"Patient-like" nude- and SCID-mouse models of human lung and pleural cancer (Review)

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Abstract. Lung cancer is one of the leading causes of cancer-related adult deaths in the world, and its incidence is rising. Patients with malignant pleural effusions are considered to be in the advanced-stage of malignant disease or in the terminal stage. For both, the lack of efficacy of non-surgical treatment modalities is related to the lack of suitable animal models for new drug discovery. Models based on athymic nude mice have been used for human cancer research. However, s.c. or i.m. xenografts usually do not metastasize, or do so at low frequencies. Conversely, human tumor cells orthotopically implanted in the corresponding organs of nude mice result in much higher metastatic rates. By avoiding disruption of tumor integrity, we have found that orthotopic implantation of histologically-intact patient specimens leads to models better reflecting the natural behavior of human cancer than models constructed by orthotopic injection of cell suspensions. With the development of a novel thoracotomy procedure, we have constructed 'patient-like' models of lung cancer (SCLC and NSCLC) with regional spread and distant metastases mimicking the clinical features of these diseases. Moreover, by implantation of histologically-intact human tumor tissue in the parietal or visceral pleura of nude mice, we were able to construct models of early- and advanced-pleural cancer, respectively. Indeed, symptoms and survival of pleural-implanted mice closely resemble the clinical situation showing a statistically-significant difference in survival between parietal- and visceral-pleural implanted mice, the latter representing an advanced-stage cancer. Thus such models, reflecting clinical features, should be of great value in the development of new drugs and treatment strategies.

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1. Introduction

Among the various forms of malignancies in man, lung cancer has been shown to be disappointingly unresponsive to treatment and the prognosis is very poor.

Small cell lung cancer (SCLC) is considered to be a disseminated disease when it is diagnosed, and although it is very chemosensitive in its initial clinical stages, the five year survival rate is only 2.5% (1-5). For patients with non-small cell lung cancer (NSCLC), surgery offers the best chance of cure (6). However, the diagnosis is frequently made in patients with advanced-stage disease (6). Response rates of less than 50% are achieved with combination chemotherapy containing cisplatin, but the duration of the response is only a few months (see refs. 3, 7, 6 for review).

Pleural cancer is a frequently-occurring tumor that is generally refractory to therapy. 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 (8,9). The lack of efficacy of non-surgical treatment modalities is related to the lack of suitable animal models for new drug discovery (10-12). In this review, we summarize what is known about the human lung and pleural cancer models constructed via implantation of tumors in immunodeficient mice. In particular we review work from our laboratory on the development of patient-like models of lung and pleural cancer constructed by orthotopic implantation of histologically-intact human malignant tissue into nude mice.

2. Early models of human lung cancer in nude mice

A number of models have been developed for human lung cancer. Carcinogen-induced animal models were developed for primary pulmonary cancer. Cigarette smoke condensate or radioactive isotopes implanted in the rat lung were used as carcinogens (13-20). However the development of models required a minimum of 6 months. In addition these models yield histological tumor cell types which are not similar to human lung cancer. Thus, the usefulness of such models for basic research and therapeutic studies is limited (21).

Subcutaneous-implant models have been used in athymic nude mice to study in vivo propagation of human lung cancers (22-26). These models are easy to construct but have limited applicability for developing treatment modalities.
since the tumors are growing heterotopically and only rarely metastasize (24-25). Giavazzi et al have examined human subcutaneously-growing colon cancer cell lines in more than 600 nude mice. The s.c. injection, although successful in initiating local tumor growth, in only one case allowed visceral metastasis. Moreover, only ten cases had tumor growth in lymph nodes draining the injection site (26,27).

In another approach, human tumor cell lines were implanted under the subrenal capsule for an 11-day growth assay in nude mice and a 6-day growth assay in normal mice (28-30). In contrast to serially-passaged tumor, this first-implant-generation tumor more closely resembled the original tumor (30). In order to retain cell membrane integrity and cell-to-cell contact to provide a tumor architecture relevant to the original tumor, tumor fragments rather than cell suspensions were implanted under the subrenal capsule. The intact fragment tumor allowed more clinically-accurate drug-response testing than a dispersed cell population implanted under the subrenal capsule (30-33). However, the disadvantage of both the subcutaneous and subrenal capsule models is that tumor cells are not orthotopically implanted and thus yield few distant metastases (28,29).

Since Paget's 'seed and soil' hypotheses that tumor cells had a specific affinity for the milieu of certain organs, it is now well-established that tumor growth and metastasis principally result when seed and soil are matched. Thus, growth and metastasis are determined not only by the characteristics of the neoplastic cells but also by the microenvironment of the host tissue (33-37). Indeed orthotopically-implanted tumors not only metastasize at a much higher rate than subcutaneously-implanted tumors but in addition they metastasize to the usual target organ seen in patients.

