Breast Cancer Research and Treatment **74:** 167–176, 2002.

Report

Regulation of MCF-7 breast cancer cell growth by β-estradiol sulfation

Josie L. Falany, Nancy Macrina, and Charles N. Falany
Department of Pharmacology and Toxicology, University of Alabama at Birmingham, Birmingham, AL, USA

Key words: breast cancer, estrogen receptor, β-estradiol, growth factors, sulfation

Summary

Estrogen stimulation is an important factor in human breast cancer cell growth and development. Metabolism of β-estradiol (E_2), the major endogenous human estrogen, is important in regulating both the level and activity of the hormone in breast tissues. Conjugation of E_2 with a sulfonate moiety is an inactivation process since the sulfate ester formed by this reaction can not bind and activate the estrogen receptor. In human tissues including the breast, estrogen sulfotransferase (EST, SULT1E1) is responsible for high affinity E_2 sulfation activity. EST is expressed in human mammary epithelial (HME) cells but not in most cultured breast cancer cell lines, including estrogen responsive MCF-7 cells. Stable expression of EST in MCF-7 cells at levels similar to those detected in HME cells significantly inhibits cell growth at physiologically relevant E_2 concentrations. The mechanism of cell growth inhibition involves the abrogation of responses observed in growth factor expression in MCF-7 cells following E_2 stimulation. MCF-7 cells expressing EST activity did not show a decrease in estrogen receptor-α levels, nor a characteristic increase in progesterone receptor or decrease in transforming growth factor-β expression upon exposure to 100 pM or 1 nM E_2. The lack of response in these MCF-7 cells is apparently due to the rapid sulfation and inactivation of free E_2 by EST. These results suggest that loss of EST expression in the transformation of normal breast tissues to breast cancer may be an important factor in increasing the growth responsiveness of preneoplastic or tumor cells to estrogen stimulation.

Introduction

Steroid hormones and peptide growth factors are involved in the complex regulation of cell proliferation of hormone sensitive tumors including breast, endometrial, ovarian, and prostate cancers. Estrogen metabolism is closely associated with the etiology and treatment of breast cancer because many breast cancers are dependent upon estrogens for both growth and progression. Factors that affect the intracellular metabolism of estrogens may be important in altering the concentration of free estrogens and thereby the effects of estrogens and anti-estrogens on breast cancer cells [1–3] The growth of many breast neoplasms and breast cancer cell lines is stimulated by estrogens via the induction of growth-stimulatory peptides, hormone receptors, and oncogenic factors [4]. Upon β-estradiol (E_2) binding, the estrogen receptor (ER) interacts with specific estrogen-response elements in the promoters of these genes and modulates their transcription resulting in accelerated cell growth [5].

Sulfation, which is a major pathway involved in both the synthesis and inactivation of steroids, is catalyzed by a family of enzymes termed the sulfotransferases (SULTs) [6, 7]. Estrogen sulfotransferase (EST, SULT1E1) is a Phase II drug metabolizing enzyme which catalyzes the sulfoconjugation of steroid hormones, particularly estrogens, at the 3-hydroxyl position [8]. Conjugation with the sulfate moiety greatly increases the aqueous solubility of the steroid as well as inhibiting its binding to the ER. EST has a Km for E_2 of 5 nM indicating that the enzyme is active at concentrations at which E_2 occurs physiologically and at which it interacts with the ER [8–10]. Thus, EST plays an important role in modulating estrogenic steroid responsiveness because sulfation inactivates E_2 by rendering it incapable of

binding to the ER and initiating ER-regulated cellular responses.

The human MCF-7 breast cancer cell line has been extensively used as a model system to study the effects of estrogens and anti-estrogens on breast cancer cell growth [11]. MCF-7 cells are ER positive and respond to estrogen treatment with an increase in cellular proliferation [11, 12]. Most human breast cancer cell lines, including MCF-7 cells, do not express EST and lack the high affinity estrogen sulfation pathway [13]. In contrast, normal human mammary epithelial (HME) cells possess endogenous EST activity and protein at physiologically significant levels [13]. Thus, the loss of EST activity may be a significant event in the transformation of normal breast cells to cancer cells by allowing increased estrogenic stimulation.

To better understand the role of E_2 sulfation in the regulation of estrogenic activity in breast tissues, we studied the effects of elevating EST levels in MCF-7 cells on the expression of growth factors involved in regulating cell growth. In a previous study, we evaluated the effects of expressing EST activity in MCF-7 cells on cellular growth rate measured by DNA synthesis and on the rate of estrogen metabolism [14]. MCF-7 cells with elevated EST levels had a decreased proliferation rate, decreased DNA synthesis and increased E_2 sulfation rate as compared to control cells [14, 15]. To further investigate the role of EST activity in regulating estrogenic activity in MCF-7 cells, the expression of estrogen-regulated hormone receptors and growth factors were studied.

The results presented in this paper demonstrate that elevated levels of EST activity in MCF-7 cells result in altered cellular responses to E_2 via modulation of the expression of both the ER and progesterone receptor (PR) as well as the expression of the estrogen-related transforming growth factor-beta1 (TGF-β1). These changes are associated with a decrease in MCF-7 cell proliferation. Therefore, EST activity, at levels analogous to those found in normal HME cells, abrogates the responsiveness of MCF-7 breast cancer cells to estrogens for each parameter evaluated.

