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PRECLINICAL STUDY

The androgen metabolite 5α-androstane-3β,17β-diol (3βAdiol) induces breast cancer growth via estrogen receptor: implications for aromatase inhibitor resistance

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Abstract The aromatase inhibitors (AIs) are used to treat estrogen receptor-positive (ER+) breast tumors in postmenopausal women, and function by blocking the conversion of adrenal androgens to estrogens by the enzyme CYP19 aromatase. Breast cancer patients receiving AI therapy have circulating estrogen levels below the level of detection; however, androgen concentrations remain unchanged. We were interested in studying the effects of androgens on breast cancer cell proliferation under profound estrogen-deprived conditions. Using in vitro models of estrogen-dependent breast cancer cell growth we show that the androgens testosterone and 5α-dihydrotestosterone induce the growth of MCF-7, T47D and BT-474 cells in the absence of estrogen. Furthermore, we demonstrate that under profound estrogen-deprived conditions these breast cancer cells up-regulate steroidogenic enzymes that can metabolize androgens to estrogens. Lastly, we found that the downstream metabolite of 5α-dihydrotestosterone, 5α-androstane-3β,17β-diol (3βAdiol), is estrogenic in breast cancer cells, and induces growth and ER-signaling via activation of ERα. In conclusion, our results show that breast cancer cells deprived of estrogen up-regulate steroidogenic enzymes and metabolize androgens to estrogen-like steroids. The generation of estrogen-like steroids represents a potential mechanism of resistance to aromatase inhibitors.

Keywords Breast cancer · 3βAdiol · Estrogen receptor

Abbreviations

ER	Estrogen receptor
3βAdiol	5α-androstane-3β,17β-diol
AI	Aromatase inhibitor
E2	17β-estradiol
TS	Testosterone
DHT	5α-dihydrotestosterone

Introduction

Estrogens play a significant role in breast cancer development and progression and many of the most potent risk factors for the development of breast cancer can be explained in terms of increased lifetime exposure to estrogen. While the contributions of estrogens to breast cancer etiology are well established, the role of androgens in breast cancer development and progression is less well understood. Current dogma holds that androgens can inhibit the growth of breast cancer cells and that this effect is mediated through the androgen receptor [1]. However, many of the in vitro studies of androgen effects on breast cancer have been conducted in the presence of low concentrations of estrogen which complicates the interpretation of the results. In the current study we were interested in evaluating the effects of

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androgens on breast cancer cell growth under conditions of profound estrogen deprivation. Examining the effects of androgens under these conditions may be clinically relevant given the extremely low concentrations of estrogen in post-menopausal breast cancer patients undergoing therapy with aromatase inhibitors (AIs).

In post-menopausal women, estrogens are generated through peripheral conversion of adrenal androgens, including testosterone and androstenedione, to estrogens by CYP19 aromatase. These peripherally generated estrogens can stimulate estrogen receptor-positive, estrogen-dependent breast cancer growth in the absence of ovarian estrogens. The third-generation AIs (letrozole, exemestane, and anastrozole) inhibit the growth of such tumors by blocking the peripheral conversion of adrenal androgens to estrogens, suppressing circulating 17β-estradiol (E2) concentrations to below that detectable by current conventional methods (low pM range) [2, 3]. Although the AIs have proven to be a highly effective therapy for post-menopausal estrogen receptor-positive (ER+) breast cancer, a significant number of patients receiving AIs will relapse within five years of treatment [4]. Thus, a better understanding of the mechanisms of AI resistance may lead to improved predictive markers of response and more effective treatment strategies.

Treatment with AIs profoundly suppresses circulating estrogen concentrations; however, the concentrations of androgens are not significantly altered [5, 6]. We were therefore interested in the possibility that androgens and/or their downstream metabolites might play a role in resistance to AI therapy. A number of studies of the effects of androgens on breast cancer cells have been published, with the majority focusing on the effects of testosterone (TS) and 5α-dihydrotestosterone (DHT) on breast cancer growth [1, 7–12]. Little is known, however, about the effects of these compounds on breast cancer cell growth under conditions of profound estrogen deprivation, similar to conditions found in women treated with AIs. Still less is known about the effects of downstream androgen metabolites, some of which have been shown to bind to estrogen receptors. TS is a relatively weak androgen and is metabolized by 5α-reductase to the potent androgen DHT, which in turn can be further metabolized by 3β-hydroxysteroid dehydrogenase (3β-HSD) to 5α-androstane-3β,17β-diol (3βAdiol). 3βAdiol has been shown to bind both ERα and ERβ, although with approximately 30-fold and 14-fold lower affinity relative to that of 17β-estradiol (E2), respectively [13]. We therefore set out to evaluate the effects of androgens and their metabolites on the proliferation of estrogen responsive breast cancer cells grown under conditions of profound estrogen deprivation, with the goal of determining if these steroids might be playing a role in resistance to AI therapy.

