Selenite-induced p53 Ser-15 phosphorylation and caspase-mediated apoptosis in LNCaP human prostate cancer cells

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Abstract

The issue of p53 requirement for the caspase-mediated apoptosis induced by selenium in a cancer chemoprevention or chemotherapy context has not been critically addressed. We and others have shown that selenite induces apoptotic DNA laddering in the p53-mutant DU145 prostate cancer cells and the p53-null HL60 leukemia cells without the cleavage of poly(ADP-ribose) polymerase (PARP; i.e., caspase-independent apoptosis), whereas selenium compounds leading to the formation of methylselenol induce caspase-mediated apoptosis in these cells. Because selenite induces DNA single strand breaks, and because certain types of DNA damage activate p53, we investigated whether the human LNCaP prostate cancer cells, which contain a wild-type p53, execute selenite-induced apoptosis through caspase pathways. The results showed that exposure of LNCaP cells for 24 hours to lower micromolar concentrations of selenite led to DNA laddering, and to the cleavage of PARP and several procaspases. In contrast to this apoptosis sensitivity, LNCaP cells were rather resistant to similar concentrations of the methylselenol precursor methylseleninic acid. Selenite treatment led to a significant increase in p53 phosphorylation on Ser-15 ($Ser^{15}P$). Time course experiments showed that p53 $Ser^{15}P$ occurred several hours before caspase activation and PARP cleavage. The general caspase inhibitor zVADfmk completely blocked PARP cleavage, and significantly decreased DNA laddering, but did not affect p53 $Ser^{15}P$. An inhibitor for caspase-8 was equally as protective as that for caspase-9 against the selenite-induced apoptosis. Attenuating p53 by a chemical inhibitor pifithrin-α decreased the selenite-induced p53 $Ser^{15}P$ and led to concordant reductions of PARP cleavage and apoptosis. In summary, selenite-induced p53 $Ser^{15}P$ appeared to be important for activating the caspase-mediated apoptosis involving both the caspase-8 and the caspase-9 pathways in the LNCaP cells. [Mol Cancer Ther 2004;3(7):877–84]

Introduction

Selenium seems to be an important preventive and possibly therapeutic agent for cancers of the prostate, lung, and colon (1-4) as well as the liver (5). Apoptosis induction may in part mediate the anticancer activity of selenium metabolites. Our earlier work (6-8) has shown that selenium forms that enter the hydrogen selenide pool, such as sodium selenite, sodium selenide, and selenocystine, induce DNA single strand breaks (i.e., genotoxic) and apoptosis in leukemia and mammary cancer cells. Recently, we and others (9-11) have shown that the apoptosis induced by selenite in the p53-mutant DU145 prostate cancer cells (12) and the p53-null HL60 leukemia cells (13) did not involve caspases. Because certain types of DNA damage induce p53-mediated apoptosis which is often executed through caspases (14-19), we sought to determine whether p53 and caspases play important roles for the selenite induction of apoptosis in the p53–wild-type LNCaP prostate cancer cells.

The p53 tumor suppressor gene product is a transcription factor that enhances the transcription of several genes known to play a critical role in transducing signals from DNA damage (18). Its expression and activity are elevated in response to ionizing radiation, UV light, or certain genotoxic chemicals and mediate DNA repair, cell cycle arrest, and apoptosis (14-16, 18-21). The functional activity of p53 is regulated through transcription, translation, protein turnover, cellular compartmentalization as well as its association with other proteins, such as MDM2. In addition, posttranslational covalent modifications, such as phosphorylation and acetylation of specific amino acids of the p53 protein, have been known to affect p53 activity (16, 19-21). Ser-15 phosphorylation ($Ser^{15}P$) of p53 was originally reported in cells exposed to ionizing radiation, UV irradiation, and the topoisomerase I inhibitor camptothecin (20, 21). Since those initial reports, many publications have defined the p53 phosphorylation sites with specificity to the types of DNA damage stimuli and the kinetics of activation (reviewed in refs. 16, 19). For example, phosphorylation on Ser^{15} and other sites has been linked to apoptosis induced by chemotherapeutic drugs and chemopreventive agents (22-24).

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The role of p53 and its phosphorylation in selenium-induced apoptosis has not been critically evaluated. In fact, the current literature espouses a notion that apoptosis induced by selenium treatment is independent of the p53 status (8, 25). We report here, to our knowledge for the first time, experimental evidence for a potent induction of p53 Ser^{15} P by selenite exposure that is causally associated with activating caspase-dependent apoptosis in the LNCaP cells.

Materials and Methods

Chemicals and Reagents

Sodium selenite was purchased from J.T. Baker, Inc., Phillipsburg, NJ. Methylseleninic acid (MSeA; CH_3SeO_2H) was synthesized (26) and kindly provided by Dr. Howard Ganther, University of Wisconsin. Once taken up by cells, MSeA is thought to readily react with reduced glutathione and NADPH to generate methylselenol CH_3SeH. The caspase inhibitors zVADfmk (general), zDEVDfmk (caspase-3 and caspase-7, ref. 27), zIETDfmk (caspase-8), and zLEHDfmk (caspase-9) were purchased from Enzyme Systems Inc., Dublin, CA. Antibodies for the poly(ADP-ribose) polymerase (PARP) and the caspases as well as phospho-specific antibodies for p53 were purchased from Cell Signaling Technology, Beverly, MA. A β-actin antibody was purchased from Sigma Chemical Co., St. Louis, MO. The p53 chemical inhibitor pifithrin-α was purchased from Alexis Biochemicals, Carlsbad, CA.