Materials and methods

Materials

MCF-7 cells were obtained from the American Type Culture Collection (Rockville, MD). Dulbecco's Modified Eagle's Medium (DMEM), geneticin, fetal bovine serum (FBS), and the RTS T4 Kinase Labelling System were obtained from Life Technologies (Grand Island, NY). E_2 was purchased from Sigma Chemical Co. (St. Louis, MO). Antibodies were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). RNA STAT-60 was purchased from Tel-Test (Friendswood, TX). Quickhyb was purchased from Stratagene (La Jolla, CA). M-PER and the SuperSignal West Pico kit were purchased from Pierce (Rockford, IL). The TGF-β1 oligonucleotide probe was from Oncogene (Cambridge, MA).

Growth and maintenance of stably transformed cell lines

The generation of MCF-7 cells stably transformed with either EST/pcDNA3 or with the pcDNA3 vector alone has been described previously [14]. The cells were maintained in 7% FBS/DMEM/geneticin (400 mg/l) at 37°C in a 95% air/5% CO_2 atmosphere. Medium was changed every 2–3 days and cells were subcultured at 80–90% confluency. For experiments, cells were grown in 7% FBS that had been stripped of endogenous steroids (sFBS) and DMEM without phenol red (PR^-DMEM) which is known to contain an estrogenic contaminant [16]. sFBS was prepared by a dextran charcoal procedure as described previously [17]. The efficiency of the procedure was monitored by adding a trace amount of radiolabeled E_2 to the serum prior to treatment. Greater than 99% of the radioactive E_2 was removed by the treatment protocol.

Cell growth experiments

To compare the E_2 growth responsiveness of EST/MCF-7 cells to that of pcDNA/MCF-7 cells, 6-well plates of cells (96,000 cells/well) were prepared for each cell line in 7% sFBS/PR^-DMEM and cells were allowed to attach for 24 h. At this point, two wells from each plate were trypsinized and resuspended in 400 μl medium. Prior to counting viable cells microscopically with a hemocytometer, cells were diluted appropriately (so that 20–50 cells were counted per square in a hemocytometer) in phosphate-buffered saline and trypan blue stain. The remaining wells of each plate were then washed twice with serum-free PR^-DMEM and treated as follows in this same medium. Two wells of each plate were treated with 0.1 nM E_2 (diluted from a 0.1 μM stock solution prepared in ethanol). The remaining two wells were treated with the appropriate volume of ethanol as controls. For each plate, an E_2 well and a control well

were counted as described above on days 3 and 6. Fresh drugs were applied every 48 h.

ER and PR immunoblots

For experiments to compare ER and PR levels of EST/MCF-7 and pcDNA/MCF-7 control cells subsequent to treatment with E_2, cells were plated in 7% sFBS/PR$^-$DMEM as described above and allowed to attach for 24 h. Cells were then quiesced for 24 h in serum-free PR$^-$DMEM, then duplicate wells were treated for 48 h with either 0.1 nM E_2, 1 nM E_2 or ethanol (control) as described above. Cell lysates were then prepared in 200 µl M-PER per well. M-PER was applied to each well for 5 min with gentle rocking then lysates were transferred to microcentrifuge tubes and centrifuged at 10,000 × *g* for 10 min at 4°C. The lysate was removed to a fresh tube and stored at −70°C until analysis. For immunoblot examination, an equivalent amount of each lysate protein was resolved via 10% SDS-polyacrylamide gel electrophoresis and electrophoretically transferred to a nitrocellulose membrane. As described previously in detail [8], the membrane was blocked with nonfat milk then incubated with a rabbit anti-ER polyclonal antibody for 1 h. Goat anti-rabbit IgG horseradish peroxidase conjugate was used as the secondary antibody. Immunoconjugates were visualized by chemiluminescence using the SuperSignal West Pico kit (Pierce). Duplicate blots were probed in the same manner with the anti-PR rabbit polyclonal antibody. Bands were quantified by densitometric scanning.

TGF-β1 Northern blot analysis

For Northern blot analysis of TGF-β1 message levels, cells were plated and treated as described for ER and PR immunoblot analysis. After 48 h of E_2 treatment, total RNA was prepared from each well by lysing cells directly into 400 µl/well RNA-STAT 60. Samples were processed as per the manufacturer's instructions. Total RNA from each well was resuspended in 10 µl RNAse-free water and quantified spectrophotometrically. For each experiment, RNA (15 µg) from each sample was resolved by agarose gel electrophoresis in the presence of formaldehyde and transferred to a nylon membrane. The blot was prehybridized at 68°C with Quickhyb then probed for 2 h at the same temperature with a [^{32}P]human TGF-β1 oligonucleotide probe (Oncogene) labeled using T4 kinase. The nylon membrane was then washed twice at room temperature with 2 × SSC (3 M NaCl, 0.3 M trisodium citrate, pH 7.0) containing 0.1% SDS and twice at 60°C with 0.1 × SSC containing 0.1% SDS for 15 min each. Autoradiography was performed at −70°C with an intensifying screen. To confirm that analogous amounts of RNA were loaded and transferred for each sample, the membrane was re-probed as described above with [^{32}P]labeled β-actin. Bands were quantified by densitometric scanning.

Results

Cell proliferation experiments

The stable expression of EST activity in MCF-7 cells has an inhibitory effect on DNA synthesis as measured by [^{3}H]-thymidine incorporation [14, 15]. To investigate whether the presence of physiological levels of EST activity also inhibited cell growth, pcDNA/MCF-7 and EST/MCF-7 cells were grown in serum-free media and serum-free media supplemented with 0.1 nM E_2. The proliferative effect of E_2 on both pcDNA/MCF-7 and EST/MCF-7 cells is presented in Figure 1. The number of pcDNA/MCF-7 cells increased approximately 2.2-fold in 2 days and 5.3-fold in 5 days in serum-free medium. We hypothesize that the pcDNA control cells grow without E_2 because there is sufficient intracellular E_2 present to support growth at this rate for the time frame involved in these studies. In the presence of 0.1 nM E_2, pcDNA/MCF-7 cells increased 4-fold and 12-fold after 2 and 5 days, respectively. In serum-free medium, no significant increase was observed in the numbers of EST/MCF-7 cells in the presence or absence of 0.1 nM E_2. Therefore, the expression of EST activity in MCF-7 cells abrogated cell growth in the presence of physiological levels of E_2.