Methods

Cell lines, culture conditions, and growth assays

Testosterone (TS), 5α-dihydrotestosterone (DHT) and 17ß-estradiol (E2), were purchased from Sigma-Aldrich Inc. (St. Louis, MO). 5α-androstane-3β,17β-diol (3βAdiol) was purchased from Steraloids, Inc. (Newport, RI). Letrozole (Femara®) was purchased from Toronto Research Chemical (Toronto, Ontario, Canada). MCF-7, T47D and BT-474 cells were obtained from the Tissue Culture Shared Resource (TCSR) at the Lombardi Comprehensive Cancer Center and were routinely cultured in modified IMEM (Biosource International Inc., Camarillo, CA) supplemented with 10% fetal calf serum (Valley Biomedical Inc., Winchester, VA), at 37°C in a humidified 5% CO_2 atmosphere. For assays in defined hormone conditions, cells were repeatedly washed and grown in steroid depleted media (phenol red-free IMEM supplemented with 10% charcoal stripped calf bovine serum—CCS) as previously described [14]. For growth assays, cells were plated in steroid-depleted media at 2×10^3 cells/well in 96-well plates (Falcon, Lincoln Park, NJ) and allowed to attach overnight before being treating with vehicle control (ethanol 0.1%), E2, androgens, and the steroid antagonists. Relative cell number was determined using the crystal violet and WST assays as described previously [15].

RNA extraction

Total RNA was isolated using TRIzol® Reagent (Invitrogen Corp., Carlsbad, CA) according to the manufacture's instructions. Yield and quality were determined by spectrophotometry (Beckman DU® 640, Beckman Coulter, Inc., Fullerton, CA) and using a Bioanalyzer RNA 6000 Nano chip (Agilent Technologies, Palo Alto, CA). All samples were stored at −80°C.

Western blot

Western blot analysis was performed on whole cell lysates from breast cancer cell lines. Cell pellets were lysed using Gold Lysis Buffer [20 mmol/l Tris (pH 7.9), 137 mmol/l NaCl, 5 mmol/l EDTA, 10% glycerol, 1% Triton X-100, with protease inhibitor cocktail, Roche, Indianapolis, IN] and total protein from cell extracts was quantified using the Bradford assay (Bradford Reagent; Bio-Rad, Hercules, CA).

Thirty micrograms of protein per lane was resolved on 4–20% gradient polyacrylamide gels (Pierce, Rockford, IL), and transferred to PVDF membrane. CYP19 aromatase

protein levels were evaluated by blotting with an anti-CYP19 antibody (ab18995, Abcam, Cambridge, MA), and 3β-HSD levels using an anti-3β-HSD antibody (P-18, Santa Cruz, Santa Cruz, CA). Secondary antibodies were obtained from Jackson Laboratories (West Grove, PA). β-Actin was used as a loading control (I-19, Santa Cruz, Santa Cruz, CA).

Real-time PCR

GREB1 mRNA expression was measured using a semi-quantitative real-time PCR assay as described previously [14]. Briefly, total RNA (1 μg) was reverse transcribed using Reverse Transcription System (Promega, Madison, WI) and the resulting cDNA amplified in a 25 μl reaction containing Platinum Supermix UDG (Invitrogen Corp., Carlsbad, CA), 250 nM of each primer (forward 5′-CAA AGA ATA ACC TGT TGG CCC TGC-3′ and reverse 5′-GAC ATG CCT GCG CTC TCA TAC TTA-3′—Integrated DNA Technologies, Inc., Coralville, IA), 10 nM fluorescein (BioRad Inc., Hercules, CA), and SYBR Green. Reactions were performed using an iCycler Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, CA). To control for RNA quality and quantity, GREB1 expression was normalized to the levels of the housekeeping genes 36B4 (forward 5′-GTG TTC GAC AAT GGC AGC AT-3′ and reverse 5′-GAC ACC CTC CAG GAA GCG A-3′) and GAPDH (forward 5′-GAA GGT GAA GGT CGG AGT C-3′ and reverse 5′-GAA GAT GGT GAT GGG ATT TC-3′) as described previously [14]. To evaluate the quality of product of real-time PCR assays, melt curve analyses were performed after each assay. Relative expression was determined using the $\Delta\Delta C_T$ method with either GAPDH or 36B4 as the reference genes [16].

