J Bioenerg Biomembr (2007) 39:91–98
DOI 10.1007/s10863-006-9065-7

MINI REVIEW

Apoptotic cellular events for selenium compounds involved in cancer prevention

Hidemi Rikiishi

Published online: 16 February 2007

Abstract Converging data from epidemiological, ecological, and clinical studies have shown that selenium (Se) can decrease the risk for some types of human cancers. Induction of apoptosis is considered an important cellular event that can account for the cancer preventive effects of Se. Prior to occurrence of apoptosis, Se compounds alter the expression and/or activities of signaling molecules, mitochondria-associated factors, transcriptional factors, tumor suppressor genes, and cellular reduced glutathione. Mechanistic studies have demonstrated that the methylselenol metabolite pool has many desirable attributes of chemoprevention, whereas the hydrogen selenide pool with excess of selenoprotein synthesis can lead to DNA single-strand breaks. To elucidate the effects of Se on cytotoxic events, it should be remembered that the chemical forms and the dose of Se, and the experimental system used, are determinants of its biological activities. This mini-review focuses on elucidation of the molecular mechanisms of cancer prevention by Se with the apoptotic approach.

Keywords Selenium compounds · Apoptosis · Chemical form · Mitochondrial pathway · Glutathione

Introduction

Cancer chemoprevention has received increasing attention in recent years. Many agents, including naturally occurring and synthetic compounds, have been demonstrated to exhibit cancer preventive activity (Kelloff et al., 2000). Of particular significance is the realization that many dietary components have cancer chemopreventive activity. Selenium (Se) is an essential dietary nutrient for all mammalian species, which is primarily taken up from the soil by plants as selenate (SeO_4^{2-}) or selenite (SeO_3^{2-}). Se is specifically incorporated into proteins in the form of selenocysteine (Se-Cys; 21st amino acid) and non-specifically incorporated as selenomethionine (Se-Met) in place of methionine, which are used for the synthesis of selenoproteins including glutathione peroxidase, thioredoxin reductase, selenoprotein P and other enzymes (Ganther, 1999). The effects of Se compounds on cancer cells are strictly compositional and concentration-dependent. At supra-nutritional dietary levels, Se can prevent the development of many types of cancer. At higher concentrations, Se compounds can be either cytotoxic or possibly carcinogenic. The cytotoxicity of Se is suggested to be associated with oxidative stress, leading to genomic instability. Accordingly, sodium selenite, an inorganic Se compound, was reported to induce DNA damage, particularly DNA strand breaks and base damage. The use of a cocktail consisting of Se, and other vitamins and minerals appears to be a promising approach to inhibit genetic damage and the development of cancer. When incorporated into selenoproteins, Se protects tissues and membranes from oxidative stress and controls the cell redox status (Rayman, 2000). In addition, epidemiological studies have suggested an inverse association of serum levels of Se with the incidence of squamous esophageal and adenomatous gastric cardia cancers (Mark et al., 2000). There is currently much interest in the potential cancer protective effects of Se. In terms of individual cancer types, evidence from the largest studies is strongest for lung, colon, prostate, and liver cancers, in which p53 mutations are frequent. Several new trials for Se are under way to validate prostate and non-small cell

H. Rikiishi (✉)
Department of Microbiology and Immunology, Tohoku University Graduate School of Dentistry, 4-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: riki@mail.tains.tohoku.ac.jp

lung cancer preventive efficacy (Klein, 2004; Karp, 2005). This review addresses the evidence for Se as a cancer preventive agent and focuses mainly on the possible mechanisms of apoptotic action of Se in cancer prevention.

Discussion

Role of chemical forms of Se and their metabolism

Se exists in mostly organic forms in normal diets. Organic Se is present in foods mainly in the forms of Se-Met, Se-Cys and Se-methylselenocysteine (CH_3SeCys), whereas inorganic Se either as selenite or selenate occurs much less frequently and in very low amounts (Fig. 1). Of the organic forms, Se-Met is the predominant form in most Se-rich diets. Both organic and inorganic forms of Se appear to be utilized with similar efficacy in the body to produce selenoproteins but Se enters at different points in metabolism depending on its chemical form. Se was thought originally to exert its cancer preventive effects via two potential mechanisms. One involves the direct actions of selenometabolites. Selenocompounds such as selenite (SeO_3^{2-}) and selenodiglutathione (the primary metabolite of selenite, GS-Se-SG), ultimately generate selenide (Se^{2-}), which is incorporated into selenoproteins by a specialized mechanism. When selenide is generated in greater amounts, it reacts with oxygen to produce superoxide and, ultimately, H_2O_2, which results in toxicity (Kim et al., 2003). Se compounds that enter either the hydrogen selenide (H_2Se) pool or the methylselenol pool undergo methylation by thiol *S*-methyltransferases and generate different methylated metabolic forms of Se that are sequentially exhaled in the breath or excreted in urine contributing to Se homeostasis of the body. Generally, the methylation pathway is considered to be the detoxification pathway for all Se in the diet or in supplements. Methylselenol (CH_3SeH) can be formed by the methylation of H_2Se as part of the excretory pathway of Se. There is fairly general acceptance that methylselenol is involved in the anti-cancer effects of Se at supra-nutritional doses. Methylselenol is almost assuredly accountable for inducing cell-cycle arrest and apoptosis in cancer cells *in vitro* and *in vivo*. Methylselenol can be formed directly from Se-Met either by the action of a γ-lyase, known as methioninase or by an α, γ-elimination reaction. Alternatively, it can be formed from a storage form of Se, i.e. γ-glutamyl-selenomethyl-Se-Cys, that is present in plants of the *Brassica* and *Allium* families and probably accounts for the anti-cancer effects of Se-enriched broccoli and garlic. Metabolism removes the γ-glutamyl group to give selenomethyl-Se-Cys, which is acted upon by a β-lyase to give methylselenol directly. There is a suggestion that the β-lyase is present at a higher level in cancer cells than in normal cells, ensuring greater exposure of the cancer cells to the anti-cancer agent (Spallholz et al., 2004).

