Review

Mechanisms of selenium chemoprevention and therapy in prostate cancer

Nagalakshmi Nadiminty and Allen C. Gao

Department of Urology and Cancer Center, University of California School of Medicine at Davis, Sacramento, CA, USA

Prostate cancer (PC) chemoprevention has generated considerable interest in the last decade and selenium and combinations of selenium have been recognized as one of the most efficacious chemopreventive agents against PC. This review focuses on a discussion of the knowledge hitherto gained about the mechanisms of action of the various *in vitro* and *in vivo* used selenium compounds and their effects on cellular processes and signaling pathways. We also describe the clinical and preclinical studies that have contributed enormously to the knowledge about dose, duration of exposure, and the chemical form of selenium effective in different scenarios. Even though the jury is still out about whether selenium can be used as a chemopreventive agent in the clinic and whether studies with cell lines and populations at low, medium, or high risk can adequately represent the physiological behavior of this micronutrient, it can safely be said to offer the most diverse spectrum of protective effects against this particular type of cancer which may augur well for its future as a chemopreventive agent.

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1 Introduction

Prostate cancer (PC) continues to be the most common malignancy among men and the third leading cause of cancer-related mortality in the United States. It is estimated by the NCI that one in six men will be diagnosed with PC and one in three will die of this disease. Despite the enormous numbers of men represented by these figures very few advances have been made in the quest for cures for PC. When primary PC arises, it is mainly androgen-dependent and the therapy of choice continues to be androgen ablation. But virtually all of the patients develop castration recurrent disease subsequent to remissions lasting 2 – 5 years after androgen ablation. There has been significant progress in the past decade that has improved our understanding of the disease but there is still much to be learned about the causes, early detection markers for diagnosis and prognosis determination, treatment, and prevention of PC. The most common and widely used strategy for reduction of PC morbidity and mortality is periodic examination of the prostate by digital rectal exam and screening for PSA. Prevalence of latent PC found in autopsies in men over 60 reaches almost 40%, emphasizing the need for development of agents that can prevent initiation of prostate carcinogenesis at the earliest stages.

Life style and dietary habits have been identified as major risk factors in PC growth and progression [1]. The major advantage of chemoprevention compared to other forms of therapy is the lack of systemic toxicity. Another advantage is that according to recent reports chemopreventive agents, when used in combination with conventional therapies, can improve therapeutic response [2]. The relatively long latency in the development of PC makes it an appropriate target for chemoprevention at an early stage. Several agents including 5-α-reductase inhibitors, selective estrogen receptor modulators, antioxidants like vitamin E, vitamin C, selenium, lycopene, and other candidates like isoflavones, natural compounds like green tea polyphenols, nonsteroidal anti-inflammatory drugs (NSAIDS) have been used in the study of chemoprevention of PC [3]. This review focuses on the advances made so far in the study of mechanisms of selenium chemoprevention, clinical trials con-

Correspondence: Dr. Allen C. Gao, Department of Urology and Cancer Center, Research III Bldg, Suite 1300, 4645 2nd Ave, Sacramento, CA 95817, USA
E-mail: acgao@ucdavis.edu
Fax: +1-916-734-8714

Abbreviations: AR, androgen receptor; **BPH,** benign prostatic hyperplasia; **GSH,** glutathione; **GPx,** glutathione peroxidase; **HGPIN,** high grade prostatic intraepithelial neoplasia; **MSC,** methylselenocysteine; **MSeA,** methylseleninic acid; **NAC,** *N*-acetylcysteine; **PC,** prostate cancer; **ROS,** reactive oxygen species; **SM,** selenomethionine

ducted and in progress and differences between various forms of selenium used in both *in vitro* and *in vivo* studies.

2 Background

Selenium is an essential micronutrient trace element and the concentration of selenium in diet depends on the soil in the region, the types of food consumed and other factors which facilitate or inhibit uptake of selenium. Following digestion, Se is metabolized to physiologically active methylselenol (CH_3SeH) or incorporated into antioxidant enzymes and other selenoproteins. The generation of methylselenol from selenium compounds is mediated by enzymes like γ-glutamyl-SeMeSeCys, β-lyase, *etc*. There is also some evidence that methylselenol can be formed directly from selenomethionine (SM) by the action of a β-lyase also called methioninase [4].

Use of Se as a chemopreventive agent requires that it be considered a pharmacological agent and supra-nutritional doses may be administered, which raises concerns about the safety margin and potential side effects [5, 6]. Administration of selenomethylselenocysteine or Se in the form of nanoparticles (Nano-Se) at a rate of 10 mg Se/kg wt in mice has shown that nano-Se exhibits lower rates of toxicity while being equally effective in increasing activities of selenoenzymes [7]. The current interest in Se stems from a trial that analyzed its effect on nonmelanoma skin cancer [8]; this study randomized 1312 participants to a dose of 200 μg of Se/day in the form of selenized yeast or placebo. The primary end point was not reached but a reduction in the rate of PC incidence at 4.5 and 7.4 years was observed. The highest association between decrease in cancer incidence and dose was noted in participants with initially low levels of Se and in men below 65 years with a PSA level <4 ng/mL. The Nutritional Prevention of Cancer study found a significant reduction (49%) in PC risk among study subjects. Kranse *et al*. [9] showed that dietary intervention with a dietary supplement containing carotenoids, selenium, and some other substances could reduce DHT and testosterone levels and increase free PSA and total PSA doubling time. Venkateswaran *et al*. [10] have shown that treatment of the 12T-10-Lady transgenic prostatic adenocarcinoma model with antioxidants (vitamin E, selenium, and lycopene) decreased the incidence of PC and increased disease-free survival. They also found that PC developed in ∼75 and ∼100% of controls in the standard and high fat diets, respectively, whereas only ∼10 and ∼15% of animals in the antioxidant-treated group developed tumors. This was accompanied by increased levels of p27(kip1) and decreased levels of PCNA expression. The nutritional benefits of Se are reportedly mediated through its incorporation into small Se-containing metabolites or its regulatory role in the synthesis of selenoproteins or antioxidant enzymes [11].