Statistical analyses and curve fitting

A two-tailed *t* test was used to compare treatments to respective controls (SigmaStat 3.5, Systat Software, Inc.). Curve fitting and effect concentration for half-maximal growth (EC_{50}) were determined using GraphPad Prism 4.03 (GraphPad Software, Inc.).

Results

Androgens induce estrogen-dependent breast cancer cell proliferation in estrogen-deprived conditions

To determine the effects of androgens on breast cancer cell proliferation in the absence of estrogen, we cultured MCF-7 cells under estrogen-free conditions and treated them with testosterone (TS) or 5α-dihydrotestosterone (DHT) at 10 nM, and measured the increase in cell number over five days of treatment as described in "Materials and Methods". Both TS and DHT induced the proliferation of MCF-7 cells under these conditions by approximately 30% and 56% over vehicle-treated controls, respectively (Fig. 1). The stimulation of proliferation by both compounds was blocked by concomitant treatment with 500 nM of the potent anti-estrogen ICI 182,780 (faslodex, checkered bars), whereas only the effects of TS were blocked by the aromatase inhibitor letrozole (500 nM) (striped bars, Fig. 1). The inability of the AI to block the stimulation by DHT suggests that this effect does not require aromatase activity. Similar data were generated using the estrogen dependent breast cancer cell lines T47D and BT474 (data not shown).

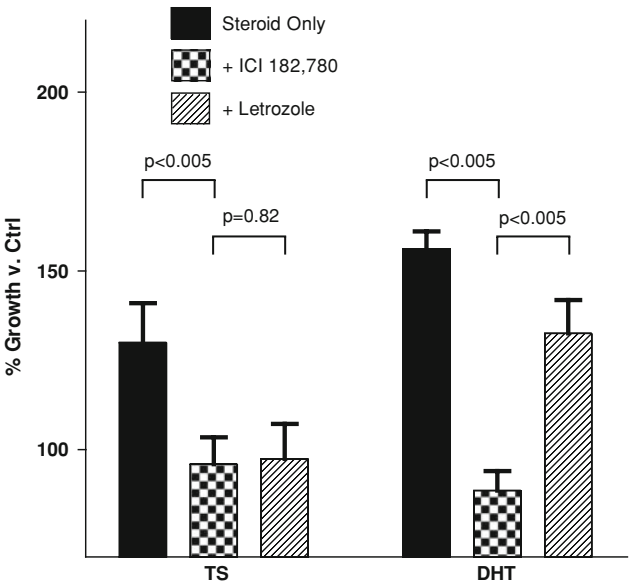

Fig. 1 MCF-7 cells were grown in E2-free conditions as described in Materials and Methods. The indicated steroids were added to a final concentration of 10 nM. ICI 182,780 (faslodex) and letrozole were added to a final concentration of 500 nM. Bars represent 5-day growth vs. vehicle-treated control ± SD

Steroid metabolizing enzymes are up-regulated under estrogen-deprived conditions

We observed that the androgens TS and DHT can stimulate the growth of estrogen dependent cells, and that this effect is apparently mediated through the estrogen receptor, since it is blocked by an estrogen receptor antagonist. These findings are surprising in light of the extremely low affinity of these androgens for ERα [13]. This led us to hypothesize that in estrogen-deprived culture conditions, the breast cancer cells are capable of metabolizing androgens into estrogens. This can be accomplished by CYP19 aromatase (TS to E2), 5α-reductase (TS to DHT) and 3β-HSD (DHT

