Intraperitoneal Xenografts of Human Epithelial Ovarian Cancer in Nude Mice

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ABSTRACT

Using continuous human ovarian cancer cell lines, i.p. xenografts were successfully established in nude mice from four of four attempts. When primary tumor material was used, xenografts grew in 8 of 10 attempts. From these eight, three passagable xenograft cell lines have been established. To our knowledge, this is the first report published of such xenografts. i.p. xenografts closely mimic the clinical behavior of human ovarian cancer, and those developed from primary tumor material maintain close morphological similarity to the parent primary tumor. When expression of placental alkaline phosphatase and the tumor associated antigens defined by the monoclonal antibodies HMFG1, HMFG2, AU/A1, and F36/22 by these models was determined, those i.p. xenografts derived from primary tumor material exactly matched the original tumor, while none of the xenografts derived from the cell lines expressed these antigens. These models will be useful for investigating the biology and treatment of ovarian cancer.

INTRODUCTION

In 1976, Davy et al. (1) described the first s.c. heterotransplants of ovarian cancer tissue into the nude mouse. Since then further descriptions of this technique have appeared and many passagable s.c. xenograft lines have been established from human ovarian cancer material. In addition, many continuous human tumor cell lines have been used to form s.c. xenografts (2).

While these s.c. xenograft tumors have been used extensively in the study of cell biology (3) and chemotherapy (4), their anatomical localization bears little relation to the natural history of human ovarian cancer. The majority of patients with this tumor present with disseminated but i.p. disease, metastases outside the peritoneal cavity being a rare event (5).

In an endeavor to model this clinical situation more closely, Cobb et al. (6) established a human clear cell carcinoma of the ovary i.p. in an immunomodulated hamster. However, apart from the description of its establishment, no further reports of this model were published. Recently, using human ovarian cancer cell lines, Hamilton et al. (7), Wahl and Piko (8), and Baumal et al. (9) have described i.p. nude mouse xenografts.

We describe here the establishment of i.p. xenografts in the nude mouse from four continuous ovarian cancer cell lines, and present the first report of the establishment of such xenografts using fresh primary tumor material.

This study was part of a larger body of work examining the role of monoclonal antibodies directed against tumor associated antigens in new treatment regimens. We were therefore particularly interested in documenting the maintenance of histological features and cell surface antigen expression between the xenografts and parent tumors.

MATERIALS AND METHODS

Establishment of Xenografts

Cell Lines. The cell line JAM was established in culture from a s.c. xenografted tumor derived from the line JA-1. This line was originally established from a patient with a poorly differentiated serous cystadenocarcinoma. The line TR170 was also derived from a patient with a poorly differentiated serous cystadenocarcinoma. Full details of these original lines are given elsewhere (10). Line OAW42 was kindly provided for these studies by Dr. A. Wilson, Birmingham, United Kingdom (11) and is described as arising from a moderately differentiated serous cystadenocarcinoma. Line SKOV3 was obtained from the American Type Culture Collection (No. HTB77) and has been fully described previously (12). This line was also derived from a serous cystadenocarcinoma of the ovary.

Three of the lines were maintained in RPM1 1640 tissue culture medium supplemented with 15% FCS, while the SKOV-3 cells were grown in Dulbecco's modification of Eagle's medium plus 10% FCS. Cells were passaged weekly at a split ratio of 1:3 or 1:4 using trypsin (0.05%):EDTA (0.02%).

Near confluent cultures were harvested by trypsin:EDTA 24 h after fresh medium had been added. Cells were washed and resuspended in tissue culture medium without FCS at a concentration of 3.3 x 10⁷ cells/ml. Of this solution, 0.3 ml was injected i.p. into each mouse using groups of 5 young (6- to 12-week-old) female random bred nu/nu nude mice from a colony of mixed genetic background bred at Imperial Cancer Research Fund, specific-pathogen-free animal breeding unit. Trypsin:EDTA was found to be nontoxic to the cell lines by assessing growth curves after harvesting (data not shown). Mice were all allowed food and water ad libitum and housed in sterile isolators at 20°C (La Calhene, Ltd., United Kingdom).

