Down-Regulation of Transforming Growth Factor β Receptors by Androgen in Ovarian Cancer Cells

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ABSTRACT

Steroid hormones have been implicated in the etiology and/or progression of epithelial ovarian cancer. As ovarian surface epithelial cells are growth inhibited by transforming growth factor β (TGF-β), we tested whether steroid hormones could regulate the expression of TGF-β1 or its receptors in ovarian cancer cells, as assessed by quantitative reverse transcription-PCR. Treatment of ovarian cancer HEY cells with 500 nM 5α-dihydrotestosterone (DHT), but not estradiol-17β or progesterone, for 60 h down-regulated the expression of mRNA for TGF-β receptors I and II (TGF-βR-I and TGF-βR-II), betaglycan, and endoglin but had no effect on TGF-β1 mRNA levels. Androgen receptor (AR) mRNA expression in HEY cells was compared to other ovarian cancer cell lines. OVCAR-3 cells expressed AR mRNA levels similar to that of androgen-responsive LNCaP cells. HEY cells expressed only 3 and 0.01%, respectively. Western blot analysis and saturation binding assays confirmed the expression of AR protein in these three cell lines, but at the limit of detection in SKOV-3 and HEY cells. Treatment of SKOV-3 and HEY cells for 24 h with 1–50 nM DHT resulted in a dose-dependent down-regulation of TGF-βR-I mRNA. The AR antagonist hydroxyflutamide did not reverse the effect of DHT on SKOV-3 cells but by itself down-regulated TGF-βR-II mRNA. This apparent androgen-mimetic action of hydroxyflutamide and the ability of SKOV-3 and HEY cells to respond to DHT may be due to their expression of AR-associating protein 70, an AR co-activator reported to amplify AR transactivation and to result in agonist activity of AR antagonists. DHT was able to reverse TGF-β1 growth-inhibitory action in SKOV-3 cells and in a primary culture of ovarian cancer cells derived from ascites. Thus, androgens may promote ovarian cancer progression in part by decreasing TGF-β receptor levels, thereby allowing ovarian cancer cells to escape TGF-β1 growth inhibition.

INTRODUCTION

The most common and lethal form of ovarian cancer is that derived from the ovarian surface epithelium. The etiology of epithelial ovarian cancer is poorly understood; however, steroid hormones have been implicated in the progression of this disease (1). Although 50–60% of ovarian cancer tumors express estrogen and/or progesterin receptors, up to 95% of ovarian cancer tumors express ARs (2–6). In a nested case-control study, an increased risk of ovarian cancer was found in women with elevated circulating levels of androgens (7). Polycystic ovary syndrome, a condition characterized by thecal cell hypertrophy and elevated circulating levels of androgens, has also been associated with an increased risk of ovarian cancer (8). Furthermore, in guinea pigs, long-term administration of testosterone, but not estrone, induced the formation of benign ovarian epithelial neoplasms (9).

A mechanism by which steroid hormones might stimulate the growth and/or progression of ovarian cancer is by blocking the action of TGF-β, a potent inhibitor of ovarian epithelial cell growth in culture (10, 11). Development of resistance to TGF-β has been observed in many cancers (12) and can occur gradually during the multistep process of tumor progression (12, 13).

TGF-β (TGF-β1, -β2, and -β3) mediate their effects on target cells by forming a heteromeric complex with transmembrane serine/threonine kinase receptors, TGF-βR-II and TGF-βR-I (14). Binding of TGF-β to constitutively active TGF-βR-II leads to recruitment, interaction, and phosphorylation of TGF-βR-I, which then propagates the signal to downstream Smad proteins (14). In addition, two other proteins can be found in TGF-β receptor complexes, betaglycan, and endoglin. Betaglycan binds TGF-β1, -β2, and -β3 directly and is referred to as type II receptor; it presents ligand to TGF-βR-II but does not have a recognizable signaling domain (15). Endoglin is a dimeric transmembrane glycoprotein that binds TGF-β1 and -β3 via its association with TGF-βR-II (16, 17). Overexpression of endoglin has been shown to modulate cellular responses to TGF-β1 (18). Thus, the down-regulation or loss of functional expression of receptors and/or signaling mediators for TGF-β would allow cancer cells to become resistant to this potent growth inhibitor.