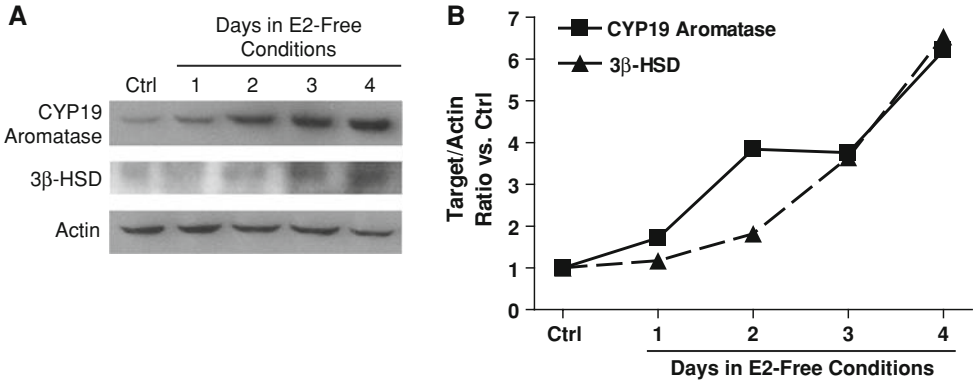

Fig. 2 CYP19 Aromatase and 3β-HSD expression increases in breast cancer cells during growth in E2-free conditions. (**a**) Western blot of cells grown in normal media with 10% FBS (lane 1), and increased time after steroid hormone removal (described in Materials and Methods) and incubation in media with 5% CCS. (**b**) Densitometry analysis of target/actin ratio vs. the normal media control (loading control)

to 3βAdiol). To test this hypothesis we used Western blot analysis to examine the expression of the steroid metabolizing enzymes CYP19 aromatase and 3β-HSD in breast cancer cells. Cells grown in standard culture conditions (media supplemented with 10% fetal bovine serum) were compared to cells grown in estrogen-free conditions (media with 5% charcoal-stripped serum) for 1–4 days. Figure 2a shows that CYP19 aromatase and 3β-HSD levels were very low in MCF-7 cells grown under standard culture conditions. However, culture under conditions of profound estrogen deprivation resulted in a dramatic induction of CYP19 aromatase and 3β-HSD expression levels in a time-dependent manner. Densitometric analysis of the target/actin (loading control) ratio shows that levels of both CYP19 aromatase and 3β-HSD in MCF-7 cells increase more than 6-fold versus control (standard conditions) after 4 days in estrogen-free conditions (Fig. 2b). Increases in CYP19 and 3β-HSD expression were also seen in T47D cells after incubation in estrogen-free conditions, though to lower levels, with approximately 2-fold increases in enzyme expression (data not shown). We observed that 5α-reductase is not expressed under estrogen-free conditions both functionally (Fig. 1; TS-induced growth is completely blocked by aromatase inhibition, suggesting that it is not metabolized to DHT or 3βAdiol) and by cDNA microarray analysis (data not shown).

3βAdiol is a weak agonist of ERα growth induction

The potential for 3βAdiol to act as an ERα agonist and induce the growth of breast cancer cells has been largely ignored in the literature, despite previous work demonstrating its binding to ERα [13]. We therefore examined the ability of the androgen metabolite 3βAdiol to induce the growth of breast cancer cells under estrogen-free conditions as described in Materials and Methods. 3βAdiol induced the proliferation of MCF-7 cells approximately 82% over vehicle-treated controls, an effect inhibited by the anti-estrogen, but not by the aromatase inhibitor (Fig. 3a), suggesting that this effect does not depend on metabolism by aromatase. Dose-response curves for growth induction were generated for TS, DHT and 3βAdiol, and compared to growth induction by E2 (Fig. 3b). 3βAdiol appears to be approximately two logs less potent than E2 with respect to induction of the growth of MCF-7 cells (EC_{50} of approximately 0.2 nM and 5.0 pM, respectively). However, 3βAdiol is substantially more potent than DHT (EC_{50}–0.8 nM) and TS (EC_{50}–2.4 nM). Interestingly, 3βAdiol appears to act as a weak agonist of ERα for growth induction, since the maximum stimulation of proliferation induction by 3βAdiol was roughly 75% of the maximal induction by E2. Similar results were observed in T47D cells (Fig. 3c).

