total Se level by 67%, the plasma SeGpx activity of selected subjects before and after Se supplementation was not increased (7). These results indicate that at most 1 μ*M* non-selenoprotein Se could be generated by the Se-yeast supplementation in otherwise Se- "adequate" individuals as judged by their lack of further plasma SeGpx response (7). Since the majority of blood Se is either incorporated into selenoproteins or bound to proteins as the free plasma Se was estimated to be 2–4% of total (7), the protein-free Se concentration in human blood is not expected to be greater than 1 μ*M in vivo*. In fact, it has been suggested that serum or tissue free Se levels of the nanomolar range are more realistic.

As discussed above, the biochemical (*e.g.,* VEGF, MMP-2, PSA expression) and cellular (*e.g.,* cell cycle arrest, apoptosis) effects of Se compounds on cancer and vascular endothelial cell lines *in vitro* were observed at Se concentrations in the lower micromolar range of MSeA or MSeCN (38), and much higher levels (50 μ*M* or greater) for other selenoamino acids or derivatives (51, 52, 61–63). Such levels used in cell culture studies and the Se levels achieved in rodent chemoprevention models were one or two orders of magnitude higher than the likely protein-free Se projected in humans. However, because nothing is known of the effective Se metabolite concentration in target organs and early lesions, it is difficult to establish how much these actions are translatable to the human prevention setting. Profiling Se metabolites in target tissues and early lesions is much needed to help address the relevance issue.

Even though the U.S. populations are considered nutritionally "adequate" for Se status based on plasma glutathione peroxidases and Se level (19–21, 54, 55), the results of the trial by Clark and co-workers concerning the significant influence of the baseline plasma Se on the preventive activity of Se-yeast supplement as described in the Introduction (Fig. 1) warrant a re-examination of the paradigm that selenoproteins are not responsible for the prevention of human cancers by supranutritional Se intake as experienced in the United States. The plasma Se and selenoprotein status do not necessarily reflect those in the transformed lesions or target tissues. Based on a consideration of the post-initiation biology of the early lesion expansion microenvironment and the absolute need of angiogenesis for lesion growth and progression and the physiochemistry of Se delivery (Fig. 6), we speculate that, like oxygen, the cellular Se content and the expression

and activity of Se-dependent enzymes in avascular lesions follow a descending gradient of distribution from the surface to the interior (Fig. 6). In other words, clonal expansion creates a "conditional Se deficiency" state in the interior hypoxic epithelial cells in comparison to their counterparts at the superficial zone even though the Se supply for the host organ is considered adequate for normal physiological functions. The rationale for focusing on the post-initiation actions was the facts that in the trial by Clark *et al.* (5) the subjects at study entry were relatively old (average age 63 years) and with a prior history of NMSC. These patients probably represented a high-risk population with existing early lesions in those organ sites, suggesting that Se could be an effective intervention agent against existing precancerous lesions as well as suppressing the initiation events.

Based on the response patterns as shown in Fig. 1, it is speculated here that Se-yeast supplementation in the trial by Clark and co-workers at a dose of 200 μg/day was not sufficient to lead to a significant generation of methylselenol. Instead, the Se-yeast supplement might have changed the relative contribution of the selenoproteins and the hydrogen selenide pool to regulate lesion growth based on the baseline Se status at study entry. Selenomethionine and SeCys, the major components of Se-yeast, undergo metabolism to generate hydrogen selenide, which provides the Se for selenoprotein synthesis (Fig. 4). In the subjects within the 1st tertile of baseline Se, supplementation of Se-yeast might be mostly for supporting selenoprotein synthesis in the early lesions, which are more "conditional Se deficient" than those in the higher tertiles, exerting a significant inhibitory effect on their growth into clinical entity. On the other hand, subjects within the 3rd tertile of baseline Se better supported expression of the selenoproteins in the early lesions; therefore, when Se-yeast was taken by these subjects, most of the Se might have enriched hydrogen selenide production. Through the production of superoxide and ROS, excess hydrogen selenide might cause DNA SSBs and mutagenesis, thereby promoting instead of inhibiting tumor development. The inhibitory action of selenoproteins might be canceled out by a promoting action of the excess hydrogen selenide. In the case of the skin, the production of hydrogen selenide might have overpowered the inhibitory action of selenoproteins and resulted in an increased rate of NMSC by Se-yeast supplement in the subjects entering the trial with baseline Se in the 2nd and 3rd tertiles.

In addition to selenoproteins, a possible modification of systemic and cellular redox toward antioxidation has been reported in a clinical pilot study with healthy young males (18), whose baseline Se level was comparable to and slightly higher than the trial by Clark and co-workers (5, 17). Se-yeast supplementation led to an increase in whole blood free glutathione (GSH) and a decrease of the glutathionylated proteins, reflecting a reduction of oxidative stress to proteins in the body. In the same study, Se supplementation for 9 months decreased the plasma level of PSA by 20% without affecting the urine level of 8-hydroxydeoxyguanosine, which is an indicator of oxidative damage to DNA. These results together suggest mechanisms independent of or in addition to selenoproteins may be involved in the protection against oxidative stress and in inhibiting androgen signaling by Se supplementation in subjects whose baseline Se was in the "nutritionally" adequate range.