Primary Tumor Samples. Tumor material was obtained at paracentesis or laparotomy. Where possible, ascites cells and solid tumor were collected from the same patient. Details of the patients from whom these samples were collected are given in Table 1.

All samples were processed within 4 h of removal from the patient after transport to the laboratory in RPM1 1640 tissue culture medium at room temperature. Ascites samples (500-1000 ml) were taken at paracentesis and centrifuged (5 min at 250 x g, Beckman F68) in 250-ml centrifuge tubes (Corning). The resultant cell pellet was then washed 3 times in RPM1 1640 tissue culture medium and resuspended in 3 ml RPM1 1640. Accurate counting was not possible due to the high proportion of cell clumps but the presence of tumor cells was determined by microscopy and confirmed by examination of a hematoxylin and eosin stained pellet aliquot of cells. Of this suspension, 0.3 ml was instilled through a 19-gauge needle i.p. as above into groups of 5 mice.

Solid tumor samples were collected under sterile conditions from the laparotomy specimen and transported to the laboratory in RPM1 1640. The samples were then minced into a slurry under sterile conditions with scalpel and scissors. This slurry was washed 3 times in RPM1 1640 and resuspended in just sufficient RPM1 1640 to allow drawing into a needle; 0.3 ml of this slurry was then instilled i.p. through a 19-gauge needle into groups of 5 mice. No attempt was made to count cells in this mixture because of cell clumps; however, 1 cm³ of tumor provided material for the injection into 5 mice. Samples of tumor were taken for histological examination and subsequent immunohistochemistry.

The abbreviations used are: FCS, fetal calf serum; PLAP, placental alkaline phosphatase; PBS, phosphate buffered saline, pH 7.2.

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2 The abbreviations used are: FCS, fetal calf serum; PLAP, placental alkaline phosphatase; PBS, phosphate buffered saline, pH 7.2.
NUDE MOUSE OVARIAN CANCER MODEL

Table 1 Characteristics of ovarian cancer patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Stage</th>
<th>Histopathology</th>
<th>Source of xenografted material</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA</td>
<td>72</td>
<td>IV</td>
<td>Poorly differentiated mucinous cystadenocarcinoma</td>
<td>Ascites</td>
</tr>
<tr>
<td>SC</td>
<td>54</td>
<td>IV</td>
<td>Moderately differentiated serous cystadenocarcinoma</td>
<td>Ascites</td>
</tr>
<tr>
<td>DO</td>
<td>63</td>
<td>III</td>
<td>Well differentiated serous cystadenocarcinoma</td>
<td>Solid tumor</td>
</tr>
<tr>
<td>CL</td>
<td>58</td>
<td>III</td>
<td>Well differentiated serous cystadenocarcinoma</td>
<td>Solid tumor and ascites</td>
</tr>
<tr>
<td>SN</td>
<td>36</td>
<td>III</td>
<td>Moderately differentiated serous cystadenocarcinoma</td>
<td>Solid tumor</td>
</tr>
<tr>
<td>PI</td>
<td>54</td>
<td>III</td>
<td>Moderately differentiated serous cystadenocarcinoma</td>
<td>Solid tumor</td>
</tr>
<tr>
<td>GI</td>
<td>57</td>
<td>III</td>
<td>Well differentiated serous cystadenocarcinoma</td>
<td>Ascites</td>
</tr>
<tr>
<td>HU</td>
<td>23</td>
<td>III</td>
<td>Moderately differentiated mucinous cystadenocarcinoma</td>
<td>Solid tumor</td>
</tr>
<tr>
<td>OS</td>
<td>51</td>
<td>III</td>
<td>Moderately differentiated serous cystadenocarcinoma</td>
<td>Ascites</td>
</tr>
<tr>
<td>ST</td>
<td>38</td>
<td>III</td>
<td>Well differentiated mucinous cystadenocarcinoma</td>
<td>Solid tumor</td>
</tr>
</tbody>
</table>

*International Federation of Gynecology and Obstetrics classification.

**Passaging of Xenografts**

Mice were monitored 3 times weekly. Passaging was performed when needle tumors grew to >1 cm³ in size, the abdomen became distended, or the mouse became ill or cachectic. The animal was killed by cervical dislocation and the tumor or ascites removed under sterile conditions and processed as for primary tumor material.

