Establishment and Characterization of a Human Ovarian Neoplastic Cell Line, DO-s

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ABSTRACT

A permanent human neoplastic cell line, DO-s, was established from ascites of a patient with a well-differentiated mucinous cyst-adenocarcinoma of the ovary. This cell line grew as vermiciform, floating colonies of epithelial cells in culture. The karyotype of DO-s was of a human female; the chromosome number ranged from 54 to 66 with several abnormalities, mainly trisomy. Epithelial-like character was confirmed by transmission electron microscopy and by the presence of cytokeratin. Inoculation of DO-s cells i.p. or s.c. in athymic nude mice resulted in, respectively, ascites and xenografts. Light and electron microscopical analysis of cultured cells and xenografts demonstrated that the cell line was derived of a mucinous adenocarcinoma biopsy. Tumor-associated antigens, cancer antigen 125 (CA 125), human milk fat globulin, and human placental alkaline phosphatase were expressed by cells in culture and in xenografts. Modulation of the antigens, CA 125 and human milk fat globulin, occurred in DO-s cells growing in athymic mice. Biochemical, immunohistochemical, and histochemical analysis showed that more than 50% of the alkaline phosphatase isoenzymes present in DO-s cells had the characteristics of human placental alkaline phosphatase and placental alkaline phosphatase-like alkaline phosphatase (AP), but fractions of intestinal AP and nonspecific AP (bone-liver-kidney) were also present. The expression of AP isoenzymes could be induced by an enhancement of the serum supplement in the culture media, and by dexamethasone, sodium butyrate, and bromodeoxyuridine. This line will be a valuable tool in studying the therapeutic effects of antibodies to tumor-associated antigens or other agents for ovarian cancer.

INTRODUCTION

Ovarian carcinoma cells propagated in long term culture are valuable for studies on the biology of human ovarian cancer. There is currently considerable interest in the use of in vitro models for the study of monoclonal antibodies to tumor-associated antigens as a tool for cancer treatment. In ovarian cancer, tumor-associated antigens as CA 125, HMFG, and HPLAP have clinical potential for detection and therapy. Antibodies to these antigens have already been used in clinical trials on patients with ovarian cancer (1-3). However, in order to realize the potential of the monoclonal antibodies to tumor-associated antigens as CA 125 and human milk fat globulin, the properties, cellular ultrastructure, neoplastic behavior in vivo in athymic nude mouse, and the expression of tumor-associated antigens. Since HPLAP is one of the few biologically well-defined tumor-associated antigens (4), this antigen was investigated in greater detail.

MATERIALS AND METHODS

Initiation and Propagation of Cell Line. Malignant ascites from a patient with a well-differentiated mucinous cyst-adenocarcinoma of the ovary (January 1985) was the source of the cells used to initiate the DO cell line. Ascites was diluted in Dulbecco's modified Eagle's MEM (GIBCO BRL, Grand Island, NY) supplemented with 15% fetal calf serum (FCS) and in RPMI 1640 supplemented with 6% FCS; antibiotics were not added to the culture medium. No attempt was made to separate the malignant cell clumps from ascites fluid, lymphocytes, macrophages and red blood cells. After 3 days, the nonadhering cell clumps were collected in a conical centrifugation tube, and were allowed to sediment. The sediments were dispersed in growth medium and added to culture flasks. The cultures were left untended until confluent, and were subcultured at split ratios of 1:2 to 1:10. After four to five passages, the FCS supplement of the growth medium was reduced. For adhering epithelial cells Dulbecco's MEM supplemented with 10% FCS was used. The nonadhering cells were less dependent on the serum supplement and could be cultured in growth medium with a serum supplement from 2 to 15%. Cells for freezing were suspended in growth medium containing 10% dimethyl sulfoxide and 10% FCS. After programmed freezing, the cells were transferred to liquid nitrogen.

Growth Parameters. The rate of cellular proliferation was measured for cultures in logarithmic growth phase in the range of 5 x 10^4-10^6 cells/ml. Population doubling times were determined from cell or nucleus counts of four culture flasks obtained at daily intervals (7).

