Novel Intrapulmonary Model for Orthotopic Propagation of Human Lung Cancers in Athymic Nude Mice


ABSTRACT

A major impediment to the study of human lung cancer pathophysiology, as well as to the discovery and development of new specific antitumor agents for the treatment of lung cancer, has been the lack of appropriate experimental animal models. This paper describes a new model for the propagation of human lung tumor cells in the bronchioalveolar regions of the right lungs of athymic NC-nu/nu mice via an intrabronchial (i.b.) implantation procedure. Over 1000 i.b. implantations have been performed to date, each requiring 3 to 5 min for completion and having a surgery-related mortality of approximately 5%. The model was used successfully for the orthotopic propagation of four established human lung cancer cell lines including an adenocarcinoma cell carcinoma (NCI-H125), a large cell undifferentiated carcinomma (A549), a large cell undifferentiated carcinomma (NCI-H460), and a bronchioalveolar cell carcinoma (NCI-H358). When each of the four cell lines was implanted i.b. using a 1.0 x 10^6 tumor cell inoculum, 100 ± 0% (SD) tumor-related mortality was observed within 9 to 61 days. In contrast, when the conventional s.c. method for implantation was used at the same tumor cell inoculum, only minimal (2.5 ± 5%) tumor-related mortality was observed within 140 days (P < 0.001). Similarly, when a 1.0 x 10^6 or 1.0 x 10^5 cell inoculum was used, a dose-dependent, tumor-related mortality was observed when cells were implanted i.b. (56 ± 24% or 25 ± 17%) as compared with the s.c. method (5 ± 7.5% or 0.0 ± 0%) (P < 0.02 and P < 0.05, respectively). Most (>90%) of the lung tumors propagated by i.b. implantation were localized to the right lung fields as documented by necropsy and/or high-resolution chest roentgenography techniques which were developed for these studies. The intrapulmonary model was also used for establishment and propagation of xenografts derived directly from enzymatically digested, fresh human lung tumor specimens obtained at the time of diagnostic thoracotomy and representing all four major lung cancer cell types as well as a bronchioalveolar cell carcinoma. Approximately 35% (10 of 29) of the fresh primary human lung tumor specimens and 66% (2 of 3) of tumors metastatic to the lung were successfully propagated i.b. at a 1.0 x 10^6 tumor cell inoculum, whereas only 20% (1 of 5) of the specimens were successfully grown in vivo via the s.c. route from a 1.0 x 10^5 tumor cell inoculum. Our experience with this in vivo intrapulmonary model for the orthotopic propagation of human lung tumor cells is consistent with the view that organ-specific in vivo implantation of human tumors facilitates optimal tumor growth. This new in vivo lung cancer model may substantially facilitate future studies of the biology and therapeutics of this catastrophic disease.

INTRODUCTION

Currently, lung cancer is the leading cause of cancer-related adult deaths in the United States, and its incidence continues to rise, especially in the adult female population (1-3). Advances in the study of the pathophysiology of human lung cancer as well as the discovery and development of new non-surgical treatment modalities for this disease may have been impeded by the lack of optimal animal models for use in these investigations (4). Animal models which use carcinogens to induce primary pulmonary tumors have been developed, but they have not been satisfactory for routine lung cancer studies (5-11). Major disadvantages of the carcinogen-induced animal models for study of primary pulmonary tumors include: they are time consuming (requiring a minimum of 6 mo for completion); and most importantly, they yield a variety of different histological tumor cell types which might not be directly relevant to human lung cancer, and thereby limit their usefulness for meaningful biochemical, cell biology, and drug-testing studies (5-10).

Athymic nude mouse models have recently been used for the in vivo propagation and study of human pulmonary cancers. The predominant prototypes which have been used for these studies are the s.c. xenograft (12-16) and the subrenal capsule (17-20) models. While these offer rapid and relatively simple methods for in vivo propagation of human lung tumors, they have limitations, particularly in their use for the study of lung cancer biology as well as for the discovery and development of effective treatment modalities for human pulmonary malignancies (12, 15, 16, 20).

