Apoptosis Is a Critical Cellular Event in Cancer Chemoprevention and Chemotherapy by Selenium Compounds

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Abstract: Epidemiological studies, preclinical investigations and clinical intervention trials support the role of selenium compounds as potent cancer chemopreventive agents; the dose and the form of selenium are critical factors in cancer prevention. Induction of apoptosis and inhibition of cell proliferation are considered important cellular events that can account for the cancer preventive effects of selenium. Toxicity should always be considered a determining factor in the selection of potential chemopreventive agents. Prior to induction of apoptosis, selenium compounds alter the expression and / or activities of a number of cell cycle regulatory proteins, signaling molecules, proteases, mitochondrial associated factors, transcriptional factors, tumor suppressor genes, polyamine and glutathione levels. Depending on the form, selenium compounds can target separate pathways but more efforts are needed to learn about disrupting different pathways converging to apoptosis. Numerous selenium compounds are known to inhibit carcinogenesis in several animal models but not all of these have been examined for their efficacy to induce apoptosis or *vice versa* in the corresponding target organ. Studies aimed at investigating the effects of selenium compounds on apoptosis in the target organ *in vivo* and *in vitro* are limited. On the basis of information provided in this review, we recommend that additional molecular markers should be added to those proposed in the Selenium and Vitamin E Cancer Prevention Trial (SELECT) on prostate cancer. Apart from the selenium compounds reviewed here, several novel synthetic organoselenium compounds need to be examined both *in vitro* and *in vivo* for their potential to induce apoptosis; such an investigation may provide better and mechanism-based cancer chemoprevention as well as chemotherapeutic agents.

INTRODUCTION

Cancer is a leading cause of death throughout the world. More than ten million new cancer cases occur annually and the disease causes over six million deaths a year. Evidence indicates that up to 80% of human cancer is potentially preventable since the factors that determine the incidence are largely environmental. These exogenous etiologic factors include, dietary factors, cigarette smoking, radiation, occupational and environmental chemicals, socioeconomic factors, specific parasites, bacteria and viruses and exogenous hormones in women [1-5]. In addition, endogenous factors, such as hereditary, immunological and hormonal factors and gene/environment interactions play critical roles in individual susceptibility [6, 7]. In some rare forms of cancer hereditary factors play a decisive role. However, the exogenous factors are clearly most amenable to interventions targeted at primary prevention of cancer.

In the United States, about two-thirds of cancer deaths are linked to tobacco use and diet. Effective control of tobacco consumption and reduction of exposure to known environmental carcinogens are important goals of primary prevention programs. Population-based programs have been developed to encourage cessation of smoking as means of primary prevention of lung cancer among men and women [8, 9]. Chemoprevention, though not a substitute for primary prevention programs, offers an attractive and mechanism-based approach to curb the cancer development. One class of promising chemopreventive agents is the selenium compounds, which have been shown to be effective in the secondary prevention of cancer of the prostate, colon and lung [10-12].

THE ROLE OF SELENIUM COMPOUNDS IN CANCER PREVENTION

Even though selenium was thought to be a carcinogen in 1943 [13], evidence of its benefits has been highlighted, including its essential role in the formation of glutathione peroxidase (GPx), an enzyme that protects against oxidative injury [14]. This view expanded with the subsequent discoveries of several selenium-dependent GPx isoforms [15-17] and of other selenoenzymes and specific selenoproteins, such as iodothyronine 5'-deiodinases [18-21], thioredoxin reductase [22], plasma selenoprotein P [23] and muscle selenoprotein W [24]. To date, about 24 selenoproteins have been characterized [25]. Each of these selenoproteins contains selenium in the form of selenocysteine (SeCys), and is considered the 21st amino acid that is incorporated by the co-translational modification of transfer RNA-bound serine at loci coded by specific uracil-guanine-adenine (UGA) codons [26, 27].

Selenium was first mentioned as possibly protective against cancer risk more than 30 years ago [28]. Epidemiological studies have shown that populations with a low selenium intake and low plasma selenium levels have an

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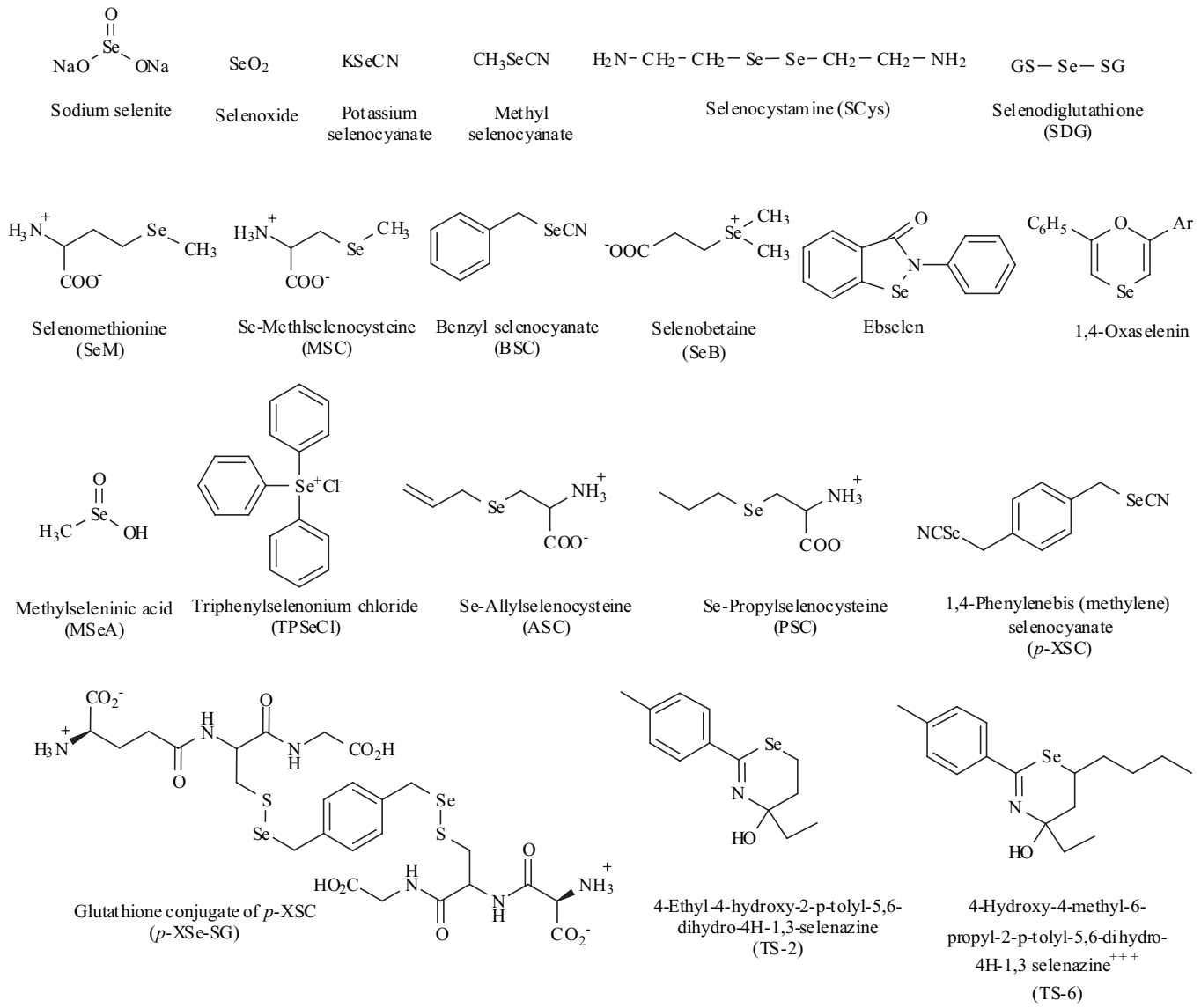

Fig. (1). Structures of inorganic and organic selenium compounds listed in Tables 1 and 2.

increased incidence of cancer, including cancer of the breast, lung, stomach, bladder, ovaries, pancreas, thyroid, esophagus, head and neck, cerebellum and melanoma [29-37]; yet, some studies found no association between selenium and cancer [38-41].