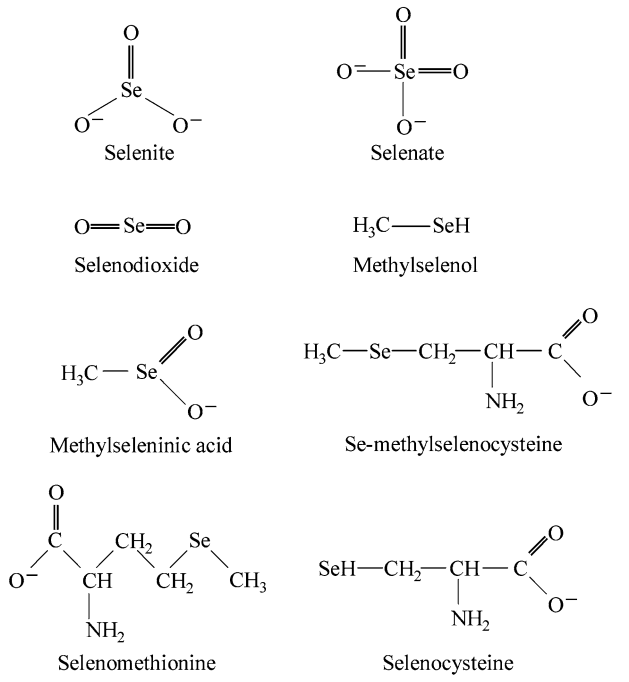

Fig. 1 Structures of Se compounds discussed in the review

The other mechanism involves the actions of selenoproteins. Se is essential to glutathione peroxidase, which protects cells from H_2O_2 and organic peroxides. Furthermore, Se-Cys was identified as the penultimate COOH-terminal residue in thioredoxin reductase, which is believed to protect cells from death. Therefore, selenoproteins serve to safeguard cells against toxicity induced by selenometabolites (Tapiero et al., 2003). However, the anti-oxidant role of Se was thought to be less plausible because the amount of Se for the synthesis of selenoproteins is already at a maximum at a much lower dietary level of Se than that required for the maximum chemopreventive effect of Se (Allan et al., 1999), suggesting that the anti-cancer effects of high levels of Se involve mechanisms unrelated to the activities of selenoenzymes. There is not enough experimental evidence to support a role for known selenoproteins in cancer prevention, and, therefore, efforts have shifted to studies of chemopreventive metabolites with low molecular weight. However, it is also important to determine how selenoproteins are related to the actions of such metabolites, because individuals differ substantially in their ability to increase selenoprotein activity in response to additional dietary Se (Brown et al., 2000).

Cancer chemopreventive activity of Se compounds

Human epidemiological studies examined the relationship between dietary intake of Se and total cancer risk, although these studies were somewhat controversial. Early studies showed a geographic correlation between low Se status and a

high incidence of certain types of cancers. Dietary intake of Se showed a significant inverse correlation with age-adjusted mortality for cancer of the colon, prostate, breast, ovary and lung as well as with hematopoietic cancers, while only a weak correlation was observed for cancers of the pancreas, skin and bladder. Although not all studies showed a correlative effect of high plasma level of Se and low cancer incidence, the epidemiological data stimulated interest in testing Se as a chemopreventive agent in human clinical trials (Meuillet et al., 2004).

Although the mechanisms underlying the cancer chemopreventive activity of Se compounds are poorly understood, selenite could significantly inhibit the proliferation of cancer cells, affect the cell-cycle distribution of cell population, and induce cellular changes characteristic of apoptotic cells. In most studies, Se has been shown to inhibit the initiation phase of carcinogenesis induced by various carcinogens in mammary, liver, colon and lung tissues in rats (El-Bayoumy, 2001). The ability of Se compounds to inhibit cell growth and to induce cancer cell apoptosis has now been widely demonstrated and is suggested to be a potential mechanism for cancer chemoprevention (Ganther, 1999). Furthermore, it is important to note that selenite induces apoptotic DNA laddering in p53-mutant DU145 prostate cancer cells and p53-null HL60 leukemia cells without the cleavage of PARP, whereas Se compounds leading to the formation of methylselenol induce caspase-mediated apoptosis in these cells (Jiang et al., 2004). Since the current literature espouses a notion that apoptosis induced by Se treatment is dependent of the p53 status, other mechanisms must be involved in inducing apoptotic events. The majority of anti-cancerous studies have employed sodium selenite (Na_2SeO_3) as the source of Se, but it is not clear to what extent such findings extend to inorganic or organic Se compounds. Methylseleninic acid (CH_3SeO_2H, MSA), a novel penultimate precursor of the putative critical anti-cancer metabolite, methylselenol in experimental *in vitro* systems, has been shown to block progression of the cell-cycle, induce apoptosis of cancer cells and inhibit the formation of new blood vessels. Processes by which these effects are achieved may involve redox cycling linked to oxidative stress-induced apoptosis, as described by Spallholz et al. (2004). Furthermore, gene profiling was performed in a time course experiment using synchronized cells. A large number of potential Se-responsive genes with diverse biological functions were identified, including changes in the expression of genes that control the cell-cycle checkpoint and regulate signaling pathways and caspase-mediated apoptosis (Dong et al., 2003). We have focused on human oral squamous cell carcinomas, which account for most malignancies that arise in the head and neck region (Takahashi et al., 2005). The main purpose of this work was to search for novel mechanisms responsible for apoptosis induced by inorganic Se compounds. This study showed that treatment with Se for 72 hr, in the form of SeO_2 and Na_2SeO_3, but not Na_2SeO_4, markedly induced apoptosis in a dose-dependent manner (Fig. 2A).

