Role of Androgens on MCF-7 Breast Cancer Cell Growth and on the Inhibitory Effect of Letrozole

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Abstract

Previous work has shown that androgens inhibit breast cancer cells and tumor growth. On the other hand, androgens can be converted to mitogenic estrogens by aromatase in breast cancer cells. Here, we report that androgens, such as the aromatizable androstenedione and the non-aromatizable 5α-dihydrotestosterone, inhibit MCF-7 cell proliferation. This effect is observed only in the absence or at a low concentration of estrogens and is evident in cells with low aromatase activity. Growth of a new aromatase stably transfected MCF-7 cell line (Ac1) was stimulated by conversion of androstenedione into estrogens and was sensitive to aromatase inhibitors. We show that blockade of the androgen receptor (AR) in these cells by the antiandrogen casodex or by the anti-AR small interfering RNA inhibited the antiproliferative effect of 5α-dihydrotestosterone and letrozole (aromatase inhibitor). We also show that suppression of the estrogen-induced anti-apoptotic protein Bcl-2 may be involved in the antiproliferative effects of androgens and letrozole. These effects can be reversed by casodex. In conclusion, the results suggest that aromatase inhibitors may exert their antiproliferative effect not only by reducing the intracellular production of estrogens but also by unmasking the inhibitory effect of androgens acting via the AR. (Cancer Res 2006; 66(15): 7775-82)

Introduction

Hormones play a pivotal role in endocrine-mediated tumorigenesis and have been shown to influence cancer cell growth and progression (1). However, androgens and estrogens exert opposing effects on the growth and development of malignant human breast tissues (2). In breast cancer cells, androgens, such as 5α-dihydrotestosterone and its precursors androstenedione and testosterone, have inhibitory effects (2–9), whereas estrogens, such as estradiol, have mitogenic effects. In fact, testosterone propionate, fluoxymesterone, and calusterone were previously used in the adjuvant therapy of breast cancer in both premenopausal and postmenopausal women with an efficacy comparable to that achieved with other types of endocrine manipulations, such as tamoxifen (10–13). The androgen receptor (AR) is present in 50% to 90% of breast tumors (14), and the AR levels are closely correlated with the content of estrogen receptor (ER) and progesterone receptor. Like the ER content, AR expression in breast cancer tissue samples has been associated with an improved response to hormone therapy and longer patient survival (14). However, the physiologic role of endogenous androgens and events following AR activation leading to inhibition of cell growth are not clearly identified in breast cancer cells. Some studies have shown that androgens modulate the expression of the Bcl-2 protein family in breast cancer cells, and that their effect is contrary to that of estrogens (15, 16). The Bcl-2 family proteins both inhibit and promote cell death (16, 17), and the Bcl-2 protein has been extensively characterized as an inhibitor of apoptosis.

Due to their adverse side effects, exogenous androgens were later replaced by other better tolerated breast cancer treatment strategies, such as ER antagonism and aromatase inhibition. Aromatase is the enzyme responsible for catalyzing the rate-limiting step in the conversion of androgens to estrogens (18, 19). In postmenopausal women, the ovary ceases to produce estrogen and the major source becomes extragonadal sites, such as breast tissue. In breast cancer patients, it was previously shown that estrogen levels in malignant tissue are higher than those in normal breast tissue and is associated with increased aromatase activity (20–24). Using an intratumoral aromatase model our group has shown that the production of estrogens in situ by the tumors can be inhibited by blocking the local aromatase activity with aromatase inhibitors (18, 25–27). Recently, the aromatase inhibitor letrozole was approved as a therapy for hormone-dependent breast cancer in numerous settings and has proved to be effective for postmenopausal patients with tumors responsive to estrogens (28).

