Flow Cytometric and Morphological Studies of Ovarian Carcinoma Cell Lines and Xenografts

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ABSTRACT

Human ovarian cancers of four different histological types have been cultured in vitro and in nude mice. Nineteen tumor specimens (11 solid tumors and eight malignant effusions) were obtained from 14 patients. Tumor lines from ten of these patients were established after several subpassages, and six xenograft lines have been grown, all of them from tumors of which a cell line exists in vitro. In all, 14 lines have been successfully cultured from the 19 tumor specimens. The morphology (studied by light and electron microscopy) of the established lines in vitro and in vivo resembles that of the original tumors in all cases.

Flow cytometric studies of DNA content of the original tumor specimens and the cell culture and xenograft lines were performed. In all lines in both culture systems, aneuploid cells became predominant after the first to fourth passages, despite an aneuploid peak having been evident in only nine of the 19 initial specimens.

Four of the original tumor specimens contained measurable estrogen receptors and five progesterone receptors, but none of the established cell lines expressed these hormone receptors.

These results indicate that, while morphological features are similar in the initial tumor specimens and in the established lines in vitro and in vivo, flow cytometric and steroid hormone receptor data suggest selection of aneuploid and receptor-negative cells.

INTRODUCTION

In recent years, the ability to culture and clone human cancer cells has allowed the identification of their requirements for nutrients and growth factors. Furthermore, the effects of cytotoxic chemotherapy on human cancer cells cultured in vitro or in vivo in immune-deprived animals can be compared with those seen in the patient (4, 7). Such studies have frequently revealed heterogeneous collections of tumor cells differing in their sensitivity to cytotoxic drugs and in their nutritional requirements for growth in vitro.

Few established xenografted or tissue culture lines of human ovarian cancer cells have been described (1, 3, 8, 10, 12, 16, 17). The original objective of this work was to develop a model in vitro and in vivo to study the heterogeneity of ovarian cancer cells with respect to their morphology, cytogenetics and cellular DNA content, and their sensitivity to chemical treatment. Sequential studies comparing cell lines established from tumors prior to treatment with those after relapse or from different metastatic sites may identify prognostically important features which will be useful in planning treatment. We now report the establishment of long-term cell cultures and xenografts derived from explants of ovarian solid tumors and malignant effusions. Morphological and flow cytometric studies have been undertaken to follow the characteristics of cells during their establishment in culture.

MATERIALS AND METHODS

Cell Culture. Tumor samples obtained at surgery or paracentesis of malignant effusions were processed within 2 hr. Solid tissues were suspended in Roswell Park Memorial Institute Tissue Culture Medium 1640 containing arginine (0.34 g/liter), asparagine (0.63 g/liter), and folic acid (0.04 g/liter) (Flow Laboratories, North Ryde, N. S. W., Australia) supplemented with 20 mM (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer, pH 7.4, 14 mM sodium bicarbonate, 6 mM l-glutamine, penicillin (100 units/ml), streptomycin (100 μg/ml), gentamicin (20 μg/ml), and 17% fetal calf serum (Flow Laboratories). The tissues were minced finely under sterile conditions in 50-mm Petri dishes, using forceps and a scalpel blade. Minced tissue and supernatant were aspirated with a 5-ml pipet and filtered through sterile gauze swabs into a universal container and washed in phosphate-buffered saline: Dulbecco's modified formula (Flow Laboratories). The cells were subsequently filtered through a sterile metal sieve with a pore size of 0.3 mm. Approximately 10⁶ cells of the filtered cell suspension were then plated in a disposable 25-sq cm tissue culture flask, 3 ml of culture medium were added, and the cells were incubated at 37°C. After 24 hr, the supernatant with nonattached cells was aspirated, centrifuged (4 min at 200 × g), and transferred to another 25-sq cm flask. Using this approach, mesothelial cells and fibroblasts were selectively eliminated (5) along with some adherent tumor cells. The reseeded cell population which was enriched with malignant cells was left undisturbed for 5 days and then fed twice weekly with fresh medium. Cells were examined using an Olympus JMT inverted microscope, and when confluent, they were harvested by incubation with 0.05% trypsin:0.02% EDTA (Flow Laboratories) for 10 min at 37°C and then subcultured after 1:2 dilution.