Preclinical Evidence

Natural and synthetic organoselenium compounds (Fig. **1**) have been examined as chemopreventive agents in several animal tumor models and were compared with inorganic selenium compounds [42-44]. Selenium in the form of sodium selenite or selenomethionine (SeM) functions as an essential micronutrient at levels of about 0.1 ppm in the animal diet but it becomes a chemopreventive agent at 3 to 5 ppm and a toxin at levels above 5 ppm [45].

Most of the selenium chemoprevention studies have used either sodium selenite or SeM as the test reagent. The effect is not organ specific because tumor inhibition has been reported in cancers of several organs including bladder, colon, liver, lung, pancreas, tongue and mammary gland [46-49]. In general, there is a dose-dependent response. Many of the earlier studies [50-54] with selenium compounds have been performed with 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary tumors (Table 1) and it was shown that the chemopreventive potential of SeM was comparable to that of selenite [55]. The chemopreventive index (CI) is defined as the numeral obtained by dividing maximum tolerable dose (MTD) by the effective dose that produces 50% inhibition of total tumor yield (ED_{50}). From the data in Table 1 it can be inferred that Se-methylselenocysteine (MSC), selenobetaine (SeB), benzylselenocyanate (BSC) and methylseleninic acid (MSeA) are equally effective against mammary tumorigenesis. Although selenobetaine is just as effective, MSC is considered to be the most promising naturally occurring selenocompound because of its predominance in selenium-enriched plants, such as broccoli [56], garlic [57] and ramps [58]. Based on the CI, the synthetic 1,4-phenylenebis- (methylene) selenocyanate (*p*-XSC) appears to be superior to all other forms of selenium against mammary

Table 1. Chemopreventive Efficacy of Selenium Compounds in the 7,12-Dimethylbenz[a]anthracene (DMBA)-Induced Rat Mammary Tumor Model Assay[a]

	Selenite	KSeCN	SeM	MSC	SeB	MSeA	BSC	*p*-XSC	TPSeCl	*p*-XSe-SG	ASC
ED_{50}[b]	3	3	4-5	2	2	2	2	5	10-20	>5-6	≤1
MTD[c]	4	4	5-6	5	5	5	5	20	>200	20	ND
CI[d]	1.3	1.3	1-1.5	2.5	2.5	2.5	2.5	4.0	>10	3.3-4.0	ND
Apoptosis	ND	ND	ND	TUNEL[e]	ND	ND	ND	TUNEL[f] Casp-3	ND	TUNEL[f] Casp-3	ND

Each compound was administered before DMBA was given and continued throughout the experiment; all selenium compounds were added to the diet. SeM and MSC are naturally occurring compounds all others are synthetic.

[a]Data were obtained from Ip *et al.* [50, 51] and Medina *et al* [52].

[b]ED_{50} is defined as the effective dose that produces 50% inhibition of the total tumor yield.

[c]MTD is the maximum tolerable dose; ND, not determined in this model.

[d]CI: Chemopreventive index equals MTD/ED_{50}

[e] MNU-induced tumoringenesis [53].

[f] Data obtained from El-Bayoumy *et al* [54].

ASC: Se-Allylselenocysteine, BSC: Benzyl selenocyanate, Casp-3: Caspase-3, KSeCN: Potassium selenocyanate, MSeA: Methylseleninic acid, MSC: Se-Methylselenocysteine, *p*-XSC: 1,4-Phenylenebis (methylene) selenocyanate, *p*-XSeSG: Glutathione conjugate of *p*-XSC, SeB: Selenobetaine, SeM: Selenomethionine, TPSeCl: Triphenylselenonium chloride, TUNEL: Terminal deoxynucleotidyl transferase [TdT]-mediated dUTP nick end labeling.

gland tumorigenesis. However, triphenylselenonium chloride (TPSeCl), another synthetic form, has the highest CI among the selenium compounds tested in the rat mammary gland tumor model. Studies aimed at understanding the mechanisms of action of TPSeCl are scarce. Table 1 also describes the effect of limited number of selenium compounds on apoptosis *in vivo*. It is important to determine the effect of selenium compounds with maximum CI on apoptosis *in vitro* and *in vivo*.

Alteration of Cellular and Molecular Targets of Cancer Prevention by Selenium Compounds

Several studies aimed at understanding the mechanisms by which selenium compounds inhibit tumor formation during the initiation and possibly during post-initiation phase of carcinogenesis, have been reported [42, 44]. Induction of apoptosis and inhibition of cell proliferation are considered important cellular events that can account for the cancer preventive effects of selenium. Selenium compounds were found to induce cell death by apoptosis and /or necrosis depending on the form of selenium and the dose of the compound used in the *in vitro* model assay systems. A comparison of known and novel selenium compounds developed so far and those that induce apoptosis in various model systems *in vitro* is lacking in the literature. The present review focuses on the cellular and molecular targets involved in the induction of apoptosis (Fig. (**2**)) by several inorganic, organic and synthetic organoselenium compounds.

Molecular targets and events modulated by selenium include selenoenzymes [59-61], selenoproteins [62, 63], protein kinase A and C [64, 65], protein phosphorylation [66, 67], phase I and phase II activities [68-70], nuclear factor κB (NF-κB) activity [71], Sp1 and Sp3 [72], *gadd* gene activation [67, 73], activator protein-1 (AP-1) binding to DNA [74], DNA cytosine methyltransferase [75], matrix metalloproteinase-2 [76], tumor suppressor gene p53 [77-79] and immune system [80, 81].

APOPTOSIS IN CELL CULTURE

Apoptosis is essential for development, maintenance of tissue homeostasis and elimination of unwanted or damaged cells from multicellular organisms [82, 83]. Apoptosis is defined by distinct morphological and biochemical changes mediated by a family of cysteine proteases called caspases, which are expressed as inactive zymogens and are proteolytically processed to an active state following an apoptotic stimulus. Two separate pathways leading to caspase activation have been characterized [84, 85]. The extrinsic pathway is initiated by ligation of transmembrane death receptors (CD95, TNF receptor and TRAIL receptor) to activate membrane-proximal (activator) caspases (caspase-8 and caspase-10), which, in turn, cleave and activate (effector) caspases like caspase-3 and caspase-7. This pathway can be regulated by c-FLIP, that inhibits upstream activator caspases, and by an inhibitor of apoptosis (IAPs) that affects both activator and effector caspases. The intrinsic pathway requires disruption of the mitochondrial membrane and the release of mitochondrial proteins including Smac/DIABLO, HtRA2 and cytochrome c. Cytochrome c functions with Apaf-1 to induce activation of caspase-9, thereby initiating the apoptotic caspase cascade, while Smac/DIABLO and HtRA2 bind to and antagonize IAPs [85, 86].

