Characterization and Properties of Nine Human Ovarian Adenocarcinoma Cell Lines

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ABSTRACT

Four series of cell lines have been derived from patients with ovarian adenocarcinoma. Nine cell lines have been established at different stages of treatment: eight from malignant effusions and one from a solid metastasis. Six lines were derived from the ascites or pleural effusion of patients with poorly differentiated adenocarcinoma: PEO1, PEO4, and PEO6 from one patient, PEA1 and PEA2 from a second, and PEO16 from a third. Three lines (PEO14 and PEO23 from ascites and TO14 from a solid metastasis) were derived from a patient with a well-differentiated serous adenocarcinoma. Each set of cell lines was morphologically distinct. The five cell lines PEO1, PEO4, PEO6, PEA1, and PEA2 had cloning efficiencies on plastic of 1-2% and only a few cells in these lines expressed alkaline phosphatase or vimentin. Only a low percentage of them grew more rapidly in the presence of autologous human ascitic filtrate. Once cell lines were established the filtrate was no longer added. Attempts to use conditioned medium derived from other cell lines proved unsuccessful. Specially coated Primaria dishes (Falcon, Oxnard, CA) were used to prevent overgrowth by fibroblasts. Once established, confluent cultures were passage by trypsinisation by 1:5 or 1:10 splits. The TO14 cell line was derived from a metastasis to the omentum and was obtained at hysterectomy. The tumor was cut into small fragments and placed into 35-mm wells (6 plate dishes, Costar, Cambridge, MA). The media described above was added. Once cells had attached and were growing successfully they were removed by trypsinization and transferred to 25-cm² flasks (Falcon, Oxnard, CA). Routine assays for mycoplasma were negative.

INTRODUCTION

Ovarian cancer is the 5th most common form of cancer in women. It is a tumor type that is sensitive to chemotherapy, but where drug resistance frequently develops and therefore sustained responses are infrequent. An improved understanding of this disease might lead to improved therapy and, to explore this, reproducible sources of material are required. A number of ovarian adenocarcinoma cell lines and xenografts have previously been established (3-23). Previous studies have concentrated on the chemosensitivity (5, 9, 10, 16, 20), the cytogenetics (5, 7, 10, 11, 17), hormone dependency (5, 7, 8, 9, 10, 13, 14), and oncogene expression (15, 24) in these models. Despite this, there are major questions concerning the biology of ovarian cancer which remain unanswered.

We have established a series of cell lines from the ascites of patients with ovarian adenocarcinoma and have characterized them while still at an early stage in culture. We have studied their growth characteristics, histochemical properties, and antigen expression and in this report describe these properties. The partial characterization of two of these cell lines, PEO1 and PEO4, has previously been reported.

MATERIALS AND METHODS

Establishment of the Cell Lines

The cell lines were derived from malignant effusions in the following manner. The freshly obtained sample was mixed with 100 units of preservative-free heparin and the cells sedimented by centrifugation (1000 x g for 10 min). These were then resuspended in PBS. Red blood cells were removed by Ficoll-Hypaque gradient separation and interface cells were washed and checked for viability by vital dye exclusion. Aliquots of 10^6 cells/ml were then cultured at 37°C, 90% humidity, and 5% CO2 in RPMI + 10% fetal calf serum with added insulin (2.5 μg/ml), streptomycin (100 μg/ml), pyruvate (2 mm), penicillin (100 IU/ml), and 3-[N-morpholino]propane sulfonic acid (12.5 mm). Other aliquots of cells were frozen in liquid nitrogen in dimethyl sulfoxide (10% v/v) in newborn calf serum. During establishment, cell lines grew more rapidly in the presence of autologous human ascitic filtrate. Once cell lines were established the filtrate was no longer added. Attempts to use conditioned medium derived from other cell lines proved unsuccessful. Specially coated Primaria dishes (Falcon, Oxnard, CA) were used to prevent overgrowth by fibroblasts. Once established, confluent cultures were passage by trypsinisation by 1:5 or 1:10 splits.

The TO14 cell line was derived from a metastasis to the omentum and was obtained at hysterectomy. The tumor was cut into small fragments and placed into 35-mm wells (6 plate dishes, Costar, Cambridge, MA). The media described above was added. Once cells had attached and were growing successfully they were removed by trypsinization and transferred to 25-cm² flasks (Falcon, Oxnard, CA). Routine assays for mycoplasma were negative.