**Necropsy**

All mice had a necropsy performed. The abdomen was opened and the organs inspected. The liver, spleen, kidneys, diaphragm, and any tumor present were fixed for histological examination in neutral buffered formalin.

**Cell Surface Antigen Expression**

Cell surface antigen expression of the tumor associated antigens defined by the monoclonal antibodies HMFG1, HMFG2, AUAI, F36/22 (Table 2), and the tumor associated enzyme PLAP was determined for the cell lines, primary tumor material, and the resultant xenografts at each passage. These surface markers have each been demonstrated to be present in 30-95% of ovarian carcinomas and provide a means of examining the persistence of cell surface phenotype throughout xenograft establishment. PLAP was detected by the monoclonal antibodies H17E2 (16) and H317 (17) on fresh specimens. However, the epitopes recognized by these antibodies are destroyed by fixation so a commercially available rabbit anti-PLAP antisemur (DakoPatts A268 Denmark) was used for examining fixed sections.

Cell surface antigen expression on ascites cells and cell lines was determined by indirect immunofluorescence while an indirect immunoperoxidase technique was used on fixed tissue sections. Briefly, paraffin embedded, formalin fixed sections of tumors to be assessed were dewaxed in xylene and rehydrated. Nonspecific protein binding was blocked by a 10-min immersion in 10% human serum in PBS while endogenous peroxidase activity was blocked by immersing in 0.6% (v/v) hydrogen peroxide (30 vols) in 80% methanol in distilled water for 30 mins. Sections were then incubated with the test monoclonal antibody (as neat hybridoma supernatant on 10 μg/ml solution of purified antibody in 5% FCS in PBS) for 40 min, washed twice in 0.5% Nonidet P-40 in PBS, then twice in PBS alone, and incubated in a 1:50 solution of peroxidase conjugated rabbit antimouse antisemur (Dako P161, Denmark) in 5% FCS in PBS for 30 min. After a further washing step, peroxidase activity was assessed by a 5-min incubation of sections in a 1 mg/ml solution of dianminobenzidine in 0.03% hydrogen peroxide in PBS (all reagents from Sigma, Poole, United Kingdom).

**Human Origin of Cell Lines and Xenografts**

In all instances, the human epithelial nature of the cell lines used in this study and the xenograft tumors created from both the cell lines and fresh tumor material was demonstrated by positive immunoreactivity with the monoclonal antibody CAM 5.2 (18). This antibody is directed against an epitope found only in human epithelial cells and is completely unreactive with mouse tissue.

**RESULTS**

**Establishment and Characterization of i.p. Xenografts**

Xenografts from Cell Lines. Within 2 months of injection, i.p. tumors appeared in all mice given injections of JAM, TR170, SKOV-3, or OAW42 continuous cell lines. In the cases of JAM and SKOV-3, these took the form of solid tumors and ascites; in TR170 and OAW42, these were solid tumors only. Passaging of these tumors has so far been attempted only for the JAM tumors where passaging was by re-injection of 0.3 ml ascites fluid. To date, passage number 17 has been reached. Passage time was approximately 6 weeks. The other cell line xenografts were not passaged, because they have not been required for other experimental purposes.

In all 4 cell lines, the resultant tumors have been completely undifferentiated. Comparison with the primary tumor from which JAM was established showed a complete loss of the differentiated elements while the tumor from which TR170 was derived was itself poorly differentiated (10). Direct comparison with the original histology from the patients from which SKOV-3 and OAW42 were derived was not possible; however, in both cases the original description of the cell line implied serous differentiation in the primary tumor.

When cell surface antigen expression was assessed, none of the cell lines and none of the resultant xenografts expressed any of these tumor associated antigens. The primary tumor from which the cell line JAM was established expressed the HMFG2 and F36/22 antigens; such expression was therefore lost in the establishment of the cell line. These results are summarized in Table 3.

When tumors developed after i.p. injection of these cell lines, they did so invariably in three regions: in the pelvis; under the diaphragm; and around and behind the stomach. Small tumor nodules were also found around the peritoneal cavity (particularly in the flanks) and tumor cells were found thickening most of the peritoneum. The tumor nodule in the pelvis formed at the site of injection, mimicking needle track recurrence after paracentesis (Fig. 1).

