Comparison of Four New Cell Lines from Patients with Adenocarcinoma of the Ovary


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ABSTRACT

Permanent human tumor cell lines COLO 110, COLO 316, COLO 319, and COLO 330 were established from four patients with serous cystadenocarcinoma of the ovary. COLO 110 was derived from primary tumor tissue; COLO 316, COLO 319, and COLO 330 were derived from cells in malignant effusions. COLO 110 and COLO 316 grew as monolayers of epithelioid cells in culture; COLO 319 and COLO 330 grew as vermiform, floating colonies of epithelioid cells in culture. Epithelial-like copy. All four cell lines had marker chromosomes and double minute chromosomes. COLO 319 and COLO 330 were established from four patients of the ovary with omental and peritoneal metastases. The patient died on April 16, 1975.

Case B. In October, 1975, a 54-year-old Caucasian female had a laparotomy with a total abdominal hysterectomy and bilateral salpingo-oophorectomy. The diagnosis was Stage IV serous cystadenocarcinoma of the ovary. Detailed pathology description of the carcinoma was not available. On March 4, 1976, a pleural effusion specimen was submitted for tissue culture. The patient died on August 23, 1976.

Case C. In June, 1976, a 69-year-old Caucasian female presented with abdominal swelling, intermittent abdominal cramping, and decreased appetite. On August 1, 1976, paracentesis was performed, and the ascitic fluid was submitted for pathology and tissue culture. Neoplastic cells and signet ring cells were observed in the fluid specimen. On August 8, 1976, the patient underwent a supracervical abdominal hysterectomy and bilateral salpingo-oophorectomy. Specimens submitted to pathology indicated that the patient had Stage III serous cystadenocarcinoma of the right ovary and metastases to the left ovary and omentum. Detailed pathological description of the carcinoma was not available. The patient died November 18, 1976.

Case D. In December, 1975, a 59-year-old Caucasian female presented with an ovarian tumor. An exploratory laparotomy in January of 1976 revealed the patient had a Stage III serous cystadenocarcinoma of the ovary. Details of the cell types observed in the pathology specimen were not available. The patient received external radiation for 6 weeks followed by a course of Alkeran. On May 23, 1977, a paracentesis fluid specimen was received for cell culture. A Giemsa-stained smear of cells from this fluid revealed numerous aggregates of neoplastic cells and signet ring cells. The patient died October 17, 1977.

INTRODUCTION

Ovarian carcinoma cells propagated in long-term culture are valuable for studies of hormones and hormone dependency, chemotherapeutic drugs, and cytogenetic alterations. Few permanent ovarian carcinoma cell lines have been described (5, 9, 14). We describe 4 human ovarian carcinoma cell lines which have been studied for hormone production, steroid hormone receptors, chromosome abnormalities, and isozyme patterns. Two of the cell lines grow as monolayer-like epithelial cells, and 2 lines grow as vermiform aggregates of epithelial cells in the supernatant. Each line is distinguishable based on cytogenetics and isozyme phenotype.

MATERIALS AND METHODS

Case A. In February, 1975, a 56-year-old Caucasian female presented with ascitic fluid containing malignant cells. On February 14, 1975, a subtotal hysterectomy and bilateral salpingo-oophorectomy were performed, and multiple specimens from the left and right ovaries, omentum, distal left fallopian tube, and serosa of the uterus were submitted for pathology and tissue culture. The pathology report indicated that the tissue specimens had neoplastic sheets of papillary aggregates, crowded cells with columnar to polygonal morphology, scattered large anaplastic cells, and psammoma bodies in the cells with large ovoid hyperchromatic nuclei with prominent nucleoli. The diagnosis of Stage III serous cystadenocarcinoma of the ovary with omental and peritoneal metastases was made. The patient died on April 16, 1975.

Cell Culture

Case A. The tumor tissue was processed for tissue culture as previously described (20).

