

# Delineating the mechanism by which selenium deactivates Akt in prostate cancer cells

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## Abstract

The up-regulation of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway is prevalent in many cancers. This phenomenon makes PI3K and Akt fruitful targets for cancer therapy and/or prevention because they are mediators of cell survival signaling. Although the suppression of phospho-Akt by selenium has been reported previously, little information is available on whether selenium modulates primarily the PI3K-phosphoinositide-dependent kinase 1 (PDK1) side of Akt phosphorylation or the phosphatase side of Akt dephosphorylation. The present study was aimed at addressing these questions in PC-3 prostate cancer cells which are phosphatase and tensin homologue-null. Our results showed that selenium decreased Akt phosphorylation at Thr308 (by PDK1) and Ser473 (by an unidentified kinase); the Thr308 site was more sensitive to selenium inhibition than the Ser473 site. The protein levels of PI3K and phospho-PDK1 were not affected by selenium. However, the activity of PI3K was reduced by 30% in selenium-treated cells, thus discouraging the recruitment of PDK1 and Akt to the membrane due to low phosphatidylinositol-3,4,5-trisphosphate formation by PI3K. Consistent with the above interpretation, the membrane localization of PDK1 and Akt was significantly diminished as shown by Western blotting. In the presence of a calcium chelator or a specific inhibitor of calcineurin (a calcium-dependent phosphatase), the suppressive effect of selenium on phospho-Akt(Ser473) was greatly reduced. The finding suggests that selenium-mediated dephosphorylation of Akt via calcineurin is likely to be an additional mechanism in regulating the status of phospho-Akt. [Mol Cancer Ther 2006;5(2):246–52]

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## Introduction

Akt/protein kinase B is a pleckstrin homology domain-containing serine/threonine kinase (1). Akt mediates survival and antiapoptotic signalings and is activated indirectly by phosphatidylinositol 3-kinase (PI3K; refs. 2, 3). PI3K converts the membrane lipid phosphatidylinositol-4,5-bisphosphate to phosphatidylinositol-3,4,5-trisphosphate; the latter then recruits various pleckstrin homology domain-containing kinases, including phosphoinositide-dependent kinase 1 (PDK1) and Akt, to the membrane. The proximity of Akt to PDK1 in the membrane microenvironment facilitates the phosphorylation of Akt at Thr308 by PDK1. Full activation of Akt requires phosphorylation at Ser473 (3) in addition to Thr308. Little is known about the kinase responsible for phosphorylation at Ser473 until recently with the identification of the rictor-mammalian target of rapamycin (mTOR) complex (4). Based on studies with PDK1-deficient cells (5) or PDK1 inhibitor (6), it seems that phosphorylation at Ser473 is not dependent on prior phosphorylation at Thr308.

Protein phosphatase is equally important in controlling the phosphorylation status of Akt. There are three major serine/threonine protein phosphatases: PP1, PP2A, and PP2B. PP1 generally targets the Ser473 site when phospho-Akt is without the protection of HSP90 (7). PP2A is a key Akt phosphatase (8, 9) and can be up-regulated by intracellular calcium (10). PP2B, also known as calcineurin, is calcium dependent (11); the relationship between calcineurin and Akt has not been clarified.

The PI3K-Akt survival pathway is a fruitful target for cancer therapy (12). This is premised on the prevalent up-regulation of PI3K-Akt activity in cancer cells due to the abnormal accumulation of phosphatidylinositol-3,4,5-trisphosphate. Phosphatase and tensin homologue (PTEN) is the 3'-phosphatase that metabolizes phosphatidylinositol-3,4,5-trisphosphate to maintain its homeostasis. In many tumors, PTEN is either absent or nonfunctional, thus leading to excessively high levels of phosphatidylinositol-3,4,5-trisphosphate (13). Consequently, Akt is hyperactivated; the outcome is to stimulate proliferation, depress apoptosis, and intensify drug resistance. In prostate cancer, PTEN deletion or mutation is frequently correlated with a high Gleason grade or poor prognosis (14, 15). The loss of PTEN suggests that targeting the PI3K-Akt pathway may have preventive and/or therapeutic value.