A potential disadvantage of both the s.c. and subrenal capsule models for the propagation and study of human primary pulmonary malignancies is that tumor cells are not orthotopically implanted (i.e., tumor cells are not implanted in the lung). Paget originally proposed that human tumor cell populations require organ site-specific interaction for optimal maintenance and progression (21). This concept has been widely supported by numerous studies using metastatic tumor models (for review, see Ref. 12) and by recent athymic nude mouse models which have been used to study the orthotopic propagation of selected human solid tumors, such as renal cell carcinoma (12, 16, 20, 22), pancreatic carcinoma (23), and certain brain tumors (24). A similar approach for the propagation of human lung neoplasms in the lungs of athymic nude mice would appear to offer a potentially very practical and relevant model for the study of this disease. This paper describes the successful development of a novel intrapulmonary model for the orthotopic propagation of human lung cancer cells in athymic nude mice using an i.b.2 implantation technique.

MATERIALS AND METHODS

Continuous Human Lung Tumor Cell Lines. Human tumor cell lines NCI-H125, NCI-H358, and NCI-H460 were isolated from fresh solid

...
Table 1 Histopathological characterization of surgically resected human lung tumor specimens propagated by i.b. or s.c. implantation

<table>
<thead>
<tr>
<th>Cell type</th>
<th>No. implanted i.b.</th>
<th>No. propagated i.b.</th>
<th>No. implanted s.c.</th>
<th>No. propagated s.c.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous cell</td>
<td>12</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Small cell</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Large cell</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Large cell-small cell</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Bronchioalveolar</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Subtotal</td>
<td>29</td>
<td>10 (35)*</td>
<td>5</td>
<td>1 (20)</td>
</tr>
<tr>
<td>Metastatic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma (prostate)</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Transitional cell (urinary bladder)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Subtotal</td>
<td>3</td>
<td>2 (66)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>12 (38)</td>
<td>5</td>
<td>1 (20)</td>
</tr>
</tbody>
</table>

* Fresh human tumor specimens were enzymatically digested and then implanted into athymic nude mice i.b. at a 1.0 × 10^5 tumor cell inoculum or s.c. at a 1.0 × 10^7 tumor cell inoculum as described in "Materials and Methods."

Fig. 1. Diagram of the i.b. implantation technique. The shaded area on the diagram represents the caudal lobe of the right lung, the area where the majority of tumor cells are localized following i.b. implantation into nude mice.

INTRAPULMONARY MODEL FOR HUMAN LUNG CANCER

tumor specimens by the NCI-Navy Medical Oncology Branch using standard procedures (25), cultivated, characterized under a variety of growth conditions (26), and kindly provided to the NCI-Developmental Therapeutics Program by Dr. J. Minna and Dr. A. Gazdar. The A549 cell line is a nude mouse ascites-passaged cell line derived at NCI-FCRF from the A549 cell line initially described by Giard et al. (27).

The cell lines used for this study were propagated in vitro utilizing conventional sterile culture techniques after recovery from cryopreserved seed stock and maintained at the NCI-FCRF tumor repository. Tumor cells were thawed under sterile conditions, washed 3 times with 0.9% saline, and counted with a hemocytometer; cell counts were corrected for viability by the trypan blue dye exclusion method. Cells were cultivated in T75 cm^2 flasks in conventional culture medium, consisting of RPMI 1640 (Quality Biologicals, Gaithersburg, MD), 10% heat-inactivated fetal bovine serum (Hyclone, Logan, UT), and 2 mM L-glutamine at a density of 0.5 × 10^6 cells/10-ml/flask. Cells were maintained at 37°C in a humidified cabinet in an atmosphere of 5% CO_2 in air. Cell monolayers approaching 75% confluency were harvested using the DeLarco formulation of trypsin-EDTA and were subcultured for a maximum of 8 to 12 serial passages. For i.b. or s.c. implantation experiments, cells were isolated by centrifugation at 500 × g for 15 min, washed 3 times in 0.9% saline, suspended in a final volume of HBSS, and adjusted to appropriate inoculation densities. All cell lines were documented to be of human origin by isoenzyme and karyotype analyses and were confirmed to be free of Mycoplasma as well as pathogenic mouse viruses at the time of study.

Preparation of Fresh Human Lung Tumors. Fresh human lung tumor specimens obtained at the time of diagnostic thoracotomy were minced into 0.5- to 1.0-mm^3 pieces and enzymatically digested as previously described (28). Cell number, viability, as well as yield were then determined, and i.b. or s.c. implantations were performed as described below. All lung tumor diagnoses were histologically confirmed and are summarized in Table 1.

