

Loss of Coordinated Androgen Regulation in Nonmalignant Ovarian Epithelial Cells with *BRCA1/2* Mutations and Ovarian Cancer Cells¹

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ABSTRACT

Epidemiological studies have implicated androgens in the etiology/progression of epithelial ovarian cancer. Because normal and malignant ovarian epithelial cells are growth inhibited by transforming growth factor (TGF) β , we tested the ability of 5 α -dihydrotestosterone (DHT) to modulate this response and the expression of TGF- β receptor types I and II. Cells derived from the ovarian surface epithelium of women undergoing oophorectomy ($n = 7$) for nonovarian indications or with a germ-line *BRCA1* or 2 mutation ($n = 9$), and from the ascitic fluid of patients with primary ovarian cancer ($n = 8$) were cultured with and without DHT. Cell proliferation after TGF- β 1 or vehicle treatment was determined, and transcripts for TGF- β receptors were measured by quantitative reverse transcription-PCR. As low levels of androgen receptor were observed in the cultures, we also measured transcript levels for steroid receptor coactivators SRC-1, ARA70, and AIB1. TGF- β 1 inhibited growth in 12 of 13 cultures tested, and DHT generally reversed this effect, demonstrating that androgens can block TGF- β -induced growth inhibition in both malignant and nonmalignant ovarian epithelial cells. Transcripts for TGF- β receptors, SRC-1, and ARA70 were found to be coordinately regulated by androgen in control cells, but not in either malignant or *BRCA1/2*-positive cell cultures. These findings raise the possibility that by modulating steroid receptor coactivator expression, androgen might affect other hormonal responses and contribute to the initiation of ovarian cancer.

INTRODUCTION

Despite the introduction of diverse and powerful chemotherapeutic agents, the survival rate for epithelial ovarian cancer has changed little over the past 20 years. In 2000 alone, ovarian cancer was diagnosed in 30,000 North American women and caused 15,400 deaths (1, 2). At the current time, a family history of ovarian or breast cancer is associated with the greatest increase in risk (3). Mutations in *BRCA1* and *BRCA2* genes account for the majority of inherited predisposition to this disease, with carriers of germ-line mutations having an estimated lifetime risk of 28% and 21%, respectively, for developing ovarian cancer (4, 5).

Numerous studies indicate that the ovarian steroidal milieu may influence the initiation or progression of epithelial ovarian cancer and support a role for androgens (reviewed in Ref. 6). The majority of ovarian cancer are diagnosed after menopause, when the balance of

ovarian steroid production shifts from estrogens to androgens. Women with high serum androgen levels (pre- or postmenopausal) or polycystic ovarian disease (associated with anovulation and overproduction of ovarian androgens) have an increased risk of developing ovarian cancer (7, 8). Ovarian epithelial cells and up to 95% of ovarian epithelial tumors express AR3 (9–12), indicating that these cells are directly responsive to androgens.

AR is a ligand-activated nuclear transcription regulatory factor that acts by binding to response elements in the regulatory region of target genes where it interacts with transcription factors to modulate gene expression. Steroid receptor coactivators, of which SRC-1 has been best studied, act as bridging proteins between the receptor and the transcription initiation complex (13, 14) and enhance the transactivational activity of the receptor in part via direct or indirect acetylation of histones (15, 16). Two steroid receptor coactivators have been reported to be up-regulated in ovarian cancer. AIB1 (amplified in breast cancer 1), which enhances ER-dependent transactivation, was amplified in 25% of sporadic ovarian cancer (17, 18) and ARA70 (AR-associating-protein 70), which has a predilection for AR, was up-regulated in ovarian carcinomas examined by *in situ* hybridization (19). Increased expression of ARA70 or AIB1 in steroid-sensitive cells could significantly magnify their steroid response pattern, thereby altering growth regulation and contributing to the development or progression of malignancy.

Additional support for a role of androgen in ovarian cancer came from the study of a polymorphic region in the AR NH₂-terminal domain, consisting of a variable number of CAG repeats. A shorter CAG repeat tract, which correlates with increased transactivational activity of the receptor (20), is associated with an earlier onset (by 7 years) of both sporadic and familial ovarian cancer (21).

One mechanism by which androgens might influence the growth of ovarian cancer cells is by modulating their sensitivity to TGF- β , a potent inhibitor of epithelial cells, including malignant and nonmalignant ovarian cells (22–24). Decreased expression or mutations in TGF- β receptor type I and/or II (T β R-I and T β R-II) have been observed in various malignancies, which render the cells unresponsive to the growth inhibitory effects of TGF- β (25–27) and may contribute to cancer initiation or progression. The vast majority of ovarian cancer cells isolated from patient ascites were growth-inhibited by TGF- β in culture (22–24) indicating an intact signaling cascade and suggesting that mechanisms to suppress the actions of TGF- β must exist *in vivo*. That androgen exposure might represent one such mechanism is supported by our previous work. We showed that treatment of HEY and SKOV-3 ovarian cancer cell lines with DHT, a nonaromatizable androgen, down-regulated the expression of TGF- β receptors (28).

To determine the effect of androgens on modulation of growth and TGF- β receptor expression, cultures of cells derived from the ovarian surface epithelium of women undergoing oophorectomy for nonovarian indications (OSE), or with a confirmed germ-line *BRCA1* or *BRCA2* mutation (OSEb), and from the ascitic fluid of patients with primary ovarian cancer were maintained in the presence or absence of 10 nM DHT, to mimic *in vivo* contact with androgen. Because these cells expressed low amounts of AR, levels of transcripts for the

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³ The abbreviations used are: AR, androgen receptor; TGF, transforming growth factor; DHT, 5 α -dihydrotestosterone; OSE, ovarian surface epithelium; MMT, malignant Mullerian mixed tumor; mAb, monoclonal antibody; RT-PCR, reverse transcription-PCR; BTSVQ, binary tree-structured vector quantization; SOM, self-organizing map; ER, estrogen receptor; PR, progesterone receptor.

coactivators ARA70, SRC-1, and AIB1 were also measured. Our results show that androgens modulate the growth response to TGF- β 1 in both malignant and nonmalignant ovarian epithelial cells, and alter the expression of T β R-I and T β R-II, as well as the coactivators studied. The expression of T β R-I, T β R-II, ARA70, and SRC-1 was coordinately regulated by androgen in OSE cultures but not in OVCAS cultures. Remarkably, this coordinate regulation was also lost in OSEb cultures. These findings suggest that altered androgen responses may occur in the ovarian epithelium of some patients with *BRCA1* or 2 mutations and play a role in ovarian carcinogenesis.

MATERIALS AND METHODS

Patients and Cell Cultures. Cell cultures were derived from surgical material obtained over an 18-month period from 24 patients. Clinical information on these patients is summarized in Table 1. The mean patient age was 59.6 ± 3.5 , 53.9 ± 3.0 , and 63.6 ± 4.2 years (\pm SE) for OSE, OSEb, and OVCAS patients, respectively. The majority of patients were postmenopausal, 7 were premenopausal, and 1 OVCAS patient (OVCAS-27) was perimenopausal (Table 1).

OSE cultures were derived from patients with nonmalignant ovaries who were undergoing surgery for uterine/cervical tumors or for a dermoid cyst. Eight OSEb samples were obtained from women undergoing prophylactic oophorectomy because of a confirmed germ-line *BRCA1* or *BRCA2* mutation. The following germ-line mutations were detected: 6174delT (OSEb-7), 983del4 (OSEb-16), and 9132delC (OSEb-17) for *BRCA2*; and 1284delA (OSEb-12), G1081A (OSEb-19), 185delG (OSEb-23), 1294del40 (OSEb-41), and ins6kb (OSEb-45) for *BRCA1*. One other patient (OSEb-14) was not tested but had a family history of germ-line *BRCA1* mutation and was diagnosed previously with breast cancer.

OVCAS cultures were derived from 5 patients with grade 2/3 serous ovarian adenocarcinoma, 1 with mixed serous/clear cell ovarian carcinoma, 1 with grade 3 endometrioid adenocarcinoma, and a final case with a MMMT of the ovary. All of the patients had stage IIIC disease. Tumor cell samples were obtained from ascitic fluid collected from patients with primary ovarian cancer before any chemotherapy except for OVCAS-33, which was obtained after recurrence, 2 years after chemotherapy.

Nonmalignant OSE cells were derived by gentle scraping of the ovarian surface at the time of oophorectomy and were immediately processed. Cells

were centrifuged at $400 \times g$ for 5 min, washed twice with PBS [0.137 M NaCl, 2.68 mM KCl, 10 mM Na₂HPO₄, and 1.76 mM KH₂PO₄ (pH 7.4)], and seeded into 60-mm tissue culture plates (Falcon Labware, Becton Dickinson Biosciences, Mississauga, Ontario, Canada). On attaining $\geq 80\%$ confluence, cells were subcultured into two 100-mm Cell⁺ tissue culture plates (Corning Inc. Life Science, VWR Canlab, Mississauga, Ontario, Canada). OSE cultures were used within four passages beyond which mesenchymal transformation was observed.