In over 30 mice on which necropsy has been performed, there have been no demonstrable metastases outside the peritoneal cavity.

Histopathological examination of the relation between tumor nodules and peritoneal surface suggests that the nodules grow on rather than through the peritoneum. Organ and serosal integrity is respected with 2 exceptions; (a) the pancreas, which in the mouse is a diffuse organ, is often infiltrated by tumor along the lines of tissue planes; (b) large nodules on the diaphragm have been shown to invade the muscles of the diaphragm.

**Xenografts from Fresh Tumor Material.** Within a period of 4 weeks to 9 weeks after the i.p. transplantation of the human ovarian cancer material into the nude mice, intraabdominal carcinomatosis developed from material from 8 of 10 patients, on 5 occasions from injected ascites (from 6 patients) and on 3 occasions from injected tumor (from 6 patients). In the 2

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patients where both ascites and tumor were injected, only the mice receiving the ascites cells established xenografts. In 6 instances where xenografts were established, all 5 mice in each group developed tumors. When intraabdominal carcinomatosis developed, it did so as discrete tumors only on 5 occasions, as tumor and ascites on 2 occasions, and as ascites only on one occasion.

Three passageable tumor lines have so far been developed and passage number has now reached at least 5. LA is passaged by transplanting 0.3 ml ascites cells; the recipient mice then developed both abdominal tumors and ascites. DO is passaged by transplanting 0.3 ml ascites cells; the recipient mice then developed tumors. When intraabdominal carcinomatosis developed, it did so as discrete tumors only on 5 occasions, as tumor and ascites only on one occasion. In more than 50 necropsies performed, lung metastases have been found in only one instance (CL) and intrahepatic metastases in only 2 instances (both DO). As a rule the tumors grew along serosal surfaces; invasion of viscera was not seen. Needle track tumors have formed in the cases of LA, DO P1, OS, and SN in addition to the i.p. tumors described.

**DISCUSSION**

I.p. xenografts were established in nude mice from the continuous human ovarian cancer cell lines JAM, TR170, SKOV-3, and OAW42. The xenografts grew along serosal surfaces, formed needle track tumors, and did not metastasize outside the peritoneal cavity. They were, therefore, shown to mimic the anatomical features of ovarian cancer closely. In the case of JAM however, the xenograft represented a section of the original tumor only. The better differentiated areas were not represented in the xenografts; this might have been due to selection pressures in the establishment of the cell line or modulation of the biological features of the cell line by the abnormal conditions associated with its propagation. The important consequence of this was that the cell line (and xenografts) did not express the tumor associated antigens found on the surface of a subpopulation of cells in the primary tumor. This observation is not original. Cell surface expression of the estrogen receptor has been found to be lost in continuous tissue culture of some human ovarian cancer cell lines (19). While primary tumor material was not available to assess antigen expression in the tumors from which the cell lines OAW and SKOV-3 were developed, in each of these instances the primary pathology was reported as a serous cystadenocarcinoma (11, 12). This would suggest that these tumors were initially differentiated and that a similar selection of poorly differentiated cells had taken place. A previous study has shown that expression of these tumor associated antigens is related to the degree of tumor differentiation. Bauml et al. (9) recently demonstrated the persistence of expression of 4 tumor associated antigens when a tissue culture cell line (HEY) was used to establish i.p. xenografts. However, data are not reported on the antigen expression patterns of the primary tumor sample and the cells in tissue culture, so it is not possible to assess the selection of cell surface antigens involved in the establishment of this cell line.

In this first description of the establishment of nude mouse...
NUDE MOUSE OVARIAN CANCER MODEL

Fig. 1. JAM i.p. ovarian carcinoma xenografts. a, liver nodules; ×100. b, stomach nodule. Note tumor deposit surrounding stomach and pancreas. ×10. c, diaphragmatic nodule. ×40. d, needle track deposit. Concomitant s.c. tumor formed at site of i.p. injection. ×10. Hematoxylin and eosin.

