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Manifestation of Metastatic Potential in Human Gastric Cancer Implanted into the Stomach Wall of Nude Mice

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The biological properties of human gastric cancer cell line G/F implanted into either the subcutis or the stomach wall of nude mice were compared. The G/F tumor in the stomach wall showed a slower growth rate than that in the subcutis. The level of carcinoembryonic antigen in serum was greater when the tumor was in the stomach wall than when it was in the subcutis. The tumor in the stomach wall invaded the surrounding tissues and metastasized to the regional lymph nodes and distant organs such as the lung and the liver in 27 of the 43 mice (68%). In contrast, the tumor in the subcutis was highly encapsulated and metastasis to other organs was not observed. These findings indicate that the stomach wall might provide a suitable microenvironment for G/F gastric cancer to exert its intrinsic properties. Therefore, implantation of human gastric cancer into the stomach wall of nude mice may provide a useful model to study the intrinsic characteristics of human cancer as well as the effectiveness of experimental chemotherapy.

Key words: Biological properties of tumor — Human gastric cancer — Nude mouse — Implantation site

Human tumor xenografts in the subcutis of athymic nude mice closely resemble the original tumors in morphological, biological and biochemical characteristics.[1-3] In contrast, the invasive and metastatic potential of tumors in the subcutis is not fully conserved.[4-6] Recent work has demonstrated that the growth, invasiveness and metastatic behaviors of xenografts depend upon the location of the implantation site, such as the subcutis,[7] the subcapsule of the kidney,[8] or the lung,[9] as well as the intrinsic biological potential of the tumor cells. However, the biological and functional properties of human tumor implanted into its original organ of an animal model are not fully understood. In the present study, the human gastric cancer G/F was implanted into either the subcutis or the stomach wall of nude mice. Biological and functional characteristics such as growth rate, invasiveness, metastasis, and production of carcinoembryonic antigen (CEA) were then compared.

MATERIALS AND METHODS

Animals Six-week-old, male athymic BALB/c nude mice were obtained from Nihon CLEA Co., Tokyo. These animals were kept in laminar flow racks under pathogen-limited conditions without antibiotic coverage. Food, drinking water and other materials coming in contact with the mice were autoclaved prior to use.

Tumor The human gastric cancer cell line G/F was derived from a poorly differentiated carcinoma in the stomach of a 72-year-old man, as described by Matsuda *et al.*[10] The tumor has been serially transplanted into the subcutis of nude mice.

Inoculation Procedure In order to prepare a single-cell tumor suspension, subcutaneous solid tumors were excised, minced with scissors, and incubated in sterile Hanks' balanced salt solution (HBSS) containing 0.1% trypsin and 0.02% EDTA for 1 hr at 37°, followed by centrifugal washing and re-suspending in HBSS. Viable cell count was estimated by using the trypan blue exclusion test. Subcutaneous tumors were established by implantation of 1×10^6 tumor cells suspended in 0.25 ml of HBSS into the left flank of mice. Tumors in the stomach wall were established as described below. Mice were anesthetized with pentobarbital and an incision was made through the median flank and peritoneum. The stomach was carefully exposed and 1×10^6 viable tumor cells suspended in 0.025 ml of HBSS were injected into the middle of the greater curvature of the glandular stomach. The stomach was then returned into the peritoneal cavity, and the abdominal wall and the skin were closed with 4–0 nylon sutures.

Determination of Tumor Growth Rate and CEA Five mice in each group were autopsied every 5 days until 50 days after the implantation, and solid

tumors in the subcutis or the stomach wall were removed and weighed. CEA levels in serum and tumor tissue were determined by an enzymeimmunoassay with an Abbott kit (DAINABOT, Tokyo) and a Roche kit (Nippon Roche, Tokyo), respectively.

Examination of Metastasis The ability of the tumor to produce invasion and visceral metastasis was examined in two groups each consisting of 50 mice. One group received implantation of tumor cells into the subcutis and the other received implantation into stomach wall. Mice were sacrificed 102 days after tumor implantation. Moribund mice were treated in the same fashion. All organs were examined macroscopically and enlarged lymph nodes, any other organs with abnormalities and all tumors including surrounding tissue were fixed in 10% buffered formalin solution. For histological examination, tissue paraffin blocks were sectioned at a thickness of 5 μm and stained with hematoxylin and eosin (H-E).

