Growth Patterns and Metastatic Behavior of Human Tumors Growing in
Athymic Mice

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ABSTRACT

The growth characteristics and metastatic behavior of human tumors growing in athymic nude mice were studied. Human tumor cell lines HEP-2 (carcinoma of larynx) and SW480 (colon carcinoma) were transplanted into athymic nude mice of BALB/c origin. Tumor cells (1 x 10^6 and 2 x 10^7) were given either s.c. or i.p.

Following s.c. injection tumors developed rapidly to become easily palpable within 2 weeks forming a s.c. tumor focus surrounded by a thick fibrous capsule. Animals with s.c. transplants were little affected by the growing tumor. At the time they were sacrificed at Day 34 (HEP-2) and 62 (SW480), a large part of the tumor was necrotic. Capsular infiltration and invasion of lymphatic vessels and perineural and perivascular lymphatic spaces were observed. Metastases to regional lymph nodes were seen in animals kept alive for up to 6 months.

Following i.p. transplantation, tumors spread widely in the peritoneal cavity, invaded intraabdominal organs, and metastasized to mediastinal lymph nodes and lungs. Fifteen of 26 animals (60%) developed metastases. Necrosis of the i.p. growing tumors was minimal. All animals in this group died as a result of tumor growth.

INTRODUCTION

Human neoplastic cells often develop into solid tumors upon s.c. transplantation into athymic nude mice (1, 2, 5, 8, 9). This provides a unique opportunity to study various aspects of human neoplasia. Thus, the effect of various chemotherapeutic agents in a large number of cancers can be tested, and the athymic nude mouse model can be used as a vehicle of human tumors in studying various immunological aspects of human neoplasia.

Our interest in the athymic nude mouse led us to investigate the growth patterns and the metastatic behavior of human tumor transplants. The latter was considered to be of particular concern since only infrequent reports of metastases have been published (3, 4, 10). Recent studies in our laboratory have demonstrated that various human tumors transplanted into athymic nude mice are characterized by progressive growth, local invasion, and distant metastases primarily to lungs and mediastinal structures and that the site of transplantation is important in the expression of these characteristics (6).

This paper presents data pertinent to the growth pattern and metastatic capabilities of human tumors growing in athymic nude mice. Both the s.c. and i.p. routes of transplantation have been investigated.

MATERIALS AND METHODS

Cell Culture. The HEP-2 cell line, derived from a carcinoma of the human larynx, was obtained from the American Type Culture Collection (CCL 23), and maintained in Minimum Essential Medium supplemented with 10% newborn calf serum (Grand Island Biological Co., Grand Island, N. Y.), and 100 I.U./ml penicillin, and 100 µg/ml streptomycin. SW480 cell line derived from a colon carcinoma in the laboratory of Dr. A. Liebowitz at Scott and White Clinic, Temple, Texas. It was kindly supplied by Dr. J. Fogh of the Memorial Sloan-Kettering Institute for Cancer Research, Rye, N. Y. McCoy's medium was used for culture of these cells and was supplemented with 10% or 15% fetal calf serum (International Biological Institutes, Rockville, Md.) and the above antibiotics.

Preparation of Cell Suspension for Injections. Tumor cells were dispersed with 0.1% trypsin, in 0.02% EDTA, washed with sterile 0.9% NaCl solution, and adjusted to 1.0 x 10^6 and 2.0 x 10^7 viable cells/ml. Cell viability was determined by trypan blue exclusion.

Nude Mouse. Congenitally athymic nude mice homozygous for the nu/nu allele were bred in our laboratory from matings of BALB/c-+nu homozygous males and BALB/c-+/nu heterozygous females.

Athymic nude mice were separated into 8 groups. Four groups received s.c. transplants, the other 4 received the tumor cells i.p. Each tumor cell line was transplanted into 2 groups of animals in the amount of 1.0 x 10^6 and 2.0 x 10^7 viable cells/ml. Cell viability was determined by trypan blue exclusion.