Intake of Se is inadequate for cancer prevention in many countries and evidence is mounting that suggests that supra-nutritional intake of Se is required for reduction in cancer risk [12]. Several large prospective studies of association between Se levels and PC risk have been published and several are still ongoing. Almost all the studies have established a significant reduced risk of PC in healthy men and men with advanced PC or those with baseline PSA level of >4 ng/mL where a significant reduction in cancer burden in the highest category of Se concentration was noted [12–14]. In a number of these studies Se was found to have a stronger protective effect against advanced PC suggesting that the primary effect of Se was on PC progression rather than initiation.

3 Clinical trials and epidemiologic studies

The phased approach for clinical trials follows a pattern of: phase I-dose-related safety and toxicity, phase II-efficacy in a small population at high risk for specific cancers or the presence of biomarkers and phase III-large, randomized, double-blind, placebo-controlled trials with a large sample size [15].

While several large-scale phase III clinical trials with clinically beneficial end points using Se are ongoing, there are a number of smaller earlier phase studies that have contributed to the planning of these trials. Four studies conducted by Shamberger and Frost [16], Schrauzer *et al*. [17, 18], Clark and Marshall [19], and Yu *et al*. [20] have found a marginal but evident inverse relationship between Se intake and overall risk of cancer. In most studies the most pronounced effect of Se intake was observed in gastrointestinal and PCs and the lowest for pancreas, skin, and bladder cancers [21, 22]. More recent epidemiologic data support the inverse correlation between Se level and PC risk [23]. This study measured serum Se in 212 men with PC and 233 age-matched controls, which showed that serum Se level was inversely associated with risk of PC with similar patterns seen in both Caucasian and African–American men. Interestingly, this study also showed that vitamin E level was also highest in men in the highest Se quartile. An interesting study among the Inuit, whose diet is rich in omega-3 PUFAs and selenium, found only 1 case of PC and no latent cancers in 61 men dying of other causes [24]. Li *et al*. [14] have shown an inverse correlation between baseline plasma Se levels and risk of advanced PC, suggesting that higher levels of Se may slow PC progression.

On the other hand, two European studies [25, 26] studied the correlation between toenail Se levels and risk of PC and found that Se may not be strongly associated with reduced PC risk since men in the highest Se quartile had only a slightly reduced risk compared to men in the lowest quartile [27]. Karunasinghe *et al*. [28] studied levels of accumulated DNA damage in a group at high risk for PC and found that

lower serum Se levels had a statistically significant inverse relationship with accumulated DNA damage. This suggests that Se intake in this population was not sufficient for adequate repair of DNA damage, thus increasing susceptibility to cancer. Another study analyzed the Se levels and glutathione (GSH) peroxidase activities in whole blood, plasma, and prostates of 32 PC and 40 benign prostatic hyperplasia (BPH) patients with 39 healthy subjects. Se concentrations in blood and plasma were found to be lower in both PC and BPH patients whereas prostate tissue Se level was higher in these groups compared to controls. In contrast, prostate tissue glutathione peroxidase (GPx) activity was lower in PC patients compared to BPH patients indicating that higher levels of Se do not necessarily mean higher efficacy in anticancer effects [29]. This was also corroborated by the finding that the level of Se in the prostate gland was similar in control subjects and BPH patients (~156 ng/g wet wt) while in PC patients this level was significantly higher (~182 ng/g wet wt) [30].

Intervention trials have also attempted to establish a correlation between Se supplementation and lower PC risk. A double blind placebo-controlled cancer prevention trial by Clark *et al*. [31] showed a 63% reduction in PC incidence in men supplemented with 200 μg/day Se (selenized yeast). A follow up of this study continues to show a significant reduction in the incidence of PC following Se supplementation [32]. The positive outcome of this study encouraged the implementation of several other trials. A study in men with normal pretreatment serum selenium levels found that 200 μg oral selenium *per* day resulted in higher levels of selenium (which were statistically significant) in prostatic tissue compared to placebo in 51 men who underwent transurethral resection of the prostate for BPH [33]. This suggests that oral administration of selenium is clinically relevant and concentrates Se in prostatic tissue [34]. A recent 4×1 wk double-blind, randomized crossover study in which healthy young men supplemented their diets with selenate and Se-enriched yeast (300 μg/day Se) and Se-enriched milk (480 μg/day Se) found that even though all sources of Se increased serum levels of Se, short-term supplementation did not modulate blood lipid markers or expression and activity of several selenoenzymes [35]. Another randomized, placebo-controlled double-blind crossover dietary intervention study in PC patients with the administration of a dietary supplement (verum, containing Se in addition to plant estrogens, antioxidants, and other putative anticancer agents, administered for 6 wk) in 37 hormonally untreated men with PC and raising PSA levels found that dietary intervention reduces DHT and testosterone levels and increases free PSA and total PSA doubling time [9]. A report by Sabichi *et al*. [36] has shown that orally administered Se can accumulate preferentially in the prostate gland as opposed to the seminal vesicles. These findings are from a randomized and controlled Southwest Oncology Group study which showed that oral administration of 200 μg/day Se in the form of L-SM increased Se levels in the prostate tissues by 22% compared to controls. These results support the hypothesis that oral Se supplementation may contribute to the PC preventive effects of Se.

