Early Resection of Primary Orthotopically-Growing Human Colon Tumor in Nude Mouse Prevents Liver Metastasis: Further Evidence for Patient-like Hematogenous Metastatic Route

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Abstract. *We have developed an orthotopic transplant model of human cancer to immunodeficient mice utilizing microsurgical techniques with intact tissue. The resulting transplanted human tumors grow locally and metastasize in a clinical-like pattern. However, there has been no definitive evidence in colon cancer that the human tumors metastasize via hematogenous route in nude mice. In the present study, in order to obtain definitive evidence of physiological spread of the human tumors, the primary tumors were resected 10 days after the initial orthotopic transplantation to the nude mice. The resection prevented metastases from forming, demonstrating that metastases of the human colon cancers occur after 10 days and by physiological and non-seeding mechanisms in the transplanted nude mice.*

Liver metastasis is one of the most important problems in the treatment of colon cancer, but the lack of relevant animals models has limited investigation. Orthotopic transplantation models have shown promise in immunodeficient mice to mimic human tumor behavior (1). We have recently developed an intact-tissue onplant method of orthotopic transplantation of human colon carcinoma, with resulting local growth and distant metastasis including in the liver, thereby reflecting the clinical situation closely (2). However, there has been no definitive evidence that the liver metastases occur via hematogenous spread, though we have observed human colon tumor cells in vessels of the nude mouse liver tissue (3).

In the present study, in order to obtain such definitive evidence, the primary tumors were resected 10 days after the initial orthotopic onplantation on the nude mouse cecum, to determine whether liver metastases occur through direct dissemination to the liver or via hematogenous spread.

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Materials and Methods

The following human colon carcinoma xenografts were used in this study: COL-3-JCK and COL-5-JCK, a poorly differentiated and a well differentiated adenocarcinoma xenograft, respectively, were established at Tokai University, Atsugi, Japan and supplied through the Central Institute for Experimental Animals. Co-3, a well differentiated adenocarcinoma xenograft, was established at the Pathology Division, National Cancer Center Research Institute, Tokyo, Japan. These xenografts were maintained at the School of Medicine, Keio University, by serial transplantation into nude mice.

Male BALB/cA nu/nu mice 6-8 weeks old were purchased from CLEA Japan Inc. for use in this study. The onplant method was carried out as reported previously (4). Tumors growing subcutaneously in nude mice were resected aseptically and the tumor tissues were scissor-minced into pieces about 3 mm in diameter, weighing about 50 mg each. Mice were anesthetized with 2.5% solution of a mixture of 2,2,2-tribromoethanol and tert-amylalcohol (1:1). An incision was made through the left lower abdominal pararectal line and peritoneum. The cecal wall was exposed and a part of the serosal membrane was carefully mechanically scraped using a 27 gauge needle, in order not to perforate the cecal wall. One of the tumor pieces was then fixed on each scraped site of the serosal surface with a 6-0 Dexon (Davis-Geck Inc., Manati, PR) transmural suture. The cecum was then returned into the peritoneal cavity, and the abdominal wall and the skin were closed with 6-0 Dexon sutures.

Ten days after the orthotopic onplantation, the mice were randomized into two groups, a control group and a group having the primary tumor resected. The treated mice were anesthetized and operated on as above, and the cecum with the growing tumor, usually about 5-6 mm in diameter, was carefully exposed and resected using a Surgiclip (Century Medical, Inc., Tokyo, Japan). The resected cecum was processed for histological examination. In initial experiments, we found that it was difficult to resect the cecum 14 days after onplantation, due to intraperitoneal adhesion. The mice were sacrificed 4-7 weeks after onplantation in the control group, and all mice treated by resection of the primary tumor were sacrificed 7 weeks after onplantation. All organs including the cecum and liver were processed for routine histological examination after careful macroscopic examination.