Results

Growth of Tumors in the Subcutis and the Stomach Wall Figure 1 shows the growth curves of G/F tumor implanted into the subcutis or the stomach wall of mice. Subcutaneous tumors had a faster growth rate than the tumors implanted into the stomach wall. The difference in growth rate was particularly distinct during the early period (10–30 days) after tumor implatation: the doubling time was 3.7 days in the subcutaneous tumors while it was 5.0 days in tumors of the stomach wall. The tumor size in the plateau phase at 35–50 days was also about 2 times greater for tumors in the subcutis than for those in the stomach wall.

CEA Levels in Serum and Tumor Tissue Serum CEA levels in mice with the subcutaneous tumor and mice with the tumor in the stomach wall were compared. As shown in Fig. 2, the level (ng/ml) in mice with the subcutaneous tumor was very low and not dependent on the growth of the tumor. In contrast, the level in mice with the same tumor in the stomach wall increased exponetially with time. At 50 days after implantation, serum CEA level was 24 times greater in the mice with stomach tumors.

A significant difference in the CEA level of the tumor tissue itself was not observed between tumors in the subcutis or the stomach wall. These levels remained low throughout the observation period.

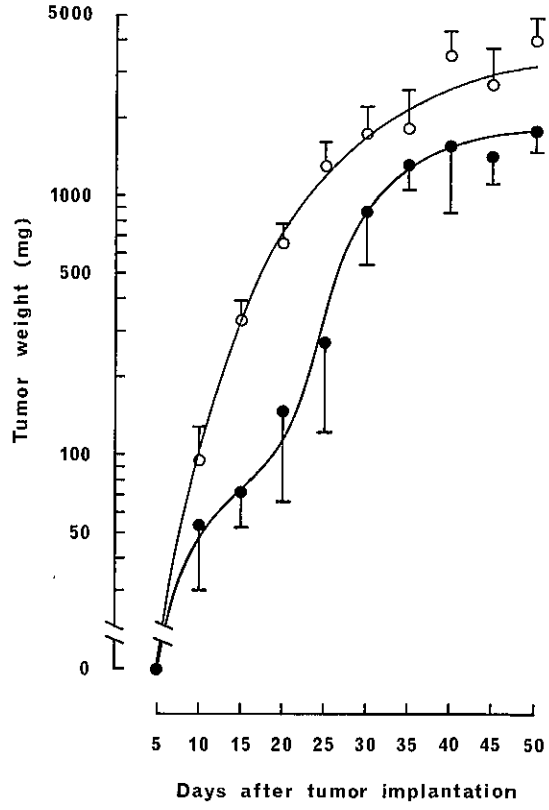

Fig. 1. Growth curves of human gastric cancer G/F implanted into the subcutis (○) or the stomach wall (●) of nude mice. Symbols and bars show mean tumor weight in 5 mice and standard deviation (SD), respectively.

Tumor Takes and Survival Time The tumor implanted into the subcutis took in all 50 mice. Survival time of the mice ranged from 45 to 102 days with a mean of 72.7 days. The tumor implanted into the stomach wall took in 43 of 50 mice (86%). The survival time of mice with the tumor ranged from 36 to 74 days with a mean of 51.8 days.

Morphology of Tumors in the Subcutis and the Stomach Wall Tumors in the subcutis grew expansively and were encapsulated with host-reactive fibrous layers (Fig. 3). Most of the tumors involved the epidermis and caused ulceration of the skin 20 to 30 days after implantation. The tumor cells in the submucosa of the stomach wall invaded the surface mucosa as well as the muscularis propria 5 to 6 weeks after implantation (Fig. 4). Most