Cell Culture and Treatments

LNCaP cells were obtained from the American Type Culture Collection, Manassas, VA, and were grown without antibiotics in the RPMI 1640 supplemented with 10% fetal bovine serum and 2 mmol/L L-glutamine. Cell cultures at 50% to 60% confluence, usually 48 hours after plating, were given a medium change and treated with selenium or other agents. To standardize selenium exposure conditions, cells were treated at a constant ratio of medium to culture surface equal to 0.2 mL/cm^2 (e.g., 15 mL for a T75 flask and 5 mL for a T25 flask). Concentrated selenium stock solutions were diluted in PBS to 1 mmol/L working solutions before addition to the culture medium. The caspase inhibitors and other hydrophobic agents were dissolved in DMSO as concentrated stocks. After necessary dilutions, the inhibitor(s) and selenite were mixed into the treatment media first and then fed to the cells. DMSO (2 μL/mL or less) was added to cells that did not receive the inhibitor to control for any solvent effects. DMSO at the concentration used did not cause any observable adverse morphologic responses.

Apoptosis Evaluation

After treatments, the adherent cells were released into the culture media by tapping the flasks hard on the side. The cells were collected by centrifugation at 200 × *g* for 5 minutes. The detection of DNA laddering was done by agarose gel electrophoresis as previously described (6, 9). In some experiments, more quantitative assessments were done using a cell death detection ELISA kit (Roche Diagnostics Corporation, Indianapolis, IN). This kit measured oligonucleosomes released by apoptotic cells after gentle lysis of the cell membrane through an antibody for the core histones and an antibody for DNA in a double sandwich arrangement. The cleavage of PARP and procaspases was detected by immunoblot as markers of caspase-mediated apoptosis (9). Flow cytometric analyses of apoptotic sub-G_1 fractions were carried out on 70% ethanol fixed cell suspension with propidium iodide staining using a Becton-Dickinson flow cytometer with MODFIT program.

Immunoblot Analyses

The cell pellet collected as above were washed in PBS twice and lysates were prepared as described previously (9). The lysates were clarified by centrifugation at 14,000 × *g* for 20 minutes at 4°C and the supernatants were recovered. The protein contents were quantified by the Bradford dye-binding assay (Bio-Rad Laboratories, Richmond, CA) or Lowry method (Sigma-Aldrich, St. Louis, MO). After size-separation by electrophoresis on SDS-polyacrylamide gels, the proteins were electroblotted onto nitrocellulose membranes and probed for total and phospho-p53 and other proteins of interest, as previously described (9).

Caspase Activity Assay

The cell pellets as harvested above were washed twice with PBS and were lysed in an ice-cold lysis buffer provided with the caspase assay kits from R&D Systems, Minneapolis, MN. The lysates were centrifuged for 20 minutes at 14,000 × *g* at 4°C. The resulting supernatants were analyzed for protein concentration by Lowry method for normalization of assay results on protein basis. The caspase fluorometric enzymatic activity assays were carried out per manufacturer's instructions. The fluorogenic substrates were DEVD-7-amino-4-trifluromethyl coumarin (AFC) (caspases-3, -7), IETD-AFC (caspase-8), and LEHD-AFC (caspase-9).

Results

Cell Death Responses of LNCaP Cells to Selenite and MSeA Exposure

The exposure of LNCaP cells for 24 hours to sodium selenite resulted in DNA laddering in a concentration-dependent manner (Fig. 1A). As little as 3 μmol/L selenite was sufficient to induce robust DNA laddering. On the other hand, LNCaP cells showed only a modest response to MSeA exposure. They required as much as 10 μmol/L MSeA to induce a detectable level of DNA laddering. Flow cytometric analyses of sub-G_1 fractions showed that 4 μmol/L selenite exposure led to approximately 3 times greater extent of apoptosis than did 10 μmol/L MSeA (Fig. 1A). The apoptosis ELISA for the oligonucleosomes confirmed the above concentration-dependent patterns of cell death responses to selenite and MSeA (Fig. 1B). These results indicated that LNCaP cells were more sensitive to apoptosis induction by selenite than by MSeA.