Cases B, C, and D. Heparinized pleural fluid was collected. Cells were recovered by centrifugation and resuspended in 3 ml of culture medium in a 4-oz culture bottle. The culture was incubated loosely capped in a moist atmosphere of 10% CO2-90% air at 37°C for 48 hr then tightly capped.

Morphology. For photography with phase optics, monolayer cultures were grown on coverslips and mounted in phosphate-
buffered saline. Replicate coverslip preparations were stained with 2% Giemsa after fixation with absolute ethanol for 3 min. For transmission electron microscopy, cell pellets were fixed in 4% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3. Embedding and staining were carried out as previously described (17).

**Chromosomes.** Three- to 7-day-old subcultures of each cell line were harvested for chromosome preparations with standard cytogenetic procedures. Trypsin-, Giemsa- and C-banding of the chromosomes were performed as previously described (27). Selected metaphase preparations were stained for nucleolar organizing regions according to the methods of Lau et al. (15).

**Isozymes.** Isozyme phenotypes of the 4 ovarian tumor cell lines were examined with the aid of W. Wright and P. Daniels in the laboratory of Dr. J. Fogh (Sloan-Kettering Institute) using starch-gel electrophoresis on tumor cell cytosols. Glucose-6-phosphate dehydrogenase isozyme mobility was monitored on each cell line by cellulose-acetate electrophoresis of the cytosol from 10^5 cultured tumor cells lysed by freeze/thaw in 0.5 ml 0.9% NaCl solution (kit from Helena Laboratories, Beaumont, Texas).

**CEA.** CEA was measured with a radioimmunoassay kit (Roche Diagnostics, Nutley, N. J.). The cell cultures were prepared as previously described (18).

**Hormone Production.** To test for hormone production, the cell lines were grown to near confluency (10 x 10^6 cells/4-oz flask). The culture medium from each cell line was replaced for 24 hr with 10 ml of RPMI Medium 1640 supplemented with 5% FBS which had been stripped of endogenous steroids by 30-min incubation at 45° with a dextran-coated charcoal pellet. FBS which had been stripped of endogenous steroids by 30-min incubation at 45° with a dextran-coated charcoal pellet (0.25% activated charcoal and 0.0025% dextran in 0.01 M Tris-HCl, pH 8.0, at 4°, 1 ml/ml FBS). RPMI Medium 1640 with 5% hormone-stripped FBS was used as control medium.

**Estrogen production** was quantitated with an Estrogen Pak (3H-labeled estrogens, estradiol and estrone; New England Nuclear, Boston, Mass.). Progesterone production was quantified with a Progesterone Pak (3H]progesterone; New England Nuclear).

The production of β-subunit of HCG was measured in spent medium from cultures and control medium (Associated Laboratories, Inc., Wichita, Kans.).

**Steroid Receptor Assays.** Tumor cells grown to near confluency were washed with PBS and placed in RPMI Medium 1640 supplemented with 10% steroid-stripped FBS for 1 week. Cells were trypsinized and washed in PBS. Fifty million or more live cells were pelleted and frozen once at −85°. An equal volume of Tris-EDTA-dithiothreitol buffer (40 mM Tris-HCl per liter, 1.5 mM EDTA per liter, 0.5 mM dithiothreitol per liter, pH 7.4) was added, the cells homogenized at 0–4°, and the cell debris pelleted (2000 x g; 15 min). The resultant supernatant (cytosol) was divided into 4 aliquots up to 0.1 ml each and incubated for 30 min at 4° with 10^-9 M of 17β-[3H]estradiol per liter or [3H]R5020-promogestone (New England Nuclear) in the presence or absence of 10^-6 M nonradioactive hormone per liter. Samples of each mixture (20 µl) were applied to a pre-cooled, equilibrated gel (G-150 Superfine; Pharmacia Fine Chemicals, Piscataway, N. J.) that was spread to a thickness of 0.6 mm on a 20- x 40-cm glass plate. The plate was inclined at 15° in a horizontal thin-layer gel apparatus (Pharmacia Fine Chemicals) at 4° until a hemoglobin marker (approximately 50 mg protein per ml) traveled 21 cm from the origin (approximately 7.5 hr). The gel was divided into 1- x 3-cm strips, each of which was counted in 10 ml Eastman Ready-to-Use II (Eastman Kodak Co., Rochester, N. Y.). Receptor proteins (4S and 8S) were calculated on a per gram of tissue basis.

