The First Patient-Derived Orthotopic Xenograft (PDOX) Mouse Models of Cancer: Cancer of the Colon, Pancreas, Lung, Breast, Ovary, and Mesothelioma

7

Robert M. Hoffman

Introduction

The introduction of the athymic *nu/nu* mouse (nude mouse) for the growth of human tumors in 1969 changed the paradigm of basic and applied cancer research. Human tumors could now be grown for the first time in a mouse model due to the nude mouse's lack of a thymus which makes T cells. In 1969, Rygaard and Povlsen [1] implanted a colon cancer from a 74-year-old patient subcutaneously (s.c.) in nude mice, which grew as a well-differentiated adenocarcinoma similar to that from the donor patient. The subcutaneously growing tumors were encapsulated and did not metastasize. The original tumor was maintained over 7 years for 76 passages. The vast majority of human solid tumors, growing s.c. in the nude mouse did not metastasize. The s.c.-transplanted tumors had noninvasive growth [1, 2].

Wang et al. [3] in 1982 were among the first to implant human tumors orthotopically (literally "correct surface") in nude mice rather than "heterotopically" (literally "different surface," such as s.c.). Human colon cancer cell suspensions were injected within the descending part of the colon of nude mice, which resulted in occasional metastasis, a big breakthrough. Fidler's laboratory and others have shown that the implantation of many types of human tumors in the orthotopic sites of nude mice resulted in metastasis of human tumors [4].

In order to overcome the low frequency of metastasis observed with orthotopic implantation of cell suspensions, our laboratory pioneered the patient-derived orthotopic xenograft (PDOX) nude mouse model with the technique of surgical orthotopic implantation (SOI) of intact colon cancer tissue [5]. A greater extent of metastasis was observed in PDOX models compared with orthotopically implanted cell suspensions.

R.M. Hoffman
AntiCancer, Inc., 7917 Ostrow Street, San Diego, CA 92111, USA

Department of Surgery, University of California, San Diego, San Diego, CA, USA
e-mail: all@anticancer.com

R.M. Hoffman (ed.), *Patient-Derived Mouse Models of Cancer*,
Molecular and Translational Medicine, DOI 10.1007/978-3-319-57424-0_7

The First PDOX Models (1991–1996)

We developed the first orthotopic metastatic model of patient colon cancer. Histologically intact human colon-cancer specimens derived surgically from patients were implanted orthotopically to the colon or cecum of nude mice. We observed extensive orthotopic growth in 13 of 20 cases of implanted patient colon tumors. These showed various growth patterns with subsequent regional, lymph node, and liver metastasis, as well as general abdominal carcinomatosis [5].

Colon-Cancer Local Growth and Abdominal Metastasis

An example is specimen case 1701, an infiltrating mucinous adenocarcinoma of the right colon (modified Duke's classification C2). Two nude mice with pre-implanted Gelfoam were used for tumor implantation, two nude mice were used for tumor implantation with an internal skin flap, and two nude mice were used for direct implantation of tumor tissue to the cecum. The mice demonstrated extensive primary growth as well as abdominal-wall metastases. All mice showed visible tumor growth in the abdomen. Autopsies were performed 113–139 days after implantation [5].

Colon-Cancer Local Growth, Abdominal Metastasis, and Lymph Node Metastasis

An example is specimen case 1707, an infiltrating adenocarcinoma of the right colon, moderately differentiated (modified Duke's classification D). Two nude mice were used for tumor and normal-surrounding-tissue co-implantation to the cecum, and two nude mice were used for tumor direct implantation to the cecum. Orthotopic primary tumor growth and abdominal metastasis occurred in three mice. A 10 × 10 mm primary tumor and 12 × 14 abdominal-wall metastasis were found at day 175 after implantation in one of the mice (tumor and normal surrounding tissue co-implanted). Lymph node metastases were noted in this animal. The histology of the original tumor and the orthotopically growing tumor both indicated adenocarcinoma [5].