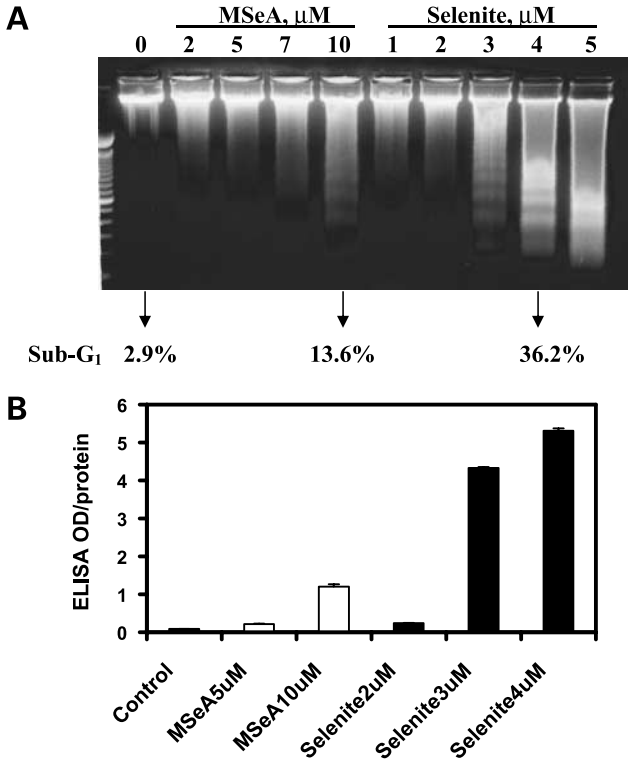

Figure 1. **A,** agarose gel (1.5%) electrophoretic detection of nucleosomal DNA fragmentation (laddering) in LNCaP cells after 24 hours exposure to increasing concentrations of MSeA or selenite. The *leftmost lane* was loaded with 100-bp DNA size markers. Flow cytometric analysis of apoptotic sub-G_1 fraction indicated the approximate extent of apoptosis of selected selenium doses. **B,** ELISA measurement of apoptotic oligonucleosomes for the estimation of overall death. Each value is the average of two flasks, each measured in duplicate.

Selenium-Induced Caspase Activation

Exposure of LNCaP cells for 24 hours to 3 μmol/L or higher concentrations of selenite led to a concentration-dependent increase of PARP cleavage (Fig. 2A). MSeA exposure at 10 μmol/L led to less PARP cleavage than that induced by 3 μmol/L selenite. To define which caspases were involved in PARP cleavage during apoptosis induced by selenite and MSeA exposure, we evaluated the cleavage status of pro-caspases-8, -9 (initiator caspases) and -3, -6, and -7 (executioner caspases). All five pro-caspases had undergone cleavages in the selenite-treated cells at the exposure concentrations of 3 μmol/L or higher (Fig. 2A). For the MSeA-treated cells, only 10 μmol/L MSeA induced a low level of cleavage of all five caspases. The measurement of DEVD-AFC hydrolysis (caspase-3 and caspase-7 activity) further supported the differential sensitivity of LNCaP cells to these two forms of selenium (Fig. 2B). These results provided solid evidence for the involvement of multiple caspases in the apoptosis induced by selenite exposure in LNCaP cells. Furthermore, LNCaP cells were relatively resistant to the induction of caspase-mediated apoptosis by MSeA.

p53 Expression and Phosphorylation

Because an obvious difference between LNCaP cells and DU145 or HL-60 cells was the p53 status (12, 13), we examined the expression and phosphorylation status of p53 in LNCaP cells after 24 hours exposure to selenite or MSeA. Data in Fig. 3 showed that selenite exposure moderately increased (~2 folds) the expression levels of total p53 protein and one of its transcriptional targets, p21cip1 (18). At the same time, the expression of a p53-independent cell cycle inhibitory protein, p27kip1, was minimally affected by selenite, thus, supporting its specific activation of p53.