In the present study, we investigated the effect of androgens on MCF-7 and Ac1 cells (MCF-7 cells stably transfected with the human aromatase gene; ref. 29). These MCF-7 and Ac1 cell lines are appropriate models for studying the balance between the androgenic and estrogenic effect in breast cancer as they express significant levels of AR and ER. In addition, the MCF-7 cells have a low rate of conversion of androgens to estrogens, whereas in the Ac1 cells, the rate is higher. Furthermore, we investigated whether the effect of androgens is mediated by their interaction with AR. Our results suggest that a change in the balance between androgenic and estrogenic influences could modify the overall growth rate of breast tumors because these hormones have opposite effects on breast cancer cell growth. We also found that letrozole, a highly specific aromatase inhibitor, impedes breast cancer cell proliferation not only by a mechanism involving reduction of estrogens biosynthesis but, additionally, by permitting endogenous androgens to exert their antiproliferative effects via the AR in a low-estrogen milieu.

Materials and Methods

Materials. Phenol red–free improved IMEM and trypsin/versene were purchased from Biosource Biofluids Cell Culture Products (Rockville, MD), DMEM, penicillin/streptomycin solution, 0.25% trypsin-EDTA (1 mmol/L) solution, Dulbecco’s PBS, and geneticin (G418) were obtained from Life
Technologies (Grand Islands, NY). Fetal bovine serum and charcoal/dextran-treated fetal bovine serum were obtained from Hyclone (Logan, UT). Androstenedione, β-estradiol, 5α-dihydrotestosterone, tamoxifen, DMSO, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma Chemical Co. (St. Louis, MO). Androst-4-ene-3,17-dione[1,2-3H(N)] (25.3 Ci/mmol, 1 mCi/mL; [1,2-3H]androstene-3,17-dione) was purchased from NEN (Boston, MA). Letrozole (Femara, CCS 20267) was provided by Dr. D. Evans (Novartis Pharma, Basel, Switzerland). Fulvestrant (Faslodex, ICI 182,780) and the pure antidirogen cosedex were supplied by Dr. A. Wakeling (AstraZeneca Pharmaceuticals, Macclesfield, United Kingdom). Enhanced chemiluminescence (ECL) kit and Hybond-ECL nitrocellulose membranes were purchased from Amersham Biosciences (Piscataway, NJ). Anti-AR and anti-ER-α antibody were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Antibodies against Bcl-2, Bcl-xL, Bad, and Bax were obtained from Cell Signaling Technology (Beverly, MA). β-Actin antibody was obtained from Oncogene Research Products (Boston, MA). MCF-7 human breast cancer cell line was originally obtained from Dr. R. Santen (University of Virginia School of Medicine, Charlottesville, VA). The 7 cc cell line was kindly provided by Dr. S. Chen (City of Hope, Duarte, CA).

Cell cultures. The MCF-7 cells were grown in DMEM supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin (will be referred to as regular medium) at 37°C with 5% CO2 atmosphere, 7 cc, Ac1, siAc1v, and siAc1 cells were grown in regular medium supplemented with 600 µg/mL of G418. Cell culture medium was changed twice weekly.

Transfection assay. The transfection experiments were carried out using FuGene 6 (Roche Molecular Biochemicals, Indianapolis, IN) following the manufacturer's protocol. The MCF-7 cells were grown in 60-mm dishes. The medium was replaced with DMEM lacking serum and antibiotics on the day of transfection. All transfection mixture contained 3.9 µl of the transfection reagents and quantified by densitometry using Molecular Dynamics software (ImageQuant).

Results

Properties of the MCF-7 cells transfected with aromatase gene (Ac1). After transfection of MCF-7 cells with the aromatase gene and selection by their resistance to G418, eight clones were isolated and screened for their aromatase enzyme activity. The Ac1 clone showed a high level of enzyme activity at 0.0462 fmol/cell/h. MCF-7 cells transfected with the empty expression plasmid (7 cc) and the parental MCF-7 cells showed no significant aromatase activity. Experiments with longer incubation time (66 hours) were used to select the cells and characterize for aromatase activity. The Ac1 clone was chosen for the future experiments.