Experimental Animals. Pathogen-free, female NCr-nu/nu mice or BALB/c × DBA/2 F1 (hereafter called CD2F1) immunocompetent mice, approximately 4 to 6 wk of age, were used. All procedures involving the animals were performed in a pathogen-free barrier facility at NCI-FCRF.

Implantation i.b. Animals were anesthetized in a Harvard small animal anesthesia chamber (Harvard Biosciences, Boston, MA) attached to a standard Sonnifer anesthesia apparatus (Richmond Veterinarian Supply Co., Richmond, VA) using a 7% flow of nebulized Metafane (Pitman Moore, Inc., Washington Crossing, NJ/100% oxygen mixture. A 1.0-cm ventral midline incision was made in the neck over the region of the trachea superior to the supraclavicular notch. The surgical procedures were facilitated by the use of a binocular ×7 to 10 magnification headset. The glandular tissue surrounding the trachea was separated by blunt dissection, and the trachea was exposed by dissection of the peritracheal muscle sheath. The tissue between the tracheal rings was then incised with the bevel of a 25-gauge needle, and a modified, 27-gauge, blunt-ended 1.2-cm needle was inserted into the trachea and advanced into the right mainstem bronchus (Fig. 1). An inoculum of 1.0 × 10^5, 1.0 × 10^6, or 1.0 × 10^7 tumor cells, in a 0.1-ml final volume of HBSS, was then introduced into the right mainstem bronchus. Animals were maintained in a reverse Trendelenberg maneuver during the inoculation to ensure that the fluid containing the cells remained in the distal airways of the right lung. The skin incision was then closed with surgical skin clips, and the animals were allowed to recover under heat lamps for approximately 10 min, were subsequently returned to their holding cages, and were followed for clinical evidence of pulmonary tumors.

Implantation s.c. Tumor cells were implanted at a 1.0 × 10^7, 1.0 × 10^6, 1.0 × 10^5, or 1.0 × 10^4 inoculum using the previously described s.c. implantation techniques (12–14) in a 0.1-ml final volume of HBSS.

Chest Roentgenographic Studies. A Senographe 500T mammographic radiological device (Thompson-CGR Medical Corporation, Columbia, MD) with a 0.1-mm focal spot with compatible screens, cassettes, and X-ray film (DuPont Lodose system; DuPont, Inc., Trenchton, NJ) was used for roentgenographic studies. (The 0.1-mm focal spot is essential for obtaining radiographs with high quality bone and soft tissue detail). Gastrovist radiopaque contrast material (Berlex Laboratories, Wayne, NJ) was used for contrast dye-enhanced radiographic studies to demonstrate localization of i.b.-implanted material to the various lung fields. The standard i.b. implantation method (described above) was used for these studies with a 0.1-ml volume of the contrast material. Animals were then immediately evaluated by anteroposterior and right lateral chest roentgenography.

 Lung Tumor Cell Autoradiography Studies. Monolayer cultures of NCI-H460 tumor cells were incubated with medium containing 10 mCi/ml [3H]thymidine (specific activity, 18.2 Ci/mmol; New England Nuclear, Boston, MA) for 48 h prior to implantation. An inoculum of 1.0 × 10^6 tumor cells was suspended in a 0.1-ml final volume of HBSS and then administered i.b. to NCr-nu/nu mice as described above. Control, radiolabeled NCI-H460 cells were incubated on glass cover-
Fig. 2. Demonstration of localization of i.b.-implanted material to the right lung by contrast dye-enhanced radiography. The chest radiograph on the left represents an anteroposterior view, and the one on the right, a right lateral view, of a mouse immediately following i.b. implantation with radiopaque dye. Note the localization of the contrast material to the caudal (lower) lobe of the right lung (arrows). This corresponds to the highlighted area in Fig. 1 representing the right lower lobe. Radiographs were performed as described in "Materials and Methods."

Fig. 3. Localization of tumor cells to the bronchioloalveolar regions of the caudal lobe of the right lung following i.b. implantation. This light micrograph illustrates a paraffin-embedded, hematoxylin- and eosin-stained histology section of the caudal lobe of the right lung of an athymic nude mouse 1 h post-i.b. implantation with $1.0 \times 10^6$ NCI-H460 tumor cells. A representative alveolus (a) and airway (b) are labeled for comparison. Note the clustering of tumor cells in the bronchioloalveolar regions with filling of the alveolar spaces by the tumor cells. Representative tumor cells are demonstrated by arrows. Normal alveoli are prominent in the lower portion of the micrograph. X300.