Animals were killed by cervical dislocation. Autopsy was performed on all animals and the grossly visible changes were recorded. Tumors, regional lymph nodes, and representative sections from various organs were taken and fixed in 10% buffered formalin solution. Particular attention was paid to the removal and processing of the lungs. In order to secure the whole lung, we removed this organ at autopsy en bloc with the trachea, paratracheal lymph nodes, great vessels and heart, and thyroid. Lungs were further processed as a whole, and sections for histological examination were cut 5 µm thick along their long axis at 3 different
levels, 1 of which was passing through the hilar region, including trachea, regional lymph nodes with the surrounding fibroadipose tissue and great vessels. All paraffin sections (tumor, lungs, and various organs) were stained with hematoxylin and eosin and were subjected to histological examination.

RESULTS

Forty-six animals received either s.c. or i.p. transplants. The number of animals in each group and the amount of the transplanted cells from cell lines HEp-2 and SW480 are given in Tables 1 and 2. Tumors grew in all tested animals regardless of the site of transplantation.

s.c. Route. Tumors transplanted s.c. were detected as early as the 13th posttransplantation day when $1 \times 10^6$ HEp-2 cells were injected, whereas transplantation of $1 \times 10^6$ SW480 cells resulted in measurable s.c. growth on Day 29. The animals tolerated the transplant well, and no adverse effect was observed. Animals bearing the HEp-2 cell line were killed on Day 34 showing an average tumor size of 600 cu mm. Those with SW480 transplants were sacrificed on Day 62. The average tumor size in this group of animals was 970 cu mm.

At autopsy the skin covering the tumor was movable and no adhesions were detected between skin and growing neoplasm. All tumors appeared well circumscribed surrounded by a capsule of dense connective tissue. On cut sections a portion of the tumor was found to be necrotic. Necrosis was evident in the central part, affecting up to 80% of its volume, whereas its peripheral portion was well preserved. The surrounding tumor capsule was invaded to a varying degree by groups of neoplastic cells (Figs. 1 and 2). Invasion of the pericapsular lymphatics and extension of the tumor to the perivasculare and perineural lymphatic spaces was seen in those cases in which capsular infiltration was extensive (Fig. 3).

Microscopically, the neoplastic cells were supported by a delicate fibrovascular stroma and exhibited morphological characteristics similar to those of the i.p. growing tumors (see microscopic description in i.p. growths). A more detailed profile of the histological features of both s.c. and i.p. growing neoplasm is given in Tables 1 and 2.

i.p. Route. Animals bearing i.p. growing tumors were sacrificed when moribund (Tables 1 and 2). At that time the peritoneal cavity was filled with approximately 5 ml of straw-colored fluid containing a large number of free neoplastic cells. The tumor grew as small or large solid nodules occupying the entire peritoneal cavity, the lower pelvis, and the retroperitoneal tissues. Groups of neoplastic cells invaded the intestinal wall, liver, and kidneys. The pancreas was extensively infiltrated, occasionally replaced completely by the growing tumor, and invasion of the liver was prominent in a number of animals. The diaphragm was extensively infiltrated, and both peritoneal and thoracic surfaces were occupied by tumor masses in the form of either thick plaques or multiple discreet nodules (Fig. 4). Tumor invaded the abdominal wall, and the anterior abdominal muscles were infiltrated to a considerable extent by neoplastic cells.

Microscopically, the tumor cells preserved their epithelial characteristics. They were round or polygonal becoming in certain areas elongated with large nuclei, prominent nucleoli, and abundant amphophilic or slightly basophilic or eosinophilic cytoplasm. Mitotic activity was prominent. They were arranged in sheets, cords, or larger compact

### Table 1

<table>
<thead>
<tr>
<th>No. of animals/Injection site</th>
<th>No. of cells injected</th>
<th>Mitotic activity</th>
<th>Supporting stroma</th>
<th>Necrosis</th>
<th>Invasiveness</th>
<th>Animal with metastases</th>
<th>LDH (units/ml)</th>
<th>Tumor wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>s.c.</td>
<td>$1 \times 10^6$</td>
<td>High</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>None</td>
<td>293$^a$ (88-974)$^b$</td>
<td>0.419$^e$ (0.194-0.919)</td>
</tr>
<tr>
<td>s.c.</td>
<td>$2 \times 10^6$</td>
<td>High</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>None</td>
<td>935$^b$ (470-1860)</td>
<td>0.874 (0.194-0.918)</td>
</tr>
<tr>
<td>i.p.</td>
<td>$1 \times 10^6$</td>
<td>High</td>
<td>±</td>
<td>+++</td>
<td>+</td>
<td>3/7 2728 (1744-4271)</td>
<td>2.152 (1.643-2.818)</td>
<td></td>
</tr>
<tr>
<td>i.p.</td>
<td>$2 \times 10^6$</td>
<td>High</td>
<td>±</td>
<td>+++</td>
<td>+</td>
<td>3/7 24301 (1626-3654)</td>
<td>2.548 (1.906-3.407)</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Geometric mean of human LDH in mouse plasma at time of sacrifice.
$^b$ Numbers in parentheses, range.
$^c$ Geometric mean of tumor weight at time of sacrifice.

