Selenium Disrupts Estrogen Signaling by Altering Estrogen Receptor Expression and Ligand Binding in Human Breast Cancer Cells

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Abstract

Cancer prevention studies suggest that selenium is effective in reducing the incidence of cancers including prostate, colon, and lung cancers. Previous reports showed that selenium inhibits premalignant human breast MCF-10AT1 and MCF10AT3B cell growth *in vitro* and reduces mammary tumor incidence after exposure to carcinogens in tumor models. Because estrogen is critical to the development and differentiation of estrogen target tissues, including the breast, the present study was designed to examine the effect of selenium on estrogen receptor (ER) expression and activation using methylseleninic acid (MSA), an active form of selenium *in vitro*. Selenium decreased the levels of expression of ERα mRNA and protein and reduced the binding of labeled estradiol to estrogen receptor in MCF-7 cells. Selenium inhibited the *trans*-activating activity of estrogen receptor in MCF-7 cells expressing functional estrogen receptor using a luciferase reporter construct linked to estrogen responsive element. Selenium decreased the binding of estrogen receptor to the estrogen responsive element site using an electrophoretic mobility gel shift assay. Selenium suppressed estrogen induction of the endogenous target gene c-myc. In contrast to the effect on ERα in MCF-7 cells, selenium increased ERβ mRNA expression in MDA-MB231 human breast cancer cells. Thus, differential regulation of ERα and ERβ in breast cancer cells may represent a novel mechanism of selenium action and provide a rationale for selenium breast cancer prevention trial. (Cancer Res 2005; 65(8): 3487-92)

Introduction

The growth of breast cancer cells is known to be regulated by estrogen through binding to estrogen receptor (ER), which affects cell growth by inducing cell proliferation (1–3) and preventing apoptotic cell death (4, 5). Estrogen receptor is a hormone-dependent transcription factor that belongs to the steroid/thyroid hormone receptor superfamily. Estrogen receptor is activated by ligand binding, followed by receptor conformational changes and dimerization and binding to estrogen response elements located in the promoter of estrogen regulated genes. The majority of the actions of estrogen are mediated by ERα and ERβ. Since estrogen effects can be modulated by the agonists and antagonists of these receptors, compounds that block estrogen signaling have proven useful in the treatment of estrogen receptor–positive breast cancer patients. Tamoxifen is widely used for the treatment of breast cancer (6).

A landmark cancer prevention trial showed that selenium supplementation was effective in reducing the incidence of cancers including prostate, lung, and colon cancers (7). This trial did not detect a statistically significant change in breast cancer risk by selenium supplementation (7). A likely explanation is the very small number of breast cancer cases in both the placebo and treatment groups, since women accounted for only about 25% of the cohort. A number of prospective case-control studies have also failed to observe decreases of breast cancer risk in women with higher blood or toenail selenium levels (8, 9). A recent epidemiologic study indicated that lower serum concentrations of selenium in women with breast cancer compared to healthy women seemed to be a consequence, rather than a cause, of cancer (10). It should be noted that these epidemiologic studies were designed to evaluate the impact of selenium within the range of dietary intake, i.e., without supplementation. Although the effect of selenium within the range of dietary intake on breast cancer risk was not observed, pharmacologic concentrations of selenium effect were detected in mammary tumor models (11, 12). Several studies showed the efficacy of selenium in the reduction of mammary tumor incidence after exposure to carcinogens, including 2-acetylaminofluorene, methylnitrosourea, and 7,12-dimethylbenz(*a*)anthracene (13–16). In addition, selenium inhibited the growth of two premalignant human breast cell lines by blocking cell cycle progression at the G_0-G_1 phase and inducing apoptotic cell death (17). Methylseleninic acid (CH_3SeO_2H, abbreviated as MSA) was developed specifically for *in vitro* studies (18), since cultured cells respond poorly to selenomethionine (a commonly used selenium reagent) due to very low levels of β-lyase activity, which is required for conversion of selenomethionine to the active methylselenol (19). The effect of physiologic concentrations of MSA on cultured cells has been documented in several publications (18, 20–22). Although selenium is an effective chemopreventive agent in mammary models, the effect of selenium on estrogen receptor signaling is currently unknown. In the present study, the effect of MSA on estrogen receptor signaling in breast cancer cells was examined. MCF-7 cells are hormone-dependent breast cancer cells that express high levels of ERα but very low levels of ERβ. MDA-MB231 human breast cancer cells are ERα-negative but ERβ-positive. MSA inhibited ERα expression and estrogen receptor–mediated gene activation and reduced the binding of ^{3}H-labeled estradiol to the estrogen receptor in MCF-7 cells. In contrast to ERα in MCF-7 cells, selenium increased ERβ expression in MDA-MB231 human breast cancer cells. Differential regulation of ERα and ERβ expression in breast cancer may represent a novel mechanism by which selenium functions as a chemopreventive agent.