The laboratory of Lu has published a series of articles showing the decrease of phospho-Akt by selenium in human prostate cancer cells and endothelial cells (16–20). However, there is scanty information available on how selenium might differentially affect the phosphorylation of Thr308 and Ser473 or whether selenium modulates primarily the PI3K-PDK1 side or the phosphatase side of phospho-Akt turnover. In the present study, we treated

PC-3 cells with methylseleninic acid (MSA) and examined its effect on (a) the phosphorylation kinetics at both the Thr308 and Ser473 sites; (b) the expression and activity of PI3K and PDK1; (c) the membrane localization of PDK1 and Akt; (d) the change in intracellular calcium; and (e) the role of calcineurin in decreasing phospho-Akt.

## Materials and Methods

### Cell Culture

The PC-3 human prostate cancer cell line was purchased from the American Type Culture Collection (Manassas, VA). The cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 100 units/mL penicillin/streptomycin, and 2 mmol/L glutamine and maintained at 37°C in an atmosphere of 5% CO<sub>2</sub> and 95% air. PC-3 cells are PTEN-null (21) and have a high expression of phospho-Akt.

### Selenium Reagent

MSA was used in all cell culture experiments. It was synthesized as previously described (22). This selenium compound was developed specifically for *in vitro* studies because of certain unique attributes (23). Seleno-amino acids are generally not suitable for cell culture experiments because many epithelial cells, including prostate cells, have a low capacity in converting seleno-amino acids to the active metabolite.

### Chemicals and Antibodies

BAPTA-AM, a cell-permeable calcium chelator, was purchased from Calbiochem (San Diego, CA). Cyclosporin A was purchased from AG Scientific, Inc. (San Diego, CA). The following antibodies were purchased from Cell Signaling Technology (Beverly, MA): phospho-Akt (Ser473), phospho-Akt(Thr308), Akt, p85 PI3K, phospho-PDK1(Ser241), and PDK1. The above antibodies are all polyclonal except for phospho-Akt(Ser473) which is monoclonal. A monoclonal glyceraldehyde-3-phosphate dehydrogenase antibody (Chemicon, Temecula, CA) was used for loading control.

### Western Blotting

Cell lysate for SDS-PAGE was prepared in 1× cell lysis buffer (Cell Signaling Technology) containing 1 mmol/L phenylmethylsulfonyl fluoride (Sigma, St. Louis, MO), 50 mmol/L NaF, and 1 tablet/7 mL of Mini Complete Protease Inhibitor (Roche Applied Science, Indianapolis, IN). Protein concentration of the lysate was determined by using the Bicinchoninic Acid Protein Assay Kit (Pierce Biotechnology, Rockford, IL). In preparing for SDS-PAGE, cell lysate was mixed with one-third volume of 4× SDS sample buffer [200 mmol/L Tris-HCl (pH 6.8), 8% SDS, 0.4% bromophenol blue, 40% glycerol, 60 μL/mL β-mercaptoethanol] and heated at 100°C for 10 minutes. Polyvinylidene difluoride membranes containing the transferred proteins were blocked in 5% nonfat dry milk in TBS-T buffer [10 mmol/L Tris-HCl (pH 7.5), 150 mmol/L NaCl, 0.1% Tween 20] at room temperature for 1 hour before probing with the primary antibodies and the horseradish peroxidase-conjugated secondary antibodies (Amersham

Biosciences, Piscataway, NJ). Protein bands were visualized by the SuperSignal West Pico Chemiluminescent Substrate kit (Pierce Biotechnology) or the Enhanced Chemiluminescence Plus Western blotting Detection System (Amersham Biosciences). Immunoreactive bands were quantitated by volume densitometry with the ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

