Glucocorticoid Sensitivity of OVCA 433 Human Ovarian Carcinoma Cells: Inhibition of Plasminogen Activators, Cell Growth, and Morphological Alterations

Waheeda Amin, Beth Y. Karlan, and Bruce A. Littlefield

Department of Obstetrics and Gynecology [W. A., B. Y. K., B. A. L.] and Yale Comprehensive Cancer Center [B. A. L.], Yale University School of Medicine, New Haven, Connecticut 06510

ABSTRACT

OVCA 433 human ovarian carcinoma cells secrete large amounts of plasminogen activator (PA), which consists of immunologically identifiable urokinase (UK) and tissue-type PA (tPA). Total extracellular PA activity is 95% inhibited by treatment of cells with $1 \times 10^{-7}$ M dexamethasone (Dex) for 3 days. This inhibition is both time and concentration dependent, with half-maximal inhibition occurring after 1.5 days with $1 \times 10^{-7}$ M Dex, or with $1 \times 10^{-6}$ M Dex for 3 days, respectively. Interestingly, the loss of UK activity precedes the loss of tPA activity, such that half-maximal inhibition of the two PA types occurs at 1 and 2 days, respectively. Dex treatment leads to approximately 50% inhibition of cell growth and pronounced morphological alterations, including marked enlargement, flattening, and multinucleation. Treatment of the cells with other classes of steroid hormones, i.e., estrogens, progestins, androgens, and mineralocorticoids, is without effect on UK and tPA activities, cell growth, or morphology. OVCA 433 cells contain about 14,000 nuclear glucocorticoid receptors (GR) per cell (measured at 37°C), with an average affinity ($K_d$) for [3H]Dex of $6.6 \times 10^{-8}$ M. Only active glucocorticoids compete with [3H]Dex for nuclear GR binding sites. Our results demonstrate steroid-specific glucocorticoid responsiveness of ovarian carcinoma cells, a tumor cell type not usually considered hormonally responsive. Since almost 90% of ovarian carcinoma tumor biopsies contain GR (M. C. Galli, et al., Cancer (Phila.), 47: 1297-1302, 1981), it is possible that glucocorticoid sensitivity could be exploited clinically, particularly following the almost universal development of resistance to the chemotherapeutics drugs commonly used in this disease.

INTRODUCTION

Ovarian carcinoma is the leading cause of death due to gynecological malignancy and the fifth most common cause of cancer-related deaths in American women (1). In 1984, the number of women dying from ovarian cancer (11,500) exceeded the number succumbing to cervical (7,000) and endometrial (3,000) cancers combined (2). Therapeutic progress in this disease has been extremely limited. Thus, despite some advances, the 5-year survival rate for ovarian carcinoma has not changed significantly in the last two decades and remains a bleak 30% (3). The general failure of traditional chemotherapy regimens to significantly increase survival rates prompted us to consider whether ovarian carcinoma cells might be hormonally responsive, and whether hormone responsive might be exploited to help control tumor progression. A number of laboratories have demonstrated the presence of estrogen, progesterone, and androgen receptor(s) in subpopulations of ovarian carcinoma biopsies (4–10). However, despite a few reports that antiestrogens and progestins may be of some limited value in the treatment of this disease (10–14), the effectiveness of steroidal therapy has in general been disappointing and such procedures are not in routine use at this time (14). We became intrigued by a report that GR were found in 88% of malignant ovarian biopsies (7). We therefore decided to examine the effects of glucocorticoids on the OVCA 433 human ovarian carcinoma cell line. In this report, we demonstrate marked steroid-specific effects of glucocorticoids on cellular morphology, growth rates, and secreted PA activity, as well as the presence of high affinity, steroid-specific GR. To our knowledge, this is the first clear demonstration and in-depth study of glucocorticoid responsiveness of ovarian carcinoma cells in vitro.