Regulation of ER expression

Treatment of MCF-7 cells with E_2 stimulates the complex regulation of hormone receptors and growth factors involved in the stimulation of cell growth [11, 18, 19]. One result of E_2 treatment of MCF-7 cells is the down-regulation of ER expression [11, 20]. Figure 2 shows the results from the immunoblot analysis of ER expression in control and E_2 treated pcDNA/MCF-7 and EST/MCF-7 cells. Both types of MCF-7 cells were maintained in serum free medium and then grown for 48 h in the presence or absence of 0.1 or 1.0 nM E_2. pcDNA/MCF-7 cells exhibited a dose-dependent decrease in ER levels in response to both concentrations of E_2. ER levels decreased 45 and

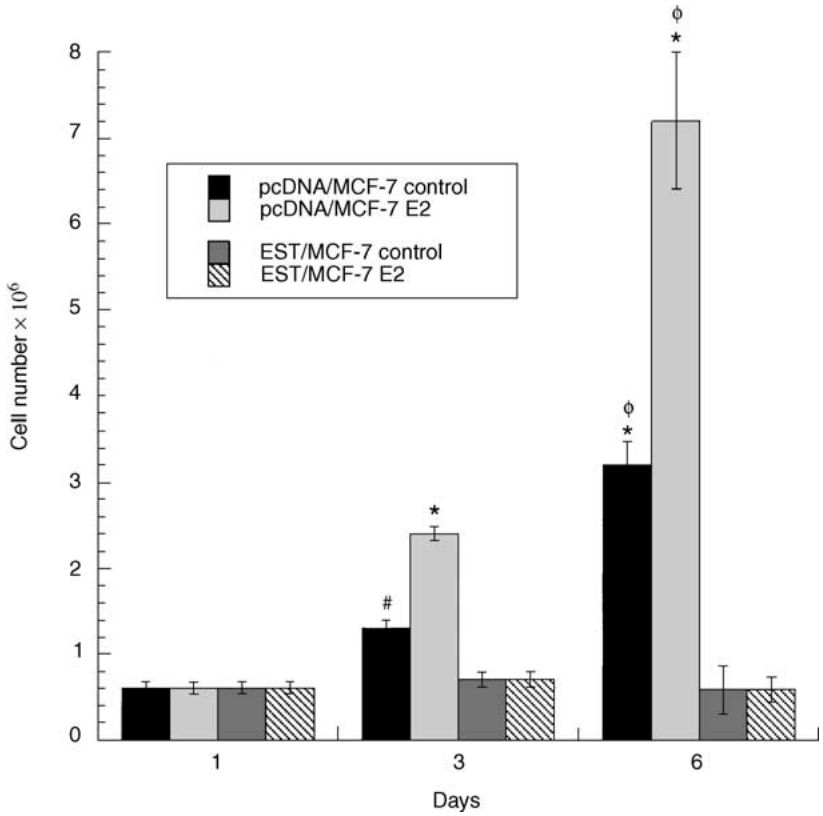

Figure 1. Effect of E_2 on pcDNA/MCF-7 and EST/MCF-7 cell growth. Cells were plated in 6-well plates and maintained in either serum-free medium or serum-free medium supplemented with 0.1 nM E_2. For each cell line, cells in a control well and an E_2-supplemented well were trypsinized and counted on a hemocytometer at days 1, 3 and 6. Results represent the mean cell number ± S.D. from three separate experiments. Number signs (#) indicate a significant difference ($p < 0.01$) between cell number as compared to control value. Asterisks (∗) indicate a significant difference ($p < 0.001$) between cell number as compared to control value. Psi (ϕ) symbols indicate a significant difference ($p < 0.01$) in cell number as compared to the previous time point.

33% following treatment with 0.1 and 1.0 nM E_2, respectively. In contrast, EST/MCF-7 cells maintained a consistent level of ER expression that was not affected by treatment with either E_2 concentration. Thus, the presence of EST activity in MCF-7 cells abolished the characteristic repression in ER expression that occurs in MCF-7 cells in response to E_2.

Regulation of PR expression

In contrast to the decrease in ER expression, MCF-7 cells respond to estrogens with an induction of the PR [21]. To investigate the effects of EST activity on the regulation of PR expression by E_2, PR expression was analyzed by immunoblot analysis in both control and E_2 treated pcDNA/MCF-7 and EST/MCF-7 cells. Figure 3 shows that E_2 treatment increased PR expression in pcDNA/MCF-7 cells as anticipated. Treatment of pcDNA/MCF-7 cells for 48 h with either 0.1 nM or 1.0 nM E_2 resulted in a 1.7 or 2.4-fold increase in PR levels, respectively. However, the EST/MCF-7 cells were not responsive to these E_2 concentrations since no changes were observed in PR expression.