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Materials and Methods

Selenium reagent, cell culture, and cell growth analysis. MSA was synthesized as described previously (18). β-Estradiol was purchased from Sigma (St. Louis, MO). The MCF-7 and MDA-MB231 human breast cancer cells were obtained from the American Type Culture Collection (Manassas, VA). The cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS), 2 mmol/L glutamine, 100 units/mL of penicillin and 100 μg/mL of streptomycin (17). In some experiments, cells were cultured in an estrogen-defined condition by using charcoal-stripped FBS in the presence of 10 nmol/L β-estradiol. For cell growth analysis by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (23), cells were seeded in 24-well plates at a density designed to reach 70% to 80% confluency at the time of assay. Cells were treated with 1, 2.5, 5, or 10 μmol/L MSA in triplicate 48 hours after seeding. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay was done after 24, 48, or 72 hours of treatment. For the quantitative determination of estrogen receptor transcripts and proteins, cells were exposed to MSA for much shorter periods of time, usually 24 hours or less. Total RNA and protein were isolated using the TRIzol reagent (Invitrogen, Carlsbad, CA), and stored at −80°C for subsequent real-time reverse transcription-PCR analysis.

Real-time reverse transcription-PCR. First-strand cDNA was synthesized from total RNA by SuperScript II reverse transcriptase (Invitrogen) following the manufacturer's protocol. Briefly, 400 ng of total RNA was mixed with 150 ng of random hexamers in a final volume of 100 μL containing 1× first-strand buffer [50 mmol/L Tris-HCl (pH 8.3), 75 mmol/L KCl, 3 mmol/L $MgCl_2$], 10 mmol/L DTT, 500 μmol/L of each deoxynucleotide triphosphate, and 200 units of SuperScript II reverse transcriptase. The samples were incubated at 42°C for 50 minutes and the reverse transcriptase was inactivated by heating at 70°C for 15 minutes.

The PCR primers and TaqMan probes for β-actin, ERα, and ERβ were Assays-on-Demand products from Applied Biosystems (Applied Biosystems, Foster City, CA). Two microliters of first-strand cDNA was mixed with 25 μL of 2× TaqMan Universal PCR Master Mix (Applied Biosystems) and 2.5 μL of 20× primer/probe mixture in a final volume of 50 μL. Temperature cycling and real-time fluorescence measurement were done using an ABI prism 7300 Sequence Detection System (Applied Biosystems). The PCR conditions were as follows: initial incubation at 50°C for 2 minutes, denaturation at 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds, and 60°C for 1 minute.

The relative quantitation of gene expression was done using the comparative C_T ($\Delta\Delta C_T$) method (21). Briefly, the threshold cycle number (C_T) was obtained as the first cycle at which a statistically significant increase in fluorescence signal was detected. Data was normalized by subtracting the C_T value of β-actin from that of the target gene. There was a match control sample for each treatment sample. Each reaction was done in duplicate and the C_T values were averaged. The $\Delta\Delta C_T$ was calculated as the difference of the normalized C_T values (ΔC_T) of the treatment and control samples: $\Delta\Delta C_T = \Delta C_{T\ \text{treatment}} - \Delta C_{T\ \text{control}}$. $\Delta\Delta C_T$ was converted to fold of change by the following formula: fold of change = $2^{-\Delta\Delta CT}$.

Western blot analysis. Details of the procedure were described previously (24). Immunoreactive bands were quantitated using volume densitometry and normalized against α-actin. The following monoclonal antibodies were used (source): anti-α-actin (Sigma), anti–estrogen receptor (BD Transduction Laboratory, San Jose, CA) and anti-c-myc (Santa Cruz Biotechnology, Santa Cruz, CA).