In the present study, we tested the effects of steroid hormones on the expression of TGF-β and its receptors in ovarian cancer cells. As steroid hormones regulate ovarian function, we hypothesize that an imbalance in their action could promote ovarian cancer progression by decreasing the action of TGF-β.

MATERIALS AND METHODS

Cell Cultures. HEY, SKOV-3, and HOC-7 cells were obtained from Dr. Alexander Marks (Banting and Best Institute, University of Toronto, Toronto, Ontario, Canada). OVCAR-429 cells were obtained from Dr. Robert Kerbel (Sunnybrook and Women’s College Health Science Center, Toronto, Ontario, Canada). OVCAR-3, LNCaP, and DU-145 cells were obtained from American Type Culture Collection (Manassas, VA). These cells were maintained in RPMI 1640 (without phenol red) supplemented with 5% (v/v) FBS, 50 units/ml penicillin, 50 μg/ml streptomycin, and 0.625 μg/ml amphotericin B (Fungizone).

All cell cultures were maintained at 37°C in a humidified 5% CO2 atmosphere. sFBS was substituted for normal FBS 48 h before beginning experiments. DHT, estradiol-17β, and progesterone (Sigma Chemical Co., St. Louis, MO) and OH-FLUT (Schering-Plow, Madison, NJ) were dissolved in ethanol before addition to culture medium. The final ethanol concentration was 0.001%. TGF-β1 (R&D Systems, Minneapolis, MN) was diluted in culture media and stored at 4°C before addition to DHT-treated cultures. Primary ovarian cancer cells (OVCAS-16) were isolated from ascites of a patient diagnosed with papillary adenocarcinoma of the ovary, as described by
Hirte et al. (19). Cells were propagated in DMEM/F12 media (without phenol red) supplemented with 3% fBS, 5 μg/ml insulin, 5 × 10^{-5} M ethanoleamine, 5 ng/ml EGF, 5 × 10^{-5} M phosphethanolamine, 10 μg/ml transferrin, penicillin-streptomycin, and fungizone. The cells were grown by flow cytometry to express the epithelial ovarian cancer antigens 2G3 and M2A (19, 20). These monoclonal antibodies were provided by Dr. A. Marks. OVCAS-16 cells were maintained in the presence or absence of 10 nM DHT added daily from the time of isolation. Experiments were performed within 2 weeks and after one passage.

**RNA Preparation, cDNA Synthesis, and Quantitative Analysis of mRNA by PCR.** Cell cultures were washed in ice-cold PBS or serum-free medium, and total cellular RNA was isolated by the guanidinium thiocyanate extraction method (21). RNA concentration in all samples was measured in two independent experiments and in two dilutions, as its accuracy is essential for quantitative RT-PCR. cDNAs for TGF-β receptors, AR, ARA70, and β-actin were generated using oligo(dT) primer, whereas an internal primer was used to generate TGF-β1 cDNA to increase the specificity of the reaction (22).

Quantitative analysis of PCR products was performed according to Murphy et al. (23). Selected primers for amplification of TGF-β1, TGF-β receptors, and β-actin have been described previously (22). Three sets of AR-specific primers spanning exons 1–4, 4–7, and 1–7 selected from the cDNA sequence (24) were originally tested in the AR-positive prostate cancer cell line LNCaP. As identical results were obtained with all three primer sets, forward primer 5′-GTCATAAGCGAATGGGCCCC-3′ and reverse primer 5′-CTTGTGAGGCTGTAGTAGAGGGC-3′, which yielded a 1031-bp PCR product, were chosen for the current study. Primers for ARA70, selected from the cDNA sequence (25), were forward primer 5′-CTTTGTCAGTGTTGTTGTGTGG-3′ and reverse primer 5′-GGGAGATTTATGCTCTTCGAT-3′, which yielded a product of 595 bp. PCR amplification conditions were as described previously (22) with an annealing temperature of 58°C for all except AR (64°C), and 28 cycles for all except betaglycan and AR (30 cycles). Aliquots (10 or 20 μl) of PCR products were fractionated on 4–20% gradient polyacrylamide gels in 0.5% Tris-borate/EDTA buffer (Helixx Technologies, Scarborough, Ontario, Canada) or on 1% agarose gels. Gels were transferred by electroblotting to nylon membranes and hybridized with specific [32P]-ATP-labeled or 32P-labeled probe sequences (26) to generate TGF-β receptors, but not TGF-β1, in the HEY Cell Line. To determine whether steroid hormones might regulate the expression of TGF-β1 and its receptors, DHT Down-Regulates mRNA for TGF-β Receptors, but not TGF-β1, in the HEY Cell Line. To determine whether steroid hormones might regulate the expression of TGF-β1 and its receptors.