Plasmids, construction of AR small interfering RNA lentivirus vector, and infection. The lentivirus vector expressing an anti-AR small interfering RNA (siRNA) was constructed as previously described (30). A modified Lentiss-Lex37.3PL/L3.7 vector, in which the sequence encoding a specific siRNA was controlled by the U6 promoter and the sequence encoding the green fluorescent protein, was removed (31). The oligonucleotides encoding AR siRNA were 5′-TGCACTGCTACTCTTCAGCATTCAAGAGTA T-3′ and 5′-TCGAGAAAAAAGCAC-3′ (32). The oligonucleotides were annealed and ligated downstream of the U6 promoter, and the lentiviruses were prepared as previously described (31). After the medium was switched to medium containing G418 (600 µg/mL). Several clones of the aromatase transfected cells were isolated and characterized for aromatase activity. The Ac1 clone was chosen for the future experiments.

Cell proliferation assay. The effect of the various drugs and hormones on MCF-7, Ac1, siAc1v, and siAc1 cells growth was examined using the MTT colorimetric assay (33). The cells growing in regular media were transferred to IMEM containing 5% charcoal-stripped serum and 1% penicillin/streptomycin (charcoal-stripped serum in medium, CSSM) for 72 hours. This medium was used for all cell growth assays. The cells were detached from their flask using trypsin/verseine and seeded in 96-well plates at 1.0 × 10^4/mL (100 µL) on day 0. Twenty-four hours later, several concentrations of the hormones or drugs were added. The cells were incubated for 6 days in the presence of the tested drugs and hormones. After the incubation period, the medium was removed, and 100 µL of a 1 mg/mL solution of MTT in IMEM was added to each well. The plates were incubated for 3 hours. At the end of the incubation period, the untransformed MTT was removed, and DMSO was added to each well (100 µL). The absorbance of each well was measured at 560 nm using a 96-well plate reader (Victor 1420 Multilabel Counter, Wallac, Turku, Finland). The absorbance values were corrected for nonspecific conversion of MTT by preparation of blank wells containing all additions apart from the cells.

Aromatase activity assay. Cells growing in CSSM were seeded in a six-well plate (1.5 × 10^5 per well). After 24 hours, the medium was removed, and the cells were washed with PBS. One percent of charcoal-stripped serum IMEM containing [1,2-3H]androstenedione in the presence or absence of letrozole was added to the cells for 2 hours. The aromatase activity was determined from conversion of [1,2-3H]androstenedione to estrogen with release of 3H2O as described previously (34). The aromatase activity was expressed as fmol/cells/h. The IC50 value was defined as the concentration of drug that reduced aromatase activity to 50% of that found for the untreated control (100%) 2 hours after adding letrozole and [1,2-3H]androstenedione.

Western blotting. The cells lysates were obtained by sonicating the cells in radioimmunoprecipitation assay buffer (35). The protein concentration was measured using the Bio-Rad method. Fifty micrograms of proteins were subjected to SDS-PAGE and then transferred to a nitrocellulose membrane. The membrane was probed for specific primary antibody as specified in the manufacturer's protocol. Immunoreactive bands were visualized using ECL detection reagents and quantified by densitometry using Molecular Dynamics software (ImageQuant).