Table 4 Establishment of i.p. xenografts of human ovarian cancer

<table>
<thead>
<tr>
<th>Xenograft</th>
<th>Establishment time</th>
<th>No. of mice</th>
<th>Morphology</th>
<th>Passage time</th>
<th>Passage no.</th>
<th>Time from first transplant* (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA</td>
<td>4 wk</td>
<td>5/5</td>
<td>Tumor and ascites</td>
<td>3-4 wk</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>SC</td>
<td>9 mo</td>
<td>2/5</td>
<td>Tumor and ascites</td>
<td>7 mo</td>
<td>2</td>
<td>23</td>
</tr>
<tr>
<td>DO</td>
<td>9 wk</td>
<td>5/5</td>
<td>Tumor</td>
<td>8 wk</td>
<td>5</td>
<td>18</td>
</tr>
<tr>
<td>CL ascites</td>
<td>4 mo</td>
<td>2/5</td>
<td>Tumor</td>
<td>1</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>SN ascites</td>
<td>8 wk</td>
<td>5/5</td>
<td>Tumor</td>
<td>3 mo</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>SN tumor</td>
<td>5/5</td>
<td>0/5</td>
<td>Tumor</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI</td>
<td>4 mo</td>
<td>5/5</td>
<td>Tumor</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GI</td>
<td>4 mo</td>
<td>5/5</td>
<td>Tumor and ascites</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HU</td>
<td>4 mo</td>
<td>0/5</td>
<td>Tumor and ascites</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OS</td>
<td>8 wk</td>
<td>5/5</td>
<td>Ascites</td>
<td>4 wk</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>ST</td>
<td>0/5</td>
<td></td>
<td></td>
<td>9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* As of June 1, 1986.

The xenografts from primary tumors, tumor material from 10 patients with human epithelial ovarian cancer was injected i.p. and in 8 of these xenografts were formed in the recipient animals. The reason for the variation in establishment time for the xenografts is unknown. This may be an indication of the viable tumor cell burden injected or an inherent biological characteristic of the particular tumor. At least 3 of these have been passaged past passage 5 and an additional 3 are at earlier passages. This rate accords with published data on the establishment of s.c. ovarian cancer xenografts in nude mice (3, 19).

I.p. xenografts, therefore, may be no more difficult to establish than s.c. xenografts. The biological behavior of the former closely mimics the spread pattern of ovarian cancer in patients. The biology of the nude mouse is of assistance in studying disease spread, because it has long been observed that metastases from xenografts in mice are rare and that this may be related to the function of circulating natural killer cells (20). The experience of Hamilton et al. (7) is unusual in that the NIH-OVCAR 3 line when transplanted i.p. forms extraperitoneal metastasis in 80% of animals given injections. This has not been seen in our tumors or in xenograft studies in general (21). In experiments where the end point is sacrifice of the animals and measurement of the tumors (or in this case antibody uptake into the tumors), i.p. models should more closely mimic the clinical situation than should s.c. xenografts. However, s.c. xenograft models are more suitable where continuous measurement of a tumor parameter (e.g., dimensions for growth curves) is required because this is not possible with i.p. xenografts.

The xenografts formed from primary tumors maintained the tumor type and cell surface antigen expression of the parent tumor, even after serial passaging. In 5 of 6 instances where the xenografts were formed from ascites cells, these cells formed solid tumors (with or without ascites) in the mouse. Although the mechanism of recruitment is unknown, these human cells are able to stimulate mouse stromal cells for their support (22).

I.p. xenografts derived from primary tumor material are being used to examine the in vivo activity of labeled monoclonal
Fig. 2. Retention of morphological characteristics from primary tumor to i.p. xenografts. a, SC primary tumor. b, SC i.p. xenograft. Immunoperoxidase staining for HMFG2 showing retention of antigen expression (hematoxylin counterstain). Antigen expression is seen as dark staining on tumor cell surfaces. c, CL primary tumor. d, CL i.p. xenograft. Hematoxylin and eosin, × 100.

antibodies and for experiments assessing other new forms of therapy. The xenografts formed from the cell lines are unsuitable for the former study because of the absence of tumor associated antigen expression but xenograft models from both sources are proving useful in experimental therapeutic studies of biological response modifiers.

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