3βAdiol growth induction is blocked by anti-estrogens

To confirm that growth stimulation by 3βAdiol is mediated through activation of ERα, we determined whether its effects could be blocked by the anti-estrogens tamoxifen, 4-hydroxytamoxifen (4-OHTam), and ICI 182,780 (faslodex). Cells were treated with either E2 or 3βAdiol (1 nM) alone or in combination with increasing concentrations of the anti-estrogens. As shown in Fig. 4, both E2- and 3βAdiol- induced MCF-7 cell proliferation was inhibited by the anti-estrogens in a dose-dependent manner (Fig. 4a and b respectively). ICI 182,780 and 4-OHTam inhibited the effects of 1 nM E2 roughly equivalently (IC_{50} of 23 nM and 47 nM, respectively), and tamoxifen only partially inhibited growth at 1 μM ($IC_{50} > 1$ μM), consistent with 2-fold lesser affinity for ERα versus ICI 182,780 and 4-OHTam. Significantly lower concentrations of the anti-estrogens were required to inhibit the effects of 1 nM 3βAdiol on cell growth. ICI 182,780 and 4-OHTam were roughly equipotent (IC_{50} of 1.0 nM and 2.0 nM, respectively), and tamoxifen inhibited 3βAdiol-induced growth at sub-micromolar concentrations (IC_{50}–0.2 μM). The anti-androgen bicalutamide did not inhibit 3βAdiol-induced growth at similar concentrations (data not shown). Similar results were observed in T47D cells (data not shown).

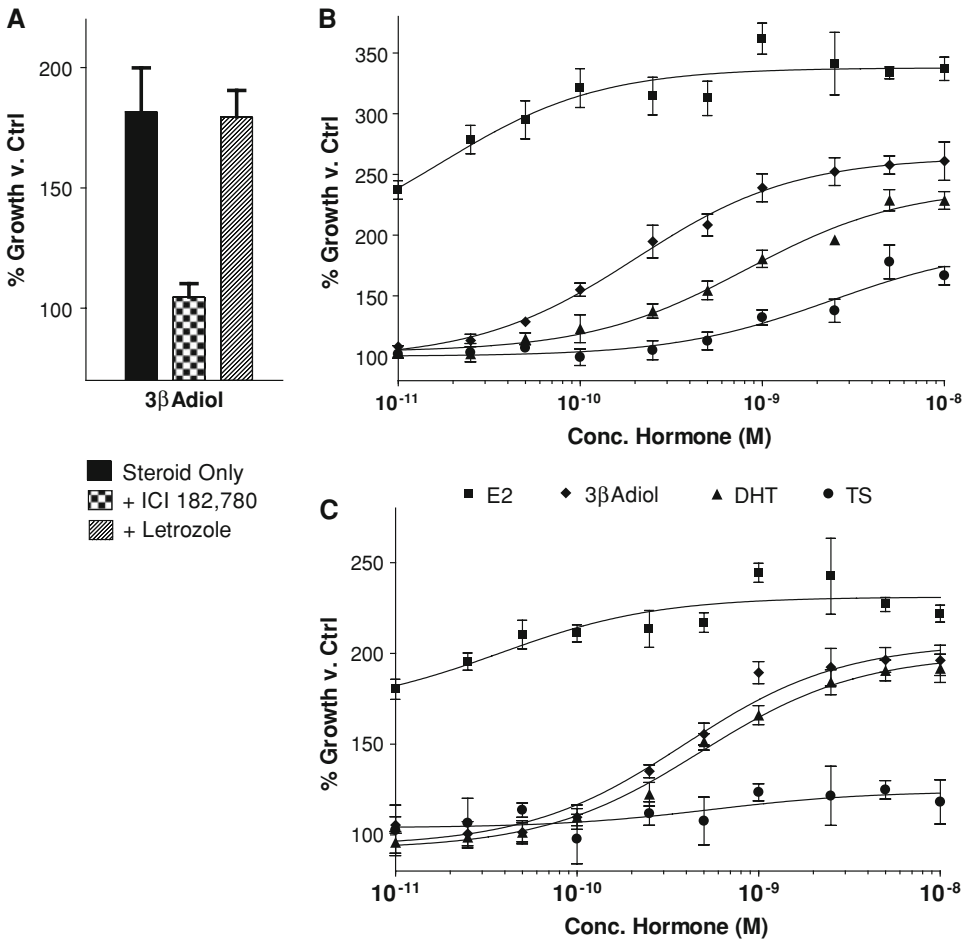

Fig. 3 Breast cancer cells were grown in E2-free conditions as described in Materials and Methods. (**a**) 3βAdiol was added to MCF-7 cells to a final concentration of 10 nM. ICI 182,780 (faslodex) and letrozole were added to a final concentration of 500 nM. Bars represent 5-day growth vs. vehicle-treated control ± SD MCF-7 (**b**) or T47D (**c**) cells were treated with the indicated steroid at concentrations from 10 pM–10 nM at half-log intervals. Points represent 5-day growth vs. vehicle-treated control ± SD