OVCAS cells were isolated from patient ascites as described by Hirte *et al.* (29). Briefly, cells were collected by vacuum filtration of ascites (250 ml to 5 liters) through 30- μ m pore size nylon mesh membranes (Spectrum Laboratories, Rancho Dominguez, CA). The number of cells recovered varied among patients and was not necessarily proportional to the ascites volume. In all of the cases, cells were collected from the filter, washed, and placed into 100-mm tissue culture plates (Falcon; $1-2 \times 10^6$ cells/plate) at 37°C for 6 h to allow fibroblasts to attach. The medium containing the nonadherent epithelial tumor cells was then transferred to 100-mm Cell⁺ tissue culture plates (Corning).

All of the cells were grown in DMEM/F12 medium without phenol red and supplemented with 3% charcoal-stripped fetal bovine serum, 5 μ g/ml insulin, 5 $\times 10^{-5}$ M ethanolamine, 5 ng/ml epidermal growth factor, 5 $\times 10^{-5}$ M phosphoethanolamine, 10 μ g/ml transferrin, 50 units/ml penicillin, 50 μ g/ml streptomycin, and 0.625 μ g/ml amphotericin B (Fungizone) at 37°C in a humidified 5% CO₂ atmosphere. All of the cultures were maintained in the presence or absence of 10 nM DHT (Sigma Chemical Co., St. Louis, MO) added daily. DHT was dissolved in ethanol and diluted with medium to a final ethanol concentration of 0.001%. All of the experiments were carried out with low passage (one to four) cultures.

Flow Cytometry. Cells were washed twice with PBS, detached with 0.25% trypsin-EDTA, and resuspended in 0.1% BSA in PBS (0.1% BSA/PBS). Cells were incubated for 45 min at 4°C with saturating amounts of murine mAbs to HLA class I (44D6; Ref. 30), MUC-1 (2G3; Ref. 31), oncofetal antigen (M2A; Ref. 32; donated by Dr. Alexander Marks, University of Toronto, Toronto, Ontario, Canada), hyaluronic acid receptor CD44 (50B4; Ref. 33), integrin subunits α 2 (P1E6; Telios, La Jolla, CA), α 3 (PIB5; Telios), α 5 (JBS5; donated by Dr. John Wilkins, University of Manitoba, Winnipeg, Manitoba, Canada), and β 1 (4B4; Coulter Clone, Fullerton, CA) or control nonimmune murine IgG1. After two washes with 0.1% BSA/PBS, the cells were incubated with FITC-conjugated affinity-purified goat F(ab')₂ antimouse IgG (BIOSOURCE International, Camarillo, CA) at 20 ng/ml for 45 min at 4°C in the dark. After washing twice with PBS, cells were stained with propidium iodide to allow for gating of viable cells during analysis by FACSscan with CellQUEST software (Becton Dickinson, Mountain View, CA). For each cell surface antigen, mean fluorescence intensity and percentage of positive cells were determined by setting the gates such that <5% cells were positive when stained with control nonimmune IgG1.

Cell Proliferation Assays. Cell proliferation was assessed using a hemacytometer with four replicates per treatment group. Cells maintained in the presence of 10 nM DHT or ethanol vehicle were seeded at 15,000–50,000 cells/well in 24-well plates. TGF- β 1 (R&D Systems, Minneapolis, MN), diluted in culture medium, was added every 48 h beginning at time zero, and 10 nM DHT was added every 24 h during the proliferation assays. A dose of 25 pM TGF- β 1 was found to be optimal for OVCAS growth inhibition (28). Results are expressed as percentage of control (no DHT or TGF- β 1).

Quantitative RT-PCR. Cell cultures were washed in ice-cold PBS and total cellular RNA was isolated using TRIzol Reagent (Invitrogen Corp., Carlsbad, CA) according to manufacturer instructions. The RNA concentration in all of the samples was measured spectrophotometrically in two independent determinations and at three dilutions. cDNAs were generated using Superscript II reverse transcriptase (Life Technologies, Inc., Burlington, Ontario, Canada) and an oligodeoxythymidylic acid primer (Amersham-Pharmacia, Piscataway, NJ).

Quantitative analysis of PCR products was performed according to Murphy *et al.* (34) with modifications. Selected primers used for amplification of T β R-I, T β R-II, ARA70, and β -actin cDNA were as reported previously (28, 35). Primers selected for amplification of SRC-1, 5'-TCTGCCTCTGCTA-ACTCTCA-3' (forward) and 5'-TCAAGGTCAGCTGTAAACTGG-3' (reverse), and of AIB1, 5'-GGCAGAATGGAACCTATGAAT-3' (forward) and 5'-AGGGAATTCCTAAGAAGAGGC-3' (reverse), produced 440 and 320 bp PCR products, respectively. PCR amplification conditions were as described previously (28, 35) with an annealing temperature of 58°C and 28 cycles. Aliquots (20 μ L) of the PCR products were fractionated by electrophoresis on

Table 1 Summary of patient clinical data

Culture	Patient age	Menopausal status	Reason for surgery/other malignancies
OSE-21 ^a	56	Pre	Cervix mucinous adenocarcinoma
OSE-22	66	Post	Cervix squamous carcinoma- <i>in-situ</i>
OSE-30	54	Post	Cervix recurrent carcinoma- <i>in-situ</i>
OSE-32	43	Pre	Right ovarian dermoid cyst
OSE-38	66	Post	Uterine adenocarcinoma/breast adenocarcinoma
OSE-43	62	Post	Uterine endometrioid carcinoma grade 1/3
OSE-48	70	Post	Uterine MMMT/breast adenocarcinoma
OSEb-7	49	Post	Prophylaxis/breast adenocarcinoma
OSEb-12	61	Post	Prophylaxis/breast adenocarcinoma
OSEb-14	61	Post	Prophylaxis/breast ductal carcinoma <i>in situ</i>
OSEb-16	46	Pre	Prophylaxis
OSEb-17	49	Pre	Prophylaxis, FBOC/breast adenocarcinoma
OSEb-19	48	Pre	Prophylaxis, FBOC, endometrial leiomyoma/ breast adenocarcinoma
OSEb-23	70	Post	Prophylaxis, FOC
OSEb-41	58	Post	Prophylaxis, FBOC, endometrial leiomyoma
OSEb-45	43	Pre	Prophylaxis, FBOC
OVCAS-16	72	Post	OC (high grade MMMT of the ovary)
OVCAS-19	80	Post	OC (grade 3 endometrioid)
OVCAS-24	60	Post	OC (grade 3 papillary serous)
OVCAS-26	67	Post	OC (grade 3 serous)
OVCAS-27	44	Peri	OC (grade 3 papillary serous)
OVCAS-29	62	Post	OC (grade 3 serous/clear cell)
OVCAS-33	73	Post	OC (grade 2 papillary serous)
OVCAS-34	51	Pre	OC (grade 3 papillary serous)

^a OSE, ovarian surface epithelium cases derived from patients with normal ovaries; OSEb, those derived from patients with *BRCA1/2* patients; OC, ovarian cancer; FBOC, familial breast/ovarian cancer history; FOC, familial ovarian cancer history; MMMT, mixed malignant müllerian tumor.

2% agarose gels and detected with SYBRgold (Molecular Probes, Eugene, OR). The gel image was digitized, and the net intensity of each band was analyzed by computer-assisted densitometry using 1DImage Analysis Software (Kodak Digital Science, Scientific Imaging Systems, Eastman Kodak Company, Rochester, NY). Data were plotted as the log of densitometric units against the log of equivalent RNA concentration to identify a linear range of exponential amplification. Linear regressions were obtained using SigmaPlot Scientific Graphing Software (version 2.00; Jandel Corporation, San Rafael, CA). The amount of total equivalent RNA estimated from the linear range of amplification after DHT treatment was expressed as a percentage of that measured in cells treated with ethanol vehicle (control). All of the measurements were corrected for β -actin expression.

For detection of AR mRNA, cDNA equivalent to 100 ng of reverse-transcribed RNA was subjected to PCR using an annealing temperature of 64°C, 30 cycles, and primers described previously (28) to yield a 1031-bp PCR product. As a control for the amount of RNA, β -actin was detected in the same cDNA samples, as described above, except that ethidium bromide was used to visualize the PCR products in the agarose gels.

Data Analysis. Data obtained from cell proliferation assays were subjected to ANOVA using SPSS for Windows statistical software (Version 10.0.7; SPSS Inc., Chicago, IL). Additional group comparisons were made using Duncan's Multiple Range Test at the $P < 0.05$ level. Quantitative RT-PCR data for the two-dimensional correlation analysis were analyzed by Simple Linear Regression to determine the correlation coefficients using SigmaStat for Windows statistical software (Version 1.0; Jandel Corporation). A pseudocolor matrix was generated using Matlab R12 software (MathWorks Inc., Natick, MA) to display all of the correlations for T β R-I, T β R-II, SRC-1, AIB1, and ARA70 mRNA expression levels within OSE, OSEb, and OVCAS cultures.

A BTSVQ method (36) was used to cluster the cultures based on the effects of DHT on T β R-I, T β R-II, SRC-1, AIB1, and ARA70 mRNA expression. This method combines a partitive k-means clustering and a SOM algorithm. Briefly, the quantitative RT-PCR data set was log-normalized and analyzed by BTSVQ. First, we organized cultures into a binary cluster tree by using k-means algorithm with $k = 2$. We then applied SOM to cluster cultures based on their quantized gene expression profiles. Partitive clustering results were cross-verified with the clusters generated by SOMs.