These equivocal results after analysis of completely different populations with varying life styles and environmental conditions prompted the initiation of large, randomized, and controlled studies using Se like the SELECT, the Watchful Waiting and the Negative Biopsy studies in the United States along with other studies including Prevention of Cancer by Intervention with Selenium (PRECISE) in three European countries and the Australian Prostate Cancer Prevention Trial using Selenium (APPOSE). The Selenium and vitamin E Cancer Prevention Trial (SELECT) sponsored by the NCI is a phase III randomized double-blind placebo-controlled trial designed to test the efficacy of Se (200 μg L-SM) and vitamin E (400 mg DL-α-tocopherol) alone and in combination in the prevention of PC [37]. Accrual of 32 400 volunteers has been completed and final results are expected by 2013. For PC hormones are a significant risk factor in addition to modifiable risk factors like diet, obesity, and screening history as well as nonmodifiable risk factors like age, race, family history, and presence of some genetic polymorphisms. SELECT will also assess, in a nested case-control study, genetic polymorphisms of four genes – androgen receptor (AR), 5α-reductase type II, cytochrome p450c 17β, and α-hydroxysteroid dehydrogenase – on PC incidence to identify potential targets for screening and intervention. If such biomarker associations with increased risk of PC are validated, targeted interventions can be developed to address disparities in PC risk [38]. A large-scale phase III study is also ongoing to examine the impact of selenium on men with high grade prostatic intraepithelial neoplasia (HGPIN) with the hypothesis that the agent decreases the risk of subsequent diagnosis of PC [19]. This trial, designed by the Southwest Oncology Group, will test the chemopreventive effect of Se in the form of SM (200 μg Se/day) to prevent the development of PC in men with HGPIN [39]. The APPOSE is a randomized, controlled chemoprevention trial with a cohort size of 2000 men at high risk for PC (with family history of PC) in each arm. The two groups are administered 200 μg Se daily with placebo being used with the control group. The subjects will be observed over a period of 10 years with annual DRE, PSA estimation, and serum Se measurement and needle biopsies will be performed after the observation of incident PC. This study is expected to provide evidence for the efficacy of Se as a chemopreventive agent in men at high risk for PC [40].

These studies are using different forms of Se: selenized yeast (200–800 μg/day) is being used in several trials currently under way like the Negative Biopsy study (200 and 400 μg/day Se) [41], the preprostatectomy study [42], and the Watchful Waiting study [43] whereas the SELECT

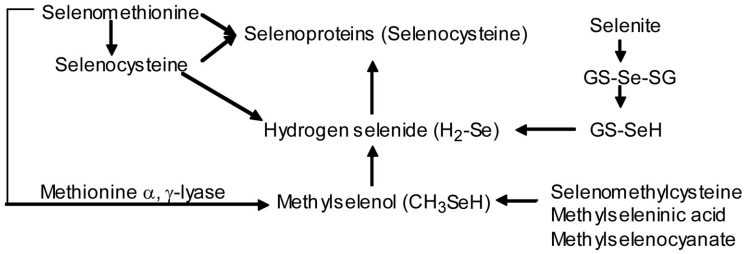

Figure 1. Schematic representation of metabolites and intermediates in the metabolism of Se and incorporation into selenoproteins (modified from [12] and [118]).

study uses SM [44]. Earlier human clinical trials which used organic forms of Se as chemopreventive agents against PC have shown promising efficacy [31, 45, 46]. These studies will shed light on the differences in bioavailability and metabolism of these forms of Se and will pave the way for the design of meaningful prevention efforts in a clinical setting. Taken together these trials and studies provide a rationale for the mechanistic evaluation of different selenium compounds in the chemoprevention of initiation and progression of PC and also in decreasing the risk in men with family history of PC.

4 Variations in the mechanisms of action of different Se compounds

Previous studies have shown that the choice of chemical form and dose of Se can strongly influence the observed biological effects. Inorganic Se compounds like selenite or selenate are known to produce genotoxic effects, whereas organic Se-containing compounds are better tolerated and exhibit anticancer activity. Organic Se is present in the forms of SM, selenocysteine, and selenomethylselenocysteine. SM is the most prevalent form of dietary selenium. The differences in anticarcinogenic activities of the various forms of Se can be attributed to their metabolism. Metabolism of selenite is tightly regulated and forms hydrogen selenide after reaction with reduced GSH. Hydrogen selenide can then act as a precursor for the synthesis of selenoproteins or can undergo enzymatic methylation to generate methylselenol. SM can either get incorporated into general body proteins, get converted to hydrogen selenide and follow the same pathway as selenite or be converted to methylselenol *via* methioninase. Synthetic Se compounds like methylseleninic acid can generate monomethylated Se easily and have been used extensively in *in vitro* studies to generate the active anticancer metabolite, methylselenol. Several other Se metabolites have been shown to have anticarcinogenic effects in cell or animal model systems: selenodiglutathione (GSSeSG), hydrogen selenide (H_2Se), and the methylated selenides ($[CH_3]_xSe$) which are the excretory forms of the element (Fig. 1).

Monomethylated Se compounds that are precursors of methylselenol, like methylselenic acid (MSeA) induce apoptosis through biochemical and cellular processes that are distinct from those induced by forms of Se that enter the hydrogen selenide pool like sodium selenite. MSeA and other precursors of methylselenol induce apoptosis *via* the caspase pathway and without induction of DNA single strand breaks, whereas sodium selenite is genotoxic with induction of single strand breaks and induces apoptosis without activation of caspases. A recent study suggests that selenite metabolism leads to generation of reactive oxygen species (ROS), mainly superoxide and hydroperoxide, which lead to DNA strand breaks and to p53 phosphorylation, ultimately leading to mitochondrial leakage and caspase activation. This explains the higher toxicity of selenite in p53-wild type PC cells like LNCaP compared to MSeA and less efficient induction of apoptosis in p53-null PC cells like DU145 and PC3 [47]. Specific organoselenium compounds have promise in delaying both early and late events in cell cycle progression of PC cells. Chemoprevention by organoselenium compounds involves apoptosis as a critical event and also exhibits inhibition of angiogenic molecules in contrast to those that enter the hydrogen selenide pool. Jiang *et al*. [48] suggested that different pathways might be activated by these two metabolite pools in exerting their antiproliferative effects on cancer cells.

