

Cancer Seed and Soil Can Be Highly Selective: Human-Patient Colon Tumor Lung Metastasis Grows in Nude Mouse Lung But not Colon or Subcutis

SHINJI TOGO^{1,2,3}, XIAOEN WANG⁴, HIROSHI SHIMADA¹, A.R. MOOSSA² and ROBERT M. HOFFMAN^{3,4}

¹Second Department of Surgery, Yokohama City University School of Medicine, Yokohama, Japan;

²Department of Surgery, University of California, San Diego Medical Center, San Diego, CA;

³Laboratory of Cancer Biology, University of California, San Diego School of Medicine, La Jolla, CA;

⁴AntiCancer Incorporated, San Diego, CA, U.S.A.

Abstract. *The question remains as to whether metastatic cells (cancer seed) that eventually colonize a particular organ (cancer soil) have specific properties that distinguish them from the other cells of the primary tumor. However until recently there have not been model systems in which this question could be fully answered. To further understand the relationship between seed and soil we have developed an orthotopic-transplantation nude-mouse model that allows human tumors to essentially replicate their behavior they had in the patient. The patient-like behavior of the transplanted human tumor in the nude mouse depends on the use of intact tumor tissue for orthotopic transplantation. Here we report that a colorectal tumor lung metastasis surgically resected from a patient could grow in nude mouse lung, but not in either the colon or the subcutis after transplantation of intact tissue. The results were striking in that the human colorectal tumor lung metastasis grew in the lung of the animals and not in the colon or in the subcutis of the animals. The results described here suggest that the lung metastasis of the patient colon tumor is distinct in its soil requirement from the majority of the cells of the original colon tumor. In contrast, in the intact-tissue orthotopic transplant model, primary human colon tumors grow when transplanted to the colon of the nude mouse. Thus the colorectal cancer "seed" which metastasized to the lung in the patient seems very selective for the "soil" of the lung of both the patient and the nude mouse.*

Paget (1) over 100 years ago observed hundreds of cases of breast cancer and noticed that the metastatic target organs are not random. From these observations, Paget formulated the

seed and soil hypothesis of metastasis. The degree of selectivity of the metastatic seed is, however, not well understood. For example, the question remains as to whether the metastatic cells that eventually colonize a particular organ have specific properties that distinguish them from the other cells of the primary tumor. However, until recently there have not been model systems in which these questions could be fully answered.

To understand further the relationship between seed and soil we have developed an orthotopic-transplantation nude-mouse model that allows human tumors essentially to replicate the behavior which they had in the patient (2-8). The patient-like behavior of the transplanted human tumor in the nude mouse depends on the use of intact tumor tissue for orthotopic transplantation. The models have already revealed highly specific interaction between tumor (seed) and the corresponding primary host organ. For example, if a human colon tumor is transplanted as intact tissue to the nude mouse colon, the tumor spreads and invades in the primary host organ and subsequently metastasizes to the lymph nodes and to the liver. In striking contrast, when the human colon tumor is transplanted to the nude mouse stomach, the tumor can grow but does not spread, even along the serosa, invade, or metastasize (10). The models have also shown that tumors may even specifically reverse metastasize, in that colon tumors implanted on the liver can metastasize back to the colon, but seemingly not to other organs.

Here we report that a colorectal tumor lung metastasis surgically resected from a patient could grow in the nude mouse lung, but not in either the colon or the subcutis, after transplantation of intact tissue, demonstrating the deviation of the metastasis from the primary tumor.

Materials and Methods

Mice. Four-week-old outbred nu nu mice of both sexes were used for tumor implantation. All animals were maintained in a sterile environment; cages, bedding, food and water were all autoclaved. All animals were maintained on a daily 12-hr light / 12-hr dark cycle. Bethaprim pedi-

Correspondence to: Robert M. Hoffman, Ph.D., AntiCancer, Inc., 7917 Ostrow Street, San Diego, CA 92111, U.S.A. Phone: 619-654-2555, FAX: 619-268-4175.