**RESULTS**

DHT Down-Regulates mRNA for TGF-β Receptors, but not TGF-β1, in the HEY Cell Line. To determine whether steroid hormones might regulate the expression of TGF-β1 and its receptors,

![Image](https://example.com/image.png)

**Fig. 1.** Effect of estradiol-17β (E2), progesterone (P4), and DHT on mRNA expression for TGF-β1 and TGF-β receptors. HEY cells were treated continuously for 60 h with ethanol vehicle (control) or 500 nM E2, P4, or DHT, and total RNA was extracted and subjected to quantitative RT-PCR using specific primers for TGF-β1, TβR-I, TβR-II, betaglycan, and endoglin cDNA. PCR products were fractionated on 4–20% gradient polyacrylamide gels, transferred onto nylon membranes, and hybridized with specific [32P]-ATP-labeled internal probes. A, TGF-β1 mRNA levels. Conditions were optimized so that an exponential range of amplification was obtained. The autoradiogram shown is representative of two or three experiments performed. B, TGF-β receptor mRNA levels, which yielded a 1031-bp PCR product, were chosen for the current study. Primers for ARA70, selected from the cDNA sequence (25), were forward primer 5′-CTTTGTCAGTGTTGTTGTGTGG-3′ and reverse primer 5′-GGGAGATTTATGCTCTTCGAT-3′, which yielded a product of 595 bp. PCR amplification conditions were as described previously (22) with an annealing temperature of 58°C for all except AR (64°C), and 28 cycles for all except betaglycan and AR (30 cycles). Aliquots (10 or 20 μl) of PCR products were fractionated on 4–20% gradient polyacrylamide gels in 0.5% Tris-borate/EDTA buffer (Helixx Technologies, Scarborough, Ontario, Canada) or on 1% agarose gels. Gels were transferred by electroblotting to nylon membranes and hybridized with specific [32P]-ATP-labeled or 32P-labeled probe sequences (26) to generate TGF-β receptors, but not TGF-β1, in the HEY Cell Line. To determine whether steroid hormones might regulate the expression of TGF-β1 and its receptors,

**Cytosol Preparation and Analysis of AR Binding and Protein Expression.** Cytosolic extracts were prepared as described previously (26). For Western blot analysis, aliquots of cytosol protein (1–50 μg) were fractionated by 8% SDS-PAGE and transferred to Immobilon polyvinylidene difluoride membranes (Millipore Corp., Bedford, MA). The membranes were preincubated for 1 h in blocking solution (50 mM Tris-HCl, 0.15 NaCl, pH 7.4, 0.2% (v/v) Tween 20, and 5% powdered skim milk) followed by 1 h with 1 μg/ml affinity-purified polyclonal antibody to AR, PAR-1 (27). Immunoreactive proteins were visualized with horseradish peroxidase-labeled goat anti-rabbit antisera (Amersham Pharmacia Biotech, Oakville, Ontario, Canada) at 1:10,000 dilution in blocking solution for 30 min at room temperature and detected by enhanced chemiluminescence (Amersham Pharmacia Biotech, Oakville, Ontario, Canada) at 1:10,000 dilution in blocking solution for 30 min at room temperature and detected by enhanced chemiluminescence (Amersham Pharmacia Biotech).

