Characterization of a Human Ovarian Carcinoma Cell Line (NIH:OVCAR-3)\(^1\) with Androgen and Estrogen Receptors


ABSTRACT

A cell line, NIH:OVCAR-3, has been established from the malignant ascites of a patient with progressive adenocarcinoma of the ovary after combination chemotherapy with cyclophosphamide, Adriamycin, and cisplatin. OVCAR-3 grows as a cobblestone-like monolayer with foci of multilayering, is tumorigenic in athymic mice, clones in agarose, and has an abnormal karyotype which includes a homogeneous staining region and a double minute chromosome. The cultured cells and xenografts contain cytoplasmic androgen- and estrogen-binding macromolecules with the specificity of the respective steroid hormone receptors. These components have sedimentation coefficients of 7 to 9S in low-salt sucrose-density gradients, have dissociation constants of 250 and 9.6 pm, and are present at concentrations of 30 and 28 fmol/mg cytosol protein characteristic of androgen and estrogen receptors, respectively. OVCAR-3 is resistant \textit{in vitro} to clinically relevant concentrations of Adriamycin (5 \times 10^{-8} M), melphalan (5 \times 10^{-6} M), and cisplatin (5 \times 10^{-7} M) with survival compared to untreated controls of 43, 45, and 77%, respectively. Furthermore, there are multiple histological similarities between the patient's original tumor, the cell line, and the transplantable tumor.

These data indicate that OVCAR-3 may be of use for investigations as to the significance of androgens and estrogens and the mechanisms of cytotoxic drug resistance in ovarian cancer.

INTRODUCTION

The search for new therapeutic modalities in ovarian cancer, including ways to increase the effectiveness of chemotherapy, and investigations into the possible role of hormones in the biology and treatment of ovarian cancer have been limited, in part, by the lack of suitable experimental models of the human disease. Relevant experimental systems are needed in which the major clinical problem of rapid emergence of primary drug resistance and associated cross-resistance can be directly investigated. In addition, although there has been an increased interest in the potential relevance of hormones in the disease\(^1\) due to the frequent identification of steroid hormone receptors in human ovarian cancer specimens\(^2\),\(^3\),\(^4\),\(^5\),\(^6\),\(^7\),\(^8\) and to continued reports of variable successes of hormonal therapy\(^2\),\(^3\),\(^4\),\(^5\),\(^6\),\(^7\),\(^8\), experimental confirmation of hormonal influence on the growth of ovarian cancer cells likewise awaits the development of appropriate model systems.

We have studied previously a murine model of ovarian cancer\(^9\) as well as the direct cloning of human ovarian cancer cells in a double-layer agar system\(^6\),\(^7\). Pharmacological studies in these systems have provided some support for new therapeutic approaches in ovarian cancer, such as i.p. chemotherapy\(^8\). However, neither of these systems was satisfactory for biochemical investigation of drug resistance and hormonal effects on ovarian cancer cells. The murine model was derived from a spontaneous teratoma, and, although the pattern of metastases is similar to that of human ovarian cancer, biological differences between murine teratoma cells and human epithelial cancer cells limit the clinical relevance of therapeutic studies in this experimental system. Although the direct cloning of human ovarian cancer cells has provided clinically relevant information about the patterns of drug resistance, the small size of tumor colonies (usually 30 to 50 cells) does not allow for direct biochemical investigations on the mechanisms of drug resistance. Accordingly, we have turned to human ovarian cancer cell lines as models with which to study endocrine relationships in ovarian cancer and the pharmacology of antineoplastic drugs.

Ovarian cancer cell lines can potentially overcome the experimental limitations inherent in both the murine model of ovarian cancer and the direct agar cloning of human ovarian cancer specimens. Cell lines may allow for the direct measurement of hormone effects on malignant cell proliferation and provide \textit{in vitro} correlation between the presence of steroid hormone receptors and the specific hormone response. In addition, cell lines should be useful for the investigation of clinically relevant biological modifiers, such as Müllerian inhibition substance\(^7\) and monoclonal antibodies\(^8\). The availability of adequate quantities of proliferating malignant cells would also facilitate the identification of potentially clinically useful dose-response relationships with antineoplastic drugs as well as a model system in which to compare the cytotoxicity of drug analogues.