Some investigators have identified parameters explaining why orthotopically-growing tumors metastasize to a much greater extent than ectopically-growing tumors (see ref. 38 for review): (i) Anatomy, which determines the local microenvironment including the location of an available capillary bed; (ii) Formation of tumor emboli; (iii) Molecules that specify attachments of tumor cells to particular cells or to extra cellular matrix molecules that are tissue specific; (iv) local growth factors; and (v) local matrix chemistries.

Recently, using the orthotopic concept, studies have demonstrated that inoculation of human lung tumor cell lines intrabronchially or intrathoracically into nude mice results in orthotopic growth (39,40). Intrabronchial implantation of a suspension of 1 x 10⁹ cells into the bronchialoveolar region of the right lung of immunodeficient mice led to a 100% mortality of the inoculated animals within 6 to 61 days. Conversely, the subcutaneous injection of 1 x 10⁹ cells led to 2.5% tumor-related mortality within 140 days.

Although organ-specific in vivo implantation of tumor facilitates optimal tumor growth, when enzymatically-dissociated fresh human lung cancer specimens obtained at the time of diagnostic thoracotomy were used for the intrabronchial technique, only 35% of the fresh primary lung tumors and 66% of the metastatic tumors grew (39). Moreover, most of the tumors that propagated intrabronchially were localized to the right lung, with only 1% metastasizing to the left lung, 2% to the trachea, 6% to the peritracheal area and only 3% spreading distantly to lymph nodes, liver or spleen.

A second orthotopic-implant model was developed by injecting lung tumor cell suspensions via an intrathoracic route through the pleural space (40). This model seems similar to the intrabronchial model in that extensive local growth occurs with little metastatic spread. Furthermore, the disadvantage of the intra-thoracic (i.t.) model was that approximately 30% of all tumors propagated by this method grew in the chest wall as well as in the pleural space and the lung parenchyma. This limits the usefulness of the i.t. model for drug discovery and tumor biology studies which require that the tumor cells grow in a localized intrapulmonary micro environment.

Taking into account that the site of implantation of human tumor cells can promote the growth of different subpopulations of cells from a heterogenous tumor and the importance of tissue architecture, we hypothesized that the appropriate lung cancer model for studying the biology and therapy should be based on orthotopic implantation of intact tumor tissue. We have shown that this approach has led to models which reflect the clinical picture of the local growth and metastasis of patient tumors (41-50).

Thus a new approach utilizing histologically-intact tumor tissue was applied to lung tumor implantation in the SCID or nude mouse left lung by a thoracotomy procedure (51). Nude and SCID mice were implanted with the histologically-intact human SCLC cell line LU-24 into the left lung. The SCID mice all developed locally growing tumors within 17 days. Involvement of the mediastinum, left chest wall, and pericardium was observed (52). Distant metastases in the opposite lung, lymph nodes, parietal pleura and diaphragm involvement were also observed.

When the same LU-24 cell line was implanted into the left lung of nude mice, 100% of the animals produced locally-growing tumors within 24 days. Regional metastases were observed in all mice, including tumor invasion of the mediastinum, chest wall and pericardium, as well as distant metastases involving the right lung, esophagus, diaphragm, parietal pleura and lymph nodes (52). The time to morbidity was shorter in SCID mice than in nude mice. The tumor maintained its oat-cell morphology while growing locally, as well as metastasizing in both types of mice.

Similar high potential for producing patient-like metastatic models of human SCLC in immunodeficient mice were observed using the LU-130 and H-69 human small carcinoma cell lines (53). Mice developed very large local growth and metastases to the opposite lung and distant lung nodes, and for LU-130 cell line, metastases to the brain. These results contrast with the orthotopic injection of human SCLC cell suspensions in nude rats which result in poor local growth and no metastases (54,55).

Table I summarizes results on the primary growth and metastases of all human lung cancer cell lines used for orthotopic implantation as histologically-intact human malignant tissue.

When large-cell squamous cell human tumor 2268 was orthotopically implanted to the left lung as histologically-intact tissue directly from surgery, 100% of the mice
Table I. Growth, loco-regional spread and distant metastases of human lung tumors after subcutaneous (s.c.), intravenous (i.v.), or orthotopic (ortho) implantation as cell suspension or histologically-intact tissue in immunodeficient mice.

