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ORTHOTOPIC TRANSPLANTATION OF HISTOLOGICALLY INTACT CLINICAL SPECIMENS OF STOMACH CANCER TO NUDE MICE: CORRELATION OF METASTATIC SITES IN MOUSE AND INDIVIDUAL PATIENT DONORS

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Fresh surgical specimens derived from 36 patients with advanced stomach cancer were orthotopically transplanted in nude mice using histologically intact tissue. Twenty of 36 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, whereas 8 of the other 16 patients whose tumors were rejected had lymph-node involvement. There was a statistical correlation ($p < 0.01$) between local tumor growth in nude mice and clinical lymph-node involvement. Of the 20 cases resulting in local growth in the nude mice, 5 had clinical liver metastases and all 5 cases resulted in liver metastases in the nude mice. Of the 20 cases, 6 had clinical peritoneal involvement of their tumor, and of these 5 resulted in peritoneal metastasis in the nude mice. There were statistical correlations ($p < 0.01$) for both liver metastases and peritoneal involvement between patients and 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.
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There is a need to develop better animal models for human cancer. Models based on athymic nude mice have been used for this purpose. However, human tumors grown s.c. in nude mice rarely metastasize, although they closely resemble the original tumors morphologically, biologically and biochemically (Fidler, 1990).

Recently, studies from a number of laboratories have indicated that implanting human tumor cells orthotopically in the corresponding organ of nude mice results in much higher metastatic rates. For example, disaggregated human colon-cancer cells injected into the cecum of nude mice produce tumors which eventually metastasize to the liver, demonstrating that orthotopic implantation can enhance the development of metastases of human cancer cells in nude mice (Fidler, 1990). Similar results have been reported following the orthotopic implantation of a variety of human cancer cell lines (Fidler, 1990), including stomach cancer (Yamashita, 1988; Hirabayashi *et al.,* 1991).

However, cell suspensions used for orthotopic implantation result in lower rates of growth and metastasis than the orthotopic transplantation of histologically intact tissue (Fu *et al.,* 1991*a*; Furukawa *et al.,* 1992*a,b*). Therefore we have established, in nude mice, new metastatic models of human cancer constructed orthotopically with histologically intact tissue, including patient specimens. These models include colon (Fu *et al.,* 1991*a*, 1992*c*; Furukawa *et al.,* 1992*b*), bladder (Fu *et al.,* 1991*b*; Fu and Hoffman, 1992), pancreas (Fu *et al.,* 1992*a*), lung (Wang *et al.,* 1992a,b,c), prostate (Fu *et al.,* 1992*b*) and stomach cancer (Furukawa *et al.,* 1992*a*). After orthotopic transplantation of histologically intact tumor tissue, these models have demonstrated local growth, regional extension and metastases, and distant metastases to other organ sites and lymph nodes, depending on the patient specimen. The models, therefore, have indicated that they are clinically relevant. This study provides further evidence for the clinical relevance of these models, by demonstrating a correlation between the clinical metastatic pattern in a series of stomach-cancer patients and those in nude mice following the orthotopic transplantation of the patients' primary tumors after resection.

MATERIAL AND METHODS

Mice

Five-week-old male BALB/cA *nu/nu* mice, which originated from the Central Institute for Experimental Animals (Kawasaki, Japan), were purchased from CLEA, Tokyo, Japan.

Stomach-cancer specimens

Fresh surgical stomach-cancer specimens were obtained from resected primary lesions of 36 patients with advanced gastric cancer who underwent surgery at Keio University Hospital between September 1991 and February 1992. Pathologically confirmed metastatic sites of these tumors are listed in Table I. During surgery, tumor specimens were aseptically removed and immediately rinsed in Hanks's balanced salt solution, and transported to the laboratory as soon as possible. After necrotic tissue and non-cancerous tissue of the specimens were carefully cut away with scissors, the remaining cancerous tissue was divided into small pieces about 2 mm in diameter.

Transplantation procedure

Tumor pieces were surgically transplanted orthotopically to the stomach serosa in nude mice as histologically intact tissue, according to the method reported for colon cancers (Fu *et al.,* 1991*a*). Mice were anesthetized with a 2.5% solution of a mixture of 2,2,2-tribromoethanol and *tert*-amylalcohol (1:1), and an incision was made through the left upper abdominal pararectal line and peritoneum. The stomach wall was carefully exposed and a part of the serosal membrane, about 2 mm in diameter, in the greater curvature of the antrum of the stomach was mechanically injured using scissors. A tumor piece was then fixed on each injured site of the serosal surface with a 6-0 Dexon transmural suture. The stomach was then returned into the peritoneal cavity, and the abdominal wall and the skin were closed with 6-0 Dexon sutures. Tumor pieces from each patient were transplanted into 3 to 5 mice, depending on the total available volume of cancerous specimen in each case. Mice were kept in laminar-flow cabinets under