Malignant effusions (both pleural and ascitic) were collected in sterile catheter bags, and aliquots were transferred to 250-ml polypropylene centrifuge tubes (Coming Glass Company, Coming, N. Y.) containing 10 units of preservative-free heparin per ml of effusion. Following centrifugation at 200 × g for 10 min, the cell pellet was washed once in phosphate-buffered saline and once in medium. The cells were then plated in 25-sq cm flasks. If necessary, contaminating RBC were removed by a Ficoll-Hypaque gradient (Ficoll-Paque; Pharmacia, Uppsala, Sweden). Interface cells were collected, and approximately 10⁶ cells/ml were then plated in 25-sq cm flasks as described previously.

Cells from the initial tumor samples and from subsequent cell passages were prepared for morphological studies by short-term cell culture in Lab-Tek Tissue Culture Chamberslides No. 4801 (Miles Laboratories, Inc., Naperville, Ill.) in a 5% CO₂ incubator at 37°C.

Ovarian Tumor Xenografts. Female BALB/c nude mice (4 to 6 weeks old) were obtained from the Australian Atomic Energy Commission, Lucas Heights, N. S. W., and housed in laminar flow racks. Tumor tissue received directly from the operating rooms was sliced finely, in medium (described above), in a 50-mm Petri dish to yield pieces approximately 2 cm mm. These fragments were inoculated s.c., using fine forceps, through...
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a small incision in the scapular region of the mouse. Bilateral implantations were performed in most instances, and ether anesthesia was used routinely. Tumor nodules were subpassaged after growth to a diameter of 6 to 9 mm. Cells from malignant ascites or pleural effusions, cultured for at least 1 day (and in some cases for several weeks), were harvested with trypsin:EDTA, washed with medium, and collected by centrifugation. Cells (10⁶) in 0.2-ml culture medium were inoculated s.c. into the scapular region of female nude mice, using an 18-gauge needle.

Light and Electron Microscopy. Short-term cell cultures on tissue culture chamberslides were fixed, when confluent, in standard formalin:acetic acid:alcohol. After staining with hematoxylin and eosin, the cultures were studied by light microscopy. Specimens for light microscopy from the original tumors and xenograft passages were fixed in formalin:acetic acid:alcohol and embedded in paraffin. After processing by conventional histological techniques, sections were stained with hematoxylin and eosin.

Tissue blocks of fresh tumor of approximately 1 cu mm were fixed for electron microscopy in cacodylate-buffered 2.5% glutaraldehyde, postosminicated, treated en bloc with 0.5% aqueous uranyl acetate, dehydrated through a graded series of ethanol, and embedded in Spurr epoxy resin (Polysciences, Inc., Warrington, Pa.). Semithin (0.5 to 1 μm) sections for orientation were examined by light microscopy after staining with toluidine blue. Ultrathin sections for electron microscopy were mounted on bare copper grids and contrast stained with saturated aqueous uranyl acetate followed by lead citrate.

Flow Cytometry. Cellular DNA content of specimens of the original tumor tissue and cell culture lines was measured using flow cytometry. The cells were stained with mithramycin and ethidium bromide using a rapid-staining technique as described previously (14, 15), and cellular DNA content was measured using an ICP22 flow cytometer (Ortho Instruments, Westwood, Mass.). The results were analyzed using a computer model (11), and ploidy was determined by reference to chick erythrocyte DNA content. Cell cycle phase distribution was calculated using a multichannel analyzer and expressed as frequency distribution histograms.