### PI3K Activity Assay

PI3K activity was assayed according to the methods of Endemann et al. (24) and Huang et al. (25). PC-3 cells were cultured on 100-mm plates to ~80% confluence. Cells were then incubated in fresh medium with or without MSA for 2 hours. The incubation cultures were washed once with ice-cold PBS and lysed in 400 μL of lysis buffer [20 mmol/L Tris-HCl (pH 8.0), 137 mmol/L NaCl, 1 mmol/L MgCl<sub>2</sub>, 10% glycerol, 1% NP40, 1 mmol/L DTT, 0.4 mmol/L sodium orthovanadate, 1 mmol/L phenylmethylsulfonyl fluoride], then solubilized on ice for 10 minutes. The lysates were centrifuged and the protein concentration of the clear lysates was determined by bicinchoninic acid assay. Cell lysates containing 300 μg of total protein were incubated overnight with agarose-conjugated anti-phospho-tyrosine monoclonal antibody Py-20 (Santa Cruz Biotechnology, Santa Cruz, CA). The agarose beads were washed twice with each of the following buffers: (a) PBS with 1% NP40, 1 mmol/L DTT; (b) 100 mmol/L Tris-HCl (pH 7.6), 0.5 mol/L LiCl, 1 mmol/L DTT; and (c) 10 mmol/L Tris-HCl (pH 7.6), 100 mmol/L NaCl, 1 mmol/L DTT. The beads were incubated for 5 minutes on ice in 20 μL of buffer 3, followed by the addition of 20 μL of 0.5 mg/mL D-myosphatidylinositol (Echelon Biosciences, Inc., Salt Lake City, UT) in 50 mmol/L HEPES (pH 7.6), 1 mmol/L EGTA, and 1 mmol/L NaH<sub>2</sub>PO<sub>4</sub>. After 5 minutes of incubation at room temperature, 10 μL of the reaction buffer [100 mmol/L HEPES (pH 7.6), 50 mmol/L MgCl<sub>2</sub>, 250 μmol/L ATP containing 5 μCi of [γ-<sup>32</sup>P]ATP] were added and the beads were incubated for 15 minutes. The reaction was stopped by adding 15 μL of 4 N HCl and 130 μL of chloroform/methanol (1:1). After vortexing for 30 seconds, 30 μL from the chloroform phase were spotted onto Silica Gel 60 TLC plates (Sigma). The plates were previously coated with 1.3% potassium oxalate and 2 mmol/L EDTA in H<sub>2</sub>O/methanol (3:2) and heated at 100°C for at least 3 hours before use. The spotted plates were placed in tanks containing chloroform/methanol/NH<sub>4</sub>OH/H<sub>2</sub>O (600:470:20:113) and developed for 40 to 50 minutes or until the solvent reached the top of the plates. The plates were dried at room temperature and autoradiographed. The PI3K product PI-3-phosphate was quantitated by densitometry of the radioactive spots. Three independent experiments were done for statistical analysis.

### Intracellular Free Calcium Measurement

The change in intracellular free calcium was measured by flow cytometry with a double fluorescent calcium indicator dye system (26). Fluo-3-AM (Molecular Probes, Eugene, OR) is a cell-permeable calcium dye. When bound to calcium, Fluo-3 emits a green fluorescent signal, the intensity of which increases with free calcium concentration. Fura Red-AM (Molecular Probes) is also a cell-permeable

calcium dye. When bound to calcium, the red fluorescence of Fura Red is weakened; the decay of the red fluorescent signal is used to monitor the increase of calcium. The green/red ratio of the fluorescent signals provides a more precise measurement than the use of a single dye because this self-normalizing system minimizes the interference from any change in cell volume during the experiment. Cells were treated with 10  $\mu\text{mol/L}$  MSA for 2 hours. The cultures were washed carefully twice with the loading buffer [calcium-free HBSS supplemented with 1 mmol/L calcium, 1 mmol/L magnesium, and 1% (v/v) of heat-inactivated fetal bovine serum], followed by incubation with 2  $\mu\text{mol/L}$  Fluo-3-AM and 5  $\mu\text{mol/L}$  Fura Red-AM at 30°C for 1 hour. The total MSA treatment time was 3 hours. After washing gently with the loading buffer, the cells were trypsinized and suspended in the loading buffer for flow cytometry. The cell suspensions were kept in a 37°C water bath during the procedure. The green/red ratio was plotted as a function of time to show the change in intracellular calcium. The samples were run for 2 minutes for the system to reach steady state before data collection for 3 minutes. At the end of each assay, a calcium ionophore, ionomycin (Calbiochem), was added at a concentration of 2  $\mu\text{g/mL}$ . Ionomycin facilitates calcium influx. If the dyes are functioning properly, the addition of ionomycin should induce an instant increase of the green/red ratio.