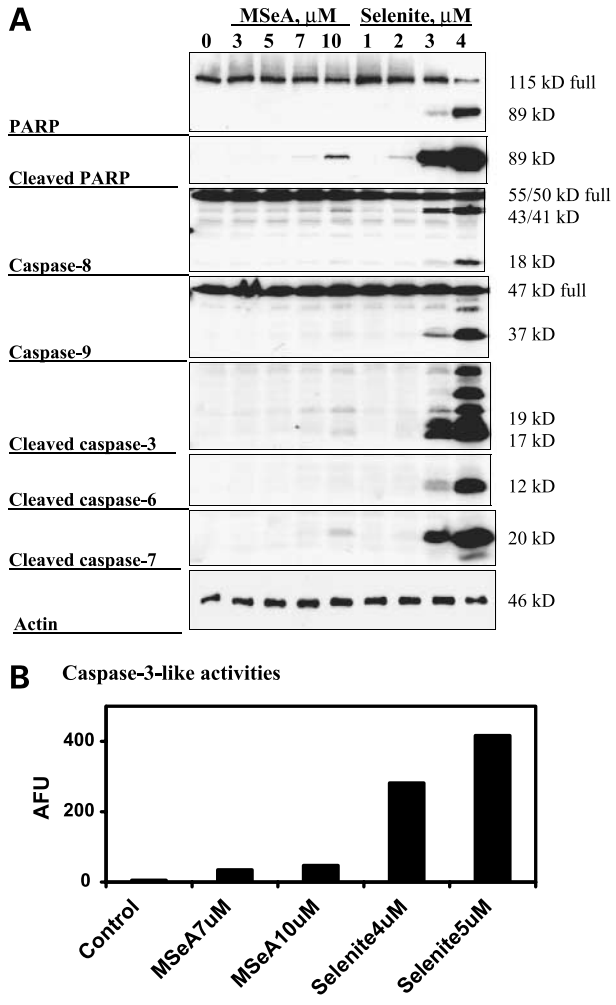

Figure 2. **A,** immunoblot detection of the cleavage of PARP and selected pro-caspases in LNCaP cells after 24 hours exposure to increasing concentrations of MSeA or selenite. Adherent cells and detached floaters were combined for cell lysate preparations for these analyses. β-Actin was probed to indicate the evenness of loading of the protein extract from each treatment. Proteins of interest are marked by size. Results were representative of two independent experiments. **B,** fluorometric measurement of caspase-3–like (DEVD-AFC hydrolysis) activities in LNCaP cells exposed to MSeA and selenite for 12 hours. *AFU*, arbitrary fluorescence unit. *Columns*, average of two T75 flasks, normalized to protein content, variability <5%.

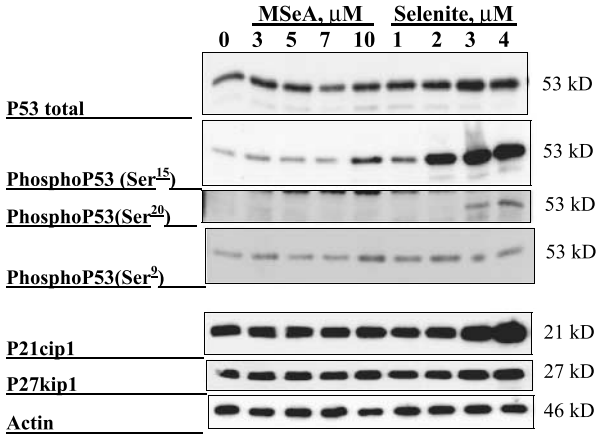

Figure 3. Immunoblot detection of the expression and phosphorylation status of p53 in LNCaP cells after 24 hours exposure to selenite and MSeA. The expression levels of a p53-target protein p21cip1 and a non-p53 target cell cycle inhibitory protein p27kip1 were also determined. β-Actin expression was probed to indicate the evenness of loading of the protein extract from each treatment. These results are representative of two independent experiments.

A significant and concentration-dependent increase of p53 $Ser^{15}P$ was detected in LNCaP cells exposed to selenite. Only weak $Ser^{20}P$ was detected in the selenite-exposed cells after a prolonged exposure of the X-ray films as evident by the darker background. Neither selenite nor MSeA altered $Ser^{9}P$. Phosphorylation at several other sites (Ser^{6}, Ser^{37}, Ser^{46}, Ser^{392}) was not detectable within the X-ray film exposure duration used for enhanced chemiluminescence (~1 hour). These results indicated that p53 $Ser^{15}P$ represented a major posttranslational modification by the selenite exposure. In contrast, the p53 expression level and $Ser^{15}P$ were minimally affected by the MSeA treatment in the concentration range tested (Fig. 3).

Time Course of p53 $Ser^{15}P$ and Caspase Activation in Selenite-Exposed Cells

A time course experiment (Fig. 4A) showed the detection of p53 $Ser^{15}P$ at 4 hours and the cleavage of pro-caspases and PARP after 8 hours of selenite exposure. A more detailed time course experiment showed that p53 $Ser^{15}P$ was observed as early as 2 hours of selenite treatment, whereas PARP cleavage was detected at 8 hours (Fig. 4B). Consistent with the kinetics of caspase cleavage patterns detected by immunobloting, the hydrolysis of DEVD-AFC (caspase-3 and caspase-7 activity) and LEHD-AFC (caspase-9) was detectable as early as 4.5 to 5 hours of exposure to selenite, whereas the hydrolysis of IETD-AFC (caspase-8) was observed by 5.5 hours (Fig. 5). Together, the results of these experiments indicated a temporal relationship of p53 $Ser^{15}P$ several hours ahead of multiple caspase activation.

Effect of Caspase Inhibitors on p53 $Ser^{15}P$ and Apoptosis Induced by Selenite

The general caspase inhibitor zVADfmk (80 μmol/L) completely blocked the PARP cleavage induced by selenite exposure but did not affect p53 $Ser^{15}P$ (Fig. 6A). However, zVADfmk did not completely prevent DNA laddering, suggesting the existence of a minor component of selenite-induced apoptosis that was independent of caspases. The inhibitor for caspase-3 and -7 (zDEVDfmk), -8 (zIETDfmk), or -9 (zLEHDfmk), when used individually at 80 μmol/L, was nearly as effective as the general caspase inhibitor at blocking PARP cleavage and decreasing DNA laddering (Fig. 6A). None of these inhibitors, however, affected the extent of the selenite-induced p53 $Ser^{15}P$.

The lack of resolving power of the above experiment on the protective efficacy of the caspase inhibitors was likely due to overlapping inhibitory spectra at the high concentration of each inhibitor used. Therefore, we examined their efficacy at lower levels to estimate the relative contribution of the caspase-8 and the caspase-9 pathways to the selenite-induced apoptosis execution. As shown in Fig. 6B, when used at 5 and 10 μmol/L, the protective efficacy of the inhibitors was as follows: zVADfmk > zDEVDfmk (caspase-3 and caspase-7) > zIETDfmk (caspase-8) = zLEHDfmk (caspase-9). Together, these data indicated that p53 $Ser^{15}P$ was upstream of caspase activation and that both caspase-8 and caspase-9 contributed about equally to the death signaling induced by selenite treatment in LNCaP cells. The experimental approaches used did not reveal any hierarchical relationship between these two initiator caspases.

