

# A patient-like orthotopic implantation nude mouse model of highly metastatic human ovarian cancer

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(Received 25 September 1997; accepted in revised form 16 September 1998)

**Clinically relevant animal models of human cancer are important for studies of cancer biology, invasion and metastasis, and for investigating new forms of prognostic diagnosis and therapy. An ovarian tumor line (RMG-1: human clear cell carcinoma of the ovary) previously grown subcutaneously was implanted orthotopically as intact tissue into the ovarian capsule of 22 nude mice. The tumors showed progressive growth at the orthotopic site in all animals. Tumor-associated serum galactosyltransferase (GAT) tended to be positive in all nude -mice. The tumors invaded or metastasized to the contralateral ovary, retroperitoneum, mesentery and peritoneum, and omentum, and metastasized to the subcutaneous tissue, lymph nodes and distant organs including the liver, kidney, pancreas, and diaphragm. In striking contrast, subcutaneous transplantation of this tumor resulted in growth in only 2 of 5 animals with local lymph node and kidney involvement but no retroperitoneal or peritoneal involvement. These findings suggest that orthotopic implantation provides a suitable micro-environment in which ovarian cancer can express its intrinsic clinically-relevant properties. This approach is relevant to the clinical features of ovarian cancer and is thought to be a useful model for studies of therapy for this cancer.**

**Keywords:** ovarian cancer, human, RMG-1, tumor-associated galactosyltransferase (GAT), orthotopic implantation, nude mouse

## Introduction

The improvement of animal models such that they mimic the situation in patients as closely as possible is important for increasing the effectiveness of treatment for ovarian cancer. Subcutaneous or intra-

muscular cancer xenografts in immunodeficient mice are known to have little metastatic capability, even when they are obtained from tumors that are highly metastatic in human patients. A number of studies [1-6] have shown that implanting human tumor cells orthotopically into the organs of nude mice corresponding to the primary sites in humans results in an increased rate of metastasis in comparison with subcutaneous or intra-muscular implantation. For example, when human colon cancer cell xenografts

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are implanted into the cecum of nude mice, the tumors eventually metastasize to the liver [6]. Similar results have also been obtained by orthotopic implantation of tumor xenografts into other organs [3,5]. One of the characteristics of the malignancy of ovarian cancers is their invasion and metastatic potential. Once the tumor cells have spread into the abdominal cavity, they form solid tumors in the proximal tissues including the mesentery and the peritoneum and can metastasize to distal organs. To understand the process of invasion and metastasis of human ovarian tumors and its treatment, it is important to establish a clinically-relevant animal model of this cancer.

In the present study, the human ovarian cancer cell line RMG-1 (clear cell carcinoma) was implanted into the ovary of nude mice, and its biological and functional characteristics were analyzed, including the tumor take rate, invasiveness and potential for metastasis. The serum level of tumor-associated galactosyltransferase (GAT), which has been characterized to be a marker useful for the diagnosis of patients with ovarian cancers, and to be secreted from human ovarian cancer cell lines including RMG-1 cells [7,8] was also characterized in this model.

## Materials and methods

### Animals

Four-week-old, female athymic BALB/c nude mice were obtained from Nihon CLEA Co., Ltd., Tokyo.

### Tumor

The human ovarian cancer cell line RMG-1 was derived from ascites of a 60-year-old woman, as described previously by Nozawa *et al.* [9]. The cells, polygonal or spindle, with neoplastic and pleomorphic features grew in multiple layers and without contact inhibition *in vitro* with a population doubling time of 60 hours. The chromosome number varied from 35 to 47, and the modal number was stable at the hyperdiploid range 47. The cultured cells produced the highest concentration of GAT of several gynecological cancer cell lines measured.