Measurement of Clonogenicity

On Plastic. Cells were plated in 35-mm wells at appropriate cell numbers in RPMI containing 10% fetal calf serum. After 2-3 weeks colonies (≥50 cells) were counted using low power inverted microscopy.

In Agar. The soft agar assay of Courtenay et al. (26) was used without the addition of irradiated feeder cells. Semiliquid cultures were set up in agar (0.3% v/v). Fresh media (1 ml) was added weekly. Colonies (≥50 cells) were scored after 28-60 days dependent on the cell line.

Measurement of Doubling Times

Cells were plated in 35-mm wells. Duplicate wells were trypsinized daily and cells counted. Population doubling times were calculated for cells in exponential phase of growth. Each experiment was repeated at least once to confirm the value.

Tumorigenicity Studies

Cells in culture were trypsinized and cell suspensions centrifuged (1000 x g for 10 min). The pellet was then washed and resuspended in PBS to give a density of 10^6 cells/ml. 2.5 x 10^6 cells (0.25 ml of cell suspension) were injected s.c. into the flanks of immunologically deprived CBA mice (27). All animals were observed weekly until their tumors grew to a mean diameter of 1 cm or for at least 4 months in the absence of tumors.

Chromosome Analysis

Exponentially growing cultures were harvested for chromosome preparations using standard cytogenetic procedures. Preparations were stained with giemsa and chromosome counts in 50 good metaphase spreads were obtained.

Received 11/6/87; revised 6/10/88; accepted 7/25/88.

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Histochemical Methods

Cell smears were stained for alkaline phosphatase by the napthol AS-BI method (28). Other histochemical procedures were performed in situ on cells (in exponential growth) attached to plastic as follows. For detection of lipid, cells were rinsed in propanol:water (3:2) and then stained with a solution of oil red O (saturated solution in propanol:water 3:2) for 15 min (29).

For detection of glycogen and acid and neutral mucin, cells were rinsed with PBS and then fixed in formalin:ethanol (1:9). The fixative was discarded and (in separate wells) acid mucin was detected with alcian blue at pH 2.5 (30), glycogen by the periodic acid Schiff method (30) and neutral mucin by a same technique after pretreatment of the cells with α-amylase solution (0.5 mg/ml in PBS) (30).

Immunoperoxidase Staining

The immunohistochemical studies of the cultured cells were performed according to the PAP method described by Sternberger (31). Mouse monoclonal antibodies were used except for detection of CEA. Briefly, cells were trypsinized from plastic and placed onto multispot slides [Hendley (Essex) Ltd., Essex, UK] at approximately $2 \times 10^4$ cells/slide. Cells were fixed in methanol:acetone (1:1) for 10 min. They were subsequently incubated at room temperature with 0.5% hydrogen peroxide in methanol for 15 min to block endogenous peroxidase. After being washed in PBS (Tris 0.5 m, pH 7.6, diluted in saline 1:10), they were incubated with sheep serum:PBS (1:5) for 10 min followed by an appropriate dilution (see below) of the mouse MoAb for 30 min. Thereafter sheep anti-mouse IgG (SAPU, Carluke, UK) was applied at a dilution of 1:5 in PBS for 30 min at room temperature. After being washed with PBS these samples were incubated with mouse monoclonal PAP complex (DAKO Ltd, High Wycombe, UK) at optimal dilution. The immunoperoxidase staining was performed using 0.05% H2O2 in PBS and 0.01% hydrogen peroxide in Tris-imidazole buffer (pH 7.6) for 10 min, and after washing with water, these samples were counterstained with hematoxylin.

For detection of CEA, rabbit antiserum was used and the method described above employed with the following changes. Donkey serum (SAPU) was used instead of sheep serum, donkey anti-rabbit IgG (SAPU) instead of sheep anti-mouse IgG, and rabbit PAP (SAPU) instead of mouse PAP.