MATERIALS AND METHODS

Cell Growth Conditions. OVCA 433 human ovarian carcinoma cells, originally derived from a human serous cystadenocarcinoma of the ovary (15), were generously provided by Drs. Vimal Band, Vincent R. Zurawski, Jr., and Robert Knapp at Harvard Medical School. These cells were grown as described previously (16). When appropriate, Dex and other steroids (Steraloids, Wilton, NH) were added to cell cultures as sterile concentrates freshly prepared in culture medium. For experiments employing only Dex (Figs. 1B, 2, 3, 4, and 7), these concentrates were made from freshly prepared solutions of Dex in water at concentrations of $5 \times 10^{-4}$ to $1 \times 10^{-4}$ M, followed by dilution to appropriate levels in culture medium. For steroid specificity studies (Fig. 1A and Table 1), the concentrates were made from $1 \times 10^{-4}$ M stock solutions prepared in 100% ethanol and stored light shielded at $-20^\circ$C. Controls for steroid specificity studies included equivalent amounts of ethanol.

Cell Growth Inhibition Studies. Effects of Dex and other steroids on OVCA 433 cells were assessed using the methylene blue-based microculture assay of Finlay et al. (17). Seeding densities in the 96-well microtiter plates were 2,000 cells/well in a total volume of 50 μl. Absorbances of the fixed, stained, and detergent-solubilized cells were determined on a Titertek Multiskan Plus enzyme-linked immunosorbent assay plate reader at a wavelength of 600 nm. Background absorbances determined from identically processed wells never receiving cells were subtracted from all readings. Initial cell plating densities were estimated in each experiment by preparing separate 96-well plates which were then harvested approximately 18 h after seeding, a time chosen to allow complete attachment of the plated cells with minimal cell division. In agreement with Finlay et al. (17), results obtained with the methylene blue-based assay were virtually identical to results obtained with electronic cell counting of harvested cells using techniques described by us previously (18, 19).

Plasminogen Activator Assay. PA activity secreted by OVCA 433 cells into serum-free medium during a 1-h collection period was quantitated following preparation of concentrated conditioned media as described (16, 18). Appropriate dilutions of concentrated conditioned media were then analyzed for PA activity using the chromogenic

Received 3/19/87; revised 7/31/87; accepted 8/10/87.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1Supported by NIH Grant CA40010 to B. A. L. and fellowships from Ortho ACOG and the James Hudson Brown-Alexander B. Coxe Fund to B. Y. K.

2To whom requests for reprints should be addressed, at Department of Obstetrics and Gynecology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510.

3The abbreviations used are: GR, glucocorticoid receptor(s); BSA, bovine serum albumin; Dex, dexamethasone (1,4-pregnadien-9-fluoro-16-methyl-11,17a,21-triol-3,20-dione); UK, urokinase; GNS, goat normal serum; GxPA, goat anti-tPA IgG; GxUK, goat anti-UK IgG; mPU, milliPloug units; PA, plasminogen activator; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; tPA, tissue-type plasminogen activator; IB, immunobuffer.
microtiter plate PA assay described previously (16). When appropriate, the presence of Dex and other steroids was maintained during the 1-h collection periods. Discrimination between UK and tPA activities based on fibrin independence and dependence, respectively, was performed as described (16).

Whole Cell Nuclear Glucocorticoid Receptor Assays. Nuclear GR binding of [3H]Dex (New England Nuclear, Boston, MA; 48.9 Ci/mmol) at concentrations of 2 x 10^{-7} to 7.8 x 10^{-10} M was determined at 37°C by saturation analysis according to the method of Scatchard (20), or, for steroid specificity studies, by a single-point assay using 1 x 10^{-6} M [3H]Dex in the presence or absence of 1 x 10^{-6} M concentrations of unlabeled steroids (Steroidal). Both procedures have been described in detail previously (18, 19).