One of the more clinically relevant studies with human cell lines relates to the mechanisms of drug resistance and cross-resistance. Ovarian cancer is clinically noted for the rapid development of primary drug resistance and a broad cross-resistance\(^8\). It would be of interest to determine if the mechanisms of cross-resistance in human ovarian cancer are similar to those reported in animal tumor cell lines, particularly with regard to the induction of certain membrane glycoproteins\(^8\). Finally, resistant cell lines may be useful to determine the mechanism of resistance to alkylating agents, anthracyclines, and cisplatin and to investigate ways in which this primary resistance can be modulated.

Although there have been several human ovarian cancer cell lines described in the literature\(^6\),\(^10\),\(^27\),\(^57\), many of these have not been fully characterized. In particular, there have been no previous reports on the presence of steroid hormone receptors in any ovarian cancer cell line, nor have there been any data on the \textit{in vitro} sensitivity of these cell lines to the standard...
chemotherapeutic agents used in the treatment of ovarian cancer. We have established a new human ovarian cancer cell line which contains cytoplasmic androgen and estrogen receptor-like macromolecules. This line was derived from the malignant ascites of a patient with progressive papillary adenocarcinoma of the ovary after combination chemotherapy. In addition to the steroid receptor status, this line has been characterized with regard to morphology, ultrastructure, karyotype, growth characteristics in vitro (including colony formation in soft agarose), tumorigenicity in athymic nude mice, and in vitro sensitivity to the chemotherapeutic drugs to which the patient was clinically resistant. Furthermore, histological and ultrastructural comparisons are made between the cell line, the nude mouse tumor, and the original specimen.

MATERIALS AND METHODS

Chemicals and Reagents

[2,4,6,7-3H]Estradiol (90 to 115 Ci/mmol), 17α-hydroxy-17α-methyltestra-4,9,11-trien-3-one (methyltestosterone, R 1881), and [17α-methyl-3H]Jr 1881 (70 to 87 Ci/mmol) were purchased from New England Nuclear, Boston, Mass. Unlabeled 5α-dihydrotestosterone, diethylstilbestrol, progesterone, and 8α-fluoro-11β,21-dihydroxy-16α,17α-isopropylidene-diol-1,4-pregnan-3,20-dione (triamcinolone acetonide) were acquired from Sigma Chemical Co., St. Louis, Mo. 1-[4-Dimethylaminoethylphosphon]-1,2-diphenylbut-1-ene (tamoxifen) was given by Stuart Pharmaceuticals (Division of ICI Americas, Inc., Wilmington, Del.). Melphalan, Adriamycin, and cisplatin were obtained from the Pharmaceutical Resources Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md.

RPMI 1640, fetal bovine serum, penicillin-streptomycin stock solution (10,000 units/ml; 10,000 μg/ml), and PBS were from Grand Island Biological Co., Grand Island, N. Y. EG and insulin were from Collaborative Research, Inc., Wattham, Mass. Agaro (type VII) was from Sigma, and lymphocyte separation medium was from Bionetics Laboratory Products, Kensington, Md. All other chemicals and reagents were of the highest purity commercially available.

Tissue Culture Medium

Medium was prepared volumetrically from RPMI 1640 powder using double-glass-distilled water. Unless otherwise stated, the formulation used for growth and passage of cells contained RPMI 1640 at unit concentration, heat-inactivated fetal bovine serum (2%, v/v), sodium bicarbonate (24 mM), insulin (10 μg/ml), penicillin (100 units/ml), and streptomycin (100 μg/ml).