**Heterologous Transplants.** For each cell line to be transplanted, 6 young female C3H/HeJ mice were immunosuppressed with 500 rads. The mice were given injections s.c. of 10^7 viable cultured tumor cells in 0.1 ml 0.9% NaCl solution. Palpable nodules were surgically excised 9 to 14 days later and sent for histology.

**Mycoplasma Tests.** Tests for Mycoplasma (pleuropneumonia-like organisms) were made by staining slide preparations of the cultured cells with bisbenzamide after the method of Chen (2).

**Cell Freezing.** Cultured cells were preserved for the cell bank by suspending in cold RPMI Medium 1640 supplemented with 20% FBS, 12.5% dimethyl sulfoxide, and antibiotics.

**RESULTS**

**Cell Line Characterization.** Cell cultures were given cell line numbers at the first successful subculture (Table 1). All cultures were maintained in RPMI Medium 1640 (19) supplemented with 10 or 20% FBS and antibiotics. Chromosome markers were described according to Paris nomenclature (21, 22).

**Case A.** Six cell lines were established from this patient, and COLO 110 was chosen for extensive characterization. This cell line grew as a semiconfluent monolayer of epithelioid cells which often formed tightly packed colonies (Fig. 2). Intercellular bridges, perinuclear granules, and large cytoplasmic vacuoles were observed by phase microscopy. Desmosomes and tight junctions were evident in electron micrographs (Fig. 3). The highly vacuolated cytoplasm had lipid deposits, dilated endoplasmic reticulum, and numerous mitochondria with dilated, often parallel, cisternae. Nuclei had diffuse chromatin.

Chromosome analyses of COLO 110 were made in 1975, 1976, and 1978. In 1975, approximately 70% of the metaphases had 65 to 68 chromosomes. Banding was poor and markers were occasionally evident. In 1976, the mode was 74; 50% of the metaphases had chromosome counts in the range of 70 to 80. The markers observed were:

$$
M_1: \{k(1;6)(xpter\rightarrow cen\rightarrow6pter) \}
M_2: \{k(1;7)(xpter\rightarrow1q43::?) \}
M_3: del(1)xpter\rightarrowq22::)
M_4: del(3)xpter\rightarrowp13::)
M_5: del(6)xpter\rightarrowq25::)
M_6: del(7)xpter\rightarrow7p11::?)
M_7: del(21;?)x21peter\rightarrow21q22::?)
$$

1 to 3 unidentified markers

A representative COLO 110 metaphase is depicted in Fig. 10. In 1978, 50 metaphases were examined and karyotyped. Markers observed in the 1976 chromosome preparations were also present in metaphases from the 1978 harvest. M_1, M_2, M_3,
and M2 were the most consistent markers. In many cells, only 1 or 2 markers were observed. Sixty % of the metaphases had no markers, only hyperdiploidy. Sixty-nine Giemsa-stained metaphases were surveyed for DM's, and 4 metaphases had 1 or 2 markers were observed. Sixty % of the metaphases had and M4 were the most consistent markers. In many cells, only mitochondria. The nuclei had diffuse chromatin. (Fig. 5). The cytoplasm contained numerous vacuoles, lipid interdigitation of microvilli, desmosomes, and tight junctions cultures were considered established cell lines from the day of first successful subculture. Case B. One cell line, COLO 316, was established from this patient. COLO 316 grew in cell aggregates that attached at a few points to the flask and extended into the supernatant as pseudovermiform cell masses. Pleomorphic cells with numerous large cytoplasmic vacuoles and nuclei with multiple nucleoli were observed (Fig. 6). Electron micrographs of COLO 319 were similar to those of COLO 316 with the exception of very large cytoplasmic vacuoles, dilated cisternal spaces in the mitochondria, and fenestrated nuclei (Fig. 7).