#### Membrane Fractionation

Membrane fraction was prepared by a method adapted from that described by Fillipa et al. (27) and Taylor et al. (28). Briefly, PC-3 cells were trypsinized and washed in cold PBS (pH 7.4) and suspended in ice-cold hypotonic buffer [20 mmol/L HEPES (pH 7.4), 5 mmol/L NaCl, 10 mmol/L  $\text{MgCl}_2$ , 1 mmol/L EDTA, 1 mmol/L DTT, 10 mmol/L NaF, 2 mmol/L orthovanadate] containing a protease inhibitor cocktail (Roche Applied Science) and 0.5 mmol/L phenylmethylsulfonyl fluoride. Cells were disrupted with a Dounce homogenizer at 4°C. The homogenate was centrifuged at  $3,000 \times g$  for 10 minutes to remove the nuclear fraction. The supernatant was centrifuged at  $100,000 \times g$  for 30 minutes at 4°C. The pellet from this high-speed centrifugation is the membrane fraction.

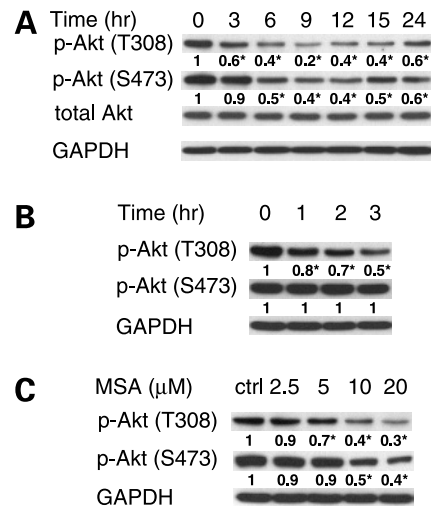
#### Statistical Analysis

The Student *t* test was used to determine statistical differences between treatment and control values;  $P < 0.05$  was considered significant.

## Results

### MSA Decreases Both Phospho-Akt(Thr308) and Phospho-Akt(Ser473)

The effect of MSA on the kinetics of Thr308 and Ser473 phosphorylation was examined by Western blot with specific antibodies. Figure 1A shows the 24-hour time course experiment with 10  $\mu\text{mol/L}$  MSA. The decrease of phospho-Akt(Thr308) was detectable as early as 3 hours (60% of control) and reached a nadir at 9 hours of treatment (20% of control). After that, it started to recover and by 24 hours, it was back to 60% of control. Phospho-Akt(Ser473)



**Figure 1.** Effect of MSA on phospho-Akt(Thr308) and phospho-Akt(Ser473), as determined by Western blotting, in PC-3 cells. **A**, response to treatment with 10  $\mu\text{mol/L}$  MSA as a function of time in a 24-h experiment. **B**, acute response to treatment with 10  $\mu\text{mol/L}$  MSA in a short-term experiment. **C**, dose-dependent response to MSA at 6 h. The numbers beneath the bands denote the fold of change relative to the zero time value or control value. \*,  $P < 0.05$  ( $n = 3$ ).

followed a similar pattern of change although there were subtle differences when compared with phospho-Akt(Thr308). A significant decrease of phospho-Akt(Ser473) was first noted at 6 hours; this was 3 hours later than the decrease of phospho-Akt(Thr308). The depressed level of phospho-Akt(Ser473) hovered around 40% to 50% of control for almost the remaining duration of treatment. Thus, phospho-Akt(Ser473) seemed to have a lower sensitivity to MSA inhibition compared with phospho-Akt(Thr308). Total Akt did not change with MSA treatment.