Initiation and Propagation of the Cell Line

Malignant ascites from a patient with poorly differentiated papillary adenocarcinoma of the ovary (Fig. 1) collected in heparin (1.0,000 units/ml) was changed at intervals of 3 to 4 days. In primary culture, 2 morphologically distinct cell types were observed by phase-contrast microscopy. The minor component consisted of elongated fibroblastoid cells which were readily removed by selective treatment with a PBS solution which contained trypsin (0.05%, w/v) and EDTA (0.02%, w/v) (33). Subculture of the remaining epithelioid cells, at split ratios of 1:2 or 1:3, was accomplished with trypsin:EDTA (as above); the suspension of detached cells was diluted with an equal volume of growth medium and centrifuged at 300 x g for 10 min at 4°C. After centrifugation, the cell pellet was suspended in growth medium and added to tissue culture flasks. Subculture was at 10- to 14-day intervals.

Tests for Mycoplasma

OVCAR-3 was tested for Mycoplasma by culture on Mycoplasma agar, Hoechst stain, and fluorescent antibody stain for Mycoplasma hyorhinis. Results were negative in all tests.

Morphology

Cells growing in tissue culture T-flasks were examined by phase-contrast microscopy. Alternatively, cells were grown on coverslips, fixed, stained in situ, and evaluated by standard light microscopy.

Ultrastructural analysis was accomplished by transmission electron microscopy on cells fixed [2.5% (v/v) glutaraldehyde in 0.1 M phosphate buffer, pH 7.4,] postfixed (osmium tetroxide), and embedded while attached to the growth surface of the tissue culture flask. Tumor tissue, 3-mm cubes, obtained from nude mice with the heterotransplanted cells (see below), was fixed and embedded in the above combination of materials prior to examination by electron microscopy.

Cytogenetic Analysis

After subculture, cells were grown for 48 to 72 hr, at which time metaphase arrest was initiated by the addition of colchicine (0.2 μg/ml), and the incubation continued for 1 hr. Chromosome preparations were made according to the air drying technique of Tjio and Whang (52). Both conventional Giemsa stain and trypsin:Giemsa banding (46) were performed. Findings are described with nomenclature established at the Paris Conference, 1971 (38).

Effect of Antineoplastic Drugs

Cells were subcultured into 25-cm2 tissue culture flasks and, 1 day postplating, were changed to media which contained carrier, 0.9% NaCl solution (control), Adriamycin (5 x 10-6 M), melphalan (5 x 10-6 M), or cisplatin (5 x 10-7 M). After 4 days of drug treatment, cells were harvested with trypsin:EDTA and counted in a hemocytometer.

% of survival = Cells remaining after drug exposure / Cells in control flask x 100

Tumorigenicity and Passage in Vivo

OVCAR-3 cells to be tested were harvested with trypsin:EDTA, and the pellet of cells obtained by centrifugation was suspended in growth medium (1.3 x 106 cells/ml). Amounts (0.1 ml) were injected s.c. into the bilateral subscapular areas of nude athymic BALB/c mice (42). Tumors which formed were heterotransplanted to subsequent hosts by s.c. surgical implantation of small 3-mm cubes of solid tumor.

Initiation of the OVCAR-3-™ Subline

Solid tumors initiated from injection of OVCAR-3 cells into nude mice were heterotransplanted once, and then the subsequent tumor was excised when it measured approximately 1 cm in its maximum plane.
The tumor was finely minced in a minimum volume of growth medium and added to a culture flask which contained 30 ml of growth medium and collagenase (250 units/ml of medium). After 24-hr incubation under culture conditions, the digest was repeatedly transferred by pipet to aid in further disaggregation of the tissue. A cell pellet obtained from the digest by centrifugation was resuspended in PBS. The tumor cells in this suspension were separated from blood cells and host stroma by a stepwise fractionation procedure which utilized lymphocyte separation medium (54). The tumor cell fraction was cultured and passed as described for the parental OVCAR-3 cell line.