Chromosome analysis of COLO 319 was based on 60 metaphases. The line was bimodal with 67 and 69 chromosomes. Forty-eight % of the metaphases had 65 to 72 chromosomes. The analysis of the marker chromosomes was particularly difficult in most cells since marker chromosomes outnumbered normal chromosomes. Our best analysis of the marker chromosomes depicted in Fig. 12 was:

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Stage of disease</th>
<th>Tissue cultured</th>
<th>Date culture initiated</th>
<th>Date culture established</th>
</tr>
</thead>
<tbody>
<tr>
<td>COLO 110</td>
<td>Stage III</td>
<td>Serosa of Fallopian tubes, paraadnexal connective tissue and uterus</td>
<td>2/14/75</td>
<td>2/16/75</td>
</tr>
<tr>
<td>COLO 316</td>
<td>Stage IV</td>
<td>Pleural fluid</td>
<td>3/4/76</td>
<td>5/23/77</td>
</tr>
<tr>
<td>COLO 319</td>
<td>Stage III</td>
<td>Ascitic fluid</td>
<td>8/1/76</td>
<td>2/19/77</td>
</tr>
<tr>
<td>COLO 330</td>
<td>Stage III</td>
<td>Ascitic fluid</td>
<td>5/23/77</td>
<td>9/1/77</td>
</tr>
</tbody>
</table>

Table 1

History of ovarian adenocarcinoma cell lines

The 4 cell lines have been in current culture since their date of initiation. All cultures were considered established cell lines from the day of first successful subculture.

M26 t(19;?)(19;?)
M27 t(19;?)(19;?)
M28 t(22;?)(22pter->22q13::?)
M29-46 unidentified markers
M33-35 silver stain positive for NOR's

Most of the depicted markers were observed in 70 to 95% of the metaphases. Often these markers were present in multiple copies; occasional variants of the markers were observed. DM's were observed in 31% of the metaphases. Most of the metaphases had 1 DM; more than 50 DM's were observed in one metaphase. In most metaphases, 3 to 5 NOR's were observed. Translocated NOR's were present on M33, M34, and M35 (Fig. 14).

Case C. One cell line, COLO 319, was established from this patient. COLO 319 grew in cell aggregates that attached at a few points to the flask and extended into the supernatant as pseudovermiform cell masses. Pleomorphic cells with numerous large cytoplasmic vacuoles and nuclei with multiple nucleoli were observed (Fig. 6). Electron micrographs of COLO 319 were similar to those of COLO 316 with the exception of very large cytoplasmic vacuoles, dilated cisternal spaces in the mitochondria, and fenestrated nuclei (Fig. 7).
With few exceptions, the depicted markers were consistently observed in different metaphases. One to 9 DM's were observed in more than 10 per metaphase. The chromosome analysis of COLO 330 was based on 50 metaphases. The chromosome mode was 57. Sixty-one percent of the metaphases had 52 to 60 chromosomes. The chromosome analysis was difficult because the cells did not readily dissociate. Hence, most metaphases were near or in cell clumps, and the chromosome morphology was poor in these areas. Our analysis was difficult because the cells did not readily dissociate. Hence, most metaphases were near or in cell clumps, and the chromosome morphology was poor in these areas. Our best analysis of marker chromosomes depicted in Fig. 13 was:

- M1: del(1)(pter→cen-2)
- M2: t(1;2)(1pter→cen;2pter)
- M3: t(1;9)(1pter→1p11::9q34→9pter)
- M4: t(2;2)(2pter→cen-2)
- M5: del(2)(2pter→p2?:)
- M6: del(3)(3pter→cen-3)
- M7: del(3)(cen-3)
- M8: del(4)(4pter→q1?)
- M9: del(5)(5pter→cen-5)
- M10: del(6)(6pter→q2?:)
- M11: del(7)(7pter→cen-7)
- M12: del(8)(8pter→cen-8)
- M13: del(9)(cen-9)
- M14: del(11)(11pter→cen-11)
- M15: del(11)(cen-11)
- M16: t(12;13)(12pter→13q21:)
- M17: t(12;13)(12pter→13q21:)
- M18: t(7;7)(qter→p2?:)

No CEA production was detected in any of the cell lines. COLO 110 was tested for CEA 4 times at yearly intervals; COLO 316, COLO 319, and COLO 330 were tested for CEA twice.

Tumor nodules developed in the C3H/HeJ mice given injections of the respective cell lines. In all cases, the pleomorphic cells, occasional giant cells, gland formation, and frequent mitoses that comprised the tumors were consistent with a diagnosis of serosal adenocarcinoma (Fig. 1).

Cell lines, negative for pleuropneumonia-like organisms, were frozen for the cell bank and successfully retrieved for active culture.

DISCUSSION

Ovarian carcinomas are common and deadly. Established, well-characterized human ovarian tumor cell lines are valuable for studies of nutritional requirements and responses to hormones and hormone antagonists, chemotherapy, and radiotherapy. Patterns of chromosomal abnormalities may reveal shared characteristics, whereas the search for common tumor antigens would have both theoretical and practical usefulness.

The characterization of the 4 ovarian carcinoma cell lines indicated that they were derived from tumor cells. This conclusion was based on morphological similarity to malignant ovarian cells (12), presence of numerical and structural chromosome abnormalities, and growth of tumor nodules in heterologous hosts. The absence of CEA in our cell lines derived from serous ovarian carcinoma tissue is in accordance with the studies of Marchand et al. (16) who reported CEA was present only in mucinous tumors of ovarian origin.

Production of estrone by 3 of our cell lines reflects the hormonal function of their tissue of origin (4). Few malignant human cell lines have been tested for hormone receptors. Horwitz et al. (11) reported no consistent pattern of estrogen and progesterone receptor proteins in 9 breast carcinoma cell lines.
lines. The lack of estrogen and progesterone receptors in our 4 cell lines contrasts with the detection of estrogen receptor proteins in some fresh ovarian carcinoma tissues studied by us. The cells from those patients did not adapt to growth in vitro.

HCG in sera and effusions has been reported as a marker of tumor burden in ovarian carcinoma patients (7,8). None of our 4 lines elaborated detectable amounts of HCG under standard culture conditions. The addition of sodium butyrate to cultured ovarian cells might stimulate HCG production as was reported by Kanabus et al. (14).

Since cell lines are established from a small proportion of tumor specimens, it is difficult to obtain in vitro an accurate reflection of the in vivo diversity within specific tumor types. We have described the characteristics of a cell line derived from a primary tumor specimen and 3 cell lines derived from pleural effusions. The growth characteristics of COLO 110 and COLO 316 were similar to previously reported cell lines derived from ovarian carcinomas (5,9). COLO 319 and COLO 330, on the other hand, did not form the typical layers of epithelial cells, but rather formed aggregates of cells in the supernatant. The variability in growth patterns and morphologies may simply reflect a difference in the level of cellular differentiation. However, processes of in vitro selection from the original heterogeneous cell population may also account for the morphological variations, or the cell lines may represent different subclasses of adenocarcinomas of the ovary which are not detectable by light microscopy alone. Such subclassifications of tumors may be important in diagnosis or selection of therapy. This has been clearly demonstrated in the case of breast carcinomas that are indistinguishable by morphological criteria but have variable susceptibility to hormone antagonists.