Immunoprecipitation of OVCA 433 Plasminogen Activators. Concentrated conditioned media from OVCA 433 cells, prepared as described previously (16, 18), or human UK and tPA standards (Calbiochem-Behring, San Diego, CA, and American Diagnostica, Greenwich, CT, respectively) were immunoprecipitated with GxUK, GtPA (both from American Diagnostica) or GNS (Grand Island Biological Co., Grand Island, NY) as follows. Fifty μl of OVCA 433 concentrated conditioned media containing 4 μg/μl total PA activity, or human UK or tPA standards each containing 10 μg/μl of PA activity, were placed in 1.5-ml polypropylene microcentrifuge tubes. Twenty-five μl of a 4 X IB/BSA buffer [1 X IB/BSA = 0.15 M NaCl, 20 mM Tris, 1 mM Na2EDTA, 0.5% Tween 20, and BSA (10 mg/ml), pH 7.4] were then added, followed by the addition of 125 μg of GxUK, GtPA, or GNS in 25-μl volumes of 10 mM NaCl, 10 mM Tris, 1 mM Na2EDTA, 0.5% Tween 20, pH 7.4. Incubations (now 100 μl) were carried out at 37°C for 2 h, followed by addition of 50 μl of a protein A-Sepharose (Sigma Chemical Co., St. Louis, MO) suspension prepared by mixing equal volumes of a packed hydrated protein A-Sepharose pellet and 1 X IB/BSA buffer. Incubations were continued for 2 h at 37°C with frequent vortexing, followed by addition of 1 ml 1 X IB/BSA buffer, centrifugation at 10,000 X g for 2 min, and aspiration of the supernatant. Protein A-Sepharose pellets were then washed once more with 1 X IB/BSA buffer, and three more times with 1 X IB without BSA. Washed pellets were then resuspended in a total volume of 75 μl of 62.5 mM Tris, 2% (w/v) SDS, pH 6.8, and boiled for 5 min. Following cooling, 30 μl of the resultant supernatants were analyzed by SDS-PAGE zymography as described below.

SDS-PAGE Zymography of Plasminogen Activators. Zymography of PAs on 10% SDS-PAGE gels polymerized in the presence of plasminogen and casein was performed as previously described (16, 18).

RESULTS

To assess the effects of different classes of steroid hormones on OVCA 433 human ovarian carcinoma cell growth, cells were either grown under control conditions or were treated with 1 x 10^{-7} M concentrations of 17β-estradiol (E2), progesterone (Pro), dihydrotestosterone (DHT), aldosterone (Ald), cortisol (Cort), and dexamethasone (Dex) were assessed using a 96-well plate microculture method (17) as described in "Materials and Methods." Data, means (± range) of two determinations from separately normalized experiments, each of which was conducted in quadruplicate. A, effects of a 4-day exposure to increasing concentrations of Dex were assessed using the 96-well plate microculture method (17). Data, means (± SE) of quadruplicate determinations from a single experiment. Dashed lines, in both panels represent initial plating densities at the start of the experiments. Details of all procedures are presented in "Materials and Methods."

While performing the experiments of Fig. 1, we noted that glucocorticoid treatment led to rather marked morphological alterations of OVCA 433 cells. As shown in Fig. 2, treatment of freshly plated cultures with 1 x 10^{-7} M Dex for 4 days led to the appearance of pronounced enlargement and flattening of most cells. In addition, giant multinucleated cells became apparent. With the possible exception of the multinucleated cells, Dex-treated cells were found to be capable of continued division (albeit at decreased rates), since such cultures have been serially passaged up to 14 times in the continuous presence of Dex.4

We previously demonstrated that OVCA 433 cells secrete relatively large amounts of PA activity (16), and in work to be presented elsewhere, that such secretion is a general property of many human ovarian carcinoma cell lines.5 As shown in Fig. 3, treatment of these cells with 1 x 10^{-7} M Dex for 0–3 days led to a marked inhibition of secreted PA activity. In this experiment, a 28% decrease in PA activity secreted during the final 1 h period was seen after the first day of Dex treatment, followed by 81 and 95% decreases by 2 and 3 days of treatment, respectively. This time-dependent inhibition of PA activity by Dex also shows dependence on the concentration of Dex employed (Fig. 4). In this experiment, cells were treated for 3 days with 1 x 10^{-6} to 1 x 10^{-4} M Dex, followed by measurement of PA activity secreted during the final hour. Half-maximal inhibition occurred at approximately 1 x 10^{-9} M, while maximal inhibition was seen at 1 x 10^{-7} M. These concentrations correspond well with the Dex concentrations required for growth inhibitory effects (Fig. 1B). As will be seen below (Table 1), inhibition of PA activity also exhibits the same specificity for glucocorticoids that was observed for growth inhibition (Fig. 1A).