Transfection and luciferase assay. An aliquot of 3×10^5 cells was placed in a six-well plate and transfected with a total amount of 5 μg of DNA using Superfect (Qiagen, Valencia, CA) according to the manufacturer's instructions. The estrogen response element-luciferase reporter plasmid was used (25). The total amount of plasmid DNA was normalized to 5 μg per well by adding empty plasmid. The DNA/liposome mixture was removed 3 hours later, and cells were treated with 10 nmol/L β-estradiol in charcoal-stripped FBS condition and different concentrations of MSA. Cell extracts were obtained after 24 hours and luciferase activity was assayed using the Luciferase Assay System (Promega, Madison, WI). Protein concentrations in cell extracts were determined using the Coomassie Plus protein assay kit (Pierce, Rockford, IL). Luciferase activities were normalized using the protein concentration of the sample. All transfection experiments were done in triplicate wells and repeated at least four times. The relative luciferase activity was averaged from at least four independent experiments each with triplicate wells. The results were expressed as the percentage of untreated control.

Nuclear lysate preparation. Nuclear protein extract was prepared as described previously (26). Cells were harvested, washed with PBS twice and resuspended in a hypotonic buffer [10 mmol/L HEPES-KOH (pH 7.9), 1.5 mmol/L $MgCl_2$, 10 mmol/L KCl, and 0.1% NP40] and incubated on ice for 10 minutes. Nuclei were precipitated by 3,000 × *g* centrifugation at 4°C for 10 minutes. After washing once with the hypotonic buffer, the nuclei were lysed in a lysis buffer [50 mmol/L Tris-HCl (pH 8.0), 150 mmol/L NaCl, and 1% Triton X-100] and incubated on ice for 30 minutes. The nuclear lysate was precleared by 10,000 × *g* centrifugation at 4°C for 15 minutes. Protein concentration was determined using the Coomassie Plus protein assay kit.

Electrophoretic mobility shift assay. Twenty micrograms of nuclear protein extract were incubated in a 20 μL solution containing 10 mmol/L HEPES (pH 7.9), 80 mmol/L NaCl, 10% glycerol, 1 mmol/L DTT, 1 mmol/L EDTA, 100 μg/mL poly(deoxyinosinic-deoxycytidylic acid), and the radiolabeled double-stranded estrogen receptor consensus binding motif 5′-GGATCTAGGTCACTGTGACCCCGGATC-3′ (Santa Cruz Biotechnologies). The protein-DNA complexes were resolved on a 4.5% nondenaturing polyacrylamide gel containing 2.5% glycerol in 0.25× Tris-borate EDTA at room temperature and the gels were autoradiographed. Quantitation of estrogen receptor DNA-binding activity in the "protein-DNA" bandshift was measured using the Molecular Imager FX System (Bio-Rad, Hercules, CA). Two- and 4-fold molar excess of cold oligonucleotide were used for competition analysis.

***In vitro* estrogen receptor binding activity.** MCF-7 cells were plated at 1×10^6 cells per dish in 60 mm dishes in DMEM (phenol red–free) + 10% charcoal-stripped FBS and incubated at 37°C for 2 days. On the third day, dishes were either treated or left untreated with 5 μmol/L MSA in 2 mL of media each and incubated at 37°C for 4 hours. Cells were scraped off dishes and homogenized in TEDG buffer [10 mmol/L Tris, 1.5 mmol/L EDTA, 1 mmol/L DTT, 10% glycerol (pH 7.4)]. Cell suspensions were passed 10 to 15 times through a 26-gauge needle and incubated on ice for 10 minutes. The homogenate was centrifuged at 13,000 × *g* for 30 minutes at 4°C. The supernatant was collected and used as the cytosol. Total protein was estimated in both the MSA-treated and untreated cell lysates and equal amounts of protein were used in the subsequent assay.

The reaction mixtures contained 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, and 1 nmol/L [^{3}H]estradiol with or without 1×10^{-6} mol/L cold estradiol and equal amounts (100-200 μg) of protein in a total volume of 250 μL. The tubes were incubated overnight at 4°C. Dextran-coated charcoal suspension (500 μL) was added to each tube and incubated for 10 minutes with vigorous shaking at 4°C. The tubes were centrifuged at 3,000 × *g* at 4°C for 10 minutes and 500 μL of the supernatant was counted in a Beckman LS 9100 liquid scintillation counter with 5 mL of scintillation fluid. The difference between cpm with [^{3}H]estradiol only and cpm with [^{3}H]estradiol + cold estradiol was calculated and taken as the amount of bound [^{3}H]estradiol. The data was analyzed by Scatchard analysis.

Statistical analysis. Student's *t* test (two-tailed) was used to determine the significance between treatments and untreated controls, and $P < 0.05$ was considered significant.