Androgen saturation binding assays were performed as reported previously (26). All samples contained 1 μM tritiated androstenedione to block possible binding of [3H]R1818 to progesterone or glucocorticoid receptor (28).

**Cell Proliferation Assays.** SKOV-3 proliferation was determined by the MTT (Sigma) reduction method as described previously (26). Cells were treated with 10 nM DHT or vehicle daily beginning 24 h prior to seeding into 96-well microtiter plates at 2500 cells/well. At time 0 (24 h after seeding) and at 48 h, 5 pm TGF-β1 (optimal dose for SKOV-3 inhibition) or vehicle was added to each well. MTT reduction was quantified using 570 nm absorbance with an ELISA microplate reader (μQuant Biomolecular Spectrophotometer, BIO-TEK Instruments, Winooski, VT). Results were expressed as the mean ± SE of 6–8 replicates.

Proliferation of the primary ovarian cancer cells (OVCAS-16) was assessed by counting the number of viable cells, as these cells did not reduce the MTT reagent. Cells maintained in the presence or absence of 10 nM DHT or vehicle were seeded at 15,000 cells/well in 24-well plates. TGF-β1 (25 pt, optimal dose for OVCAS-16 inhibition) and/or 10 nM DHT was added every 24 h during proliferation assays. Cell counts were obtained using a hemacytometer, with four replicates per treatment group.
we examined the effect of estradiol-17β, progesterone, and DHT treatment in HEY cells. Cells were grown in serum-free DMEM for 2 days, then treated with 500 nM estradiol-17β, progesterone, DHT, or ethanol vehicle (control), which was replaced every 12 h. Cells were harvested at 60 h, and total RNA was extracted and subjected to quantitative RT-PCR. TGF-β1 mRNA levels were unaffected by treatment with 500 nM of estradiol-17β, progesterone or DHT (Fig. 1A). However, the autoradiograms from a representative experiment revealed that DHT down-regulated mRNA for TβR-II, TβR-I, beta-glycan, and endoglin, whereas estradiol-17β and progesterone did not (Fig. 1B). The expression of β-actin mRNA was unaffected by treatment with steroids, suggesting that a concentration of 500 nM had no nonspecific inhibitory effects on mRNA synthesis.

A summary of the results from 3–5 independent experiments is shown in Fig. 1C. TGF-β receptor mRNA levels within the exponential range were quantified for each treatment and expressed as percentage of that measured in control cells. Expression of steady-state mRNA levels of TβR-I, TβR-II, beta-glycan, and endoglin were markedly reduced to 9–18% that of control cells. Treatment of HEY cells with estradiol-17β or progesterone did not affect mRNA levels for any of the TGF-β receptors (Fig. 1C).

Variable Expression of AR in Ovarian Cancer Cell Lines. Because androgenic responses should be mediated by AR, we examined its expression in HEY cells, as well as in several other ovarian cancer cell lines. RNA was extracted from HEY, SKOV-3, OVCAR-3, HOC-7, and OVCA-429 ovarian cancer cell lines, and AR mRNA was detected by RT-PCR. The AR-positive LNCaP and AR-negative DU-145 prostate cancer cell lines, and AR mRNA was detected by RT-PCR. The AR-positive LNCaP and AR-negative DU-145 prostate cancer cell lines were included as positive and negative controls, respectively. A 1031-bp PCR product, corresponding to exons 1–7 of AR, was amplified in all of the ovarian cancer cell lines tested (Fig. 2A); however, the apparent levels of expression were variable. High levels of AR mRNA were observed in LNCaP and OVCAR-3 cells, and intermediate levels were found in SKOV-3 cells. HEY, HOC-7, and OVCA-429 cells gave markedly less intense signals and required 100 ng instead of 20 ng per lane for detection (Fig. 2A). As expected, no AR mRNA expression was detected in DU-145 cells (Fig. 2A).