A whole cell saturation binding study with [3H]Dex, analyzed according to the method of Scatchard (20), indicated that OVCA 433 cells contained 14,270 nuclear GR per cell (measured at 37°C with an estimated affinity (Ka) for [3H]Dex of 5.0 x 10^{9} M (Fig. 5). In a series of four such experiments, we found a mean of 14.085 ± 1.594 (SE) nuclear GR per cell with

4 Personal observations.
an average $K_d$ for $[^3]$H$\text{Dex}$ of $6.6 \pm 0.9 \times 10^{-9}$ M (data not shown). The specificity of nuclear GR binding was then examined by assessing the capacity of various steroids to compete with $[^3]$H$\text{Dex}$ for nuclear GR binding sites (Table 1). Only the natural glucocorticoid cortisol and the synthetic glucocorticoid Dex proved to be highly effective competitors. Other steroids tested ($17\beta$-estradiol, progesterone, dihydrotestosterone, and aldosterone) were ineffective competitors for nuclear GR binding sites. The abilities of the various steroids to bind to nuclear GR were paralleled closely by their abilities to inhibit secreted PA activity (Table 1). Thus, Dex and cortisol, the most effective competitors for nuclear $[^3]$H$\text{Dex}$ binding sites, were the only steroids capable of reducing secreted PA activity. The specificity for active glucocorticoids in both GR binding and PA inhibition (Table 1) was identical to that previously seen for growth inhibition (Fig. 1A).

To lay a foundation for future experiments designed to examine the mechanisms of PA inhibition by glucocorticoids, the specific effects of Dex on the two PA types secreted by OVCA 433 cells were assessed. The fibrin-dependent nature of tPA has been known for some time (21–23). Previous studies from our laboratory (16) have shown that OVCA 433 PA activity consists of both a fibrin-independent UK-like species which comigrates with a human UK standard, and a fibrin-dependent tPA-like species which comigrates with a human tPA standard. Immunoprecipitation studies with goat antibodies to human UK and tPA were undertaken to immunologically identify these two species as true UK and tPA, respectively. As previously reported (16), total PA secreted by OVCA 433 cells migrated as two distinct species (Fig. 6, lane A), one of which comigrated with a human UK standard (lane B) and the other with a human tPA standard (lane C). These two distinct species were selectively immunoprecipitated by GxUK (lane E) or GxtPA (lane F), but not by GNS (lane D). The specificity of GxUK and
duplicate determinations. Binding by the radial subtraction method of Rosenthal (39). Points, mean of according to the method of Scatchard (20), with correction for nonsaturable described previously (18, 19) and in “Materials and Methods.” Results are plotted in OVCA 433 cells. Whole cells were incubated at 37°C for 45 min with 2.0 x 10^-7 M to 7.8 x 10^-7 M [3H]Dex, followed by quantitation of nuclear binding as described previously (18, 19) and in “Materials and Methods.” Results are plotted according to the method of Scatchard (20), with correction for nonsaturable binding by the radial subtraction method of Rosenthal (39). Points, mean of duplicate determinations.

![Equilibrium saturation analysis of nuclear [3H]dexamethasone binding in OVCA 433 cells. Whole cells were incubated at 37°C for 45 min with 2.0 x 10^-7 M to 7.8 x 10^-7 M [3H]Dex, followed by quantitation of nuclear binding as described previously (18, 19) and in “Materials and Methods.” Results are plotted according to the method of Scatchard (20), with correction for nonsaturable binding by the radial subtraction method of Rosenthal (39). Points, mean of duplicate determinations.](image-url)