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Procedure</th>
<th>Mouse</th>
<th>Cell number or size of implanted tumor</th>
<th>Growth time (days)</th>
<th>Number of mice</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-69</td>
<td>iv</td>
<td>nude</td>
<td>5x10^6</td>
<td>ND</td>
<td>0/4</td>
<td>0/4 (50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SCID</td>
<td>5x10^4</td>
<td>21</td>
<td>4/4</td>
<td>0/4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5x10^6</td>
<td>14</td>
<td>4/4</td>
<td>2/4</td>
</tr>
<tr>
<td></td>
<td>ortho</td>
<td>nude</td>
<td>1-1.5mm^3 (6pieces)</td>
<td>18.5</td>
<td>8/8</td>
<td>4/8   (53)</td>
</tr>
<tr>
<td></td>
<td>s/c</td>
<td>nude</td>
<td>1-1.5mm^3 (10-30pieces)</td>
<td>24</td>
<td>4/4</td>
<td>0/4   (53)</td>
</tr>
<tr>
<td>Lu-130</td>
<td>iv</td>
<td>nude</td>
<td>5x10^3</td>
<td>ND</td>
<td>0/4</td>
<td>0/4   (50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SCID</td>
<td>5x10^6</td>
<td>21</td>
<td>4/4</td>
<td>0/4</td>
</tr>
<tr>
<td></td>
<td>ortho</td>
<td>nude</td>
<td>1-1.5mm^3 (6pieces)</td>
<td>62</td>
<td>5/5</td>
<td>4/5   (53)</td>
</tr>
<tr>
<td></td>
<td>s/c</td>
<td>nude</td>
<td>1-1.5mm^3 (10-30pieces)</td>
<td>82</td>
<td>0/2</td>
<td>0/2   (53)</td>
</tr>
<tr>
<td>Lu-24</td>
<td>ortho</td>
<td>nude</td>
<td>1-1.5mm^3 (5pieces)</td>
<td>24</td>
<td>5/5</td>
<td>5/5   (52)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SCID</td>
<td>1-1.5mm^3 (5pieces)</td>
<td>17</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#2268</td>
<td>ortho</td>
<td>nude</td>
<td>1-1.5mm^3 (6pieces)</td>
<td>61</td>
<td>5/5</td>
<td>4/5   (52)</td>
</tr>
<tr>
<td></td>
<td>s/c</td>
<td>nude</td>
<td>1-1.5mm^3 (10-30pieces)</td>
<td>85</td>
<td>2/4</td>
<td>0/4</td>
</tr>
<tr>
<td>A549</td>
<td>cell inj.</td>
<td>nude</td>
<td>ND</td>
<td>ND</td>
<td>5/5</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>ortho</td>
<td>nude</td>
<td>1-1.5mm^3</td>
<td>ND</td>
<td>20/20</td>
<td>6/20</td>
</tr>
</tbody>
</table>

developed locally-growing tumors in an average time of 61 days (52). Mediastinal lymph-nodes and opposite-lung metastases also occurred. When the same tumor was grown subcutaneously, the take rate was 50% and no metastases were observed.

Orthotopic implantation of intact tissue of the human lung adenocarcinoma A549 has resulted in local growth and metastases to the opposite lung and lymph nodes (56). Table I also summarizes behavior of the latter two tumors after orthotopic implantation in nude mice.

Thus, orthotopic implantation of histologically-intact tissue may be critical in allowing the full metastatic potential of transplanted tumors to be expressed in immunodeficient mice.

3. 'Patient-like' nude mouse models of pleural cancer

Previously pleural cancer models in rodents have been constructed by intratracheal instillation or intrapulmonary injection of suspensions of tumor cell lines (21,39,40). These models had a relatively low take rate and with regard to local, regional and distant growth were not representative of the clinical picture.

In our laboratory, we have constructed a nude-mouse pleural cancer model using a novel thoracotomy procedure by which intact human lung adenocarcinoma tissue was successfully implanted to the parietal and visceral pleura. Autopsy carried out on day 31 after tumor implantation demonstrated a 100% take rate. Tumor spread indicated chest wall invasion in all mice, ipsilateral involvement of the lung in 9 mice, diaphragm in 6 mice, pericardium in 4 mice, and mediastinum in 7 mice. Pleurisy was observed in 7 mice. No distant metastases were noted.

Implantation in the posterior and low part of the parietal pleura was chosen because of the presence of pleural stomas previously described (57). Indeed, such structures are considered to be a gate through which small particles (e.g. malignant cells) are absorbed from the pleural cavity to the lymphatic circulation via submesothelial lymphatic vessels, and also have a connection with subperitoneal lymphatics (58,59). Furthermore, these stomas are surrounded by macrophages and lymphocytes and are principally located on the inferior part of the mediastinal pleura, on the surface of the diaphragm, and the lower part of parietal pleura whereas relatively few of them are found in other areas (58). These
Table II. Loco-regional growth and metastases after pleural implantation of human tumors as histologically-intact tissue in nude mice.