DNA damage by Se exposure: Positive and negative action

It has been previously hypothesized that the cancer-protective effects of Se are related to its ability to limit the accumulation of genotoxic damage within the aging prostate. By studying only non-human species (elderly beagle dogs) that develops spontaneous prostate cancer, an U-shaped dose-response relationship was shown between dietary intakes of the essential trace mineral Se and the extent of DNA damage within the aging prostate gland (Waters et al., 2005). In New Zealand, the single cell gel electrophoresis (comet) assay was reported to show a significant inverse relationship with overall accumulated DNA damage in blood leucocytes from subjects with serum levels of Se below the mean (Karunasinghe et al., 2004). Women born with constitutional heterozygous mutations of the BRCA1 gene carry a lifetime risk of breast cancer of 80% and of ovarian cancer of 40% (Kowalska et al., 2005). The BRCA1 gene product is involved in maintaining the integrity of the human genome and helps repair of double-strand breaks. When blood lymphocytes from BRCA1 carriers are exposed to bleomycin, a known mutagen that induces double-strand breaks, an increased frequency of chromosome breaks per cell occurs. In thirty-two female BRCA1 carriers supplemented with Na_2SeO_3 (276 μg/d) for 1–3 months, the frequency of chromosome breaks was found to be reduced from 0.63 per cell before supplementation with Se to 0.40 per cell after supplementation, bringing it to the level in non-carrier controls. Thus, Se may have the potential to reduce breast cancer risk in these women.

Inversely, it has been known for a long time that some Se compounds have the potential to induce DNA damage. These data clearly indicated that DNA damage is implicated in the genotoxicity of Se and that Se-induced toxicity is mediated by its pro-oxidant activity connected with ROS formation. For example, selenite chosen as the source of Se in a majority of DNA damaging experiments, activates DNA damage signals and induces apoptosis in NIH 3T3 cells, suggesting that DNA damage plays a major role in selenite-induced apoptosis (Zhou et al., 2003). The DNA damaging activity of Se-Met has recently been demonstrated in prostate cells of dogs fed high doses of this dietary amino acid (Waters et al., 2005). Generally, compounds that are directly converted to methylselenol, are supposed to be superior to the inorganic selenite as well as to the organic Se-Met due to their significant preventive efficacy and the minimal side effects on DNA stability and toxicity (Combs and Gray, 1998). These findings indicate that Se in low concentrations may have anti-carcinogenic effects, whereas in high concentrations it