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TABLE I – LOCAL TUMOR GROWTH IN NUDE MICE AND CORRELATION OF METASTATIC SITES IN STOMACH-CANCER PATIENTS AND IN NUDE MICE AFTER TRANSPLANTATION OF PRIMARY TUMORS

Patient tumor number	Local tumor growth in nude mice[1]	Metastases in patients/nude mice[2]		
		Lymph nodes	Liver	Peritoneum
1	+	+/+	−/−	−/−
2	+	+/+	+/+	+/+
3	+	+/−	−/−	−/−
4	−	−	−	−
5	−	+	−	−
6	+	+/−	−/−	−/−
7	−	−	−	−
8	+	+/+	−/−	+/+
9	−	−	−	−
10	+	+/+	−/+	−/−
11	−	−	−	−
12	−	+	−	−
13	+	+/−	−/−	−/−
14	+	+/−	−/−	+/+
15	−	+	−	−
16	−	−	−	−
17	−	+	−	+
18	+	+/+	−/−	−/−
19	−	+	−	−
20	+	+/−	−/−	−/−
21	−	+	−	−
22	+	+/+	−/−	−/−
23	−	−	−	−
24	+	+/+	−/−	+/+
25	+	+/−	−/−	−/−
26	−	−	−	−
27	−	+	−	+
28	+	+/+	+/+	−/−
29	−	+	+	−
30	+	+/−	−/−	+/−
31	+	+/−	−/−	−/−
32	+	+/+	+/+	+/+
33	+	+/+	+/+	−/−
34	−	−	−	−
35	+	+/−	−/−	−/−
36	+	+/+	+/+	−/−

Primary stomach cancer tissue of patient was resected and transplanted as histologically intact tissue to the serosa of the nude-mouse stomach, using a group of 3 to 5 mice for each patient, as described in the text. After 8 to 24 weeks, the mice were autopsied and analyzed histologically for the presence of metastases. Metastases were considered to have occurred if at least one microscopic lesion was found in any of the recipients. [1]+, local tumor growth observed in nude mice; −, tumor rejected in nude mice.–[2]+/+, metastases observed both in patient and in nude mice; −/−, metastases observed neither in patient nor in nude mice. +/−, metastases observed in patient, but not in nude mice. −/+, metastases not observed in patient, but observed in nude mice.

specific-pathogen-free conditions and were inspected every day.

Evaluation of growth and metastases of orthotopically transplanted tumors

Mice were killed if they developed signs of distress. At autopsy, the stomach, lymph nodes, liver and other organs were resected and processed for routine histological examination for tumors after careful macroscopic examination. Metastases were considered to have occurred if at least one microscopic metastatic lesion was found in any of the recipients.

Chromosome analysis

The human origin of the tumors growing in the nude mice was confirmed by chromosome analysis. Tumor masses were finely minced and incubated for 2 hr at 37°C in RPMI-1640 medium with 10% FCS and 8 mg/ml of collagenase II. The tissues were then washed repeatedly in a Petri dish with fresh medium, and filtrated through a 100-mesh screen. The cell suspensions were incubated for 48 hr at 37°C in RPMI-1640 supplemented with FCS, and colcemid was added at a concentration of 0.001 μg/ml 12 hr before harvest. The G-banding technique was used for the analysis of karyotypes (Ochi *et al.*, 1984).

RESULTS

Local tumor growth in nude mice and lymph-node involvement

Out of a total of 36 transplanted tumors, 20 gave rise to locally growing tumors in the mice ranging in size from 5 mm to 2 cm over a period from 8 to 24 weeks after transplantation (Table I). All 20 patients whose stomach tumors resulted in local growth in the nude mice after orthotopic transplantation had clinical lymph-node involvement, whereas 8 of the other 16 patients whose tumors were rejected had lymph-node involvement. There was a statistical correlation ($p < 0.01$) between local tumor growth in nude mice and clinical lymph-node involvement (Table II). However, there was no significant correlation between local tumor growth in the mice and clinical liver metastases or peritoneal involvement (Table II). As shown in Figure 1, chromosome analysis confirmed the human origin of the tumors grown in nude mice. Of the 20 cases with clinical lymph-node involvement that resulted in local tumor growth in the mice, 11 resulted in lymph-node

TABLE II – CORRELATION OF LOCAL TUMOR GROWTH IN NUDE MICE AND METASTATIC SITES IN PATIENTS

Metastatic site in patients	Metastases[1]	Local tumor growth in nude mice[2] (number of cases)	
		(+)	(−)
Lymph node	(+)	20	8
	(−)	0	8*
Liver	(+)	5	1
	(−)	15	15 NS
Peritoneum	(+)	6	2
	(−)	14	14 NS

NS, not significant, *$p < 0.01$ by chi-squared test. [1](+) and (−) indicate that metastases were positive or negative in patients.– [2](+) and (−) indicate that metastases were positive or negative in nude mice.