RESULTS

Cell Culture and Xenografts. Nineteen specimens (8 malignant effusions and 11 solid tumors) were received from 14 patients with ovarian carcinoma, the pathological subtypes being serous carcinomas (9 patients), endometrioid carcinoma (2 patients), and one each of clear cell carcinoma, dysgerminoma, and malignant mixed Müllerian tumor. Fourteen cell culture lines were established from 10 patients, including 7 of the malignant effusions and 7 of the solid tumors. In the 3 cases (one serous carcinoma, one endometrioid carcinoma, and the dysgerminoma) in which cell line could not be established, the available specimens were small with low cell counts. Xenografts were established from 6 patients, 2 from effusions and 4 from solid tumor specimens. Clinical details of the patients from whom the original tumor samples were obtained together with the time to first passage of each cell culture and xenograft are shown in Table 1. All of the malignant effusions were obtained from patients who had relapsed following chemotherapy using a combination of chlorambucil and cis-platinum, but the solid tumors were from patients who had not been exposed to cytotoxic chemotherapy (except for Patient M. W.). The time to first passage for cells in culture varied widely from 2 to 16 weeks and for xenografts, from 10 to 41 weeks. Generally, once cultures had been passaged at least 3 times, the malignant cells became established.

Table 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Histological analysis</th>
<th>Grade</th>
<th>Initial treatment</th>
<th>Tumor source</th>
<th>Outcome of culture</th>
<th>Time (wk) to first passage</th>
<th>Steroid hormone receptor concentration (fmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. G.</td>
<td>Clear cell</td>
<td>IV</td>
<td>Cisplatin, chlorambucil</td>
<td>Acetates</td>
<td>+ +</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>H. C.</td>
<td>Dysgerminoma</td>
<td>III</td>
<td>Cisplatin, chlorambucil</td>
<td>Acetates</td>
<td>+ +</td>
<td>12</td>
<td>120</td>
</tr>
<tr>
<td>V. B.</td>
<td>Endometrioid</td>
<td>III</td>
<td>Cisplatin, chlorambucil</td>
<td>Acetates</td>
<td>+ +</td>
<td>2</td>
<td>40</td>
</tr>
<tr>
<td>M. C.</td>
<td>Endometrioid</td>
<td>III</td>
<td>Cisplatin, chlorambucil</td>
<td>Acetates</td>
<td>+ +</td>
<td>2</td>
<td>1364</td>
</tr>
<tr>
<td>H. M.</td>
<td>Serous</td>
<td>IV</td>
<td>Cisplatin, chlorambucil</td>
<td>Acetates</td>
<td>+ +</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>J. N.</td>
<td>Serous</td>
<td>III</td>
<td>Cisplatin, chlorambucil</td>
<td>Acetates</td>
<td>+ +</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>D. D.</td>
<td>Serous</td>
<td>II</td>
<td>Cisplatin, chlorambucil</td>
<td>Acetates</td>
<td>+ +</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>J. C.</td>
<td>Serous</td>
<td>IV</td>
<td>Cisplatin, chlorambucil</td>
<td>Acetates</td>
<td>+ +</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>M. W.</td>
<td>Serous</td>
<td>III</td>
<td>Cisplatin, chlorambucil</td>
<td>Acetates</td>
<td>+ +</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>S. K.</td>
<td>Serous</td>
<td>II</td>
<td>Cisplatin, chlorambucil</td>
<td>Acetates</td>
<td>+ +</td>
<td>6</td>
<td>26</td>
</tr>
<tr>
<td>J. V.</td>
<td>Serous</td>
<td>III</td>
<td>Cisplatin, chlorambucil</td>
<td>Acetates</td>
<td>+ +</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>E. H.</td>
<td>Serous</td>
<td>III</td>
<td>Cisplatin, chlorambucil</td>
<td>Acetates</td>
<td>+ +</td>
<td>3</td>
<td>29</td>
</tr>
<tr>
<td>N. W.</td>
<td>Serous</td>
<td>III</td>
<td>Cisplatin, chlorambucil</td>
<td>Acetates</td>
<td>+ +</td>
<td>4</td>
<td>41</td>
</tr>
<tr>
<td>A. M.</td>
<td>Mixed Müllerian</td>
<td>II</td>
<td>Cisplatin, chlorambucil</td>
<td>Acetates</td>
<td>+ +</td>
<td>6</td>
<td>16</td>
</tr>
</tbody>
</table>

* Cisplatin, chlorambucil.
* Not established.
* These ascites specimens were obtained 1, 2, and 4 months after the initial specimen.
Flow Cytometry of Cultured Ovarian Cancers