Results

Methylseleninic acid inhibits MCF-7 cell growth. Table 1 shows the results of the effect of MSA treatment on cell growth. The data were expressed as percentages of the untreated control. A concentration of 1 μmol/L MSA did not alter cell growth, even after 3 days of treatment. MSA did not affect cell growth at concentrations between 1 and 5 μmol/L at the 24-hour time point. MCF-7 cell growth at the 24-hour time point was inhibited 24% by 10 μmol/L MSA. Increasing the concentration of MSA to 2.5 μmol/L

Table 1. Effect of MSA on the accumulation of MCF-7 cells

Treatment, MSA (μmol/L)	Treatment duration (h)*		
	24	48	72
1	105.1 ± 5.2	101.4 ± 5.3	86.7 ± 5.7
2.5	99.3 ± 4.9	92.4 ± 3.2	48.6 ± 6.3†
5	95.5 ± 3.9	68.4 ± 5.7†	36.3 ± 3.8†
10	76.1 ± 7.9†	54.5 ± 7.8†	32.2 ± 3.1†

NOTE: The effect of MSA on cell proliferation was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays and expressed as a percentage of untreated control.
*Results are expressed as mean ± SE (n = 3 independent experiments with triplicate wells, each reflect three treatment versus three nontreatment experiments).
† Significantly different compared with the corresponding control value ($P < 0.05$).

had no effect on cell growth until the 48-hour time point, but cell growth was inhibited by about 51% at the 72-hour time point. MCF-7 cell growth was inhibited by 5 μmol/L MSA by 32% and 64% at the 48 and 72-hour time points, respectively. Increasing MSA concentration to 10 μmol/L inhibited the growth of MCF-7 cells further by 46% and 68% at the 48- and 72-hour time points, respectively.

Methylseleninic acid suppresses ERα mRNA and protein expression in MCF-7 cells. We next examined the effect of MSA on the expression of estrogen receptor. MCF-7 cells express endogenous ERα. The expression of ERα mRNA in response to MSA was measured using real-time reverse transcription-PCR. Figure 1*A* shows the time course of MCF-7 cells in response to 10 μmol/L MSA. The levels of ERα mRNA decreased significantly after 2 hours of treatment with 10 μmol/L MSA and continued to decreased to 30% after 16 hours of treatment. The dose response to MSA was done at the 6-hour time point (Fig. 1*B*). Increasing the concentration of MSA to 5 μmol/L or above decreased ERα transcripts to 20% or less of controls, although 2.5 μmol/L MSA slightly increased the level of ERα mRNA. We next examined ERα protein expression in response to increasing doses of MSA treatment. MSA produced a graded suppression of the estrogen receptor protein in a dose-dependent manner (Fig. 1*C*). The changes in protein levels were consistent with changes in mRNA levels in MCF-7 cells in response to MSA treatment.

Methylseleninic acid increases ERβ mRNA expression in MDA-MB231 cells. MCF-7 cells are hormone-dependent breast cancer cells that express high levels of ERα but very low levels of ERβ. To examine the effect of selenium on the expression of endogenous ERβ, ERα-negative but ERβ-positive MDA-MB231 human breast cancer cells were tested by quantitative reverse transcription-PCR. Treatment with MSA increased ERβ mRNA expression in a dose- and time-dependent manner in MDA-MB231 cells (Fig. 2). Within the first hour, there was a 50% increase in ERβ mRNA expression in response to 10 μmol/L MSA (Fig. 2*A*). The levels of ERβ mRNA continued to increase to about 250% after 16 hours of treatment with MSA. Treatment with MSA in a concentration of 2.5 μmol/L or above increased ERβ mRNA expression by 2.5-fold compared with the untreated control at the 6-hour time point (Fig. 2*B*).

Methylseleninic acid inhibits estrogen receptor *trans*-activating activity. In an attempt to determine the ability of MSA to affect estrogen receptor *trans*-activating activity, we tested the effect of MSA on MCF-7 cells transiently transfected with an