A recent study suggests that the molecular structure in which Se resides determines the induction of physiological responses at the cellular and molecular level by modulating signaling factors as well as transcription factors that regulate proliferative processes leading to cancer prevention [49]. This study compared two naturally occurring Se compounds, SM and Se-methylselenocysteine (MSC) with two synthetic ones 1,4-phenylenebis(methylene)selenocyanate (*p*-XSC) and *p*-xylylbis(methylselenide) (*p*-XMS). Corcoran *et al*. [50] have shown that inorganic selenium in the form of sodium selenate was more effective in inhibiting the progression of hormone refractory PC compared to SM, MSC, and selenized yeast. This was found to be due to a reduction in the growth of primary prostatic tumors as well as retroperitoneal lymph node metastases accompanied by a decrease in angiogenesis.

Methylseleninic acid (CH_3SeO_2H) was developed specifically for *in vitro* studies since cultured cells do not metabolize SM adequately due to very low levels of β-lyase activity [51]. *In vitro* studies showed that 10 μM MSeA can inhibit LNCaP, DU145, and PC-3 PC cell growth and *in vivo* studies have shown that tumor growth is also decreased in xenografts treated with MSC [52, 53]. MSeA (1 – 10 μM) disrupts androgen signaling by inhibiting AR mRNA and pro-

tein expression and reducing the expression of AR target genes [54]. A study by Zhao *et al*. [55] has suggested that 3 – 30 μM MSeA inhibits proliferation by reducing the expression levels of several cell cycle regulated genes. MSeA also regulated the expression of AR, PSA, and phase II detoxification enzymes, thereby inducing cellular defenses against carcinogens. A study by Zu and Ip [56], demonstrated that combining vitamin E succinate and MSeA produced a synergistic effect on cell growth inhibition and augmented apoptosis.

SM and MSeA arrest LNCaP cells at different phases of the cell cycle [57, 55]. Both agents induce changes in the transcriptional program in LNCaP cells. In LNCaP cells *in vitro* 10 μM MSeA produces 50% inhibition in cell growth after 48 h of treatment while SM requires doses of 130 μM to achieve similar growth inhibition [57, 55]. Androgen regulated genes like KLK3, NKX3.1, and GUCY1A3 are downregulated over different time points by MSeA but not by SM [58]. Several pathways like cell cycle, apoptosis, and signal transduction are affected similarly by both compounds. The differences in these two compounds have been attributed to differences in their rates of conversion to methylselenol or the depletion of SM by nonspecific incorporation into proteins. MSeA decreases transcription of genes from all phases of the cell cycle suggesting that it causes LNCaP cells to exit cell cycle rather than inhibit a specific phase of the cell cycle. On the other hand, SM enriches transcripts in the G1/S phase and downregulates transcripts involved in the G2/M progression suggesting that SM induces a G2/M arrest in LNCaP cells. Treatment with 1 μM SM resulted in G2/M arrest in LNCaP, PC-3, and DU145 cells accompanied by the phosphorylation of cdc2 [57] and decreased expression of cyclins D1 and D3 [59]. It did not affect the expression levels of cdk2, cdk4, and cyclin E. It has also been shown that the anticancer efficacy of SM can be enhanced by methioninase treatment and that apoptosis induced by SM and methioninase is superoxide-mediated and p53-dependent in human PC cells [4]. Wang *et al*. [60] have shown that addition of methioninase increases the efficacy of SM in generation of methylselenol and induction of apoptosis. As low as 1 μM SM was sufficient to induce antiproliferative effects and inhibition of Akt phosphorylation in DU145 cells. SM also protects cells from DNA damage through a p53-dependent mechanism by promoting reduction of 2 cysteine residues in p53 [61].

In vitro studies have shown that micromolar concentrations of SM inhibit growth and induce apoptosis in LNCaP, PC-3, and DU145 PC cells but not in primary fibroblast cultures or primary prostate epithelial cells [52, 62]. MSeA induces apoptosis and inhibits growth of PC-3 cells at a concentration of 5 – 10 μM and similar to SM the mechanisms of apoptosis induction include DNA fragmentation and PARP cleavage [52, 63]. But the products of PARP cleavage induced by these two compounds are different in DU145 cells. The androgen responsive LNCaP cells are more sensitive to SM than the androgen-independent cells PC-3 and DU145. This has been suggested to be due to the inhibition of the interaction of the AR with the early growth response protein 1 (EGR-1) by SM [64]. Cho *et al*. [65] have shown that methylselenol or MSeA specifically and rapidly inhibit PSA expression through induction of PSA protein degradation and suppression of androgen-stimulated PSA transcription in LNCaP cells. This inhibitory effect was not observed in the case of sodium selenite or SM.

A study by Husbeck *et al*. [66] has also shown that MSeA and selenite inhibit AR signaling by different mechanisms. They found that selenite reacts with reduced GSH in cells and produces superoxide radicals. This was confirmed by use of the antioxidant *N*-acetylcysteine (NAC), which blocked the downregulation of AR and PSA expression by selenite. MSeA did interact with reduced GSH but did not produce superoxide radicals as shown by the fact that NAC did not inhibit AR downregulation by MSeA. They also found that selenite decreased AR expression by decreasing Sp1 expression which reduced the binding of Sp1 to its target site in the AR promoter and this effect was not seen with MSeA. Jiang *et al*. [67] have shown that selenite induces Ser15 phosphorylation of p53 and thereby activates caspase-mediated apoptotic pathway involving both caspase 8 and caspase 9 in LNCaP cells. MSeA inhibited the activation of the ERK and Akt pathways leading to the inhibition of cell survival, whereas selenite activates phospho- and total Akt, phospho-JNK, and phospho-p38 MAPK [68 – 70].

Selenite has been shown to regulate signal molecules, especially those involved in apoptotic and antiapoptotic signals. For example, the activities of NF-κB, AP-1, p53, HIF-1α, JNK, Akt/PI3K pathways are inhibited by redox regulation of their reactive cysteine residues. Treatment with MSeA causes G1 arrest in DU145 cells whereas selenite causes S phase arrest. G1 arrest induced by MSeA is accompanied by the increased expression of cdk inhibitors such as p27kip1 and p21cip1 and downregulation of cdk2 [71]. Gasparian *et al*. [72] have studied the effects of MSeA and selenite on the inhibition of NF-κB activity in JCA1 and DU145 PC cells. MSeA produced a rapid but transient inhibition whereas selenite produced a much slower but more consistent inhibitory effect on NF-κB. Selenite, which is reductively metabolized to methylselenides, was found to be effective in inhibiting both primary prostatic tumors as well as retroperitoneal lymph node metastases in nude mice with orthotopically implanted PC-3 tumors [50].