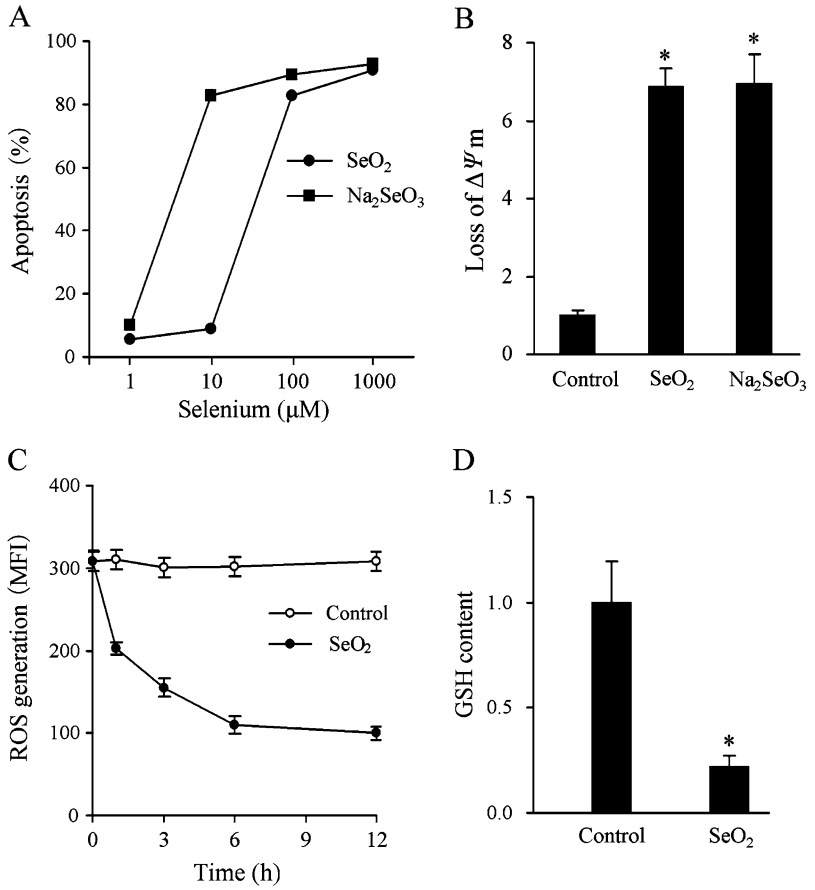

Fig. 2 Apoptotic events induced by inorganic Se compounds. (A) HSC-3 cells were treated with SeO_2 or Na_2SeO_3 for 72 hr at a concentration range of 1–1,000 μM. The degree of apoptosis after treatment was measured by TUNEL staining. (B) Cells treated with SeO_2 (50 μM) or Na_2SeO_3 (10 μM) for 24 hr, were incubated with JC-1 (potential sensor of $\Delta\Psi$m) for 20 min, and then subjected to flow cytometric analysis. (C) Cells were incubated for 12 hr with 50 μM SeO_2. Intracellular ROS accumulation was measured for the indicated periods. Cells were incubated with 20 μM H_2DCF-DA (ROS probe) for 50 min at 37°C and analyzed by flow cytometry. Results are expressed as the means of fluorescence intensity (MFI). (D) GSH was measured in cells treated with SeO_2 (50 μM) for 18 hr using monochlorobimane. Results are expressed as the ratio to the untreated group (B, D). The means ± SD of triplicate wells are given (B, C, D). $^*P < 0.01$ vs. untreated group (B, D)

can be genotoxic and carcinogenic. It follows that too much Se supplementation might be harmful.

Mechanisms of Se-induced apoptosis

To better understand the mechanism of Se-induced apoptosis in oral squamous cell carcinoma cells, we wanted to know whether this type of apoptosis involves the activation of caspases and is dependent on caspase activity (Takahashi et al., 2005). We observed that SeO_2 induces the activation of caspase-3. Moreover, SeO_2-induced apoptosis was interrupted by a broad-spectrum caspase inhibitor, z-VAD-fmk, suggesting that this type of apoptosis is dependent on caspase-3 activity. Although upstream signaling events that lead to the activation of caspase-3 after Se treatment are not exactly known, the co-activation of caspase-9 suggests that a mitochondrial pathway is involved in this process. Selenomethyl-Se-Cys also activates caspase-3 in mouse mammary epithelial tumor cells *in vitro* while MSA is known to activate caspases-1, 3, 6–8, 10, and 12. Apoptosis induced by MSA in DU-145 and PC-3 human prostate cancer cells is principally initiated by caspase-8 and involves cell detachment as a prerequisite (Zu and Ip, 2003). Recent reports that selenite-induced apoptosis was observed in the absence of the activation of caspases in prostate cancer cells (Jiang et al., 2001) are not consistent with the general notion that the activation of caspases is required for apoptosis induced by various stimuli, and for the development of certain aspects of apoptosis, indicating that distinct pathways for cell death are likely involved in apoptosis induced by some types of Se compounds. It is noteworthy that, in a PC-3 human prostate cancer cell line treated with organic Se, the inactivation of caspase-9 may be due to Akt-mediated phosphorylation of the caspase (Cardone et al., 1998). Inactivation of the Akt negative regulator gene PTEN is common in prostate cancer. Interestingly, a decrease of Akt phosphorylation by MSA

(Hu et al., 2005b) might be one of the mechanisms for its sensitization of LNCaP cells to apoptotic signals. However, an increase of Akt phosphorylation by selenite suggests that selenite sensitization of LNCaP cells to TRAIL was independent of Akt activity (Hu et al., 2006). Further study is needed to clarify the relation between the kinase activity and caspase activation in Se-induced apoptosis. Furthermore, caspase-12, an endoplasmic reticulum (ER)-resident caspase essential for ER stress-induced apoptosis, is activated during apoptosis induced by MSA in PC-3 cells. The discovery of an active caspase-12 during Se-induced apoptosis is novel, suggesting that disruption of the microenvironment of the ER as a trigger of apoptosis may be integral to the molecular mechanism of the chemopreventive activity of Se (Jimbo et al., 2003). Selenoprotein (15 kDa) has been implicated in the chemopreventive effect of dietary Se and is expressed at high levels in the normal liver and prostate but at reduced levels in the corresponding malignant organs. It is located in the ER, and tightly complexed to UDP-glucose: glycoprotein glucosyltransferase, an enzyme involved in the quality control of protein folding (Labunskyy et al., 2005). These observations suggest a novel mechanism for the chemopreventive effects of dietary Se.

