Malignant Behavior and Resistance to Cisplatin of Human Ovarian Carcinoma Xenografts Established from the Same Patient at Different Stages of the Disease

Gabriella Masazza, Valeria Lucchini, Antonella Tomasoni, Fedro Peccatori, Vito Lampasona, Giovanni Giudici, Costantino Mangioni, Andrea Biondi, and Raffaella Giavazzi

INTRODUCTION

Ovarian cancer is a major cause of female cancer deaths, yet little is known of the progression of this disease (1, 2). Ovarian carcinoma is responsive to a variety of chemotherapeutic agents, cisplatin and its analogues being among the most active. However, despite the success of chemotherapy the remission which results from treatment is often short and after relapses patients are usually resistant to subsequent chemotherapy.

Primary resistance has been studied with human ovarian tumor lines derived from patients who responded differently to therapy (3–5). In addition, experimental models have been developed to study acquired resistance in ovarian cancer (6). With few exceptions drug-resistant tumor lines have been developed in laboratories by continuous or intermittent exposure to a single drug (6–8). Although these cell lines are considered representative of the phenomenon of acquired resistance and have been a useful tool for studying mechanisms associated with drug resistance, it is questionable whether the high level of drug resistance obtained by the selection in vitro is indeed representative of the behavior in patients.

ABSTRACT

Three human ovarian carcinoma lines (HOC8) derived from the same patient before (P-HOC8) and after (R-HOC8 and Y-HOC8) cycles of chemotherapy were established i.p. in nude mice. The biological characterization showed that these tumor lines had various features in common. Cytological and histopathological characteristics and the expression of tumor-associated antigens OC125 and MOV18 were maintained in the three variants and were comparable to the patient's primary tumor. The HOC8 variants were aneuploid with a chromosome mode number of 80–81.

All three tumor lines grew better i.p. than s.c. in nude mice. After i.p. injection the HOC8 lines produced ascites in all the mice, infiltration of pancreas, liver, diaphragm, and lung metastases. The sensitivity to cisplatin was evaluated for HOC8 lines growing in nude mice and mirrored the clinical development of resistance. Treatment with cisplatin of mice transplanted i.p. with P-HOC8 (obtained before the patient received chemotherapy) resulted in a significant increase in survival time; the R-HOC8 and Y-HOC8 lines (obtained after chemotherapy) were less sensitive.

HOC8 xenografts, which represent the course of a single patient's disease, are a useful model for investigating the development of drug resistance in ovarian carcinoma.

In this report we describe the establishment in nude mice of three tumor lines obtained from a patient with ovarian carcinoma before any treatment and after three cycles of chemotherapy. The changing response to DDP1 was maintained in the tumor lines transplanted in nude mice.

Patients with advanced ovarian cancer develop ascites and tumor disseminating the peritoneum that becomes the target of therapy (2). Since it was first demonstrated that human tumors can grow in nude mice several attempts have been made to establish animal models that mimic the malignant pathogenesis of human disease (9, 10). Human ovarian carcinoma xenografts that grow i.p. into nude mice, causing ascites and carcinomatosis, have been described (11–15) and recently we have shown that i.p. growth can be used to define the malignancy of ovarian tumor lines (11). Major phenotypic changes may occur during neoplastic progression, so we analyzed a number of parameters including growth pattern, histology, karyotype, and antigen expression, in addition to the metastatic behavior of the three HOC8 lines established i.p. in nude mice.

MATERIALS AND METHODS

Patient's History. P-HOC8 was derived in 1986 from a malignant pleural effusion removed from a previously untreated 58-year-old patient with serous ovarian adenocarcinoma, FIGO grade 3, Broder's grade IV, FIGO stage IV. The patient received six courses of carboplatin-based chemotherapy as single agent, for a total of 2346 mg/m². Second look laparotomy showed a partial response with a residual 2-cm nodule in the pelvis. Second line chemotherapy consisted of six cycles of epi-DX, CTX, and DDP with total delivered doses of, respectively, 306, 3800, and 320 mg/m². After complete remission of the pelvis nodule was achieved the patient remained disease free for 1 year. In 1988 she relapsed with ascites and a s.c. 3- x 4-cm nodule. R-HOC8 was derived from the peritoneal effusion at this time. The patient underwent 12 more cycles of epi-DX, CTX, and DDP with total delivered doses of 416, 3166, and 660 mg/m², respectively, achieving another apparent clinical complete response for 6 months. In 1989 the patient had an abdominal relapse with ascites, which was the source of Y-HOC8. She died of her disease at the end of 1989.