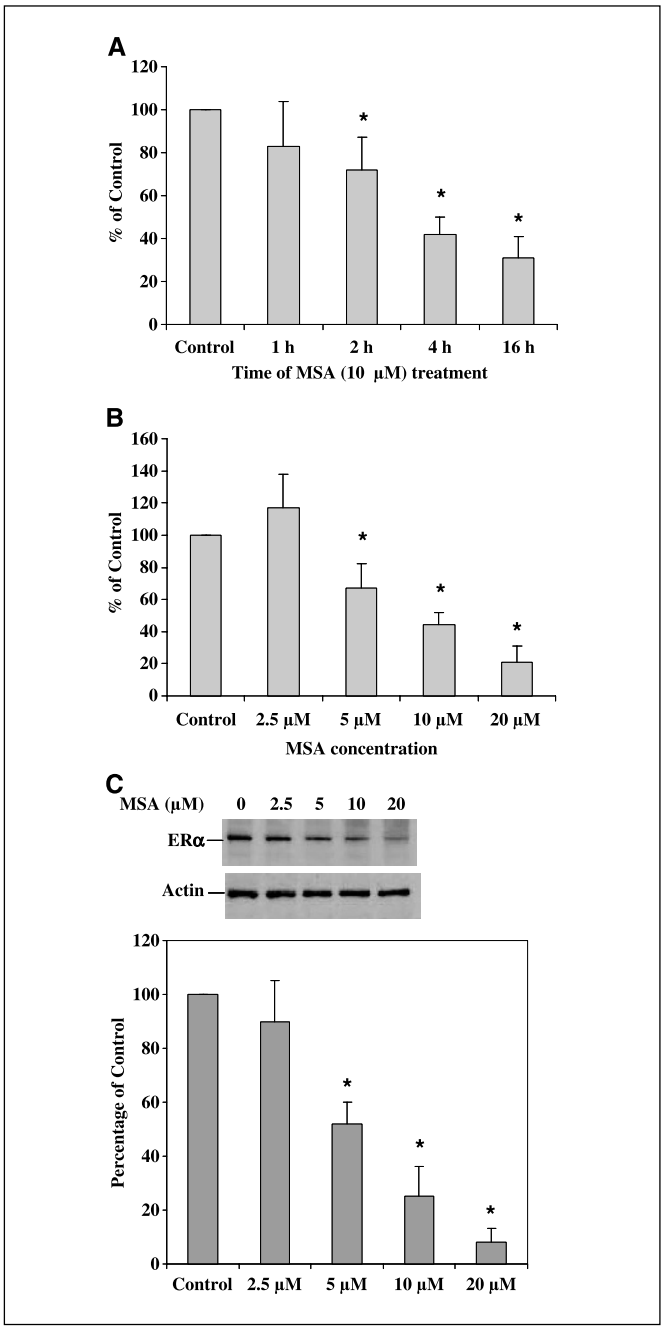

Figure 1. Effect of MSA on ERα expression. MCF-7 cells were cultured in phenol red–free DMEM medium in 10% charcoal-stripped FBS in the presence of 10 nmol/L β-estradiol. The cells were treated either with increasing doses of MSA or variable time points. Total RNAs were extracted for quantitative reverse transcription-PCR analysis and whole cell protein extracted for protein expression assay. *A* and *B,* change in ERα mRNA, as determined by quantitative reverse transcription-PCR, as a function of time of treatment with 10 μmol/L MSA (*A*), or as a function of MSA concentration at 6-hour treatment (*B*); *C,* effect of increasing concentrations of MSA treatment at the 6-hour time point on the levels of ERα protein expression. The percentage of control is represented as the mean ± SE of four independent experiments; *, significantly different from the control ($P < 0.05$), which is set as 100%. *Bars,* SE. Actin as a loading control.

estrogen response element-luciferase reporter plasmid (25). MSA inhibited the luciferase reporter in a dose-dependent manner (Fig. 3). The estrogen response element promoter activity decreased by 18%, 50%, 64%, or 88% in the presence of 2.5, 5, 10, or 20 μmol/L MSA, respectively.

Methylseleninic acid decreases binding of estrogen receptor to estrogen response element. Electrophoretic mobility shift assay was done using radiolabeled oligonucleotides of estrogen response element with nuclear extracts from MCF-7 cells treated with 10 μmol/L MSA for 30 minutes to determine whether MSA reduces DNA binding activity of estrogen receptor protein to estrogen response element. Estrogen receptor-estrogen response element complex formation decreased with MSA treatment compared with the untreated control (Fig. 4*A*). Cold competitor oligonucleotides blocked the complex formation (Fig. 4*B*), indicating that estrogen receptor protein specifically binds the estrogen response element region.

