A Metastatic Orthotopic - Transplant Nude-Mouse Model of Human Patient Breast Cancer

XINYU FU1, PHUONG LE1 and ROBERT M. HOFFMAN1,2

1AntiCancer Inc., 5325 Metro Street, San Diego, CA 92110; 2Laboratory of Cancer Biology, Department of Pediatrics, 0609F, School of Medicine, University of California, San Diego, La Jolla, CA 92030609, U.S.A.

Abstract. We report here the development of an orthotopic-transplant model of human patient breast cancer in nude mice. Histologically-intact patient breast tumor tissue was transplanted as intact tissue to the mammary fat pad of nude mice where the tumor tissue grew extensively and metastasized to the lungs. This is the first orthotopic-transplant metastatic model of human breast cancer. The potential clinical and basic-science uses of the model are discussed.

Breast cancer is one of the most devastating diseases to women and, in the Western world, affecting one out of nine women in their lifetime. Metastatic breast cancer has a poor prognosis, especially if the tumor is hormone-independent.

A number of animal models of breast cancer have been developed over the past years, especially using cell lines. Metastasis of xenografted tumors can occur if human breast cancer cell lines such as MCF-7 injected in the mammary fat pad of female nude mice supplemented with estrogen. These orthotopically-injected breast cancer cell lines can metastasize to the lungs and lymph nodes (1, 2, 3, 4). One estrogen-receptor (ER)-negative cell line, MDA-MB-231, has been reported to form lung metastases after i.v. injection (5). Price et al (6) have reported that another ER-negative cell line, MDA-MB-435, after injection in the mammary fat pad, produces metastases in several different organs in the nude mice. The orthotopic injection model of murine breast cancer cell line was also shown by Elliott et al (7,8) to allow both local growth and metastases as well, for example, to the lung of the nude mouse.

However, human breast carcinoma patient specimens have previously had a low tumor take rate in nude mice (9). Although Outzen and Caster (10) orthotopically implanted human breast cancer-patient surgical specimens and local growth occurred, no metastases were observed. In the other experiments reported above only cell lines were used. Thus, there is a critical need for metastatic rodent models of human patient breast cancer. In this light, we have developed orthotopic-transplant nude-mouse models of human cancers of the colon (11, 12), stomach (13), pancreas (14), bladder (15, 16), prostate (17) and lung (18, 19, 20) that utilized microsurgery for transplantation of intact tissue, including patient specimens. In at least bladder cancer (15, 16), lung cancer (18, 19, 20), and stomach cancer (13) intact-tissue orthotopic transplants seem to result in considerably more metastatic potential than orthotopic injections of cell suspensions. We describe here the orthotopic transplantation of a human breast cancer patient specimen to the nude mouse mammary fat pad which subsequently led to orthotopic growth and metastases to the lung of the nude mice. The results described here demonstrate it is possible to develop metastatic models for breast - cancer patient tumors in immunodeficient mice for basic and treatment studies.

Materials and Methods

Four-week old outbred female nu/nu mice were used for tumor transplantation. All nude mice were bred and maintained in a separate specific pathogen-free facility with controlled light/dark cycle, temperature and humidity. Cages, bedding, food and water were all autoclaved. A surgical specimen of a poorly-differentiated ductal carcinoma of human breast (AntiCancer #2468) was used for tumor transplantation. The tumor specimen was inspected, and grossly necrotic and suspected necrotic tissue was first removed. The tumor specimen was then equally divided into six parts, and each part was subsequently cut into small pieces about 1 mm³. Tumor pieces for each transplantation were taken from each of the six parts of the specimen equally. In this way, different areas of the heterogeneous cancer tissue can be equally selected, and the chance for viable tissue to be transplanted is also maximized.

For orthotopic transplantation, nude mice were anesthetized with isoflurane inhalation before surgery. After a proper state of anesthesia was induced, the nude mice were put in a supine position. The second right mammary gland was chosen for orthotopic transplantation because it has anatomical resemblance to the anatomical position of the human breast. The surgical region was sterilized with iodine and alcohol swabs.
Table I. Comparison of growth and metastasis in nude mice of orthotopically-transplanted and subcutaneously-transplanted histologically-intact surgical specimens of human breast cancer.