OVCAR-3, SKOV-3, and HEY cells expressing high, moderate, and low AR mRNA, respectively, were further analyzed by quantitative RT-PCR (Fig. 2, B and C). A linear range of exponential amplification was obtained with less than 0.4 ng of equivalent RNA for both LNCaP and OVCAR-3 cells, whereas the linear range of amplification for SKOV-3 cells was 0.8–20 ng of equivalent RNA. In comparison, HEY cells had barely detectable AR expression (Fig. 2B). By determining the relative amount of RNA needed to obtain similar densitometric units, SKOV-3 and HEY cells were found to express 3 and 0.01%, respectively, of the level of AR mRNA measured in OVCAR-3 cells (Fig. 2C). OVCAR-3 cells expressed levels of AR mRNA similar to that expressed by LNCaP cells. DU-145 cells did not express detectable AR mRNA. β-Actin levels were comparable for all cell lines, indicating an equivalent amount of RNA was amplified (Fig. 2C).

Cytosol extracts from ovarian cancer cell lines were analyzed for AR protein by Western blot analysis and by saturation androgen binding assay (Fig. 3 and Table 1). LNCaP and DU-145 cells were included as positive and negative controls, respectively. Western blot analysis revealed a 110-kDa immunoreactive protein in OVCAR-3 and LNCaP cells, whereas no AR immunoreactive protein was de-
Cells were grown in culture medium supplemented with 5% sFBS for 48 h before harvesting. Cytosol was extracted and incubated overnight at 4°C with a range of \([^3H]R1881\) concentrations (0.1–12.0 nM) in the presence or absence of 1 μM unlabeled DHT. Values for \(B_{\text{max}}\) (saturation of binding) and \(K_d\) (binding affinity) represent the mean ± SE from five independent experiments, except for OVCAR-3 cells (n = 2). For SKOV-3 and HEY cells, specific \([^3H]R1881\) binding was not observed in three of the five experiments; therefore, a value of 0 was assigned for these experiments for statistical purposes. In the two experiments in which binding was detected, \(B_{\text{max}}\) values (fmol/mg cytosol) were 7.06 and 7.33 for SKOV-3, and 11.11 and 3.51 for HEY. ND, binding not detected.

### Table 1  Saturation binding analysis of AR in cytosol extracts

<table>
<thead>
<tr>
<th>Cell line</th>
<th>(B_{\text{max}}) ± SE (fmol/mg cytosol protein)</th>
<th>(K_d) ± SE (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LNCaP</td>
<td>314 ± 71.6</td>
<td>0.324 ± 0.082</td>
</tr>
<tr>
<td>OVCAR-3</td>
<td>20.6 ± 2.57</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>SKOV-3</td>
<td>2.88 ± 1.76</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>HEY</td>
<td>2.92 ± 2.16</td>
<td>0.10 ± 0.03</td>
</tr>
<tr>
<td>HOC-7</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>OVCA429</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>DU-145</td>
<td>ND</td>
<td></td>
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</table>

Saturation binding assays were performed with \([^3H]R1881\), a synthetic radiolabeled androgen (28), to further characterize AR in ovarian cancer cell lines and to determine whether the receptor was capable of ligand binding. Scatchard analysis revealed a single class of high-affinity \((K_d < 0.1 \text{ nM})\) and low capacity \((B_{\text{max}} < 30 \text{ fmol/mg cytosol protein})\) androgen binding sites in OVCAR-3, SKOV-3, and HEY cytosol extracts (Table 1). The mean androgen binding capacity in OVCAR-3 cells was approximately 7% of that measured in the AR-positive LNCaP cells (Table 1). SKOV-3 and HEY cells had much lower levels of binding activity, near the limit of sensitivity of the assay (Table 1).