TABLE III – CORRELATION OF METASTATIC SITES IN STOMACH-CANCER PATIENTS AND IN NUDE MICE IN 20 CASES WITH LOCAL TUMOR GROWTH

Metastatic site	In patients[1]	In nude mice[2] (number of cases)	
		(+)	(−)
Lymph node	(+)	11	9
	(−)	0	0 NS
Liver	(+)	5	0
	(−)	1	14*
Peritoneum	(+)	5	1
	(−)	0	14*

NS, not significant; *$p < 0.01$ by chi-squared test. [1](+) and (−) indicate that metastases were positive or negative in patients.– [2](+) and (−) indicate that metastases were positive or negative in nude mice.

FIGURE 1 – Karyotype of a metaphase with 73 chromosomes from a locally growing tumor on the nude-mouse stomach 12 weeks after orthotopic transplantation of a patient tumor. The human origin was confirmed morphologically.

metastases in the mice, ranging in size from 1 mm to 2 mm (Table I).

Liver metastases in patients and mice

Of the 20 cases resulting in local growth in the nude mice, 5 had clinical liver metastases, and all 5 cases gave rise to liver metastases in the nude mice, ranging in size from 1 mm to 3 mm (Table I). 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 (Table I). There was a statistical correlation ($p < 0.01$) in liver metastases between patients and mice (Table III). Figure 2*a–d* shows comparison of histological views of local tumor growth and liver metastasis in a stomach-cancer patient and in a nude mouse 12 weeks after orthotopic transplantation of the histologically intact primary tumor. The histology both of primary growth and of metastases in the patient were found to be reproduced in the nude mice.

Peritoneal involvement in patients and mice

Of the 20 cases resulting in local growth in the mice, 6 had clinical peritoneal involvement of their tumor; of these, 5 gave rise to peritoneal metastases in the nude mice, ranging in size from 1 mm to 5 mm (Table I). Of the 14 patients without peritoneal involvement whose primary tumor grew locally in the mice, none gave rise to peritoneal involvement in the mice (Table I). There was a statistical correlation ($p < 0.01$) in peritoneal involvement between patients and mice (Table III). In this series of patients, 2 had metastases in the liver, peritoneum and lymph nodes. In both cases, after orthotopic transplantation of the primary tumor, some mice were found to be positive at all 3 metastatic sites (Table I).

DISCUSSION

Tumorigenicity of various human tumors in nude mice correlates with poor clinical outcome of the patients (Jessup *et al.,* 1989; Friedlander *et al.,* 1985; Rousseau-Merck *et al.,* 1985). In this study, local tumor growth after orthotopic transplantation of human stomach cancer into nude mice correlated with clinical lymph-node involvement. Since lymph-node involvement is one of the most significant prognostic factors of stomach cancer (Ichiyoshi *et al.,* 1990; Maehara *et al.,* 1991), tumorigenicity in nude mice in this study seems also to correlate with clinical outcome.

All of 5 cases with clinical liver metastases resulted in liver metastases in nude mice, and 5 of 6 cases with peritoneal involvement resulted in peritoneal involvement in nude mice. These results indicate that, after orthotopic transplantation of histologically intact primary tumors from stomach-cancer patients into nude mice, the subsequent metastatic behavior of the tumors in the mice closely correlates with the course of the tumor in the patient. There was only 1 case without clinical liver metastasis which produced liver metastasis in nude mice. This patient is still alive without liver metastasis in the 12th month after gastrectomy, although it is possible that he may develop liver metastases in the future.

Because of experimental limitations, the human stomach tumor was transplanted on the serosal side stomach of the nude mouse, even though tumors in the patients originated from the mucosal side. However, transplanted tumor cells were found to have invaded the muscular and sub-mucosal layers at the time of autopsy (Furukawa *et al.,* 1992*a*). Even if it were possible to transplant the tumors on the mucosal side, tumor cells would have to invade the sub-mucosal and muscular layer in order to produce metastases. Therefore metastases after serosal transplantation seem to reflect metastases that occur after mucosal origin.