Figure 1. Characterization of Ac1 Cells. A. effect of increasing concentrations of letrozole on Ac1 aromatase activity. Letrozole was coincubated with [1,2-3H]androstenedione for 2 hours. Columns, mean of three experiments, each with four replicates; bars, SE. B. Western immunoblotting analysis of whole-cell lysates from MCF-7 and Ac1 cells for ER-α and AR. Blot shows ER-α and AR proteins expression. The experiment was performed using the Bioblotter II system. Blot shows for the untreated control (100%) 2 hours after adding letrozole and [1,2-3H]androstenedione. Western blotting. The cells lysates were obtained by sonicating the cells in radioimmunoprecipitation assay buffer (35). The protein concentration was measured using the Bio-Rad method. Fifty micrograms of proteins were subjected to SDS-PAGE and then transferred to a nitrocellulose membrane. The membrane was probed for specific primary antibody as specified in the manufacturer's protocol. Immunoreactive bands were visualized using ECL detection reagents and quantified by densitometry using Molecular Dynamics software (ImageQuant).
showed that MCF-7 cells present a significant but lower aromatase activity of 8.0 fmol/150 × 10⁵ cells/h (data not shown). Increasing concentrations of letrozole caused a dose-dependent decrease in the aromatase activity of Ac1 cells (Fig. 1A). Letrozole showed an IC₅₀ of 40 ± 3 pmol/L and 90% inhibition of aromatase activity at 1 and 10 nmol/L. Higher concentrations of letrozole did not cause complete inhibition of the enzyme in these cells (data not shown).

The expression of ER-α and AR in the MCF-7 and Ac1 cells was examined by Western blot (Fig. 1B). Whole-cell lysates from both cell lines were used. The aromatase transfection of MCF-7 cells did not cause any significant change in the levels of ERα and AR expression as shown by the densitometric analysis in Fig. 1B.

**Growth effect of estradiol on MCF-7 and Ac1 cells.** To examine the effect of estradiol on the proliferation of MCF-7 and Ac1 cells, they were cultured in estrogen-deprived media for 4 days before treatment. The response of the cells to estradiol was measured after 6 days of treatment in charcoal-stripped medium (Fig. 2A). This protocol for cell treatment was used for all future experiments with other hormones and drugs. The pure antiestrogen ICI 182,780 (ICI) and tamoxifen, respectively, in the presence of 1 nmol/L estradiol on MCF-7 and Ac1 cells. Cell proliferation is expressed as the percentage of the cells compared with the control wells (untreated cells). Columns, mean of two to four experiments, each with six replicates; bars, SE. B and C, antiproliferative effect of increasing concentrations of ICI (fulvestrant, Faslodex) and tamoxifen, respectively, in the presence of 1 nmol/L estradiol on MCF-7 and Ac1 cells. Cell proliferation is expressed as the percentage of the cells compared with the control wells (1 nmol/L estradiol-treated cells). Columns, mean of two to six experiments, each with six replicates; bars, SE.

**Growth effects of androgens in the absence and presence of estradiol in MCF-7 and Ac1 cells.** To evaluate the role of androgens on cell growth, MCF-7 and Ac1 cells were treated with the aromatizable androgen, androstenedione, and non-aromatizable androgen 5a-dihydrotestosterone. Increasing concentrations of androstenedione caused an inhibitory effect on MCF-7 cells growth when compared with control (untreated cells; Fig. 3A). Thus, androstenedione at 1 and 10 nmol/L resulted in 13% and 30% inhibition of proliferation. On the other hand, in Ac1 cells (has a high aromatase enzyme activity), androstenedione lead to a stimulation in growth that was 50% higher than the control cells (Fig. 3A). Androstenedione at the physiologic concentration of 1 nmol/L showed similar results as estradiol in inducing Ac1 cell proliferation (53% and 58% of the control, respectively). In contrast, the non-aromatizable androgen dihydrotestosterone (1 nmol/L) inhibited MCF-7 and Ac1 cells growth by 49% and 43%, respectively (Fig. 3A). Significant growth inhibition by dihydrotestosterone in both cell lines was observed at concentrations as low as 0.01 nmol/L, whereas concentrations higher than 1 nmol/L did not yield any additional effect (data not shown). Dihydrotestosterone was more potent than the aromatizable androstenedione in inhibiting MCF-7 cell growth. The physiologic concentration of 1 nmol/L androstenedione and dihydrotestosterone was selected for further experiments.