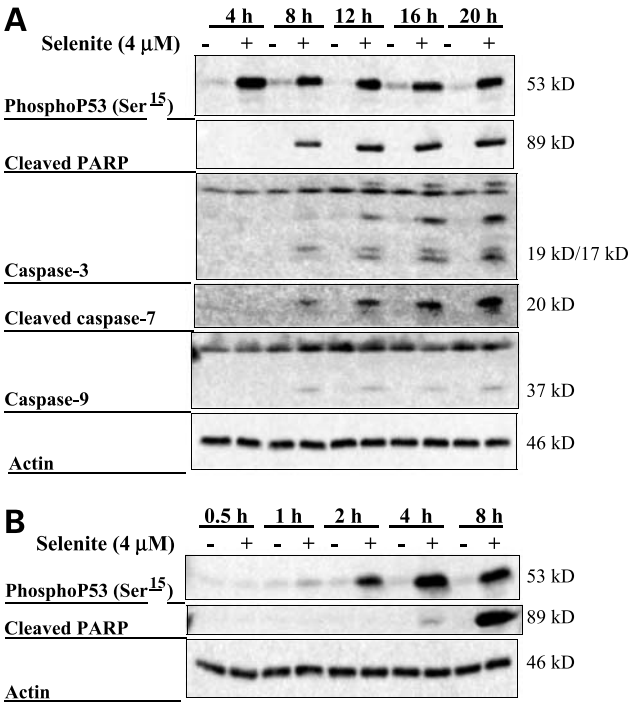

Figure 4. Time course of selenite induction of p53 $Ser^{15}P$ and the cleavage of PARP and selected pro-caspases in LNCaP cells detected by immunobloting. β-Actin expression was probed to indicate the evenness of loading of the protein extract from each treatment. **A,** 4 to 20 hours exposure duration; **B,** 0.5 to 8 hours exposure duration.

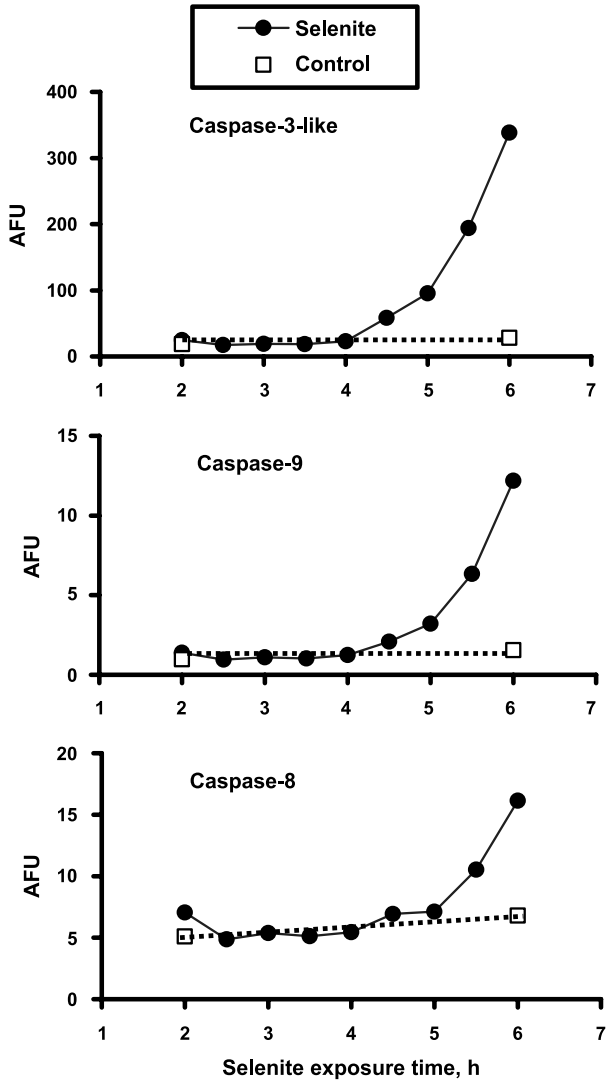

Figure 5. Kinetics of selenite induction of the hydrolytic activity of LNCaP cells on DEVD-AFC (caspases-3, -7), LEHD-AFC (caspase-9), and IETD-AFC (caspase-8) substrates detected by a fluorometric assay. *AFU*, arbitrary fluorescence unit. *Points*, average of duplicate measurements of a single T75 flask, variability <2%. The activation patterns are representative of two independent experiments.