For chromosome analysis, 2 h after addition of colcemid (0.5 μg/ml) cells were harvested and incubated in 75 mm potassium chloride at room temperature. Ten min later they were fixed with 25% acetic acid in anhydrous methanol. Slides were stained with 10% Giemsa, and the karyotype was determined on 50 metaphase spreads (8).

Cell cycle phase distribution analysis and DNA-ploidy measurements were performed with a cytofluorograf 50-H (Ortho instruments, Westwood, MA) flow cytometer. Nuclei were isolated and DNA stained according to the method of Vindelov et al. (9). At least 10,000 nuclei were analyzed in each sample.

To analyze tumorigenicity, DO-s cells were harvested, and the cells obtained by centrifugation (10 min, 150 x g) were suspended in growth medium (1 to 5 x 10^7 cells/ml). Amounts of approximately 0.1 ml were injected i.c. into the area of the left hind leg, or i.p. of 6-20-week-old athymic Swiss nu/nu mice (Iffa Credo, France). Tumors growing s.c. were measured weekly, when they achieved a size of 3-5 mm in diameter, using graduated calipers. Growth curves were expressed as tumor size versus time, where the area (mm^2) was calculated using the formula for an ellipse.

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2 Department of Nephrology and Hypertension, University Hospital Antwerp, Wilrijkstraat 10, B-2520 Edegem, Belgium. The abbreviations used are: CA, cancer antigen; AP, alkaline phosphatase; CEA, carcinoembryonic antigen; EM, electron microscopy; FCS, fetal calf serum; HMFG, human milk fat globulin; HPLAP, human placental alkaline phosphatase; MEM, minimal essential medium; PAS, periodic acid-Schiff; RER, rough endoplasmic reticulum.
OVARIAN NEOPLASTIC CELL LINE. DO-s

Fig. 1. A, ascites smear of DO-s patient; May-Grünwald Giemsa, × 350; B, morphological features of DO-s cells; arrow, signet cell, × 300; C, ascites smear originating from a athymic mouse injected with DO-s cells; i.p. May-Grünwald Giemsa. × 350. Bar, 50 μm.

with d₁ and d₂ representing the maximum diameters of the tumors measured at right angles (10).

Morphology. Cells growing in tissue culture flasks were examined by standard light microscopy. Cells in suspension were prepared as blood smears or fixed and embedded in paraplast prior to staining (Shorr's stain and Carazzi's haematoxilin and eosin, H & E). Papanicolaou, periodic-acid-Schiff, May-Grünwald Giemsa, and Alcian blue stains were used occasionally. Ultrastructural analysis was accomplished by transmission EM on cells fixed (3%, v/v, glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, containing 0.1% CaCl₂, postfixed (2%, v/v, osmiumtetroxide in 0.1 M sodium cacodylate, pH 7.4), and embedded in EPON. Tumor tissue (3 mm³), obtained from xenografts (see below), was fixed and embedded in the above combination of materials prior to examination by EM.

Immunohistochemistry. The distribution in cultured cells and in xenografts of HPLAP (17E3, supernatant 1:80; E6, supernatant 1:80, Dr. W. Fiers, Department of Molecular Biology, State University of Ghent, Belgium), carcinoembryonic antigen (CEA; purified 1:100000, 36079 Hybritech), HMFG (HMFG 1 & 2, supernatant 1:100 & 1:50, Dr. Taylor-Papadimitriou, Imperial Cancer Research Fund, London, UK), CA 125 (OC125, ascites 1:3000, Dr. R. Bast, Department of Medicine, Duke University, Durham, NC), CA 19-9 (111NS 19-9, supernatant 1:80, Dr. H. Koprowsky, The Wistar Institute, Philadelphia, PA), and intermediate filament proteins (anti-cytokeratin No 18, 1:1, 814385; Boehringer Mannheim, DBR; DAKO-CK-1, 1:5, M717; DAKO-Desmin, 1:10, M725; DAKO-Vimentin, 1:5, M725; Dakopatts, Santa Barbara, CA) were determined if possible in adjacent sections. The immunohistochemical staining procedure has been described in detail elsewhere (11).

