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PROSPECTS

Metastatic Models of Human Cancer Xenografted in the Nude Mouse: The Importance of Orthotopic Transplantation

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Abstract Metastatic model of human tumor xenografts have been developed using orthotopic transplantation of histologically intact tissue (onplantation) of lung, stomach, colon, pancreatic, prostate and bladder carcinomas. These models represent the entire process of the metastasis, consisting of local tumor growth, vascular and lymphatic invasion at the local site, flow in the vessels and lymphatic, extravasation at the metastatic organs, and seeding and growth at relevant metastatic sites. Orthotopically transplanted human small-cell lung carcinoma displayed a different chemosensitivity pattern compared with the subcutaneous transplanted model, suggesting different pharmacodynamics between the orthotopic lung and the ectopic subcutaneous sites. The intact-tissue orthotopic-onplantation model seems to be useful to study the mechanism of metastasis for discovery of antimetastatic agents and for the patient tumors and for this treatment design. © 1994 Wiley-Liss, Inc.

Key words: metastatic model, orthotopic transplantation, nude mouse

Spontaneous metastasis has rarely been observed in the conventional subcutaneous-transplant model of human tumor xenografts in nude mouse models. Sordat and Fidler developed the dissociated-tissue model using human tumor cells intra-splenic-injection as well as the orthotopic injection mode of the single cell suspensions which sometimes metastasize after local tumor growth. However, the splenic injection model does not represent the whole process of the metastasis. In addition, the metastatic rate of orthotopically-injected single cell suspensions is limited. To develop a model that accurately reflects the cancer patient, we have further developed the intact-tissue orthotopic transplantation model initiated by R.M. Hoffman and Vezerides. This model includes lung [Kuo et al., 1992; Kuo et al., 1993d; Wang et al., 1992a,b], stomach [Furukawa et al., 1993a; Furukawa et al., 1993c; Furukawa, 1993f] colon [Fu et al., 1991a; Fu et al., 1992c; Furuakawa et al., 1993d; Furuakawa et al., 1993e; Kuo et al., 1993a; Kuo et al., 1993b] pancreatic [Fu et al., 1992a; Furuakawa et al., 1993g] prostate [Fu et al., 1992a; Furuakawa et al., 1993g] and bladder [Fu et al., 1991b] carcinomas. These models represent the whole process of metastasis, consisting of local tumor growth, vascular and lymphatic invasion at the local site, flow in the vessels and lymphatic, extravasation at the relevant metastatic organs, and seeding the growth at the metastatic sites. These models should be useful as therapeutic models for metastasis which is one of the most difficult and important problems in the control of cancer.

METASTATIC MODEL OF HUMAN GASTRIC CANCER

Male nude mice with a genetic background of BALB/cA were purchased from Nihon Co. Ltd. They were maintained in a specific pathogen-free conditions using an Isorack in the experimental animal center at the Keio University Medical School and fed sterile food and water ad libitum. Six week old mice weighing 20–22 g were used for the experiments described in this report.

Tumors used for the experiments described here are human stomach cancer xenograft lines St-40, H-111, and SC-1-NU. The histological features of the strains are poorly differentiated adenocarcinoma for St-4 and SC-1-NU, and well differentiated adenocarcinoma for St-40 and

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H-111. H-111 and SC-1-NU were kindly supplied by Dr. M. Fujita, Osaka University and Dr. M. Yamauchi, Nagoya University respectively. St-4 and St-40 were established at the Pathology Division, National Cancer Center Research Institute, Tokyo, Japan.

The experimentally growing tumors were removed aseptically. The necrotic parts were cut away, and the remaining healthy tumor tissues were scissors minced into pieces about 5 to 7 mm in diameter in Hanks' balanced salt solution. Pieces of tumor were cut to 150 mg units with a scissors.

Mice were anesthetized with 2.5% Avertin® and an incision approximately 3 mm in diameter was made through the left upper abdominal pararectal line and the serosal membrane in the middle of the greater curvature of the glandular stomach which was mechanically injured using a scissors. The 150 mg tumor pieces were then fixed on each injured serosal surface with a 4-0 Dexon® (Davis-Greck, Manati, PR) transmural suture. The stomach was then returned to the peritoneal cavity, and the abdominal wall and skin were closed with 4-0 Dexon® sutures. The 150 mg gastric tumor pieces were also minced with scissors, and incubated with 0.1 mg/ml DNase, 0.5 mg/ml actinase and 0.2 mg/ml collagenase for 30 min. at 37°C. The tumor cell suspension was passed through a stainless mesh (200 m/s) resulting in 1–4 × 10^7/0.1 ml cell suspension, which was injected into the middle of the greater curvature of the stomach.

Mice were observed daily after the implantation, and the mice were sacrificed when they developed signs of distress. The experiments were terminated 12 weeks after intact tissue transplantation and 24 weeks after inoculation of single cell suspension.