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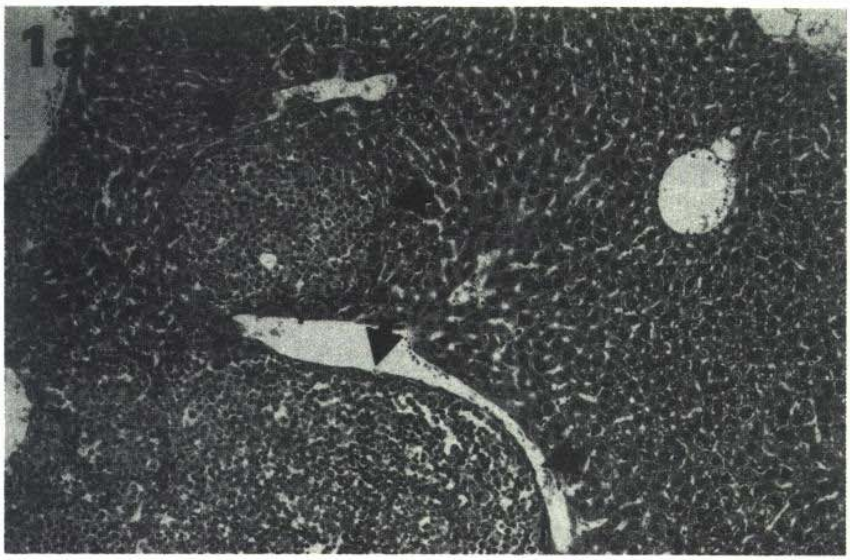

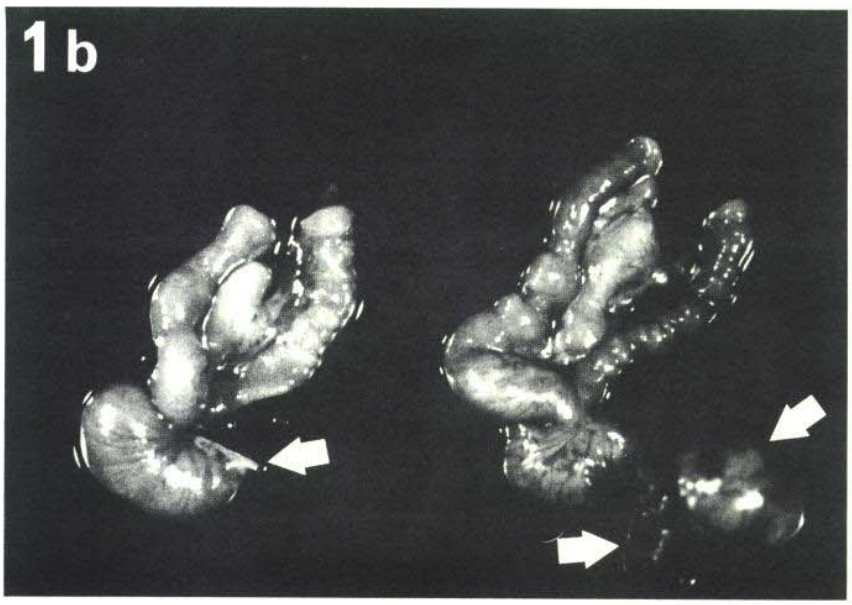

Figure 1. *a) Liver metastases of COL-3-JCK 4 weeks after orthotopic onplantation (x100, HE). Arrows indicate the metastases. b) Extensive local growth in cecum (right) and no local recurrence in cecum after resection of the primary tumor (left), both 4 weeks after orthotopic onplantation. Arrows indicate the tumor mass in cecum (right) and arrow indicates the Surgiclip used for the resection (left).*