Recently, it has been shown that a prototype monomethylated Se, MSA, but not the inorganic sodium selenite, specifically enhances apoptosis induced by diverse classes of chemotherapeutic drugs, such as paclitaxel (microtubule inhibitor), SN-38 (topoisomerase I inhibitor), and etoposide (topoisomerase II inhibitor), in DU145 and PC-3 prostate cancer cells (Hu et al., 2005a). MSA, but not selenite, has been also reported to enhance apoptosis induced by TRAIL in DU145 cells (Yamaguchi et al., 2005). In addition, the synergistic effects between MSA and TRAIL resulted from activation of the mitochondrial pathway-mediated amplification loop, which was accompanied by the induction of mitochondrial permeability transition and release of apoptogenic cytochrome *c* and Smac/DIABLO proteins from the mitochondria into the cytosol. These results suggest that Se-based dietary compounds may help to overcome resistance to TRAIL-mediated apoptosis in prostate cancer cells. MSA sensitizes DU145 cells to TRAIL-induced apoptosis through a decrease of c-FLIP and TRAIL-medicated phosphorylation of Bad. *In vitro* evidence suggests that Se and vitamin E work synergistically to cause cell-cycle arrest, induce caspase-mediated apoptosis, and act as antiandrogens in arresting clonal expansion of nascent tumors. The Selenium and Vitamin E Cancer Prevention Trial (SELECT) is an intergroup Phase III, randomized, double-blind, placebo-controlled, population-based clinical trial designed to test the efficacy of Se and vitamin E alone and in combination in the prevention of prostate cancer (Klein et al., 2000). As mentioned above, it is expected that combining various types of Se with chemotherapeutic drugs may widen the spectrum of cancers responsive to combination therapy.

Modulation of Se-induced apoptosis by cellular glutathione

It is well known that reduced glutathione (GSH), the major intracellular anti-oxidant, is closely involved in the metabolism and bioactivity of Se. It has been reported that the cytotoxicity of selenite involves the status of cellular GSH (Tapiero et al., 2003). Selenite undergoes thiol-dependent reduction to selenide before being incorporated into Se-Cys in the synthesis of specific Se proteins or being methylated to a variety of excretion products, thereby consuming intracellular GSH (Shen et al., 2000). GSH was demonstrated to play a dual role in selenite-induced oxidative stress and apoptosis in human hepatoma HepG2 cells. GSH acts as a pro-oxidant, facilitating Se-induced oxidative stress, and GSH acts as an anti-oxidant, protecting against Se-induced oxidative stress and apoptosis. It is assumed that the further-oxidized form of selenate (SeO_4^{2-}) exerts cytotoxicity without reduction to selenite, resulting in no effect on the cellular GSH level. An intermediate in this reduction pathway is GS-Se-SG, which is formed by a reaction with GSH either intra- or extra-cellularly. By the reaction with GSH, SeO_2 as a metabolite of H_2Se was shown to deplete intracellular GSH rapidly (Fig. 2D), and also formed GS-Se-SG through a different pathway from selenite. The fact that the cytotoxicity of selenite was increased by the addition of purified GS-Se-SG (Fleming et al., 2001) suggested that the active inhibitor might be GS-Se-SG. However, it remains a critical subject that GS-Se-SG is unstable and undergoes a stepwise reduction to selenide in the presence of excess GSH. *N*-acetylcysteine (NAC) increases the intracellular GSH content because it is a precursor of GSH and because it facilitates the synthesis of GSH by increasing the supply of cysteine. On the other hand, L-buthionine-[S,R]-sulfoximine (BSO) is a specific inhibitor of γ-glutamylcysteine synthetase and can deplete intracellular GSH in many cells and tissues. NAC or GSH pretreatment significantly inhibited SeO_2- or Na_2SeO_3-induced apoptosis, whereas BSO treatment enhanced it (Takahashi et al., 2005), suggesting that alterations in GSH levels are associated with Se metabolism and bioactivity. MSA was also shown to deplete intracellular GSH rapidly, preceding typical apoptotic changes such as DNA fragmentation in HepG2 cells with a higher level of intracellular GSH. NAC markedly augmented MSA-induced apoptosis, while BSO significantly inhibited MSA-induced apoptosis. These observations suggest that intracellular GSH mainly acts as a co-factor to facilitate MSA-induced apoptosis, while its anti-oxidant function becomes largely irrelevant (Shen et al., 2002). Understanding such a unique association between GSH and Se compounds may help to explain the controversy in the literature over the complex

relationship, and ultimately the capability of Se to prevent cancer.