Methylseleninic acid suppresses c-myc protein expression in MCF-7 cells. The expression of c-myc is regulated by estrogen receptor signaling. To examine whether MSA affects the expression of c-myc protein, Western blot analyses were done. Cells were treated with increasing concentrations of MSA (0, 2.5, 5, 10, and 20 μmol/L) for 6 hours, and nuclear proteins were prepared and analyzed. The c-myc protein expression in response to β-estradiol was blocked by MSA treatment (Fig. 5*A* and *B*). The level of c-myc protein expression was barely detected after treatment with 20 μmol/L MSA.

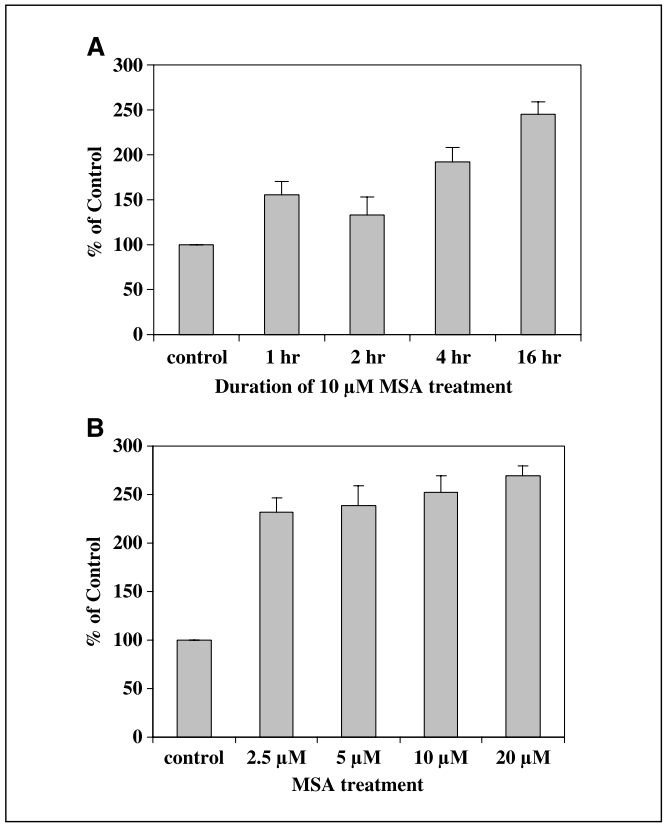

Figure 2. Effect of MSA on ERβ expression. MDA-MB231 cells were cultured in DMEM medium with 10% FBS. The cells were treated either with increasing doses of MSA or in variable time points. Total RNAs were extracted for quantitative reverse transcription-PCR analysis. *A,* time course of treatment with 10 μmol/L MSA as determined by quantitative reverse transcription-PCR; *B,* as a function of increasing MSA concentrations at 6-hour treatment by quantitative reverse transcription-PCR. The percentage of control is represented as the mean ± SE of four independent experiments.

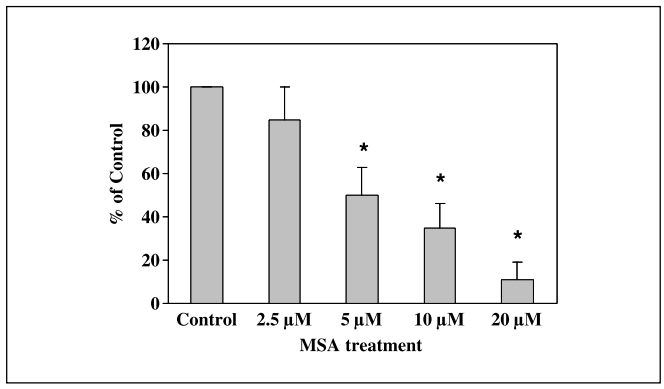

Figure 3. Effect of MSA on estrogen response element promoter activity. The cells were cultured in phenol red–free media with charcoal-stripped FBS containing 10 nmol/L of β-estradiol. The results are expressed as percentages of untreated control. *, significantly different from the control ($P < 0.05$); *bars,* SE.

Methylseleninic acid reduces the binding of labeled estradiol to the estrogen receptor in MCF-7 cells. MCF-7 cells were subjected to Scatchard analysis in the absence and presence of 5 μmol/L MSA to examine whether MSA affects estradiol binding to the estrogen receptor (Fig. 6). MSA treatment decreased B_{max} 44% from 59.1 ± 4.2 to 35.4 ± 3.9 fmol ($n = 3$; $P < 0.05$). Treatment with 5 μmol/L MSA altered the kinetics of estradiol binding to estrogen receptor in MCF-7 cells. K_d decreased 50% in the presence of MSA (0.22 ± 0.04 nmol/L) compared with untreated controls (0.41 ± 0.08 nmol/L).