Immunohistochemistry at the ultrastructural level (CA 125, HMFG 2, and HPLAP; CA 19-9 was used as a negative control) was performed on the glutaraldehyde fixed cells (fixation time 30 min) in suspension by an immunohistochemical staining method described elsewhere (diamino benzidine stain (11)).

Histochemistry. AP isoenzymes were localized by the method of Gossrau (12) with 5-bromo-4-chloro-3-idoxylphosphate-p-toluidine salt as the substrate and nitro blue tetrazolium as the capturing agent. L-p-Bromotetramisole (0.5 mivi) was used as an inhibitor of nonspecific AP and L-phenylalanine (50 mivi) was used as inhibitor of intestinal AP and HPLAP; the D-enantiomers were used as controls. For temperature inhibition of nonspecific and intestinal AP, slides were incubated at 65°C in 0.1 M Tris-HCl, pH 9.4, for 90 min.

Treatment with Sodium Butyrate, Dexamethasone, and Bromodeoxyuridine. Sodium butyrate was added at a concentration of 3 mM to the growth medium. After 3 days, the cells were harvested and extracted (see extraction of AP isoenzymes). Dexamethasone, a glucocorticoid, was added at concentrations ranging from 0.13 up to 25 μM to the growth medium. Cells were cultured in the growth medium containing dexamethasone until confluency. They were harvested and extracted. Bromodeoxyuridine (5-bromodeoxyuridine) was used at a final concentration of 33 μM. The cells were harvested after 1 week.

Determination of AP Isoenzymes. For the extraction of AP isoenzymes, cultured cells were harvested (scraping), and were washed thrice with phosphate buffered saline containing Dulbecco's salts. The cells

Fig. 2. Representative karyotype of DO-s. Formula: 59,XX,+4A,+6C,+E,+F,+G. Giemsa stain.
were lysed in a hypotonic buffer (Tris-HCl 10 mM, pH 7.5, containing 10 mM sodium chloride and 10 mM sodium azide). The further extraction procedure has been described in detail elsewhere (13).

The total AP activity was determined kinetically by the method of Van Belle et al. (14). Temperature inactivation of nonspecific and intestinal AP was performed at 65°C for 15 min; at 56°C the activity of the intestinal fraction was not inhibited. The concentration of the inhibitors l-phenylalanine and l-p-bromotetramisole was, respectively, 5 and 0.01 mM.

The immunological detection of AP isoenzymes by a enzyme-antigen immunoassay has been described in detail elsewhere (13). Monoclonal antibodies to HPLAP (327, Innogenetics Inc., Ghent, Belgium; 7E8, 15) and to intestinal AP (250) were developed by our research group.

RESULTS

Primary Culture

The primary culture, DO, originated from ascites of a patient with a well-differentiated mucinous cystadenocarcinoma (January 1985; Fig. 1A). The serum (0.145 milliunits/ml) and especially the ascites (1.500 milliunits/ml) of the patient contained a high HPLAP activity. The ascitic fluid contained numbers of neutrophils, macrophages and lymphocytes, and numerous large free floating clusters of neoplastic cells were present (Fig. 1A). The primary culture produced two sublines: (a) cells of the subline DO-m formed a monolayer on the plastic surface of the culture flasks, and (b) cells of the subline DO-s remained in suspension (Fig. 1B).

Subline DO-s

The subline DO-s resembled the large free floating clusters of cells of parent ascitic fluid (Fig. 1, A & B). DO-s has been in culture for over 25 months (≥70 passages), and the culture has been cryopreserved. The cells can be cultured using RPMI 1640 or Dulbecco’s MEM, and serum supplements may range from 2 to 15% (20% was already toxic for the cells). DO-s grew in cell aggregates that attached at a few points to the culture flask and extended into the supernatant as pseudopods. (a) Cells of the subline DO-m formed a monolayer on the plastic surface of the culture flasks, and (b) cells of the subline DO-s remained in suspension (Fig. 1B).

Light microscopical analysis of sections showed that many of the clusters were not just multilayered masses of cells, but formed a lumen. The presence of intermediate filament proteins was evaluated on paraffin sections. DO-s cells were positive for cytokeratin (anticytokeratin 15, strong staining, all cells positive; DAKO-CK1, moderate staining, less than 50% of the cells) and vimentin (weak staining, less than 50% of the cells). The sections were negative for Desmin.