A report by Hu *et al*. [73] has shown that inorganic Se sensitizes PC cells to TRAIL-induced apoptosis through superoxide/p53/Bax-mediated activation of the mitochondrial pathway. Selenite induces a rapid superoxide burst and p53 activation, leading to Bax upregulation and translocation into mitochondria which restore the crosstalk with stalled TRAIL signaling for synergistic caspase 9/3 cascade-mediated apoptosis execution. Another report has shown that MSeA is also capable of sensitizing PC cells to TRAIL-

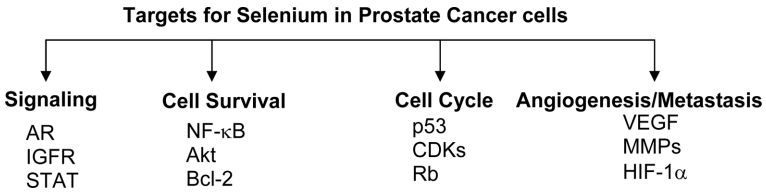

Figure 2. Schematic representation of cellular processes targeted by Se and some specific molecular targets in each pathway.

mediated apoptosis [74]. Akt and ERK have been shown to play a major role in the regulation of apoptosis sensitivity of LNCaP and DU145 cells to MSeA, whereas these kinases did not significantly regulate caspase-mediated apoptosis by selenite [75]. MSeA (10 μM) has been shown to deactivate Akt by reducing phosphorylation at Ser473 and Thr308 in PC-3 cells which are PTEN null [76]. Husbeck *et al*. [77] have tested the effects of selenite on normal and malignant cells derived from the same patient. They used selenite to treat three patient-matched pairs of primary prostatic epithelial cells from normal and cancerous tissues. They found that tumor-selective cell killing was achieved by selenite and this was facilitated by higher MnSOD expression in normal cells compared to cancerous ones.

We have shown that monomethylated Se in the form of MSC at pharmacological doses is able to inhibit the growth of LNCaP xenografts in nude mice, which was accompanied by a decrease in AR and PSA expression. These findings indicate that in addition to the chemopreventive role of Se it can also act as an antiandrogen and as a therapeutic agent for PC [53].

MSeA has been shown to potentiate apoptosis induced by chemotherapeutic agents in androgen-independent PC cells [78]. MSeA increased the potency of SN38 (topoisomerase I inhibitor), etoposide (topoisomerase II inhibitor), and paclitaxel in inducing apoptosis of DU145 and PC-3 cells and this was dependent on interactions with JNK-dependent targets to amplify the caspase 8-initiated apoptotic cascades. Ip *et al*. [51] have studied the *in vitro* and *in vivo* effects of MSeA and Se-MSC on apoptosis, cell cycle, and tumor inhibition. MSeA has been found to be more powerful *in vitro* than Se-MSC whereas tumor-inhibitory activities of both compounds were comparable *in vivo*.

One of the mechanisms involved in prevention of PC by Se is the prevention of DNA damage in prostate cells [79]. Waters *et al*. [80] suggested that DNA damage and apoptosis are Se-responsive events that may be important regulatory points in the multistep carcinogenesis. He *et al*. [81] showed that the upregulation of the membrane death receptor is coupled with the activation of caspase 8 and Bid cleavage and is accelerated by Se. Gene expression profiling provides an opportunity to unravel whole genome level changes, even very subtle ones, in cancer cells. Oligonucleotide array analysis of MSeA-treated DU145 cells showed altered expression of proteins involved in cell cycle, angiogenesis, apoptosis, cell–cell adhesion, tumor suppressors, DNA repair proteins, and several transcription factors [71]. Schlicht *et al*. [82] have found that the genes IGFBP3 (insulin-like growth factor-2 binding protein 3) and RXR-α (retinoid X receptor-α) were expressed in both human and rat prostate, induced by selenium and downregulated in PC. These changes were observed after 6 h and 5 days of treatment with 25 μM DL-SM, respectively. The different effects of different chemical forms of Se on signaling and expression of transcripts in PC cells might have important implications in the outcome of ongoing PC prevention clinical trials.

5 Mechanisms of Se anticancer effect

Several mechanisms have been suggested to mediate the anticancer effects of Se. The major ones are reduction of DNA damage [12, 47, 58, 83], oxidative stress [84, 49], inflammation; induction of phase II conjugating enzymes that detoxify carcinogens; enhancement of immune response [85, 86]; incorporation into selenoproteins; alteration in DNA methylation status of tumor suppressor genes [87, 88]; inhibition of cell cycle [37]; and angiogenesis and induction of apoptosis [89]. Mechanisms specific for PC are the inhibition of AR signaling, reduction in the mRNA, and protein levels of the androgen receptor, recruitment of corepressors to the AR elements in the promoters of androgen responsive genes, inhibition of signaling pathways like NF-κB, IL-6, Stat3, and induction of apoptosis (Fig. 2). Molecular and cellular bases for Se action in cancer prevention are still emerging and provide plausible rationale for clinical trials like SELECT.

Precursors of methylselenol in *in vitro* systems like MSeA have been shown to block cell cycle, induce apoptosis and inhibit angiogenesis by induction of caspases 1, 8, 10, and 12 [56, 71]. Gene expression studies using cDNA microarrays in both AR-positive (LNCaP) and AR-negative (PC-3) PC cells have indicated that Se exerts its effects at physiological levels by a dose-dependent inhibition of growth, cell cycle progression, and induction of apoptosis [55, 71]. These authors also found that Se downregulated genes like CYCLIN A, CDK1, CDK2, CDK4, DHFR, PCNA while upregulating genes like GADD153, CASPASE-9, CHK2, P19, P21, RXR, and several zinc finger proteins in PC-3 cells. On the other hand, Se also modulated AR signaling and expression and decreased expression of AR-regulated genes in AR-positive LNCaP cells, which was not seen in AR-negative PC-3 cells. In another study, Schlicht *et al*. [82] used human PC-3 and rat PAII PC cell

lines and found an association between upregulation of IGFBP3 and RXR-α with progression of PC.