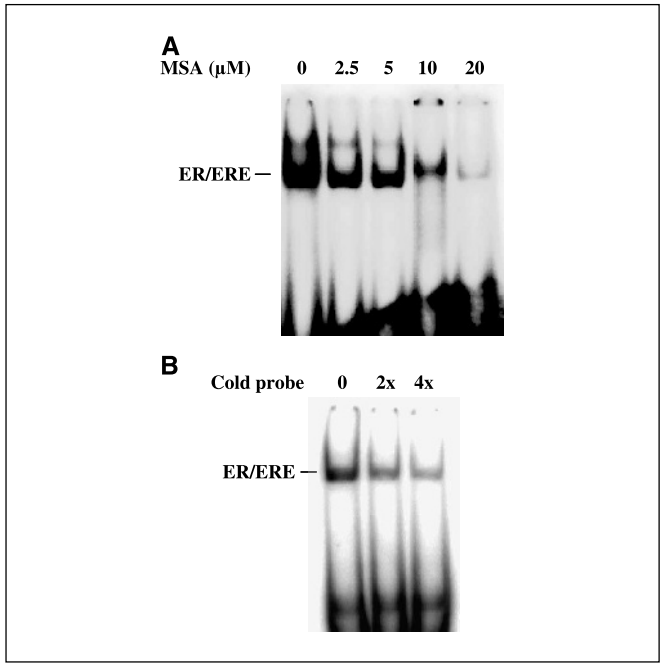

Figure 4. Effect of MSA on estrogen receptor binding to estrogen response element. *A,* electrophoretic mobility shift assay results of estrogen receptor binding to estrogen response element as a function of different concentrations of MSA at 3-hour treatment; *B,* competition analysis was done with 2-fold (2×), 4-fold (4×) excess of unlabeled competitor probe or no competitor (0) in the reaction mixture containing the labeled probe.

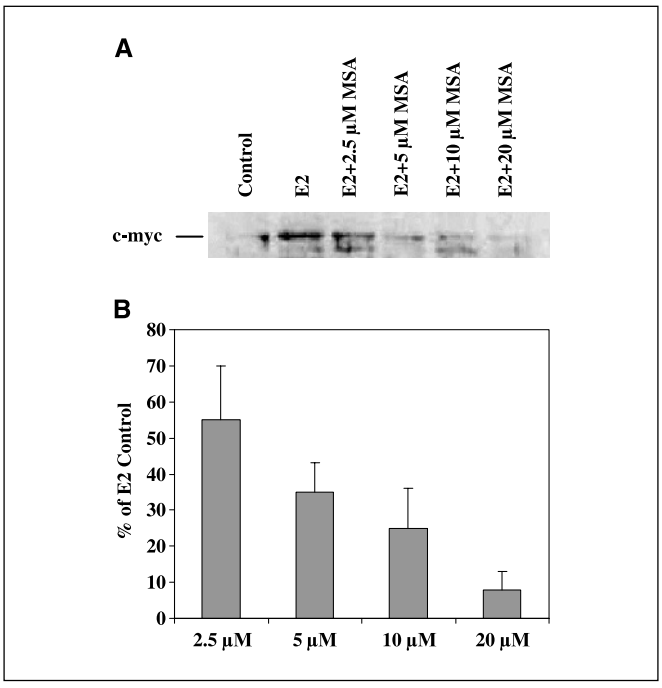

Figure 5. Effect of MSA on c-myc protein expression. *A,* Western blot analysis of the levels of c-myc protein expression in increasing concentrations of MSA at 6 hours of treatment. Twenty micrograms of nuclear proteins were loaded and equal protein loading was confirmed by Ponceau stain of the membrane after blotting; *B,* c-myc expression normalized quantitative changes compared with the β-estradiol control value of 100%.

Discussion

In this study, we showed that selenium disrupted estrogen receptor signaling in breast cancer cells *in vitro*. The anticancer activity of pharmacologic doses of selenium has been shown in mammary tumor models and cell lines (12, 17). Although epidemiologic studies have yet to show the chemopreventive activity of selenium in breast cancer, accumulating epidemiologic and molecular evidence suggest that selenium protects against the development of a variety of cancers including prostate, colon, esophagus, lung, and gastric cardia (7–9, 27). The apparent disconnect between results from studies in cell lines and the epidemiologic studies in breast cancer may be due to the different doses of selenium used in cell lines (pharmacologic doses) and in the epidemiologic studies (nutritional range). To put things in perspective, we need to take our *in vitro* data and design the appropriate *in vivo* experiment in order to determine the dose sensitivity of estrogen signaling modulation by selenium treatment.