**Table 1** Steroid specificity of nuclear GR binding and inhibition of OVCA 433 PA activity

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Nuclear-bound [3H]Dex (100 ± 5)</th>
<th>PA Activity (% control) (100 ± 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100 ± 5</td>
<td>100 ± 6</td>
</tr>
<tr>
<td>17β-Estradiol</td>
<td>101 ± 20</td>
<td>110 ± 37</td>
</tr>
<tr>
<td>Progesterone</td>
<td>67 ± 7</td>
<td>109 ± 9</td>
</tr>
<tr>
<td>Dihydrotestosterone</td>
<td>83 ± 17</td>
<td>112 ± 4</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>78 ± 9</td>
<td>98 ± 13</td>
</tr>
<tr>
<td>Cortisol</td>
<td>20 ± 2</td>
<td>34 ± 24</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>12 ± 1</td>
<td>3 ± 1</td>
</tr>
</tbody>
</table>

*Whole cells were incubated for 45 min at 37°C with 1 x 10^-7 M [3H]Dex alone (control) or in the presence of 1 x 10^-6 M concentrations of the indicated steroids. Nuclear binding of [3H]Dex was then determined as described previously (18, 19) and in “Materials and Methods.” Values, mean (±SE) of four determinations from four separate experiments, each experiment having been performed in quadruplicate. 100% represents 1,763 (±93) dpm/10^6 nuclei.

The experiments described in this report have demonstrated steroid-specific effects of glucocorticoids on OVCA 433 human ovarian carcinoma cells. These effects encompass growth inhibition, morphological alterations, and inhibition of immunologically identifiable UK and tPA activities. Moreover, the concentration dependence and steroid specificity characteristics of these effects are consistent with mediation by the high affinity, steroid-specific GR which we have demonstrated in these cells. Treatment of the cells with other classes of steroid hormones, notably estrogens and progestins, was without effect on any of the processes under consideration. However, responsiveness to other steroids cannot be ruled out, because regulated processes other than those considered here may have gone unobserved. Moreover, steroids present in fetal calf serum, and potential estrogenic effects of the pH indicator phenol red (24), may have already led to maximal stimulation of the cells, although control experiments with 1 x 10^-6 M concentrations of the antiestrogen tamoxifen failed to support this possibility (data not shown). Nevertheless, our studies do not rule out responsivity to other steroid classes, and experiments designed to specifically address this issue are currently underway in our laboratory.

An interesting aspect of the effects of glucocorticoids on OVCA 433 cell morphology and PA activity is that these effects show only limited reversibility once cells are allowed to reach confluence. OVCA 433 cells are largely contact inhibited at confluence and appear to reside primarily in the G0 phase (25). An interesting aspect of the effects of glucocorticoids on OVCA 433 cell morphology and PA activity is that these effects show only limited reversibility once cells are allowed to reach confluence. OVCA 433 cells are largely contact inhibited at confluence and appear to reside primarily in the G0 phase (25).

We previously demonstrated that OVCA 433 cells produce large amounts of UK-like and tPA-like PA activities (16). We have now immunologically identified these two species as UK and tPA, and have shown them both to be inhibited by glucocorticoids. Such inhibition is probably a direct effect of glucocorticoids on UK and tPA regulation rather than an effect secondary to growth inhibition, since treatment of cells with concentrations of doxorubicin (Adriamycin) sufficient to inhibit cell growth by 90% did not lead to inhibited PA production on a per cell basis (data not shown). Moreover, confluent G0 phase cells secrete UK and tPA at a rate similar to subconfluent,
Fig. 6. Immunological identification of the two OVCA 433 plasminogen activators as urokinase and tissue-type plasminogen activator. SDS-PAGE zymography was performed on concentrated serum-free conditioned media from OVCA 433 cells (lane A); a human UK standard (lane B); a human tPA standard (lane C); OVCA 433 conditioned medium immunoprecipitated with GNS (lane D), GxUK (lane E), or GxtPA (lane F); human UK standard immunoprecipitated with GNS (lane G), GxUK (lane H), or GxtPA (lane I); human tPA standard immunoprecipitated with GNS (lane J), GxUK (lane K), or GxtPA (lane L). Details of immunoprecipitation procedures and SDS-PAGE zymography are presented in “Materials and Methods.”