RESULTS

Phenotypic Characterization of Low Passage Ovarian Epithelial Cell Cultures. The epithelial nature of all of the ovarian cell cultures was analyzed by flow cytometry using mAbs 2G3 and M2A, which react specifically with epithelial cell surface MUC-1 and oncofetal antigens, respectively. The mAb 2G3 reacts with a carbohydrate epitope of normal MUC-1 and with hypoglycosylated forms on malignant cells (37–39), whereas mAb M2A, produced against an ovarian epithelial carcinoma cell line, reacts with the monomeric sialoglycoprotein (32). Representative profiles of 2G3 and M2A immunostaining are shown in Fig. 1. All of the OSE, OSEb, and OVCAS cultures tested were positive for 2G3 and M2A surface antigens, confirming their epithelium origin. The level of oncofetal antigen was generally higher than the MUC-1 level, and DHT treatment did not alter their expression. All of the cell cultures analyzed also expressed HLA class I antigen, used as a positive control in the experiments.

The metastatic spread of ovarian cancer cells into the peritoneal cavity is dependent on β 1-integrins and CD44 (40, 41). We characterized by flow cytometry the expression of α 2-, α 3-, α 5-, and β 1-integrins, and CD44 on the surface of OSE, OSEb, and OVCAS cells maintained in the presence or absence of DHT. Representative profiles are shown in Fig. 1. All of the cultures analyzed expressed high levels of β 1-, α 2-, and α 3-integrins, as well as CD44. However, OVCAS cultures consistently expressed higher levels of α 5-integrin than did OSE and OSEb cultures, which showed very low to nondetectable levels of this integrin. These data support previous observations of expression of functional α 5 β 1 integrin on ovarian cancer cells

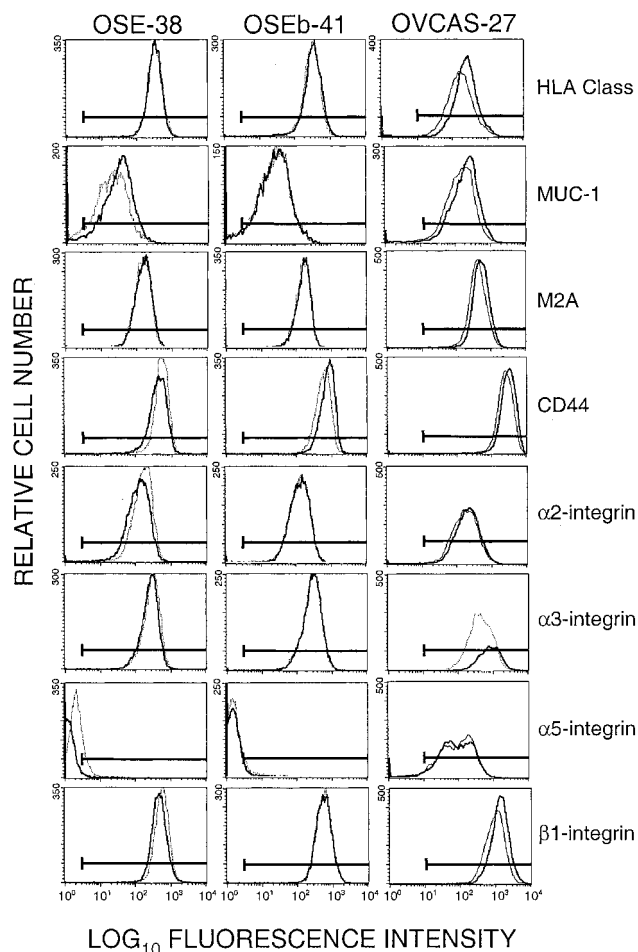


Fig. 1. Representative flow cytometry profiles of MUC-1, M2A oncofetal antigen, CD44, and integrins on ovarian epithelial cells from a control (OSE-38), a patient with a *BRCA1* mutation (OSEb-41), and from ascites of a patient with ovarian cancer (OVCAS-27), maintained in the presence (heavy lines) or absence (dotted lines) of 10 nM DHT. HLA class I antigen staining defines the region of positive cells. The gate was set using isotype control IgG1 such that $<5\%$ cells fell within the positive gate. Cells were analyzed immediately using propidium iodide to allow for gating of viable cells.

(40, 41). DHT treatment did not affect integrin and CD44 levels in any of the cases studied.

Expression of AR was assessed by RT-PCR in all of the cultures but OSEb-16, -17, and -19. A low level of expression was found in all of the cultures tested, consistent with our previous findings in ovarian cancer cell lines (28). We demonstrated previously that identical results were obtained using sets of primers spanning AR exons 1–4, 4–7, and 1–7, respectively (28), and use routinely the 1031-bp PCR product, corresponding to exons 1–7. This product was detected after staining of the gel with SYBRgold (Fig. 2), a sensitive fluorescent DNA dye, but not with ethidium bromide (data not shown). β -Actin mRNA expression was readily detected with ethidium bromide confirming RNA integrity and efficient reverse transcription in all of the samples.

Reversal of TGF- β 1 Growth Inhibition by Androgen. The ability of DHT treatment to reverse the growth-inhibitory effect of TGF- β 1 was tested in several cultures of ovarian epithelial cells. In control cultures maintained in the absence of DHT, TGF- β 1 inhibited the growth of 5 of 5 OVCAS by 40–50%, of 5 of 6 OSE by 30–60%, and of 2 of 2 OSEb cultures by 40–50% (Fig. 3). Maintenance of the cells in DHT blocked the growth-inhibitory effect of TGF- β 1 in 4 of 5 OVCAS, 3 of 5 OSE, and 1 of 2 OSEb cultures (Fig. 3). A significant increase in proliferation by DHT was observed for OSE-30

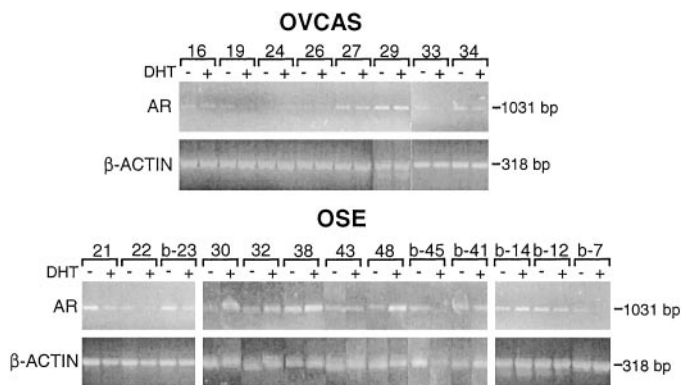


Fig. 2. AR and β -actin mRNA expression in malignant (OVCAS) and nonmalignant ovarian epithelial (OSE/OSEb) cell cultures. Total RNA was extracted from cultures maintained in the presence (+) or absence (-) of 10 nM DHT and subjected to reverse transcription. An amount of cDNAs corresponding to 100 ng RNA was subjected to PCR using primers specific for AR and β -actin. PCR products resulting from 20 ng RNA equivalents were fractionated on 2% agarose gels and stained with SYBRgold for detection of AR mRNA and ethidium bromide for β -actin mRNA. OSEb cultures are indicated with a *b* before the culture number.

(Fig. 3), which was attenuated by TGF- β 1. The ability of DHT to reverse the growth-inhibitory effects of TGF- β 1 thus appears to be a normal regulatory mechanism, because it was observed in both malignant and nonmalignant ovarian epithelial cells.

Androgen-induced Modulation of TGF- β Receptors. The effect of DHT on expression of T β R-II and T β R-I mRNA levels was examined by quantitative RT-PCR analysis. A summary of the results for all of the cultures is shown in Table 2, whereas representative experiments for T β R-II and β -actin are shown in Fig. 4.

Treatment with 10 nM DHT down-regulated the expression of T β R-II mRNA in several cell cultures. For example, in OVCAS-16 and OVCAS-24 cultures, T β R-II mRNA levels were reduced to <10% of controls (Fig. 4). In OSEb-16 and 3 OSE cultures, T β R-II mRNA was down-regulated to <40% of controls (Table 2). DHT also reduced the expression of T β R-I mRNA levels to <50% of controls in 1 OVCAS culture, 1 OSEb, and 2 OSE cultures. In some cases, DHT increased the levels of mRNA for T β R-I and/or T β R-II; both receptors were up-regulated in OSE-32, OSEb-12, and OVCAS-27. In OVCAS-16, a 2.9-fold increase in T β R-I was observed, whereas T β R-II was decreased by 17-fold.

Androgen-induced Modulation of Steroid Receptor Coactivators ARA70, SRC-1, and AIB1. Several coactivator proteins have been identified that can interact with steroid receptors to increase their transcriptional activity, including ARA70, SRC-1, and AIB1. We examined the effect of DHT on the levels of mRNA for these steroid receptor coactivators. A summary of these results is shown in Table 2, and representative experiments are illustrated in Fig. 5. In general, the levels of ARA70 and AIB1 mRNA expression in controls were ~2-fold higher in OVCAS than in OSE or OSEb cultures, whereas levels of SRC-1 did not appear to change significantly between these groups.

