Metastatic Human Pleural Ovarian Cancer Model Constructed by Orthotopic Implantation of Fresh Histologically-Intact Patient Carcinoma in Nude Mice

PHILIPPE ASTOUL1,2,3, HENRI G. COLT1, XIAOEN WANG2 and ROBERT M. HOFFMAN2,4,5

1Department of Pulmonary and Critical Care Medicine, UCSD Medical Center, San Diego, California 92103-8380
2AntiCancer, Inc., 7917 Ostrow St., San Diego, California 92111, U.S.A.;
3Department of Pulmonology (Pr Boutin), Hopital de la Conception, 147 bd Baille, 13385 Marseilles, France;
4Laboratory of Cancer Biology, UCSD, La Jolla, California 92093-0609, U.S.A.

Abstract. Pleural cancer is a frequently-occurring tumor that is generally refractory to therapy. Clinically-relevant animal models of human pleural cancer are greatly needed for testing experimental and standard treatments, as well as for understanding the clinical features of this disease. We report the first orthotopic transplant model for human patient pleural cancer. Fresh histologically-intact patient specimens of human pleural ovarian adenocarcinoma were implanted onto the visceral and parietal pleura of nude mouse. The human tumors grew locally and regionally mimicking the usual human clinical features of this disease. Moreover, only visceral pleural implantation subsequently involved mediastinal lymph nodes corroborating clinical observations suggesting that visceral pleural involvement in pleural cancer represents an advanced-stage disease. This model should facilitate basic research of pleural malignancies, and stimulate studies of pleural-tumor response to cytotoxic treatment, biologic modifiers, and other modalities of therapy.

The prognosis of metastatic pleural effusions is very poor. The median survival time of patients usually ranges between 6 and 12 months despite chemotherapy and/or radiotherapy, except in breast cancer where it exceeds 1 year (1). Experimental research of pleural-based malignancies could be enhanced by the development of suitable animal models. Subcutaneous implantation of tumors has been used for establishing animal models of human cancer in nude mice (2,3). However, the disadvantages of such techniques are low take rates. When the tumor grows subcutaneously, it is usually encapsulated and fails to develop regionally and distally (4,5). Orthotopic implantation of cells, on the other hand, allows a high take rate, with local and regional tumor growth and distant metastases (6,7,8). However, recent studies have indicated that cell suspensions may not express the full metastatic potential of the original tumor (9,10). Metastatic potential is enhanced by the construction of orthotopic transplant models in nude mice using intact tumor tissue obtained from patients with colon (11,12), pancreas (13), stomach (10), bladder (14), ovarian (15), prostate (16), and lung cancer (17,18) which allow growth and metastatic patterns resembling clinical features.

Previously, pleural cancer involvement in rodents had been obtained by intratracheal instillation of tumor cell lines or after intrapulmonary implantation of tumor cell lines (7,19). Besides having the disadvantage of a low take rate, these models do not offer a realistic animal model of pleural cancer with regard to local and regional growth.

Recently we have developed a thoracotomy procedure through which intact human lung adenocarcinoma tissue from a xenograft was successfully implanted onto the pleura of nude mice with a 100% take rate. The same specimens implanted onto the parietal pleura or visceral pleura of two groups of nude mice led to local and regional tumor spread closely resembling the clinical picture. Moreover, in comparison to the parietal-pleural implanted group, weight-loss, survival, and the presence of mediastinal lymphadenopathies in the visceral-pleural implanted group suggested a model of advanced-stage disease (Astoul, Ph., Colt, H.G., Wang, X., Boutin, C. and Hoffman, R.M., unpublished data).

In this report, we describe a pleural adenocarcinoma model in nude mice that can be constructed directly from patients with metastatic pleural cancer.

Materials and Methods

Animals. Three-week-old outbred female nu/nu mice were utilized for tumor implantation. All animals were maintained in a sterile environment. Cages, bedding, food and water were all autoclaved and changed regularly. All the mice were maintained on a daily cycle of 12 hr light and 12 hr darkness.

Tissue procurement. Fresh pleural adenocarcinoma specimens were
Table I. Local and regional growth of human pleural adenocarcinoma after orthotopic implantation as intact tissue in nude mice.

<table>
<thead>
<tr>
<th>Implantation Site</th>
<th>Local tumor growth</th>
<th>Tumor spread</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Chest wall</td>
</tr>
<tr>
<td>Parietal pleura</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>Visceral pleura</td>
<td>2/3</td>
<td>3/3</td>
</tr>
</tbody>
</table>

¹Number of mice with tumor/number of mice transplanted
²Including involvement of the pericardium

obtained from the parietal pleura of a 65-year-old white female presenting with persistent and recurrent right-sided pleural effusion secondary to metastatic ovarian carcinoma. Large parietal pleural biopsies were obtained by thoracoscopy from the UCSD Medical Center, San Diego, California, and kept in Hank's Balanced Salt Solution at 4°C.