Effect of a p53 Inhibitor on PARP Cleavage and Apoptosis Induced by Selenite

To test the hypothesis that p53 $Ser^{15}P$ is causally linked to the caspase activation and apoptosis induced by selenite exposure, we examined the effects of pretreatment with pifithrin-α, a chemical inhibitor of p53 activities (28), on the caspase-mediated PARP cleavage and overall apoptosis. Pifithrin-α decreased the selenite-induced p53 $Ser^{15}P$ and decreased PARP cleavage in concordant extents (Fig. 7A). These changes were accompanied by similar extents of reduction of the overall apoptosis detected by ELISA (Fig. 7B). The data were consistent with a cause-effect relationship between p53 $Ser^{15}P$ and the caspase-mediated apoptosis induced by selenite in LNCaP cells.

Discussion

The present study investigated the role of p53 and its phosphorylation in apoptosis induction in the LNCaP prostate cancer cells by two selenium compounds with opposite genotoxicity profiles (7, 8, 10). The data presented here show that the LNCaP cells are very sensitive to apoptosis induction by selenite exposure (Fig. 1). These results agreed well with two recent studies that reported similar sensitivity of this cell line to the selenite-induced apoptosis (29, 30). We provided convincing evidence that the selenite-induced apoptosis was predominantly executed through caspases (Figs. 2 and 6). Yet the failure of the general caspase inhibitor zVADfmk to completely suppress overall apoptosis induced by selenite exposure also supported the existence of a minor yet caspase-independent apoptosis pathway (Fig. 6). The finding of caspase-mediated apoptosis induction by selenite in LNCaP cells is novel in contrast with previous studies about the role of caspases in selenite-induced apoptosis (9, 11). In our earlier work with the p53-mutant DU145 prostate cancer cells, caspase activities were not observed in apoptosis induced by selenite (9). The absence of caspase involvement was independently confirmed in apoptosis of the p53-null

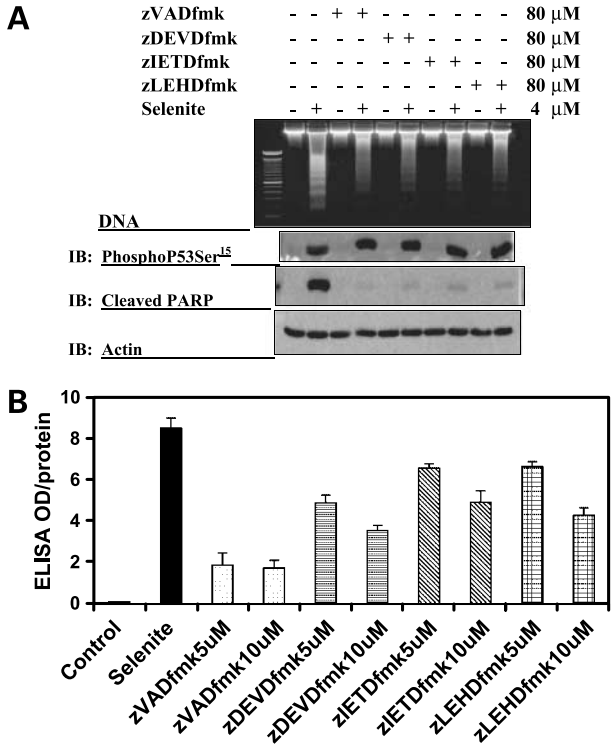

Figure 6. **A,** effects of the general caspase inhibitor zVADfmk and inhibitors of caspases-3 and -7 (*zDEVDfmk*) and for caspase-8 (*zIETDfmk*) and caspase-9 (*zLEHDfmk*) on the selenite-induced DNA fragmentation, PARP cleavage, and p53 $Ser^{15}P$ in LNCaP cells at 16 hours of exposure. Each inhibitor was used at a final concentration of 80 μmol/L. **B,** protective efficacy of lower concentrations of caspase inhibitors against the selenite-induced apoptosis detected by ELISA after 24 hours selenite exposure. *Columns*, average of two T25 flasks, each measured in duplicates.

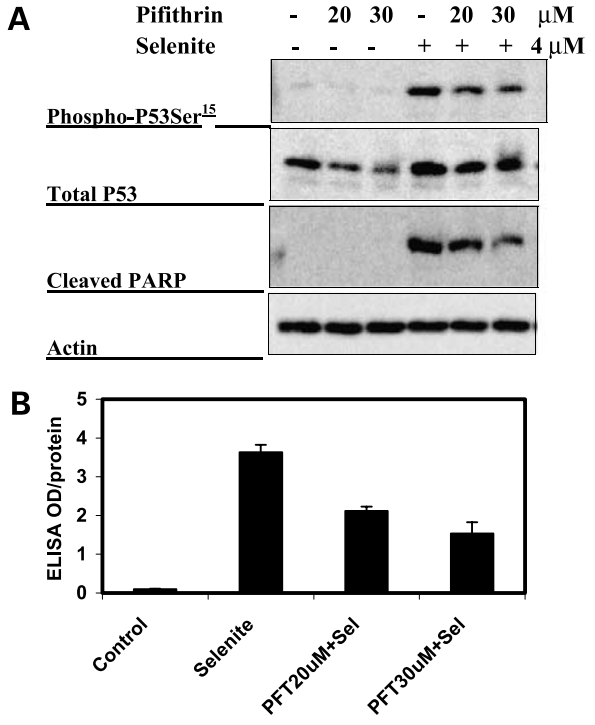

Figure 7. **A,** effect of pifithrin-α (*PFT*) on selenite-induced p53 $Ser^{15}P$ and PARP cleavage. LNCaP cells were pretreated with pifithrin-α for 24 hours and the medium was replaced with fresh medium containing pifithrin-α with or without selenite. Cells were harvested 16 hours after initiation of selenite exposure. **B,** protective efficacy of pifithrin-α against selenite-induced apoptosis detected by ELISA for oligonucleosomes. *Columns*, mean of three independent experiments; *bars*, SD. The protective effects of both doses of pifithrin-α were statistically significant, $P < 0.01$.