DHT reduced ARA70 and SRC-1 mRNA levels in 3 OVCAS and AIB1 mRNA in 5 OVCAS to \leq 60% of control values. In contrast, DHT up-regulated the expression of AIB1 mRNA by ~3-fold in OVCAS-34. In OSE cultures, treatment with DHT down-regulated the expression of ARA70, SRC-1, and AIB1 mRNA levels in 3 cases to \leq 60% of control. ARA70 mRNA was increased >2-fold in 2 cases, whereas SRC-1 mRNA was increased 4-fold in OSE-32, and AIB1 mRNA was increased 2-fold in OSE-30. For the OSEb cultures, DHT treatment increased ARA70 mRNA >2-fold in OSEb-23, SRC-1 mRNA by 2.7-fold in OSEb-41, and AIB1 mRNA by 2–6-fold in 3 cases.

Correlation between Androgen-induced Modulation of TGF- β Receptors and Steroid Receptor Coactivators in OSE, OSEb, and OVCAS Cultures. In view of the multiple effects of DHT observed on TGF- β receptors and AR coactivators, the percentage of changes in mRNA levels were subjected to linear regression analysis. In OSE cultures, the change in expression of T β R-I was significantly correlated with that of T β R-II (Fig. 6). However, this correlation was not observed in OSEb or OVCAS cultures. In addition, changes in SRC-1 mRNA levels correlated with changes in T β R-II and ARA70 in OSE cultures (Fig. 6). Again, these correlations were not observed in OSEb and OVCAS cultures. These results suggested that TGF- β receptors and the AR coactivators ARA70 and SRC-1 were coordinately regulated by androgen in OSE but that this normal mechanism was lost in OSEb and OVCAS.

To visualize the complex pattern relating the effects of DHT on TGF- β receptor and steroid receptor coactivator gene expression, a pseudocolor matrix of correlation values was constructed (Fig. 7A). An intriguing pattern emerged between the different groups of patients. The effects of DHT on the expression levels of all of the genes except AIB1 were highly correlated in OSE cultures but not in OVCAS cultures. Interestingly, OSEb cultures displayed a pattern similar to OVCAS and distinct from OSE cultures. This suggests that ARA70, SRC-1, T β R-I, and T β R-II are coordinately regulated by androgen in nonmalignant ovarian epithelial cells, and that this coordination is lost in malignant cells and in ovarian epithelial cells with a predisposition to malignant transformation.

To determine how individual cultures are distributed relative to one another based on their altered expression of AIB1, ARA70, SRC-1, T β R-I, and T β R-II in response to DHT, a tree-structured visualization method of data clustering was used (36). A tree-structured visualization revealed that, with few exceptions, OSE and OVCAS cultures partitioned to separate arms of the tree (Fig. 7B). OSEb cultures partitioned with both

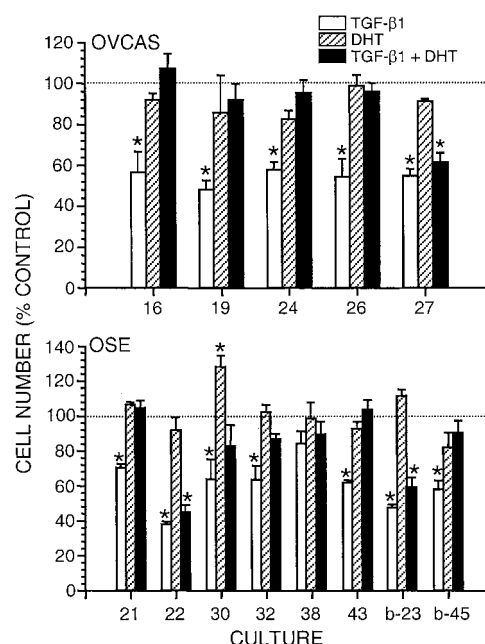


Fig. 3. Reversal of the growth-inhibitory action of TGF- β 1 by DHT in malignant and nonmalignant ovarian epithelial cells. OVCAS, OSE, and OSEb cells were maintained in culture with daily addition of 10 nM DHT or vehicle (0.001% ethanol). After seeding in 24-well plates, cells continued to be treated daily with 10 nM DHT or vehicle beginning at time zero with concomitant treatment with 25 pM TGF- β 1 or vehicle at 0, 48, and 96 h in quadruplicate. Viable cells were counted, and the 96 h time point is shown. Bars represent the cell number expressed as % control (vehicle, indicated by the dotted line at 100%) for each group, \pm SD. OSEb cultures are indicated with a *b* before the culture number. *, significantly different from control ($P < 0.05$).

Table 2 Expression of *TβR-I*, *TβR-II*, *ARA70*, *SRC-1*, and *AIB1*^a

Culture	Vehicle treated (% β-actin)					DHT treated (% β-actin)				
	<i>TβR-I</i>	<i>TβR-II</i>	<i>ARA70</i>	<i>SRC-1</i>	<i>AIB1</i>	<i>TβR-I</i>	<i>TβR-II</i>	<i>ARA70</i>	<i>SRC-1</i>	<i>AIB1</i>
OSE-21	0.32	14.39	17.29	3.63	0.14	0.09	2.53	5.22	1.18	0.15
OSE-22	0.64	2.37	3.29	1.76	0.34	0.03	0.59	1.01	0.29	0.17
OSE-30	0.09	1.48	1.50	0.28	0.16	0.13	0.89	3.14	0.37	0.35
OSE-32	0.13	0.42	0.49	0.33	0.12	0.20	0.56	1.38	1.29	0.09
OSE-38	0.21	16.69	4.14	0.29	0.78	0.17	6.01	4.38	0.32	0.77
OSE-43	0.22	1.05	0.76	0.9	1.31	0.12	0.62	0.39	0.55	0.17
OSE-48	0.24	1.09	0.64	0.16	0.67	0.34	0.96	0.54	0.19	0.40
OSEb-7	1.90	1.72	5.34	1.69	1.72	1.84	1.33	4.45	1.17	1.33
OSEb-12	0.53	2.56	3.73	0.33	1.26	0.68	3.77	6.05	0.52	2.50
OSEb-14	1.36	2.51	3.76	0.23	0.98	0.80	2.11	4.28	0.19	0.68
OSEb-16	0.96	0.50	2.23	0.27	0.94	0.94	0.17	1.90	0.20	0.31
OSEb-17	1.07	0.55	0.40	0.43	0.17	0.51	0.56	0.33	0.34	0.21
OSEb-19	3.50	0.25	0.34	0.21	0.08	0.19	0.22	0.23	0.29	0.46
OSEb-23	2.78	0.54	3.96	0.22	1.00	1.61	0.31	8.91	0.19	2.93
OSEb-41	0.23	6.1	1.38	0.18	0.23	0.19	3.59	1.28	0.49	0.32
OSEb-45	0.57	5.99	10.27	1.26	1.15	0.46	3.76	8.08	1.79	1.74
OVCAS-16	0.07	11.24	1.52	0.45	0.74	0.20	0.71	2.61	0.67	1.19
OVCAS-19	0.11	2.15	2.15	0.98	0.49	0.09	2.98	2.98	0.95	0.48
OVCAS-24	0.03	4.79	1.31	1.46	1.12	0.02	0.15	0.67	0.09	0.51
OVCAS-26	0.22	0.97	20.92	0.12	2.88	0.06	0.89	12.22	0.19	1.05
OVCAS-27	0.04	0.86	16.92	0.15	2.40	0.11	1.09	22.23	0.10	0.05
OVCAS-29	0.41	0.72	3.9	0.06	1.21	0.37	1.88	7.22	0.08	0.73
OVCAS-33	0.55	0.4	3.72	2.42	4.46	0.34	0.41	5.33	1.15	1.82
OVCAS-34	0.34	0.84	7.14	2.64	0.65	0.23	0.60	4.01	1.55	1.90

^a mRNA levels were determined by RT-PCR and calculated as described in "Materials and Methods."

OSE and OVCAS cultures, 3 clustered tightly with 5 of the OVCAS cultures, whereas the remainder clustered with the OSE cultures (Fig. 7B).

A pseudocolor distance matrix shown in Fig. 7C was used to visualize the partitioning of individual OSEb cultures relative to OSE and OVCAS using a SOM algorithm. OSEb cultures partitioned as three distinct groups. One group of 3 OSEb cultures (OSEb-7, -12, and -14) were indistinguishable from 2 malignant cell cultures (OVCAS-19 and -29), and clustered closely with 2 more OVCAS cultures (-26 and -16) and more distantly with OVCAS-27. Another group consisting of OSEb-17, -23, and 19 clustered loosely with a cancer cell culture (OVCAS-34). In the third group, OSEb-16 and -41

were indistinguishable and clustered with 2 OSE cultures (-30 and -36), and less closely with OSEb-45 and OVCAS-24 cultures. The 3 OSEb cultures with *BRCA2* mutations (7, 17, 16) did not partition as a distinct group.