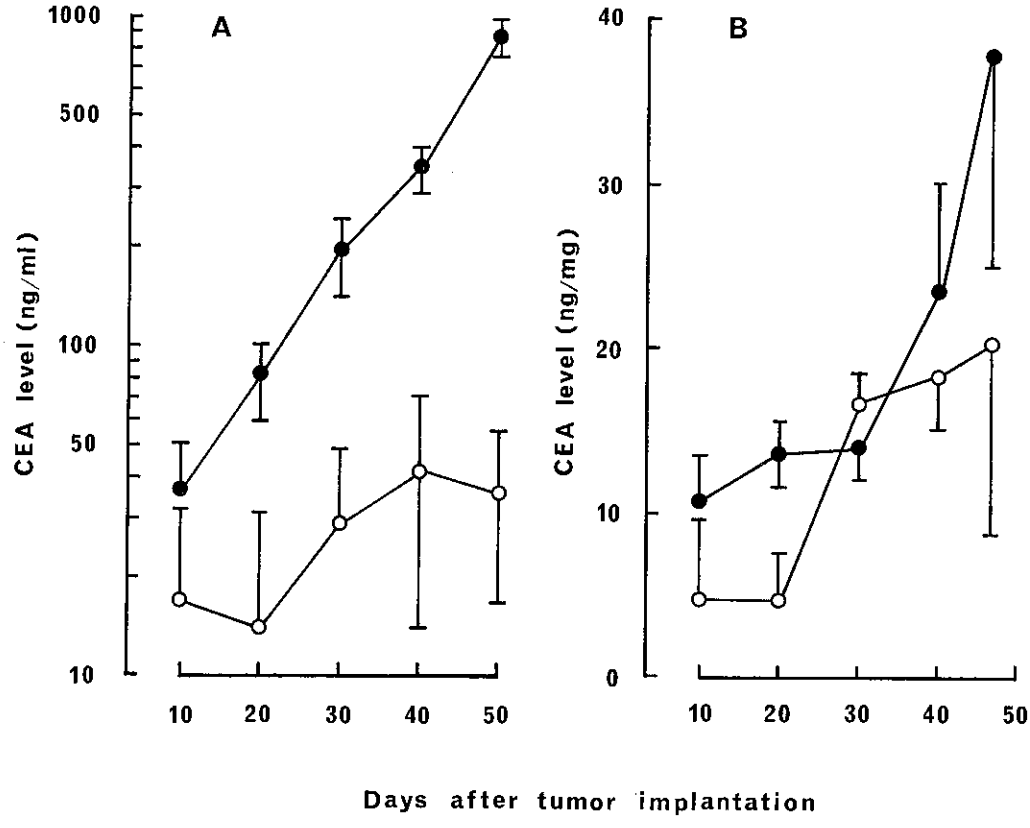

Fig. 2. CEA levels in the serum (A) and the tumor tissue (B) of nude mice with G/F tumor in the subcutis (○) or the stomach wall (●). Symbols and bars show mean value of CEA levels in 5 mice and SD, respectively.

of the tumors caused stomach ulceration, bleeding and obstruction of the pyloric antrum cavity leading to death.

Metastases of Tumors in the Subcutis and the Stomach Wall The metastatic behavior of tumors in the stomach wall is shown in Table I. Twenty-seven of the 43 mice (63%) which carried growing tumors had evidence of metastatic foci in various organs. In the early stage the metastases were observed in the regional lymph nodes. Distant metastases increased in the later stage from 45 days after tumor implantation. The lung metastases seemed to be established earlier than the liver metastases (Table I and Fig. 5). No metastasis from the subcutaneously implanted and developed tumors in 50 mice was observed.

Discussion

Studies on tumors orthotopically implanted into several organs such as the brain,[11] pancreas,[12] renal subcapsule[8] and lung[9] of nude mice raised the possibility that microenvironmental factors such as stroma reaction[13] or vascularity[7] might influence the biological properties of tumors. Naito *el al.*[8] reported that a human renal cell carcinoma implanted into the renal subcapsule showed faster growth and more systemic metastases than the same tumor implanted into the subcutis. In the present study, G/F human gastric cancer implanted into the stomach wall of nude mice exhibited a slow growth rate, invasive growth and systemic metastases, while