### Implantation procedure

Subcutaneous tumors were established by implantation of  $5 \times 10^6 - 1 \times 10^7$  tumor cells suspended in 1.0 ml of Ham's F12 medium into the left flank of the mice. When the subcutaneous tumor had grown to approximately 1 g in weight, 3-mm<sup>3</sup> pieces of tumor tissue were obtained. For orthotopic implan-

tation, the mice were anesthetized with nembutal, and a right lateral dorsal incision was made. The retroperitoneum was then opened, and part of the right ovary was well exposed. One tissue block was implanted on the ovarian capsule with a 7-0 surgical suture. The retroperitoneum and skin were then closed with a 7-0 surgical suture.

### Evaluation of tumor growth

The mice were divided into two groups: 12 mice in the first group were necropsied 6 weeks after implantation, and 10 mice in the second group at 12 weeks, for tumor removal and measurement to assess tumor growth.

### Examination of metastasis

Local tumorigenicity and metastatic potential were compared between the two groups. All organs were examined macroscopically, and enlarged lymph nodes, any other organs showing abnormalities, and all tumors with surrounding tissue were fixed in 10% buffered formalin solution, sectioned at a thickness of 5  $\mu$ m, and stained with hematoxylin and eosin.

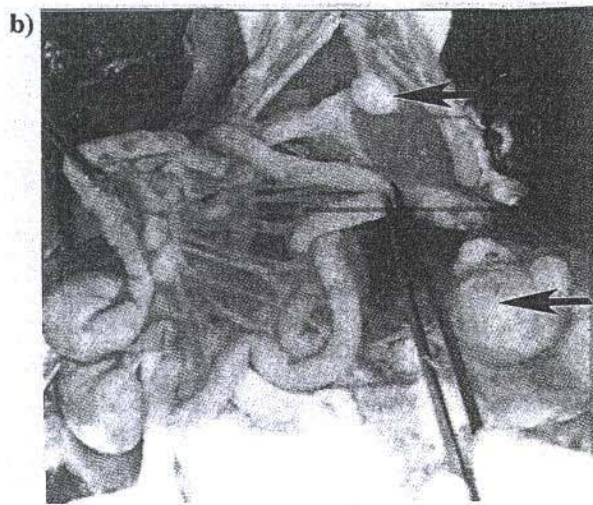
### Assay of serum GAT

We have previously characterized several monoclonal antibodies (Mab) which recognize GAT using a galactosyltransferase purified from the ascitic fluid of an ovarian cancer patient [9]. In brief Mab8513 reacted only with polymeric GAT, which is an aggregated form of GAT obtained by mercaptoethanol treatment, and another antibody, MabS62S, bound to both GAT polymer and galactosyltransferase (Gal T) monomer. These two Mabs were used for double-determinant sandwich-enzyme immunoassay to quantify the serum GAT level in the tumor-bearing nude mice.

## Results

### Local tumor growth

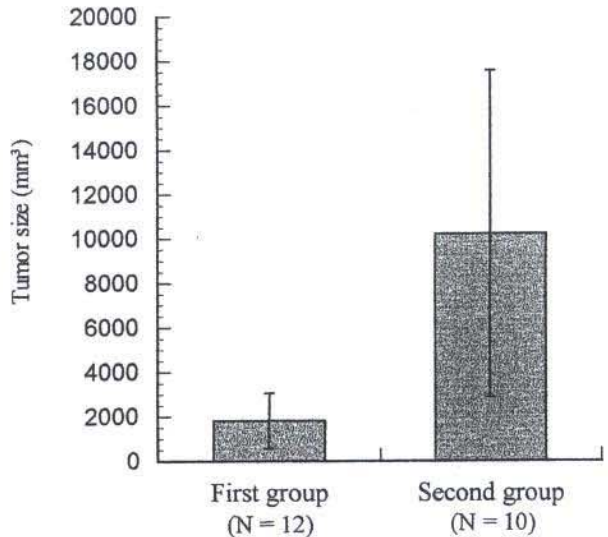
The orthotopically implanted tumors grew successfully in the ovaries of all the mice implanted, demonstrating a 100% (22/22) take rate. Figure 1 (a) shows the typical local growth of an implanted tumor on the right ovary of a mouse in the first group necropsied 6 weeks after implantation. Figure 1 (b) shows peritoneal tumor spread in a mouse in the second group necropsied 12 weeks after implantation. Figure 2 shows the sizes of the tumor localized to the ovary. The sizes ranged from 250 to 4,027 mm<sup>3</sup> in the first group and from 1,382 to 26,108 mm<sup>3</sup> in the second group.