TGF-β1 expression

TGF-β1 is an important growth factor in breast cells and exhibits a growth inhibitory effect in MCF-7 cells [19]. One characteristic of the growth stimulation of E_2 in MCF-7 cells is the down-regulation of TGF-β1 expression in the presence of E_2 [19]. To investigate whether the presence of EST activity could prevent this effect, the level of TGF-β1 mRNA was investigated in both control and E_2-treated pcDNA/MCF-7 and EST/MCF-7 cell lines treated with E_2 using northern blot hybridization. Figure 4 shows that both pcDNA/MCF-7 and EST/MCF-7 cells possessed similar constitutive TGF-β1 mRNA levels. TGF-β1

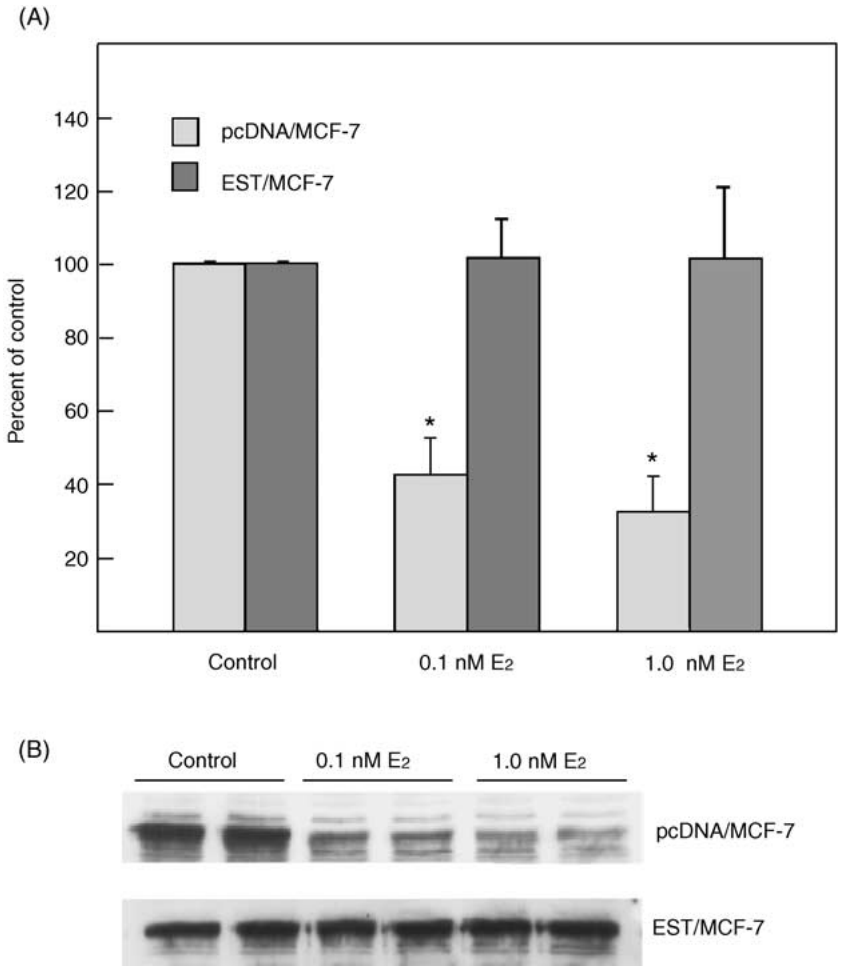

Figure 2. Immunoblot analysis of the effect of E_2 on ER expression in pcDNA/MCF-7 and EST/MCF-7 cells. (A) For each cell line, cells were plated in a 6-well plate and treated in duplicate with ethanol (control), 0.1 nM E_2 or 1.0 nM E_2. Immunoblot analysis was performed with an ER antibody and chemiluminescent visualization. The results from densitometric analysis of the autoradiograph film are presented in panel (A) and represent mean values from three separate experiments. Control values were designated as 100% and E_2-treated wells were compared to these values for each cell line. Asterisks (∗) indicate a significant difference ($p < 0.001$) of ER expression as compared to control value. (B) This figure shows a typical ER immunoblot from this series of experiments; the upper blot contains pcDNA/MCF-7 lysates and the lower blot contains EST/MCF-7 lysates. Lanes 1 and 2 are control lanes, lanes 3 and 4 are lysate from cells treated with 0.1 nM E_2 and lanes 5 and 6 are lysates from cells treated with 1.0 nM E_2.

mRNA expression in pcDNA/MCF-7 cells was decreased 44 and 61% following treatment with 0.1 and 1.0 nM E_2, respectively. However, the level of TGF-β1 mRNA remained constant in EST/MCF-7 cells treated with either E_2 concentration.

Discussion

The development of breast cancer is closely associated with the responsiveness of mammary tissues to estrogenic stimulation [1]. All breast cancers at some time in their development are dependent upon estrogen stimulation for growth, suggesting that changes in the levels of active estrogens or the loss of estrogen responsiveness are important factors in their transformation to a malignant tumor. E_2 is the major endogenous estrogen and its tissue levels may be regulated by increased synthesis, protein binding, or metabolism. Therefore, E_2 metabolism is a major factor involved in regulating the levels of active estrogens in both normal and cancerous breast tissue [22–25].