3βAdiol induces expression of the ERα-responsive gene GREB1

To determine whether 3βAdiol activation of ERα induced ERα-mediated gene transcription, we examined the expression of GREB1 in breast cancer cells treated with 3βAdiol. We have previously demonstrated that GREB1 is an ERα-specific downstream target critically involved in the estrogen induced growth of breast cancer cells [14]. Cells were grown in estrogen-free conditions as described above, and GREB1 expression was measured 24 h after the addition of 3βAdiol, alone or in combination with ICI 182,780 as described in "Materials and Methods". Cells treated with 1 nM 3βAdiol exhibited modest induction of GREB1 expression (~2.5-fold) over vehicle-treated controls (Fig. 5), however, 10 nM and 100 nM 3βAdiol substantially induced GREB1 expression (17.5- and 56.4-fold vs. control, respectively). Treatment with 100 nM 3βAdiol resulted in GREB1 expression levels comparable to those produced by treatment with 1 nM E2, which caused a 49.0-fold induction in GREB1 expression (dashed line). Concomitant treatment with the anti-estrogen ICI 182,780 completely blocked the induction of GREB1 expression by both 3βAdiol and E2 (Inhibition of E2 stimulation not shown).

Discussion

The use of AI as first line endocrine therapy for post-menopausal, estrogen receptor positive breast cancer has increased dramatically over the last few years with the publication of clinical trials suggesting that these compounds may be more effective than the anti-estrogen tamoxifen [4]. Although the AIs are effective, well tolerated drugs, a significant percentage of patients experience disease relapse during AI therapy, suggesting that resistance to this class of compound is a significant problem. Anecdotal evidence suggests that some women that fail to respond to AIs may still respond to other modes of endocrine therapy, even though their serum estrogen concentrations have been successfully suppressed by AI therapy. This suggests that at least a proportion of these resistant tumors in these women are still estrogen dependent, and implies that non-classical estrogens may be playing a role in resistance to AI therapy.

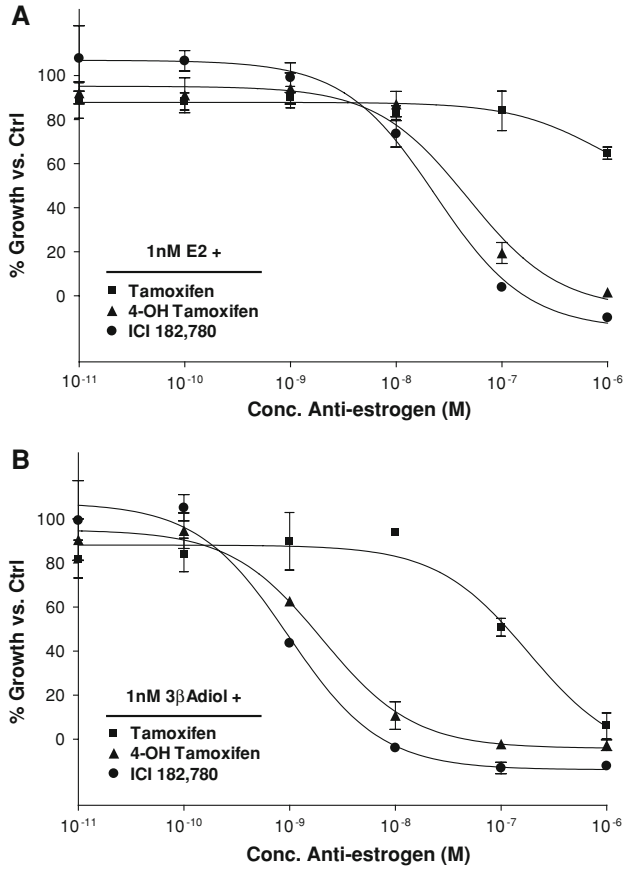

Fig. 4 MCF-7 cells were grown in E2-free conditions as described in "Materials and Methods". Growth induction by (**a**) 1 nM E2 or (**b**) 1 nM 3βAdiol was antagonized by the anti-estrogens tamoxifen, 4-hydroxy-tamoxifen (4-OH Tamoxifen), and ICI 182,780 (Faslodex). Anti-estrogens were added to final concentrations from 10 pM–1 μM at log intervals. Points represent 5-day growth vs. vehicle-treated control ± SD