![Graph](image)

**Fig. 7.** Kinetics of inhibition of OVCA 433 urokinase and tissue-type plasminogen activator by dexamethasone. Serum-free conditioned media obtained during the last 1-h period of a 0-3-day treatment with 1 × 10^{-7} M Dex were analyzed for UK and tPA activities based on fibrin independence and dependence characteristics, respectively, as described previously (16) and in “Materials and Methods.” The addition of Dex was staggered so that the collection of conditioned media from the individual flasks and subsequent PA activity measurements were all performed at the same time. Data, means (± SE) of three determinations from three separate experiments, each of which was performed in triplicate. Left, absolute UK and tPA values; right, values normalized to percentage of control (day 0) values. Differences between percentage of UK and tPA values at days 1 and 2 were statistically significant (+) at p < 0.025 and p < 0.05, respectively.

rapidly dividing cells (data not shown). Thus, the inhibitory effects of glucocorticoids on UK and tPA regulation do not appear to be mechanistically secondary to glucocorticoid-induced growth inhibition.

We and others have previously demonstrated glucocorticoid inhibition of PAs in a variety of cell types (18, 19, 26–31). The production of both UK and tPA, and the glucocorticoid inhibition of both, makes the OVCA 433 cell line a good model system in which to study possible relationships between the regulation of these two PA types. In this regard, it is of interest that following Dex treatment, decreased UK activity preceded decreased tPA activity by 24 h, suggesting that while some mechanisms of glucocorticoid inhibition may be common to both UK and tPA, other mechanisms specific to tPA may also exist. Studies examining glucocorticoid effects on UK and tPA biosynthesis, mRNA levels, and gene transcription are currently underway in our laboratory.

Our results with the OVCA 433 cell line represent the first demonstration of glucocorticoid inhibition of ovarian carcinoma cell growth or cellular processes. We have confirmed these results in the SK-OV-3 human ovarian carcinoma cell line, which responds similarly to glucocorticoids with inhibited cell growth and PA activity (data not shown). Like OVCA 433 cells, SK-OV-3 cells contain high affinity, steroid-specific GR (data not shown). Although our studies with both OVCA 433 and SK-OV-3 cells suggest that glucocorticoid responsiveness may be a common characteristic of this cell type, an examination of additional established cell lines, primary cultures from patient tumors, and in vivo animal models will be required before this conclusion can be reached.

Little has been published regarding glucocorticoid responsiveness of ovarian carcinoma cells, despite the fact that GR are known to be present in a high percentage of ovarian carcinoma tumor biopsies (7) as well as normal ovarian surface epithelium (32), the tissue from which most ovarian carcinomas arise (33). In one study of four ovarian carcinoma cell lines (34), significant effects of cortisol on cell growth were found in only one line. In this case, cortisol treatment of EFO-47 cells led to a 2.8-fold increase in cell growth. This observation, which contrasts with our results, may reflect the fact that EFO-47 cells were derived from a poorly differentiated clear cell carcinoma (34), a relatively uncommon form of ovarian carcinoma (33). Other detailed studies, such as concentration-dependence experiments, full steroid specificity characteristics, and GR information, were not reported for EFO-47 cells (34). To our knowledge, other reports of glucocorticoid responsiveness of ovarian carcinoma cells have not been published.

While our data indicate that inhibition of OVCA 433 cell growth by glucocorticoids is not complete, such inhibition might still be of use clinically, particularly if it persisted following the almost universal development of resistance to more commonly used chemotherapeutic agents. Also, there is much evidence linking PA production by cancer cells to metastasis and invasion (35–38). These processes might be suppressed in ovarian carcinoma patients by treatment with glucocorticoids. It is clear that much work will be required for an assessment of the clinical utility of glucocorticoids in ovarian carcinoma. However, our findings with the OVCA 433 cell line should provide a reference point for further work in this area.

**ACKNOWLEDGMENTS**

We thank Donna Manzer and Mary Amthor for technical assistance and Rachel Bobst and Diana Dye for their help in preparing the manuscript.