Fig. 4. Autoradiographic demonstration of radiolabeled tumor cell distribution in the caudal lobe of the right lung. This light micrograph illustrates a periodic acid-Schiff-stained, hematoxylin-counterstained serial section of the caudal lobe of the right lung 1 h following i.b. implantation with a $1.0 \times 10^6$ NCI-H460 tumor cell inoculum which was labeled for 48 h prior to implantation with $[^3H]$thymidine as described in "Materials and Methods." Heavily radiolabeled tumor cells are easily identified in the bronchioloalveolar regions (representative cells are demonstrated by arrows). Inset, control radiolabeled cells, placed on a glass coverslip for 1 h, shown for comparison. A representative alveolus (a) and airway (b) are also labeled in the figure. Normal alveoli are present in the upper left and lower right portions of the micrograph.

slips at 37°C in the presence of 5% CO₂ for 1 h and then processed for autoradiographic evaluation. Mice that received i.b. implants with radiolabeled cells were anesthetized with 0.1 ml of sodium pentobarbital (Richmond Veterinary Supply Company, Richmond, VA) 1 h after the i.b. implantation, and lung tissues were then fixed in situ by vascular perfusion with 10% low-molecular-weight dextran (Abbott Lab., Chicago, IL) (w/v) in 0.9% saline followed by 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) as previously described (11). Individual lung lobes were collected and immersed in the above glutaraldehyde solution for 2 h, followed by further fixation in 10% buffered formalin for 2
days. Glass slides (Fisherbrand; Fisher Scientific, Fairlawn, NJ) were precleaned for 5 min with 70% ethyl alcohol containing 1% hydrochloric acid, rinsed well with running distilled water, placed in a 95% ethyl alcohol rinse for 5 min, and subsequently air dried. Serial 5-μm sections of each lung lobe were placed onto precleaned glass slides at three levels approximately 100 μm apart. Duplicate sections were taken at each level. The fixed lung tissues were subsequently embedded in paraffin, and autoradiography and staining procedures were performed as previously described (29). Lung tissue sections were then microscopically evaluated for radionuclide tumor cell localization.

Lung Tumor Cell Localization Studies. Athymic nude mice were implanted i.b. with 1.0 × 10⁶ NCI-H460 cells, and animals were then fixed in situ by vascular perfusion as described above at 1 h, 7 and 10 days following implantation. Individual lung lobes were removed and separated, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and microscopically evaluated for tumor cell localization.

Monitoring of Animals for Tumor Development. Animals that received either i.b. or s.c. implants were carefully followed daily for signs of tumor development. Throughout the observation period, animals developing respiratory distress were killed by cervical dislocation. All those animals remaining after 140 days were also killed, and the major organs (lung, liver, spleen, kidney, brain) were removed, fixed in 10% buffered formalin, and then processed for histopathological evaluation. Paraffin sections were stained with hematoxylin and eosin and then evaluated for the presence of tumor or other histopathology. Mortality data were based on only those animals that died from direct effects of tumor growth and/or metastatic disease before 140 days.

RESULTS

The i.b. implantation method (Fig. 1) provides for the inoculation of human lung tumor cell suspensions into the right lower lobe of the nude mouse lung. Figure 1 shows light micrographs of NCI-H460 tumor nodules in the right lower lobe following i.b. implantation. These micrographs demonstrate NCI-H460 tumor nodules (arrows) at 7 (A) and 10 (B) days post-i.b. implantation. Note the location of the nodules in the peripheral regions of the right lower lobe. A, × 500; B, × 200.

Fig. 5. Light micrographs of microscopic NCI-H460 tumor nodules in the right lower lobe 7 and 10 days following i.b. implantation. These micrographs demonstrate NCI-H460 tumor nodules (arrows) at 7 (A) and 10 (B) days status post-i.b. implantation. Note the location of the nodules in the peripheral regions of the right lower lobe. A, × 500; B, × 200.
sections of individual mouse lung lobes following i.b. implantation of 1.0 \times 10^6 NCI-H460 cells demonstrated localization of the majority of the tumor cells to the bronchioalveolar regions of the caudal lobe of the right lung (Fig. 3). Localization of tumor cells to this region of the right lung 1 h after i.b. implantation was more clearly demonstrated when 1.0 \times 10^6 radiolabeled NCI-H460 cells were used (Fig. 4). Again, only the caudal lobe of the right lung consistently contained radiolabeled tumor cells when individual lung lobes were separately evaluated. In addition, microscopic tumor nodules were observed predominantly in the right lower lobe 7 and 10 days following i.b. implantation when individual lung lobes were again evaluated at these longer time intervals (Fig. 5). These different studies all clearly demonstrated that the majority of tumor cells initially resided in the bronchioalveolar regions of the caudal lobe of the right lung following i.b. implantation.