The ability of androstenedione to increase Ac1 cell proliferation was consistent with its metabolic transformation into estrogens by the cells than from its direct interaction with the AR. This was confirmed by the inhibition of androstenedione-induced proliferation of Ac1 cells by the aromatase inhibitor letrozole (Fig. 3B). This inhibitor reduced Ac1 cell growth with an IC₅₀ of 1.5 ± 0.4 nmol/L. A higher concentration of 10 nmol/L did not result in greater inhibition than 5 nmol/L letrozole, and cell growth was
equivalent to that of untreated cells in CSSM (P > 0.06). These results were similar to those reported for MCF-7Ca cells, a previous cell line we have used transfected with the aromatase gene (36). Additionally, increasing concentrations of the ER antagonist ICI reduced the androstenedione-stimulated Ac1 cell growth (Fig. 3C). ICI at 10 nmol/L caused a 75% inhibition of Ac1 cell proliferation induced by androstenedione. ICI at 10 nmol/L caused only 44% inhibition on Ac1 cells growth induced by estradiol.

MCF-7 cells possess much lower aromatase activity than Ac1 cells and were inhibited by lower concentration of letrozole (Fig. 3D). The 20% inhibition of 1 nmol/L androstenedione on cell growth was further increased by 50% with the addition of letrozole at a concentration as low as 0.01 nmol/L, resulting in a total 60% inhibition in comparison with the control (untreated cells; Fig. 3D). These results suggest that letrozole inhibiting aromatase in MCF-7 cells reduced the conversion of androstenedione to estradiol, thereby increasing the bioavailability of androgens. In this low-estrogen milieu, androgens could then exert an even greater inhibitory effect on cell growth. We also confirm that part of androstenedione in MCF-7 cells is being converted to estrogens by showing that the inhibitory effect of androstenedione was further increased by addition of the pure antiestrogen ICI (Fig. 3C). The maximal effect of ICI on MCF-7 cells was obtained at 10 nmol/L, which caused a 50% increase in androstenedione cell growth inhibition.

The results shown in Fig. 3 suggest that there is a balance between the mitogenic effect of estradiol and the inhibitory effect of the aromatase precursor androgen androstenedione and the non-aromatizable androgen dihydrotestosterone on breast cancer cell growth. This hypothesis was further elucidated by the treatment of MCF-7 and Ac1 cells with androgens at 1 nmol/L and increasing concentrations of estradiol (Fig. 4). For both cell lines, the inhibitory effect of dihydrotestosterone at 1 nmol/L alone was overcome by addition of increasing concentrations of estradiol. This induced a proliferative effect that was similar to 1 nmol/L estradiol alone (Fig. 4A). The same result was observed for the treatment of MCF-7 cells with androstenedione at 1 nmol/L, where the addition of estradiol at concentrations as low as 0.01 nmol/L caused a reversal of the inhibitory effect of androstenedione (Fig. 4B).

Elucidation of the role of androgens on letrozole-mediated growth inhibition. To verify that the inhibitory effect of androgens on cell growth was mediated by the interaction of androgens with the AR, we added increasing concentrations of the antiandrogen casodex to androstenedione- and dihydrotestosterone-treated cells (Fig. 5A and B). A concentration of 0.01 μmol/L casodex was enough to reverse the inhibitory effect of androstenedione on MCF-7 proliferation that returned to the basal level of the untreated cells growth (100% of control; Fig. 5A). The androstenedione-induced cell proliferation of Ac1 cells that is mediated by an estrogen-mitogenic action was not changed with increasing concentrations of the antiandrogen casodex. In addition, increasing concentrations of casodex (0.01-10 μmol/L) added to estradiol (1 nmol/L)-treated cells did not show any significant effect (data not shown). However, to reverse the inhibitory effect of dihydrotestosterone on MCF-7 and Ac1 cell growth to the basal level, it was necessary