DISCUSSION

Our results demonstrate that androgens can suppress the growth inhibitory actions of TGF- β 1 in most ovarian cell cultures tested, indicating that this may be a normal regulatory effect of androgens that persists in the cancer cells. Androgen levels are high in follicular fluid and in the ovarian stroma (42, 43). Thus, ovarian epithelial cells would normally be exposed to the paracrine action of androgens at ovulation, when the expelled follicular fluid bathes the surrounding ovarian surface, or as these cells form deep invaginations leading to inclusion cysts within the ovarian stroma. These inclusion cysts, which are thought to be normally short-lived, have been implicated as the primary site of origin for epithelial ovarian cancer (44, 45). Therefore, androgen-induced suppression of growth-inhibitory signals could contribute to the persistence of these cells within the stroma and the development of ovarian cancer.

TGF- β signaling requires the combined action of *TβR-I* and *TβR-II*, and occurs principally through SMAD 2/3 mediated pathways (46) and to some degree via mitogen-activated protein kinase pathways (47). Maintenance of ovarian cell cultures in DHT was capable of either decreasing or increasing transcript levels for these receptors. Furthermore, the loss of TGF- β -induced growth suppression was not always associated with a down-regulation of the receptors (*e.g.*, OSE-32), suggesting that androgen effects on downstream members of the signaling cascade or opposing signaling pathways may be involved.

With the exception of a single culture, DHT treatment did not alter growth in the absence of TGF- β . This finding is consistent with that of Karlan *et al.* (48), who reported a lack of effect of androgen on growth of OSE cells. However, two recent studies reported androgen-stimulated DNA synthesis in low-passage OSE cultures (12, 49). The differences in the studies likely reflect the various growth conditions, treatment paradigms, and/or methods of measuring proliferation. In the present study, cultures were maintained continuously in medium supplemented with charcoal-stripped fetal bovine serum, epidermal

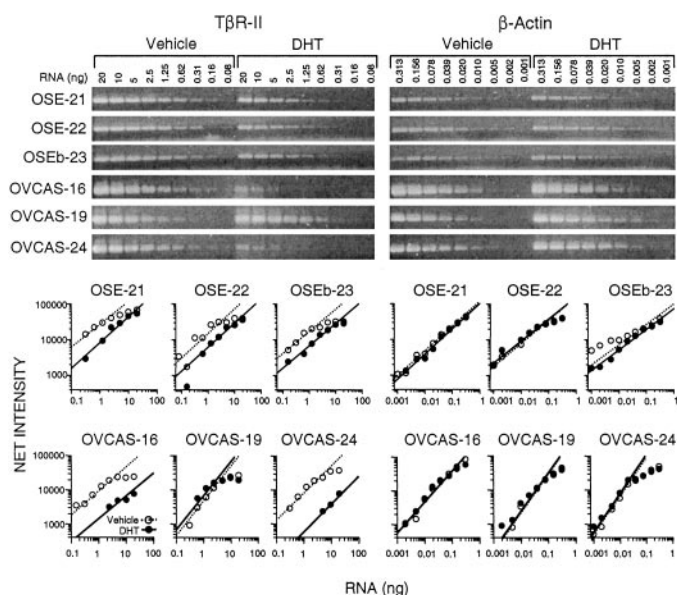


Fig. 4. Androgen-induced modulation of *TβR-II* mRNA expression in representative cultures as determined by quantitative RT-PCR. Total RNA was extracted from cultures maintained with or without 10 nM DHT and reversed transcribed. Serial dilutions of the cDNAs were subjected to PCR using primers specific for *TβR-II* and β -actin. PCR products stained with SYBRgold are shown and RNA equivalents loaded per lane indicated. Graphs of *TβR-II* and β -actin mRNA levels expressed as band net intensity values *versus* equivalent RNA concentrations are shown.

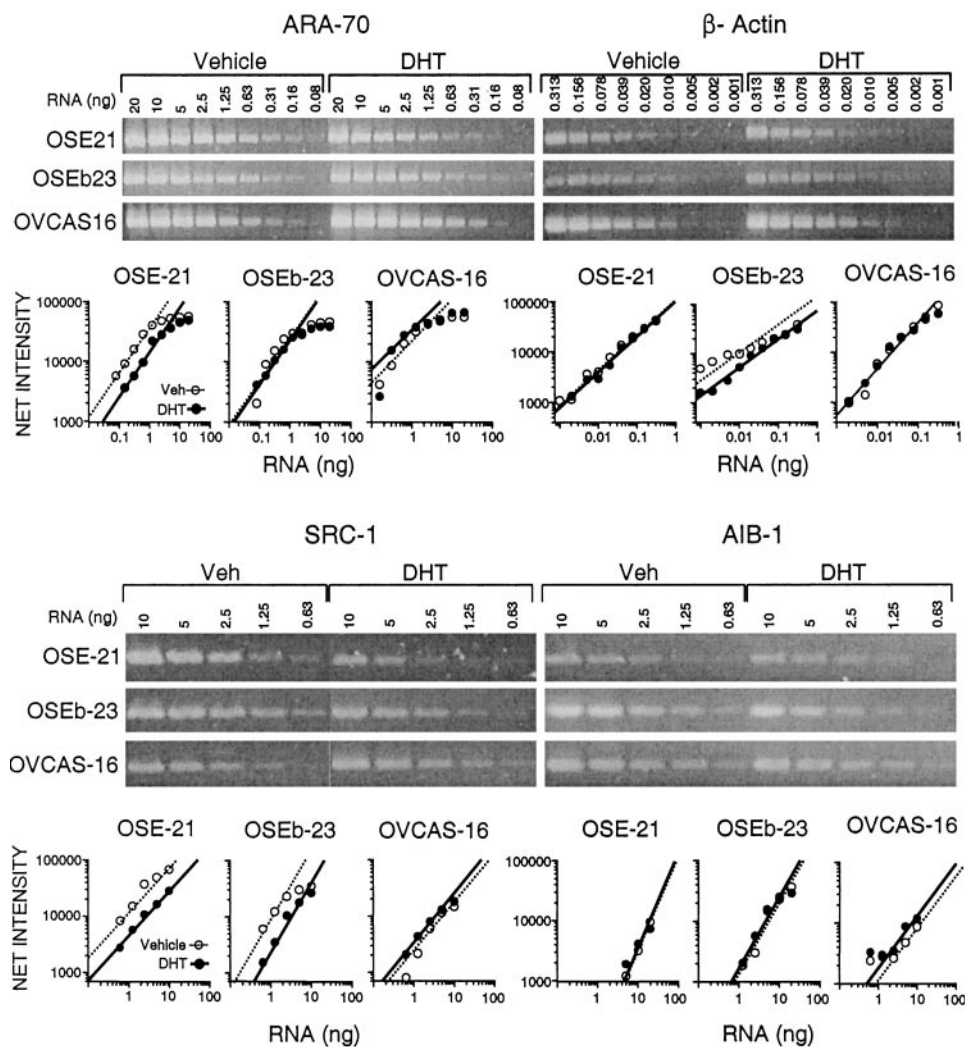


Fig. 5. Androgen-induced modulation of expression of steroid receptor coactivators ARA70, SRC-1, and AIB1 in OSE-21 control cells, in OSEb-23 *BRCA1* case, and in OVCAS-16 ovarian cancer cells, as determined by quantitative RT-PCR. Serial dilutions of cDNAs were subjected to PCR using primers specific for ARA70, β -actin, SRC-1, and AIB1 cDNAs. PCR products were stained with SYBRgold and the respective graphs are shown.

growth factor, insulin, and either DHT or ethanol vehicle. These conditions were chosen to reflect more closely the *in vivo* situation and test the ability of androgen to block TGF- β -induced growth inhibition.

The most striking findings of this study were the coordinate regulation by DHT of ARA70, SRC-1, T β R-I, and T β R-II transcripts in OSE cultures, and the loss of this coordination not only in malignant cells, but also in OSEb cells with a genetic predisposition to malignant transformation. That androgen could regulate the expression of several steroid coactivators was unexpected. Although it has been shown that androgens modulate ARA70 mRNA expression in prostate cancer cell lines (50), its effect on AIB1 and SRC-1 has not been reported previously. However, nucleotide sequences consistent with androgen (and estrogen) response elements are present in the 5 kb immediately upstream of the coding regions of these coactivators and of TGF- β receptors, as determined using MatInspector v. 2.0 software for transcription factor binding site prediction (51),⁴ raising the possibility of direct regulation of these genes by AR.

What can be the influence of *BRCA1* or *BRCA2* breast/ovarian susceptibility genes in androgen regulation of TGF- β receptors and steroid receptor coactivators? The structurally distinct proteins encoded by *BRCA1* and *BRCA2* have both been implicated in DNA repair and homologous recombination, cell cycle checkpoint progres-

sion, and transcription (52, 53). Although expression of the remaining wild-type allele is usually lost in tumor cells, mutation in only one *BRCA* allele is sufficient to predispose a carrier to breast and/or ovarian cancer. Decreased expression of *BRCA1* has been shown to increase the proliferation of malignant and nonmalignant breast mammary cells (54), whereas overexpression led to decreased growth of both breast and ovarian cancer cells (55). *BRCA1* has also been implicated in transcriptional regulation by steroids. Interaction of *BRCA1* with ER inhibited signaling pathways involved in ER-mediated growth (56, 57), whereas interaction with AR increased the transactivational activity of this receptor in prostate cancer cell lines (58, 59). Interestingly, the cultures bearing confirmed *BRCA2* mutations in this study did not cluster separately from those bearing *BRCA1* mutations, suggesting that the observed altered androgen responses were not *BRCA1* protein-specific and, therefore, not because of altered coactivator function of *BRCA1*. Nevertheless, it remains possible that the disruption of coordinated androgen responses in OSEb cultures results from other regulatory factors that are present in *BRCA* heterozygotes that affect normal hormonal mechanisms.