Table 2
Flow cytometric analysis of cellular DNA content of initial specimens of ovarian tumor cells and established cell and xenograft lines

<table>
<thead>
<tr>
<th>Patient</th>
<th>Tumor source</th>
<th>Initial G&lt;sub&gt;i&lt;/sub&gt; peaks (ratio to chick RBC)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Established line Cell</th>
<th>Xenograft</th>
<th>Passage no. of ploidy change in cell culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. G.</td>
<td>Ascites</td>
<td>2.9, 4.7</td>
<td>5.15</td>
<td>4.8</td>
<td>2</td>
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<tr>
<td>H. C.</td>
<td>Tumor</td>
<td>4.3</td>
<td>4.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>V. B.</td>
<td>Tumor</td>
<td>2.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. C.</td>
<td>Tumor</td>
<td>2.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H. M.</td>
<td>Ascites</td>
<td>2.7</td>
<td>4.4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ascites</td>
<td>2.7</td>
<td>4.8</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ascites</td>
<td>2.7</td>
<td>4.5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ascites</td>
<td>3.0</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>J. N.</td>
<td>Ascites</td>
<td>2.7, 7.9</td>
<td>8.6</td>
<td>8.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Ascites</td>
<td>2.9, 8.6</td>
<td>8.3</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>D. D.</td>
<td>Tumor</td>
<td>2.8</td>
<td>4.8</td>
<td>5.3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3</td>
</tr>
<tr>
<td>J. C.</td>
<td>Tumor</td>
<td>2.8, 3.0</td>
<td>4.7</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pleural effu-</td>
<td>2.8, 3.0</td>
<td>4.5</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>sion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. W.</td>
<td>Tumor</td>
<td>2.5</td>
<td>4.8</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>S. K.</td>
<td>Tumor</td>
<td>2.7, 3.8</td>
<td>4.8</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>J. V.</td>
<td>Tumor</td>
<td>2.5, 2.7</td>
<td>4.6</td>
<td>2.5</td>
<td>3</td>
</tr>
<tr>
<td>E. H.</td>
<td>Tumor</td>
<td>2.8, 4.6</td>
<td>4.4</td>
<td>4.6</td>
<td>3</td>
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<tr>
<td>N. W.</td>
<td>Tumor</td>
<td>3.0</td>
<td>4.9</td>
<td>4.5</td>
<td>1</td>
</tr>
<tr>
<td>A. M.</td>
<td>Tumor</td>
<td>2.8, 4.7</td>
<td>4.8</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Normal diploid cells have a ratio of 2.7 to 3.1.

<sup>b</sup> Not established.

<sup>c</sup> Cell lines also established from xenograft; DNA ratio, 8.7 (Patient J. N.) and 4.5 (Patient D. D.).

and the cell cultures could then be passaged at 1- to 3-week intervals and xenografts, after the first graft passage, at 4- to 12-week intervals.

**Flow Cytometry.** Table 2 summarizes the flow cytometric DNA profiles of the initial specimens of ovarian tumor cells and of the established cell cultures and xenograft lines. Nine of the 19 original specimens showed aneuploid peaks in addition to cells with a normal diploid DNA content. Although there were initially 10 diploid tumors, all of these lines have now become aneuploid, the majority being somewhere between triploid and tetraploid. Aneuploid cells became predominant after one to 4 passages (median, 3).

Chart 1 shows the flow cytometric DNA histograms of the cultured cells of the first ascites specimen from Patient H. M., contrasting the appearance of the initial ascites cells with their appearance after 4, 5, and 8 passages. Cell passages were made at approximately 2-week intervals. The overgrowth of aneuploid cells, which occurred between the fourth and fifth passages, was followed after the sixth passage by a doubling of the DNA content of the aneuploid cell population. In the second ascites preparation from this patient, the change from diploid to aneuploid cells was also detected by the fourth passage.