Mitochondrial membrane permeabilization is regulated by the opposing actions of proapoptotic and antiapoptotic Bcl-2 family members. Multidomain proapoptotic Bcl-2 proteins (Bak and Bax) can be activated directly following interaction with the BH3-only proteins (Noxa, Puma, Bad and Bim) to antiapoptotic Bcl-2 proteins (Bcl-2 and Bcl-X_L) and results in activation of Bak and Bax [87, 88]. The net effect is the regulated release of proapoptotic factors from mitochondria, induction of downstream caspases, and potential loss of mitochondrial function.

Disruption of the intrinsic apoptotic pathway is extremely common in cancer cells [reviewed in 89]. The p53 tumor suppressor gene is the most frequently mutated in

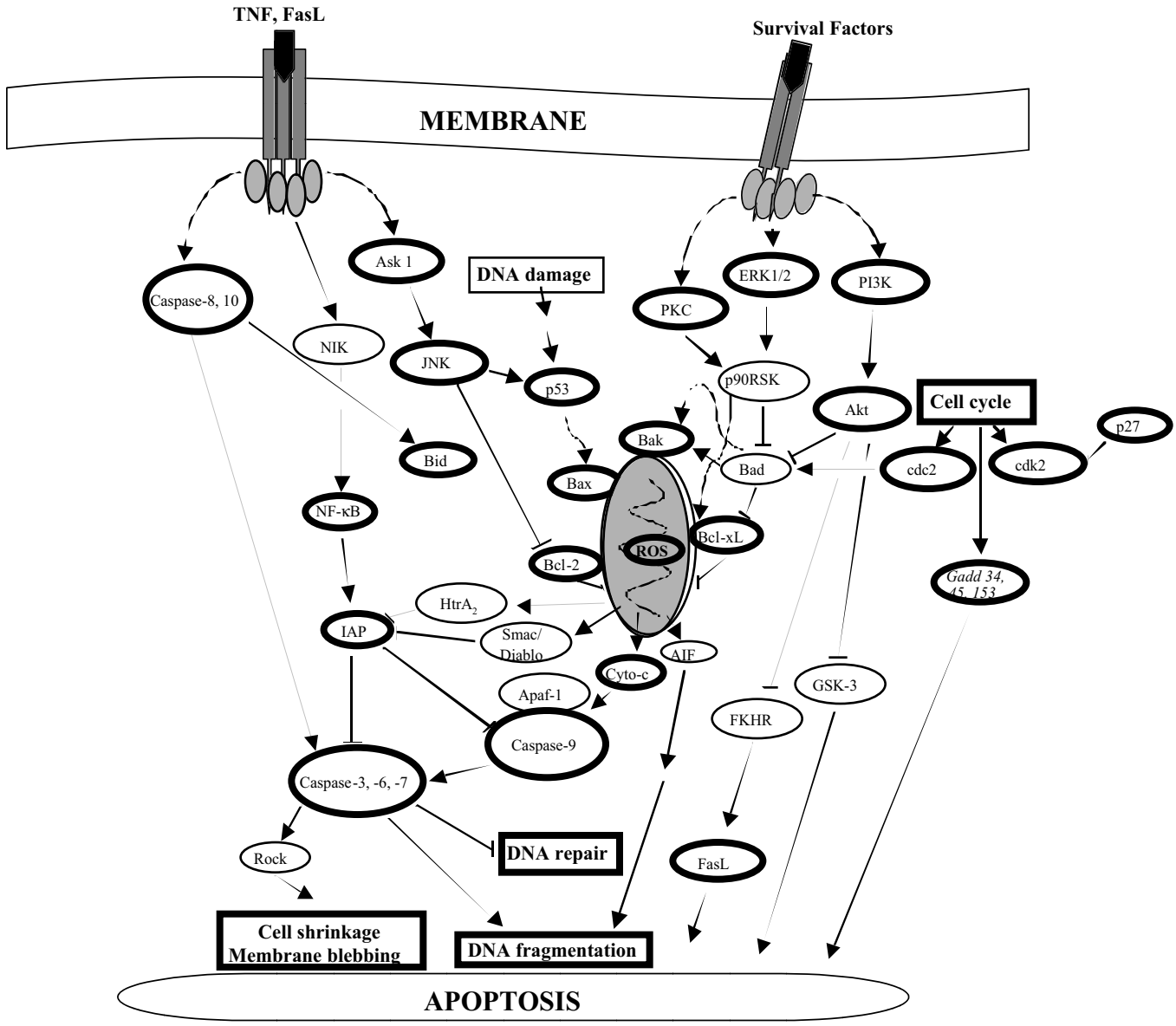

Fig. (2). Cellular and molecular alterations in selenium-induced apoptosis. Several targets and events altered by selenium are enclosed in bolded-ovals.

human tumors and loss of p53 function can both inhibit apoptosis and accelerate tumor development in transgenic mice [90, 91]. The presence of wild-type p53 does not necessarily indicate that the pathway is intact whereby p53-dependent as well as p53-independent apoptosis is observed in a variety of cell types. In addition, mutations or altered expression of upstream regulators of Bcl-2 proteins are associated with cancer. For example, the Bad-kinase Akt is positively regulated by the PTEN tumor suppressor [92]. Amplified Akt and mutated PTEN have been found with high frequency in a variety of solid tumors, indicating the importance of this pathway in regulating tumorigenesis [89]. Some DNA-damaging agents are effective antitumorigenic drugs because they damage malignant tissues more than normal tissues [93]. A recent report [94] suggests that chemotherapy induces deamidation of the antiapoptotic Bcl-X_L in tumor cells but not in normal cells.

EFFECT OF SELENIUM COMPOUNDS ON NORMAL VERSUS TUMOR CELLS

The chemopreventive efficacy of selenium compounds in preclinical models depends on the chemical form in which it is administered thus, indicating that the metabolism of the compounds is a prerequisite for its chemopreventive effect [59]. It has also been shown in several *in vitro* studies, that not only the form of selenium but also the dose is a critical determinant related to its cellular and molecular responses [44]. Several studies performed *in vitro* suggest that selenium may exert its chemopreventive action via inhibition of cell growth and / or induction of apoptosis in the transformed cells [95-97]. However, studies aimed at assessing the sensitivity of normal cells versus transformed cells to various forms and doses of selenium are limited and future experiments in this area of research are recommended.

Cell culture experiments with mammary and prostate epithelial cells have shown that low concentrations of selenium in the range of 10^{-8}M can stimulate growth of normal, preneoplastic and neoplastic cells. In contrast, high concentrations of 10^{-6} M to 10^{-4}M selenium were growth inhibitory for normal and in some cases for tumor cells [98-101]. At concentrations ranging from 6 x 10^{-9} M to1.2 x 10^{-5}M selenite was not toxic and in many instances increased the colony forming efficiency in primary rat tracheal epithelial cells [102].

Normal and malignant prostate cell lines were examined for evidence of apoptosis after exposure to 1-500 μM of selenite or SeM [103]. Selenocompounds induced less apoptosis in normal prostate cells compared with prostate tumor cells and this difference was mainly attributed to intact poly (ADP-ribose) polymerase (PARP). The untreated primary prostate cells did not express PARP while tumor cell lines expressed distinct baseline levels.

Normal oral mucosa cells (NOMC) were less sensitive to selenodiglutathione (SDG) than squamous cell carcinoma (SCC) cultures, whereas following treatment of both normal and cancer cells with *p*-XSC any differences were small [104]. Furthermore, the extent of Fas-L induction by SDG and *p*-XSC correlated closely with the level of apoptosis induced in NOMCs or carcinomas. Since both NOMCs and SCCs express the Fas receptor constitutively, induction of Fas-L may explain why selenium compounds induce apoptosis in oral cells. In addition, induction of Jun NH_2-terminal kinase (JNK) by SDG in SCCs is more marked than in NOMCs, which may explain the increased sensitivity of SCC to induction of Fas-L/apoptosis by SDG. SDG induced more Fas-L in SCC than normal.