### Table 2

<table>
<thead>
<tr>
<th>No. of animals/Injection site</th>
<th>No. of cells injected</th>
<th>Mitotic activity</th>
<th>Supporting stroma</th>
<th>Necrosis</th>
<th>Invasiveness</th>
<th>Animal with metastases</th>
<th>LDH (units/ml)</th>
<th>Tumor wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>s.c.</td>
<td>$1 \times 10^6$</td>
<td>High</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>None</td>
<td>148$^a$ (52-419)$^b$</td>
<td>0.942$^e$ (0.304-2.881)</td>
</tr>
<tr>
<td>s.c.</td>
<td>$2 \times 10^6$</td>
<td>High</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>None</td>
<td>284 (191-421)</td>
<td>0.940 (0.533-1.655)</td>
</tr>
<tr>
<td>i.p.</td>
<td>$1 \times 10^6$</td>
<td>High</td>
<td>±</td>
<td>+++</td>
<td>+</td>
<td>3/6 215 (115-403)</td>
<td>ND$^d$</td>
<td></td>
</tr>
<tr>
<td>i.p.</td>
<td>$2 \times 10^6$</td>
<td>High</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>6/6 No survivors$^e$</td>
<td>ND$^e$</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Geometric mean of human LDH in mouse plasma at time of death.
$^b$ Numbers in parentheses, range.
$^c$ Geometric mean of tumor weight at time of death.
$^d$ ND, weight ranged from 8 to 12 g. However, due to the extensive involvement of peritoneal cavity exact weight could not be determined.
$^e$ All animals in this group died.
masses supported by a scant, finely delicate connective tissue stroma. Small and medium-sized tumor nodules were free of necrosis. Larger tumor masses exhibited central necrosis affecting no more than one-sixth of the entire tumor mass (Tables 1 and 2). Tumors growing i.p. metastasized by the lymphatic route to the mediastinal lymph nodes and by both the hematogenous and lymphatic routes to the lungs. Metastatic foci were usually multifocal composed of small groups of cells or larger tumor masses showing a close morphological resemblance to those of the primary tumor mass. Early lymph node involvement was characterized by the presence of neoplastic cells into the peripheral sinus. At a later stage the nodal architecture was completely effaced, and the entire lymph node was replaced by metastatic tumor (Fig. 5). Invasion of the capsule of the lymph nodes and infiltration of the surrounding fibroadipose tissue did occur in certain instances. In the lungs groups of tumor cells were found within the great vessels, and the metastatic foci occupied mainly the alveolar capillaries and the small and medium-sized branches of the pulmonary artery (Figs. 6 and 7). Frank infiltration of the lung parenchyma was observed in certain areas (Fig. 8).

**DISCUSSION**

For the purpose of this study, tumor cell lines HEp-2 and SW480 were selected for their consistently reproducible growth pattern in tissue culture and upon transplantation into athymic mice. Furthermore the use of tumor cells growing in tissue culture made possible the precise quantitation of the transplanted number of tumor cells and gave a more accurate picture of their viability.

The presented data show that both s.c. and i.p. routes of transplantation of human tumors into athymic mice result in tumor growth. However, growth rates, invasiveness, and metastatic behavior of the transplanted tumors differ depending on the route of transplantation.