![Figure 3. Proliferative response of MCF-7 and Ac1 cells to androgens. Cells were cultured in IMEM steroid-free medium without phenol red for 3 days before plating.](image-url)
to add a 1 μmol/L concentration of casodex (100 times more than required to reverse the effect of androstenedione; Fig. 5B). Dihydrotestosterone is a more potent antiproliferative androgen than androstenedione and binds the AR with high affinity and may explain why higher casodex concentrations are necessary to reverse the dihydrotestosterone inhibitory effect. These results indicate that the AR is involved in androstenedione and dihydrotestosterone inhibitory effects on breast cancer cell proliferation.

Letrozole inhibits cell growth by inhibiting conversion of androgens to estrogens, thereby making androgens available to exert their antiproliferative effect. This androgenic effect seems to involve the activation of the AR. To confirm this hypothesis, we treated androstenedione-stimulated Ac1 cells with 5 nmol/L of letrozole and increasing concentrations of casodex. As shown in Fig. 5C, 0.1 μmol/L casodex completely reversed the inhibitory effect of letrozole, restoring growth to the basal level (control, untreated cells). The addition of 1 μmol/L of casodex to the letrozole-treated Ac1 cells resulted in cell growth levels that were equivalent to the 1 nmol/L estradiol and androstenedione treatments alone.

Effects of androgens, estradiol, and letrozole on AR and Bcl2 family proteins expression. Androgens and estrogens are known to modulate the expression of antiapoptotic proteins, such as the Bcl-2 family, in a variety of breast cancer cells lines (15, 16). Therefore, the levels of expression of the AR and the Bcl-2 family proteins in Ac1 and MCF-7 cells was determined to further elucidate the relationship between the actions of androgens and letrozole. Protein expression was identified by Western blot of the whole-cell lysates after a 6-day treatment in charcoal-stripped medium (control C, untreated cells; Fig. 5D).

AR expression was up-regulated by all treatments of both cell lines in comparison with the untreated control. As shown in Fig. 5D, the expression of Bcl-2 was greatly stimulated by 1 nmol/L estradiol in MCF-7 cells (Fig. 5D, top) and by both androstenedione and 1 nmol/L estradiol in Ac1 cells (Fig. 5D, bottom). The up-regulation of antiapoptotic Bcl-2 in Ac1 cells by androstenedione at 1 nmol/L is probably due to the conversion of androstenedione to estradiol by aromatase that is highly expressed in this cell line. On the other hand, androstenedione elicited a reduction in Bcl-2 expression in MCF-7 cells (with low aromatase activity) compared with estradiol (Fig. 5D, top). The addition of 0.1 μmol/L of the antiandrogen casodex to MCF-7 and androstenedione-treated cells reversed this effect. The non-aromatizable androgen dihydrotestosterone also suppressed Bcl-2 in both cell lines. This effect of dihydrotestosterone was observed previously in ZR-75-1 breast cancer cells (15). The androstenedione-mediated up-regulation of Bcl-2 in Ac1 cells was inhibited in a dose-dependent manner by letrozole at 0.1 and 5 nmol/L (Fig. 5D, bottom). The level of expression of Bcl-2 in MCF-7 cells treated with 1 nmol/L dihydrotestosterone, and 0.1 and 5 nmol/L letrozole was equivalent (Fig. 5D, top). The expression of the proapoptotic proteins Bad and Bax and antiapoptotic Bcl-xL was not altered by androgens or estradiol (data not shown).

