Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 5645–5649, June 1992
Medical Sciences

A metastatic nude-mouse model of human pancreatic cancer constructed orthotopically with histologically intact patient specimens

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Communicated by Sheldon Penman, March 2, 1992

ABSTRACT **Pancreatic cancer is one of the most intractable and least understood of all human cancers. Pancreatic cancer is the fourth-leading cause of cancer-related mortality in the United States with <2% of the patients surviving for 5 yr. In an effort to help develop more effective treatment modalities for pancreatic cancer and improve detection, we report an animal model for individual human pancreatic-cancer patients. The model involves orthotopic transplantation of histologically intact pancreatic-cancer specimens to the nude-mouse pancreas, which can result in models that resemble the clinical picture including (*i*) extensive local tumor growth, (*ii*) extension of the locally growing human pancreatic cancer to the nude-mouse stomach and duodenum, (*iii*) metastases of the human pancreatic tumor to the nude-mouse liver and regional lymph nodes, and (*iv*) distant metastases of the human pancreatic tumor to the nude-mouse adrenal gland, diaphragm, and mediastinal lymph nodes. In a series of five patient cases, a 100% take rate has been demonstrated, and of 17 mice transplanted, 15 supported tumor growth. Immunohistochemical analysis of the antigenic phenotype of the transplanted human pancreatic tumors showed a similar pattern of expression of two different human tumor-associated antigens, such as tumor-associated glycoprotein 72 and carcinoembryonic antigen in the transplanted tumors when compared with the original surgical biopsy, suggesting similarity between the two. This model should, therefore, prove valuable for treatment evaluation of individual cancer patients, as well as for evaluation of experimental treatment modalities for this disease.**

Cancer of the pancreas is one of the most intractable cancers and is the fourth-leading cause of cancer death in the United States (1–3). At surgery, most patients are found to have unresectable disease with no imminent effective treatment strategies; <2% of patients survive 5 yr (3). Despite the potential value of a relevant model for human pancreatic cancer, no animal model is available, to our knowledge, for the individual pancreatic-cancer patient.

Models based on athymic nude mice have been used for human cancer. However, metastatic rates from subcutaneous or intramuscular xenografts have been low or nonexistent, even from tumors that were highly metastatic in the patient from whom the tissues were derived (4–8).

Recent work from a number of laboratories has indicated that implanting human tumor cells orthotopically in the corresponding organ of nude mice resulted in much higher metastatic rates. Dissociated human colon cancer cells, when grown in culture and subsequently injected into the cecum of nude mice, produced tumors that eventually metastasized to the liver, showing that orthotopic implantation can enhance the metastatic capability of human tumor cells in nude mice (8–16). Similar results also have been achieved for orthotopic implantation of cell lines of human lung cancer (17), bladder cancer (18, 19), melanoma (20, 21), breast cancer (22–25), and head and neck cancer (26). Marincola and coworkers (27, 28), Pan and Chu (29), and Vezeridis *et al.* (30) have established orthotopically growing pancreatic tumors in nude mice, but these tumors were derived from cell lines.

Our approach is to avoid disruption of tumor integrity and to orthotopically implant histologically intact patient tumor tissue directly after surgery or biopsy. Such a model should better resemble the original properties of the human cancer and could be of great value in developing additional drugs and treatment strategies for cancer. Guided by this overall strategy, we have used nude mice to construct human colon-cancer models that directly use surgical specimens and can exhibit the variety of clinical behaviors seen in human subjects (31). These behaviors include (*i*) local growth, (*ii*) abdominal metastasis, (*iii*) general abdominal carcinomatosis with extensive peritoneal seeding, (*iv*) lymph-node metastasis, (*v*) liver metastasis, and (*vi*) colonic obstruction: a tumor-establishment rate of 13 cases in 20 attempts was found (31). We have constructed a similar set of models for human bladder cancer (32).

We report here the use of the orthotopic-transplant strategy of histologically intact patient specimens to develop a human pancreatic-cancer model with a 100% take rate and subsequent growth and metastatic behavior while retaining human tumor-associated antigens (TAAs), thereby resembling the clinical picture.

MATERIALS AND METHODS

Mice. Four-week-old outbred *nu/nu* mice of both sexes were used for tumor implantation. All animals were maintained in a sterile environment; cages, bedding, food, and water were all autoclaved. All animals were maintained on daily 12-hr light/12-hr dark cycle. Bethaprim pediatric suspension (containing sulfamethoxazole and trimethoprim) was added to the drinking water. Four- to-6-week-old outbred *nu/nu* mice of both sexes were used for the orthotopic-transplantation experiments.