A recent study showed that 30 nM – 7.6 μM Se in the form of MSeA eliminated NF-κB DNA binding without altering IκB levels. This was explained as possibly an interaction of Se with the NF-κB protein or an effect on recruitment of coactivators or corepressors. Inhibition of transcription factor DNA binding and antiapoptotic gene expression may be one of the mechanisms for the chemopreventive effects of Se on PC [90]. Ghosh [91] has shown that the apoptosis-inducing effect of Se is modified by metabolites of the arachidonate 5-lipoxygenase pathway suggesting that Se anticancer action may involve 5-lipoxygenase as a target. Venkateswaran *et al*. [2] have shown that Se potentiates vitamin E-induced inhibition of LNCaP cell growth *in vitro* by inducing a G1 phase arrest and decreasing the percentage of cells in the S phase. There have been several reports which show that Se alters methylation status of tumor suppressors and antioxidant enzymes in PC cells. In contrast, a study looking at methylation status of GSTP1 and RASSF1A genes after Se treatment concluded that Se did not alter the methylation status or expression of these genes [88]. Thus, the emerging picture of the action of Se includes the induction of pathways resulting in growth arrest, caspase-mediated apoptosis, reduced androgen signaling, and impaired angiogenesis by Se metabolites [92].

There are conflicting reports about the stages at which Se inhibits tumorigenesis. Several *in vitro* studies and some clinical intervention trials like the NPC found that Se protection is largely due to reduction of local disease and inhibition of early steps in tumorigenesis. These effects were found to be limited to men with PSA levels <4 ng/mL or less. Others and observation studies like that of Li *et al*. [14] found that Se affects PC progression rather than the primary disease and the effects are limited to men with PSA levels >4 ng/mL [93]. It has been reported that oral selenium accumulates preferentially in the substance of the prostate [36]. There has also been speculation about whether Se is involved in delaying or preventing transformation to cancer as opposed to inhibition or treatment of subclinical, microscopic cancer, but most reports agree that either effect would be largely beneficial with the alarming rate of latent disease found in men with PSA levels <4 ng/mL.

6 Selenoproteins

The human selenoproteome consists of 25 selenoproteins. The main groups are GSH peroxidases 1 – 5, iodothyronine deiodinases 1 – 3, thioredoxin reductases, selenoprotein P, and other proteins with mostly unknown functions [94]. The incorporation of Se into selenoproteins has been studied extensively and the effects of selenoproteins can vary by cell type, physiologic status, or the presence or absence of incorporated selenocysteine. Genetic variations (SNPs) in selenoproteins have been reported [95 – 98]. Different selenoprotein SNPs may respond differently to Se supplementation thus providing an explanation for the pharmacogenetic differences in selenium's preventive effects. Identification of a 15 kDa selenoprotein in the rat prostatic glandular epithelium and the discovery that it bound the UDP-glucose/glycoprotein glucosyltransferase that is involved in protein folding led to speculation that Se may have a role in the regulation of protein folding [99] but this does not explain the relatively high amounts of Se in the prostate.

Selenoproteins have received a great deal of attention due to the finding that selenoprotein-P is downregulated in PC cell lines and in the progression from high grade prostatic intraepithelial neoplasia (PIN) to metastatic PC [100]. A recent study has reported that selenoprotein deficiency accelerates prostate carcinogenesis in a transgenic model (with targeted expression of SV40 large T-antigen and an altered selenocysteine tRNA) [101]. In this study, selenoenzymes like GSH peroxidase and thioredoxin reductase have been shown to be important in the protective effects of Se against cancer. Another selenoprotein, Selenoprotein H, has been shown to be overexpressed in LNCaP cells and mouse lung cancer cells, LCC-1 [102]. Selenoprotein synthesis is a complex and highly regulated process. Se is incorporated as selenocysteine (Sec) at specific UGA codons in 25 human proteins. Selenoprotein mRNAs all contain a SECIS element needed for the incorporation of Sec into selenoproteins. Selenium supplementation may also modulate selenoprotein expression in PC cells. In an analysis of the modulation of selenoprotein expression in nontumorigenic prostate epithelial cells (RWPE-1) *versus* PC cells (LNCaP and PC-3) GPX-1 and GPX-4 were shown to be elevated to higher levels in LNCaP and PC-3 cells compared to RWPE-1 cells in response to supplemental Se (0 – 250 nM sodium selenite, sodium selenate, or SM). Significant differences are proposed to exist between PC cells and normal epithelial cells in their ability to utilize organic sources of Se. These results demonstrate that selenoproteins and selenium metabolism are differentially regulated at multiple levels in prostate cells [103]. Clinical observations which suggest that Se supplementation is only effective when baseline Se is at a level where selenoprotein production is regulated lend credence to the hypothesis that selenoprotein production is a likely mediator of the effects of Se chemoprevention [103].

7 Effects of Se on signaling pathways and cellular processes

7.1 AR

In PC ligand-associated AR signaling drives cell proliferation and many signaling pathways are constitutively active leading to persistent growth of cancer cells. Prostate cells go on accumulating genetic and epigenetic changes leading

to neoplastic transformation in normal cells and metastatic disease in the case of cancer cells. PC in the initial stages is completely androgen-dependent and androgen ablation is the therapy of choice after surgery or radiation. But several recent studies have suggested that androgen ablation does not constitute "androgen absence," and that low levels of androgen produced by adrenal glands or the tumor itself are sufficient to activate AR signaling [104, 105]. This has been postulated variously to be due to hypersensitivity of the receptor to low levels of androgens, AR gene amplifications, or mutations leading to gain-of-function of AR [106 – 108] as well as ligand-independent activation of the AR. These studies imply that AR signaling is important in the development as well as progression of PC and chemopreventive agents that can block AR signaling would be beneficial in a clinical setting.