Fifty banded metaphases were analyzed. The number of chromosomes in the majority of the cells clustered around 54 to 66 chromosomes, and the modal number was determined as 59 (hypotriploid). A small percentage of chromosome counts clustered around 46 (4%). Unfortunately the origin of these cells could not be ascertained. The representative karyotype was consistent with that of a human female (Fig. 2). The karyotype had several abnormalities: 59XX,+4A,+6C,+E,+F,+G. Noticeable was trisomy in the A and c groups. Cell cycle analysis (Fig. 3) revealed that the DNA content was aneuploid (DNA index of 1.53) and variable as was shown by the SD of the G1/G0 peak (8.8 versus 3.9 for the diploid control), what clearly correlated with the chromosome counts. There was a dominant S-phase (22%, Fig. 3). The flow cytometric analysis indicated that a small percentage of cells had a DNA index of approximately 1. The low intensity peak below channel 50 was due to nuclear debris.

Ultrastructural Analysis of DO-s. Cultured DO-s cells contained large amounts of both mitochondria (large, ovoid or elongated) and vacuoles often very large (Fig. 4, A–E). The cells frequently contained large multivesicular bodies that appeared as spherical formations packed with numerous, round vesicles, some of which contained a dense center. The endoplasmic reticulum and the Golgi bodies were well developed. The nuclei were irregular and had usually several indentations; they had a diffuse chromatin and a prominent nucleolus. The plasma membrane facing the growth medium was covered by microvilli, but the membrane facing the lumen had very few microvilli. The cells were interconnected by frequent desmosomes (macula occludens). Tight junctions (zonula occludens) were found at the side nearest the growth medium.

Tumorigenicity of DO-s. Tumor nodules developed in athymic Swiss nu/nu mice given injections s.c. (Fig. 5). When cells were injected i.p., athymic mice developed ascites (Fig. 5); tumor nodules were only found in regions where clusters of cells became trapped. With both ways of injection there was a Lag phase (40 days for s.c.; 22 days for i.p.) before the neoplastic population began to increase significantly. Mortality occurred earlier when the cells were injected i.p. (approximately 70 days, ≥100 days when injected s.c.).

Light microscopical analysis of the xenografts showed the presence of pleomorphic cells and occasionally giant cells (Fig. 6G). Often large parts of the cytoplasm of cells, especially giant cells, were visualized as an empty space (H & E). None of the xenografts studied could clearly prove the existence of cyst
formation, nor were papillary structures obvious. Many cells were positive for PAS and Alcian blue staining. Xenografts from i.p. or s.c. origin did not differ in their morphological features. The xenografts were mostly arranged as groups of cells interspaced with stromal tissue and capillaries. The evaluated tissue resembled an undifferentiated mucinous adenocarcinoma. Ascites produced in athymic mice resembled well ascites of which the cell line was derived (Fig. 1, A and C). This ascites fluid contained also a high HPLAP activity (ranging from 0.99 up to 5.90 milliunits/ml).
Ultrastuctural analysis showed cells that were variable both in size and shape (Fig. 4, F-H). The intercellular spaces were filled by numerous microvilli and sometimes resembled intercellular canaliculari. Intracellular lumen were also present. Desmosomes, desmosome-like structures, and tight junctions were found in regions of cellular contact. Nuclei were irregular and invaginations were found, often seen as cytoplasmic inclusions. The nucleolus was often marginated, and there was a variable heterochromatin content with prominent perichromatin granules. The many mitochondria were pleomorphic. There was an abundance of polysomes; there was a fair amount of RER, and Golgi complexes were prominent. Secretory granules were numerous and appeared spherical. They were membrane bound and filled with low density, flocculent material. The cells frequently contained large multivesicular bodies that resembled spherical formations stuffed with round vesicles, some of which contained a dense center.