Estrogen-responsive breast cancer cell lines, and most prevalently the MCF-7 cell line, have been used

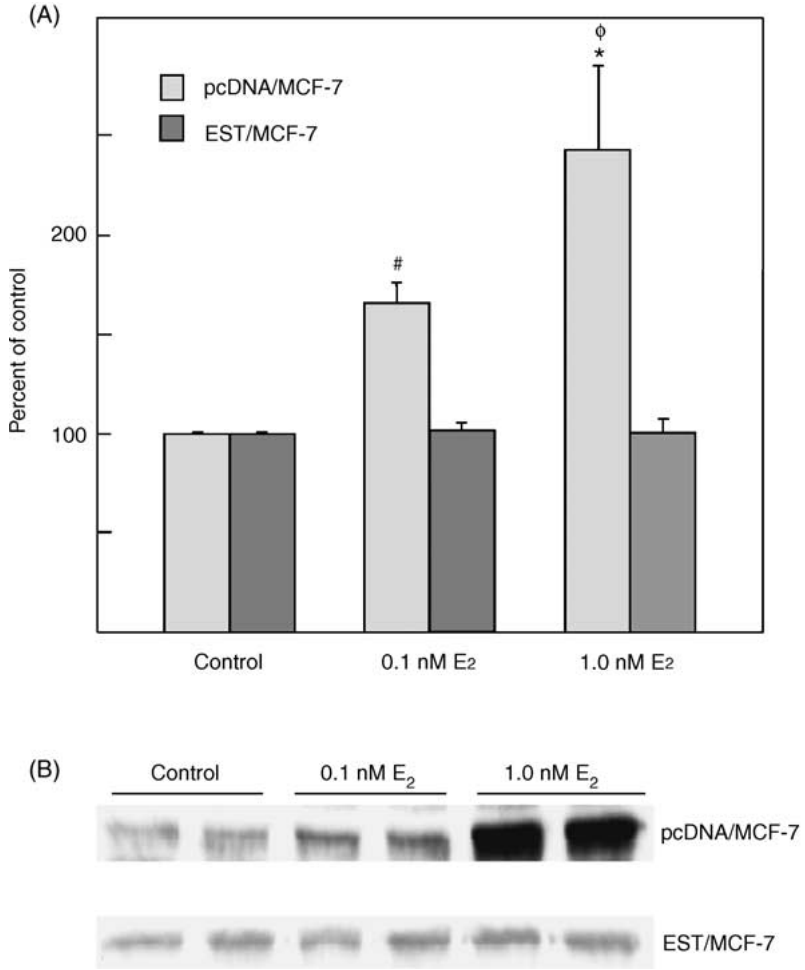

Figure 3. Immunoblot analysis of the effect of E_2 on PR expression in pcDNA/MCF-7 and EST/MCF-7 cells. (A) For each cell line, cells were plated in a 6-well plate and treated in duplicate with ethanol (control), 0.1 nM E_2 or 1.0 nM E_2. Immunoblot analysis was performed with an ER antibody and chemiluminescent visualization. The results from densitometric analysis of the autoradiograph film are presented in panel (A) and represent mean values from three separate experiments. Control values were designated as 100% and E_2-treated wells were compared to these values for each cell line. Number signs (#) indicate a significant difference ($p < 0.01$) in PR expression as compared to control value. Asterisks (∗) indicate a significant difference ($p < 0.001$) as compared to control value. Psi (ϕ) symbols indicate a significant difference ($p < 0.01$) as compared to results of the previous E_2 concentration. (B) This figure shows a typical PR immunoblot from this series of experiments; the upper blot contains pcDNA/MCF-7 lysates and the lower blot contains EST/MCF-7 lysates. Lanes 1 and 2 are control lanes, lanes 3 and 4 are lysate from cells treated with 0.1 nM E_2 and lanes 5 and 6 are lysates from cells treated with 1.0 nM E_2.

in studies designed to elucidate the mechanisms by which hormones affect cell proliferation and protein synthesis [11, 26]. Since metabolism will greatly affect active estrogen levels in breast cancer cells, understanding the role of sulfation in E_2 metabolism is important. At physiologically relevant E_2 concentrations, sulfation is a major mechanism for the inactivation of E_2 and EST is the primary SULT involved with the sulfation and inactivation of E_2 at these concentrations [9, 14, 15]. The conversion of E_2 to estrone catalyzed by 17β-hydroxysteroid dehydrogenase occurs at significantly higher concentrations [27]. Therefore, the lack of EST expression in MCF-7 cells and other breast cancer cells is responsible for part of the increased estrogen levels observed in breast cancer tissues.

We hypothesize that EST is responsible for the sulfation of E_2 in normal breast tissue and that the sulfation of E_2 is involved in regulating the activation of the ER. At some point in the transformation process, breast preneoplastic cells or tumors lose EST activity which results in increased levels of free E_2 and