Mitochondrial dysfunction by Se exposure

Caspase-mediated apoptosis in most cells is induced through the activation of either the mitochondrial (intrinsic) pathway or the death receptor (extrinsic) pathway (Wang, 2001). Mitochondria have been reported to play a key role in the regulation of apoptosis and to also be one of the targets of Se compounds (Shen et al., 2001). Mitochondrial dysfunction including the loss of mitochondrial membrane potential ($\Delta\Psi m$), permeability transition, and release of cytochrome *c* from the mitochondria into the cytosol is associated with physiological or chemotherapy-induced apoptosis, the latter of which activates the caspase mediators of apoptotic cell death. In our study, the rapid loss of $\Delta\Psi m$ coincided with the onset of apoptosis in Na_2SeO_3- or SeO_2-treated cells, but not in Na_2SeO_4-treated cells (Fig. 2B). Thus, a mitochondrial damage-dependent pathway might be involved in inorganic Se-induced apoptosis in cancer cells with mutated p53, but be independent of the generation of reactive oxygen species (ROS) (Takahashi et al., 2005). As both Na_2SeO_3 and SeO_2 are easily diffusible, they may reach the mitochondria and react with mitochondrial GSH. GSH in mitochondria plays an important role in maintaining the integrity of mitochondrial proteins and lipids known to have a vital role in the permeabilization of mitochondrial membranes and release of pro-apoptotic factors (Costantini et al., 2000). It is speculated that Se compounds enhance the oxidative tonus of a responding cell without requiring an increase in the generation of ROS. In fact, depletion of cellular GSH by oxidation may occur during apoptosis, and lower the reducing capacity of the cell, thereby enhancing oxidative stress (Coppola and Ghibelli, 2000). Hence, it is possible that Na_2SeO_3 and SeO_2 mediate transition of mitochondrial permeabilization by modulating mitochondrial GSH, which is accompanied by the dissipation of $\Delta\Psi m$ (Wojtczak et al., 1998). The unique association between mitochondrial GSH and Se may be crucial to explain the chemopreventive capability of Se against cancer. On the other hand, selenite induces a rapid superoxide burst and p53 activation in prostate cancer cells, leading to Bax up-regulation and translocation into mitochondria, which restores the cross-talk with stalled TRAIL signaling for synergistic execution of apoptosis mediated by caspase-9/3 cascade (Hu et al., 2006). It was also shown that superoxide production by selenite was p53 dependent via mitochondrial pathways in wild-type p53-expressing LNCaP cells (Zhao et al., 2006). Selenite, Se-Cys, and SeO_2 induced apoptosis in HepG2 cells and all mediated oxidation of protein thiol groups in both HepG2 cells and isolated mitochondria. Se compounds capable of oxidizing thiol groups also induced transition of mitochondrial permeability in isolated mitochondria (Kim et al., 2003). Both organic and inorganic Se compounds are able to induce apoptosis by triggering transition of mitochondrial permeability, which results in a loss of $\Delta\Psi m$ and the release of cytochrome *c* into the cytosol.

Role of ROS in Se-induced apoptosis

Reduction of O_2 produces superoxide, peroxides, and hydroxyl free radicals, each of which has various essential cellular functions, including the oxidation of fatty acids and alcohols, hydroxylation reactions, and the implementation of phagocytosis. With regard to tumor development, ROS have been considered as DNA-damaging agents that increase the mutation rate and promote oncogenic transformation (Jackson and Loeb, 2001). Excessive cellular levels of these ROS may produce oxidative damage, induce mutations of nucleic acids, and are limited by anti-oxidants, such as GSH, and the selenoenzyme, glutathione peroxidase. In contrast, the cytotoxicity of Se compounds is thought to result from an ability to catalyze the oxidation of thiol groups and generation of the superoxide anion (Drake, 2006). As another mechanism for the generation of ROS, it was assumed that selenite is reduced to selenide (Se^{2-}) in the presence of GSH, and Se^{2-} reacts with oxygen to generate ROS. Nevertheless, a defined mechanistic model of how ROS contribute to the cascade of apoptotic events is still lacking. Consistent with a previous study (Saito et al., 2003), however, our data show a significantly decreased accumulation of ROS in Se-treated cells as compared with untreated cells (Fig. 2C) (Takahashi et al., 2005), indicating that anti-oxidative rather than pro-oxidative properties of Se compounds (selenite or SeO_2) best account for their observed anti-cancer effects. In addition to oral squamous cell carcinoma cells (HSC-3), we also found that HeLa cells retained comparatively high levels of ROS under non-stimulated conditions. An elevated oxidative status has been found in many types of cancer cells, and the introduction of chemical and enzymological anti-oxidants can inhibit cancer cell proliferation, pointing to a critical role of ROS in mediating growth control (Behrend et al., 2003). Although Se compounds such as selenite and selenate have strong inhibitory effects, particularly on mammalian tumor cell growth, the mechanism of action of these Se compounds appears to be distinct. Cells treated with selenite accumulated in the S-phase, and selenite-mediated growth inhibition was irreversible, whereas selenate treatment lead to cell accumulation in G_2 and the effect on cell growth can be reversed (Spyrou et al., 1996). Selenate did not generate ROS in the presence of clinical concentration of cysteine or GSH, suggesting that selenate is not reduced to selenite with clinical concentrations of Cys or GSH under physiological pH. Moreover, Se-Met did not generate ROS under the same condition (Terada et al., 1999). We also confirmed that selenate does not change cellular levels of ROS in HSC-3 cells.

Although inorganic forms of Se compounds enter the cellular metabolic pathway of Se at different points, it is assumed that selenate or Se-Met directly enters this metabolic pool. Therefore, the mechanism of the cytotoxic action of these Se compounds on mammalian tumor cells appears to be distinct from that of selenite.