HL-60 leukemia cells induced by selenite (11). These two studies support caspase-independent apoptosis by selenite exposure in cancer cells with a nonfunctional p53 background, consistent with a prevailing notion in the selenium literature that the induction of apoptosis by selenium is independent of wild-type p53 (8, 25). In light of our findings reported here, more systematic investigations with respect to the selenium metabolites and the p53 functional status in caspase-dependent and caspase-independent apoptosis signaling are warranted.

There are several novel aspects about our findings. First, our data support a crucial involvement of caspases in LNCaP cells for the execution of apoptosis on selenite exposure. Multiple caspases were activated as detected by pro-caspase cleavages (Figs. 2 and 4) and activity assays (Fig. 5). The general caspase inhibitor led to a major protection against overall apoptosis (Fig. 6), indicating that the caspase-mediated apoptosis accounts for the bulk of death signaling induced by selenite (Fig. 8). Our data suggest that the caspase-8 and the caspase-9 activation pathways contributed about equally to the activation of the major executioner caspases, such as caspases-3 and -7. This pattern of caspase activation differed from that induced by MSeA in DU145 cells wherein caspase-8 is a major initiator caspase that is upstream of and activates caspase-9 (9). In LNCaP cells, the protective effect of zDEVDfmk (caspase-3 and casapse-7) was less than that of the general caspase inhibitor zVADfmk (Fig. 6B), hence indicating that additional caspases (such as caspase-6 detected in Fig. 2A) in concert with these major executioner caspases (i.e., -3 and -7) fully execute the death program. In view of the equal contribution of the caspase-8 and the caspase-9 pathways, future work will examine the detailed mechanics of caspase activation with respect to the death receptor-DISC-caspase-8 and the mitochondria-cytochrome *c*-caspase-9 cascades (31, 32) as well as the endoplasmic reticulum–stress activated caspases (33).

The second novel aspect is the induction of p53 $Ser^{15}P$ by selenite exposure. Our data documented for the first time this posttranslational modification of p53 in the selenium-treated cancer cells. The time course experiments (Figs. 4 and 5) supported a temporal sequence as follows: Selenite→p53 $Ser^{15}P$→caspase activation→DNA laddering/death execution. Consistent with this scheme, p53 $Ser^{15}P$ induced by selenite exposure was not affected by inhibiting caspases with the general or the specific inhibitors (Fig. 6A). Furthermore, attenuation of p53 activity with pifithrin-α

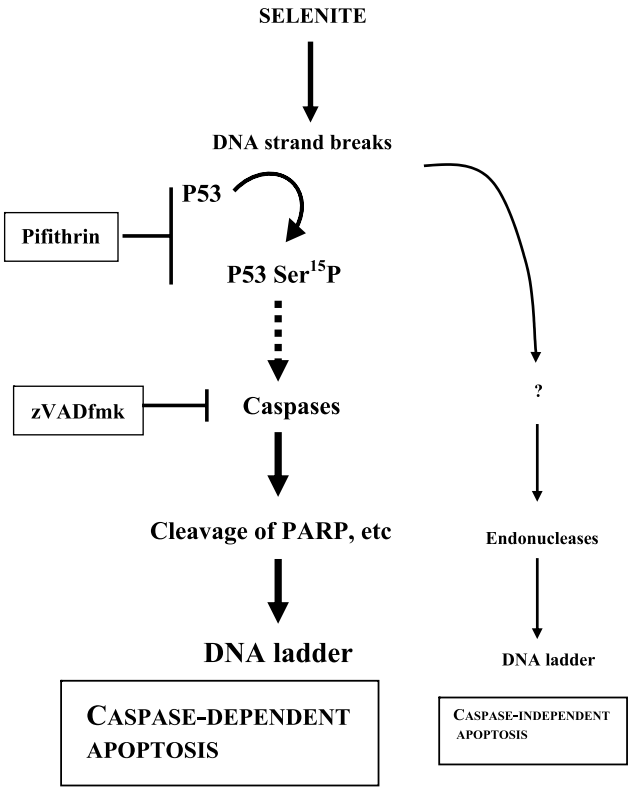

Figure 8. Proposed caspase-dependent and caspase-independent pathways of apoptosis induction by selenite in LNCaP cells. Extensive literature supports the selenite induction of DNA strand breaks in a variety of cell types including mammary, leukemia, colon, liver, and fibroblast cells. This model proposes a central role of the wild-type p53 and its $Ser^{15}P$ for signaling to the caspase pathways in LNCaP cells. The general applicability of this model requires confirmation.

decreased p53 $Ser^{15}P$ and led to concordant reductions of the PARP cleavage and overall death (Fig. 7), consistent with a possible cause-effect relationship between p53 $Ser^{15}P$ and the activation of caspases. It could be postulated that a p53-dependent transcription of pro-apoptotic genes (17, 18) in both the caspase-8 cascade (death receptor signaling) and the caspase-9 cascade (mitochondria signaling) may be involved in signaling from p53 $Ser^{15}P$ to caspases. The time lag of several hours between p53 $Ser^{15}P$ and caspase activation was compatible with a transcriptional mechanism.