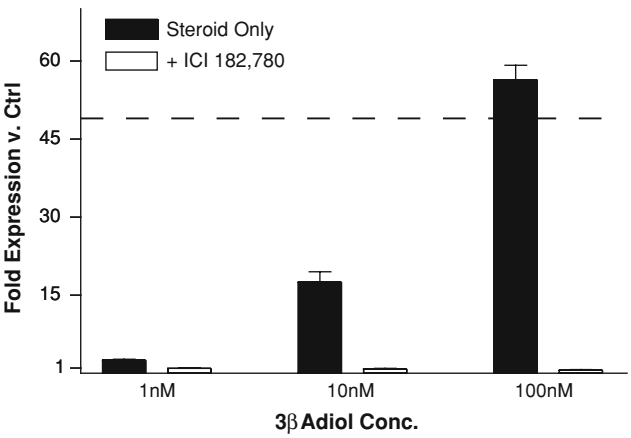

Fig. 5 MCF-7 cells were grown in E2-free conditions as described in "Materials and Methods". Cells were treated with the indicated concentrations of 3βAdiol in the presence (light bars) or absence (dark bars) of 1 mM ICI 182,780 for 24 h before harvest. Bars represent GREB1 expression vs. vehicle-treated control ± SD. The dashed line represents GREB1 expression induced by 1 nM E2

Women on AI therapy have circulating concentrations of estrogen that are below the limit of detection (low pg/ml), however, the concentrations of androgens remain unchanged (low nM range) [5, 6]. We hypothesized that under conditions of profound estrogen deprivation, the weak estrogenic activity of other steroids might be sufficient to drive the proliferation of estrogen dependent breast cancer, thereby providing a mechanism for AI resistance. Specifically, we hypothesized that androgens and their metabolites, generated independent of aromatase activity, may contribute to breast cancer growth in the absence of estrogens.

A considerable amount of work has been done over the years studying the effects of androgens on the proliferation of breast cancer cells [1, 7–12]. The literature is, however, somewhat confusing, with conflicting data coming from relatively similar experimental systems. For example, 30 years ago we demonstrated that under serum-free conditions, androgens stimulate thymidine incorporation in breast cancer cell lines apparently through the androgen receptor [8, 12]. Similarly, the testosterone metabolite DHT was shown by Birrell et al. to inhibit the growth of some breast cancer cell lines, but induce the growth of others [7]. Anti-androgens demonstrated mixed ability to inhibit the effects of DHT on growth, and this was attributed to the potential activity of un-indentified DHT metabolites [7]. Macedo et al. later showed that DHT is growth-inhibitory in MCF-7 cells under low-estrogen conditions, and that this effect was mediated by the androgen receptor [1]. One common thread of much of this work is that many of the studies use culture systems in which it is possible that low, but significant, amounts of residual estrogen remain, and so may not adequately model the conditions present in a woman on AI therapy. We have previously made use of a culture system in which residual estrogen concentrations are extremely low (sub pM) [14, 17], and decided to make use of this system to revisit the effects of androgens and their metabolites on the proliferation of estrogen dependent breast cancer cells.

In this study we have demonstrated that profound estrogen depravation results in the up-regulated expression of two important steroid metabolizing enzymes, CYP19 aromatase and 3β-HSD. MCF-7 and T47D cells are generally considered to express very low levels of aromatase and the finding that estrogen withdrawal can substantially increase expression levels has important implications. The induced expression of aromatase may not be important in the context of AI therapy, since the newly expressed enzyme should be efficiently inhibited by the drug. However, the induction of 3β-HSD and potentially other enzymes raises the possibility of significant local metabolism of androgens and other steroids, and generation of estrogens, by the breast cancer cells.