Mn-containing superoxide dismutase (MnSOD) converts ROS to oxygen and hydrogen peroxide, and the latter is catalyzed into water by catalase and glutathione peroxidase. Previously, some investigators found that high MnSOD expression correlated with poor prognosis, advanced stages of progression and an invasive and metastatic phenotype. These data indicate that abnormally high levels of MnSOD activity, while suppressing cell growth, increase the invasive potential of cancer cells (Mantovani et al., 2002). On the other hand, MnSOD as anti-oxidant enzyme, has been shown to be generally scarce in most types of cancers compared with normal cells. A polymorphism encoding for either valine or alanine at codon 16 in the mitochondrial targeting sequence of the human MnSOD gene was previously described (Shimoda-Matsubayashi et al., 1996). It is suggested that this polymorphism alters the secondary structure of the protein, and hence may affect the efficiency of mitochondrial transport of MnSOD. The Ala-containing MnSOD is transported more efficiently through the mitochondrial membrane, suggesting that, compared with those with the Val/Val or Val/Ala genotype, individuals with the homozygous Ala/Ala genotype may have higher MnSOD activity. Although little overall association was found between MnSOD polymorphism and prostate cancer risk, among men with the Ala/Ala genotype, high Se status was shown to be associated with a significantly lower risk (Li et al., 2005). Both endogenous and exogenous anti-oxidants play an important and interdependent role in preventing clinically significant prostate cancer. As described above, it had been thought that selenoenzymes were not involved in anti-cancer mechanisms because the level of Se supplementation that reduced cancer risk was greater than the amount then believed to be needed to optimise selenoenzyme activity (Combs and Gray, 1998). However, this anti-oxidant function of Se is at least considered to be the probable mechanism of the loss of cellular ROS. Furthermore, it is reasonable that a permanent oxidative shift in the redox status could be a crucial event in the appearance of the malignant phenotype (Suh et al., 1999).

Recent evidence indicates that ROS may function as intracellular messengers to modulate signaling pathways (Benhar et al., 2002). Many protein kinases and transcription regulatory factors are activated under the conditions of oxidative stress. The cellular processes linked to ROS functions are mitogenic signals, one of which is the activation of effector kinases of the mitogen-activated protein kinases (MAPKs) family. MAPK cascades are protein kinase promoting signal transduction pathways that are differentially used to relay numerous extracellular signals within cells. These MAPK cascades have been found to be involved in such diverse cellular functions as proliferation, differentiation, stress responses, and apoptosis. JNK is generally regarded as a mediator of apoptotic cell death in many cell types. In contrast to JNK, ERKs are regarded generally as anti-apoptotic kinases. ERK1/2 activation is strictly dependent on ROS production. An MSA-induced activation of ERK1/2 in LNCaP cells contributed to resistance to apoptosis. In DU145 cells, the apoptotic enhancing effect was primarily through interactions between MSA and JNK-dependent targets to amplify the cascades initiated by caspase-8; however, these kinases did not significantly regulate caspase-mediated apoptosis induced by selenite. These findings support the differential involvement of these protein kinase pathways in regulating apoptosis induction by different forms of Se (Hu et al., 2005b).

Conclusions

Se compounds were reported to suppress the growth of cancers, including those of the prostate, lung, colon, and liver. Several possible mechanisms have been proposed to explain the cancer-protective effects of Se compounds. A valid hypothesis is that Se compounds act as anti-promotion agents, possibly by inducing apoptosis via a mitochondrial pathway in malignant cells. Apoptotic responses were demonstrated in many types of human cancer cells treated with high levels of Se compounds. It is also consistent with the finding that, in general, the relative efficacy of Se derivatives as chemopreventive agents *in vivo* parallels their growth inhibitory effects *in vitro* and their ability to induce apoptosis. However, despite the substantial progress in our knowledge about the protective role of Se compounds for cancer prevention, the molecular mechanisms of Se cytotoxicity in association with apoptosis remain undefined. We have examined the ability of inorganic Se compounds selenite, selenate, and selenodioxide to induce apoptosis and found that the percentage of apoptotic cells reached more than nearly 80% when human oral squamous cell carcinoma cell line was treated with selenite or selenodioxide for 72 hr, indicating that apoptosis is the dominant form of cell death caused by these Se compounds. There is no doubt that Se is a promising chemopreventive agent for human cancers, including squamous cell carcinoma of the head and neck. However, all potential preventive interventions will need to be rigorously evaluated before they can be advocated for cancer prevention, because all these attributes of Se mainly depend upon the concentration, the chemical form and metabolic activity of the compounds.

Acknowledgments I apologize to all colleagues whose work may not have been cited for space reasons. I thank D. Mrozek for editing the

manuscript. Our work described in this article was supported in part by a Grant-in-Aid for Scientific Research (16390577) from the Japan Society for the Promotion of Science.