The results of gastric cancer transplantation are shown in Table I. The local tumor growth and the metastatic foci were confirmed histologically. Local tumor growth of the gastric cancer xenografts was observed in 26 out of 26 animals, after orthotopic transplantation of intact-tissue, while only 50% (15/30) of the animals developed local tumor growth after single cell suspension injection. The take rate of single-cell-suspension injection depended on the tumor cell type with the rapidly growing SC-1-NU demonstrating a 100% (6/6) take rate, while the take rates of the other strains was less than 50%. Lymph nodes metastases were observed in 23 of 23 cases for intact tissue transplantation, while only one case, SC-1-NU, injected as a single cell suspension developed lymph node metastasis. Liver metastases were observed in 18 of 26 animals (70%), after orthotopic transplantation of intact-tissue, while no hepatic metastases were found after single-cell suspension injection. Distant metastasis to the lung, pancreas and adrenal glands were also observed after intact-tissue transplantation of SC-1-NU and St-4, while no distant metastases were observed after orthotopic transplantation of all cell suspensions.

METASTATIC MODELS OF HUMAN COLON CANCER

Tumors used for the experiments were human colon tumor xenograft lines Co-3, Col-3-JCK and Col-5-JCK. Co-3, a well differentiated adenocarcinoma of colon was established at the Pathology Division, National Cancer Center Research Institute, Tokyo, Japan. Col-3-JCK, a

TABLE I. Local Tumor Growth and Metastasis of Orthotopically Transplanted Human Gastric Cancer Xenografts in Nude Mice

Tumor	St-4[a]	St-40	H-111	SC-1-NU[b]	Total (%)
Intact-tissue implantation					
Local tumor growth	5/5[c]	4/4	4/4	13/13	26/26 (100%)
Lymph node metastasis	5/5	3/3	4/4	11/11	23/23 (100%)
Liver metastasis	2/4	4/4	1/4	11/13	18/26 (70%)
Cell suspension implantation					
Local tumor growth	3/10	3/8	3/6	6/6	15/30 (50%)
Lymph node metastasis	0/3	0/3	0/3	1/6	1/15 (7%)
Liver metastasis	0/3	0/3	0/3	0/6	0/15 (0%)

[a]The metastasis to lung, pancreas, kidney, and adrenal gland was observed 1 of 5 mice in which St-4 was orthotopically transplanted.

[b]The metastasis to lung was observed 3 of 9 mice in which SC-1-NU was orthotopically transplanted.

[c]Data are shown as the number of mice with local tumor growth or metastases/number of mice evaluable.

poorly differentiated adenocarcinoma of colon and Col-5-JCK, a well differentiated adenocarcinoma were kindly supplied from Dr. T. Nomura, Central Institute for Experimental Animals, Tokyo, Japan. Tumors growing subcutaneously in nude mice were removed aseptically, the necrotic parts were cut away, and the remaining healthy tumor tissues were scissors minced into pieces about 5 to 7 mm in diameter in Hanks' balanced salt solution. Fifty mg pieces of tumor were obtained with scissors. Mice were anesthetized with 2.5% Avertin®. An incision was made through the lower middle abdominal line and the cecum was extracted extra-abdominally. The serosal membrane of the cecum was mechanically injured using a scissors. Fifty mg tumor pieces were then fixed to the serosal surface with 4-0 Dexon® sutures. The cecum was then returned to the peritoneal cavity, and the abdominal wall and skin were closed with 4-0 Dexon® sutures. Ten days after inoculation, the treated mice were re-anesthetized and a laparotomy carried out to resect the cecum which included the transplanted tumor. The untreated and treated mice were observed for an additional 6 weeks until the termination of the experiments. At autopsy, all the primary and metastatic lesions were confirmed histologically.

Local tumor of the human colon cancer xenografts was observed in all 25 cases, with liver metastases occurring in 14 of 25 cases (Table II). All the metastatic lesions were nodular including those at the surface of the liver. The number of metastatic lesions ranged from 1 to 5 per mouse. However, no metastases to the liver developed if the cecum was surgically removed 10 days after tumor transplantation. No lymphatic or vessel invasions were observed after resection, while all control cases showed vessel invasion of the tumor at the transplantation site.

DISCUSSION

Human tumor xenografts transplanted subcutaneously resemble benign tumors since they are often encapsulated and rarely metastasize.