In normal human keratinocytes (NHK), selenite at 127 μM induces DNA oxidative damage measured as 8-hydroxy-2'-deoxyguanosine (8-OHdG) lesions probably because of its capability of redox cycling and superoxide production. Whereas, SeM is not cytotoxic to NHK since it is a nonreducing selenium compound and does not produce superoxide [105].

Selenium compounds may be inducing apoptosis by altering the redox status of cells by manipulating levels cellular reducing agent thioredoxin, implicated in growth control and over expressed in many tumors [reviewed in 59, 61]. Furthermore, the differential effects of selenium compounds on apoptosis and chemosensitivity in normal versus transformed cells may in part be related to the differential effects of these compounds on the expression of BCl-X_L, BCl-2 and Bax expression in these cells.

The differences between normal and the neighboring tumor cells are often small and a part of a continuum where a gene product may be expressed more or less in tumor compared with the surrounding normal tissue but rarely turned off completely. The chemopreventive properties of selenium compounds may relate to their differential effects on apoptotic pathways in normal versus transformed cells. An in-depth side-by-side comparison in several cell culture model systems is required to understand the selective sensitivity of tumor cells towards selenium compounds as compared to the corresponding normal cells.

Although, various selenium compounds with diverse chemical structures are known to inhibit cell proliferation *in vitro*, little is known of selenium effects on cell proliferation in normal growing cells or in neoplastic cells of the same organ following carcinogen treatment. Ip *et al* [106] described that the effect of selenium on cell proliferation and cell cycle biomarkers varies depending upon the form and whether cells are normal or transformed. Findings of this study further suggested that early transformed cells are sensitive to selenium intervention, whereas normal cells are not. These types of investigations are important in the development of selective biomarkers that will help to translate laboratory findings into human application.

INHIBITION OF TUMORIGENESIS AND INDUCTION OF APOPTOSIS BY SELENIUM COMPOUNDS

Selenium compounds inhibit the development of chemically induced tumorigenesis in several animal models [42, 44] and induce apoptosis in a variety of cell culture systems *in vitro* (Table 2). Induction of apoptosis by selenium compounds may occur as a result of interference with the activation of mitogenic or survival pathways by growth factors (Fig. **2**). In addition several intermediate cellular and molecular targets and events are altered following selenium treatment (Table 2). The target proteins and related cellular events involved in cell death are discussed below for each of the selenium compounds listed in Table 2.

Sodium Selenite

Sodium selenite, one of the earliest and most extensively studied selenium compound, has been shown to inhibit carcinogenesis in several animal models. On the basis of cytotoxicity, it was not recommended to be included as a representative selenium compound in chemoprevention research [107]. In this review, however, we include it for comparison with various forms of selenium since it has been shown to induce both apoptosis and necrosis.

Several reports have demonstrated morphological signs of apoptosis [73, 108-110] and accumulation of cells in sub-G1 fraction of cell cycle [103, 109] following selenite treatment. The ultrastructure of mitochondria in selenite-treated glioma cells was studied in depth to demonstrate induction of apoptosis [111]. Selenite induces mitochondrial permeability transition and provokes the release of cytochrome c [112]. Selenite induced DNA fragments have been determined in prostate cells [103]; keratinocytes [113] and hepatoma cells [114] by terminal deoxynucleotidyl transferase [TdT]-mediated dUTP nick end labeling (TUNEL) assay. Furthermore, selenite like several other selenium compounds (Table 2) demonstrates formation of DNA fragments of multiple of 50-300 bp that appear as a ladder following DNA gel electrophoresis [73, 103, 108, 115-118]. Selenite-induced DNA strand breaks are oxygen-dependent and may act as a signal for a cell to undergo apoptosis [119]. In addition, the cell death induced by selenite may involve activation [120] or suppression [121] of p38MAP kinase and the JNK depending upon the cell type.

Table 2. Selenium-induced Apoptosis in Mouse, Rat and Human Cell Types: Examination of Apoptotic Assays and Molecular Targets

Agent[1]	System used[2]	Assays for apoptosis and molecular targets examined[3]	References
Inorganic selenium compounds			
Sodium selenite	Mouse keratinocytes (MK2)	TUNEL, 8-OHdG	[113]
	Mouse mammary cancer cells (MOD)	Morphology, DNA fragmentation, *gadd153*	[73, 108]
	Human colon carcinoma cells (SW480)	Morphology, $\Delta\Psi_m$, ROS	[110]
	Human colon carcinoma cells (HT29)	DNA fragmentation, ROS, GSH	[117]
	Human glioma cells (A172, T98G)	Comet, DNA fragmentation	[116]
	Human glioma cells (T98G, U87MG, U373MG)	Ultrastructure	[111]
	Human hepatoma cells ($HepG_2$)	Morphology, Comet, TUNEL, ROS, GSH	[114]
	Human normal keratinocytes	Death-ELISA	[105]
	Human ovarian cancer (SKVO3)	PARP-cleavage; cyt c release	[157]
	Human promyelocytic leukemia cells (HL-60)	Death-ELISA, DNA fragmentation	[115]
	Human prostate cancer cells (LNCaP)	Morphology, sub-G1 peak, p21	[109]
	Human prostate cancer cells (LNCaP, PC-3, DU145)	Sub-G1 peak, DNA fragmentation, TUNEL, caspase-3 co-localized with TUNEL, PARP-cleavage, cdc2, XIAP	[103]
	Human prostate cancer cells (DU145)	DNA fragmentation, p21, p27, Akt, JNK, p38MAPK	[118]
	Human prostate cancer cells (DU145)	DAPI staining, DR5	[122]
Selenoxide	Human hepatoma cells (HL-7702, SMMC-7721)	p53, bcl-2	[78]
	Human osteosarcoma cells (MG-63, U2-OS, Saos-2)	Sub-G1 peak, TUNEL, ultrastructure bcl-2 family, Fas-ligand	[134]
	Human osteosarcoma (KOS)*	Morphology	[134]
Inorganic selenium metabolites			
SDG[a]	Mouse erythroleukemia cells (MEL)	Sub-G1 peak, DNA fragmentation, p53	[77]
	Mouse mammary epithelial cells (C57/MG)	Morphology, DNA fragmentation	[138]
	Human oral squamous carcinoma cells (SCC)	Annexin-V staining, TUNEL, Fas-ligand, JNK, p38 kinase, ERKs 1& 2, Akt	[104]
	Human ovarian cancer cells (A2780)	Sub-G1 peak, DNA fragmentation, p53	[77]
	Human promyelocytic leukemia cells (HL-60)	Death-ELISA, DNA fragmentation	[115]
Organic selenium compounds (natural and synthetic)			
ASC[b]	Mouse mammary tumor cells (TM2H, TM12)	Morphology, Comet, Rb, p53, p21 p27, cyclin D1, cdk4	[140, 141]
Ebselen[c]	Human hepatoma cells ($HepG_2$)	Sub-G1 peak, DNA fragmentation, TUNEL, GSH	[145]
Kappa-S[d]	Human hepatoma cells (SMMC-7721, H22*)	Morphology, Sub-G1 peak, DNA fragmentation, NO	[146]
MSC[e]	Mouse mammary hyperplasia (TM6)*	TUNEL, caspase-3 activity	[Footnotes[1, 3]]
	Mouse mammary tumor cells (TM6)	Sub-G1 peak, DNA fragmentation, PARP-cleavage, caspase-3, 6, 8, PKC, cyclin E, cyclin A, cdk2, cdk4, *gadd34, 45 & 153*	[66, 153, 154]
	Rat mammary gland*	TUNEL, cyclin D1, cyclin A, p27, p16, Bcl-2, bax, bak	[53]
	Human breast cells (MCF10A)	PARP-cleavage	[Footnote[4]]
	Human ovarian cancer cells (SKOV-3)	Sub-G1 peak, caspase-3 activity, PARP-cleavage, cyt c release, HIAP1, XIAP, survivin, bax, calpain, PLC-γ1 cleavage	[157]

(Table 2). contd.....