**Low-Dose DHT Down-Regulates TβR-II mRNA Expression in Several Ovarian Cancer Cell Lines.** Because TβR-II is the ligand-binding receptor and its expression and function are more frequently altered in many human cancers (12), we examined the effect of DHT
Treatment on its expression in ovarian cancer cell lines. OVCAR-3, SKOV-3, and HEY cells were treated with ethanol vehicle or with 1, 10, or 50 nM DHT, and TβRII mRNA levels were compared by quantitative RT-PCR. LNCaP and DU-145 prostate cancer cells were included as controls. Autoradiograms from a representative experiment, performed after 24 h of DHT treatment, are shown in Fig. 4. A linear range of exponential amplification for TβRII mRNA expression was detected between 0.08 and 10 ng of equivalent RNA per lane (Fig. 4). Reduction in TβRII mRNA levels was observed in SKOV-3 and HEY cells with 1 and 10 nM DHT (Fig. 4B). With OVCAR-3 cells, the effect was less apparent, whereas with DU-145 cells, no effect was noted, as expected (Fig. 4B). LNCaP cells did not express detectable TβRII mRNA (Fig. 4A), in agreement with previous reports (29). Androgen treatment did not alter β-actin mRNA levels in the different cell lines tested, confirming the specificity of the TβRII down-regulation (Fig. 4, A and B).

The results of several experiments are combined in Fig. 5. DHT down-regulated the expression of TβRII mRNA in a dose-dependent manner. Treatment with 1, 10, or 50 nM DHT down-regulated the expression of TβRII mRNA levels by 30, 80, and 90% in HEY cells and by 70, 90, and 99%, respectively in SKOV-3 cells measured 24 h after addition of DHT to the culture medium (Fig. 5). With OVCAR-3 cells, a decrease in TβRII mRNA expression was only noted after treatment with 50 nM DHT; however, this did not attain statistical significance (Fig. 5). By 48 h after addition of DHT to the culture medium, the suppression of TβRII mRNA levels was no longer observed, as shown for SKOV-3 cells (Fig. 5).

**OH-FLUT Down-Regulates TβRII mRNA in SKOV-3 Cells.** To further demonstrate that down-regulation of TβRII mRNA by DHT is mediated by AR, SKOV-3 cells were treated with ethanol vehicle (control) or with 1 or 10 nM DHT in the presence or absence of 1 or 0.1 μM OH-FLUT, an AR antagonist. Additional OH-FLUT was added 12 h later, and cells were harvested 24 h after addition of DHT. OH-FLUT at either dose did not block the effect of DHT on TβRII mRNA expression. Surprisingly a 90% inhibition of TβRII mRNA expression was observed with 0.1 and 1.0 μM OH-FLUT in the absence of DHT (Fig. 6), suggesting that OH-FLUT may act as an AR agonist in SKOV-3 cells.

**DISCUSSION**

In this study, we demonstrate that treatment of ovarian cancer cell lines with the nonaromatizable androgen DHT decreased mRNA expression for TGF-β receptors. DHT treatment also reversed the growth-inhibitory effect of TGF-β1 on SKOV-3 cells, as well as in a primary culture of ovarian cancer cells isolated from patient ascites. Because TGF-β is a potent inhibitor of cell proliferation, including that of ovarian surface epithelial cells (10) and ascites-derived ovarian...
cancer cells (11, 32), the attenuation of TGF-β-induced growth inhibition by a reduction in receptor expression may represent an important mechanism by which androgens promote ovarian cancer progression.

The ability of DHT to down-regulate TβR-II mRNA levels in SKOV-3 and HEY cells was somewhat surprising given the near-nondetectable levels of AR expressed by these cells. Our findings that high levels of estradiol or progesterone did not inhibit expression of TGF-β receptor mRNAs in HEY cells indicate that the effect of DHT is specific. Although we were unable to block DHT-induced down-regulation of TβR-II mRNA expression with OH-FLUT, this cannot be taken as evidence of an AR-independent mechanism, as treatment of cells with the AR antagonist alone also resulted in decreased TβR-II expression.

The ability of HEY and SKOV-3 cells to respond to DHT, and the apparent androgen-mimetic action of OH-FLUT on TβR-II mRNA expression, may involve the actions of ARA70. This putative coactivator has a predilection for AR (25) and is highly expressed in ovarian cancer cell lines tested here and in ovarian cancer tissue.5 Expression of ARA70 in prostate cancer cells has been shown to increase AR transactivation potential by approximately 10-fold (25), although the magnitude of this effect is controversial (33, 34). Thus, in ovarian cancer cell lines expressing low AR levels, ARA70 could increase their sensitivity to physiologically relevant levels of androgens. Furthermore, the observation that OH-FLUT acts as an androgen agonist in ovarian cancer cells is consistent with the finding by Miyamoto et al. (30) that ARA70 promotes agonist activity by antiandrogens when overexpressed in transfected DU-145 prostate cancer cells. These data raise the possibility that modulation of TβR-II mRNA by androgens may include potentiation of transcription involving ARA70, leading to increased androgen sensitivity of ovarian cancer cells.