<table>
<thead>
<tr>
<th>Transplantation route</th>
<th>No. of mice</th>
<th>No. of mice with primary tumor</th>
<th>No. of mice with lung metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orthotopic transplantation</td>
<td>8</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Subcutaneous transplantation</td>
<td>7</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Histologically-intact human-patient breast cancer specimens from patient AC # 2468 were transplanted orthotopically to the 2nd mammary fat pad of nude mice or subcutaneously in the flank of nude mice and were allowed to grow for three months. (For details, see text).

An incision of about 1.5 cm was made along the medial side of the nipple. After blunt dissection, the fat pad was exposed. A small incision was made on the fat pad and a small pocket was formed. Six pieces of tumor tissue, previously prepared as described above, were transplanted into the pocket and a 3-0 suture was made to close the pocket. The skin layer was closed by 6-0 sutures.

For subcutaneous transplantation, after the nude mice were anesthetized and the surgical region sterilized, six pieces of tumor specimen were transplanted into the flank of the nude mice. Incisions were closed with 6-0 surgical sutures.

For histological studies at the time of sacrifice, primary tumors grown in the nude mice were removed, major organs and lymph nodes were inspected and removed and put into 10% formalin. All the tissues were subsequently processed through alcohol dehydration, chloride and paraffinization. Tissues were embedded in paraffin and sectioned at 5 μm. All slides were stained by hematoxylin-eosin and examined microscopically.

Figure 2. Nude mouse bearing human breast cancer after orthotopic transplantation of histologically-intact surgical specimens from patient (AC # 2468). The histologically-intact human breast cancer tissue was transplanted to the right second mammary gland fat pad of the nude mouse and was allowed to grow for three months.

Figure 3. In situ hybridization of human genomic DNA probe to lung metastasis in nude mouse after orthotopic transplantation of human breast cancer. Brown stain indicates positive probe hybridization and human origin of cells.
For *in situ* hybridization studies to demonstrate the presence of human genes in the breast tumors growing in the nude mice, paraffin-embedded tissue blocks were sectioned at 4 μm and mounted on silanized slides. After deparaffinization and enzymatic protein digestion, a biotinylated human DNA probe ( Oncor, Gaithersburg, MD 20877) was used for hybridization. Avidin and anti-avidin-antibody, along with avidin-horseradish peroxidase and 3,3'-diaminobenzidine tetrahydro-chloride (DAB) as the substrate, were subsequently applied for the detection of the hybridization which was visualized by brown staining. Hematoxylin and eosin were used for counterstaining.

**Results and Discussion**

Eight mice were used for orthotopic transplantation and seven mice were used for subcutaneous transplantation of the breast cancer specimen. All 15 mice had primary tumor growth after transplantation. The subcutaneously-growing tumors were encapsulated with no local invasion or distal organ metastasis observed. For mice with orthotopic transplantation, the local tumor grew in the mammary gland into a very large mass (Figure 2). The locally-growing tumor was anaplastic and poorly differentiated (Figure 1B) and was very similar to the pretransplantation patient's tumor (Figure 1A). No local invasion and infiltration of the tumor, and no axillary lymph node metastasis were observed. However, six out of eight (75%) mice in the orthotopic transplantation group had multiple metastatic nodules in the lung (Table I, Figure 1C).

The metastatic nodules in the lung, when examined histopathologically, were seen also to be poorly differentiated and (Figure 1D) very similar to the locally-growing tumor. *In situ* hybridization experiments with a human genomic-wide probe were positive for the locally-growing tumor and lung metastasis demonstrating their human origin (Figure 3). Thus, the results described here demonstrate that human breast cancer obtained directly from surgery can grow orthotopically in nude mice and metastasize to a clinically-important organ, in this case the lung. This model opens the way toward achieving the goal of being able to transplant, as standard procedure, human breast cancer patient specimens, to obtain clinically-relevant models in an appropriate animal system. Achievement of this goal should greatly enhance our understanding of breast cancer and lead to more effective treatment.

**Acknowledgements**

This work was supported by U.S. National Cancer Institute Small Business Innovation Research Grants R44 CA35963 and R44 CA38139. We thank Ms. Polly Jayne Pomeroy for expert word processing of the manuscript and for many years of devoted service.

**References**


Received February 23, 1993
Accepted March 30, 1993