Agent[1]	System used[2]	Assays for apoptosis and molecular targets examined[3]	References
	Human promyelocytic leukemia cells (HL-60)	DNA fragmentation, TUNEL, PARP- cleavage, caspase-3 activity, ROS	[155, 156]
	Human umbilical vein endothelial cells (HUVEC)	Morphology, VEGF, IMVD, MMP-2	[76]
MSeA[f]	Mouse mammary tumor cells (TM2H, TM12)	Morphology, Comet	[147]
	Human breast cells (MCF10AT1, MCF10AT3B)	Annexin-V staining, TUNEL, genes by cDNA array	[159]
	Human breast cells (MCF10A, MCF10DCIS.com)	Morphology, PARP-cleavage	[Footnote[4]]
	Human hepatoma cells ($HepG_2$)	DNA fragmentation, TUNEL, GSH	[161]
	Human prostate cancer cells (DU145)	DNA fragmentation, VEGF, ERKs 1 & 2, PARP-cleavage, caspase-3, 7, 8, 9, cyt c release, p21, p27, JNK, p38MAPK	[118, 120, 160]
	Human prostate cancer cells (PC3)	Annexin-V staining, *gadd153*, p21, DHFR, cyclin A	[163]
	Human umbilical vein endothelial cells (HUVEC)	DNA fragmentation, PARP-cleavage, caspase activation, ERKs 1& 2, PI3-K, JNK, p38MAPK, Akt	[158]
CH_3SeH[g]	Human prostate cancer cells (DU145)	DNA fragmentation, PARP- cleavage, ERKs 1 & 2, Akt	[164]
Oxaselenins	Human ovarian cancer cells (SKOV-3)	Annexin-V staining	[166]
PSC[h]	Mouse mammary tumor cells (TM2H, TM12)	Comet	[140]
p-XSC[i]	Mouse mammary cancer cells (MOD)	Morphology, DNA fragmentation	[108]
	Rat mammary cancer cells (RBA)	Morphology, DNA fragmentation, mitochondrial activity	[178]
	Human colon cancer cells (Col2)	Sub G1-peak, death-ELISA, DNA fragmentation	[176]
	Human oral squamous carcinoma cells (SCC)	Annexin-V staining, TUNEL, Fas-ligand, JNK, p38MAPK, ERKs 1& 2, Akt	[104]
Selenazine derivatives[j]	Human fibrosarcoma cells (HT-1080)	DNA fragmentation	[179]
(TS-2, TS-6)	Human gastric cancer cells (TMK-1)	Morphology, DNA fragmentation, caspase-3 activity	[180]
Se-yeast	Human breast cancer cells (SKBR-3)	Morphology, DNA fragmentation	[188]
SCys[k]	Mouse keratinocyte (MK2)	TUNEL, 8-OHdG	[113]
SeM[l]	Human breast cells (MCF-7)	Morphology, TUNEL	[196]
	Human colon cancer cells (HT29)	Morphology, polyamine level	[197]
	Human colon cancer (HCT116)	Morphology, cyclin B1, cdc2	[198]
	Human lung cancer cells (A549)	Morphology, polyamine level	[197]
	Human melanoma cells (UACC-375)	Morphology, TUNEL	[196]
	Human prostate cancer cells (LNCaP, PC-3, DU145)	Sub-G1 peak, DNA fragmentation, TUNEL, caspase-3 co-localized with TUNEL, PARP-cleavage, cdc2, XIAP	[103]
	Human prostate cancer cells (DU145)	Morphology, TUNEL	[196]

[1a]: Selenodiglutathione, [b]: Se-Allylselenocysteine, [c]: 2-Phenyl-1, 2-benzisoselenazol-3-(2H)-one, [d]: Kappa-selenocarrageenan (organic selenium containing 1% selenium and polysaccharide solid powder), [e]: Se-Methylselenocysteine, [f]: Methylseleninic acid, [g]: Methylselenol generated by seleno-L-methionine as a substrate for methioninase, [h]: Se-Propylselenocysteine, [i]: 1,4-Phenylenebis (methylene) selenocyanate, [j]: 4-Ethyl-4-hydroxy-2-p-tolyl-5, 6-dihydro-4H-1,3-selenazine (TS2), 4-Hydroxy-4-methyl-6-propyl-2-p-tolyl-5,6-dihydro-4H-1,3 selenazine^{++} (TS6), [k]: Selenocystamine, [l]: Selenomethionine

[2] *: *in vivo.* [3] $\Delta\Psi_m$: mitochondrial membrane potential, cyt c: cytochrome c, DHFR: dihydrofolate reductase, DR5: death receptor 5, ELISA: enzyme-linked immunosorbent assay, ERK: extracellular regulated kinase, *gadd*: growth arrested DNA damage, GSH: glutathione, 8-OHdG: 8-hydroxy-2'-deoxyguanosine, HIAP1: human inhibitor of apoptosis protein 1, IMVD: intratumoral microvessel density, JNK: Jun-N terminal kinase, MAPK: mitogen activated protein kinase, MMP-2: matrix metalloproteinase-2, NO: nitric oxide, PARP: poly (ADP-ribose) polymerase, ROS: reactive oxygen species, TUNEL: terminal deoxynucleotidyl transferase [TdT]-mediated dUTP nick end labeling, VEGF: vascular endothelial growth factor, XIAP: x-linked inhibitor of apoptosis protein.

Recently, selenite has been shown to up-regulate DR5 (death receptor) in DU145 cells [122] and to induce caspase-8 and Bid cleavage; these results suggest the existence of a potential cross talk between the DR5 and the mitochondrial pathways.

Selenite, at concentrations of 5 to10 μM in the medium, caused extensive cytoplasmic vacuolization of cells as well as cell detachment from the culture dish [123]. It is known that the effects of selenite can be influenced by cell type [101], plating density [124] and intracellular glutathione (GSH) level [124, 125]. It is possible that variability of response of different cell types to selenite *in vitro* is due to variation in cellular regulation of GSH levels since this is thought to influence the rate of clearance of potentially toxic SDG conjugates [125]. Furthermore, there are indications of oxidative damage to DNA by selenite, evident from accumulation of 8-OHdG lesions [113]. Selenite appears to kill by necrosis caused by its pro-oxidant and toxic effects at high dose [126]. It seems that the chemopreventive effects of selenite are related in part to the generation of reactive oxygen species (ROS) [110, 114, 116]. The toxic effects of ROS generally result in induction of necrosis and / or apoptosis. Various cell signaling proteins, transcription factors, and cell cycle regulatory proteins are known to be redox sensitive, e.g., p53 [127], $p21^{Waf1/Cip1}$ [128], AP-1 [129] and NF-κB [130]. Selenite-mediated cell growth inhibition has been reported to involve NF-κB and AP-1 [74, 96, 131, 132]. Upregulation of $p21^{Waf1/Cip1}$ was reported in selenite-inhibited cell growth in prostate cancer cells [109].