Whereas as a group, OSEb cells responded more like cultures of malignant cells than OSE cells, cluster analysis indicated that there is heterogeneity in this group. Interestingly, three OSEb cultures partitioned strongly with the malignant cell cultures (Fig. 7, B and C), whereas the remaining OSEb cultures partitioned loosely with OSE

⁴ Internet address: <http://www.gsf.de/biodv/matinspector.html>.

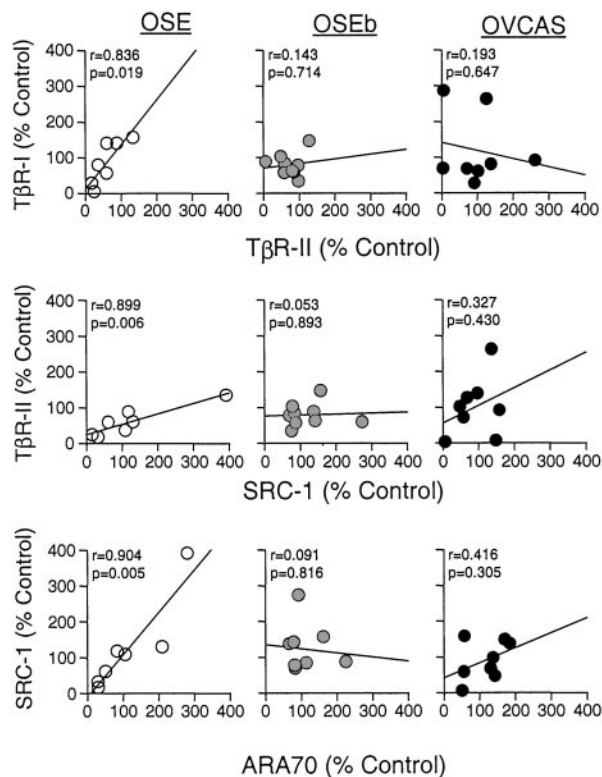


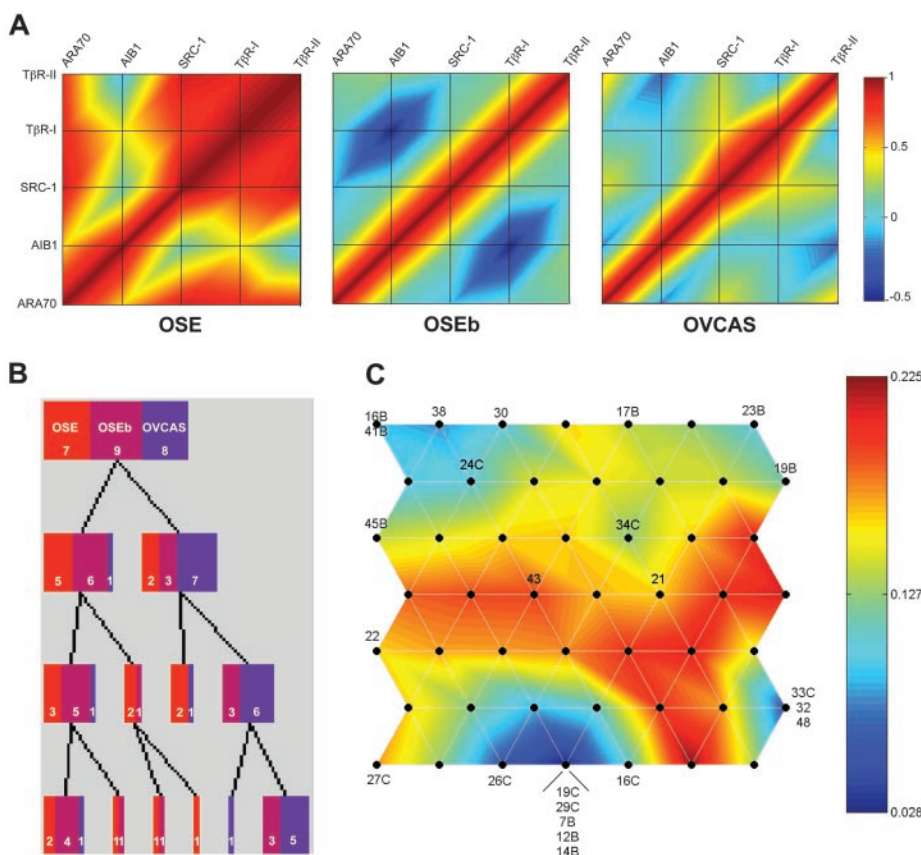
Fig. 6. Correlation between androgen-induced modulation of TGF-β receptors and steroid receptor coactivators. Transcript levels measured after DHT treatment and expressed as % control were plotted for TβR-I versus TβR-II, TβR-II versus SRC-1, and SRC-1 versus ARA70. Linear regression analysis was performed using SigmaStat software. The resulting regression coefficients (r) and p-values are indicated.

cultures. This is perhaps not surprising given that only a third of women with BRCA mutations develop epithelial OVCAS. Although it is generally accepted that specific BRCA1/2 mutations influence the risk of ovarian cancer, little is known of the penetrance of specific mutations. Thus, it is presently difficult to speculate on whether these three cases represent patients within the OSEb cohort most likely to develop ovarian cancer. However, one of these cases (OSEb-7) contained a BRCA2 mutation within the ovarian cancer cluster region (nucleotides 4075–6503), which is associated with the greatest risk of ovarian cancer (60).

The modulation of ARA70, AIB1, and SRC-1 by DHT represents a mechanism whereby androgen can modify sensitivity to itself as well as to other steroids. ARA70 has been shown to interact with PRs and ERs (61). SRC-1 and AIB1 interactions have been best characterized for ER, but also occur with multiple members of the steroid hormone receptor superfamily, including AR and PR (62–64). ERα and β expression has been reported in ovarian cancer tumor specimens as well as in OSE (11, 65), and estradiol has been shown to stimulate growth of both malignant and nonmalignant ovarian epithelial cells (49). OSE cells and most ovarian tumors also express PR (11, 66). Progesterone in micromolar quantities has been shown to induce apoptosis in two immortalized ovarian cancer cell lines (67). The modulation of multiple steroid receptor coactivators by androgen suggests a mechanism whereby androgen could modify responses to estrogens, progestins, and possibly other nuclear receptor ligands. Through such action, androgen could alter the cancer-promoting or protective effects of these steroids.

The study of androgen effects on ovarian epithelial cells requires the use of cultured cells, which represents a more controllable but somewhat artificial environment. The inherent limitations of the use of cell cultures include the removal of various endocrine and paracrine factors secreted from multiple cell types present *in vivo* and changes

Fig. 7. A, pseudocolor correlation matrix representation for TβR-II, TβR-I, ARA70, SRC-1, and AIB1 mRNA percentage change in expression induced by DHT in OSE, OSEb, and OVCAS cultures. The color map corresponds to the scale of correlation coefficients, noncorrelated data show with a coefficient of zero (light blue), negative correlation dark blue, and positive correlation ranging from yellow to red. The diagonal of the symmetric correlation matrix represents self-correlation and thus is equal to one (dark red). B, clustered tree-structured visualization of the 24 cultures using BTSVQ (36). Cultures were clustered on the basis of the effect of DHT on expression of TβR-I, TβR-II, ARA70, SRC-1, and AIB1 using a partitive k-means clustering algorithm. Cultures are indicated as OSE (red), OSEb (brown), and OVCAS (blue). C, pseudocolor distance matrix illustrating the partitioning of individual cultures using a SOM algorithm to cluster samples based on their quantized gene expression profiles. The bar scale indicates arbitrary distance units with scale from blue to red. The location of the individual clusters is indicated by the culture number(s) adjacent to the node. B indicates an OSEb culture, C indicates an OVCAS culture.



in the extracellular microenvironment, and likely altered production of specific matrix proteins, and integrin binding and cell signaling. Although the possibility that the androgen responses of ovarian epithelial cells *in vivo* may differ from those observed *in vitro*, our studies clearly reveal novel and interesting differences between malignant and nonmalignant cells, which should provide insight in both disease processes and hormone effects. The derivation of cultures from patient material was not always successful: 60% of ascites and 50% of ovarian surface scrapings yielded growing cells. Thus, potential bias introduced to the study through the removal of nonproliferating cultures cannot be excluded. However, differences were found between OSE and OSEb cultures, which are both derived by the same procedures. Exclusion of OVCAS-16 and OVCAS-33, a rare poorly differentiated epithelial type cancer and a recurrent case, respectively, because they differ from the other OVCAS, did not alter the outcome of the study.