Colon-Cancer General Abdominal Carcinomatosis with Extensive Peritoneal Seeding

An example is specimen case 1935, infiltrating mucinous adenocarcinoma, moderately differentiated. Extensive carcinomatosis was found with small tumors growing all over the peritoneum and abdominal organs [5].

Colon-Cancer Liver Metastasis

One nude mouse with pre-implanted Gelfoam and an orthotopically-implanted colon tumor was found to have extensive primary tumor growth and multiple liver metastasis. Histology studies on the original tumor tissue, abdominal masses, and multiple liver lesions indicated adenocarcinoma [5].

Colon-Cancer Sequential Appearance of Primary Tumor and Metastasis

Laparotomy was performed on day 26 on the nude mouse implanted with patient tumor 1594. Primary tumor growth was observed. No local or distal organ metastases were observed. The animal was returned for further observation on day 78 when the second laparotomy was performed. Primary growth was found. No liver or other distal organ metastasis was found. On day 160, the mouse was sacrificed. Primary tumor growth, local invasion, and liver metastasis were found at autopsy [5].

PDOX Model of Lung-Metastatic Colon Cancer

The human-patient colorectal tumor lung metastasis grew in the lung in 2 out of 2 animals and not in the colon in 4 out of 4 animals nor in the subcutis in 2 out of 2 animals. Histology showed a 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 from which the lung metastasis occured [6]. The lung metastasis appeared to be altered to such an extent that it lost the ability to grow in the colon.

PDOX Model of Pancreatic Cancer

We developed the first orthotopic metastatic mouse model of patient pancreatic cancer. Orthotopic transplantation of histologically intact pancreatic-cancer specimens to the nude-mouse pancreas was performed. The results reflected clinical pancreatic cancer including extensive local tumor growth; extension of the locally growing human pancreatic cancer to the nude-mouse stomach and duodenum; metastases to the nude-mouse liver and regional lymph nodes; and distant metastases to the nude-mouse adrenal gland, diaphragm, and mediastinal lymph nodes. In a series of five patient cases, there was a 100% take rate. Of 17 mice transplanted, 15 had tumor growth. Immunohistochemical analysis of the antigenic phenotype of the transplanted human pancreatic tumors showed a similar pattern of expression of two human

tumor-associated antigens, tumor-associated glycoprotein 72, and carcinoembryonic antigen (CEA) in the transplanted tumors, similar to the original surgical biopsy [7].

Pancreatic-Cancer Distant Metastasis to Liver

Metastases to the liver surface were seen in case 2020 [7].

Pancreatic-Cancer Very Distant Metastases

Case number 2020 involved metastasis to mesenteric lymph nodes, and case number 2008 involved distant metastases to the diaphragm, adrenal glands, mesenteric lymph nodes, and mediastinal lymph nodes and iliac lymph nodes. Metastasis to distant sites such as mediastinal lymph nodes demonstrated that actual metastasis occurred in the model, as opposed to just extension or seeding [7].

PDOX Model of Lung Cancer

We developed the first orthotopic metastatic model of patient lung cancer. Poorly differentiated large-cell squamous-cell lung cancer from a patient was transplanted orthotopically to the left lung as histologically-intact tissue directly from surgery. All five implanted mice produced locally grown tumors in an average time of 61 days. Opposite-lung metastases occurred, as well as lymph node metastases. The primary tumor and metastases faithfully maintained its large cell-squamous cell morphology. When grown s.c., this tumor grew only locally in 2 of 4 animals, and no metastases were observed [8].

PDOX Model of Mesothelioma

We developed the first orthotopic metastatic model of patient mesothelioma. Fresh specimens derived from four patients with malignant mesothelioma were implanted on the parietal pleura of nude mice using SOI. All xenografted tumors resulted in locally growing tumors in the mice. The transplants had extensive tumor spread in the ipsilateral and contralateral pleural cavity as well as mediastinal lymph nodes. When the tumors were confined to the ipsilateral parietal pleura, the implanted animals were in good physical condition. The macroscopic features usually found in mesothelioma patients were also found in the implanted animals, such as nodules, masses, and pleural thickness. Histology of the mousegrown tumor was similar to the original tumor [9].