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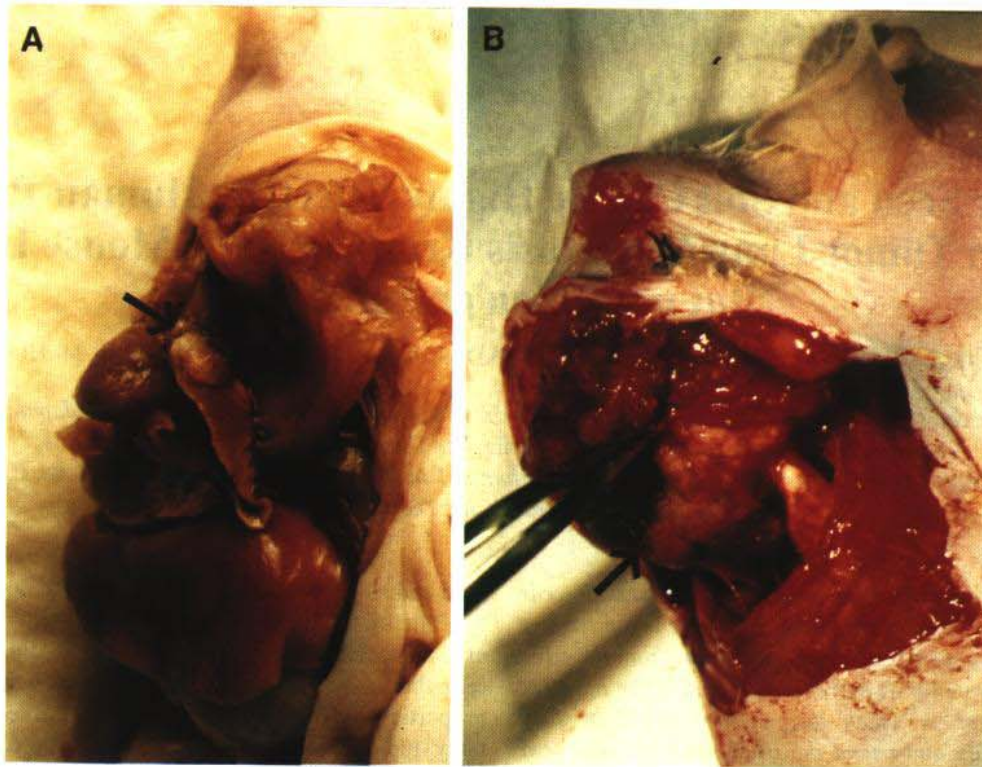


Figure 1. Gross finding in nude mice of lung-implanted human colon tumor lung metastasis. Implanted human colon tumor lung metastasis growth in the left lung of the nude mouse (indicated by the black arrow). A=mouse 1, B=mouse 2
For transplantation procedures see text.

atric suspension (containing sulfamethoxazole and trimethoprium) was added to the drinking water. NIH guidelines were followed for all animal experimentation.

Human cancer tissue. A 66-year-old man underwent a low anterior resection due to rectal cancer and simultaneous partial hepatectomy for a liver metastasis in January 1993. A partial lung resection was performed for a lung metastasis of the colorectal tumor in May 1994. The original colon tumor, liver metastasis and subsequent lung metastasis specimens were all moderately differentiated adenocarcinoma.

Tumor tissue implantation. The lung metastasis specimen was inspected, and grossly necrotic and suspected necrotic tissues were cut away. The remaining healthy tumor tissues were scissor minced into pieces about 1-2 mm³ size in Hanks' balanced salt solution.

Nude mice were anesthetized with isoflurane (Forane) inhalation.

For implantation on the colon, an incision was made through the left upper abdominal pararectal line and peritoneum. The cecum wall was carefully exposed, and a part of the serosal membrane, about 3 mm in diameter, was mechanically injured using forceps. Five to 7 pieces of 1-2 mm³ size were implanted on the nude mouse colon where the serosa had been injured. An 8-0 suture was used to penetrate these small pieces and suture them on the wall of the colon. The colocecal part of the intestine was then returned to the peritoneal cavity, and the abdominal wall and skin were closed with 6-0 black-silk sutures.

Tumor tissue was also transplanted onto the left lung of nude mice (5). Before starting the operation, 3-5 tumor pieces (1-2 mm³ per piece) were sewn together with a 7-0 nylon surgical suture and were fixed by making one knot. Experimental animals were put in a position of right lateral decubitus, restraining four limbs properly. A 0.8 cm transverse incision of skin was made in the chest wall. Chest muscles were separated by a sharp dissection. The costal and intercostal muscles were then exposed. A 0.5 cm intercostal incision between the third and fourth costa on the chest

Table I. Site of growth of human-patient colon tumor lung metastasis in nude mice.

Transplantation site	Number of mice with tumor growth / mice transplanted
Lung	2 / 2
Colon	0 / 4
Subcutis	0 / 2

The lung metastasis of a 66 year old patient who originally had a colorectal tumor was surgically transplanted as intact tissue to the nude mouse lung, colon and subcutis as described in the text. Tumor growth was analysed at autopsy 12-15 weeks after transplantation as described in the text.

wall was made and the chest wall was opened. The left lung was taken up by a forceps and the tumor was sewn onto the left upper lung promptly by one suture. All lung tissue was returned into the chest cavity. The incision of the chest wall was closed by a 6-0 suture.