Athymic nude mice implanted i.b. with human lung tumor cells demonstrated clinical symptoms of cachexia and dyspnea as the pulmonary xenografts became progressively larger. These were similar to the symptoms frequently observed in human lung cancer patients. The tumor cells grew most frequently in the peripheral regions of the right lung following i.b. implantation (Fig. 5); however, certain tumors did grow predominately in the airways (Fig. 9). Implantation i.b. produced tumors that ultimately led to the death of the animals (Fig. 6). Lung tumor progression could also be readily followed roentgenographically (Fig. 7), thus allowing tumor-bearing animals to be monitored noninvasively. When the 251 tumor-bearing animals represented in Fig. 6 were studied at necropsy following i.b. implantation, 91% of the tumors implanted by this method were localized to the right lung. Other tumor locations included the left lung (1%), trachea (2%), or peritracheal (6%) area. Local mediastinal invasion was observed at necropsy in approximately 90% of the tumor-bearing animals following i.b. inoculation. In 3% of the i.b.-implanted, tumor-bearing animals, distant metastases were also observed at necropsy in the left lung, lymph nodes, liver, or spleen.

The histologies of tumors obtained from the i.b. implantation model were consistently similar to the parent human lung tumor cell lines from which they were derived (n = 251). As an example, a tumor obtained from the right lung of a nude mouse 19 days following i.b. implantation with the NCI-H460 cell line demonstrated characteristic histopathological features of a human large cell undifferentiated carcinoma of the lung (Fig. 8). The reproducibility of this model among different cell lines was demonstrated in that it produced similar pulmonary tumors and death patterns for the 4 lung tumor cell lines tested (Fig. 6). Reproducibility within cell lines was also demonstrated for the NCI-H460 cell line when it was implanted i.b. multiple times over a 6-mo interval (coefficient of variation for mean survival was 24% for this cell line implanted on 4 separate occasions).

The intrapulmonary model was also compared to the conventional s.c. model for effectiveness in propagation of human lung tumor cells. When the 4 different human lung tumor cell lines were implanted i.b. at 3 different tumor cell inocula, 100% tumor-related mortality was observed at the 1.0 \times 10^6 tumor cell inoculum, and a dose-dependent response was noted with lower (1.0 \times 10^5 or 1.0 \times 10^4) tumor cell suspensions (Fig. 6). In contrast, minimal (5%) tumor-related mortality was observed within 140 days for the s.c. model for the 4 cell lines at all tumor cell inocula tested (Fig. 6). When tumor cells from the 4 cell lines were implanted i.b. or s.c. in CD2F1, immunocompetent mice at a 1.0 \times 10^6 tumor cell inoculum, no tumors were mainstem bronchus of athymic nude mice. Over 1000 i.b. implants have been performed to date, each requiring an average of 3 to 5 min for completion and having a surgery-related mortality of approximately 5%. This model provides an orthotopic site for tumor implantation in that the majority of tumor cells are deposited in the bronchioalveolar regions of the right lung, as verified by radiopaque dye-enhanced radiographic studies, routine histology, and autoradiographic evaluation (Figs. 2 to 4). Radiopaque dye-enhanced radiographic studies were initially performed, whereby radiopaque contrast material was implanted i.b., and low-dose, high-resolution chest radiographs were then immediately performed. These studies clearly demonstrated radiographic localization of the radiopaque contrast material predominantly to the caudal (lower) lobe of the right lung (Fig. 2). Similarly, microscopic evaluation of histological
Fig. 7. Anterioposterior chest radiographic comparison of a normal and a lung tumor-bearing athymic nude mouse. A normal mouse radiograph is depicted on the left, and a mouse 19 days status post-i.b. implantation with $1.0 \times 10^6$ NCI-H358 tumor cells is shown on the right. A large tumor involving the right lung and mediastinum is easily identified (arrow) and accounts for the volume loss in the left lung field. Radiographs were obtained using the techniques described in “Materials and Methods.”