Orthotopic implantation in nude mice of mesothelioma resulted in growth in all four cases attempted. All patient specimens showed regional spread as well as orthotopic primary tumors [9]. Histologic examination as well as immunohistochemical profiling revealed malignant pleural mesothelioma similar to the original

tumor specimen [9]. In case AC 3157, the implanted tumor was located only on the parietal pleura in one mouse, and in the other mouse, the tumor invaded the visceral and mediastinal pleura. Moreover, in one mouse, a huge nodule developed on the chest wall due to tumor invasion from the pleura. Contralateral invasion of the mediastinal pleura as well as ipsilateral metastatic lymph nodes were observed [9]. In case AC 3208, parietal and visceral-pleural involvement was observed in one transplanted mouse without any other signs of tumor-related disease. Macroscopic examination demonstrated tumor involvement of the visceral and mediastinal pleura as well as the diaphragm. The mediastinum was invaded by the tumor, and ipsilateral and contralateral metastatic mediastinal lymph nodes were observed [9]. In case AC 3083, two mice were shown to have tumor spread in the ipsilateral as well as contralateral pleural cavity [9]. Visceral, diaphragmatic, and mediastinal pleura were involved with tumor in the transplanted mice. In some mice, the tumor grew through the mediastinum and invaded the contralateral mediastinal pleura as well as contralateral visceral and parietal pleura. Ipsilateral and contralateral metastatic mediastinal lymph nodes were seen [9]. Orthotopic implantation of patient tumors allowed growth in 100% of the cases. At autopsy, mice were shown to have an extensive tumor spread in the ipsilateral and contralateral pleural cavity as well as mediastinal lymphatic nodes. These results are in agreement with clinical studies showing that mediastinal and visceral-pleural invasion occur in advanced-stage patient cases of mesothelioma [10]. When the lesions were still confined to the ipsilateral parietal pleura, the implanted animals remained in good condition [9].

PDOX Model of Ovarian Cancer

We developed the first orthotopic metastatic model of patient ovarian cancer. Histologically intact patient specimens of ovarian cancer were transplanted by microsurgical techniques under the capsule of the nude mouse ovary. The human tumors grew locally and gave rise to a patient-like metastatic pattern including the parietal peritoneum, colon, omentum, and ascites. Five cases of human ovarian cancer were transplanted into nude mice, two of which gave rise to tumors. In the first case with patient specimen #1943 of stage II cancer, the largest growth was an encapsulated cyst. No rupture or intraperitoneal seeding was observed. This tumor grew with a cystadenocarcinoma growth pattern. In the second case with patient specimen #2443 of stage IV cancer, extensive solid primary tumor growth was observed along with ascites with extensive metastasis to the colon, omentum, and parietal peritoneum of two of the nude mice. It should be noted that in the patient, there was also metastasis to the bowel and omentum [11].

PDOX Model of Pleural-Metastatic Ovarian Cancer

We developed the first orthotopic metastatic model of patient pleural ovarian cancer. Fresh histologically intact patient specimens of human pleural ovarian adenocarcinoma were implanted onto the visceral and parietal pleura of nude mice.

The human tumors grew locally and regionally, mimicking the usual human clinical features of this disease. A pleural adenocarcinoma specimen was obtained from a patient with a 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. Local and regional spread was observed 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 [12].

PDOX Model of Breast Cancer

We developed the first orthotopic metastatic model of patient breast cancer. 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 lung. This was the first orthotopic transplant metastatic model of human patient breast cancer [13]. 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. In contrast, six out of eight (75%) mice in the orthotopic transplantation group had multiple metastatic nodules in the lung [13]. The metastatic nodules in the lung, when examined histopathologically, were seen also to be poorly differentiated and very similar to the locally growing tumor and to the patient's original 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 [13].