In summary, our results demonstrate that androgens regulate the growth of ovarian epithelial cells *in vitro* by preventing inhibition by TGF- β 1 and coordinately modulate TGF- β receptors and steroid receptor coactivators in these cells. This coordinated effect of androgen was lost in tumor cells and in some cells bearing a *BRCA1/2* mutation, raising the possibility that altered androgen response may influence the predisposition to cancer or reflect a consequence of regulatory changes brought about by both ovarian carcinogenesis and *BRCA* heterozygosity.

REFERENCES

- Greenlee, R. T., Hill-Harmon, M. B., Murray, T., and Thun, M. Cancer statistics, 2001. *CA Cancer J. Clin.*, *51*: 15–36, 2001.
- National Cancer Institute of Canada. Canadian Cancer Statistics 2001. Toronto, Ontario: National Cancer Institute of Canada, 2001.
- Modugno, F., Ness, R. B., and Wheeler, J. E. Reproductive risk factors for epithelial ovarian cancer according to histologic type and invasiveness. *Ann. Epidemiol.*, *11*: 568–574, 2001.
- Whitmore, A. S., Gong, G., and Itnyre, J. Prevalence and contribution of *BRCA1* mutations in breast cancer and ovarian cancer: results from three U. S. population-based case-control studies of ovarian cancer. *Am. J. Hum. Genet.*, *60*: 496–504, 1997.
- Cancer risks in *BRCA2* mutation carriers. The Breast Cancer Linkage Consortium. *J. Nat. Cancer Inst.*, *91*: 1310–1316, 1999.
- Risch, H. A. Hormonal etiology of epithelial ovarian cancer, with a hypothesis concerning the role of androgens and progesterone. *J. Nat. Cancer Inst.*, *90*: 1774–1786, 1998.
- Schildkraut, J. M., Schwingl, P. J., Bastos, E., Evanoff, A., and Hughes, C. Epithelial ovarian cancer risk among women with polycystic ovary syndrome. *Obstet. Gynecol.*, *88*: 554–559, 1996.
- Helzlsouer, K. J., Alberg, A. J., Gordon, J. B., Longcope, C., Bush, T. L., Hoffman, S. C., and Cornstock, G. W. Serum gonadotropins and steroid hormones and the development of ovarian cancer. *JAMA*, *274*: 1926–1930, 1995.
- Chadha, S., Rao, B. R., Slotman, B. J., van Vroonhoven, C. C. J., and van der Kwast, T. H. An immunohistochemical evaluation of androgen and progesterone receptors in ovarian tumors. *Hum. Pathol.*, *24*: 90–95, 1993.
- Kühnel, R., De Graaff, J., Rao, B. R., and Stolk, J. G. Androgen receptor predominance in human ovarian cancer. *J. Steroid Biochem.*, *26*: 393–397, 1987.
- Lau, K-M., Mok, S. C., and Ho, S-M. Expression of human estrogen receptor- α and- β , progesterone receptor, and androgen receptor mRNA in normal and malignant ovarian epithelial cells. *Proc. Natl. Acad. Sci. USA*, *96*: 5722–5727, 1999.
- Edmondson, R. J., Monaghan, J. M., and Davies, B. R. The human ovarian surface epithelium is an androgen responsive tissue. *Br. J. Cancer*, *86*: 879–885, 2002.
- Shibata, H., Spencer, T. E., Onate, S. A., Jenster, G., Tsai, S. Y., Tsai, M. J., and O'Malley, B. W. Role of co-activators and co-repressors in the mechanism of steroid/thyroid receptor action. *Recent Prog. Horm. Res.*, *52*: 141–164, 1997.
- Jenster, G. Coactivators and coreceptors as mediators of nuclear receptor function: an update. *Mol. Cell. Endocrinol.*, *143*: 1–7, 1998.
- Spencer, T. E., Jenster, G., Burcin, M. M., Allis, C. D., Zhou, J., Mizzen, C. A., McKenna, N. J., Onate, S. A., Tsai, S. Y., Tsai, M. J., and O'Malley, B. W. Steroid receptor coactivator-1 is a histone acetyltransferase. *Nature (Lond.)*, *389*: 194–198, 1997.
- Kim, M. Y., Hsiao, S. J., and Kraus, W. L. A role for coactivators and histone acetylation in estrogen receptor α -mediated transcription initiation. *EMBO J.*, *20*: 6084–6094, 2001.
- Anzick, S. L., Kononen, J., Walker, R. L., Azorsa, D. O., Tanner, M. M., Guan, X-Y., Sauter, G., Kallioniemi, O-P., Trent, J. M., and Meltzer, P. S. AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. *Science (Wash. DC)*, *277*: 965–968, 1997.
- Tanner, M. M., Grenman, S., Koul, A., Johannsson, O., Meltzer, P., Pejovic, T., Borg, Å., and Isola, J. J. Frequent amplification of chromosomal region 20q12–q13 in ovarian cancer. *Clin. Cancer Res.*, *6*: 1833–1839, 2000.
- Shaw, P. A., Rittenberg, P. V. C., and Brown, T. J. Activation of *ARA70* mRNA expression in ovarian cancer. *Gynecol. Oncol.*, *80*: 132–138, 2001.
- Chamberlain, N. L., Driver, E. D., and Miesfeld, R. L. The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. *Nucleic Acids Res.*, *22*: 3181–3186, 1994.
- Levine, D. A., and Boyd, J. A. The androgen receptor and genetic susceptibility to ovarian cancer: results from a case series. *Cancer Res.*, *61*: 908–911, 2001.
- Berchuck, A., Rodriguez, G., Olt, G., Whitaker, R., Boente, M. P., Arrick, B. A., Clarke-Pearson, D. L., and Bast, R. C. Regulation of growth of normal ovarian epithelial cells and ovarian cancer cell lines by transforming growth factor- β . *Am. J. Obstet. Gynecol.*, *166*: 676–684, 1992.
- Havrilesky, L. J., Hurteau, J. A., Whitaker, R. S., Elbendary, A., Wu, S., Rodriguez, G. C., Bast, R. C., and Berchuck, A. Regulation of apoptosis in normal and malignant ovarian epithelial cells by transforming growth factor β . *Cancer Res.*, *55*: 944–948, 1995.
- Hurteau, J., Rodriguez, G. C., Whitaker, R. S., Shah, S., Mills, G., Bast, R. C., and Berchuck, A. Transforming growth factor- β inhibits proliferation of human ovarian cancer cells obtained from ascites. *Cancer (Phila.)*, *74*: 93–99, 1994.
- Pasche, B., Kolachana, P., Nafa, K., Satagopan, J., Chen, Y-G., Lo, R. S., Brenner, D., Yang, D., Kirstein, L., Oddoux, C., Ostrer, H., Vineis, P., Varesco, L., Jhanwar, S., Luzzatto, L., Massagué, J., and Offit, K. TBR-1 (6A) is a candidate tumor susceptibility allele. *Cancer Res.*, *59*: 5678–5682, 1999.
- Wang, J., Sun, L., Myeroff, L., Wang, X., Gentry, L. E., Yang, J., Liang, J., Zborowska, E., Markowitz, S., Wilson, J. K., and Brattain, M. Demonstration that mutation of the type II transforming growth factor β receptor inactivates its tumor suppressor activity in replication error-positive colon carcinoma cells. *J. Biol. Chem.*, *270*: 22044–22049, 1995.
- Abe, T., Ouyang, H., Migata, T., Kato, Y., Kimura, M., Shiiba, K., Sunamura, M., Matsuno, S., and Horii, A. The somatic mutation frequency of the transforming growth factor β receptor type II gene varies widely among different cancers with microsatellite instability. *Eur. J. Surg. Oncol.*, *22*: 474–477, 1996.
- Evangelou, A., Jindal, S. K., Brown, T. J., and Letarte, M. Down-regulation of TGF- β receptors by androgen in ovarian cancer cells. *Cancer Res.*, *60*: 929–935, 2000.
- Hirte, H. W., Clark, D. A., Mazurka, J., O'Connell, G., and Rusthoven J. A rapid and simple method for the purification of tumor cells from ascitic fluid of ovarian carcinoma. *Gynecol. Oncol.*, *44*: 223–226, 1992.
- Quackenbush, E. J., and Letarte, M. Identification of several cell surface proteins of non-T, non-B acute lymphoblastic leukemia by using monoclonal antibodies. *J. Immunol.*, *134*: 1276–1285, 1985.
- Linsley, P. S., Brown, J. P., Magnani, J. L., and Horn, D. Monoclonal antibodies reactive with mucin glycoproteins found in sera from breast cancer patients. *Cancer Res.*, *48*: 2138–2148, 1988.
- Marks, A., Sutherland, D. R., Bailey, D., Iglesias, J., Law, J., Lei, M., Yeger, H., Banerjee, D., and Bauman, R. Characterization and distribution of an oncofetal antigen (M2A antigen) expressed on testicular germ cell tumours. *Br. J. Cancer*, *80*: 569–578, 1999.
- Letarte, M., Iturbe, S., and Quackenbush, E. J. A glycoprotein of molecular weight 85,000 on human cells of B-lineage: detection with a family of monoclonal antibodies. *Mol. Immunol.*, *22*: 113–124, 1985.
- Murphy, L. D., Herzog, C. E., Rudick, J. B., Fojo, A. T., and Bates, S. E. Use of the polymerase chain reaction in the quantitation of *mdr-1* gene expression. *Biochemistry*, *29*: 10351–10356, 1990.
- Jindal, S. K., Ishii, E., Letarte, M., Vera, S., Teerds, K. J., and Dorrington, J. H. Regulation of transforming growth factor α gene expression in an ovarian surface epithelial cell line derived from a human carcinoma. *Biol. Reprod.*, *52*: 1027–1037, 1995.
- Sultan, M., Wigle, D., Cumbaa, C. A., Maziarz, M., Glasgow, J., Tsao, M. S., and Jurisica, I. Binary tree-structured vector quantization approach to clustering and visualizing microarray data. *Bioinformatics*, *18*(Suppl. 1): S111–S119, 2002.
- Linsley, P. S., Ochs, V., Horn, D., Ring, D. B., and Frankel, A. E. Heritable variation in expression of multiple tumor associated epitopes on a high molecular weight mucin-like antigen. *Cancer Res.*, *46*: 6380–6386, 1986.
- Auersperg, N., Maines-Bandiera, S., Booth, J. H., Lynch, H. T., Godwin, A. K., and Hamilton, T. C. Expression of two mucin antigens in cultured human ovarian surface epithelium: influence of a family history of ovarian cancer. *Am. J. Obstet. Gynecol.*, *173*: 558–565, 1995.
- Auersperg, N., Maines-Bandiera, S. L., Dyck, H. G., and Kruk, P. A. Characterization of cultured human ovarian surface epithelial cells: phenotypic plasticity and pre-malignant changes. *Lab Invest.*, *71*: 510–518, 1994.
- Strobel, T., and Cannistra, S. A. β 1-Integrins partly mediate binding of ovarian cancer cells to peritoneal mesothelium *in vitro*. *Gynecol. Oncol.*, *73*: 362–367, 1999.
- Casey, R. C., and Skubitz, A. P. CD44 and β 1 integrins mediate ovarian carcinoma cell migration toward extracellular matrix proteins. *Clin. Exp. Metastasis*, *18*: 67–75, 2000.
- Haning, R. V., Jr., Flood, C. A., Hackett, R. J., Loughlin, J. S., McClure, N., and Longcope, C. Metabolic clearance rate of dehydroepiandrosterone sulfate, its metabolism to testosterone, and its intrafollicular metabolism to dehydroepiandrosterone, androstenedione, testosterone, and dihydrotestosterone *in vivo*. *J. Clin. Endocrinol. Metab.*, *72*: 1088–1095, 1991.
- Morgan, P. M., Boatman, D. E., and Bavister, B. D. Relationships between follicular fluid steroid hormone concentrations, oocyte maturity, *in vitro* fertilization and embryonic development in the rhesus monkey. *Mol. Reprod. Dev.*, *27*: 145–151, 1990.