Localization of Tumor-associated Antigens of the Subline DO-s. DO-s was investigated for the presence of tumor-associated antigens (Fig. 6, A–F). Only CA 19-9 and CEA were not positive. DO-s did express almost homogeneously HMFG (2) and CA 125, especially the plasma membrane facing the growth medium was stained (Fig. 6, C and D). Staining for HMFG (1) was less abundant and was also only membranous. Most of the HPLAP present was membranous, although intracellular granules often stained for HPLAP. Membranous HPLAP was not only found on the plasma membrane facing the growth medium; it was also apparent on the plasma membrane between adjacent cells (Fig. 6A). Ultrastructural analysis gave the same results (Fig. 4, E and D), and showed that the membranous staining was particularly found on the microvilli of the cells (Fig. 4E).

The same distributions shown for the cell line were also found for the xenografts and for subcutances from the xenograftic material (Fig. 6, G–M, Table 1). However, CA 125 and HMFG (2) were no longer homogeneously expressed; regions of the xenografts showing strong staining for these markers were interspaced by regions that were negative. However, this was not observed for the expression of HPLAP; the distribution was fairly similar with the occurrence in vitro, and in some xenografts staining was even stronger. There was a marked decrease in expression of HMFG (1 and 2) when DO-s was injected i.p., and this decrease was retained after subcuturing this material (Table 1).

Histochemistry of AP Isoenzymes of the Subline DO-s. Histochemistry for the presence of AP isoenzymes revealed that the majority of the AP present is l-phenylalanine sensitive, and l-p-bromotetramisole resistant, or the major part of the AP isoenzymes present in DO-s cells was of the intestinal AP and HPLAP type (Fig. 7). AP staining was found on the plasma membrane as well as intracellular. Temperature inhibition at 65°C for 90 min (intestinal AP is not completely inhibited when the time of incubation is less than 40 min) showed that most of the activity present was of the HPLAP type. When temperature inhibition was combined with l-leucine inhibition, a large part of HPLAP disappeared. This result pointed to the presence of PLAP-like AP. The same results of distribution of AP isoenzymes were found for xenografts. AP staining was predominantly found on the plasma membrane and some intracellular staining was also present. The inhibition with l-phenylalanine, l-p-bromotetramisole, and l-leucine, and the temperature resistance also showed that HPLAP and PLAP-like AP were the main AP isoenzymes present.

Biochemistry of the Subline DO-s. The effect of increased temperature and specific inhibitors on the AP activity, and the reactivity of antibodies to AP isoenzymes for the AP isoenzymes of DO-s was analyzed. From these results could be concluded that the AP isoenzyme profile expressed by the DO-s cell line was not homogenous. The inhibition at 56°C (inhibition of nonspecific AP; 56%) pointed to the presence of nonspecific AP in the DO-s extracts, as was also shown by the l-bromotetramisole treatment (inhibition of nonspecific AP; 88% remaining activity), or the l-phenylalanine treatment (inhibition of intestinal AP and HPLAP; 20% remaining activity). Heating at 65°C (inhibition of nonspecific and intestinal AP) resulted in a further decrease of the AP activity (49%), showing that some activity was due to intestinal AP; this correlated well with the reactivity to the anti-intestinal AP antibody (250; 24%). The remaining AP activity at 65°C was due to HPLAP and PLAP-like AP. The PLAP-like AP activity could be demonstrated by the l-leucine sensitivity (38%). The reactivities to the anti-HPLAP antibodies (327, 33%; 7E8, 27%) were in agreement with the temperature sensitivity experiments.

In conclusion of the histochemical and biochemical analysis of DO-s cells, the major part of the isoenzymes present in these cells had the characteristics of HPLAP (and PLAP-like), but fractions of intestinal and nonspecific AP were also present.

Induction of AP Isoenzyme(s) Expression in DO-s. The subline DO-s, could be cultured in growth medium supplemented with FCS in concentrations ranging from 2 to 15%; although FCS concentrations higher than 10% resulted in a lower growth rate of the cells. Because FCS contains growth factors as well as hormones, it was necessary to establish the effect of the serum supplement on the AP expression (Table 2). When the FCS supplement was raised from 2% up to 6%, there was a marked but not significant increase of the total AP activity of the cells, and there was no specific stimulation of HPLAP.