Anchorage-independent Growth

The capacity for anchorage-independent growth of OVCAR-3 and OVCAR-3™ was assessed in medium modified to contain agarose (0.3%, w/v) and EGF (10 ng/ml) (15, 39). A single-cell suspension of trypsin:EDTA-harvested cells was suspended in the above medium (100,000 cells/ml), and 1-ml amounts were plated over a layer (1 ml) of 0.6% (w/v) agarose in growth medium in 35-mm tissue culture dishes. After solidification of the cell-containing upper layer, plates were first examined to ascertain that only single cells were present and then incubated at 37° in a humidified atmosphere of 5% CO₂ in air. At 5- to 7-day intervals, 0.8 ml of growth medium with EGF (10 ng/ml) was layered onto the agarose surface. After 21 days of incubation, cultures were scored for colonies, which were defined as aggregates containing more than 30 cells.

Assessment of Steroid Hormone Receptor Status

Buffer. The buffer used in the following procedures consisted of Tris-HCl (10 mM), pH 7.4, containing EDTA (1 mM), dithiothreitol (1 mM), phenylmethylsulfonyl fluoride (1 mM), and sodium molybdate (10 mM).

Protein Estimation. Cytosol protein was measured by a modification (21) of the method of Lowry et al. (31) with bovine serum albumin as standard.

Preparation of Cytosol. OVCAR-3 cells grown to confluence were switched to and maintained for 6 to 8 days in a medium prepared with charcoal-treated fetal bovine serum (49), after which time, the cells were harvested with trypsin:EDTA, and the pellet obtained by centrifugation was washed once with the above medium. Cytosol was then prepared from the cell pellet as described previously (18) in detail. In brief, the cell pellet was suspended in buffer and homogenized with a motor-driven glass-Teflon homogenizer. The homogenate was then centrifuged at 100,000 x g for 1 hr at 3°. The supernatant was the tumor cell cytosol.

Sedimentation Analysis. Cytosol was labeled with [³H]estradiol or [³H]R 1881 in the absence or presence of various potential competitors for 3 to 5 hr at 4° and then treated with charcoal (18) prior to analysis of 200-µl amounts on 4-ml linear (5 to 20%, w/v) gradients of sucrose ([3H]R 1881 in the absence or presence of various potential competitors were analyzed according to the method of Scatchard (43).

Saturation Analysis. Androgen binding was assessed in cytosol from OVCAR-3-induced mouse tumors with [³H]R 1881 as labeled ligand and 5α-dihydrotestosterone as competitor at a 100-fold molar excess. Estrogen binding was studied with [³H]estradiol and diethylstilbestrol. The assay protocols were as was described previously for assessment of estrogen binding using charcoal to separate free from bound steroid (17). The data for specific binding (difference between binding in the presence and absence of unlabeled competitor) were analyzed according to the method of Scatchard (43).

Measurement of Radioactivity. Samples containing tritium were mixed with 10 ml of scintillation fluid (Aquassure; New England Nuclear) and counted at an efficiency of approximately 40%.

RESULTS

Morphology and Growth Characteristics. OVCAR-3 cultures consisted of cells with well-defined, closely approximating margins which grew as a monolayer giving the typical cobblestone-like appearance ascribed to epithelium in vitro (Fig. 2). OVCAR-3 has been in continuous culture for >10 months and has been passed 20 times. The cells have a doubling time of 48 hr. Cells have been cryopreserved using a programmed freezer, and cultures were subsequently established from the frozen cells. Additionally, it is noted that OVCAR-3 cultures left at confluence for extended periods develop foci of "piled-up" cells. These foci seem morphologically more complex than simple multilayering; observation of carefully handled cultures revealed fragile 3-dimensional papillary-like structures. Closer examination of this feature was possible in culture material prepared for electron microscopy. One-µm plastic-embedded vertical sections of the culture examined on the light microscope showed cells in single layers continuous with the grape-like clusters of cells (Fig. 4).

High-magnification light microscopy revealed the rounded free surfaces of the cells to have microvilli. The cell cytoplasm was often vacuolated, and round intercellular spaces were seen.

Stained coverslip cultures permitted more detailed examination of OVCAR-3. Papnicolaou-stained cells (Fig. 3) appeared variable in both size and shape and contained large strikingly pleomorphic nuclei with prominent multiple nucleoli. These nuclear abnormalities contributed to an evident alteration in the nuclear-cytoplasmic ratio for the cells. The cells were also studied with regard to their staining characteristics with periodic acid-Schiff pre- and post-diastase treatment and were mucin positive. This was confirmed in mucicarmine-stained coverslip cultures. The OVCAR-3™ subline was morphologically indistinguishable from the parental OVCAR-3 line.