Selenoxide

Selenoxide administered in the drinking water inhibited mammary tumor formation in DMBA-treated mice and MMTV-induced alveolar hyperplasia [133]; its effects on apoptosis in this target organ have not been examined. However, selenoxide (3 to 30 μM) induced apoptosis in a dose-dependent manner in both immortal hepatic cells and hepatoma cells [78] with down-regulation of bcl-2. In this study, selenoxide was reported to up-regulate p53 expression in hepatoma cells [78]. In a recent study, selenoxide inhibited tumor volume but not the incidence of osteosarcoma [134]. Apoptosis was visible *in vitro* as determined by sub-G1 fraction of cell cycle; TUNEL assay and ultrastructure but no apoptotic bodies were detected *in vivo* [134]. The induction of apoptosis in these cells was independent of p53 and not accompanied by changes in bcl-2 expression.

Selenodiglutathione (SDG)

SDG, the primary metabolite of selenite, has not been tested against chemically induced carcinogenesis but has been shown to inhibit the propagation of Ehrlich ascites tumor in Swiss ICR mice [135]. SDG has proven to be a more powerful inhibitor of cell growth *in vitro* than selenite itself [77]. This was demonstrated using MEL mouse erythroleukemia cells [77]. These data also suggest that SDG-induced apoptosis (at 3 μM concentration) was not solely dependent on the p53 response pathway. It is likely that oxidative stress is induced, since one consequence of selenite reduction by GSH is the generation of H_2O_2 [136], which has also been shown to induce apoptosis [137]. However, it was suggested that SDG-induced cell death (at 8 μM concentration) requires signals other than the generation of H_2O_2 [138]. A more recent report described that SDG (5 to10 μM) induces the expression of Fas ligand (Fas-L) in oral cells to a degree that correlates with the extent of apoptosis induction [104]. These data also demonstrate that SDG induces JNK and p38 kinase and activates extracellular regulated kinases 1& 2 (ERKs 1 & 2) and Akt.

Se-Allylselenocysteine (ASC) and Se-Propylselenocysteine (PSC)

ASC and PSC are chemopreventive at a dose of 2 ppm Se in the methylnitrosourea (MNU)-induced rat mammary tumor model [139]. This study clearly suggests that Se-alkylselenoamino acids can serve as precursors that deliver the Se-alkyl moiety. In mouse mammary epithelial cells ASC inhibited cell growth and induced alkaline-labile DNA damage as well as an increased rate of apoptosis in the range of 12.5 to 50 μM [140], while PSC did not induce alkaline-labile DNA damage and the rate of apoptosis was increased only marginally at the highest level of exposure (50 μM). ASC also reduced the levels of phosphorylated Rb protein and increased levels of p53, p21 and p27 proteins favoring an arrest of cell growth and increase in apoptosis [140, 141].

Ebselen [2-Phenyl-1,2 benziselenazol-3(2H)one]

Ebselen, a synthetic heterocyclic organoselenium compound exhibits weak GPx activity [142] and inhibits aflatoxin-B1 (AFB1)-induced hepatocarcinogenesis in rats [143]. This anticarcinogenic property is attributed to its antioxidant capability and reduction of AFB1-DNA adducts. Ebselen was not effective against DMBA-induced rat mammary tumors [144].

In vitro, ebselen (50 to 75 μM) induces apoptosis in hepatoma cells ($HepG_2$) through rapid depletion of intracellular thiols [145]. Even though it has both anti-inflammatory and anti-oxidant properties, ebselen has not been widely explored as an anticarcinogenic agent.

Kappa-Selenocarrageenan (Kappa-S)

Carrageenan is a family of linear sulfated food-grade polysaccharides obtained from red seaweeds. They have the unique ability to form an almost infinite variety of gels at room temperature. Kappa carrageenans produce strong rigid gels and Kappa-S is an organic selenium compound containing 1% of the element (the form of selenium is not known) and polysaccharide solid powder prepared by the Shanghai Medical University. An orally fed Kappa-S was able to suppress the growth of subcutaneously inoculated hepatoma (H22) cells in mice [146]. The same compound induced apoptosis in SMMC-7721 human hepatoma cells possibly by inducing nitric oxide with increasing doses (concentration: 20-150 μg/ml Se).

Se-Methylselenocysteine (MSC)

MSC, a naturally occurring chemopreventive agent, has been documented to be effective against carcinogen-induced mammary tumors in rats [53, 139, 147-149] and in mice[1]. However, a dose of 4 ppm as selenium, MSC had no inhibitory effect on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced lung tumors in mice[2]. It has been proposed [149, 150] that selenium compounds that directly enter the methylated pool, are more effective chemopreventive agents than selenium compounds that are

metabolized through the hydrogen selenide (H_2Se) pool. This hypothesis gained further support from recent studies involving methylseleninic acid (described below), a direct precursor of methylselenol, which acts much more rapidly than MSC in inhibiting DNA synthesis *in vitro* [147, 151]. MSC is readily converted to the methylated metabolite (methylselenol) in mammalian cells via β-lyase [139, 152]. In the MNU mammary carcinogenesis model in rats MSC is known to inhibit intraductal proliferations (IDPs). In the small IDP lesions MSC significantly inhibited BrdU labeling, cyclin D1 and cyclin A expression but increased p27 protein levels, while in larger IDP lesions p27 was upregulated but BrdU labeling and cyclin levels were not affected [53]. Furthermore, MSC stimulated apoptosis as determined by TUNEL assay (by 3 to 4 fold) in both the small and large IDP lesions. This is one of the few studies in which MSC actually demonstrated apoptosis (Table 1) in the IDP lesions along with a reduced expression of bcl-2. MSC was recently reported to induce caspase-3 activity in the mammary tumor model in mice[3].

In cell culture MSC has been shown to induce apoptosis in several model systems (Table 2). Treatment with 50 to 100 μM MSC is sufficient to induce apoptosis in mouse mammary tumor cells [153, 154] and several human cell types including breast[4], promyelocytic leukemia [155, 156], ovarian cancer [157], and human umbilical vein endothelial cells (HUVEC) [76]. In all of these studies, MSC has been shown to specifically activate caspase-3, followed by PARP-cleavage. ROS [156] and other target molecules being affected by MSC treatment include HIAP1, XIAP, survivin and Bax [157], PKC, cdk2 and *gadd34, 45 and 153* [153], as well as PI-3K, Akt and p38MAPK[5].

It has been hypothesized that MSC may be able to activate the cysteine protease whose active site is blocked by zinc. The higher affinity of methylselenolate for zinc could result in release of the cysteinethiolate-bound zinc and activate the enzyme [59]. This hypothesis needs to be tested.

Methylseleninic acid (MSeA)

MSeA is a strong oxidizing agent that is able to readily react with reduced GSH to form methylselenol in mammalian cells. Methylselenol is a highly reactive intermediate that cannot be isolated as such but is now believed to be responsible for the anticancer effect of methylated selenium compounds that readily deliver it [147, 151]. MSeA represents a simplified version of MSC without the amino acid moiety, thereby obviating the need for β-lyase action. MSeA is more potent than MSC *in vitro*. Interestingly, the distinction between these two compounds disappeared *in vivo* since both were equally effective as cancer chemopreventive agents [147].