DO-s cells were cultured with varying concentrations of the glucocorticoid, dexamethasone, and were harvested when confluent (10–14 days). The results of these experiments (n = 5) are shown in Fig. 8. There was an optimal concentration of 0.64 μM, and the increase from 0.13 up to 0.64 μM was significant (P < 0.01). The decrease thereafter was obvious although it was not significant. Dexamethasone seemed to induce preferentially HPLAP (Table 2), however it had no effect on the l-leucine sensitivity of this AP fraction (Fig. 8).

When DO-s cells were harvested 72 h after a treatment with 3 mM of sodium butyrate, the total AP activity was clearly increased (4-fold) as compared with the control (Table 2). There was a marked increase of HPLAP. l-Leucine inhibition of the
heat stable AP (control, 0.073 ± 0.015 units/mg protein; 3 mM sodium butyrate 0.337 ± 0.058 units/mg protein) showed a significant difference in inhibition of the heat stable AP between the controls (29 ± 4.5%) and the treated cell cultures (40 ± 3%). Sodium butyrate seemed to induce the total AP activity including HPLAP but also to have some preferential stimulation of the L-leucine sensitive fractions.

DO-s cells were cultured for 1 week in growth medium supplemented with 33 μM bromodeoxyuridine. A large increase in total AP (14-fold) was observed in the treated cultures as compared with the controls. The same relative increase was found for HPLAP (15-fold). L-Leucine treatment of this fraction pointed to a not significant decrease of the PLAP-like fraction (control 34 ± 1% versus treated 27 ± 4%).

Immunohistochemical and histochemical staining of treated and nontreated cell cultures showed that the increase in AP activity was not only due to an enhancement of the AP production in cells that already expressed AP isoenzymes, but that there was also an increase of the population of AP isoenzyme-positive cells.
Table 1  Immunohistochemical analysis of tumormarkers in the tumor cell line, DO-s

Light microscopical analysis was performed on paraffin sections using an indirect avidin-biotin-peroxidase complex staining with 3-amin-9-ethylcarbazole as a substrate (counterstaining with methyl green).

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* Route of administration.

DISCUSSION

The cell line, DO-s, has been maintained in culture for more than 2 years. It was derived from ascites of a patient with a mucinous cystic adenocarcinoma of the ovary. Its morphology, ultrastructure, chromosomal picture, the expression of tumor-associated antigens, and the tumorigenicity in athymic nude mice indicated that the cell line consists of malignant ovarian neoplastic cells.

The use of cells present in body fluids as a starting material for cell culture provides many technical advantages, and has already been used for the establishment of several ovarian cancer cell lines (16–18). However, body fluids contain mesothelial cells which are difficult to discern from malignant epithelial cells by conventional cytology (19). As for fibroblasts, mesothelial cells tend to attach faster to the plastic surface of the culture flask, and the elimination of the mesothelial cells is possible by a serial passage of free-floating cells (19, 20). The application of this technology resulted in two sublines, a monolayer forming line and a line that consisted of cells growing in clusters. The suspension culture DO-s excluded by its way of growing a fibroblastic or mesothelial origin. Ultrastructurally, the presence of microvilli, desmosomes, and tight junctions, and the presence of the intermediate filament protein, cytokeratin, confirmed the epithelial character of the DO-s cells (21–23).

Ultrastructural features were observed that were consistent with a cell culture derived from a mucinous adenocarcinoma based on descriptions by loachim et al. (21) and Fenoglio (22). These characteristics are: cells containing large amounts of mitochondria, an apical margin covered by microvilli, a well-developed RER, Golgi complexes, and large multivesicular bodies. The morphology of the xenografts did not resemble the tumor of origin; it did resemble a poorly differentiated carcinoma. We were not able to show the existence of cyst formation in the xenografts, and papillary structures were also not obvious. The presence of tight junctions and desmosomes were a clear indication of adenocarcinoma (23) and PAS and Alcian blue positivity as well as the EM features (22, 24) pointed to a mucinous type.

The use of tumor-associated antigens for diagnosis of certain cancer types, imaging of tumors, and cancer therapy is a major issue of debate in the oncological research field. However, only a few studies have been performed on the expression and comparison of different cell surface antigens and tumor markers in neoplastic epithelial cell lines and xenografts (25–28). In this
study we used a panel of tumor markers; CA 125, HMFG, and HPLAP were expressed by the cell line DO-s. However, the expression of other antigens may have been modulated by \textit{in vitro} culture, as was shown by Friedman et al. (27) for primary cultures of human normal and neoplastic epithelial cells. Unfortunately we were not able to collect enough ascites or tumor tissue of the patient to determine immunohistochemically the presence and distribution of the different antigens on the original material.