Electron microscopic examination of the culture material confirmed and expanded on the light microscopic observations (Figs. 5 and 6). Ultrastructural examination confirmed the presence of microvilli which were sometimes branched. Cell-cell attachments were abundant, but true individual desmosomes were uncommon. Junctional complexes demarcated intercellular spaces into which long microvilli often protruded, and rare intracellular spaces were seen. Distinct from intracellular spaces, there were intracellular vacuoles which were membrane bound and contained no discernible contents. The cytoplasm also contained irregular electron-dense bodies consistent with lysosomes. Small elongated mitochondria with shelf-like cristae were evident in the cells as were abundant polyribosomes. The Golgi complex was not prominent, nor were rough or smooth endoplasmic reticula abundant. The nuclei were seen to contain only limited heterochromatin but did contain abundant euchromatin and one to 2 nucleoli.

Tumorigenicity of OVCAR-3. The cultured cells injected s.c. into immunodeficient heterologous hosts formed expansile masses in the dermis and subcutis which grew progressively to >1 cm within 10 to 12 weeks in all animals. At this stage, the tumor was excised, prepared for examination by light or electron microscopy, or implanted into subsequent hosts. Light microscopy showed that the malignant cells formed glands and papillary structures (Fig. 7) but did not invade the skeletal muscle beneath the subcutis. Mitotic figures were common in the tumor. Numerous vacuoles were seen within tumor cells, and these contained mucicarmine-positive material. Grimelius stain for argyrophil (en-
terochromaffin) granules was negative. This pattern of staining was the same as in the original solid tumor of the patient. There was only limited stromal involvement with no malignant-appearing connective tissue cells.

Electron microscopy of the tumor growing in nude mice showed that carcinoma cells had an extensive array of branching microvilli, numerous junctional complexes, and relatively straight borders with peg-like interdigitations (Fig. 8). Although OVCAR-3 cells as a tumor in vivo contained a full complement of organelles consistent with their cultured progenitors, differences in degree of organelle development suggestive of more active cells were apparent. In contrast to the cultured cells, the Golgi complex was prominent, and the rough endoplasmic reticulum was more highly developed. Polyribosomes were abundant also. Patches of glycogen were seen in some of the tumor cells, and areas of the tumor had cells with large vacuoles, which in some sections were seen to be membrane bound, often empty, but rarely contained reticular, partially eluted material consistent with mucin. Scattered among the predominant tumor cells were dark cells and rare multinucleated cells. At the interface of the tumor and mouse tissue, tumor cells formed a well-developed external lamina, and the cells resting on this external lamina often had small, pleomorphic dense granules consistent with lysosomes (Fig. 9).

Anchorage-independent Growth. The cloning efficiency of OVCAR-3 cells was 0.01% when suspended in semisolid agarose. OVCAR-3 also demonstrated the capacity for substrate-independent growth but with a 10-fold-greater cloning efficiency (0.1%), compared to the parent cell line. The colonies which formed contained numerous balloon-like cells similar to the appearance of ovarian carcinoma cells after direct cloning of malignant ascites cells in the double-layer agar system (35).

Cytogenetic Analysis. The conventional Giemsa-stained metaphases examined revealed 20% of the cells to have chromosome numbers near the 16-ploid region with extensive fragmentation present in 10% of the metaphases. Fifty banded metaphases were analyzed. In this group, the number of chromosomes in the majority of cells clustered around 60 to 70 chromosomes, but a range of 54 to 160 chromosomes per cell was found. The representative karyotype consistent with that of a human female is shown in Fig. 10. Nearly every pair of chromosomes had some abnormalities, either numerical or structural, with origins of some of them not identifiable. All contained one or 2 small acrocentric chromosomes, and 5% of preparations also had an additional small acentric fragment which resembled a single DM. The common chromosome markers were 1p+, 1p+q31−, 7p+, 11p+, 12q+, 19q+, 20q+, 20p12−q12− (small acrocentric), and one small acrocentric with an unknown origin. The additional material on 19q is consistent with a HSR.