Sordat et al. [1982] and Fidler [1990] first developed orthotopic and intrasplenic transplantation models of human tumor cell suspensions which in some cases resulted in metastatic lesions in the liver of nude mice. Although, the intrasplenic injections method was useful to reproduce "metastasis" in vivo, the whole process is not represented in this model. The intrasplenic injection model lacks the process of (1) local tumor growth and (2) vessel and lymphatic invasion of tumor cells which are considered to be key processes of the metastasis. Thus, the intrasplenic model was thought to be a seeding model rather than a "metastatic" model. The "orthotopic" onplantation models were also developed using single cell suspension of gastric, [Yamashita, 1988] colon [Brezelier et al., 1987; Fidler, 1986; Giavazzi et al., 1986] and pancreatic carcinomas [Morikawa et al., 1988; Vezeridis et al., 1988]. Tumor cells were injected in the orthotopic organs, resulting in metastases. Although this model showed a superiority over the intrasplenic injection model in that local tumor growth occurred before metastasis, the problem remained that the dissociated tumor cells lacked normal cell interactions necessary for the metastatic processes of lymphatic and vessel invasion at the primary site as well as anglo genes. Indeed our results indicated that the orthotopically-transplanted dissociated gastric cancer cells had only a 50% local tumor growth take rate without liver metastasis. In striking contrast, 100% local tumor take rates and 100% hepatic and 70% lymphatic metastatic rates were observed in the intact-tissue orthotopic transplant models. Since the number of tumor cells was equivalent in the single cell suspension and intact tissue ortho-

TABLE II. Prevention of Liver Metastases by Resection of Primary Human Colon Carcinoma Xenografts Orthotopically Transplanted on the Cecum of Nude Mice

Tumor	Co-3	Col-3-JCK	Col-5-JCK	Total (%)
Local growth	9/9[a]	8/8	8/8	25/25 (100)
Liver metastasis[b]	4/7	5/8	5/10	14/25 (50)
After local resection[b]	0/9	0/8	0/8	0/25 (0)

[a]Data are shown as the number of mice with local tumor growth or metastases/number of mice evaluable.
[b]$P < 0.05$ by chi squared test.

topic transplant models and almost all the tumor cells were viable in the single-cell suspensions which was implanted to the stomach of nude mice, the low metastatic rates in single-cell suspension orthotopic transplantation seemed not due to low cell numbers or cell viability of the inoculated tumor cells. The growth rate of the locally growing tumor that formed after orthotopic transplantation of the SC-1-NU cell suspension was less than that of the tumor that formed after orthotopic transplantation of intact tissue. However, the locally growing tumors of all tumor lines that formed after orthotopic implantation of cell suspensions were allowed to attain, before sacrifice, a weight equivalent to those tumors that formed after orthotopic implantation of intact tissue. These findings indicated that the resultant local tumor size did not determine metastatic capacity. In addition, even first injuring the serosa, as was done for intact tissue transplantation, did not increase the metastatic rate after orthotopic onplantation of cell suspensions of stomach tumor [Furukawa et al., 1993a]. Thus, the very low metastatic rate observed after orthotopic implantation of cell suspensions may be due to the disruption of native cell to cell interactions necessary for full expression of the malignant characteristics of human cancer cells.

We have utilized the metastatic model of colon cancer xenograft to elucidate the metastatic pathway of this model. Since the specimens were inoculated onto the serosal side, spontaneous dissemination of the tumor cells could have been the origin of metastases to the liver. However, when the cecum with tumor specimens was removed 10 days after transplantation, no hepatic metastases were observed and no lymphatic nor vessel invasion of the tumor cells were observed in the resected local tissues. Since all the tumors showed vessel and lymphatic invasion when hepatic metastases were observed, the metastatic pathway in this model was thought to be hematogenous or lymphatic as is also the case clinically.

Thus, the orthotopic onplantation model of the tissue seems to represent the entire metastatic process, including local tumor growth, vessel or lymphatic invasion, hematogenous or lymphatic flow, seeding and growth at the metastatic site. This is in contrast to the intra-splenic injection model which is thought to be a seeding model. The intact-tissue orthotopic transplantation models of gastric and colon carcinoma xenografts are promising experimental models to elucidate the mechanism of the metastasis and develop therapy to prevent and treat metastatic foci.

We have also reported that orthotopically-transplanted human small-cell lung carcinoma revealed a different chemosensitivity pattern compared with the subcutaneously-inoculated model [Kuo et al., 1993c]. This suggested different pharmacodynamics in the orthotopic lung and the subcutaneous tissue. Thus the orthotopic models may overcome the deficiency of the conventionally-available subcutaneous models of human tumor xenografts in nude mice for effective drug discovery. Differential chemosensitivity of local and metastatic tumors was observed in the gastric cancer xenografts after orthotopic transplantation of histologically-intact tumor tissue in nude mice [Furukawa et al., 1993b], suggesting that this model might be useful in evaluating anti-metastatic agents as well as anti-tumor drugs. Most importantly, we have observed 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 correlates with the course of the tumors in the patients [Furukawa et al., 1993c]. Thus, the intact-tissue orthotopic transplantation model seems to be useful not only to study the mechanism of the metastasis, but to evaluate the biological malignancy of the individual patients and to treat them with adequate therapy.

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