In DO-s, the expression of the different tumor-associated antigens could not readily be attributed to different cell types. CA 125 and HMFG (2) were quite homogenously distributed over the plasma membrane of most cells, so that at least the great majority of the cells express both antigens. It could not be ascertained if HMFG- and CA 125-positive cells also experienced HPLAP, but the homogenous distribution of HMFG indicated that HMFG-positive cells could also express HPLAP. Multiple tumor-specific antigens have been shown to be expressed by a single tumor cell (29). The same antigens were present in xenografts. However here, CA 125 and HMFG were no longer homogenously distributed. Therefore, it is plausible that, in view of the homogenous expression in cell culture, modulation of CA 125 and HMFG occurred. However, this was not so for the expression of HPLAP, although it has been demonstrated for other models (30, 31). Individuality of xenografts in and between different animals was also clearly present, this is in agreement with studies on human neoplasms where heterogeneity and individuality in tumor and secondaries was clearly shown to be present (32, 33). The expression of these different tumor-associated antigens makes this cell line an ideal tool for the study of their clinical value. To our knowledge this is the first ovarian neoplastic cell line that has been shown to express CA 125, HMFG, and HPLAP. It enables comparison of the use of antibodies to different markers as well as the selection of monoclonal antibody “cocktails” for antibody-mediated imaging of tumors or for eradication of neoplastic cells, and allows to investigate the effect of cellular heterogeneity and antigen modulation when antibody mediated imaging or therapy is tried (5).

The AP activity contained within DO-s cells is composed of several isoenzymes. HPLAP forms the main component and small amounts of intestinal and nonspecific AP are also present. The tissue sensitivity of this cell line indicates that part of the heat-stable form is PLAP-like AP. We were not able to determine the adult or fetal type of intestinal AP. It seems that most of the AP isoenzyme expressing neoplastic cells cultured \textit{in vitro} do not solely express one AP isoenzyme-type, but do express different types (25, 34). The AP-isoenzyme profile of DO-s corresponds well with what is found for JEG-3 cells (34). The main difference with the JEG-3 cell line is the AP inducibility by glucocorticoids of DO-s cells. In JEG-3 cells, an induction of AP activity due to glucocorticoids could not be demonstrated, whereas this did occur when sodium butyrate or bromodeoxyuridine were added to the culture medium (35). Therefore, DO-s cells seem to have a membranous steroid receptor which is lacking in JEG-3 cells. A primary action of these compounds lies in blocking the cell cycle in a specific phase by an action on the genome (6), for sodium butyrate the late G1-phase (36) and for dexamethasone the G0,G1-phase (37). This effect is accompanied by a marked increase not only of AP-isoenzymes but also of other proteins (38, 39), by a decrease of synthesis of certain other proteins (40), and even by an induction of a cytoarchitectural reorganization (41, 42). By their combined activity in arresting cells in the G1-phase and increasing the HPLAP expression, glucocorticoids and butyrate may prove valuable tools for the therapeutic treatment of cancer patients in combination with cytostatica (37, 43).

The tumorigenicity of the DO-s cell line, the production of ascites after i.p. injection in athymic animals, and especially the expression of several putative tumor markers suggest that the model may be useful for the evaluation of therapeutic effects of antibodies alone, conjugated to radioisotopes, toxic agents, factors that activate the immunological defence and other therapeutic agents (44–46). The sensibility of DO-s cells for steroid hormones suggests its relevance for the evaluation of hormonal and antihormonal therapy (e.g., Ref. 47). Because DO-s cells grow in large clusters with often a luminal space, a threedimensional model with typical cell-cell interactions (e.g., tight junctions, desmosomes) and a microenvironment that simulates conditions in microregions of tumors or micrometastatic foci is obtained (48). Therefore, the multicellular DO-s model intermediates between standard monolayer or suspension cultures \textit{in vitro} and tumor cells \textit{in vivo}.

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