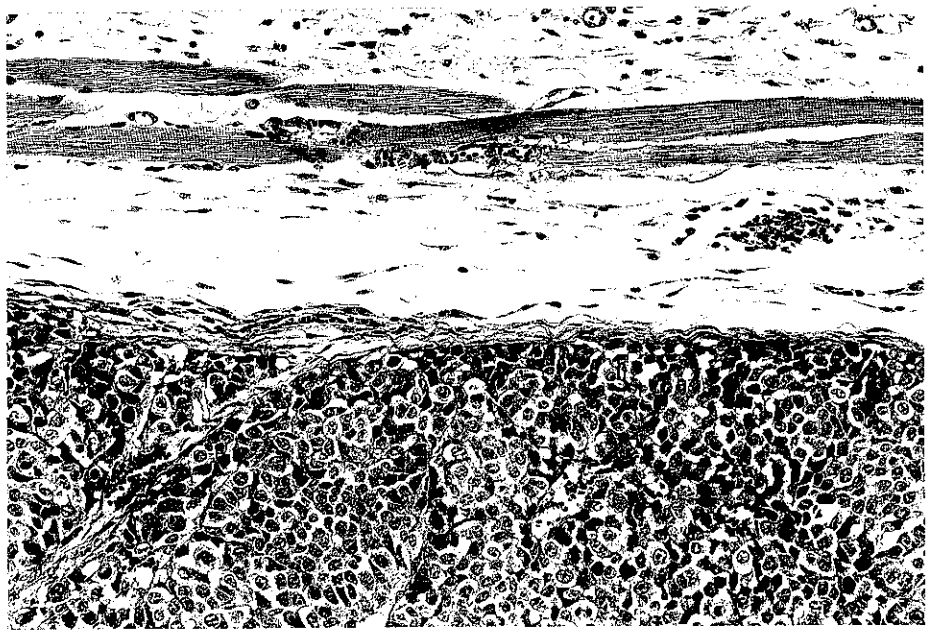

Fig. 3. G/F human gastric cancer implanted into the subcutis. The preparation was obtained 50 days after implantation. The tumor nodule is encapsulated with fibrous layers. (H-E stain, ×100)

Fig. 4. G/F human gastric cancer implanted into the stomach wall of mouse. The preparation was obtained 40 days after implantation. The tumor invaded the mucosa, causing ulceration, and also invaded throughout the muscularis propria and protruded into the subserosa. (H-E stain, ×20)