A characteristic feature of the s.c. transplanted tumors is that they are completely encapsulated. The fibrous capsule that can be seen as early as the first posttransplantation days seems to interfere with the growth of the tumor. This can explain the difference in tumor size when s.c. and i.p. growing tumors are compared despite the fact that both are characterized by high mitotic activity. Furthermore it may affect the vascularization of the implant thus contributing to tumor cell necrosis that was observed in all s.c. growing tumors. In addition it may represent an important factor in preventing distant metastases. In our experiments metastases were to be anticipated in cases in which invasion of the capsule was extensive and lymphatic involvement was present (Figs. 2 and 3). In order to test this observation we kept 3 animals with s.c. transplanted tumors for a period of 6 months in a pathogen-free environment. At the time the animals were sacrificed, 2 showed extensive capsular infiltration and metastases to the regional lymph nodes. Systemic metastases, however, were not found in any of these animals. In contrast only minimal involvement of the tumor capsule was present in the animal that failed to show metastases.

It is evident that when growth patterns of s.c. and i.p. growing tumors are compared there are certain features that are characteristic for the i.p. growth. The tumor grows in a multifocal pattern throughout the peritoneal cavity, and it is characterized by minimal necrosis. Tumor masses are not encapsulated although few nodules are covered by a flattened mesothelium. The neoplastic growth exhibits its true malignant behavior as it is seen in the capability of the tumor to infiltrate various organs, invade lymphatic and blood vessels, and metastasize. This is more dramatically expressed in the extensive metastases found in the mediastinal lymph nodes and the lungs in a large number of animals. This last observation was made possible by serial sectioning of the whole lung and the mediastinal tissues, a procedure that is crucial in such a study, whether or not macroscopic changes are present (see "Materials and Methods"). The failure to detect a number of metastases by gross examination was due to the fact that in many cases metastatic foci, although multifocal, were small. Their small size might be attributed to the short time interval between metastases and death of the animals, the low growth rate of the metastatic lesion per se and/or the emergence of certain immunological factors that may appear at some time during the tumor growth. We have obtained similar results with a number of other human tumor cell lines such as Clouser, a breast adenocarcinoma; KAPAN-1, a pancreatic carcinoma; and T-24, transitional cell carcinoma of the urinary bladder.

These observations indicate that human tumors grow exceedingly well when transplanted i.p., and unlike s.c. transplants imitate more closely the biological characteristics of the malignant growths in man as they are expressed in their tendency to spread over a wide area, infiltrate, and give distant metastases by both the lymphatic and hematogenous routes.

**REFERENCES**


*Provided by Dr. D. P. Houchens of Battelle, Columbus Laboratories.

Provided by Dr. J. Fogh of the Sloan-Kettering Institute for Cancer Research, New York.
Figs. 1 to 4. HEp-2 and SW480 tumors transplanted s.c.

Fig. 1. HEp-2 tumor growing s.c. The tumor, supported by a delicate fibrovascular stroma (short arrows) is surrounded by a well-defined fibrous capsule (long arrows). Invasion of the capsule is evident. H & E, x 200.

Fig. 2. Extensive capsular invasion of a s.c. growing SW480 tumor. Tumor cells are oval or rounded showing large nuclei and prominent nucleoli. Mitotic activity is conspicuous. H & E, x 312.5

Fig. 3. The capsule of a s.c. growing tumor has been extensively infiltrated and tumor is seen invading the extracapsular fibroadipose tissue. Large tumor emboli within lymphatics are conspicuous (arrows). H & E, x 125.

Fig. 4. Extensive infiltration of the diaphragm. The tumor has reached the thoracic surface (upper right). H & E, x 200.
Figs. 5 to 8. Growth of HEp-2 and SW480 tumors, i.p.

Fig. 5. Paratracheal lymph node showing complete replacement of lymphoid tissue by tumor cells. H & E, × 31.2.

Fig. 6. Tumor cells within alveolar capillaries of lungs. Note anisocytosis and cellular pleomorphism. Empty spaces represent distorted alveoli. H & E, × 312.5

Fig. 7. Microscopic multifocal lung metastases occupying blood vessels adjacent to terminal bronchioles. H & E, × 312.5

Fig. 8. Section of the lungs close to the hilar region. Tumor growing within large vessels. Endothelial cells lining the lumen of a vessel are clearly distinguished (arrow). Infiltration of the lung parenchyma is evident (upper part). H & E, × 200.
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