Figure 1B shows the acute response to MSA. In this experiment, cells were treated for 1, 2, or 3 hours. Phospho-Akt(Thr308) started to decrease as early as 1 hour. In agreement with the results of Fig. 1A, the phosphorylation level was markedly reduced at 3 hours. In contrast to phospho-Akt(Thr308), phospho-Akt(Ser473) remained unchanged during this early time interval.

Figure 1C shows a dose dependence of phospho-Akt down-regulation. Cells were treated for 6 hours with MSA at 2.5, 5, 10, or 20  $\mu\text{mol/L}$ . Both phospho-Akt(Thr308) and phospho-Akt(Ser473) were resistant to 2.5 or 5  $\mu\text{mol/L}$  MSA. Raising the concentration of MSA to 10 or 20  $\mu\text{mol/L}$  produced a pronounced inhibition. Consistent with the above observations, phospho-Akt(Thr308) was more sensitive to MSA inhibition than phospho-Akt(Ser473).

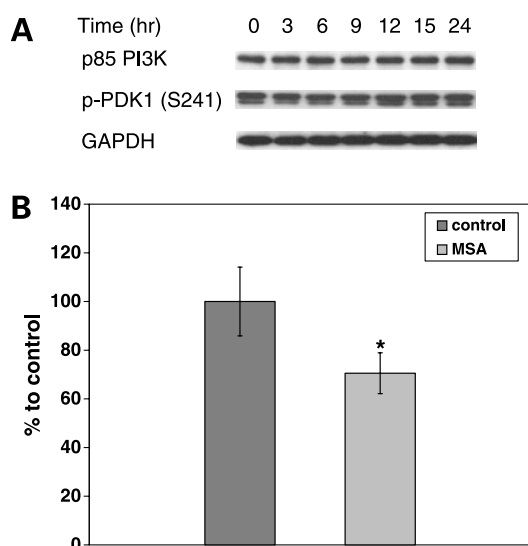
### MSA Inhibits PI3K Activity

The Thr308 site is known to be a direct target of PDK1. Our next step was to find out whether PI3K and PDK1 are inhibited by MSA. Cells were treated with 10  $\mu\text{mol/L}$  MSA for 24 hours. The p85 subunit of PI3K was used to determine the protein level of PI3K whereas the activation status of PDK1 was quantified by phosphorylation at

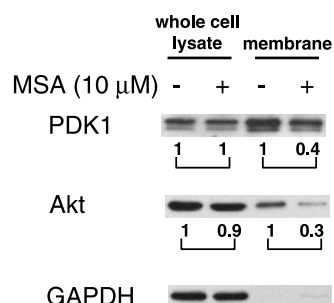
Ser241 (29). Figure 2A shows the Western blot results of p85 PI3K and phospho-PDK1(Ser241). The amount of p85 PI3K protein remained unchanged with time. Likewise, phospho-PDK1(Ser241) was not affected by MSA. As noted in Introduction, the rictor-mTOR complex is the newly identified kinase responsible for Akt phosphorylation at Ser473. We failed to find a significant reduction of mTOR protein by MSA nor could we find any change in phosphorylation of mTOR at Ser2448 and Ser2481 (data not shown). The antibody to rictor is not available commercially. Thus, we were not able to study the effect of MSA on the rictor-mTOR complex. Because neither PI3K protein, PDK1 activation, nor total mTOR was modulated by MSA, we decided to use the PI3K activity assay, which measures the formation of PI-3-phosphate, to determine whether the function of PI3K might be compromised. Figure 2B shows the results of the PI3K activity assay. Cells were treated with 10  $\mu\text{mol/L}$  MSA for 2 hours before the assay. The activity of PI3K was decreased by  $\sim 30\%$  in the MSA-treated cells.