Response to Antineoplastic Drugs. Table 1 summarizes the effects of Adriamycin, melphalan, and cisplatin upon the survival and proliferation of OVCAR-3 cells. The cells were most resistant to melphalan, with a 45% survival following continuous exposure to the drug at a concentration 3 times greater than the peak achievable plasma level. At concentrations of cisplatin and Adriamycin approximately one-tenth the peak-achievable plasma levels, i.e., chosen to reflect clinically relevant drug doses (36), treated cultures were 77 and 43% of untreated controls, respectively. These results of in vitro resistance are in marked contrast to the in vivo sensitivities for these same drugs in ovarian cancer cell lines 1847 and 2780, which were established from patients previously untreated (8). At the drug concentrations shown in Table 1, there was complete inhibition of colony formation in cell line 2780. Likewise, for cell line 1847, these concentrations of Adriamycin, melphalan, and cisplatin resulted in 15, <2, and 60% survival, respectively.

Steroid Hormone Receptor Status. Cytosol prepared from OVCAR-3 cells when examined by sedimentation analysis was found to contain a component which bound synthetic (R 1881) and natural androgens (5α-dihydrotestosterone) and with a sedimentation coefficient of 7 to 9S (Chart 1A). A separate and distinct moiety with similar sedimentation characteristics but with specificity for estrogenic compounds was present, also, in the cytosol of OVCAR-3 (Chart 1B). Under these conditions, apparent androgen receptor-like binding was greater than estrogen binding site levels based on displaceable tritiated steroid binding in the 8S region of gradients. Cytosol prepared from OVCAR-3 had the same pattern of binding (Chart 2, A and B) as did the parental line, OVCAR-3.

The ability to grow OVCAR-3 in nude mice simplified accumulation of sufficient cell mass for more extensive characterization of these steroid hormone receptor-like macromolecules and had the advantage of a more physiological growth environment for the cells than that obtainable in the tissue culture flask. The specificities of the androgen and estrogen binding entities observed by sucrose density gradient analysis were more completely investigated in tumor cytosol. Chart 3A shows that the 7 to 9S [3H]R 1881 binding species only bound androgenic compounds with the exception of partial competition by progesterone at a 100-fold molar excess. The component which bound [3H] estradiol was seen to bind nonsteroidal estrogenic and antiestrogenic molecules but showed no ability to bind the synthetic androgen receptor ligand, R 1881, or triamcinolone acetonide (Chart 3B). When the specific binding data generated by saturation analysis were analyzed by the method of Scatchard (43), apparent and distinct single classes of binding sites for androgen and estrogen were present with dissociation constants of 250 and 9.6 pM, respectively. Saturation analysis, also, demonstrated that the levels of androgen and estrogen receptor-like binding in tumor cytosol (30 and 28 fmol/mg cytosol protein, respectively) were consistent with our previous results (17) on fresh human ovarian tumors [range (fmol/mg cytosol protein): androgen, 4 to 88; estrogen, 15 to 136].

DISCUSSION

The morphological, ultrastructural, and biochemical features of OVCAR-3 and the corresponding human tumor grown in the nude mouse are consistent with a derivation from a malignant common epithelial tumor of the human ovary. The demonstrations that OVCAR-3 cells both in culture and as tumors in nude mice possess steroid hormone-binding macromolecules provide

Table 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>m dose (µg/ml)</th>
<th>% of control</th>
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<tbody>
<tr>
<td>Adriamycin</td>
<td>5 x 10^-7 (0.03)</td>
<td>43</td>
</tr>
<tr>
<td>Melphalan</td>
<td>5 x 10^-7 (1.1)</td>
<td>45</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>5 x 10^-7 (0.15)</td>
<td>77</td>
</tr>
</tbody>
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* Numbers in parentheses, µg/ml.