Taken together, the data reported here and previously (6-10) lead to a hypothetical model as depicted in Fig. 8. The model proposes that selenite treatment induces DNA single strand breaks, which in turn trigger the p53 phosphorylation and caspase-mediated apoptosis pathway as well as the caspase-independent apoptosis pathway in the LNCaP cells. In the absence of a wild-type p53, DNA strand breaks induce caspase-independent apoptosis as has been reported for the DU145 cells (9) and HL60 cells (11). Although the p53 chemical inhibitor data (Fig. 7) were consistent with a cause-effect relationship between p53 $Ser^{15}P$ and signaling to the caspases in LNCaP cells, genetic approaches through re-introduction of wild-type p53 into DU145 and other p53 null prostate cancer cells and p53 knockdown in LNCaP cells will be necessary to critically affirm the p53 requirement for caspase activation. It is imperative that in future experiments, the kinetics of selenite induction of DNA single strand breaks be validated in relationship to p53 phosphorylation in the LNCaP cells. It will also be important to test whether selenite-induced p53– and caspase-mediated apoptosis is specifically confined to the prostate cancer cells only. If so, a prostate specificity may help to explain the human prevention trial data that have shown the prostate as the most responsive organ site for the selenium prevention of cancer (1, 2, 4).

Further work is also needed to elucidate the protein kinase(s) involved in the selenite-induced p53 phosphorylation. In this regard, the highly specific nature of p53 $Ser^{15}P$ with a minimal involvement of other serine residues (Fig. 3) suggests that the likely candidate protein kinases are ATM and/or ATR (34, 35), rather than the DNA-dependent protein kinase which catalyzes the phosphorylation of p53 on both Ser^{15} and Ser^{37} (20). Consistent with this hypothesis, a recent report showed that ATM mediates the selenite-induced apoptosis in NIH3T3 mouse embryonic fibroblasts (36).

In addition to these major novel points discussed above, our data also showed that LNCaP cells, in spite of their sensitive apoptosis response to selenite exposure, were rather resistant to the induction of apoptosis by MSeA, requiring as much as 10 μmol/L MSeA to induce a detectable level of DNA nucleosomal fragmentation and PARP cleavage. In contrast, under the same growth conditions, MSeA induces caspase-mediated apoptosis in DU145 cells at only 4 μmol/L through a process resembling anoikis (9), which is a mode of apoptosis due to the deprivation of adherent cells to the extracellular matrix support (37). A plausible explanation may reside in the constitutively active AKT survival pathway in LNCaP cells because of a mutation in PTEN (38), which is a lipid phosphatase that negatively regulates AKT activity. The phosphatidylinositol 3-kinase (PI3K)/AKT survival pathway protects cells against apoptosis in many cell types (39). DU145 cells possess a wild-type PTEN and much lower basal AKT activity than that observed in LNCaP cells. Work is in progress to critically test the hypothesis that the high AKT activity in LNCaP cells is responsible for conferring a resistance to apoptosis induction by methylselenol or its precursors.

Prostate cancer is the second leading cause of cancer deaths among North American men. The landmark prevention trial by Clark et al. (1-4) has indicated a particular sensitivity of prostate cancer to selenium intervention. The work presented here illustrates a causal association of p53 $Ser^{15}P$ and caspase-mediated apoptosis in a p53–wild-type prostate cancer cell line on exposure to selenite, which is a representative of the genotoxic hydrogen selenide pool. Our results imply that the functional integrity of the p53 tumor suppressor protein in the prostate cancer cells may be a determinant of the chemopreventive and/or chemotherapeutic efficacy of selenium. The selenium in the selenized yeast used in the Clark trial (1-4) was mostly selenomethionine, accounting for 85% of total selenium (40). *In vivo* catabolism of selenomethionine will likely undergo *trans*-selenation to selenocysteine, analogous to the *trans*-sulfuration from methionine to form cysteine. Selenocysteine is acted upon by cysteine β-lyase to release hydrogen selenide. Therefore, we speculate that in early stage prostate cancer development, such as in the cancer prevention setting, a wild-type p53 protein may confer to the target prostate epithelial cells a greater sensitivity for caspase-mediated apoptosis induced by the *in vivo* metabolism of these seleno amino acids. A testable prediction is that the prostate cancers that had appeared in the selenium prevention group would more likely bear mutant or nonfunctional p53 than those in the placebo group in this remarkable study (1). The merit of this prediction could be tested using archival samples from the Clark trial and prospective samples in the ongoing Selenium and Vitamin E Cancer Prevention Trial (SELECT), which aims to validate the prostate cancer preventive efficacy of selenomethionine (41).

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