#### MSA Decreases Membrane Localization of PDK1 and Akt

Inhibition of PI3K activity causes a decrease of phosphatidylinositol-3,4,5-trisphosphate and is expected to impede the recruitment of PDK1 and Akt to the membrane. To test the hypothesis, membrane fraction was prepared from control cells or MSA-treated cells. The amount of Akt in whole-cell lysate or membrane fraction was determined by Western blot using a total PDK1 antibody and a total Akt antibody. An equal amount of protein from each fraction was loaded on the gel. The results are shown in Fig. 3. Cytosolic contamination of the membrane fraction was ruled out by the absence of glyceraldehyde-3-phosphate dehydrogenase. In whole-cell lysate, there was no signif-



**Figure 2.** **A**, effect of 10  $\mu\text{mol/L}$  MSA on protein levels of PI3K and phospho-PDK1 as a function of time. **B**, effect of 10  $\mu\text{mol/L}$  MSA on PI3K activity as determined by production of PI-3-phosphate. Results expressed as percent of untreated control. \*,  $P < 0.05$  ( $n = 3$ ).



**Figure 3.** Effect of MSA on membrane localization of PDK1 and Akt after treatment for 3 h. The numbers beneath the bands denote the fold of change relative to the untreated control value from either the whole-cell lysate or membrane fraction ( $n = 3$ ).

icant difference in PDK1 and Akt between the control and MSA-treated cells. However, in the membrane fraction from MSA-treated cells, the amount of PDK1 and Akt was markedly decreased to 40% and 30% of control, respectively.

#### MSA Elevates Intracellular Free Calcium

It has been reported that selenium triggers calcium release from the endoplasmic reticulum, thereby increasing free calcium in the cytosol (30). The elevation of free calcium may activate calcineurin, which is calcium dependent. Calcineurin might decrease phospho-Akt by dephosphorylation. To explore this possibility, we first tested the ability of MSA to increase intracellular free calcium with the use of flow cytometry. Cells were treated for 3 hours with 10  $\mu\text{mol/L}$  MSA and the change in free calcium was followed by the change in the ratio of green and red emissions from two fluorescent calcium indicator dyes. The results are shown in Fig. 4. Compared with the baseline value in untreated cells, the calcium level in MSA-treated cells was elevated by 2-fold. This increase lasted for several minutes under the experimental condition. A longer measurement was not possible due to the limitation of the flow cytometry instrumentational setup. Nonetheless, the increase of calcium by MSA was highly reproducible. As a positive control, we spiked cells with the ionophore ionomycin. The immediate surge of calcium confirmed the proper functioning of the calcium dyes.

#### Calcium and Calcineurin May Cause Decrease of Phospho-Akt(Ser473) by MSA

Elevated calcium may contribute to the decrease of phospho-Akt by activating calcineurin. BAPTA-AM, a cell-permeable calcium chelator, and cyclosporin A, a specific calcineurin inhibitor, were used to further investigate the role of calcium. The concentration of each chemical was optimized to eliminate any confounding effect on Akt phosphorylation in the absence of MSA treatment. Cells were treated with 10  $\mu\text{mol/L}$  MSA for 6 hours with or without BAPTA-AM (15  $\mu\text{mol/L}$ ) or cyclosporin A (50  $\mu\text{mol/L}$ ). The change in phospho-Akt(Thr308) and phospho-Akt(Ser473) was determined by Western blot. As shown in Fig. 5A and B, neither BAPTA-AM nor cyclosporin A significantly prevented the decrease of