Fig. 8. Light micrograph of a large NCI-H460 tumor following i.b. implantation. This represents a paraffin-embedded histology section of a large tumor located in the right lung of a nude mouse 19 days following i.b. implantation with $1.0 \times 10^6$ NCI-H460 tumor cells. The tumor histology is characteristic of the large cell undifferentiated lung carcinoma cell line from which it was derived. H & E, x 500.

observed at necropsy after 160 days in either the lungs or the skin ($n = 10$ for the number of animals implanted i.b. or s.c. for each cell line).

Enzymatically dissociated, fresh human lung cancer specimens, obtained at the time of diagnostic thoracotomy, were also successfully propagated using the i.b. technique at a $1.0 \times 10^6$ tumor cell inoculum. Approximately 35% (10 of 29) of the fresh primary lung tumors and 66% (2 of 3) of the tumors metastatic to the lung were successfully propagated in vivo by this technique, whereas only 20% (1 of 5) of these same tumors were successfully established and propagated using the s.c. method at a $1.0 \times 10^7$ tumor cell inoculum (Table 1). Primary lung tumors which were successfully propagated i.b. were representative of all 4 major lung tumor histological cell types as well as one relatively rare human pulmonary tumor, a bronchioloalveolar cell carcinoma. Metastatic lung tumors which were successfully propagated i.b. included a transitional cell carcinoma of the urinary bladder as well as an adenocarcinoma of the prostate (Table 1). Tumors were easily passaged i.b. using the enzymatic dissociation technique described in “Materials and Methods.”

The tumors which were propagated in the mouse i.b. were remarkably similar in histological appearance to the original human lung tumor specimens from which they were derived. This is exemplified by comparison of the histological appearance of a superficially invasive squamous cell carcinoma with a prominent endobronchial exophytic component after its removal from the patient’s lung and its appearance after being propagated i.b. in the lung of an athymic nude mouse (Fig. 9). Note the squamous cell characteristics in both tumor specimens as well as the striking endobronchial polypoid growth of the tumor propagated i.b. in the right lung of the nude mouse.

**DISCUSSION**

This paper describes a novel intrapulmonary model for the orthotopic implantation and propagation of human lung cancers in athymic nude mice. The procedure is relatively easy to perform, is reproducible, and requires a small number of animals to obtain adequate tumor tissue for experimentation. The model uses human lung tumor material rather than less ideal carcinogen-induced animal tumors (5–11) or other nonhuman lung tumors (30) and potentially affords investigators with an improved opportunity for the study of human lung cancer. The present i.b. studies support the concept of the importance of organ site-specific tumor implantation for optimal tumor growth which was originally proposed by Paget (21) and subsequently supported by numerous other studies using metastatic tumor models (for review, see Ref. 12) as well as orthotopic models for certain human cancers (12, 16, 20, 22–24). Positive and/or negative selection factors which are present in various mouse tissues may be major determinants of the success or failure of human tumor cells to propagate in vivo (for discussion, see Ref. 12). These factors are probably relevant for both established human lung tumor cell lines as well as for fresh human lung tumor suspensions implanted orthotopically in athymic nude mice. Many tissue characteristics, including the abundant blood supply in the lung, the relatively high compliance of lung tissue, and the availability of numerous endogenous factors in the pulmonary microenvironment (31–37), conceivably might be responsible for the enhanced survival and growth of tumor cells in the immunodeficient mouse lung. This is further supported by the observation that lung tumor cells retained their original histological characteristics when they were grown i.b. It is also supported by the reduced lung tumor cell inoculum requirement which was observed for the i.b. model as compared to the previously described s.c. model which requires a tumor cell inoculum of $1.0 \times 10^7$ cells for optimal
Fig. 9. Comparison of the histological appearance of a human squamous carcinoma of the lung propagated i.b. with the original primary tumor from which it was derived. The original primary human lung tumor specimen is a poorly differentiated squamous cell carcinoma which is invading the wall of the bronchus and adjacent lung (A, lower left), but which is growing primarily as a bulky intrabronchial exophytic tumor (A, center). It contains scattered foci of squamous differentiation (B, center). The tumor which was propagated in the athymic nude mouse from the original lung tumor specimen was present in the right lung at necropsy 25 days following i.b. implantation and is located on the mucosal surface of a bronchiole and has a large polyoid endobronchial component (C) similar to that of the tumor from which it was obtained. The tumor implant is growing predominantly as undifferentiated malignant cells with scattered individual cell keratinization (arrow) (D). A, × 6; B, × 600; C, × 100; D, × 600.
tumor development (12–15), and many of these previous s.c. studies have used human tumor fragments which usually contain >1.0 x 10^7 cells/inoculum. The lower tumor cell inoculum requirements for the i.b. model appear consistent with an organ site-specific implantation theory for optimal tumor growth; however, it is important to note that comparably low lung tumor cell inocula have been shown to be effective for propagation of certain small cell lung carcinoma cell lines implanted intracranially in immunodeficient rodents (38, 39). Additional i.b. studies using nonpulmonary human tumors will be necessary to further evaluate this organ-site specificity concept.