Selenium has been reported to inhibit AR activity in LNCaP cells which is accompanied by a reduction in cell proliferation [109]. Dong *et al*. [54] have shown that 10 μM Se in the form of MSeA downregulates PSA expression by disruption of AR signaling. Se was able to inhibit PSA transcript and protein levels as well as AR protein and transcript levels. Se also inhibited the transactivating activity and DNA-binding ability of AR. When prostate epithelial cells were treated with 100 – 200 μM selenium or SM, DNA synthesis was dose-dependently reduced accompanied by a concomitant reduction in AR-activity [109]. Chun *et al*. [52] have shown that Se (10 μM MSeA) reduces the recruitment of coactivators to the promoters of AR-dependent genes while simultaneously enhancing the recruitment of corepressors. The mechanisms through which different Se compounds inhibit the AR signaling pathway vary; for example, MSeA and SM both produce mixed effects on the expression of androgen responsive genes. MSeA affects the expression of more androgen responsive genes than SM and also to a greater degree. Whereas 10 μM MSeA after 48 h can reduce the expression of both AR and PSA, 10 μM SM does not show any effect on these parameters. It requires higher concentrations or longer exposures for the effect of SM to manifest itself. Mechanisms involved in reduction of AR signaling include reduction in AR mRNA stability, increase in AR protein turnover, reduction in AR translocation, and inhibition of coactivator recruitment followed by corepressor recruitment to promoters of AR target genes [52]. These results are interesting in light of the fact that Se has been shown to be able to act as an antiandrogen in addition to its role as a chemopreventive agent [53]. It is tempting to speculate that doses of Se that are too low to show full chemopreventive activity may be used to antagonize AR signaling in a clinical setting.

7.2 Cell cycle

Deregulated cell cycle progression is one of the hallmarks of cancer cells. Cell cycle progression is regulated by the activity of CDKs, cyclins, and CDK inhibitors. CDKs are activated at different phases of the cell cycle, for example, CDK4 and CDK6 are activated in early and mid-G1 phase along with D-type cyclins, whereas CDK2-cyclin E and CDK1(cdc2)-cyclin B are activated during the S and G2/M phases, respectively [1]. Several genetic or epigenetic changes are known to deregulate cdc2 kinase activity in cancers including PC [110, 111]. Many prostate tumors harbor mutations in at least one of the CDKs, most frequently CDK2, CDK4, CDK6, or CDK1. Taken together, agents that provide checks in the process of deregulated cell proliferation due to aberrant activation of CDKs warrant further attention as chemotherapeutic or chemopreventive agents. Se has been shown to modulate the expression levels as well as activities of cyclins and cyclin-dependent kinases. Treatment with MSeA or selenite causes G1 and S arrest in DU145 cells. G1 arrest induced by MSeA is accompanied by the increased expression of cdk inhibitors such as p27kip1 and p21cip1 and downregulation of cdk2 [48, 71, 112]. MSeA (2 – 10 μM) was recently shown to induce G1 arrest in immortalized human microvascular endothelial cells and halt progression to S-phase [89]. This study also showed that MSeA was able to reduce tumor microvessel density in xenografts of human PC DU145 cells. These effects of MSeA were attributed to induction of increased binding of CDK inhibitors to CDKs 2, 4, and 6, and inhibition of hyperphosphorylation of Rb, thereby increasing steady-state levels of Rb-E2F1 complexes.

7.3 NF-κB

NF-κB has been implicated in the initiation as well as progression of several types of cancer, including PC [113]. Targets of the NF-κB pathway include cytokines, chemokines, cell adhesion molecules, survival and growth factors, genes involved in metastasis, angiogenesis, *etc*. Aberrant activation of the NF-κB transcription factor is found in hormone-refractory PC [114] and agents that can inhibit the NF-κB pathway may prove beneficial in the treatment of PC. Se inhibits NF-κB activity in androgen-dependent as well as androgen-independent PC cells [72]. A recent report has found that higher concentrations of Se (7.6 μM MSeA) compared to lower concentrations (50 nM MSeA) drastically reduced NF-κB DNA binding and consequently reduced the rates of transcription and mRNA levels of NF-κB-regulated genes [90]. This occurred in the absence of effects on the activation or translocation of the NF-κB transcription factor itself, suggesting that Se may interact with the protein itself or may affect the recruitment of coactivators.

7.4 Epidermal growth factor receptor

EGFR has been identified as being involved in the pathogenesis of many solid tumors including PC [115]. Many

studies have shown that increased expression of ligands for the EGFR pathway as well as high levels of EGFR lead to the formation of a constitutively active mitogenic signaling loop in androgen-independent PC cells [116]. Human PIN and primary as well as metastatic PC show frequent overexpression of the EGFR family members [117]. Few reports exist about the effects of Se on EGFR signaling. One study found that 5 μM organic Se in the form of SM (24 h) was able to enhance EGFR expression in androgen-responsive PC cells and not in androgen-independent cells [49].

7.5 Generation of reactive oxygen species (ROS)

ROS and the intracellular redox state have emerged as important determinants of cell signaling [66]. Alterations in the intracellular redox state can affect the activity of redox sensitive proteins *via* the oxidation of critical cysteine residues which may have downstream effects on signal transduction and gene transcription. The generation of ROS is a well-known and important mechanism of selenium- and ionizing radiation-induced cytotoxicity, in which ROS are scavenged by antioxidants like GSH. The intracellular level of GSH plays a major role in the ability of cells to withstand oxidative stress induced by ROS. Levels of GSH are normally elevated in cancer cells compared to normal cells and the antitumor activities of selenium compounds are dependent on the dose and chemical form of Se which can react with intracellular levels of GSH.