* T. C. Hamilton and R. F. Ozols, manuscript in preparation.
Cytosol protein (6.2 mg/ml). After centrifugation, gradients were fractionated bottom to top (shown left to right). Indicators of comparative sedimentation are bovine catalase (a, $s_{20w} = 11.3S$), human immunoglobulin G (b, $s_{20w} = 6.9S$), and bovine serum albumin (c, $s_{20w} = 4.6S$).

Morphologically, cultured cells were epithelial and malignant in nature and continued to show the focal presence of mucin. Ultrastructurally, the presence of microvilli, occasional desmosome-like junctions, and abundant tight junctions confirmed the epithelial and supported the ovarian character of the cells (9, 27). The intercellular and rare intracellular spaces described were consistent with the gland-forming propensity of adenocarcinoma cells (14), and the striking histological similarities between the tumor induced in athymic mice and the original solid tumor of the patient were noteworthy. The patient’s tumor, although classified as poorly differentiated and often exhibiting as solid sheets of malignant cells, did show the occasional tendency to form glandular and papillary structures (Fig. 1) as did the tumor when grown in nude mice (Fig. 7). The presence of mucin in the tumor, in culture, and in the nude mouse tumor provides further support for the biological similarities between the original tumor and the experimental models.

Epidemiological evidence has long implicated hormones in the etiology of ovarian cancer (16); an example is the increased incidence of the disease at or near the climacteric. We (17, 19) and others (2, 11, 12, 22, 24, 28, 40, 45) have reported recently the existence of steroid hormone receptors in fresh epithelial ovarian tumors. In addition, our previous studies have shown that the surface germinal epithelium of the rat ovary has at least 2 classes of steroid hormone receptors, estrogen and glucocorticoid (18, 19). If this pattern is maintained across species bounds as may be expected, then the surface epithelium of the human ovary, the accepted normal progenitor of common epithelial tumors, would have such receptors. The clinical significance of these potential control systems is unknown in both the normal and malignant cells. The presence of steroid receptors in ovarian tumors, however, has been used as the rationale for hormonal therapy in the disease (44). In the present study, cell culture and in vivo growth were used as sources of material to demonstrate that binding site concentrations, sedimentation characteristics, specificity, and dissociation constants of OVCAR-3 cytosol were characteristic of androgen and estrogen receptors in classical target tissues (5, 25, 47). Unfortunately, the receptor status of the patient’s primary tumor was not established at the time of diagnosis. We have shown, also, that the peaks of radioactivity seen on sedimentation analysis of $^3$H-steroid-labeled cytosol are shifted from the BS region of low-salt gradients to the 4 to SS region in high-salt (0.6 M KCl) sucrose density gradients and that the material which binds $^3$H-estradiol interacts with a monoclonal antibody to the estrogen receptor as manifested by a change in sedimentation profile of $^3$H-estradiol-labeled cytosol in the presence of the antibody (20).
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The characteristics of OVCAR-3 in culture and as xenografts make these systems suitable for investigations into the biology of ovarian carcinoma both in vivo and in vitro. Studies on the effects of androgens, antiandrogens, estrogens, and antiestrogens on cell growth of OVCAR-3 in vitro and tumor growth in vivo are in progress. The results of these studies may provide further insight into the molecular basis for endocrine therapy in ovarian cancer. The effects of steroids on macromolecular synthesis are also under investigation. Of particular interest are studies on the induction of progesterone receptors with estrogen as occurs in breast cancer (26) and has been speculated to occur in ovarian cancer (22). These model systems should also prove useful in the study of other factors of regulatory significance. Indeed, the shift in pattern of binding site concentration for androgen and estrogen receptors between the cultured cells and the mouse tumor indicates that these systems are subject to regulation by as yet unidentified factors. The impact of the dynamic milieu in vivo on organelle development in OVCAR-3 provides further evidence for the regulation of ovarian tumor cell function by unknown physiological agents.