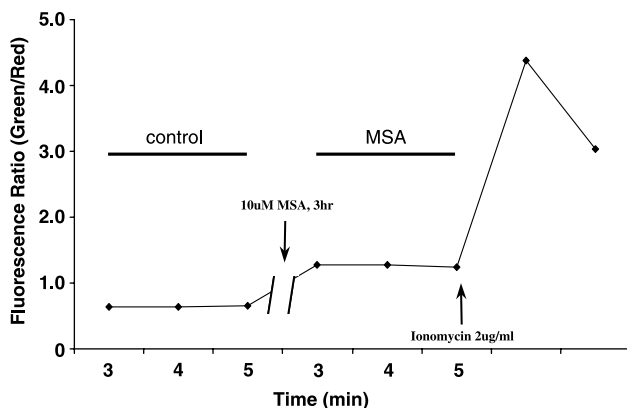


phospho-Akt(Thr308) by MSA. On the other hand, both BAPTA-AM and cyclosporin A successfully attenuated the decrease of phospho-Akt(Ser473) by MSA. MSA alone caused a 60% decrease whereas in the presence of BAPTA-AM or cyclosporin A, MSA caused only a 20% or 30% decrease, respectively. Figure 5C shows that neither BAPTA-AM nor cyclosporin A significantly affected total Akt.

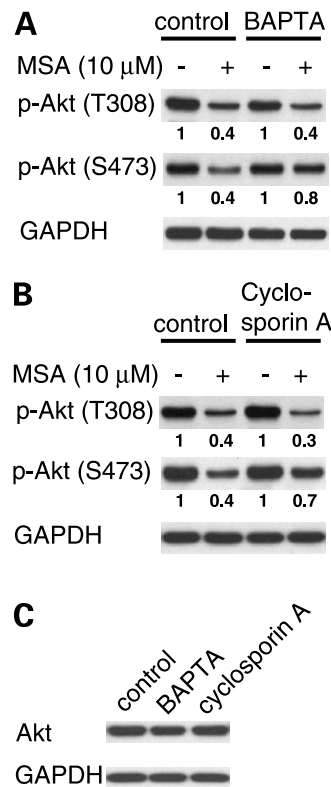
Two other phosphatases, PP1 and PP2A, have been implicated in Akt dephosphorylation. We tried two PP1 and PP2A inhibitors, calyculin A and okadaic acid, in an attempt to see whether they could weaken MSA depression of phospho-Akt. At a concentration high enough to block both phosphatases, neither inhibitor was able to counteract the effect of MSA on Akt phosphorylation (data not shown). Thus, at least in PC-3 cells, PP1 and PP2A do not seem to play a role in the decrease of phospho-Akt by MSA.

## Discussion

We report for the first time that the down-regulation of phospho-Akt by selenium is due to a decrease in PI3K activity (not protein level) and an increase in the dephosphorylation mechanism which may potentially involve calcineurin. Our findings are illustrated in the schematic diagram of Fig. 6. A low PI3K activity means low abundance of phosphatidylinositol-3,4,5-trisphosphate, and this condition does not favor the recruitment of PDK1 and Akt to the membrane. Because PDK1 is the kinase that phosphorylates Akt at Thr308, limiting the contact of Akt with PDK1 is expected to block Akt activation. The full activation of Akt requires phosphorylation at both Thr308 and Ser473. A recent study identified rictor-mTOR as the kinase responsible for phosphorylating Ser473 (4). Although neither total nor phosphorylated mTOR was affected by selenium, it is possible that selenium may down-regulate the rictor-mTOR complex.



**Figure 4.** Elevation of intracellular free calcium by MSA. The results are expressed as the change in the green/red fluorescent signal ratio in flow cytometry as described in Materials and Methods. Ionomycin is a positive control to confirm the proper functioning of the calcium indicator dyes.

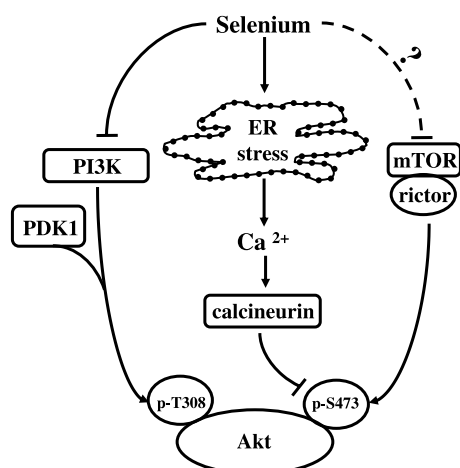


**Figure 5.** **A**, effect of BAPTA, a calcium chelator, on the down-regulation of phospho-Akt by MSA. **B**, effect of cyclosporin A, a specific inhibitor of calcineurin, on the down-regulation of phospho-Akt by MSA. **C**, effect of BAPTA or cyclosporin A alone on total Akt.