Resistance of the AR knockdown Ac1 cell line (siAc1) to letrozole and dihydrotestosterone. To further show the importance of the AR signaling for the inhibitory effects of androgens and for the mechanism of action of letrozole, we used the AR down-regulated siAc1 cell line. These siAc1 cells were obtained by the infection of Ac1 cells with a lentivirus vector expressing an anti-AR siRNA. The decrease in the AR expression in siAc1 cells was ~86% of the control Ac1 and Ac1 cells transfected with the empty vector (siAc1v; Fig. 6A). The Ac1, siAc1v, and siAc1 cells did not show significantly different levels of expression of ER-α. The down-regulation of the AR persisted throughout all of the cell proliferation experiments. The siAc1v and Ac1 cells yielded the same results in the growth assays (data not shown). The growth of siAc1 cells was stimulated equally by androstenedione and estradiol at 1 nmol/L (Fig. 6B), and the results were similar to the ones obtained for the Ac1 cells (~156% of the control; Fig. 3A). In contrast, unlike the parental Ac1 cells, siAc1 cells were not inhibited by the non-aromatizable androgen dihydrotestosterone. The knocking down of the AR resulted in resistance of the AC1 cells to letrozole treatment with growth responses similar to those obtained by androstenedione and estradiol alone at 1 nmol/L (~146% of the control).

Discussion

The results showed that MCF-7 breast cancer cells are responsive to androgens and estrogens. Estrogens stimulate MCF-7 and Ac1 (MCF-7 aromatase transfected) cell proliferation over a physiologic range of concentrations, and their effects are mediated by the ER. In contrast, androgens show the opposite effect and inhibit cell growth. MCF-7 cell proliferation was inhibited by androstenedione and 5α-dihydrotestosterone at concentrations found in women and
hypothesized that the letrozole antiproliferative mechanism of action may be due not only to the reduction in estrogens biosynthesis but also to the unmasking of the inhibitory effect of androgens acting via the AR. In fact, AR antagonism by 0.1 μmol/L casodex reversed the inhibitory effect of 5 nmol/L letrozole on androstenedione-treated Ac1 cells to the control level. A higher concentrations of casodex (1 μmol/L) or down-regulation of the AR in siAc1 cells restored cell growth to the level induced by estrogens. This result may be explained by the fact that letrozole at 5 nmol/L did not inhibit completely the aromatase activity in Ac1 cells. It is possible that although the estrogen production was dramatically reduced by the 5 nmol/L letrozole dose treatment, some of the androstenedione added was still converted to estrogen. The presence of these estrogen compensated for the androstenedione inhibitory effect and impaired androstenedione (or dihydrotestosterone obtained from androstenedione) from exerting a stronger inhibition. When the Ac1 cells were released from the inhibitory androgenic effect with increasing concentrations of casodex or down-regulation of the AR in siAc1 cells, this permitted cell proliferation stimulated by estrogens converted from androstenedione. The fact that estradiol even at low concentrations possesses marked proliferative action on Ac1 cells further confirmed this hypothesis. It has been reported that androstenedione can be converted to dihydrotestosterone in the cells in vitro and then metabolized to 3β-androstenediol, an androgen that possesses...
Fig. 6. Characterization of AR knockdown siAc1 cells and growth response to estradiol, androgen, and letrozole treatment. A. Western immunoblotting analysis of whole-cell lysates from Ac1, siAc1v, and siAc1 cells cultured in vitro for AR and ERα. Blot shows ERα and AR proteins expression. Experimental protocol was as described in Materials and Methods. First lane, Ac1; second lane, siAc1v; third lane, siAc1. Blots were also probed for β-actin (bottom) to verify equal amount of protein loaded in each lane. B. Effect of 1 nmol/L estradiol, androstenedione, and 5α-dihydrotestosterone and combination of 1 nmol/L androstenedione and increasing concentrations of letrozole on siAc1 cell growth. Cells were cultured in IMEM steroid-free medium without phenol red for 3 days before plating. Cells were seeded in 96-well plates and, 24 hours after, were exposed for 6 days to the specific treatments. Cell proliferation was measured using the MTT assay as described in Materials and Methods. Cell growth is expressed as the percentage of the cells compared with the control wells (untreated cells). Columns, mean of four to seven experiments, each with 6 to 12 replicates; bars, SE.
References