Abnormalities of chromosome 1 are the most common cytogenetic abnormalities in ovarian cancer; they are present in over 80% of reported cases (10, 29, 30, 51, 53, 55, 57) and are common (30 of 44 patients) in our own studies performed on fresh tissue (56) as well as in the present study on OVCAR-3. Unfortunately, such changes are not unique and thus cannot be considered markers in the classical sense. It was noted, also, that about 50% of the cells examined were missing chromosome 6; in cases where the chromosome was present, however, no abnormalities were evident. Of special interest were the observations of a HSR and one DM in the karyotype of OVCAR-3. Trent and Salmon (53) noted either HSRs or DMs in 20% of their agar cultures of ovarian cancer specimens, and Woods et al. (57) found such changes in their ovarian cancer cell lines. The relevance of such changes in human cancer has yet to be delineated, but HSRs and DMs have been postulated to have a role in the mechanism of drug resistance (3, 4). It is possible that these karyotypic changes in OVCAR-3 relate to mutations favorable to cancer cell survival in the face of extensive but unsuccessful chemotherapy in the patient from whom this line originated. Studies in progress should clarify whether the pattern of resistance on OVCAR-3 is related to the cytotoxic drugs to which the patient was exposed. If this is the case, the mechanisms of drug resistance in OVCAR-3 are likely to be analogous to those which develop in vivo in the course of treatment of human ovarian cancer. The preliminary results of in vitro drug sensitivity for melphalan, Adriamycin, and cisplatin (Table 1) suggest that OVCAR-3 is resistant to these drugs at clinically relevant drug levels. The exact magnitude of the resistance awaits comparative cytotoxicity studies with human ovarian cancer cell lines established from untreated patients and cell lines made resistant by exposure to drugs in vitro. It is also possible that drug resistance in OVCAR-3 may be transitory for some of the drugs, since it has been reported previously that both drug resistance and DMs can disappear when cells are maintained in the absence of drug (4). Since OVCAR-3 does appear to be resistant to those drugs with which the patient was treated, this cell line should be a useful model in which to study the role of membrane glycoproteins in development of resistance and cross-resistance (41) and potential modulators of drug resistance, such as verapamil (48) and glutathione (50). Knowledge gained from such studies in OVCAR-3 and other appropriate model systems will hopefully lead to a better understanding of ovarian cancer and ultimately to new treatment approaches for this disease in which current therapeutic modalities are not successful for the majority of patients.

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REFERENCES


Fig. 1. Surgical specimen of ovarian carcinoma. Malignant cells invade the ovarian stroma and form glands. H & E, × 160.

Fig. 2. OVCAR-3 cells as a confluent monolayer. Phase contrast, × 118.

Fig. 3. Coverslip culture of OVCAR-3 prepared for light microscopy. Papanicolaou, × 400.
Fig. 4. One-μm plastic section shows that malignant cells in vitro form clusters of varying sizes. The lower edge of the section corresponds to the bottom of the culture flask. Methylene blue, Azur II, × 1,080.

Fig. 5. Low-magnification electron micrograph shows microvilli at the free cell surface. × 4,400.

Fig. 6. Cell cluster in vitro. A mitotic figure is present at the upper center. Intercellular spaces are wide and are bounded by junctional complexes (arrows). × 9,800.
Fig. 7. Malignant cells after injection into a nude, athymic mouse form a tumor which invades the subcutis and encroaches on underlying skeletal muscle. H & E, × 160. Inset, papillary clusters of malignant cells. H & E, × 320.

Fig. 8. Tumor cells in the nude mouse show well-developed Golgi complex (G) seen in one cell above and below the nucleus and also in an adjacent cell. A junctional complex (c) is present. × 10,500.
Fig. 9. Cells at the invading edge of the tumor in the nude mouse form a well-developed external lamina (arrow). Lysosome-like granules are present in these cells, $\times 9,240$. Two membrane-bound coalescing vacuoles (v) are shown. A solitary vacuole with flocculent material (m) is also present in this vacuolated cell, $\times 8,400$.

Fig. 10. Representative karyotype of OVCAR-3. Arrows indicate sites of additions or deletions on involved individual chromosomes.
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