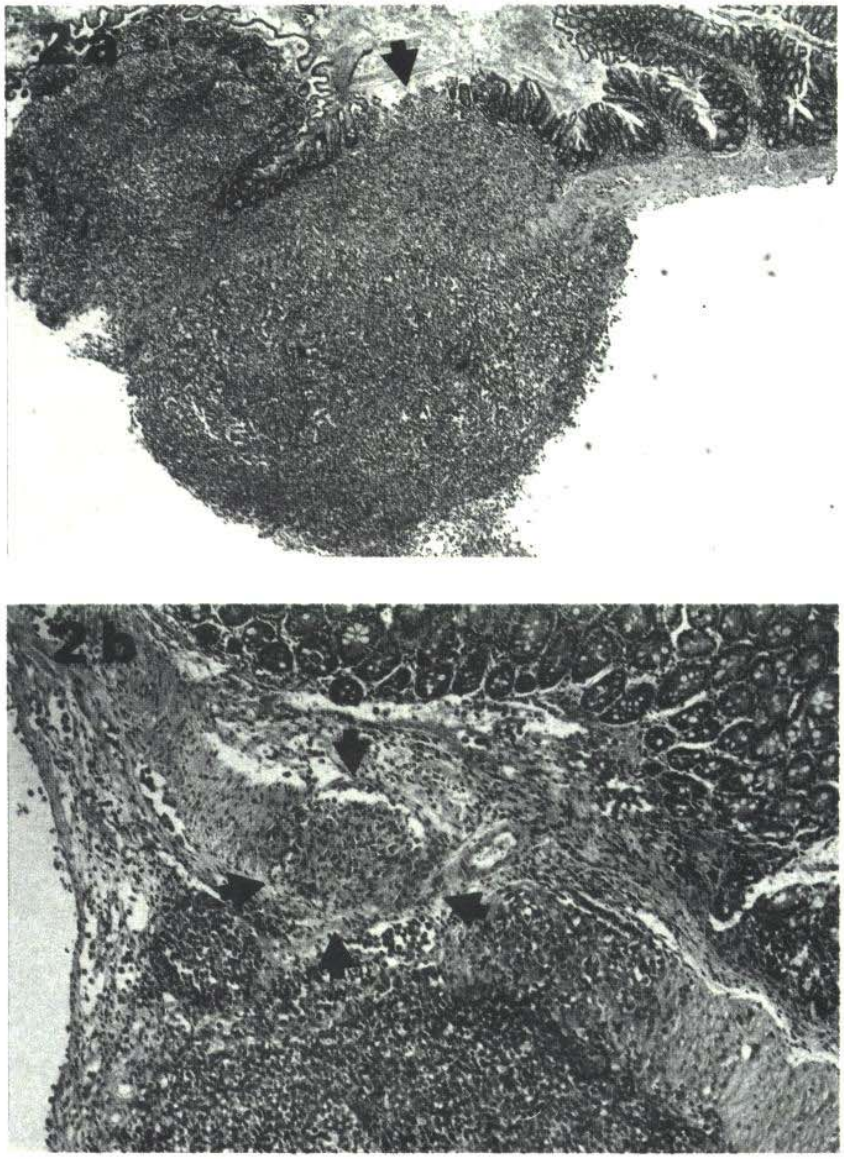

Figure 2. *Pathohistology of COL-3-JCK invading the cecal wall 4 weeks after onplantation. a) Arrow indicates the tumor mass invading and penetrating into the mucosal layer (x40, HE). b) Arrows indicate the invasive tumor cells in vessels (x100, HE).*