We have previously shown that pharmacologic doses of selenium causes G_1 cell cycle arrest, induction of apoptosis, and modulation of cell signaling molecules such as cyclins A and D1, p16 and p27, which resulted in reduction in the size of intraductal papillary lesions in a rat mammary cancer model (12, 17). Besides induction of apoptosis and inhibition of cell proliferation, other potential mechanisms of the anticancer activity of selenium include suppression of angiogenic activity and modulation of oxidative stress (17, 28, 29). Estrogens exert their proliferative effect on hormone-dependent breast cancer cells by stimulating cell cycle progression and protecting cells from apoptotic death. It is possible that selenium-mediated growth arrest and apoptosis is mediated in part through disruption of the estrogen signaling in estrogen receptor–positive breast cancer cells. This report shows a novel mechanism through modulation of estrogen receptor expression whereby selenium may serve as a chemopreventive agent for breast cancer. Using both MCF-7 (ERα-positive) and MDA-MB231 (ERα-negative but ERβ-positive) cells, we show that MSA disrupts estrogen receptor signaling by decreasing the levels of ERα and increasing the levels of ERβ expression, inhibiting estrogen receptor *trans*-activating activity and estrogen receptor–mediated gene expression, and reducing estrogen receptor-ligand binding. It would be interesting to further validate these findings in mammary cell lines that produce approximately equal amounts of ERα and ERβ proteins.

c-Myc is a well-characterized β-estradiol target gene, which plays a critical role in the ability of β-estradiol to enhance the proliferation of MCF-7 cells. The effects of selenium on the expression of c-myc were used as a model to assess its activity against endogenous estrogen targets. Treatment with MSA inhibited β-estradiol-induced expression of c-myc in a dose-dependent manner as early as 6 hours. Since MSA had no effect on MCF-7 cell number at the 6-hour treatment at a concentration of 5 μmol/L, the reduction of c-myc expression was not due to cytotoxicity.

Estrogens signal through two distinct receptor pathways, ERα and ERβ, which show differential tissue distribution (30), affinity for coactivators (31), and responses to hormones (32). ERα and ERβ even shows opposing activation and repression activities (33). It is generally recognized that estrogen transcriptional activities implicated in breast cancer cell proliferation are mediated through ERα (34, 35). In contrast, ERβ inhibits the transcriptional activity of ERα and negatively affects cell proliferation in breast and uterus (34–36). In this study using cell lines, selenium inhibited expression of ERα and increased expression of ERβ. If selenium modulates ERα and ERβ expression in clinical specimens similar to breast cancer cell lines, these actions on estrogen receptor would characterize an ideal agent for evaluation in breast cancer chemoprevention.

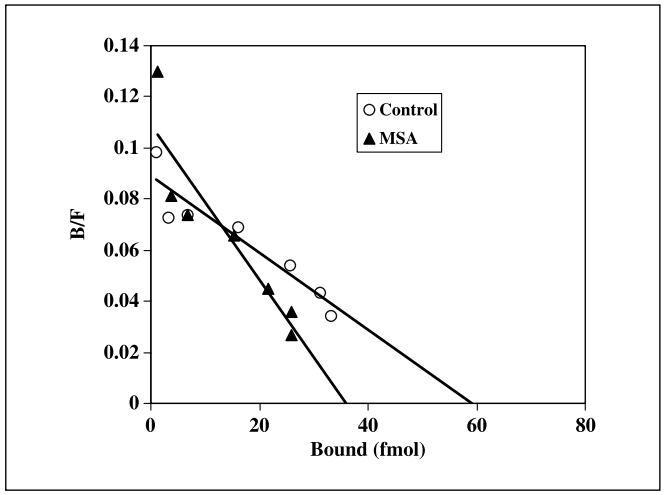

Figure 6. Scatchard analysis of specific estradiol binding to estrogen receptor in MCF-7 cells in the absence and presence of MSA (5 μmol/L). The kinetic constants for the data presented are K_d values for untreated control (0.41 ± 0.08 nmol/L) and with MSA 0.22 ± 0.04 nmol/L, whereas B_{max} values for untreated control was 59.1 ± 4.2 fmol and with MSA 35.4 ± 3.9 fmol. Values are mean ± SE for triplicate experiments.

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