Clinical Correlation of PDOX Model of Stomach Cancer

We developed the first orthotopic metastatic model of stomach cancer. Fresh surgical specimens derived from 36 patients with advanced stomach cancer were orthotopically transplanted in nude mice using histologically intact tissue. Twenty of thirty-six patient tumors gave rise to locally-growing tumors in the mice. All 20 patients whose stomach tumors resulted in local growth in the nude mice had clinical lymph node involvement. In contrast, only 8 of the other 16 patients whose tumors were rejected had lymph node involvement. Of the 20 cases resulting in local growth in the nude mice, five had clinical liver metastases and all five cases resulted in liver metastases in the nude mice. Of the 15 patients without liver metastases whose primary tumor grew locally in the mice, only one case gave rise to a liver metastasis in a mouse. Of the 20 cases whose tumors grew in nude mice, six had

clinical peritoneal involvement of their tumor and of these five resulted in peritoneal metastasis in the nude mice. Chromosome analysis confirmed the human origin of the tumors grown in nude mice. These results indicate that, after orthotopic transplantation of histologically intact stomach cancers from patients to nude mice, the subsequent metastatic behavior of the tumors in the mice closely correlated with the course of the tumors in the patients [14]. There was a statistical correlation in metastases between patients and mice. The histology both of primary growth and of metastases in the patient were found to be reproduced in the nude mice [14].

Pleural Lung Cancer PDOX

We developed the first orthotopic metastatic model of pleural lung cancer. A patient lung adenocarcinoma was implanted in the parietal pleura of 11 mice [15]. Implantation in the posterior and low part of the parietal pleura was chosen because of the presence of pleural stomas previously described [16]. Such structures are considered to be a gate through which malignant cells are absorbed from the pleural cavity to the lymphatic circulation via sub-mesothelial lymphatic vessels and also have a connection with sub-peritoneal lymphatics [16–18]. All mice were moribund by days 28–31 after surgery. Performance status was stable until shortly before death. Weight loss, however, was constant [15]. Parietal tumor growth was noted on autopsy in all 11 animals transplanted with patient lung cancer on the parietal pleura. All animals had evidence of chest wall invasion. In addition, the lung was also involved in nine, the mediastinum in seven, the diaphragm in six, and the pericardium in four mice. The mouse-grown tumors had similar histology to the original tumor specimens that were derived. Enlarged lymphadenopathy was not observed. Small, ipsilateral pleural effusions were observed in seven mice. No metastases were observed in the kidneys, adrenal glands, liver, or contralateral lung. There was no evidence of abdominal or contralateral lung metastasis. Rapid tumor growth led to cachexia and death in a relatively short time [15]. In another study, both in the visceral- and parietal-pleural implanted groups, tumor grew in all ten mice transplanted in each group. The median survival time was 27.9 days for the visceral-pleural implanted group and 31 days for the parietal-pleural implanted group. The body weights of pleural-implanted mice decreased from the 14th day until day 31 post-transplantation for the mice remaining alive at that time. The visceral-pleural implanted group had the most weight loss. In contrast, no body weight loss was observed in the subcutaneous-implanted group. The mouse-grown tumors had adenocarcinoma histology similar to the original patient tumor specimen that was derived [19]. Although all pleural-implanted animals showed local and regional spread, no macroscopic and microscopic metastases were observed either on ipsi- or contralateral lung as well as in other organs. However, 5/10 visceral-pleural implanted mice developed metastases involving contralateral mediastinal lymph nodes [19]. Thus, visceral-pleural involvement represents an advanced-stage disease with respect to greater tumor metastases as well as a shorter mean survival time than observed in the parietal-pleural implanted group which is an early-stage

disease. These two components of pleural cancer are mimicked in their respective models described here. Moreover, these models contrast with the symptom-free survival of subcutaneous-implanted mice [19].

These early PDOX models were largely forgotten for nearly 20 years as genetically-engineed and subcutaneous cancer models dominated the field. It was not until Nature Reviews Cancer published a comment comparing orthotopic and subcutaneous cancer models did the PDOX model start to make a come back [2].