Subcutaneous transplantation was also carried out. Seven-10 pieces of 1-2 mm³ tumor tissue were transplanted subcutaneously in the left flank with a 6-0 suture.

Evaluation of growth and metastasis of orthotopically implanted tumors. The animals were sacrificed and autopsied 12-15 weeks after transplantation. The gross tumor growth was observed and all major organs were fixed in formalin and prepared for sectioning and staining with hematoxylin and eosin by standard procedures.

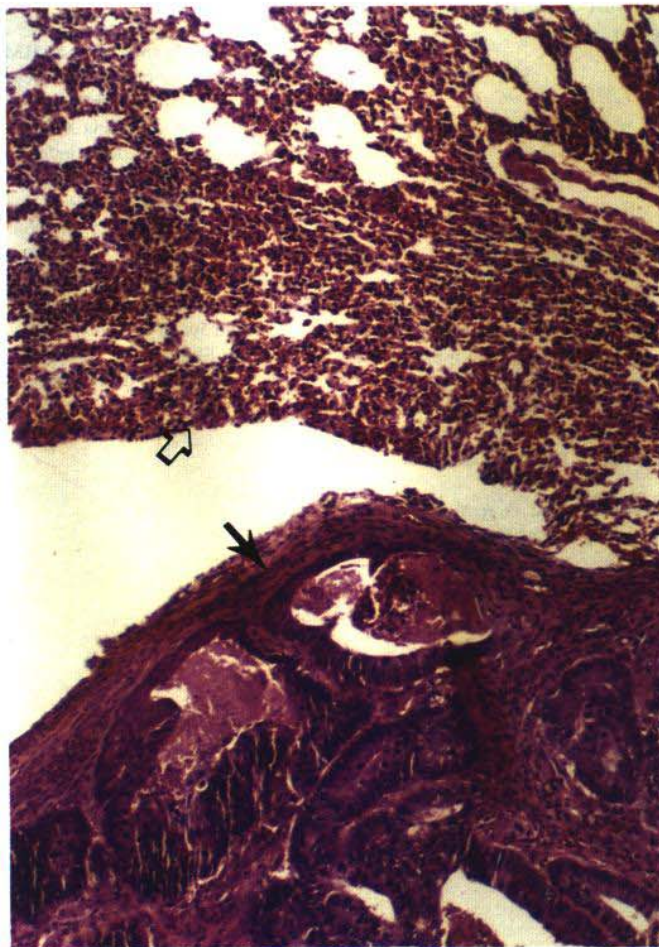


Figure 2. Microscopic finding of human colon lung metastasis implanted on the lung of the nude mouse. Growth of human colon tumor lung metastasis indicated by the black arrow Mouse lung tissue indicated by the open arrow For transplantation procedures see text.

Results and Discussion

The results were striking in that the human colorectal tumor lung metastasis grew in the lung in 2 out of 2 animals and not the colon in 4 out of 4 animals nor the subcutis in 2 out of 2 animals (Table I). Tumor growth on the lung ranged from 2 x 2 mm to 25 x 22 mm (Figure 1A, B). Figure 2 is a photomicrograph which shows the moderately differentiated transplanted human colorectal cancer lung metastasis grown on the nude mouse lung. The resected lung metastasis from the patient and the growing lung metastasis in the mouse were both identical histologically to the patient's original rectal tumor (data not shown).

The results described here suggest that the lung metastasis of the patient's colon tumor is distinct in its "soil" requirement from the majority of the cells of the original colon tumor. In

contrast, in the intact-tissue orthotopic transplant model, primary colon tumors grow when transplanted to the colon. Thus the colorectal cancer "seed" which metastasized to the lung in the patient seems very selective for the "soil" of the lung of both the patient and the nude mouse.

In earlier studies, Fodstad's group demonstrated that a lung metastasis of a human melanoma showed a preference for the lung when injected into the nude mouse by several routes. In contrast, cells from a patient with lymphatic spread of a melanoma gave lymph node metastasis when injected in nude mice (9). The orthotopic transplant studies described in our report now give direct evidence that metastatic tumors can become highly selective for the target organ. Future studies should focus on the selective properties that tumors acquire in order to grow in the metastatic target organ and which selective properties are lost that eliminate the tumor's ability to grow on the primary organ. The potential difference in the

metastatic and primary tumors should be considered in treatment strategies for metastatic cancer.

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