MoAbs were obtained from the following sources and used at these dilutions. 123C3 and 123A8 were used at a dilution of 1:100 of the ascites (32, 33). 123A8 was obtained from the same fusion as 123C3 and thus far has never reacted any differently from 123C3. Antivimentin was obtained from Boehringer (Mannheim) and used at a dilution of 5 μg IgG/ml Tris (34). OC 125 (35) and OC 19/9 (36) were obtained from kits supplied by HistoCis, Gif-sur-Yvette, France, and used at the recommended dilutions. CAM 5.2 (37), AUAI (38), HMPG1 (39), HMF2 (39), LEG1 (40), and the MoAbs raised against placental alkaline phosphatase (H17E2, 3F6, 11F7, and 8B6) (41) were gifts from ICRF, London, UK, and were used as supernatants. Anti-CEA was obtained from DAKO and used at a dilution of 1:50. CR343 (42) and 92.1 were gifts from Drs. C. M. Steel and K. Guy, MRC Clinical and Populations Cytogenetics Unit, Edinburgh, UK. Leukocyte common antigen (DAKO-TC), used as a negative control throughout, was obtained from DAKO and used at a 1:20 dilution of supernatant.

Studies of the Ascites

Aliquots of the ascites from which these cell lines were obtained were stored in liquid nitrogen. These were removed from liquid nitrogen in order to compare the histochemical and immunohistochemical staining patterns of the cell lines and the relevant ascites. Multiphotol slides of the ascites ($2 \times 10^4$ cells/slide) were prepared and cells stained by the techniques described above.

RESULTS

Establishment of the Cell Lines. Nine cell lines have been derived from 4 patients at different times during treatment. Details of the patients' histories from whom the cell lines were derived are summarized in Table 1. Of the nine cell lines, six were derived from patients with poorly differentiated adenocarcinoma while three were derived from a patient with a well-differentiated tumor. Seven of the nine were obtained from peritoneal ascites, one from a metastasis to the omentum (TO14), and one from a pleural effusion (PEA1). The series of cell lines represent different stages of treatment. The cell lines PEA1, PEO14, and TO14 were all obtained prior to therapy while the rest were obtained after either chemotherapy (PEO1, PEO4, PEO6, PEA2, and PEO23) or radiotherapy (PEO16). Details of the treatments are given in Table 1. The time taken for each cell line to reach the earliest passage number used in these studies is recorded in Table 2.

Morphological Characteristics of the Cell Lines. The four sets of cell lines were all morphologically distinct (Fig. 1). All grew as monolayers. The set PEO1, PEO4, and PEO6 all grew as islands of uniform polygonal cells (Fig. 1, a–c). PEA1 and PEA2 cells grew in a swirling pattern (Fig. 1, d and e). PEO16 cells were stellate in shape (Fig. 1f) and produced hemispheric colonies which grew upward from the plastic surface and pinched off to float away as spheres, a property also shown by PEO14, TO14, and PEO23. These cell lines grew as tight foci with smaller cells in the centre of the colonies and large vacuolated cells towards the edges of the islands (Fig. 1, g–i).

Electron microscopy revealed desmosomes between PEO16 cells confirming their epithelial origin (Fig. 1, j).

Growth Characteristics of the Cell Lines. Some of the growth characteristics of the cell lines are summarized in Table 2. The CFE values of the cell lines on plastic ranged between 0.01 and 8.1% for the early passages. With time, however, the CFE does increase as demonstrated for PEO1 and PEO4 which have CFE values of 1.0 and 1.5, respectively, in early passages and values of 3.9 and 8.1 in later passages (Table 2). All cell lines produced recognizable colonies when grown on either plastic or in culture rendered semisolid by agar. Frequency of colony growth could be linearly related to the number of cells plated. It is unusual that CFE values for the cell lines in agar tended to be higher than values on plastic. This may be due to differences in oxygen tension since plastic assays were conducted at 20% O2 and agar assays at 5% O2. Population doubling times on plastic for the cell lines varied between 36 and 130 h (Table 2). Aliquots ($2.5 \times 10^6$) of cells from PEO1, PEO4, PEO6, and PEO16 were injected into the flanks of immunologically deprived CBA mice. All four cell lines produced pathologically confirmed tumors.

Modal Chromosome Numbers. Chromosome counts for the cell lines are illustrated in Fig. 2. All cell lines were aneuploid and counts varied over a large range. A modal chromosome number could only be assigned to PEO1 and PEO4, both with a value of 41.