Animals. Female Ncr-nu/nu mice obtained from the Division of Cancer Treatment, National Cancer Institute (Frederick, MD) were used when they were 6–8 weeks old. Throughout the experiments, mice were housed in laminar flow racks under specific-pathogen-free conditions. Mice received proper care and maintenance in accordance with institutional guidelines.

Establishment of HOC8 Lines. Ascites and pleural effusions from the patients were centrifuged, washed three times, and then resuspended in HBSS. An accurate count was not possible, because of cell clumps; thus an approximate number of 10 x 10^6 tumor cells, shown to be

Received 6/3/91; accepted 9/24/91.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This research was supported by grants from the Italian Association for Cancer Research, Milan, Italy, the National Research Council, Rome, Italy, and the Developmental Therapeutics Program DCT, NCI. Drs. A. Biondi and G. Giudici are supported by Fondazione Tettamanti.

2 To whom requests for reprints should be addressed, at Istituto di Ricerche Farmacologiche Mario Negri, Via Gavazzeni 11, 24100 Bergamo, Italy.
were unsuccessful. Stocks of HOC8 cell lines at different passages in
of 10 × 10^6 tumor cells i.p. Portions of such cell suspensions were
were abdominal distension and palpable tumor masses in the peritoneal
with calipers and the tumor weight was calculated from length × width^2/
diameters of tumors growing s.c. were measured in mm once a week
with calipers and the tumor weight was calculated from length × width^2/

s.c. Growth. Ascitic effusions were aseptically collected from the
peritoneum of nude mice, washed three times, and resuspended in
HBSS. One and 10 × 10^6 tumor cells in suspension, shown to be viable
by trypan blue exclusion, were then injected s.c. in 0.2 ml HBSS. The
diameters of tumors growing s.c. were measured in mm once a week
with calipers and the tumor weight was calculated from length × width^2/

i.p. Growth. Ascites-derived tumor cell suspensions were injected i.p.
into nude mice at concentrations of 1 and 10 × 10^6 cells in 0.2 ml
HBSS. Mice were monitored twice a week; criteria for growing tumors
were abdominal distension and palpable tumor masses in the peritoneal
cavity. Mice were killed and autopsied when they became moribund or
had an evident heavy tumor burden. To study the malignant behavior
liver, pancreas, spleen, kidney, diaphragm, lungs, and all other organs
suspected of containing metastases were processed for histological
examination.

Cytohistopathological Studies. Xenografts of tumor tissue and organs
involved in metastasis were fixed in 10% phosphate-buffered formalin,
embedded in paraffin, cut in 5-μm-thick sections, and stained with
hematoxylin and eosin by a standard technique. All specimens from
patient and xenograft samples were classified by routine histopatholog-
ical examination according to the FIGO classification for ovarian
tumor. Cell suspensions from ascites were cyt centrifuged, fixed with
Spray fix (Novachimica, Milan, Italy), and stained according to Papan-
icolau. For immunohistochemical analysis cyt centrifuged samples
were acetone fixed and analyzed by the avidin-biotin technique for
cytokeratins (Dako, Golstrup, Denmark), vimentin (Dako), OC125,
and Mov18 (Cis, Siena, Italy).