The downstream metabolite of DHT, 5α-androstane-3β,17β-diol (3βAdiol), is generated by the action of 3β-HSD. It has been known for some time that 3βAdiol can bind to both ERα and ERβ with approximately 30-fold and 14-fold lower affinity relative to that of E2, respectively, suggesting slight specificity for ERβ [13]. 3βAdiol has been extensively characterized as an ERβ ligand in in vitro ERβ-promoter driven luciferase assays [18, 19], gene expression assays [20, 21], and in vivo prostate and prostate cancer models [22, 23]. 3βAdiol has been shown to play a well defined role in prostate cancer etiology as an ERβ ligand. Weihua et al. demonstrated that 3βAdiol is anti-proliferative in prostate cancer via activation of ERβ [22, 23]. The cytochrome P450 CYP7B1 has been shown to be the primary enzyme responsible for the inactivation and elimination of 3βAdiol [24, 25]. Activation of ERβ by 3βAdiol and elimination of 3βAdiol by CYP7B1 have been shown to be critical regulators of prostate cancer growth as an anti-proliferative pathway [22, 26]. Recently, increased CYP7B1 levels were correlated with increased prostate cancer grade, suggesting that increased elimination of 3βAdiol removes tumor growth inhibition by ERβ [26]. Surprisingly, in spite of this work elucidating a role for 3βAdiol in prostate cancer, little is known about the importance of this steroid in breast cancer. Reporter studies have suggested that androgen metabolites (largely undefined in these studies) can induce the expression of an estrogen-responsive luciferase construct, but little further analysis of the function of these metabolites has been reported [11, 27]. Interestingly, female knockout mice generated by Omoto et al that lack expression of CYP7B1 (the enzyme responsible for the elimination of 3βAdiol) showed increased proliferation of both mammary and other reproductive tissues, as well as early onset of puberty and early ovarian failure, suggesting that 3βAdiol is indeed estrogenic in the breast and reproductive tissues [28].

In this study, we report for the first time that 3βAdiol can induce the proliferation of breast cancer cells through direct activation of ERα. This growth-stimulation is antagonized by the anti-estrogens 4-hydroxytamoxifen and ICI 182,780. In addition to inducing growth, 3βAdiol also induces the expression of the ERα-specific downstream gene GREB1 which we have previously shown is a critical mediator of estrogen stimulated proliferation. These findings raise the possibility that in the absence of conventional estrogens, 3βAdiol may be an important mediator of estrogen dependent breast cancer growth. We hypothesize that the generation of 3βAdiol from testosterone via aromatase-independent pathways represents a potential mechanism for resistance to AIs. The enzymes required for generation of 3βAdiol, 5α-reductase and 3β-HSD, are both expressed in a wide variety of tissues, primarily the adrenal glands and liver [29]. In addition, we have demonstrated that 3β-HSD is expressed in estrogen-deprived breast cancer cells. Thus, in the context of AI therapy, while circulating testosterone cannot be converted to 17β-estradiol due to inhibition of aromatase activity, it may readily be converted to 3βAdiol both systemically and, potentially, locally in the mammary tumor. In one study, plasma concentrations of 3βAdiol in humans were reported to be approximately 1.5 nM [30]. These relatively low concentrations of circulating 3βAdiol may be sufficient to drive tumor growth in the absence of estrogen, particularly in women undergoing treatment with AIs. In addition, prior reports have demonstrated that breast cancer cells can become hypersensitive to extremely low concentrations of estrogens after long-term estrogen deprivation [31, 32]. These data suggest that tumors may be sensitive to very low concentrations of a weak ERα agonist such as 3βAdiol. Further, the reported 3βAdiol plasma concentrations are greater than the calculated EC_{50} values for growth induction of breast cancer cells in culture (as shown in Fig. 3).

In summary, these data demonstrate the important concept that the metabolism of testosterone by aromatase does not represent the only mechanism by which estrogen-like steroids may be generated in post-menopausal women. While inhibition of aromatase may be sufficient to block the productions of the more well know estrogens in the majority of patients treated with AIs, conditions causing an increase in activity of the enzymes responsible for 3βAdiol production, particularly locally within the tumor, may lead to production of estrogen-like steroids independent of aromatase. These pathways may represent an important mechanism for resistance to AI therapy and a more thorough understanding of the diversity of hormone metabolism may be extremely valuable in the refinement of optimal endocrine therapy for breast cancer.

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