Histochemical Properties of the Cell Lines. Several histochemical properties of the cell lines were examined. >90% cells were positive for alkaline phosphatase in the PEO14, TO14, PEO23, and PEO16 lines while <10% cells were positive in the other five lines. The percentage of cells positive for glycogen was also higher in the PEO14, TO14, and PEO23 lines (28–39%) than in the other lines. The cell lines contained varying numbers of cells positive for lipid (2–14%) and acid mucin (1–36%) and some contained cells staining for neutral mucin (0–4%). When these properties were examined in the ascites samples from which the cell lines were derived the results (not shown) were similar results were obtained indicating that the cell lines are a
acted with a large percentage of PEO6 and PEA1 cells. Expression, was generally negative while MoAb CR343-indicating major histocompatibility complex Class I on the same mucin) reacted with very few cells. The MoAb except for PEO16. The MoAb HMFG1 (against the mucin, weight cytokeratins) and AU Al (against an epithelial mem

The reverse situation was found for CA125 (detected by OC125). MoAbs LE61 and CAM5.2 (against low molecular weight cytokeratins) and AU1 (against an epithelial membrane antigen) were expressed in almost all cells in the cell lines except for PEO16. The MoAb HMFG1 (against the mucin, human milk fat globulin) stained varying percentages of cells ranging from 2 to 90% while HMFG2 (against a second epitope on the same mucin) reacted with very few cells. The MoAb 92.1, indicating major histocompatibility complex Class I expression, was generally negative while MoAb CR343-indicating major histocompatibility complex Class II expression reacted with a large percentage of PEO6 and PEA1 cells. Examination of the ascites from which the cell lines were derived indicated similar patterns for 123C3, vimentin, OC125, CAM5.2, AU A1, and LE61. However in some, e.g., HMFG1 and HMFG2, expression was decreased and occasionally lost as the cell line developed. This might be due either to clonal selection or decreased expression in culture. The antibody OC 19/9 detected only a subset of PEA2 cells although it was present in many of the parent ascites.

The expression of placental alkaline phosphatase was also examined in these cell lines using a panel of MoAbs (H17E2, 3F6, 11F7, and 8B6) reacting with this isozyme. The PEO23 line contained 4–9% cells positive for this isozyme depending on which MoAb was used, the PEA1, PEA2, PEO14, TO14, and PEO16 lines contained about 1–2% cells positive while PEO1, PEO4, and PEO6 were all negative.

**DISCUSSION**

We have derived and characterized nine human ovarian carcinoma cell lines. These represent multiple sets from three patients and a single line from a fourth. Most ovarian carcinoma cell lines available have been passaged for prolonged periods and may therefore have lost certain characteristics. For this reason the cell lines were studied while still at early passage number and stored in liquid nitrogen to maintain a source of cells. Comparison of the properties of the cell lines and the ascites from which they were derived suggests that at least in their histochemical and immunohistochemical properties the cell lines are a reasonable representation of the tumors in their natural state. While it was not possible to make a comparison between the cell lines and the original primary tumor, a comparison with parent metastatic (ascitic) cells is valid, in that it is these cells that present the most significant therapeutic challenge.

All the cell lines produced colonies when grown on plastic or in agar. The set PEO1, PEO4, and PEO6 grew well on plastic or in agar; PEA1 and PEA2 grew better on plastic and PEO14, TO14, PEO23, and PEO16 in agar. The four lines tested for tumorigenicity all produced tumors in immune-deprived mice. The reverse situation was found for CA125 (detected by OC125). MoAbs LE61 and CAM5.2 (against low molecular weight cytokeratins) and AU1 (against an epithelial membrane antigen) were expressed in almost all cells in the cell lines except for PEO16. The MoAb HMFG1 (against the mucin, human milk fat globulin) stained varying percentages of cells ranging from 2 to 90% while HMFG2 (against a second epitope on the same mucin) reacted with very few cells. The MoAb 92.1, indicating major histocompatibility complex Class I expression, was generally negative while MoAb CR343-indicating major histocompatibility complex Class II expression reacted with a large percentage of PEO6 and PEA1 cells. Examination of the ascites from which the cell lines were derived indicated similar patterns for 123C3, vimentin, OC125, CAM5.2, AU A1, and LE61. However in some, e.g., HMFG1 and HMFG2, expression was decreased and occasionally lost as the cell line developed. This might be due either to clonal selection or decreased expression in culture. The antibody OC 19/9 detected only a subset of PEA2 cells although it was present in many of the parent ascites.