DNA Analysis. High-molecular-weight DNA was extracted from the
patient's primary tumor and HOC8 ascites, according to a standard
protocol, and digested to completion with EcoRI (Bethesda Research
Laboratories BRL, Gaithersburg, MD), size-fractionated by electropho-
resis through a point 8% agarose gel, and transferred to a nylon
membrane (Hybond-N\(^+\) plus; Amersham). Prehybridization, hybridi-
zation, and washing were done as described previously in detail (16).
To detect RFLPs, PAW 101 (17), a probe that recognizes a highly
resistance through a point 8% agarose gel, and transferred to a nylon
membrane (Hybond-N\(^+\) plus; Amersham). Prehybridization, hybridi-
zation, and washing were done as described previously in detail (16).
To detect RFLPs, PAW 101 (17), a probe that recognizes a highly

RESULTS

Characteristics of the Cell Lines. Three cell lines (P-HOC8,
R-HOC8, Y-HOC8) derived from a patient with ovarian carcino-

Table 1. Characteristics of HOC8 xenografts

<table>
<thead>
<tr>
<th>Tumor origin</th>
<th>Antibody recognition</th>
<th>Chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OC125</td>
<td>MOV18</td>
</tr>
<tr>
<td>Patient</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>P-HOC8</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>R-HOC8</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Y-HOC8</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Analysis was done on ascites from the patient and from nude mice.
+ + no staining; +, positive staining.
* Fifty metaphases; n.v., not evaluated.
growth i.p., the s.c. injection of the same concentration of cells (10 x 10^6) produced a slow growing tumor and only in 67–83% of the injected mice. The doubling time of the s.c. growing tumor was not significantly different among the lines (Table 2). The growth rate s.c. was no faster after transplanting tumor cell suspensions obtained from late passages in nude mice. The tumor growing s.c., examined histologically, showed viable neoplastic cells with few mitosis (data not shown). Although the growth behavior was not significantly different for the HOC8 lines, a tendency for a higher growth rate was in general observed for the Y-HOC8 lines.

Malignant Behavior of HOC8 Lines in Nude Mice. Representative results of the malignant behavior of the HOC8 lines growing i.p. in nude mice is shown in Table 3 and Fig. 2. Autopsies were done when mice were moribund. HOC8 lines produced ascites in all mice. Tumor deposits were grossly visible on liver, pancreas, and diaphragm in almost all animals. The ovaries were sometimes involved, but never the kidneys or spleen. Mesenteric lymph nodes were often involved. Histological examination revealed invasive tumor cells with marked anaplastic features such as nuclear atypia (Fig. 2). Gross lung metastases were never observed, but histological analysis indicated lung metastases in almost all the mice, often endolymphatic metastases (Fig. 2D). There were no significant differences in metastatic capacity in nude mice for the three HOC8 tumor variants (Table 3) and the metastatic behavior was maintained at different passages in nude mice.

All mice bearing sc tumors were autopsied and no gross metastases were observed.

Drug Sensitivity to DDP. The sensitivity of HOC8 variants growing i.p. to DDP is shown in Table 4. A single dose of 5 mg/kg was sufficient to delay tumor growth in mice bearing the P-HOC8 (ILS 30%) and the survival time (ILS 150%) was significantly prolonged after three weekly treatments of 2.5 mg/kg. R-HOC8 and Y-HOC8 variants treated with 5 mg/kg DDP were unresponsive, while the repeated treatment with 2.5 mg/kg induced an ILS of 66 and 21%, respectively, for R-HOC8 and Y-HOC8. The differences in sensitivity was maintained for all three cell lines (P-HOC8 > R-HOC8 > Y-HOC8) when drug sensitivity was tested at a comparable passage and growth rate in nude mice (data not shown).

DISCUSSION

The model of ovarian HOC8 variants described here is representative of the clinical history of patients with ovarian carcinoma. After an initial good response to chemotherapy, often resulting in complete remission, patients with ovarian carcinoma relapse and further chemotherapy is less or completely ineffective. In this study tumor effusions were obtained in the course of the disease of an individual patient and established as continuous lines in the peritoneum of nude mice. The P-HOC8 line was derived from the pleural effusion of the untreated patient at the initial discovery of the tumor, the R-HOC8 was obtained from ascites at relapse after 2 years of chemotherapy, and Y-HOC8 after additional chemotherapy when the patient had become refractory.

The three tumor variants were studied s.c. and i.p. in nude mice, but while tumor cells injected i.p. grew in all the mice, after s.c. injection of the same cell suspension fewer mice showed a tumor take and developed a growing tumor. Transplantation studies thus suggested a preferential growth of HOC8 lines in the peritoneal cavity of nude mice.

