A Patient-Like Metastasizing Model of Human Lung Adenocarcinoma Constructed via Thoracotomy in Nude Mice

XIAOEN WANG¹, XINYU FU¹ and ROBERT M. HOFFMAN¹,²

¹AntiCancer, Inc., 5325 Metro Street, San Diego, California 92110;
²Laboratory of Cancer Biology, University of California, San Diego, La Jolla, California 92030-069, U.S.A.

Abstract. A new nude-mouse metastasizing orthotopic transplant model of human adenocarcinoma of the lung is described. Histologically-intact human A549 adenocarcinoma 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 described indicate the model developed could have clinical relevance and contrast with models of the A549 lung adenocarcinoma constructed orthotopically with injections of cell suspensions which result in low metastatic potential.

Adenocarcinoma of the lung in humans is a frequently occurring tumor that is generally refractory to therapy. New treatment of this disease could be enhanced by development of animal models representative of this type of tumor. In this light, Howard et al. (1) injected suspensions various of types of the human adenocarcinoma cell line A549 intrabronchially via the trachea in nude mice. This method of orthotopic transplantation resulted in local growth in the right lung with metastases to mediastinal lymph nodes but no metastases to the ipsilateral or contralateral lung, both important sites of clinical metastases in adenocarcinoma.

Other 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 (2). The studies of McLemore et al. (2,3) 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. (3) who utilized the growth of human lung cancer cell lines in the bronchioloalveolar region of the right lung of nude mice implanted via an intrabronchial injection. Suspensions of disaggregated fresh tumor specimens were also implanted intrabronchially by this group. The tumors grew intrabronchially much more extensively than the same tumors that inoculated subcutaneously. However, 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, 5% to the peritracheal area and only 3% spreading distantly to lymph nodes, liver or spleen. McLemore et al. (2) subsequently developed a second model by injecting lung tumor cells via an intrathoracic route into the pleural space. This model seems similar to the intrabronchial model in that extensive local growth occurs with relatively metastatic spread. Intrabronchial models using injection of cell suspensions various types of human lung tumor cells have been used in the nude rats (1,4) with similar results.

Continuing our approach of orthotopically transplanting intact tissue to construct clinically-relevant metastasizing models in nude mice of human colon, pancreas, prostate and bladder cancers (5-9,11), 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 lung adenocarcinoma.

Materials and Methods

A549 human lung adenocarcinoma cells were grown on monolayers and then 10⁶ cells were injected into the flank of nude mice. When the tumor was approximately 1 cm in diameter, the tumor was harvested and subdivided into 1 mm³ pieces.

Male and female athymic nu/nu and scid mice at approximately 4-6 weeks of age were used. First, experimental animals were put in a glass chamber and are anesthetized with an inhalation anesthetic, isoflurane. When the animals were fully anesthetized, they were taken out and restrained. Continuous 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 (1-1.5 mm$^3$ 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 ensure a quick operation, which is necessary for the survival of the animals. Experimental animals were put in a position of right lateral decubitus, restraining the four lungs properly. An 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 intercostal incision on the third or fourth costa on the chest wall was made and the chest wall was opened. The left lung was taken up by a pair of 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 in 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 was closed completely. After closing the chest wall, an intrathoracic puncture was made by using a 3 ml syringe and 25 G1/2 needle to withdraw the remaining air in the chest cavity. After the withdrawal of air, a completely inflated lung through the thin chest wall of the mouse could be seen. Then the skin and chest muscles were closed with 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 begins to decrease the animals were sacrificed and autopsied. Evidence for gross tumor was obtained and all major organs were fixed in formalin 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 cosin by standard procedures.

Results and Discussion

After orthotopic transplantation of histologically-intact tissue of A549 into the left lung of 5 nude mice via thoracotomy, tumors resulted in 3 of the animals. The animals became symptomatic of apoxia with an elevated breathing rate and became cachectic. The primary tumor reached about 1 cm in diameter and also was found widely disseminated on the chest wall (Figure 1a).

The histopathology revealed an adenocarcinoma phenotype (Figure 1c). Opposite lung metastases were also observed (Figure 1b) with the histopathology revealing an adenocarcinoma phenotype (Figure 1d). The tumor also metastasized to lymph nodes. A mediastinal lymph node is shown in Figure 1e and histopathology revealed an adenocarcinoma phenotype (Figure 1f).

Thus, the method for using intact tissue for orthotopic transplantation is more effective than using cell suspensions for lung carcinoma (1-4). While the cell-suspension method of orthotopic transplantation resulted in local tumor growth and some lymph node metastases, no contralateral or ipsilateral lung metastases resulted as they did for our method utilizing intact tissue.

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

The orthotopic transplant human lung adenocarcinoma model described here should be of use for pre-clinid evaluation of new therapeutics and for research into the biology of lung adenocarcinoma growth and metastasis.

References


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