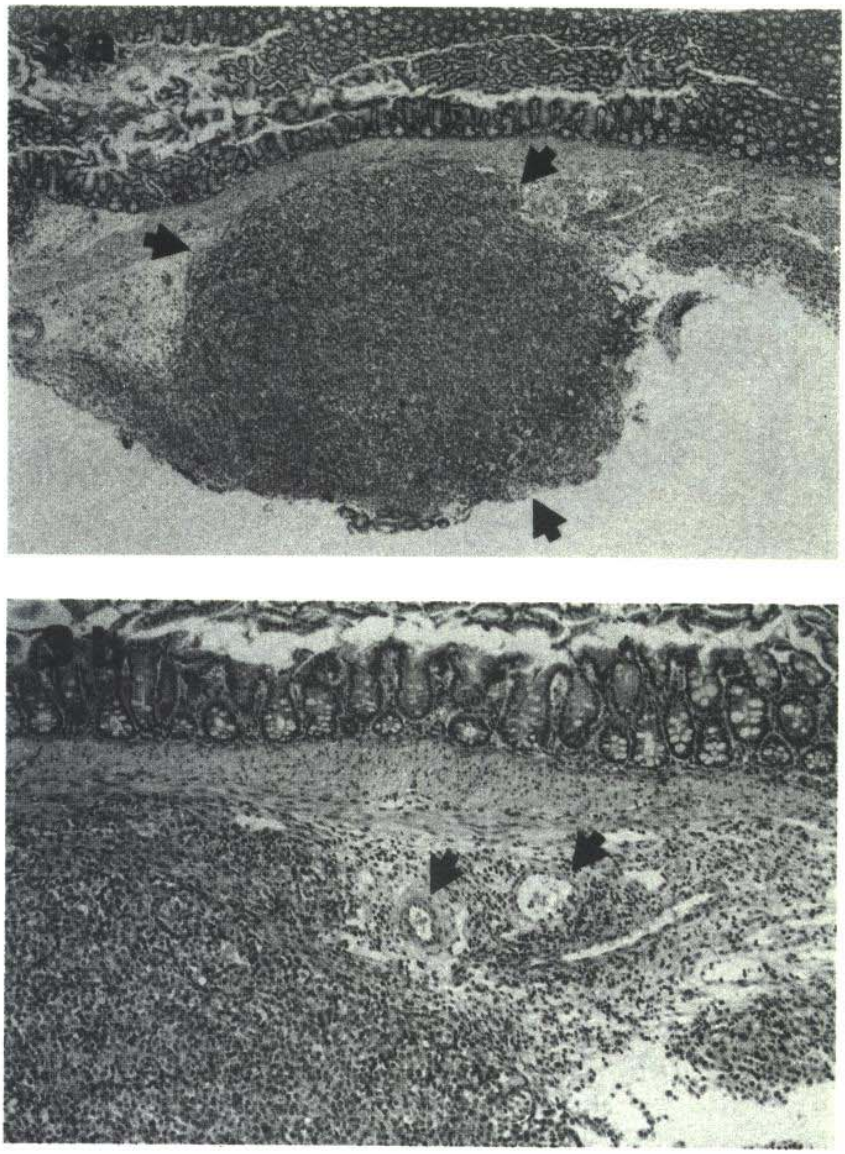

Figure 3. *Pathohistology of growing COL-3-JCK in the resected cecum 10 days after the onplantation. a) Arrows indicate the tumor mass within the propriate muscle layer (x40, HE). b) Arrows indicate no invasive tumor cells in vessels (x100, HE).*

Table I. *Prevention of liver metastases by resection of primary human colon carcinoma xenografts orthotopically onplanted on the cecum of nude mice.*

Tumor	No. of Mice	Resection of primary tumor[1]	Local tumor growth[2]	Liver metastasis[3]
COL-3	8	+	0/ 8	0/ 8 *
	8	—	8/ 8	5/ 8 *
COL-5	8	+	0/ 8	0/ 8 **
	10	—	10/10	5/10 **
Co-3	9	+	0/ 9	0/ 9 **
	7	—	7/ 7	4/ 7 **

[1]Histologically intact tissue of human colon carcinoma was orthotopically onplanted on the cecum of nude mice with(+) or without(—) resection of primary tumor 10 days after onplantation. Mice were sacrificed 4-7 weeks after the initial onplantation.

[2]Data are shown as number of mice with local tumor growth after orthotopic onplantation or mice with local recurrence after resection of primary tumor/number of mice evaluated.

[3]Data are shown as number of mice with liver metastases/number of mice evaluated. *, ** Statistically significant by chi-squared test: *$p<0.01$, **$p<0.05$

Results and Discussion

As shown in Table I, we observed extensive local growth on the cecum in all the control mice and liver metastases in over half of controls after orthotopic onplantation (Figure 1a), whereas neither local recurrence nor liver metastases were observed in the mice treated by resection of the primary tumor (Figure 1b). We also observed extensive invasion of tumor cells into vessels of the cecal wall 4-7 weeks after onplantation in the control mice (Figure 2). However, no vessel invasion was observed in the resected cecum 10 days after onplantation (Figure 3), which was relevant to the result that no mice developed liver metastases even 12 weeks after resection of the primary tumor (data not shown). Thus we surmised that vessel invasion occurred more than 10 days after onplantation, resulting in liver metastases via hematogenous spread and not by seeding during the operative procedure.

It is imperative to study the metastatic process of colon cancer in order to establish therapeutic strategies for preventing liver metastases. In this context, the orthotopic onplant model, which shows full expression of the metastatic process including hematogenous spread and resembles the clinical situation, would be useful for further study of the metastatic process of colon cancer.

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