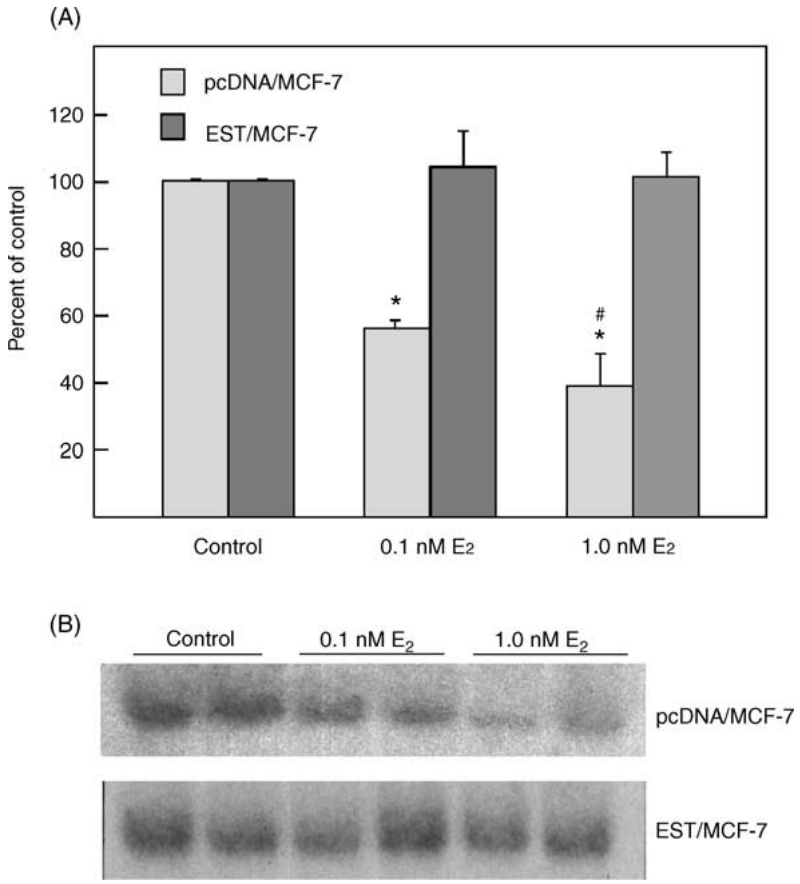

Figure 4. Northern blot analysis of the effect of E_2 on TGF-β1 mRNA expression in pcDNA/MCF-7 and EST/MCF-7 cells. (A) For each cell line, cells were plated in a 6-well plate and treated in duplicate with ethanol (control), 0.1 nM E_2 or 1.0 nM E_2. RNA was prepared from each well then 15 μg of RNA from each well was resolved by agarose gel electrophoresis in the presence of formaldehyde, and transferred to a nylon membrane. The membrane was probed with a [^{32}P]human TGF-β1 oligonucleotide probe then analyzed by autoradiography. The results from densitometric analysis of the autoradiograph film are presented in panel (A) and represent mean values from three separate experiments. Control values were designated as 100% and E_2-treated wells were compared to these values for each cell line. Asterisks (∗) indicate a significant difference ($p < 0.001$) between TGF-β1 expression as compared to control value. Number signs (#) indicate a significant difference ($p < 0.05$) as compared to the previous E_2 concentration. (B) This figure shows a typical TGF-β1 Northern blot from this series of experiments; the upper blot contains pcDNA/MCF-7 RNA and the lower blot contains EST/MCF-7 RNA. Lanes 1 and 2 are control lanes, lanes 3 and 4 contain RNA from cells treated with 0.1 nM E_2 and lanes 5 and 6 contain RNA from cells treated with 1.0 nM E_2.

greater estrogenic growth stimulation. Our laboratory has previously reported that EST is expressed in primary human mammary epithelial (HME) cells but not in human breast cancer cell lines, including MCF-7 cells [13]. Preliminary studies also indicate that EST expression is detectable in only a fraction of human breast cancer specimens [28]. Since E_2 plays an important role in regulating the proliferation of some breast tumors via the induction or suppression of key growth regulatory factors, the presence of EST activity in MCF-7 cells should be reflected in changes in cell proliferation as well as in the regulation of important hormone receptors and growth factors, such as the ER, PR, and TGF-β1.

Our laboratory as well as others [14, 15] have reported that the growth of EST/MCF-7 cells is decreased compared to that of pcDNA/MCF-7 cells by analyzing [^{3}H]thymidine uptake. This study demonstrates that the presence of EST activity in MCF-7 cells is also associated with decreased cell proliferation. This can be explained by the fact that EST sulfates and thereby inactivates estrogens in the pico to nanomolar concentration range rendering them physiologically inactive. This unresponsiveness of the

EST/MCF-7 cells to estrogens could be overcome by administering micromolar concentrations of E_2, resulting in growth responsiveness similar to that found with control pcDNA/MCF-7 cells (data not shown). This results from the fact that EST is functional at nanomolar concentrations of estrogens but is inefficient at sulfation in the micromolar concentration range [8, 10]. With sufficiently high levels of E_2, the effect of elevated cellular EST activity can be overcome, restoring the E_2 growth response.

Estrogens play a very important role in regulating the proliferation of breast tumors via the induction or suppression of key growth regulatory factors [29]. E_2 has been shown to be autoregulatory, down-regulating the expression of its own receptor in MCF-7 breast tumor cells [30]. Also, estrogens induce the expression of the PR and inhibit expression of the potent growth repressor, TGF-β1 [18, 20, 21, 31]. The expression of EST activity in MCF-7 cells inhibited the regulation of these growth factors by E_2 which is consistent with the hypothesis that EST is capable of inactivating estrogens and abolishing their cellular effect. In Ishikawa endometrial adenocarcinoma cells, EST expression has been reported to inhibit ER activation at picomolar and nanomolar E_2 concentrations indicating that the modulation of growth factor expression is regulated via a decrease in ER activation [9].

In breast tissues, the sulfation and inactivation of physiological concentrations of E_2 is associated with EST activity. Multiple human SULT isoforms are capable of conjugating E_2 including SULT1E1 (EST), SULT1A1 (PPST-1) and SULT2A1 (dehydroepiandrosterone (DHEA)-ST) [8, 32, 33]. Only EST is capable of sulfating E_2 and preventing activation of the ER at physiological E_2 concentrations [10, 14, 15]. Of the other isoforms, MCF-7 cells express SULT1A1 (PPST-1) but not SULT2A1 (DHEA-ST). However, no effects on ER, PR or TGF-β1 expression which could be associated with PPST-1 activity were observed. It has been shown that there is no correlation of PPST-1 activity with the regulation of E_2 activity in human endometrial tissues [34] and Ishikawa endometrial adenocarcinoma cells [9, 17]. Also, 10-fold over-expression of PPST-1 in MCF-7 cells had no effect on the growth responsiveness of MCF-7 cells or E_2 sulfation at physiological concentrations [14]. Although PPST-1 is capable of sulfating E_2, it apparently does not have a role in regulating E_2 activity in MCF-7 cells.