Our findings of low levels of AR expression in ovarian cancer cell lines are compatible with those of Lau et al. (35), who recently reported little or no AR mRNA expression in ovarian cancer cells by semiquantitative RT-PCR. We detected AR mRNA in all five ovarian cancer cell lines tested, likely because our quantitative RT-PCR method is more sensitive, due to detection of PCR products by

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Fig. 7. ARA70 mRNA expression in ovarian and prostate cancer cell lines. A, detection of ARA70 mRNA by RT-PCR. Total RNA was subjected to RT-PCR using primers specific for ARA70 cDNA. The PCR products were fractionated on 1% agarose gels, stained with ethidium bromide, and photographed. B, quantitation of ARA70 mRNA. The PCR products were transferred and detected by chemiluminescence with a specific digoxigenin-11-ddUTP-labeled internal probe. C, graphical analysis of ARA70 and β-actin mRNA levels.

Fig. 8. Reversal of the growth-inhibitory action of TGF-β1 in ovarian cancer cells by DHT. A, SKOV-3 cell cultures were treated daily with 10 nM DHT beginning 48 h before the treatment with 5 ps TGF-β1 at 0 and 48 h. Growth of SKOV-3 cells was assessed by the MTT reduction assay with 6–8 replicates per treatment group. Histogram at right shows the percentage of control (vehicle only) for each treatment group. B, growth of primary ovarian cancer cells, OVCAS-16, derived from patient ascites was measured by counting viable cells using four replicates per treatment group. TGF-β1 (25 ps) and 10 nM DHT were added every 24 h to the culture medium, which was replaced after 3 days. The histogram at the right shows the percentage of control for each group. *, significantly different from control (P < 0.05) as assessed by ANOVA followed by Tukey’s multiple range test. White columns, with DHT; gray columns, without TGF-β1; black columns, with both DHT and TGF-β1.
hybridization with labeled AR probes. Two of our cell lines, HOC-7 and OVCA-429, expressed very low levels of AR mRNA and barely detectable levels of receptor protein, at the limit of sensitivity of both Western blot and binding assays.

Whereas normal ovarian surface epithelium cells respond to TGF-β by growth inhibition, many immortalized ovarian cancer cell lines are relatively resistant (10, 32). The growth inhibition of SKOV-3 cells in this study was not observed until 96 h after initiation of TGF-β1 treatment. In contrast, growth inhibition by TGF-β1 was observed after 24 h in ovarian cancer cells isolated from patient ascites. Hurteau et al. (11) have shown that 19 of 20 primary ovarian cancer cells isolated from ascites fluid of patients were growth inhibited by TGF-β1. These findings were confirmed by Havrilesky et al. (32), who reported that 10 of 11 primary cultures were growth inhibited by TGF-β1. TGF-β1 has been shown to be present in ascites derived from patients with epithelial ovarian carcinoma (36). Furthermore, androgens are produced and secreted by ovarian adenocarcinomas into the ascites fluid (37) and may decrease the expression of TGF-

The androgen-induced down-regulation of TGF-β1 mRNA by androgens requires sustained levels of DHT, as indicated by return to control levels 48 h after addition of DHT to SKOV-3 cell cultures. Thus, once removed from the ascites and cultured in the absence of androgens, ovarian cancer cells may regain their sensitivity to TGF-β1.

In summary, our data provide a novel mechanism whereby androgens may act to promote ovarian cancer cell growth and progression. The androgen-induced down-regulation of TGF-β receptors occurred in ovarian cancer cells that expressed very low AR levels. Further studies are therefore warranted to define the role of AR co-activators, such as ARA70, in modulating the sensitivity of ovarian cancer cells to DHT.

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