The fact that BAPTA-AM and cyclosporin A could not fully reverse the decrease of phospho-Ser473 suggests that an effect of selenium on rictor-mTOR should not be excluded.

Our study provides evidence that selenium-mediated calcium release is involved in controlling the phosphorylation status of Akt. Previously, Xia et al. (30) reported that in skeletal muscle cells, selenium causes calcium release from the endoplasmic reticulum through thiol oxidation of the ryanodine receptor. We also have data showing that selenium is a potent inducer of endoplasmic reticulum stress in PC-3 cells (31) and endoplasmic reticulum stress is often accompanied by calcium release from the endoplasmic reticulum (32). It is interesting that both BAPTA-AM and cyclosporin A attenuate the decrease of phospho-Akt by selenium. BAPTA-AM prevents the increase of intracellular free calcium by chelation whereas cyclosporin A is a specific inhibitor of calcineurin, which is a calcium-dependent phosphatase. Taken together, these two pieces of information suggest that calcineurin might be a direct phosphatase of phospho-Akt.

The discovery that selenium could down-regulate PI3K activity is important. PI3K mediates multiple signals in addition to PDK1-Akt (33, 34). A number of transducer molecules containing the pleckstrin homology domain are



**Figure 6.** Schematic diagram showing the various mechanisms by which selenium deactivates Akt. The narrative to this diagram is summarized in the first two paragraphs of Discussion.

regulated by binding to phosphatidylinositol-3,4,5-trisphosphate, as exemplified by Rac, Rho, and cdc42. These molecules are known to modulate cytoskeletal rearrangement and actin polymerization associated with cell migration. Thus, inhibiting PI3K could prevent tumor invasion and metastasis. How might selenium interfere with the activity of PI3K? Selenium is a potent redox modulator. Recent evidence showed that selenium is capable of causing global protein thiol/disulfide interchange which may lead to alterations of protein function (35). Previous studies describing the redox modification of protein kinase C and p53 by selenium and their resulting changes in activity are supportive of this explanation (36–38).

What is the implication of targeting Akt in cancer control strategy? The Akt pathway is constitutively active in some prostate cancer due to mutations of PTEN (incidentally, PC-3 cells are PTEN-null). However, selenium is also effective in inhibiting Akt phosphorylation in cells expressing the wild-type PTEN (16, 18, 20). Thus, our present observation is not unique to PTEN-mutant or PTEN-null cells. The downstream targets of Akt include BAD, caspase-9, FKHR, and glycogen synthase kinase 3 $\beta$ , just to name a few. Phosphorylation of BAD by Akt interferes with the binding of BAD to Bcl-2 or Bcl-X<sub>L</sub>, thereby preventing apoptosis (39). The proapoptotic activity of caspase-9 is also diminished by phosphorylation (40). When FKHR is phosphorylated, it is sequestered in the cytosol and is unable to carry out its function as a transcription factor in facilitating the expression of proapoptotic genes, such as *FasL*, as well as cell cycle inhibitory genes, such as *p27<sup>Kip1</sup>* (41). The role of glycogen synthase kinase 3 $\beta$  is to suppress the positive proliferation regulators, such as c-myc and cyclin D, by phosphorylation. Glycogen synthase kinase 3 $\beta$  activity is turned off when it is phosphorylated by Akt, thus releasing the cells from growth arrest (42). In prostate cancer cells, glycogen synthase kinase 3 $\beta$  phosphorylation of  $\beta$ -catenin leads to its accumulation; the latter is an

androgen receptor coactivator (43). In summary, Akt activation forges a tempo which accelerates cell proliferation and decelerates apoptosis. Finding a way to slow down this tempo would be desirable. Our future research will be focused on validating the changes in some of these Akt targets when phospho-Akt is down-regulated by selenium and evaluating their functional significance in selenium-mediated growth inhibition.

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