**Figure 1.** (a) Massive tumor that developed in the right ovary of a nude mouse in the first group after orthotopic implantation of the human ovarian tumor line RMG-1. (b) Peritoneal and retroperitoneal metastases of human ovarian tumor line RMG-1 in a nude mouse in the second group, after orthotopic implantation into the right ovary.

#### *Invasion and metastasis*

Invasion and metastasis were observed in 15 of the 22 mice (68.2%): 6/12 in the first group and 9/10 in the second. The sites of invasion or metastasis were the contralateral ovary (1/10, second group); retroperitoneum (4/12, first group, 2/10, second group); mesentery (2/10, second group); peritoneum (1/10, second group); and omentum (1/12, first group, 5/10, second group); sites of distant metastases were lymph nodes (9/10, second group); subcutaneous tissue (1/12, first group); liver surface (1/12, first group, 1/10, second group); pancreas (1/10, second group); kidney (1/10, second group); and diaphragm (7/10,



**Figure 2.** Mean local tumor size in the two groups.

second group). In addition, serous fluid was observed in 1 of the 10 mice in the second group.

Table 1 shows the sizes of all the tumors, including the 'primary' and invasive or metastatic tumors, in the mice. The tumors ranged in size from 385 to 10,045 mm<sup>3</sup> in the first group and from 3,063 to 26,108 mm<sup>3</sup> in the second.

#### *Tumor histopathology*

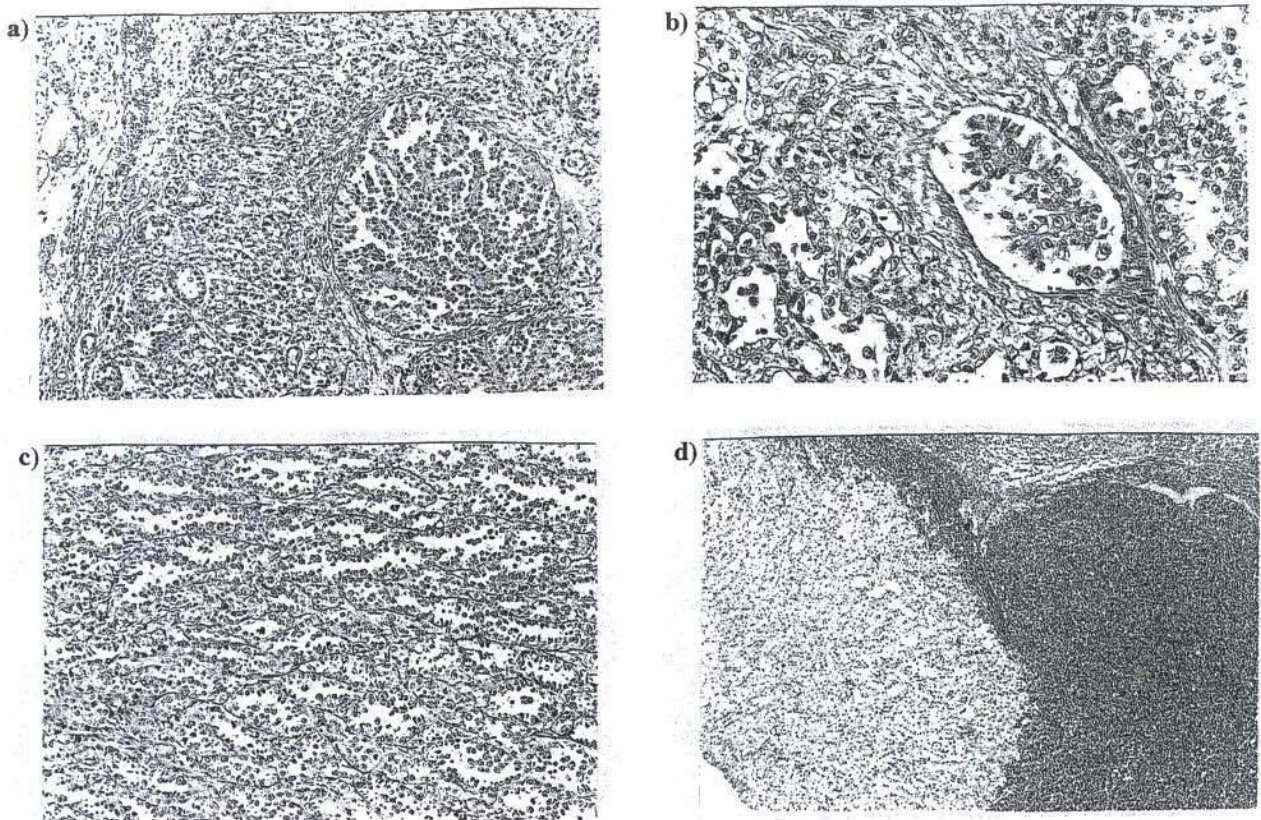
Both the 'primary' and invasive or metastatic tumors demonstrated the same histological features of moderately differentiated clear cell carcinoma, as shown in Figure 3 (a-d). Figure 3 (b) shows a typical example of the vascular invasion which was found in 2 of 12 and 6 of 10 mice in the first and second groups, respectively. The histological features were essentially the same in the nodules found in the peritoneum and in the lymph node metastases, as shown in Figures 3 (c) and 3 (d). When the tumor exceeded 10<sup>4</sup> mm<sup>3</sup> in size, central necrosis was frequently observed.