FUTURE RESEARCH

Selenium metabolite profiling and status assessment

A number of circulating forms of Se are predicted to exist *in vivo*. These include the selenoanimo acids from digestion of food proteins (selenomethionine, SeCys, and perhaps CH_3SeCys), and the intermediary and excretory metabolites (hydrogen selenide, methylselenol, and perhaps $[CH_3]_2Se$ and $[CH_3]_3Se^+$) (Fig. 4). Because hydrogen selenide and methylselenol have been shown to exert differential anti-cancer actions (Fig. 5), there would be obvious utility for methods that measure these metabolites and their ratios in plasma or target tissues. These will also be critical to test the plausibility of excess hydrogen selenide as a mutagenic agent for increasing cancer risk through ROS and genotoxicity-mediated genetic and epigenetic pathways.

Roles of selenoenzymes

A better understanding is needed of the roles, if any, of selenoproteins in the cancer preventive effects of Se. As we proposed, a conditional Se deficiency in early lesions may create a need for additional Se to support selenoproteins in the trial by Clark and co-workers, in which the subjects within the low tertile of baseline Se experienced the greatest reduction in cancer risk. This differs from the current paradigm that the protective effect did not involve the selenoproteins based on the two-stage model proposed by Combs and Gray (7). It has been reported that a polymorphism of codon 198 of the cellular SeGpx gene is strongly associated with risk to lung cancer among male smokers (56). This appears to be consistent with a protective role of SeGpx and other selenoproteins in suppressing risk of cancers with a strong oxidative etiology. Additional genetic polymorphisms in other selenoproteins may help to further build the case. In addition, the application of gene knock-out and knock-down technology for selenoproteins in carcinogenesis models may help to address this issue.

Define optimal Se dosage and interactions with baseline Se

It is important to establish the Se dosage that will be both safe and effective in reducing cancer risk in accord with the baseline Se. The SELECT (44) and lung cancer trial (see http://www.cancer.gov/clinicaltrials/) will provide valuable data to validate the influence of baseline Se on the response of the target cancers to Se supplementation. Such interactions should also be tested in future randomized trials with methylselenium precursors and other organoselenium compounds. It is important to understand the safety issues regarding Se doses and the possibility of increased cancer risk by too much supplementation to people with high baseline Se.

Speciation of Se compounds

Sensitive and specific methods are needed to characterize the chemical forms of Se present in foods and supplements. Besides selenomethionine and SeCys, which are the major Se in Se-yeast, additional Se forms may contribute to the observed anti-cancer activity. A few groups (2, 34, 46) have attempted to develop effective methods for characterizing the various chemical species of Se present in complex biological matrices such as Se-garlic. The ability to quantitatively identify Se species will be important for the evaluation of food sources of Se in cancer prevention, and for the management and breeding of "functional food" crops to enhance the values of foods for cancer prevention.

Randomized cancer prevention trials with methylselenol precursors and organoselenium

Although preclinical studies have strongly indicated many desirable attributes of the methylselenol precursors, randomized cancer prevention trials are needed to validate their efficacy. Methylselenocysteine, MSeCN, and MSeA have been shown to have equal chemopreventive efficacy against chemically induced mammary carcinogenesis in a rodent model (28, 30, 31, 35). The recent clearance of toxicology tests in dogs for methylselenocysteine (C. Ip, personal communication) should prepare the way for Phase I trials to establish the tolerated dose in humans before its implementation into large-scale randomized prevention trials. Similarly, clinical trials are necessary for testing the applicability of 1,4-phenylbis(methylene)selenocyanate and related organoselenium compounds for human cancer chemoprevention.

SUMMARY AND IMPLICATIONS

The clinical trial results of Clark and co-workers have strengthened the plausibility of Se supplementation for decreasing the risk of solid cancers of several organ sites. The significant interaction between baseline Se and the efficacy of the Se-yeast for reducing cancer risk, if validated, has important implications for the tailor-designing of delivery of Se to target populations for cancer prevention. Mechanistic studies have indicated that the methylselenol metabolite pool has many desirable attributes for chemoprevention and therapy, targeting cancers of both epithelial cells and vascular endothelial cells. Future randomized cancer chemoprevention trials are necessary to test the efficacy of methylselenium compounds in different organ sites. Speciation (profiling) methods for Se metabolites are much needed for hypothesis testing and for developing mechanism-based Se status markers for future prevention trials. We proposed a new paradigm to integrate the roles of selenoproteins and specific Se metabolites to account for cancer risk reduction in some organ sites and risk enhancement in others.

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ABBREVIATIONS

GSSeSG, selenodiglutathione; MMP, matrix metalloproteinase; MSeA, methylseleninic acid (CH_3SeO_2H); MSeCN, methylselenocyanate (CH_3SeCN); NHANES III, Third National Health and Nutrition Examination Survey; NMSC, nonmelanoma skin cancers; PSA, prostate-specific antigen; ROS, reactive oxygen species; RR, relative risk; Se, selenium; SeCys, selenocysteine; SeGPx, selenium-dependent glutathione peroxidases; SELECT, Selenium and Vitamin E Cancer Prevention Trial; SOD, superoxide dismutase; SSB, single strand break; VEGF, vascular endothelial growth factor.

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