A New Patient-Like Metastatic Model of Human Small-Cell Lung Cancer Constructed Orthotopically with Intact Tissue Via Thoracotomy in Nude Mice

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Abstract. A new nude-mouse metastasizing orthotopic transplant model of human small-cell carcinoma of the lung is described. Histologically-intact human small-cell lung tumors were transplanted to the left lung of nude mice via a thoracotomy procedure we have developed. The transplanted tumors grew extensively locally and metastasized to the opposite lung, lymph nodes and other clinically-relevant sites. The results indicated that the model developed could have clinical relevance and contrast with models of small-cell carcinoma constructed with injections of cell suspensions which result in few or no metastases.

A number of xenograft models have been developed for human lung cancer. These include subcutaneous-implant models and implantation under the renal capsule, but these models have not been sufficiently representative of the clinical situation (1). The studies of McLemore et al (1, 2) have utilized the orthotopic transplantation concept to develop more relevant lung-tumor models in nude mice. The first orthotopic-transplant model of human lung cancer was developed by McLemore et al (2) who utilized the growth of human lung cancer cell lines in the bronchial/pleural region of the right lung of nude mice implanted via an intratracheal injection. Suspensions of disaggregated fresh tumor specimens were also implanted intrathecally by this group. The tumors grew intrathecally much more extensively than the same tumors inoculated subcutaneously. However, most of the tumors propagated intrathoracically were localized to the right lung, with only 1% metastasizing to the left lung and only 2% to the trachea, 6% to the peri-tracheal area and only 3% spreading distantly to lymph nodes, liver or spleen. McLemore et al (1) subsequently developed a second model by injecting lung tumor cells via an intrathoracic route into the pleural space. This model seems similar to the intratracheal model in that extensive local growth occurs with little metastatic spread. Intratracheal models using injection of cell suspensions of human lung tumor cells have been used in the nude rats (3, 4) with similar results.

Continuing our approach of orthotopically transplanting intact tissue to construct clinically-relevant metastasizing models in nude mice of human colon, pancreas and bladder cancers (5-9), we have developed a thoracotomy procedure in nude and severe-combined - immunodeficient (scid) mice to implant histologically-intact lung cancer tissue (10). This model, as described in this report, is the first to allow extensive local growth, opposite lung metastases and metastases to regional and distant lymph nodes of human small-cell lung carcinoma.

Materials and Methods

Male and female athymic nude and scid mice at approximately 4-6 weeks of age were used. First, experimental animals were put in a glass chamber and were anesthetized with an inhalation anesthetic, isoflurane. When the animals were fully anesthetized, they were taken out and re-stained. Continued anesthesia was maintained with an inhalation tube. Tumors were transplanted into the left lung in all these experimental animals. The reason that the left lung was used for tumor transplantation is based on two aspects:

1) The loss of lung function is relatively smaller than in right lung operations, and the left-lung-operated animals survive better.

2) The left lung in mice has only one lobe, and tumors can develop easily after implantation.

Before starting the operation, 1-6 tumor pieces (1-1.5mm3 per piece) were sewn together with a 7-0 nylon surgical suture and were fixed by making one knot. Putting all pieces of the tumor on one suture before the operation can induce a quick operation, which is necessary for the survival of the animals. Experimental animals were put in a position of right lateral decubitus, retaining four limbs properly. A 0.8 cm transverse incision of skin was made in the left chest wall. Chest muscles were separated by a sharp dissection and costal and intercostal muscles were exposed. A 0.4-0.5 cm intercostal incision between the third and fourth costal wall was made and the chest wall was opened. The left

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Figure 1. Growth and metastases of human Lu-130 small-cell lung tumor line in nude mice after orthotopic transplantation of histologically-intact human tissue.

A. Implanted human lung tumor grown in left lung of nude mouse (indicated by black arrow).
B. Histopathology of implanted human lung tumor in left lung of nude mouse. Photomicrograph shows typical small cell histology.
C. Metastasis of human lung tumor to mediastinal lymph node. The metastatic lymph node is indicated by open arrow.
D. Photomicrograph shows that the tumor invaded a blood vessel and traveled inside the blood vessel. Open arrow indicates the tumor in the vessel. Solid arrow indicates the locally-growing tumor.
E. Histopathology of metastasis of human lung tumor in mediastinal lymph node.
F. Histopathology of metastasis of human lung tumor in contralateral lung of nude mouse. Open arrow indicates normal lung tissue. Black arrow indicates the metastatic tumor.