The origin of the three tumor lines from patient’s effusions (pleural or ascites) and the fact that the peritoneum is the site of growth for ovarian cancer could account for their preferential growth in the peritoneum of nude mice. The site of human tumor injection in nude mice may influence metastatic spread as well and it may be related to the orthotopic transplantation of tumor cells into the mice (9, 19). We ourselves have previously shown that i.p. injection of ovarian tumors can distinguish tumor populations with different tumorigenic and malignant properties (11). The HOC8 variants that did not metastasize after s.c. injection in nude mice developed invasive tumors in the peritoneal visceras and distant lung metastases after i.p. growth.

Xenografts must not change their characteristics in comparison to the original patient’s tumor (10, 20, 21). In this study the three HOC8 variants in nude mice were all similar and resembled the patient’s tumor as regards cytohistopathological characteristics, antigen expression, karyotype, and malignant behavior. RFLP analysis confirmed the common origin of the HOC8 lines. These results indicate that direct transplantation of human ovarian tumor in the nude mouse did not cause important changes at least in the phenotypes analyzed. The similarity in several phenotypes makes the HOC8 variants appropriate for the study of drug sensitivity. The patient from which the HOC8 variants were derived was treated with a combination of drugs, including epi-DX, DDP, CTX, carboplatin. We have previously shown that DX and CTX had no significant effect on the growth of P-HOC8 in nude mice (11), while DDP significantly inhibited its growth. The antitumor activity of DDP on HOC8 variants was evaluated on the basis of its ability to prevent tumor growth in the peritoneum of nude mice. The P-HOC8 line was the most sensitive to DDP and R-HOC8 was more sensitive than Y-HOC8. P-HOC8 was derived from patient at the initial discovery of the tumor, the R-HOC8 was obtained from ascites at relapse after 2 years of chemotherapy, and Y-HOC8 after additional chemotherapy when the patient had become refractory.
Table 2 Growth behavior of HOC8 lines in nude mice

<table>
<thead>
<tr>
<th>Tumor line</th>
<th>P</th>
<th>Tumor growth/ injected mice</th>
<th>MST*</th>
<th>Tumor growth/ injected mice</th>
<th>Median days to reach 100 mg (range)</th>
<th>Mean doubling time (days ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-HOC8</td>
<td>4</td>
<td>6/6</td>
<td>105 (79-123)</td>
<td>21/31 (67%)</td>
<td>121 (55-144)</td>
<td>47.3 ± 18.2</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>7/7</td>
<td>86 (65-100)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-HOC8</td>
<td>3</td>
<td>6/6</td>
<td>124 (101-158)</td>
<td>13/18 (72%)</td>
<td>109 (109-177)</td>
<td>46 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>8/8</td>
<td>71 (52-92)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y-HOC8</td>
<td>3</td>
<td>6/6</td>
<td>121 (94-137)</td>
<td>15/18 (83%)</td>
<td>103 (72-127)</td>
<td>35.5 ± 18.4</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>8/8</td>
<td>52 (49-56)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Cells (10 x 10⁶) were injected i.p. or s.c. in the flanks.
* P, number of passages in nude mice.
* Tumor growth/injected mice.
* MST, median survival time (range).
* Results are from two experiments with tumor cells obtained between passages 3 and 9 in nude mice.

Table 3 Malignant behavior of HOC-8 lines injected i.p. in nude mice

<table>
<thead>
<tr>
<th>Tumor line</th>
<th>Median autopsy day (range)</th>
<th>No. of mice with ascites</th>
<th>No. of mice with tumor</th>
<th>No. of mice with lung metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-HOC8</td>
<td>86 (78-120)</td>
<td>6/6</td>
<td>6/6</td>
<td>2/6</td>
</tr>
<tr>
<td>R-HOC8</td>
<td>114 (82-136)</td>
<td>6/6</td>
<td>5/6</td>
<td>4/6</td>
</tr>
<tr>
<td>Y-HOC8</td>
<td>81 (70-86)</td>
<td>8/8</td>
<td>8/8</td>
<td>8/8</td>
</tr>
</tbody>
</table>

* Tumor cells (10 x 10⁶) were injected i.p. and autopsies done when animals were moribund.
* Gross tumor deposit confirmed by histological analysis.
* By histological examination.