References

1. Rygaard J, Povlsen CO. Heterotransplantation of a human malignant tumor to 'nude' mice. Acta Pathol Microbiol Scand. 1969;77:758–60.
2. Hoffman RM. Patient-derived orthotopic xenografts: better mimic of metastasis than subcutaneous xenografts. Nat Rev Cancer. 2015;15:451–2.
3. Wang WR, Sordat B, Piguet D, Sordat M. Human colon tumors in nude mice: implantation site and expression of the invasive phenotype. In: Sordat B, editor. Immune-deficient animals—4th international workshop on immune-deficient animals in experimental research. Basel: Karger; 1982. p. 239–45.
4. Fidler IJ. Critical factors in the biology of human cancer metastasis: twenty-eighth G.H.A. Clowes memorial award lecture. Cancer Res. 1990;50:6130–8.
5. Fu X, Besterman JM, Monosov A, Hoffman RM. Models of human metastatic colon cancer in nude mice orthotopically constructed by using histologically intact patient specimens. Proc Natl Acad Sci U S A. 1991;88:9345–9.
6. Togo S, Wang X, Shimada H, Moossa AR, Hoffman RM. Cancer seed and soil can be highly selective: human-patient colon tumor lung metastasis grows in nude mouse lung but not colon or subcutis. Anticancer Res. 1995;15:795–8.
7. Fu X, Guadagni F, Hoffman RM. A metastatic nude-mouse model of human pancreatic cancer constructed orthotopically from histologically intact patient specimens. Proc Natl Acad Sci U S A. 1992;89:5645–9.
8. Wang X, Fu X, Hoffman RM. A new patient-like metastatic model of human lung cancer constructed orthotopically with intact tissue via thoracotomy in immunodeficient mice. Int J Cancer. 1992;51:992–5.
9. Astoul P, Wang X, Colt HG, Boutin C, Hoffman RM. A patient-like human malignant pleural mesothelioma nude-mouse model. Oncol Rep. 1996;3:483–7.
10. Boutin C, Rey F, Gouvernet J, Viallat JR, Astoul P, Ledoray V. Thoracoscopy in pleural malignant mesothelioma: a prospective study of 188 consecutive patients. Part 2: Prognosis and staging. Cancer. 1993;72:394–404.
11. Fu X, Hoffman RM. Human ovarian carcinoma metastatic models constructed in nude mice by orthotopic transplantation of histologically-intact patient specimens. Anticancer Res. 1993;13:283–6.
12. Astoul P, Colt HG, Wang X, Hoffman RM. Metastatic human pleural ovarian cancer model constructed by orthotopic implantation of fresh histologically-intact patient carcinoma in nude mice. Anticancer Res. 1993;13:1999–2002.
13. Fu X, Le P, Hoffman RM. A metastatic-orthotopic transplant nude-mouse model of human patient breast cancer. Anticancer Res. 1993;13:901–4.
14. Furukawa T, Kubota T, Watanabe M, Kitajima M, Fu X, Hoffman RM. Orthotopic transplantation of histologically intact clinical specimens of stomach cancer to nude mice: correlation of metastatic sites in mouse and individual patient donors. Int J Cancer. 1993;53:608–12.

15. Astoul P, Colt HG, Wang X, Hoffman RM. A “patient-like” nude mouse model of parietal pleural human lung adenocarcinoma. Anticancer Res. 1994;14:85–91.
16. Wang NS. The preformed stomas connecting the pleural cavity and the lymphatics in the parietal pleura. Am Rev Respir Dis. 1975;111:12–20.
17. Wang NS. Morphological data of pleura. Normal conditions. In: Chretein J, Hirsch A, editors. Diseases of the pleura. New York: Masson; 1977. p. 10–24.
18. Feldman GB. Lymphatic obstruction in carcinomatous ascites. Cancer Res. 1975;35:325–32.
19. Astoul P, Colt HG, Wang X, Boutin C, Hoffman RM. A “patient-like” nude mouse metastatic model of advanced human pleural cancer. J Cell Biochem. 1994;56:9–15.