Chart 2 shows the DNA histograms of the cell culture line from the first ascites specimen from Patient J. N. at the time of the original culture and after 5 passages. The histogram of the fresh ascites cells shows a small diploid and a larger aneuploid peak which persisted until the third passage. Subsequently, the aneuploid cell peak overgrew, and diploid cells disappeared from passaged cultures. A similar transfer in ploidy was seen with the second ascites specimen from this patient (data not shown). The DNA histograms of the xenografted tumor as well as xenografted cell line from this patient at the time of first passage and after 5 passages are also shown in Chart 2, C and D. Aneuploidy of the xenograft line was seen from the first passage and has remained stable up until the eighth passage (the most recently measured). This xenograft cell line has only a single large peak of aneuploid cells. No murine stromal or vascular cell components have been detected.

**Light and Electron Microscopy.** The cytomorphology of the cell lines and the histological appearances of the xenografts closely resembled the parent tumors. The striking similarity, for

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*Chart 1.* DNA histograms of serous adenocarcinoma, Patient H. M. Flow cytometry was performed at the time of original culture (A) and at the fourth (B), fifth (C), and eighth (D) passages (P<sub>4</sub>, P<sub>5</sub>, and P<sub>8</sub>, respectively). The left-hand peak represents chick RBC (CRBC). The calculated proportions of G<sub>i</sub>, S, and G<sub>i</sub> + M are shown, except for B where the calculation was not possible due to the overlapping cell populations. C. V., coefficient of variation.

*Chart 2.* DNA histograms of the cultured cells of the first ascites specimen from Patient J. N. Flow cytometry was performed at the time of original culture (A) and at the fourth (B), fifth (C), and eighth (D) passages (P<sub>4</sub>, P<sub>5</sub>, and P<sub>8</sub>, respectively). The left-hand peak represents chick RBC (CRBC). The calculated proportions of G<sub>i</sub>, S, and G<sub>i</sub> + M are shown, except for B where the calculation was not possible due to the overlapping cell populations. C. V., coefficient of variation.
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Chart 2. DNA histograms of serous adenocarcinoma. Patient J. N. Flow cytometry was performed on the first ascites specimen (A), the cell line at the fifth passage (B), the xenografted tumor at the first (C) and fifth (D) passages, and the cell line established from the xenografted tumor at the first (E) and fifth (F) passages. In C and D, the peaks at Channels 29 and 27, respectively, represent normal diploid mouse cells. CRBC, chick RBC; C.V., coefficient of variation; P1 and P5, passages 1 and 5, respectively.

Chart 3. DNA histogram of a typical serous adenocarcinoma (Patient D. D.) from this series at second passage (P2) of cells in vitro. C.V., coefficient of variation; CRBC, chick RBC.

example, between the original clear-cell carcinoma from the patient and that from the xenograft is illustrated in Fig. 1, each showing a typical tubulopapillary pattern with the only difference being a minor depletion in the amount of intracellular glycogen. The original specimen was not studied by electron microscopy, but the xenograft showed the typical features of ovarian clear-cell carcinoma (Fig. 1D). Xenograft transplants examined by electron microscopy showed a mixture of papillary and solid growth patterns. Tumor cells were joined by desmosomes with junction complexes at their apical part adjacent to glandular lumina. The cytoplasm generally appeared undifferentiated with free ribosomes and scanty elongated cisternae of rough-surfaced endoplasmatic reticulum being the predominant organelles. However, occasional cells contained the clusters of small dense granules that are a component of serous tumors. The DNA histograms (Charts 3 and 4) show cell cultures in the second and seventh subpassages. The change in ploidy of the cell cultures was evident by the third cell passage and after the fourth cell passage; the DNA histogram shows a doubling of the DNA content of the previous aneuploid cell population. The doubled DNA content has persisted for a further 8 passages, and the cells have now been stored in liquid nitrogen. The aneuploidy was also apparent in the first xenograft passage and has remained stable up until the eighth subpassage in mice (data not shown). A long-term cell culture from this xenograft tumor has also been established.

Of the other tumors studied, 3 were of note. The endometrioid carcinoma was moderately differentiated with a typical microglandular pattern. The malignant mixed Müllerian tumor had a carcinomatous component which was serous in type and a very minor sarcomatous component of endometrial stromal type. This latter fraction of the neoplasm was not seen in either the cell cultures or xenografts, presumably due to sampling. The dysgerminoma exhibited in the original tumor the typical trabecular pattern of large, uniform polyhedral cells with numerous mitoses and thin connective tissue septa containing lymphocytes. The xenograft was distinguishable from the original tumor only by a
relative lack of this septal lymphocytic infiltrate. No significant ultrastructural differences were observed between the original tumor and the xenografted dysgerminoma.