MSeA has been reported to induce apoptosis in several cell types (Table 2). MSeA (5 to 10 μM) can induce cell growth arrest in the G1 phase prior to apoptosis in mouse mammary TM6 cells [151], in HUVEC [158], in MCF10AT1 and MCF10AT3B human breast cells [159]. MSeA induced PARP-cleavage in MCF10A and MCF10ADCIS.com human breast cells[4] and in DU145 prostate cancer cells [120, 160] as a result of caspase-3 activation *in vitro*. Molecular targets that are modulated by MSeA include ERKs 1 & 2, VEGF, JNK, p38MAPK and PI3-K/Akt [158, 160]. Intracellular GSH has been implicated as a cofactor in MSeA-induced apoptotic cell death of human hepatoma ($HepG_2$) cells [161]. But the data need to be interpreted with caution, as the concentration of MSeA used in this study may be cytotoxic to other cell types. The advent of microarray technology has led to a study of differential expression signals of several genes in human breast cells [159], in mammary cells of mice [151], and rats [162]. Rat cDNA array experiments revealed that MSeA might have a widespread effect on the gene expression of epithelial cells along with the adipocytes and stromal cells in the mammary gland [162]. Furthermore, there is a considerable overlap of the MSeA-modulated genes or signaling pathways identified in prostate cells [163] with those identified in breast cells [159] using the microarray approach. Similar selenium targets were identified, including g*add 153*, cyclin A, cdk1, cdk2, cdk4, cdc25, E2Fs as well as components of the MAPK, JNK and PI3-K pathways.

Since methylselenol is highly reactive and cannot be isolated, experiments were performed using methylselenol generated from seleno-L-methionine as a substrate for L-methionine-alpha-deamino-gamma-mercaptomethane lyase (EC4.4.1.11) (methioninase). Exposure of DU145 cells to methylselenol generated in the sub-micromolar range led to caspase-mediated cleavage of PARP and DNA fragmentation. In addition the experiment resulted in a profile of biochemical effects similar to that of MSeA [164] namely, dephosphorylation of Akt and ERKs 1 & 2. The results support that methylselenol is an active selenium metabolite that induces caspase-mediated apoptosis and cell cycle G1 arrest. Methioninase cancer gene therapy uses the same principle; employing SeM as a prodrug provides a new approach for cancer gene therapy [165].

MSeA inhibited NF-κB DNA binding induced by TNF-α and lipopolysaccharide in prostate cells at the concentration that induced apoptosis in these cells [96]. A key to NF-κB regulation is the inhibitory κB (IκB) protein that in response to diverse stimuli are rapidly phosphorylated by IκB kinase complex, ubiquitinated, and undergo degradation, releasing NF-κB factor. In the same study, MSeA inhibited IκB kinase activation and IκB-α phosphorylation and degradation induced by TNF-α and lipopolysaccharide in prostate cells. These results suggested that selenium might target the NF-κB activation pathway to exert, at least in part, its cancer chemopreventive effect in prostate.

1,4-Oxaselenins

Oxaselenins are a new class of organoselenium compounds that have not been extensively examined *in vivo*. Oxaselenins (15 to 60 μM) have been shown to induce apoptosis (Table 2) in SKOV-3 ovarian cancer cells in a dose-dependent manner [166]. These cells were treated with the oxaselenins in serum-free medium; the data need to be interpreted with caution because growth factor and serum starvation may lead to apoptosis on their own.

1,4-Phenylenebis (methylene) Selenocyanate (p-XSC)

p-XSC is a synthetic organoselenium compound that has been shown to be effective against carcinogen-induced

tumorigenesis of the colon, lung, tongue and mammary glands [167]. We have observed that *p*-XSC significantly suppressed AOM-induced colon carcinogenesis in rats during both the initiation and the post-initiation stage [168] and that it was equally effective in inhibiting small intestinal tumors and colon tumors in the APC^{min} mouse model [169]. Inhibition of cyclooxygenase (COX-2) activity in these intestinal polyps may be a possible target for *p*-XSC's antitumor activity. *p*-XSC also has a strong antitumor activity against NNK-induced lung cancer in the A/J mouse [170]. Preliminary findings suggest that the inhibition of NNK-induced DNA adduct formation in the lung may, in part, account for the chemopreventive effect of *p*-XSC. It inhibits NNK-induced 8-OHdG lesions in rat and mouse lung [171]. Recently, *p*-XSC was reported to inhibit lung tumors induced by a mixture of NNK and benz(a)pyrene [B(a)P] in A/J mice [172]; both carcinogens are considered to be relevant to the development of lung cancer in smokers. Furthermore, diet supplementation with *p*-XSC in mice reduced the pulmonary metastases of B16BL6 melanoma cells and inhibited the growth of these metastatic tumors in the lung, in part by inducing apoptosis [173]. In addition, *p*-XSC can effectively block 4-nitroquinoline-1-oxide-induced oral carcinogenesis [70]. *p*-XSC inhibited DMBA-induced rat mammary tumors [174] and, when compared to BSC, methylselenocyanate, potassium selenocyanate and selenite, *p*-XSC was found to have a considerably higher chemopreventive index (Table 1). In the same model system *p*-XSC induced apoptosis as measured by TUNEL assay and caspase-3 gene expression [54]. The GSH conjugate of *p*-XSC (*p*-XSe-SG), one of its putative metabolites, inhibited colon carcinogenesis when administered during the post-initiation stage and down-regulated COX-2 activity [175].

When *p*-XSC (5-20 μM) was evaluated against mouse mammary epithelial tumor cells [108], SCC [104], and human colon cancer cells [176], the mechanism of growth inhibition was attributed to *p*-XSC-induced apoptosis (Table 2). In addition, *p*-XSC has been documented to inhibit thymidine kinase in mammary tumor cells of rats and humans [177]. *p*-XSC also inhibited mitochondrial membrane potential prior to apoptosis in rat mammary cells [178]. *p*-XSC induces expression of Fas-L, JNK and p38MAPK at concentrations of 30-50 μM, causing apoptosis in oral cancer cells [104]. Furthermore, *p*-XSC activated the ERKs 1 & 2 and Akt in the same cell type [104].

1,3-Selenazine Derivatives

As a novel class of selenium compounds, 1,3-selenazine derivatives (TS-2 and TS-6) at a concentration of 5 to 20 μM, have been found to induce apoptosis in HT-1080 human fibrosarcoma cells [179] and TMK-1 human gastric cancer cells [180] (Table 2). These compounds specifically inhibit elongation factor-2 kinase (EF-2K) [181]. Details of the mechanisms underlying the apoptosis induced by these chemicals need to be determined along with efficacy studies *in vivo*.

Selenium-Yeast

Dietary supplementation with selenium-enriched yeast for a mean of 4.5 years resulted in reduced incidence of lung, colon and prostate cancers by 46%, 58% and 64% respectively among American subjects [10-12]. The mechanism responsible for the protective effect remains elusive and as an initial investigation towards this end, we have demonstrated that supplementation of selenium-enriched yeast to healthy men at 200 μg/day for 9 months reduced markers of oxidative stress as well as prostate specific antigen [182]. In animal model studies selenium yeast moderately inhibited chemically induced hepatocarcinogenesis [183] and mammary carcinogenesis [184] but had no effect on tumorigenesis in the forestomach [185], in the lung [186] and in the prostate [187]. The anticarcinogenic potential of selenium-enriched yeast at the molecular level has not been reported.

Yeast-derived selenium has been shown to be effective against several tumor cell types *in vitro* including cells from the breast, lung, liver and small intestine [188]. The study demonstrated that selenium yeast-induced apoptosis at 40 μg/ml concentration in SKBR-3 human breast cells by DNA fragmentation. The lack of knowledge on solubility of the selenium-yeast in the above experiment makes it difficult to interpret the results.