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A number of histochemical properties within these cells were examined. The four cell lines with low CFE values on plastic (PEO14, TO14, PEO23, and PEO16) were also the four which...
Fig. 1. Plates of cell lines. a, PEO1 cells; A, PEO4 cells; c, PEO6 cells; d, PEA1 cells; e, PEA2 cells; f, PEO16 cells; g, PEO14 cells; h, TO14 cells; i, PEO23 cells; j, Electron micrograph of PEO16 cells showing a desmosome (arrow) between two cells. Bars, 40 μm.
OVARIAN ADENOCARCINOMA CELL LINES

Fig. 2. Histogram of chromosome counts for each cell line.

contained >90% cells positive for alkaline phosphatase. All the cell lines contained some cells positive for lipid, acid mucin, and glycogen and these markers may reflect differences in growth properties [e.g., levels of glycogen are cell-cycle related in colon lines (43)].

Antigen expression varied considerably between the cell lines. MoAbs 123C3 and 123A8 were raised against a small cell lung cancer specimen and in normal tissues react predominantly with endocrine and neuron supporting tissue (32, 33). A large percentage of cells in the cell lines PEO 14, TO 14, PEO23, and PEO 16 reacted with 123C3 and 123A8 and also with the antivimentin MoAb, while <5% cells in the PEO1, PEO, PEO6, PEA1, and PEA2 lines reacted with these MoAbs. Conversely OC 125 (detecting the antigen CA 125) reacted with <35% cells in the former four lines but with the majority of cells in the latter five. The cytokeratin markers (LE61 and CAM5.2) were positive in almost all cells in all the lines consistent with their epithelial nature. The PEO16 line however contained very few cells reacting with these markers. The epithelial marker AU1 was also unreactive with these cells. Electron microscopy, however, demonstrated the presence of desmosomes between PEO16 cells indicating epithelial origin (Fig. 1j).

Clearly there is marked heterogeneity of antigen expression in these cell lines. The most probable reasons for this are either the presence of variable cellular differentiation, clonal heterogeneity, or changes due to cell cycle. Buick has suggested that antigen expression changes with cellular differentiation in ovarian carcinoma (44, 45). Alkaline phosphatase [a marker of differentiation in colorectal, prostatic, and embryonal carcinoma cell lines (46–50)] and the determinants recognized by 123C3 and 123A8 may perhaps be markers of differentiation though our data are insufficient to support this hypothesis. However we have observed that all three of these markers are induced in PEO1 and PEO4 cells after treatment with sodium butyrate, an agent known to modulate differentiation in many tissue systems (51). We are currently examining primary tumors and further ascites to see how these markers relate to pathological differentiation.

A number of markers are currently under study as possible serum indicators of active ovarian carcinoma. Among these are CA125, HMFG1, HMFG2, and placental alkaline phosphatase (52–55). CA125 (detected with OC125) was present in at least some cells in all cell lines except PEO16. These cell lines showed marked variability for HMFG1 while only a few cells reacted for HMFG2. Importantly though, examination of the ascites samples from which the cell lines were derived indicated that most cells in all these samples (apart from perhaps PEO1) were strongly positive for both HMFG1 and HMFG2. The reason for this loss of reactivity in culture is unclear. The MoAbs detecting placental alkaline phosphatase reacted with very few cells in either the ascites or lines. The reason for this heterogeneity of expression in the cell lines is being explored and novel markers are currently being sought.

These models are also being used to study chemosensitivity in this tumor type. The cell lines were derived at different stages of treatment. Some were obtained prior to therapy and others reflect sequential samples after treatment. The chemosensitivity of two of these cell lines has previously been described (PEO1 and PEO4) representing a model of resistance acquired during treatment of the patient (25).

Table 3 Antigen expression of the cell lines

<table>
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<tr>
<th>Cell line</th>
<th>123C3</th>
<th>123A8</th>
<th>Vimentin</th>
<th>OC125</th>
<th>CAM5.2</th>
<th>LE61</th>
<th>AU1</th>
<th>CEA</th>
<th>HMFG1</th>
<th>HMFG2</th>
<th>CR343</th>
<th>92.1</th>
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<td>0</td>
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<td>++++</td>
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* Passage numbers.

* Percentage of cells positive: 0, 0%; +, 1-25%; ++, 26-50%; ++++, 51-75%; +++, 76-100%.
ACKNOWLEDGMENTS

We gratefully acknowledge Dr. M. A. McIntyre for assistance with the pathology of these tumors.

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