**Estrogen and Progesterone Receptors.** Four primary tumors contained detectable estrogen receptors, and 5 contained progesterone receptors (Table 1). However, no cell line established from these tumors expressed measurable quantities of estrogen receptors (progesterone receptors not measured).

**DISCUSSION**

We describe the establishment of cell culture in and nude mice of tumor lines derived from patients with ovarian cancer. A large proportion (14 of 19, 74%) of the tumor specimens received were successfully cultured in vitro. Cell lines were established from both solid tumors (7 of 11) and malignant effusions (7 of 8). All cell lines formed a monolayer in culture rather than aggregates of cells in the supernatant as described by Woods et al. (17). Cell cultures as well as xenograft lines were established from both well- and poorly differentiated tumors and from untreated and drug-treated patients. Our results contrast with the reports of Engel et al. (5) as well as others who found that malignant effusions had a higher take rate than specimens from solid tumors. The rapid initial processing of specimens and the nutrient medium used in our study may have contributed to this different experience. We have not studied the role of growth factors or used other media because of our success with the conditions described.

Flow cytometry was used to follow the cellular DNA content of cells from the tumors and malignant effusions of the patients after culture in vitro and in vivo. Solid tumors and malignant effusions from ovarian cancer usually contained predominantly diploid or mixed diploid and aneuploid tumor cell populations, but tumor cell lines derived from these sources invariably became aneuploid during serial passage. Sequential flow cytometric studies showed that aneuploid cells became dominant, usually between the first and fourth passage in vitro and following the first passage in xenografts. Interestingly, in the patient (J. C.) from whom tumor cells were obtained for culture from 2 different metastatic sites, the cell lines established from both sources became aneuploid at the same (second) passage. In this patient, only diploid cells were apparent in the original specimens, although it is likely that some aneuploid cells were not detected due to dilution by the diploid cells. Higher growth rates of aneuploid populations or tumor cell fusion-hybridization with other cells may have caused this phenomenon.

Some primary ovarian adenocarcinomas have been reported previously (2) to be estrogen receptor positive. Four of the 11 primary tumors described here contained measurable estrogen receptors, and 5 contained progesterone receptors. However, none of the cell lines established in vitro or in vivo demonstrated estrogen receptor, indicating some selection during culture. Selection of cells during early growth was also evident on light microscopy, because there were tumor cells among the population of adherent cells.

Cell lines can be established from a high proportion of ovarian tumors. Although these cells retain the morphological appearances of their parent tumor, selection takes place during the cell culture procedure with the out growth of aneuploid and hormone receptor-negative clones in those cultures which were initially diploid or receptor positive. The basis for this development is uncertain but may reflect their higher growth rate or metabolic adaptability to cell culture conditions.

The development of cloning assays for ovarian cancers will allow the further dissection of factors contributing to the emergence of tumor cell populations with different physical and biochemical properties. These studies are being pursued in our laboratory.

**ACKNOWLEDGMENTS**

We wish to thank Dr. Leigh Murphy and Terry Foo for their assistance with the hormone receptor studies and Dr. Ian Taylor for his advice and assistance with flow cytometric studies. We are grateful also to the Gynecological Oncology Unit at King George V Hospital for provision of the tumor specimens from patients under their care.

**REFERENCES**


Fig. 2. Morphology of a typical serous adenocarcinoma (Patient D. D.) from this series. Photomicrographs: A, original tumor, x 100; B, cell line (seventh passage), cultured on a chamberslide, x 400.
Fig. 1. Morphology of clear-cell ovarian carcinoma from Patient G. G. A, original tumor, × 100; B, xenograft (sixth passage), × 100; C, cell line (seventh passage), cultured on a chamberslide, × 400; D, xenograft (sixth passage), electron micrograph, × 17,000.
Flow Cytometric and Morphological Studies of Ovarian Carcinoma Cell Lines and Xenografts


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