Before implantation, no later than 12 hr after thoracoscopy, specimens were inspected, and grossly necrotic and suspected necrotic tissue was removed. Each specimen was equally divided into five separated parts, and each part was subsequently cut into smaller pieces of 1 to 1.5 mm³. Tumor pieces for each implantation were taken from each of the five parts of the specimen equally. In this way, the chance for viable tissue to be implanted was maximized.

Surgical microprocedure. For implantation, nude mice were anesthetized by isoflurane inhalation. The animals were then placed in the right lateral decubitus position with all four limbs restrained. A small, one-centimeter transverse incision was made on the left-lateral chest of the nude mouse via the fourth intercostal space and an access to the pleural space was provided resulting in total lung collapse. Five tumor pieces were implanted onto the posterior inferior part of the left parietal pleura of three mice and onto the left visceral pleura of three others. The chest wall incision was closed with a 7-0 nylon suture. A sterile 2 cc syringe with an attached 25 G 1.5 inch needle was then inserted into the pleural cavity in order to remove air and actively reinflate the lung. Chest muscles and skin were closed with a single layer of 6.0 silk suture. This procedure takes approximately 15 minutes.

Monitoring animals. Animals were kept in a sterile environment. They were followed daily after surgery, and monitored for signs of infection, decreased physical activity, and chest wall invasion. Potential tumor growth was determined by evidence of weight-loss, tumor-related respiratory distress or dehydration. Mice were sacrificed by CO₂ inhalation when moribund.

Tumor was harvested immediately after death for gross examination prior to formalin fixation for microscopic tissue examination. Hematoxylin and eosin staints were performed using standard procedures. The lung, mediastinal lymph nodes, liver, kidney, and adrenal gland were also processed for macroscopic and histologic examination. Any signs of local, regional, or metastatic spread of human pleural cancer were noted.

Results and Discussion

After orthotopic implantation of fresh histologically-intact human pleural adenocarcinoma in nude mice, continuous weight-loss was observed from day-fifty six, suggestive of tumor growth or disease.

Mice were sacrificed between day sixty and day seventy four after implantation. At autopsy, tumor-growth was noted in all cases.

As shown in Table I, all animals but one (in the visceral-pleura implanted group) had evidence of chest wall invasion. Local and regional spread on macroscopic examination included involvement of the ipsilateral lung, diaphragm, mediastinum, and pericardium. Interestingly, enlarged contralateral mediastinal lymphadenopathy was only observed in the case of visceral-pleural implantation (Figure 1). This finding may corroborate clinical observations showing that visceral-pleural involvement in pleural cancer represents an advanced-stage disease (20,21).

Implantation onto the median lung and visceral pleura was chosen because of the presence of pleural stomas previously described (22). Indeed, such structures are considered to be a gate through which small particles (e.g., malignant cells) are absorbed from the pleural cavity into the lymphatic circulation via sub-mesothelial lymphatic vessels, and also connect with sub-peritoneal lymphatic vessels (23,24). Furthermore, stoma are surrounded by macrophages and lymphocytes and are mostly located on the
inferior portion of the mediastinal pleura, on the surface of the diaphragm, and on the lower aspect of parietal pleura. Relatively few are found in other areas (23). These structures look like the "milky spots" previously described in the peritoneum, which are initially infiltrated in the early stage of neoplastic peritoneal dissemination (25,26).

No metastases were observed in the contralateral lung, liver, kidneys, or adrenal glands in any case. In order to explain this finding, we hypothesize that after pleural implantation, an inflammatory response may cause occlusion of the pleural stomas connecting the pleural cavity with submesothelial lymphatic vessels (22,23). The blockage may delays access of tumor cells to lymphatic vessels. The presence of pleural effusion in 4 of 6 mice may indirectly result from lymphatic obstruction (26).

In summary, the pleural cancer models constructed directly from patient tumor specimens have a high take rate. In addition, the local and regional spread of implanted tumor closely mimics the clinical picture of human pleural cancer. A "patient-like" nude mouse model of parietal-pleural and visceral pleural cancer should facilitate basic research of pleural malignancies and prompt studies of pleural tumor response to cytotoxic treatment, biologic modifiers, and other therapeutic modalities.

Acknowledgements

This study was support in part by National Cancer Institute (SBIR grant R43CA53963) and by la Fondation pour la Recherche Medicale.

References


Received June 29, 1993
Accepted August 27, 1993