E_2 is the primary endogenous estrogenic hormone associated with the stimulation of breast cancer growth, and although it has been difficult to relate breast cancer development to steroid levels in the circulation [35], in recent studies, serum estrogen levels have been associated with an increase in breast cancer in postmenopausal women [36, 37]. However, intracellular metabolism of estrogens within breast cancer cells is important for the synthesis and activity of E_2 as well as to the stimulation of breast cancer cell growth. There are multiple pathways for synthesizing and inactivating E_2 [22–24]. E_2 in breast cancer cells can originate either from the aromatization of androgens or the conversion of estrone sulfate to E_2 by sulfatase and 17β-dehydrogenase activity [1, 22, 25, 38]. These pathways may act to increase E_2 levels without the counterbalance of EST activity to sulfate and inactivate E_2. Therefore, the lack of EST expression in breast cancer cells is involved in maintaining of high levels of estrogens.

Free E_2 levels in MCF-7 cells may represent a balance between estrogen sulfatase activity and EST activity. Since EST is not expressed in MCF-7 cells [13–15], these cells lack high affinity E_2 sulfation activity which would enhance the conversion of estrogen-sulfates to free E_2. The level of EST activity in the stably transformed EST/MCF-7 cells in this study was 4.47 pmol/min/mg cytosolic protein [14]. Because of the low levels of estrone sulfatase activity (1 pmol/min/mg prot. [38]) and the high Km (6 μM) of estrone sulfatase for E1-sulfate in MCF-7 cells [39], the expression of low levels of EST activity should convert the majority of the free E_2 to E_2-sulfate. Because the Km of EST for E_2 is 5 nM [10], the presence of even low levels of EST activity in MCF-7 cells will therefore have an effect on estrogen regulated cellular processes as exemplified by decreased growth rates (Figure 1).

If the loss of EST activity in HME cells *in vivo* is associated with increased sensitivity of the cells to estrogen stimulation during the transformation process, then the introduction of EST activity into breast cancer cells should confer protection from estrogen responsiveness to these cells. It is evident that elevated EST in the estrogen-dependent MCF-7 cell line confers protection from the cell-proliferative effects of E_2 with respect to both the initial ER binding step and subsequent downstream effects. Potentially, this information can be utilized to affect the etiology of this disease by decreasing breast cancer cell growth. Increasing estrogen sulfation activity may provide a potential mechanism by which stimulation of estrogen-responsive breast tumors could be abrogated.

Acknowledgement

This research was supported in part by PHS grant GM38953 to C.N. Falany.