#### *Tumor volume and serum GAT level*

Every nude mouse demonstrated a positive serum GAT level ranging from 2.7 to 35.5 units/ml, as shown in Table 1. There was a statistically significant correlation between tumor size and the serum GAT level in the first group, as shown in Figure 4 (a), but not in the second group (Figure 4 (b)) perhaps due to necrosis of these larger tumors.

#### *Subcutaneous vs. orthotopic implantation*

Five animals were transplanted subcutaneously with 5 × 10<sup>6</sup> cells. The tumors grew very slowly, and in 3 of



**Figure 3.** (a) Histopathology of human ovarian tumor line RMG-1 in the ovary of a nude mouse after orthotopic implantation (first group). (b) Histopathology of human ovarian tumor line RMG-1 in the ovary of a nude mouse after orthotopic implantation (second group). Note the vascular invasion of RMG-1, indicating hematogenous spread. (c) Histopathology of omental metastasis human ovarian tumor line RMG-1 in a nude mouse (first group). (d) Histopathology of human ovarian tumor line RMG-1 metastatic to the pelvic lymph node of a nude mouse.

the 5 mice the tumors regressed after approximately 4 months after transplantation. In the other two mice, the primary tumor weighed approximately 2.3 g in one animal and 0.38 g in the other. Neither positive animal had any peritoneal or retroperitoneal involvement. In the animal with the larger primary tumor, there were lymph nodes involved in the region of the primary subcutaneous tumor as well as growth on the surface of the kidney with peri-renal lymph node involvement. In the animal with the smaller tumor, there were also two lymph nodes involved in the region of the subcutaneous tumor but no other involvement.

## Discussion

In order to develop new therapeutic strategies for cancer, appropriate animal models that mimic the biological behavior of human cancers are required. Hoffman [1] reported that histologically-intact human cancer specimens obtained from patients could be successfully implanted orthotopically into

the corresponding organs of immunodeficient mice. This procedure was termed surgical orthotopic implantation (SOI). These included eight major types of human cancer including those of the colon, bladder, pancreas, head and neck, stomach, lung, prostate, and ovary. It was also observed that these tumors were able to mimic the biological behavior of human cancer in terms of local growth and invasion or metastasis. In a brief previous study when tumor tissue from three cases of human ovarian cancer was implanted orthotopically into the ovarian capsule of nude mice, tumor growth was observed in the ovary, as well as invasion and metastasis to the lung, to the peritoneum, and major organs such as the colon [1,13]. This biological behavior was similar to that of human ovarian cancer, indicating that this animal model of the disease was an appropriate one for therapeutic investigations. In fact, it was noteworthy that one mouse showed extensive distant subcutaneous metastasis, a feature which was also observed in the original patient.