Fig. 2. Photomicrographs of HOC8 secondary growth in nude mice after i.p. injection. a, diaphragm showing neoplastic cell nests invading and deranging smooth muscle fibers; b, liver with intraparenchymal metastasis (lower right), showing marked cytological anaplasia; c, pancreas with an intraparenchymal metastasis (top center); d, lung with endolymphatic neoplastic emboli (left) and normal alveoli (right). H & E, x 200.


Table 4  Effect of cisplatin on HOC-8 lines growing i.p.

<table>
<thead>
<tr>
<th>Line (a)</th>
<th>Dose (mg/kg)</th>
<th>Schedule (day)</th>
<th>MST*</th>
<th>% ILS</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-HOC8</td>
<td>Vehicle</td>
<td>3</td>
<td>3 (54-70)</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>3</td>
<td>81 (71-117)</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>2, 5</td>
<td>3, 10, 17</td>
<td>74 (58-88)</td>
<td>150</td>
</tr>
<tr>
<td>R-HOC8</td>
<td>Vehicle</td>
<td>3</td>
<td>48 (31-65)</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>3</td>
<td>45 (37-108)</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>2, 5</td>
<td>3, 10, 17</td>
<td>45 (38-90)</td>
<td>66</td>
</tr>
<tr>
<td>Y-HOC8</td>
<td>Vehicle</td>
<td>3</td>
<td>80 (64-94)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>3</td>
<td>72 (69-94)</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>2, 5</td>
<td>3, 10, 17</td>
<td>52 (49-56)</td>
<td>2</td>
</tr>
</tbody>
</table>

* Tumor cells (10 x 10^6) were injected i.p. in nude mice. Results are representative of two different experiments obtained between passages 5 and 10 in nude mice. N = 6.
* MST, median survival time (range).
* P < 0.05 compared to control mice.

from the patient before chemotherapy and R-HOC8 and Y-HOC8 were established from subsequent relapses after chemotherapy; therefore the drug response of these tumor lines established in nude mice reflects the clinical development of drug resistance. The HOC8 lines described here were studied at different passages in nude mice (now at passages 10–12). Despite a faster growth in nude mice after initial passages (Table 2) all the other phenotypes were maintained with no major differences (data not shown). An association between drug response and growth rate has been reported (22), with the faster tumors often being more responsive than the slow ones. The response to DDP of the HOC8 lines transplanted i.p. slightly increased after a few passages in nude mice (data not shown) in accordance with the faster growth rate, but the differences in drug response remained among the HOC8 variants when tested at comparable passages and growth rate in nude mice (Table 4). These results suggest that differences in DDP response are a characteristic of the tumor population rather than an artifact of the growth condition in nude mice.

Malignancy and drug resistance are most likely the most devastating aspect of tumor progression, but the direct relation to the growth condition in nude mice confirms the validity of the orthotopic tumor transplant for studying human ovarian carcinoma in particular and the malignant behavior of human tumors in general. The availability of tumor variants derived from the same patient, similar in several phenotypes, but with different sensitivity to DDP, makes this model a unique tool for investigating the acquisition of drug resistance and the development of new therapy.

**ACKNOWLEDGMENTS**

We thank Drs. M. D'Incalci and P. Allavena for helpful discussion, Drs. B. J. Abbott and J.G. Mayo for enthusiastic support, J. D. Baggott for style editing and G. Tassi and L. Piccoli for typing the manuscript.

**REFERENCES**


Malignant Behavior and Resistance to Cisplatin of Human Ovarian Carcinoma Xenografts Established from the Same Patient at Different Stages of the Disease

Gabriella Masazza, Valeria Lucchini, Antonella Tomasoni, et al.


Updated version  Access the most recent version of this article at:  
[http://cancerres.aacrjournals.org/content/51/23_Part_1/6358](http://cancerres.aacrjournals.org/content/51/23_Part_1/6358)

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.