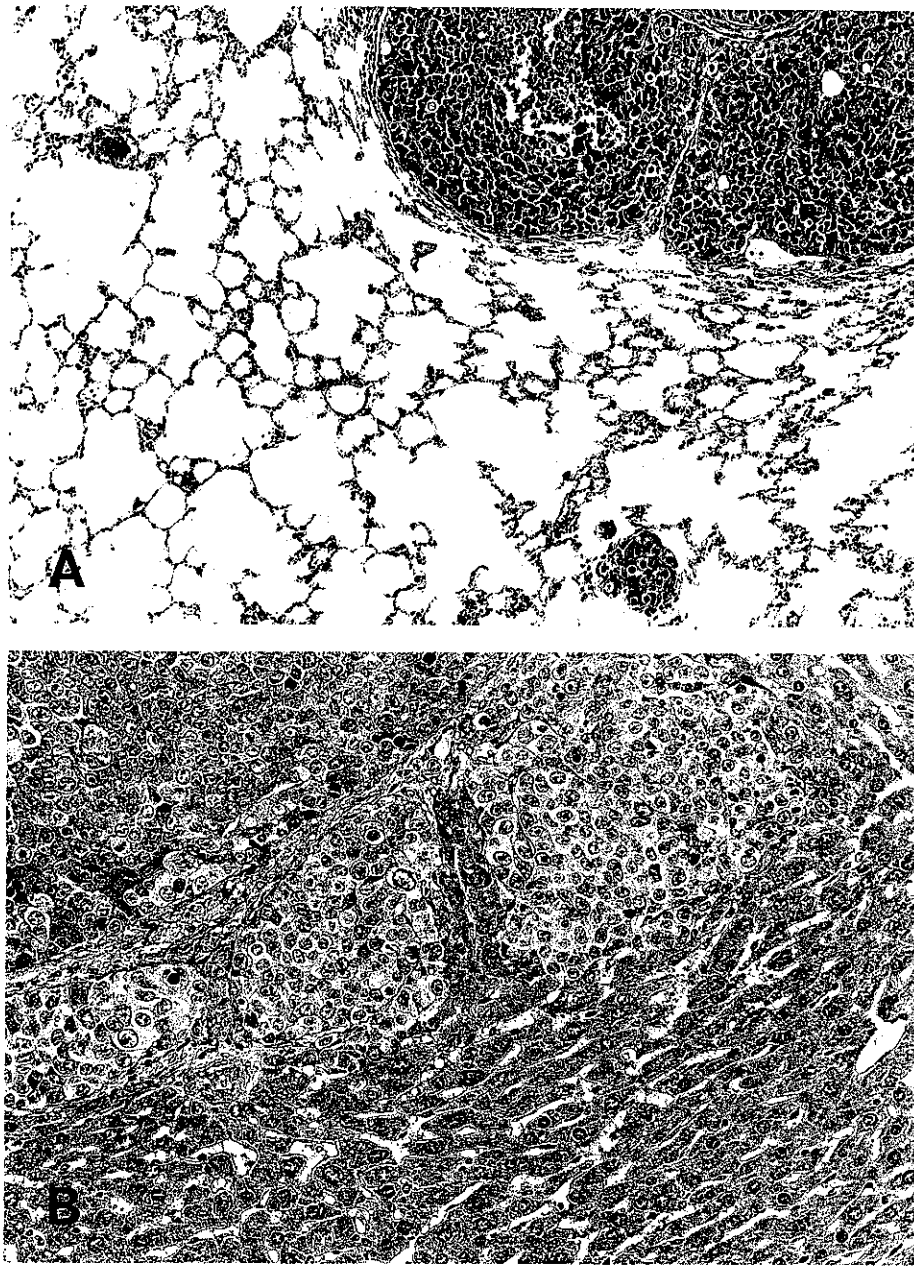

Fig. 5. Metastatic lesions in the lung observed at 53 days (A) and in the liver observed at 55 days (B) after implantation of G/F tumor into the stomach wall. (H-E stain, ×50 and ×100)

the same tumor implanted into the subcutis showed an expansive and faster growth without metastases. These results suggest that implantation into a model of the original organ provides a suitable microenvironment for the gastric cancer to exert its intrinsic properties.[8, 9, 11, 12] The present results appear to support the so-called seed and soil hypothesis

Table I. Metastatic Behavior of Human Gastric Cancer G/F Implanted into the Stomach Wall of Nude Mice

Animal No.	Days to autopsy	Lungs	Liver	Diaphragm	Lymph nodes[a]
14	39	−	−	−	+
6	40	−	−	−	+
7	40	−	−	−	+
16	41	−	−	−	+
18	43	−	−	−	+
42	45	+	−	−	−
37	47	−	−	−	+
44	47	−	−	−	+
48	47	+	−	−	+
38	51	−	−	+	+
22	53	+	−	−	+
23	53	+	−	+	+
24	53	+	−	−	+
21	55	−	−	−	+
26	55	−	+	−	−
27	55	−	+	−	−
4	63	−	−	−	+
35	64	−	−	−	+
45	64	−	+	−	+
46	64	−	−	+	+
47	64	−	+	+	+
11	73	+	+	−	−
31	74	−	+	−	−
32	74	+	+	−	−
34	74	−	−	+	+
36	74	+	−	−	+
39	74	−	+	−	+
Incidence (%)		30	30	19	78

a) Metastatic foci were observed in the gastric, pulmonary, hepatic, iliac and mesenteric lymph nodes.

which was originally proposed by Paget.[14] However, there is the possibility that the high metastatic potential of G/F tumor is not simply due to implantation into the stomach but may be due to implantation into an internal organ where invasive growth is allowed for unknown structural and/or immunological reasons. In order to verify the hypothesis, the metastatic potential of G/F tumor should be examined by implantation into other internal organs.

Motoyama and Watanabe[15] found that two cloned gastric cancer cell lines implantd intraperitoneally produced higher levels of serum CEA than the same tumors in the subcutis in nude mice. They suggested that the transport of CEA to the systemic blood flow may be difficult in the case of subcutaneous implantation. In this study, high serum levels of CEA were observed when implantation was done into the stomach wall, while low levels were observed when implantation was done into the subcutis. Because the CEA content in the tumor tissue was not significantly different between the two implanted sites, the high serum level of CEA produced by the stomach tumors is difficult to explain solely in terms of vascular supply or the function of capillaries for transporting CEA. Perhaps the microenvironment of the implantation site influences the functional action of tumor cells for production and secretion of CEA.

These results indicate that the implantation of human gastric cancer into the stomach wall of nude mice can provide a model for studies on the intrinsic characteristics of human cancer. In addition, this gastric cancer model is worthy of further investigation to establish its value as a screening system to find new antitumor agents effective against human gastric cancer.

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