<table>
<thead>
<tr>
<th>Tumor #2572</th>
<th>Implantation site</th>
<th>Size of implanted tumor</th>
<th>Growth time</th>
<th>Survival time</th>
<th>Tumor growth</th>
<th>Distant metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>#2572</td>
<td>PP</td>
<td>1-1.5mm³ (5 pieces)</td>
<td>14</td>
<td>31</td>
<td>11/11</td>
<td>0/11</td>
</tr>
<tr>
<td></td>
<td>VP</td>
<td>1-1.5mm³ (5 pieces)</td>
<td>14</td>
<td>27.9</td>
<td>10/10</td>
<td>5/10</td>
</tr>
<tr>
<td></td>
<td>SC</td>
<td>1-1.5mm³ (10 pieces)</td>
<td>14</td>
<td>*</td>
<td>5/5</td>
<td>0/5</td>
</tr>
<tr>
<td>#2870</td>
<td>PP</td>
<td>1-1.5mm³ (5 pieces)</td>
<td>65</td>
<td>ND</td>
<td>3/3</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>VP</td>
<td>1-1.5mm³ (5 pieces)</td>
<td>65</td>
<td>ND</td>
<td>3/3</td>
<td>2/3</td>
</tr>
<tr>
<td></td>
<td>SC</td>
<td>1-1.5mm³ (10 pieces)</td>
<td>-</td>
<td>*</td>
<td>0/3</td>
<td>0/3</td>
</tr>
</tbody>
</table>

*no mice were moribund; *Astouol Ph, Colt H, Wang X and Hoffman RM (unpublished data); *PP= parietal pleura; VP= visceral pleura; SC= subcutaneous.

structures look like 'milky spots' previously described in the peritoneum which are initially infiltrated in the early stages of peritoneal dissemination of cancer (60,61).

The same tissue was used for visceral-pleural implantation in 10 mice. It resulted in a 100% take rate. Pleurisy was observed in 6 mice. The chest wall was involved in 10/10 mice, diaphragm in 8/10, and mediastinum in 10/10. Contralateral mediastinal lymph nodes were involved in 5/10 mice.

Interestingly, when the parietal-pleural and visceral-pleural implanted groups were compared: (i) Mean survival was 27.9 and 31 days in visceral-pleural, and parietal-pleural implanted group, respectively, with a statistically significant difference. Moreover, the visceral-pleural implanted mice mimicked advanced-stage pleural diseases compared to the parietal-pleural implanted group regarding greater weight loss, greater tumor spread and lower survival. Thus, these 'patient-like' nude mouse models of human pleural cancer closely resemble the clinical situation.

Some authors have speculated that primary pleural cancer develops from the parietal or diaphragmatic pleura and invades the visceral pleura (62,63). Such a hypothesis takes into account the difference in prognosis of malignancies localized on the parietal pleura or those invading the visceral pleura, both of which are reflected in the mouse models.

Table II summarizes loco-regional growth and metastases after visceral- and parietal-pleural implantation of histologically-intact human lung adenocarcinoma.

Recently, the same procedure of implantation was developed using fresh histologically-intact patient tissue. A pleural adenocarcinoma specimen (#2870) was obtained from a patient with metastatic pleural tumor from a primary ovarian cancer. Five tumor pieces were implanted to the visceral pleura of three mice and to the parietal pleura of three others. Tumor growth was noted in all mice at autopsy. Mean average growth time was 65 days. Table II shows local and regional spread on macroscopic examination which included involvement of the ipsilateral lung, diaphragm, mediastinum, and pericardium. Enlarged contralateral lymphadenopathies were only observed in mice that were visceral-pleural implanted corroborating clinical observations that visceral-pleural involvement in pleural cancer represents an advanced-stage disease.

4. Clinical applications

Lung cancer is one of the leading causes of cancer-related adult deaths in the world, and its incidence is rising, particularly in the adult female population. Patients with malignant pleural effusions are considered to be in the advanced-stage of malignant disease.

Except for surgery of early-stage lung cancer, therapy is not very effective. This lack of efficacy of non-surgical treatment modalities is related to the lack of suitable animal models. The development of orthotopically-implanted human models constructed with histologically-intact human tissue should be of particular value for future studies of lung and pleural cancer biology and treatment. Advantages include the following: (i) These models can be constructed directly from patient tumor specimens; (ii) Local and regional spread are observed with a high take rate; (iii) The tumors can metastasize distally. These models closely reproduce clinical features and should facilitate further studies of lung and pleural cancer biology, pathophysiology and lead to new treatment modalities.
References