There were 11 cases in which at least one metastatic site was positive in nude mice. In 2 cases, mice which had received tumor tissue derived from the same patient tumor had fewer

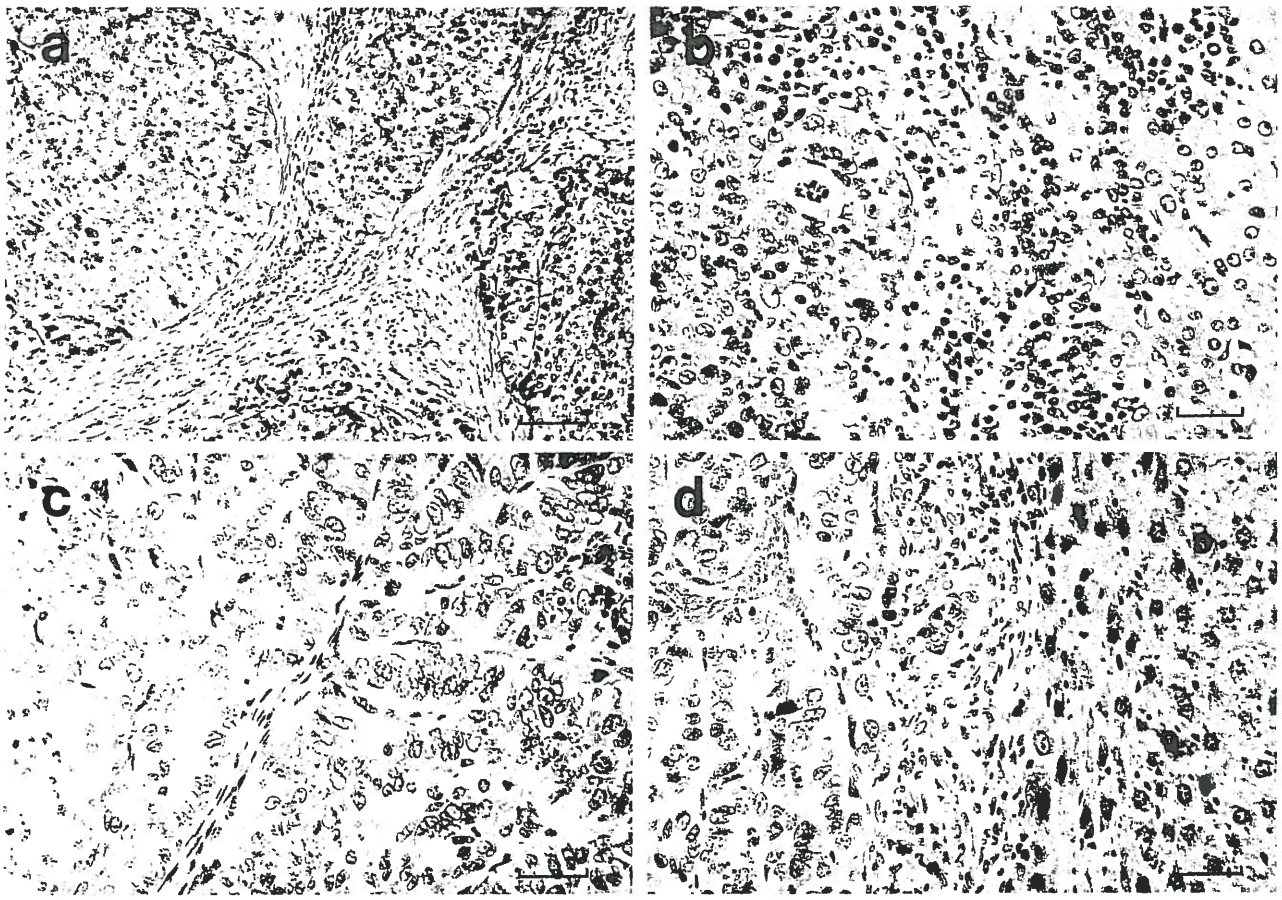

FIGURE 2 – Comparison of histological views of local tumor growth and liver metastasis in a stomach-cancer patient and in nude mouse after orthotopic transplantation of the histologically-intact patient primary tumor (H+E staining). *(a)* Local tumor growth in patient; *(b)* liver metastasis in patient; *(c)* local tumor growth in nude mouse; *(d)* liver metastasis in nude mouse. Primary stomach-cancer tissue from patient was resected and transplanted as histologically intact tissue to the serosa of nude-mouse stomach, as described in text. After 12 weeks the mice were autopsied, and the local tumor and the liver were resected and analyzed histologically. For *(a)* Bar = 20 μm. For *(b-d)* Bar = 10 μm.

metastatic sites than the other mice, possibly reflecting the tumor heterogeneity in the patient tumors. However, in 9 other cases, each of the mice receiving tissue from each case had the same pattern of metastases. Tumor heterogeneity does not appear, therefore, to be a major factor in this study.

In conclusion, the orthotopic transplantation of histologically intact clinical specimens of stomach cancer into nude mice produces a metastatic pattern that correlates with the individual patient. This model would facilitate the study of metastasis of human stomach cancers.

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