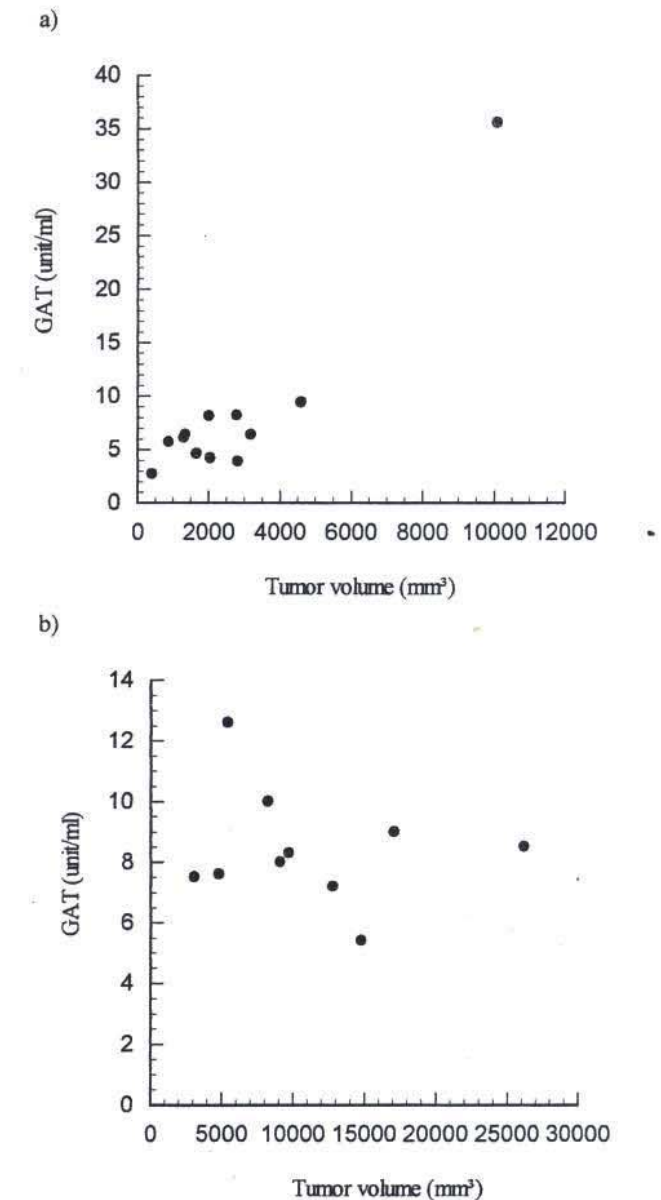
**Table 1.** Tumor sizes and tumor-associated serum galactosyltransferase (GAT) levels in the two groups of nude mice with orthotopically-transplanted ovarian cancer

Group	Mouse	Weeks	Tumor size (mm <sup>3</sup> ) <sup>a</sup>	GAT (units/ml)
First	1	6	385	2.7
	2	6	871	5.7
	3	6	1300	6.1
	4	6	1336	6.4
	5	6	1653	4.6
	6	6	2004	8.1
	7	6	2048	4.2
	8	6	2762	8.2
	9	6	2797	3.9
	10	6	3167	6.4
	11	6	4578	9.4
	12	6	10045	35.5
Second	13	12	3063	7.5
	14	12	4789	7.6
	15	12	5354	12.6
	16	12	8203	10.0
	17	12	9063	8.0
	18	12	9704	8.3
	19	12	12782	7.2
	20	12	14742	5.4
	21	12	17062	9.0
	22	12	26108	8.5

<sup>a</sup>Tumor size represents the summation of primary and metastatic lesions.