References

Allan CB, Lacourciere GM, Stadtman TC (1999) Annu Rev Nutr 19:1–16
Behrend L, Henderson G, Zwacka RM (2003) Biochem Soc Trans 31:1441–1444
Benhar M, Engelberg D, Levitzki A (2002) EMBO Rep 3:420–425
Brown KM, Pickard K, Nicol F, Beckett GJ, Duthie GG, Arthur JR (2000) Clin Sci 98:593–599
Cardone MH, Roy N, Stennicke HR, Salvesen GS, Franke TF, Stanbridge E, Frisch S, Reed JC (1998) Science 282:1318–1321
Combs GF, Jr, Gray WP (1998) Pharmacol Ther 79:179–192
Coppola S, Ghibelli L (2000) Biochem Soc Trans 28:56–61
Costantini P, Belzacq AS, Vieira HL, Larochette N, de Pablo MA, Zamzami N, Susin SA, Brenner C, Kroemer G (2000) Oncogene 19:307–314
Dong Y, Zhang H, Hawthorn L, Ganther HE, Ip C (2003) Can Res 63:52–59
Drake EN (2006) Med Hypotheses 67:318–322
El-Bayoumy K (2001) Mutat Res 475:123–139
Fleming J, Ghose A, Harrison PR (2001) Nutr Can 40:42–49
Ganther HE (1999) Carcinogenesis 20:1657–1666
Hu H, Jiang C, Ip C, Rustum YM, Lu J (2005a) Clin Can Res 11:2379–2388
Hu H, Jiang C, Li G, Lu J (2005b) Carcinogenesis 26:1374–1381
Hu H, Jiang C, Schuster T, Li GX, Daniel PT, Lu J (2006) Mol Can Ther 5:1873–1882
Jackson AL, Loeb LA (2001) Mutat Res 477:7–21
Jiang C, Wang Z, Ganther H, Lu J (2001) Can Res 61:3062–3070
Jiang C, Hu H, Malewicz B, Wang Z, Lu J (2004) Mol Can Ther 3:877–884
Jimbo A, Fujita E, Kouroku Y, Ohnishi J, Inohara N, Kuida K, Sakamaki K, Yonehara S, Momoi T (2003) Exp Cell Res 283:156–166
Karunasinghe N, Ryan J, Tuckey J, Masters J, Jamieson M, Clarke LC, Marshall JR, Ferguson LR (2004) Can Epidemiol Biomarkers Prev 13:391–397
Karp DD (2005) Clin Adv Hematol Oncol 3:313–315
Kelloff GJ, Crowell JA, Steele VE, Lubet RA, Malone WA, Boone CW, Kopelovich L, Hawk ET, Lieberman R, Lawrence JA, Ali I, Viner JL, Sigman CC (2000) J Nutr 130:467S–471S
Kim TS, Yun BY, Kim IY (2003) Biochem Pharmacol 66:2301–2311
Klein EA, Thompson IM, Lippman SM, Goodman PJ, Albanes D, Taylor PR, Coltman C (2000) Prostate Can Prostatic Dis 3:145–151
Klein EA (2004) Ann NY Acad Sci 1031:234–241
Kowalska E, Narod SA, Huzarski T, Zajaczek S, Huzarska J, Gorski B, Lubinski J (2005) Can Epidemiol Biomarkers Prev 14:1302–1306
Labunskyy VM, Ferguson AD, Fomenko DE, Chelliah Y, Hatfield DL, Gladyshev VN (2005) J Biol Chem 280:37839–37845
Li H, Kantoff PW, Giovannucci E, Leitzmann MF, Gaziano JM, Stampfer MJ, Ma J (2005) Can Res 65:2498–2504
Mantovani G, Maccio A, Madeddu C, Mura L, Gramignano G, Lusso MR, Mulas C, Mudu MC, Murgia V, Camboni P, Massa E, Ferreli L, Contu P, Rinaldi A, Sanjust E, Atzei D, Elsener B (2002) Int J Can 98:84–91
Mark SD, Qiao YL, Dawsey SM, Wu YP, Katki H, Gunter EW, Fraumeni JF, Jr, Blot WJ, Dong ZW, Taylor PR (2000) J Natl Can Inst 92:1753–1763
Meuillet E, Stratton S, Prasad CD, Goulet AC, Kagey J, Porterfield B, Nelson MA (2004) J Cell Biochem 91:443–458
Rayman MP (2000) Lancet 356:233–241
Saito Y, Yoshida Y, Akazawa T, Takahashi K, Niki E (2003) J Biol Chem 278:39428–39434
Shen H, Yang C, Liu J, Ong C (2000) Free Radic Biol Med 28:1115–1124
Shen HM, Yang CF, Ding WX, Liu J, Ong CN (2001) Free Radic Biol Med 30:9–21
Shen HM, Ding WX, Ong CN (2002) Free Radic Biol Med 33:552–561
Shimoda-Matsubayashi S, Matsumine H, Kobayashi T, Nakagawa-Hattori Y, Shimizu Y, Mizuno Y (1996) Biochem Biophy Res Commun 226:561–565
Spallholz JE, Palace VP, Reid TW (2004) Biochem Pharmacol 67:547–554
Spyrou G, Bjornstedt M, Skog S, Holmgren A (1996) Can Res 56:4407–4412
Suh YA, Arnold RS, Lassegue B, Shi J, Xu X, Sorescu D, Chung AB, Griendling KK, Lambeth JD (1999) Nature 401:79–82
Takahashi M, Sato T, Shinohara F, Echigo S, Rikiishi H (2005) Int J Oncol 27:489–495
Tapiero H, Townsend DM, Tew KD (2003) Biomed Pharmacother 57:134–144
Terada A, Yoshida M, Seko Y, Kobayashi T, Yoshida K, Nakada M, Nakada K, Echizen H, Ogata H, Rikihisa T (1999) Nutrition 15:651–655
Wang X (2001) Genes Dev 15:2922–2933
Waters DJ, Shen S, Glickman LT, Cooley DM, Bostwick DG, Qian J, Combs GF, Jr, Morris JS (2005) Carcinogenesis 26:1256–1262
Wojtczak L, Wieckowski MR, Schonfeld P (1998) Arch Biochem Biophys 357:76–84
Yamaguchi K, Uzzo RG, Pimkina J, Makhov P, Golovine K, Crispen P, Kolenko VM (2005) Oncogene 24:5868–5877
Zhao R, Xiang N, Domann FE, Zhong W (2006) Can Res 66:2296–2304
Zhou N, Xiao H, Li TK, Nur EKA, Liu LF (2003) J Biol Chem 278:29532–29537
Zu K, Ip C (2003) Can Res 63:6988–6995