---

**Fig. 7.** Kinetics of inhibition of OVCA 433 urokinase and tissue-type plasminogen activator by dexamethasone. Serum-free conditioned media obtained during the last 1-h period of a 0-3-day treatment with 1 × 10^{-7} M Dex were analyzed for UK and tPA activities based on fibrin independence and dependence characteristics, respectively, as described previously (16) and in “Materials and Methods.” The addition of Dex was staggered so that the collection of conditioned media from the individual flasks and subsequent PA activity measurements were all performed at the same time. Data, means (± SE) of three determinations from three separate experiments, each of which was performed in triplicate. Left, absolute UK and tPA values; right, values normalized to percentage of control (day 0) values. Differences between percentage of UK and tPA values at days 1 and 2 were statistically significant (+) at p < 0.025 and p < 0.05, respectively.

rapidly dividing cells (data not shown). Thus, the inhibitory effects of glucocorticoids on UK and tPA regulation do not appear to be mechanistically secondary to glucocorticoid-induced growth inhibition.

We and others have previously demonstrated glucocorticoid inhibition of PAs in a variety of cell types (18, 19, 26–31). The production of both UK and tPA, and the glucocorticoid inhibition of both, makes the OVCA 433 cell line a good model system in which to study possible relationships between the regulation of these two PA types. In this regard, it is of interest that following Dex treatment, decreased UK activity preceded decreased tPA activity by 24 h, suggesting that while some mechanisms of glucocorticoid inhibition may be common to both UK and tPA, other mechanisms specific to tPA may also exist. Studies examining glucocorticoid effects on UK and tPA biosynthesis, mRNA levels, and gene transcription are currently underway in our laboratory.

Our results with the OVCA 433 cell line represent the first demonstration of glucocorticoid inhibition of ovarian carcinoma cell growth or cellular processes. We have confirmed these results in the SK-OV-3 human ovarian carcinoma cell line, which responds similarly to glucocorticoids with inhibited cell growth and PA activity (data not shown). Like OVCA 433 cells, SK-OV-3 cells contain high affinity, steroid-specific GR (data not shown). Although our studies with both OVCA 433 and SK-OV-3 cells suggest that glucocorticoid responsiveness may be a common characteristic of this cell type, an examination of additional established cell lines, primary cultures from patient tumors, and in vivo animal models will be required before this conclusion can be reached.

Little has been published regarding glucocorticoid responsiveness of ovarian carcinoma cells, despite the fact that GR are known to be present in a high percentage of ovarian carcinoma tumor biopsies (7) as well as normal ovarian surface epithelium (32), the tissue from which most ovarian carcinomas arise (33). In one study of four ovarian carcinoma cell lines (34), significant effects of cortisol on cell growth were found in only one line. In this case, cortisol treatment of EFO-47 cells led to a 2.8-fold increase in cell growth. This observation, which contrasts with our results, may reflect the fact that EFO-47 cells were derived from a poorly differentiated clear cell carcinoma (34), a relatively uncommon form of ovarian carcinoma (33). Other detailed studies, such as concentration-dependence experiments, full steroid specificity characteristics, and GR information, were not reported for EFO-47 cells (34). To our knowledge, other reports of glucocorticoid responsiveness of ovarian carcinoma cells have not been published.

While our data indicate that inhibition of OVCA 433 cell growth by glucocorticoids is not complete, such inhibition might still be of use clinically, particularly if it persisted following the almost universal development of resistance to more commonly used chemotherapeutic agents. Also, there is much evidence linking PA production by cancer cells to metastasis and invasion (35–38). These processes might be suppressed in ovarian carcinoma patients by treatment with glucocorticoids. It is clear that much work will be required for an assessment of the clinical utility of glucocorticoids in ovarian carcinoma. However, our findings with the OVCA 433 cell line should provide a reference point for further work in this area.

**ACKNOWLEDGMENTS**

We thank Donna Manzer and Mary Amthor for technical assistance and Rachel Bobst and Diana Dye for their help in preparing the manuscript.
REFERENCES


Glucocorticoid Sensitivity of OVCA 433 Human Ovarian Carcinoma Cells: Inhibition of Plasminogen Activators, Cell Growth, and Morphological Alterations

Waheeda Amin, Beth Y. Karlan and Bruce A. Littlefield


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/47/22/6040

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/47/22/6040.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.