Several reasons have been suggested as to why orthotopically-growing tumors can invade or metastasize to a much greater extent than ectopically-growing tumors. Reid and Zvibel [10] summarized the related factors as (i) anatomy, which determines the local microenvironment including the location of an available capillary bed, (ii) formation of tumor emboli, (iii) molecules that specify the attachment of tumor cells to particular cells or tissue-specific extracellular matrix molecules, (iv) local growth factors, and (v) local matrix chemistry. Colon tumor cells implanted intracecally grew, metastasized, and also produced high levels of heparanase and type IV collagenase of the 92-kDa and 64-kDa forms, compared with tumors planted subcutaneously which did not metastasize [11]. We have suggested that in addition, maintenance of the tissue architecture of the orthotopically-implanted tissue is critical for metastatic potential [1].

In the present study, a human ovarian tumor (clear cell carcinoma) was implanted orthotopically as intact tissue into the ovarian capsule in 22 nude mice after serial passage in subcutaneous tissue. Orthotopically growing tumors were established in all mice, and the tumors invaded or metastasized to the contralateral



**Figure 4.** (a) Tumor size and serum GAT level in the first group necropsied 6 weeks after implantation. (b) Tumor size and serum GAT level in the second group necropsied 12 weeks after implantation.

ovary, retroperitoneum, mesentery, peritoneum, omentum, and metastasized to the subcutaneous tissue, regional lymph nodes, and distant organs such as the kidney, liver, pancreas and diaphragm.

Invasion and metastasis observed after orthotopic transplantation appear not random and not due to leakage, since the pattern is so different than after subcutaneous transplantation. This result is consistent with our orthotopic implantation studies with colon tumors [14] in which resection by day 10 after implantation precluded liver metastasis, also

indicating seeding did not take place during the transplantation process. In conclusion, the ovarian tissues are a superior host for this ovarian cancer than in the subcutaneous space as evidenced by a 22 out of 22 take rate in the ovary as opposed to a 2 of 5 take rate in the subcutaneous site. The subcutaneous dissemination or metastasis was limited to regional lymph nodes and on the kidney as opposed to extensive invasion dissemination, and distant metastasis after orthotopic transplantation.

All the nude mice demonstrated positive serum GAT levels, and a close correlation was found between tumor volume and serum GAT in the group necropsied 6 weeks after implantation. Since Mab8626 is human specific and RMG-1 cells are strongly positive for GAT, these results indicated that human ovarian clear cell carcinoma in nude mice produces human GAT, which could be an appropriate marker of tumor growth. Clinically, a poor prognosis is indicated when the concentration of GAT becomes elevated in the sera of patients with ovarian cancer, even if they have no clinical evidence of invasion or metastasis, since it is considered that neoplasms producing a high amount of GAT are more malignant than those which do not produce GAT. GAT was previously shown to correlate with CA125, another ovarian cancer marker [7-9].

The tumor growth, invasion, metastasis and tumor marker data presented in this report suggest this is the first animal model of ovarian cancer that reflects the natural history and physiology of clinical ovarian cancer. We have also found that orthotopically-growing and invading or metastasizing human tumors in nude mice maintain their characteristics after serial passage (data not shown). Thus, the model described here for orthotopic implantation of human ovarian cancer tissues into nude mice appears promising for the evaluation of new *in vivo* treatment modalities, such as responses to drugs in individual cancer patients and prediction of their clinical course.

This procedure should yield a library of specific tumor types and subtypes, according to stage, grade, and drug-response spectrum, which can be used in the study and treatment of human ovarian cancer. Orthotopic implantation of this cancer into mice has the potential of providing almost every patient with an 'individualized' tumor model. Thus this animal model should significantly enhance our understanding of human ovarian cancer and its treatment.

## Acknowledgements

We thank Mr. Y. Nagashima, Ms. H. Terui, and Ms. M. Saito for their excellent technical assistance

and Ms. Y. Mochizuki for her excellent secretarial assistance.

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