The inorganic form of Se, selenite, undergoes thiol-dependent reduction to selenide, which supplies Se for the synthesis of selenoproteins [118]. Even though at lower concentrations selenite acts as a micronutrient, at higher concentrations it is a potent prooxidant by producing superoxide ions. The addition of superoxide dismutase can abolish the cytotoxicity induced by selenite, confirming the importance of oxidative stress in the mechanism of selenite-induced cell death [57, 119]. Organic forms of selenium have been shown to have a predominantly antioxidant effect due to the incorporation of Se into selenoproteins and antioxidant enzymes like GSH peroxidase, GSH-s-transferase, *etc*., in the form of selenocysteine [118, 119]. Se has also been shown to induce ER stress in p53-null PC cells by redox modification of thiol-disulfide interchange in proteins leading to protein unfolding [83, 120]. MSeA induced several markers of ER stress like phosphorylated forms of PERK, eIF2a, GRP-78, and CHOP/GADD153. Selenite (10 μM) has been shown to enhance the radiosensitization of human PC cells LAPC-4 and DU145 [69]. GSH-depleted cells will be unable to eliminate ROS and repair DNA damage induced by ionizing radiation, whereby in addition to other mechanisms of cytotoxicity, selenite can have clinical implications as a radiosensitizer. But one needs to keep in mind that although selenite, SM, methylselenol, and other anticancer metabolites of Se induce cancer-preventive effects in cell culture, they are present at very low concentrations in plasma and tissues [62, 118, 121]. Hence, it is very important to determine whether these effects occur *in vivo* at bioavailable concentrations of Se and whether they can translate to clinically relevant findings.

7.6 Angiogenesis

Very little information is available as to the precise targets of Se in inhibition of angiogenesis in PC. A few reports state that inorganic Se (sodium selenate) inhibits the progression of experimental hormone refractory PC which is accompanied by inhibition of angiogenesis [50] and that monomethyl-Se can inhibit MMP-2 and VEGF expression, which was not seen with selenite [63]. A recent report has shown that treatment with an oral dose of 1 – 3 mg/kg wt of Se can reduce tumor microvessel density in DU145 xenografts [89]. VEGF is the most important mitogenic and survival factor for vascular endothelial cells in both normal and tumor environments. Binding of VEGF to its receptors stimulates receptor dimerization and activation of a signaling cascade leading to the proliferation of endothelial cells. Targeting VEGF signaling may provide an attractive target for chemoprevention since angiogenesis is the cornerstone of solid tumor development. MSeA, at lower concentrations than needed to induce apoptosis, has been shown to inhibit VEGF expression both in the cellular as well as secreted forms in DU145 PC cells [63, 122]. Effects on VEGF expression may precede the effects on the subsequent signaling molecules, leading to a rapid and sustained reduction in tumor angiogenesis.

The breakdown of tissue matrix to facilitate tumor cell migration is performed by matrix metalloproteinases and several tumor cell types, including PC cells, have been shown to overexpress MMPs. MMP-2 and MMP-9 are overexpressed in invasive PC [122, 123]. MSeA and methylselenocyanate have been shown to inhibit MMP-2 activity in a dose-dependent manner [63]. This was due to a decrease in expression level of MMP-2 and occurred after 0.5 h of exposure. Exposure to MSeA also decreased HUVEC cell tube formation ability *in vitro*, suggesting that Se is able to reduce angiogenesis *in vitro.* Information on the effect of Se on integrin signaling is lacking and needs to be addressed to adequately assess whether the observed effects of Se on VEGF and MMPs are specific to cancer cells or the results of a general inhibitory effect on cell migration and invasion.

8 Conclusions and future directions

How can the normal metabolic role of Se in the prostate be rationalized with the proapoptotic and antiproliferative efficacy of physiologically relevant Se metabolites? The answer possibly lies with the dose of Se. Even though normal serum/plasma Se concentrations in a person with nor-

mal Se-diet range between 70 and 200 ng/mL, most of this Se seems to be complexed with proteins and would be unavailable for the production of anticarcinogenic metabolites. When higher doses of Se are administered it increases the uptake of Se by organs such as the prostate, which would be consistent with the fact that supra-nutritional doses of Se are required to show efficacy in cell culture systems [124]. In a recent report in the JNCI, analysis of men participating in the National Institutes of Health (NIH)-AARP Diet and Health Study revealed that even though regular use of multivitamins is not associated with the risk of early or localized PC, there was an increased risk of developing advanced and fatal PCs among men taking high levels of multivitamin supplements along with other micronutrient supplements like selenium, β-carotene, and zinc [125]. This raises concerns about the dosage and combinations of multivitamins or micronutrients being administered to patients and to healthy men. Controversy about the efficacy and mechanism of action of Se still exists, so due caution is warranted in devising chemopreventive strategies to be used with established therapies to prevent unforeseen adverse effects.

Current cancer prevention strategies have been facilitated by advances in basic research into the genetic, environmental, nutritional, and epidemiologic factors that modulate cancer risk. Multifocal signal modulation therapy is one of the new strategies being visualized for PC therapy by targeting multiple signaling pathways. Androgen ablation is the standard signal modulation measure that is already in use. Additional molecular targets like the type I insulin-like growth factor receptor, hsp90, cyclooxygenase-2, protein kinase A type I, vascular endothelial growth factor, 5-lipoxygenase, 12-lipoxygenase, angiotensin II receptor type I, bradykinin receptor type I, c-Src, IL-6, ras, MDM2, bcl-2/bcl-xL, vitamin D receptor, estrogen receptor, and PPAR are being studied and validation of these molecular targets would greatly improve the efficacy of clinical regimen [126]. Targeted therapeutic strategies are not always successful due to the development of resistance to drugs as well as the development of alternative pathways for aberrant activation of signal molecules. Se has been shown to be effective against a variety of PC cells and to target several different signal cascades, thereby giving rise to the hope that it may prove to be a great tool in the hands of oncologists in the fight against PC [127]. Further research into the stage and dose at which Se is most effective against PC would hopefully result from the clinical trials and studies currently under way and may provide the groundwork for the use of Se for effective chemoprevention and/or therapy.

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