References

1. Miller W, O'Neill J: The significance of steroid metabolism in human cancer. J Steroid Biochem Mol Biol 37: 317–325, 1990
2. Roy AK: Regulation of steroid hormone action in target cells by specific hormone-inactivating enzymes. Proc Soc Exp Biol Med 199: 265–272, 1992
3. Pasqualini J, Chetrite G: Estrone sulfatase versus estrone sulfotransferase in human breast cancer: potential clinical applications. J Steroid Biochem Mol Biol 69: 287–292, 1999
4. Safe S: Transcriptional activation of genes by 17beta-estradiol through estrogen receptor-Sp1 interactions, Vitam Horm 62: 231–252, 2001
5. Pietras RJ, Arboleda J, Reese DM, Wongvipat N, Pegram MD, Ramos L, Gorman CM, Parker MG, Sliwkowski MX, Slamon DJ: HER-2 tyrosine kinase pathway targets estrogen receptor and promotes hormone-independent growth in human breast cancer cells. Oncogene 10: 2435–2446, 1995
6. Falany CN: Enzymology of human cytosolic sulfotransferases. FASEB J 11: 206–216, 1997
7. Glatt H: Bioactivation of mutagens via sulfation. FASEB J 11: 314–321, 1997
8. Falany CN, Krasnykh V, Falany JL: Bacterial expression and characterization of a cDNA for human liver estrogen sulfotransferase. J Steroid Biochem Mol Biol 52: 529–539, 1995
9. Kotov A, Falany JL, Wang J, Falany CN: Regulation of estrogen activity by sulfation in human Ishikawa endometrial adenocarcinoma cells. J Steroid Biochem Mol Biol 68: 137–144, 1999
10. Zhang H, Varmalova O, Vargas FM, Falany CN, Leyh TS: Sulfuryl transfer: the catalytic mechanism of human estrogen sulfotransferase. J Biol Chem 273: 10888–10892, 1998
11. Katzenellenbogen BS, Kendra KL, Norman MJ, Berthois Y: Proliferation, hormonal responsiveness, and estrogen receptor content of MCF-7 human breast cancer cells grown in the short-term and long-term absence of estrogens. Cancer Res 47: 4355–4360, 1987
12. Chen I, Hsieh T, Thonas T, Safe S: Identification of estrogen-induced genes downregulated by AhR agonists in MCF-7 breast cancer cells using suppression subtractive hybridization. Gene 262: 207–214, 2001
13. Falany JL, Falany CN: Expression of cytosolic sulfotransferases in normal mammary epithelial cells and breast cancer cell lines. Cancer Res 56: 1551–1555, 1996
14. Falany JL, Falany CN: Regulation of estrogen activity by sulfation in human MCF-7 breast cancer cells. Oncology Res 9: 589–596, 1998
15. Qian Y, Deng C, Song W: Expression of estrogen sulfotransferase in MCF-7 cells by cDNA transfection suppresses the estrogen response: potential role of the enzyme in regulating estrogen-dependent growth of breast epithelial cells. J Pharm Exp Ther 286: 555–600, 1998
16. Berthois Y, Katzenellenbogen JA, Katzenellenbogen BS: Phenol red in tissue culture media is a weak estrogen: implications concerning the study of estrogen-responsive cells in culture. Cancer Res 83: 2496–2500, 1986
17. Falany JL, Falany CN: Regulation of estrogen sulfotransferase in human endometrial adenocarcinoma cells by progesterone. Endocrinology 137: 1395–1401, 1996
18. Knabbe C, Lippman ME, Wakefield LM, Flanders KC, Kasid A, Derynck R, Dickson RB: Evidence that transforming growth factor-beta is a hormonally regulated negative growth factor in human breast cancer cells. Cell 48: 417–428, 1987
19. Jeng MH, ten Dijke P, Iwata KK, Jordan VC: Regulation of the levels of three transforming growth factor beta mRNAs by estrogen and their effects on the proliferation of human breast cancer cells. Mol Cell Endocrin 97: 115–123, 1993
20. Eckert RL, Katzenellenbogen BS: Effects of estrogens and antiestrogens on estrogen receptor dynamics and the induction of progesterone receptor in MCF-7 human breast cancer cells. Cancer Res 42: 139–144, 1982
21. Cho H, Aronica SM, Katzenellenbogen BS: Regulation of progesterone receptor gene expression in MCF-7 breast cancer cells: a comparison of the effects of cyclic adenosine 3′,5′-monophosphate, estradiol, insulin-like growth factor-I, and serum factors. Endocrinology 134: 658–664, 1994
22. Evans T, Rowlands M, Silva M, Law M, Coombes R: Prognostic significance of aromatase and estrone sulfatase enzymes in human breast cancer. J Steroid Biochem Mol Biol 44: 583–587, 1993
23. Santner S, Masamura S, Wang J, Demers L, Hamilton C, Santen R: Determinants of tissue estradiol levels and biologic responsiveness in breast tumors. Breast Cancer Res Treat 49: S1–7, 1998
24. Reed MJ, Purhoit A: Aromatase regulation and breast cancer. Clin Endo 54: 563–571, 2001
25. Purohit A, Singh A, Reed M: Regulation of steroid sulphatase and oestradiol 17 beta-hydroxysteroid dehydrogenase in breast cancer. Biochem Soc Trans 27: 323–327, 1999
26. Soule HD, McGrath CM: Estrogen responsive proliferation of clonal human breast carcinoma cells in nude mice. Cancer Lett 10: 177–189, 1980
27. Hata H, Holinka CF, Pahuja SL, Hochberg RB, Kuramoto H, Gurpide E: Estradiol metabolism in Ishikawa endometrial cancer cells. J Steroid Biochem 26: 699–704, 1987
28. Falany C, Wang J, Falany J, Frost A: Sulfotransferase expression in normal and cancerous human breast tissues. Breast Cancer Res Treat 64: 28, 2000
29. Molis TM, Spriggs LL, Jupiter Y, Hill SM: Melatonin modulation of estrogen-regulated proteins, growth factors, and proto-oncogenes in human breast cancer. J Pineal Res 18: 93–103, 1995
30. Saceda M, Lippman ME, Chambon P, Lindsey RL, Ponglikitmongkol M, Puente M, Martin MB: Regulation of the estrogen receptor in MCF-7 cells by estradiol. Mol Endocrinol 2: 1157–1162, 1988
31. Read LD, Snider CE, Miller JS, Greene GL, Katzenellenbogen BS: Ligand-modulated regulation of progesterone receptor messenger ribonucleic acid and protein in human breast cancer cell lines. Mol Endocrinol 2: 263–271, 1988
32. Falany CN, Vazquez ME, Kalb JM: Purification and characterization of human liver dehydroepiandrosterone sulfotransferase. Arch Biochem Biophys 260: 641–646, 1989
33. Falany CN, Wheeler J, Oh TS, Falany JL: Steroid sulfation by expressed human cytosolic sulfotransferases. J Steroid Biochem Mol Biol 48: 369–375, 1994
34. Falany JL, Azziz R, Falany CN: Identification and characterization of the cytosolic sulfotransferases in normal human endometrium. Chem-Bio Interact 109: 329–339, 1998

35. van Landeghem AAJ, Poortman J, Nabuurs M, Thijssen JHH: Endogenous concentration and subcellular distribution of estrogens in normal and malignant human breast cancer. Cancer Res 45: 2900–2906, 1985
36. Dorgan JF, Longcope C, Stephenson Jr HE, Falk RT, Miller R, Franz C, Kahle L, Campbell WS, Tangrea JA, Schatzkin A: Serum sex hormone levels are related to breast cancer risk in postmenopausal women. Environ Health Perspect 105: 583–585, 1997
37. Hankinson SE, Willet WC, Manson JE, Colditz GA, Hunter DJ, Spiegelman D, Barbieri RL, Speizer FE: Plasma sex hormone levels and risk of breast cancer in postmenopausal women. J Natl Cancer Instit 90: 1292–1299, 1998
38. James MR, Skaar TC, Lee RY, MacPherson A, Zwiebel JA, Ahluwalia BS, Ampy F, Clarke R: Constitutive expression of the steroid sulfatase gene supports the growth of MCF-7 human breast cancer cells *in vitro*. Endocrinology 142: 1497–1505, 2001
39. MacIndoe JH, Woods G, Jeffries L, Hinkhouse M: The hydrolysis of estrone sulfate and dehydroepiandrosterone sulfate by MCF-7 human breast cancer cells. Endocrinology 123: 1281–1287, 1988

Address for offprints and correspondence: Charles N. Falany, Department of Pharmacology, 101 Volker Hall, University of Alabama at Birmingham, Birmingham, AL 35294; *Tel.:* (205) 934-9848; *Fax:* (205) 934-8240; *E-mail:* Charles.Falany@CCC.UAB.EDU