Isozyme "fingerprinting" is a useful tool to identify both the possibility of intraspecific cell line contamination and the uniqueness of a cell line based on the genetic diversity in man (6,23). COLO 110, COLO 316, COLO 319, and COLO 330 are distinguishable based on their isozyme phenotypes.

Cytogenetic analysis also provides an excellent means of identifying specific cell lines. We are not aware of any other cell lines with complements of chromosome markers similar to these 4 cell lines.

In addition to utilizing cytogenetic analysis for identification, we were interested in assessing nonrandom patterns of chromosome involvement in markers. All 4 cell lines had markers involving Chromosomes 1, 3, 6, and 7. Chromosomes 2, 4, 5, 9, 11, and 15 were involved in markers of at least 3 of 4 cell lines. Based on the analyses of our 4 cell lines and on reports by Freedman et al. (9), Kakati et al. (13), and Tiepolo and Zuffardi (28), Chromosome 1 appears to be present frequently in markers of ovarian carcinomas. Chromosome 1 has been most often reported in abnormalities associated with malignant cells (3,13,24,25,29).

A specific chromosome rearrangement, an i(q9), reported by Freedman et al. (9) in an ovarian carcinoma cell line was similar to M10 of COLO 316 and to M13 of COLO 330. Possibly the t(17q;21) in the same report is similar to M25 of COLO 319. Comparisons of specific chromosome aberrations with published reports are lacking because complete karyotypic analyses are lacking.

The presence of DM’s was noted in 6% of 75 metaphases from COLO 110 and in 27 to 31% of the other 3 cell lines. Hamburger et al. (10) reported DM’s in cultures from effusions of 2 patients with ovarian carcinoma. It has been our observation that DM’s are ubiquitous, but greatly variable in number, in human tumors and tumor cell lines.

The M29 and M30 chromosome markers of COLO 319 have possible HSR’s. Recent reports imply that true HSR’s are associated with gene amplification (1,26). The development of more discriminating means of analyses are necessary before we can assess the nature of these peculiar chromosome aberrations.

The 4 ovarian carcinoma cell lines described in this report should provide a valuable resource and are available to other investigators.

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REFERENCES

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Fig. 2. Case A, COLO 110. Colony formation of cells. Phase microscopy. × 320.
Fig. 3. Case A, COLO 110. Cells with cytoplasmic vacuoles, lipid deposits, and dilated endoplasmic reticulum. Electron microscopy. × 3600.
Fig. 4. Case B, COLO 316. Cells in acinar formation. Phase microscopy. × 320.
Fig. 5. Case B, COLO 316. Cells with desmosomes and bizarre mitochondria. Electron microscopy. × 14,800.
Fig. 6. Case C, COLO 319. Colony formation. Phase microscopy. × 320.
Fig. 7. Case C, COLO 319. Cells with highly vacuolated cytoplasm. Electron microscopy. × 4000.
Fig. 8. Case D, COLO 330. Vermiform formation of cell colonies. Phase microscopy. × 320.
Fig. 9. Case D, COLO 330. Cells with multiple intercellular junctions. Electron microscopy. × 4050.
Fig. 10. A karyotype of a cell from COLO 110 (Case A) with G-banded normal and marker chromosomes.

Fig. 11. A karyotype of a cell from COLO 316 (Case B) with G-banded normal and marker chromosomes.
Fig. 12. A karyotype of a cell from COLO 319 (Case C) with G-banded normal and marker chromosomes.

Fig. 13. A karyotype of a cell from COLO 330 (Case D) with G-banded normal and marker chromosomes.
Fig. 14. a, Giemsa-stained metaphase of COLO 316. Solid arrows, translocated NOR's and satellites; Open arrow, DM; b, AgNO3-stained metaphase of COLO 316. Solid arrows, translocated NOR's.
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