In addition to its utility for propagating continuous long-term human lung tumor cell lines, the intrapulmonary model also provides the basis for orthotopic propagation of enzymatically dissociated fresh human lung tumor tissue in athymic nude mice. This might offer a useful avenue for clinical investigators to expand relatively small surgically excised biopsy specimens into a tumor volume of sufficient size to provide fresh tumor tissue for biochemical, histopathological, and cell biology studies. This procedure might also allow for propagation and expansion of lung tumor tissue from those patients who are not candidates for surgical resection, but who undergo diagnostic procedures such as bronchoscopy or needle biopsy which yield small tumor samples. Since 70 to 80% of all lung cancer patients are inoperable at the time of diagnosis (40, 41), this would allow potential access to a much larger lung cancer patient population for a variety of clinical research and/or diagnostic studies.

The intrapulmonary model also offers a potentially attractive system for in vivo testing and experimental therapeutics of new potential antitumor agents. This model might provide pharmacokinetic and pharmacodynamic features more characteristic of human lung cancer than s.c. or intrarenal implanted tumors. In addition, it frequently demonstrates local mediatinal invasion which is characteristic of certain human lung cancers (41). It also allows for the experimental use of a lethality end point which might be particularly convenient for large scale drug-testing studies.

In addition, noninvasive X-ray procedures can be used to periodically monitor lung tumor size, and this is especially attractive for experimental therapies studies. Implantation of tumors in the right lung of nude mice is particularly advantageous, since no other major anatomical structures (e.g., the heart) are located in this area which might interfere with the radiographic evaluation of small human tumor xenografts. Thus, by using both anteriorioposterior (or posteroi anterior) and right lateral radiographic views, a semiquantitative, three-dimensional, radiographic approximation of tumor size can be obtained (Figs. 2 and 7). Such technology could ultimately provide an orthotopic in vivo drug-testing model which closely resembles the clinical setting, in that it makes possible accurate, periodic, noninvasive monitoring of human lung tumors in an animal host following treatment with different experimental therapeutic modalities.

In summary, this intrapulmonary model has several advantages over other currently available models and should be of particular value for future studies of lung cancer biology and treatment. Advantages include the following. (a) The procedure provides the first short-term, reproducible, orthotopic in vivo animal model for the study of human lung cancer. (b) Lung tumor cells are implanted at the organ site of tumor origin. (c) The i.b. procedure is relatively easy to perform in a modified conventional laboratory setting and uses a smaller tumor cell inoculum than that required by other models. (d) It allows for propagation of fresh human lung tumor specimens. (e) It potentially provides a suitable in vivo model for the study of human lung tumor biology, and (f) it offers a potentially attractive and relevant model for in vivo testing of the efficacy of potential therapeutic agents for the treatment of lung cancer.

ACKNOWLEDGMENTS

The authors express their appreciation to Dr. J. Minna and Dr. A. Gazdar, NCI Navy Oncology Branch, Bethesda, MD, for providing tumor lines; to Dr. I. J. Fidler, M.D. Anderson Hospital and Tumor Institute, Houston, TX, for his helpful advice regarding this manuscript; and to Kathy Gill for the typing and organization of this text for publication.

REFERENCES

22. Naito, S., von Eschenbach, A. C., Giavazzi, R., and Fidler, I. J. Growth and metastasis of tumor cells isolated from a human renal cell carcinoma im...


Novel Intrapulmonary Model for Orthotopic Propagation of Human Lung Cancers in Athymic Nude Mice


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/47/19/5132

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.