Selenocystamine (SCys)

SCys inhibits DMBA-induced rat mammary tumors [189]. It induces apoptosis in MK2 mouse keratinocytes (5 to 250 μg/ml Se concentrations) as measured by TUNEL [113]. 8-OHdG lesions were observed in these cells following treatment with SCys. It is possible that metabolism of this compound may lead to the formation of ROS that, in turn, can damage DNA resulting in the subsequent formation of 8-OHdG.

Selenomethionine (SeM)

SeM is the major Se component of selenium-enriched yeast [184]. While it inhibits metastasis of B16BL6 murine melanoma cells to the lung [190], SeM had no effect on B(a)P-induced forestomach tumors [185], AOM-induced colon tumors [191], NNK-induced lung tumors [192], prostate cancer in Wistar-Unilever rat model [193] and DMBA-induced mammary tumors in mice [194]. However, SeM was weakly active in DMBA-induced mammary tumors in the rat [144]. In addition, a recent report provided evidence that dietary selenium in the form of either selenomethionine or selenized yeast decreased DNA damage and increased epithelial cell apoptosis within the aging canine prostate [195].

Interestingly, SeM induces apoptosis in cancer cell lines of breast, colon, lung, melanoma and prostate (Table 2). Doses that can be used *in vivo* are limited by the MTD and its effect on apoptosis *in vitro* is achieved at levels that may not be possible to use *in vivo*. SeM has been shown to inhibit growth of several human cell lines in the μM range (45 to 150 μM) while growth inhibition of normal diploid fibroblasts required 1mM SeM, i.e., levels approximately 1000-fold higher than for the cancer cell lines [196]. Selenium metabolism and polyamine synthesis are linked since both processes require S-adenosylmethionine as a cofactor, SeM was tested against A549 lung and HT29 colon cancer cells and was found to decrease polyamine levels in

these cells. The decline in polyamine levels prompted apoptosis in these cells [197]. In addition, p53 status in these cells did not affect the SeM-induced apoptosis. Further experiments have indicated that SeM induces apoptosis in HCT116 colon cancer cells by modulation of mitotic cyclin expression and inhibition of cdc2 kinase activity [198]. A recent study compared the effects of SeM on normal prostate cells and on prostate cancer cells [103], and showed that SeM selectively induces apoptosis in prostate cancer cells (LNCaP, PC3 and DU145). In contrast, using similar doses of SeM, both LNCaP and PC3 were arrested without apoptosis [199]. The difference may be attributed to the form of SeM as the former used DL-selenomethionine and the latter used L-selenomethionine. Furthermore, the ability of both types of cells to metabolize SeM can be a determining factor. Both studies however, reported that SeM was more effective against the androgen-sensitive LNCaP cells. This selective effect can be due to the HSP56 protein present only in the androgen-sensitive LNCaP cells [200].

SeM has been shown to regulate p53 by Ref1-dependent redox mechanism [79], thereby converting p53 into a form that does not have any growth suppressing effect but can effectively induce DNA repair [201]. Furthermore, SeM-induced cell growth inhibition may be, in part, mediated by COX-2 dependent mechanism [202]. Since polyamine may mediate post-transcriptional regulation of COX-2 [203], the role of polyamine depletion by SeM leading to apoptosis needs to be studied in depth.

SUMMARY AND FUTURE RECOMMENDATIONS

Epidemiological studies, preclinical investigations and clinical intervention trials support the role of selenium compounds as potent cancer chemopreventive agents. The dose and the form of selenium are critical factors in cancer prevention. Induction of apoptosis and / or inhibition of cell growth can account for cancer prevention by selenium compounds. Sodium selenite, selenoxide, SDG, ASC, ebselen, kappa-S, MSC, MSeA, oxaselenins, PSC, *p*-XSC, selenazine derivatives, SCys and SeM have the ability to induce cell death by apoptosis in a number of cancer cell types at 5 to 100 μM concentration.

Prior to induction of apoptosis, selenium compounds alter the expression and / or activities of a number of cell cycle regulatory proteins (Rb, p53, cyclins A, B, D1 and E, p16, p21 and p27, cdk2, cdk4 and cdc2, *gadd34, 45 and 153*), mitochondria-associated factors (Bcl-2, bax, bak, bid, cytochrome c, ROS), signaling proteins (PKC, PI3-K, Akt, ERKs 1 & 2, p38MAPK, JNK), proteases (such as caspase-3, 6, 7, 8 and 9, calpain), angiogenic factors (VEGF, MMP-2, IMVD), transcription factors (NF-κB and AP-1), tumor suppressor genes (e.g., p53), polyamine and GSH levels. Several selenium compounds can arrest cells in either phase of the cell cycle but it is not yet established if a cell cycle arrest by selenium compounds is a necessary or sufficient event to induce apoptosis. Furthermore, selenium compounds induce apoptosis by both p53-dependent and -independent pathways. This differential effect may be explained on the basis of structural differences in the selenium compounds examined, but the phenomenon needs to be studied in greater detail.

Learning more about the initial and intermediate targets that may be altered by selenium, such as protein kinases [for a recent review see 204], will assist in determining multiple steps that can be modulated to induce the execution phase of cell death. In addition, depending on the form of the compound these agents can target separate pathways but a detailed study needs to be performed in order to find an appropriate approach that would disrupt different pathways converging to apoptosis.

Most of the selenium compounds induced apoptosis in cell culture model systems (Table 2). MSC and *p*-XSC have been shown to inhibit carcinogenesis and induce apoptotic cell death *in vivo* (Table 1). Analyzing the ability to induce apoptosis is certainly a key factor in selecting potential chemopreventive agents but the toxicity of the selenium compounds needs to be considered in long-term efficacy studies in rodents and certainly in human clinical intervention trials. These studies reiterate that compounds such as synthetic TPSeCl with a CI of more than 10 should be considered for human breast cancer prevention trials after it has fulfilled the toxicological clearance for human use. Further studies are warranted in order to delineate molecular targets and associated pathways that are being altered by TPSeCl *in vivo*. Measurement of apoptotic cells in tumors is difficult to quantify with accuracy because their half-life of histologically recognizable apoptosis is short and cell samples are often heterogeneous; therefore, an assay that could quantitate caspase-3 activity in the tumor tissue would provide a better estimate of apoptosis.

The Selenium and Vitamin E Cancer Prevention Trial (SELECT) on prostate cancer funded by the National Cancer Institute, and coordinated by the Southwest Oncology Group will be testing the hypothesis that selenium (provided as L-selenomethionine) or vitamin E (provided as DL-α-tocopherol acetate) alone or in combination can reduce the clinical incidence of prostate cancer in a population-based cohort of healthy men [205]. Potential molecular and genetic markers that will be studied include proliferation markers such as Ki-67, invasion markers such as MMP2, cathepsin D and E-cadherin, cell cycle proteins such as cyclin A, cyclin D, cyclin E, p21, p16, p27, p53 and Rb [206]. On the basis of the information reviewed here we would recommend that polyamine levels, *gadd153*, bcl-2, Akt, caspase-3 activity, PARP-cleavage, as well as blood GSH levels and protein glutathiolation [182] should be included as ancillary markers nested within the SELECT study.

Apart from the selenium compounds reviewed here, several novel synthetic organoselenium compounds have been examined for their growth inhibitory properties in a variety of cell culture systems [reviewed in 207], but there is a need to test these compounds for their potential to induce apoptosis; such an investigation may provide better and mechanism based cancer chemopreventive as well as chemotherapeutic agents.

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FOOTNOTES

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