Table I. Growth and metastases of human small-cell lung carcinoma cell line Lu-130 in nude mice after orthotopic transplantation of histologically-intact tissue.

<table>
<thead>
<tr>
<th>TPTR*</th>
<th>No. of mice</th>
<th>No. of mice with local growth</th>
<th>No. of mice with metastases</th>
<th>Size of implanted tumor</th>
<th>Average diameter of primary tumor</th>
<th>Average growth time</th>
<th>Regional metastases</th>
<th>Distant metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orthotopic</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>1-1.5 mm^3</td>
<td>9.9 mm^3</td>
<td>62 days</td>
<td>mediastinum</td>
<td>None</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1-1.5 mm^3</td>
<td>ND</td>
<td>82 days</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

*TPTR = Transplantation Route
lung was taken up by a forceps and the tumor was sewn into the left upper lung promptly by one suture. Two knots were made and rest of the suture was cut off. All of the lung tissue was returned into the chest cavity. The incision of the chest wall was closed by a 6-0 surgical suture. Then the closed condition of the chest wall was examined immediately. If a leak exists, it must be closed by adding sutures until the incision is closed completely. After closing the chest wall, an intrathoracic puncture was made by using a 3 ml syringe and 25G1/2 needle to withdraw the remaining air in the chest cavity. After the withdrawal of air, a completely inflated lung could be seen through the thin chest wall of the mouse. Then the skin and chest muscles were closed with a 6-0 surgical suture in one layer. All procedures of the operation described above were performed with a 7x magnification microscope. When palpable tumor was formed and the performance status of the mice began to decrease the animals were sacrificed and autopsied. Evidence for gross tumor was obtained and all major organs were fixed in formalin and prepared for sectioning and staining with hematoxylin and eosin by standard procedures.

Results and Discussion

Table I and Figure 1 demonstrate that for the human
Table II. Growth and metastases of human small-cell lung carcinoma cell line H-69 in nude mice after orthotopic transplantation of histologically-intact tissue.

<table>
<thead>
<tr>
<th>TPTR*</th>
<th>No. of mice</th>
<th>No. of mice with local growth</th>
<th>No. of mice with metastases</th>
<th>Size of implanted tumor</th>
<th>Average diameter of primary tumor</th>
<th>Average growth time</th>
<th>Regional metastatic sites</th>
<th>Distant metastatic sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ortho-</td>
<td>8</td>
<td>8</td>
<td>4</td>
<td>1-1.5 mm³ 6 pieces</td>
<td>10.4 mm</td>
<td>18.5 days</td>
<td>mediastium</td>
<td>chest wall</td>
</tr>
<tr>
<td>Subcu-</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>1-1.5 mm³ 10-30 pieces</td>
<td>ND</td>
<td>24 days</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

*TPTR = Transplantation Route

Small-cell carcinoma xenograft Lu-130, when implanted into the left-lung of nude mice as intact tissue via thoracotomy, extensive local growth occurred with metastases to the contralateral lung and mediastinal lymph nodes. Average time to obvious symptoms in the mice was 62 days. Subcutaneous implantations of this tumor resulted in local growth with no metastases.

Table II and Figure 2 demonstrate that for the small-cell carcinoma xenograft H-69, when implanted into the left lung of the nude mice via thoracotomy, extensive local growth occurred with metastases to the mediastinum, chest wall, ipsilateral lung, contralateral lung and mediastinal lymph nodes. The growth of this tumor was exceedingly rapid with time to symptoms only 18.5 days as compared to 62 days for Lu-130.

The results described here for Lu-130 and H-69, as well as Lu-24 from our previous publication (10), indicate that all three xenografts explanted as intact tissue into the left lung of nude mice via thoracotomy grew extensively locally with a highly metastatic pattern that reflects the clinical picture. Our results contrast with Howard et al. where an intrabronchial injection method was used to implant a cell suspension of the NIH-H-345 small-cell carcinoma line with resulting low-level local growth and no metastases.

Thus, as shown for our bladder-tumor results (5, 7), orthotopic implantation of histologically-intact tissue may be critical in allowing the full metastatic potential of tumors transplanted to immunodeficient mice.

The orthotopic-transplant human small-cell carcinoma model described here should be of use for pre-clinical evaluation of new therapeutics and for research into the biology of small-cell carcinoma growth and metastasis.

References