44. Scully, R. E. Pathology of ovarian cancer precursors. *J. Cell. Biochem.*, 23(Suppl.): 208–218, 1995.
45. Feeley, K. M., and Wells, M. Precursor lesions of ovarian epithelial malignancy. *Histopathology*, 38: 87–95, 2001.
46. Wrana, J. L., Attisano, L., Wieser, R., Ventura, F., and Massagué, J. Mechanism of activation of the TGF- β receptor. *Nature (Lond.)*, 370: 342–347, 1994.
47. Mulder, K. M. Role of Ras and Mapks in TGF β signaling. *Cytokine Growth Factor Rev.*, 11: 23–35, 2000.
48. Karlan, B. Y., Jones, J., Greenwald, M., and Lagasse, L. D. Steroid hormone effects on the proliferation of human ovarian surface epithelium *in vitro*. *Am. J. Obstet. Gynecol.*, 173: 97–104, 1995.
49. Syed, V., Ulinski, G., Mok, S. C., Yiu, G. K., and Ho, S. M. Expression of gonadotropin receptor and growth responses to key reproductive hormones in normal and malignant human ovarian surface epithelial cells. *Cancer Res.*, 61: 6768–6776, 2001.
50. Tekur, S., Lau, K. M., Long, J., Burnstein, K., and Ho, S. M. Expression of RFG/ELE1 α /ARA70 in normal and malignant prostatic epithelial cell cultures and lines: regulation by methylation and sex steroids. *Mol. Carcinog.*, 30: 1–13, 2001.
51. Quandt, K., Frech, K., Karas, H., Wingender, E., and Werner, T. MatInd and MatInspector: new fast and versatile tools for detection of consensus matches in nucleotide sequence data. *Nucleic Acids Res.*, 23: 4878–4884, 1995.
52. Venkitaraman, A. R. Cancer susceptibility and the functions of BRCA1 and BRCA2. *Cell*, 108: 171–182, 2002.
53. Monteiro, A. N. BRCA1: exploring the links to transcription. *Trends Biochem. Sci.*, 25: 469–474, 2000.
54. Thompson, M. E., Jensen, R. A., Obermiller, P. S., Page, D. L., and Holt, J. T. Decreased expression of BRCA1 accelerates growth and is often present during sporadic breast cancer progression. *Nat. Genet.*, 9: 444–450, 1995.
55. Holt, J. T., Thompson, M. E., Szabo, C., Robinson-Benion, C., Arteaga, C. L., King, M. C., King, M. C., and Jensen, R. A. Growth retardation and tumour inhibition by BRCA1. *Nat. Genet.*, 12: 298–302, 1996.
56. Fan, S., Wang, J.-A., Yuan, R., Ma, Y., Meng, Q., Erdos, M. R., Pestell, R. G., Yuan, F., Auborn, K. J., Goldberg, I. D., and Rosen, E. M. BRCA1 inhibition of estrogen receptor signaling in transfected cells. *Science (Wash. DC)*, 284: 1354–1356, 1999.
57. Fan, S., Ma, Y. X., Wang, C., Yuan, R. Q., Meng, Q., Wang, J. A., Erdos, M., Goldberg, I. D., Webb, P., Kushner, P. J., Pestell, R. G., and Rosen, E. M. Role of direct interaction in BRCA1 inhibition of estrogen receptor activity. *Oncogene*, 20: 77–87, 2001.
58. Park, J. J., Irvine, R. A., Buchanan, G., Koh, S. S., Park, J. M., Tilley, W. D., Stallcup, M. R., Press, M. F., and Coetzee, G. A. Breast cancer susceptibility gene 1 (BRCA1) is a coactivator of the androgen receptor. *Cancer Res.*, 60: 5946–5949, 2000.
59. Yeh, S., Hu, Y. C., Rahman, M., Lin, H. K., Hsu, C. L., Ting, H. J., Kang, H. Y., and Chang, C. Increase of androgen-induced cell death and androgen receptor transactivation by BRCA1 in prostate cancer cells. *Proc. Natl. Acad. Sci. USA*, 97: 11256–11261, 2000.
60. Thompson, D., and Easton, D. Variation in cancer risks, by mutation position, in BRCA2 mutation carriers. *Am. J. Hum. Genet.*, 68: 410–419, 2001.
61. Alen, P., Claessens, F., Schoenmakers, E., Swinnen, J. V., Verhoeven, G., Rombauts, W., and Peeters, B. Interaction of the putative androgen receptor-specific coactivator ARA70/ELE1 α with multiple steroid receptors and identification of an internally deleted ELE1 β isoform. *Mol. Endo.*, 13: 117–128, 1999.
62. Yeh, S., Miyamoto, H., Shima, H., and Chang, C. From estrogen to androgen receptor: a new pathway for sex hormones in prostate. *Proc. Natl. Acad. Sci. USA*, 95: 5527–5532, 1998.
63. Suen, C. S., Berrodin, T. J., Mastroeni, R., Cheskis, B. J., Lyttle, C. R., and Frail, D. E. A transcriptional coactivator, steroid receptor coactivator-3, selectively augments steroid receptor transcriptional activity. *J. Biol. Chem.*, 273: 27645–27653, 1998.
64. Bevan, C. L., Hoare, S., Claessens, F., Heery, D. M., and Parker, M. G. The AF1 and AF2 domains of the androgen receptor interact with distinct regions of SRC-1. *Mol. Cell. Biol.*, 19: 8383–8392, 1999.
65. Choi, K.-C., Kang, S. K., Tai, C.-J., Auersperg, N., and Leung, P. C. K. Estradiol up-regulates antiapoptotic Bcl-2 messenger ribonucleic acid and protein in tumorigenic ovarian surface epithelium cells. *Endocrinology*, 142: 2351–2360, 2001.
66. Akahira, J., Inoue, T., Suzuki, T., Ito, K., Konno, R., Sato, S., Moriya, T., Okamura, K., Yajima, A., and Sasano, H. Progesterone receptor isoforms A and B in human epithelial ovarian carcinoma: immunohistochemical and RT-PCR studies. *Br. J. Cancer*, 83: 1488–1494, 2000.
67. Bu, S.-Z., Yin, D.-L., Ren, X.-H., Jiang, L.-Z., Wu, Z.-J., Gao, Q.-R., and Pei, G. Progesterone induces apoptosis and up-regulation of p53 expression in human ovarian carcinoma cell lines. *Cancer (Phila.)*, 79: 1944–1950, 1997.