Pancreatic-Cancer Specimens. Each pancreatic-cancer surgical specimen was cut into pieces of ≈1-mm^3 size. For each transplantation tumor, pieces were sampled from different regions of the specimen equally to take into account zonal heterogeneity in the tumor (31, 32). All cases analyzed in this report were adenocarcinomas.

Surgical Microprocedures. Nude mice were anesthetized with isoflurane (Forane) inhalation. A left lateral abdominal incision was made, the peritoneum was opened, and the part of the pancreas near the portal area of the spleen was well

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Abbreviations: CEA, carcinoembryonic antigen; mAb, monoclonal antibody; TAA, tumor-associated antigen; TAG-72, tumor-associated glycoprotein 72.

exposed. The pieces of 1-mm^3 tumor tissue were transplanted on the pancreas with 8-0 surgical sutures. The peritoneum and the skin were then closed in one layer with 7-0 surgical suture (31).

Monoclonal Antibodies (mAbs). The generation, characterization, and purification of murine mAbs CC49 and COL-1 to the human TAA tumor-associated glycoprotein 72 (TAG-72) and carcinoembryonic antigen (CEA), respectively, have been well described (33, 34). The murine IgG1, MOPC-21 (Litton Bionetics), was used as a negative control.

The detailed method for protein biotinylation has been described (35). Briefly, purified mAbs CC49 and COL-1 were dialyzed vs. 0.1 M sodium bicarbonate, pH 8.5; 150 μg of *N*-hydroxysuccinimidobiotin (bio-NHS, from Sigma), dissolved in dimethyl sulfoxide, was added per mg of protein and incubated at room temperature for 2 hr. The unincorporated bio-NHS was separated from the labeled antibody by gel filtration through Sephadex G-25 (10-ml column), and immunoreactivity of the mAbs was determined by ELISA by using cell extracts with known binding to these mAbs. MOPC-21 was biotinylated by using the same methods.

Immunohistochemical Methods. Expression of two human-TAAs TAG-72 and CEA was studied by using mAbs CC49 and COL-1, respectively. Tissues obtained from the mice and from human surgical biopsy were fixed in formalin and embedded in paraffin. Sections of 5 μm were assayed for TAG-72 and CEA expression by using a modification of described ABC immunohistochemical methods (36). Briefly, tissue sections were deparaffinized in xylene, rehydrated in graded ethanol, and treated for 20 min with 0.3% H_2O_2/methanol to block endogenous peroxidase. The sections were then incubated with 10% normal horse serum for 20 min and then incubated for 1 hr at room temperature with biotin-labeled mAbs CC49 and COL-1, as well as with control biotinylated IgG MOPC-21, at 40 μg/ml. After the phosphate-buffered saline (PBS) rinse, avidin dehydrogenase and biotinylated horseradish peroxidase H complex were added for 30 min at room temperature. The slides were then rinsed in PBS/0.06% diaminobenzidine (Sigma), and 0.01% H_2O_2 was added for 3–5 min to initiate the peroxidase reaction. PBS washes were then followed by counterstaining with hematoxylin (3 min).

RESULTS AND DISCUSSION

Five different cases of human pancreatic cancer were transplanted directly from surgery to the nude-mouse pancreas. Orthotopically growing tumors were established in all cases. Table 1 shows that not only were all cases established orthotopically in the nude mice, but of the total of 17 mice used, tumors were established in 15 mice. In addition, four out of five human cases demonstrated metastases. Nude-mouse models were constructed for pancreatic cancer that included (*i*) local growth, (*ii*) regional invasion of the stomach and duodenum, (*iii*) metastasis to regional lymph nodes, and (*iv*) distant metastasis to the liver, diaphragm, adrenal glands, mediastinal lymph nodes, and mesenteric lymph nodes.

Table 1. Human pancreatic cancer growing and metastasizing in nude mice after orthotopic transplantation of intact tissue

Case	Tumor growth	Local invasion	Metastasis	
			Lymph node*	Organ†
1957	+	+	+	+
2008	+	+	+	+
2020	+	+	+	+
2060	+	+	+	+
2090	+	+	−	−

*Lymph-node metastases include mediastinal lymph node, mesenteric lymph node, iliac lymph node, and inguinal lymph node metastasis.

†Organ metastases include liver, diaphragm, adrenal gland, stomach and duodenum, and abdominal-wall metastases.

In the clinical situation, the sites most frequently involved with pancreatic tumors include regional lymph nodes, liver, lung, peritoneum, duodenum, adrenal gland, stomach, gallbladder, spleen, kidney, intestine, and mediastinal lymph nodes (3). Of these 12 sites, our relatively small sample of cases in nude mice involved 8 of these sites, resembling the clinical picture of pancreatic-cancer spread in the nude mice after orthotopic transplantation of intact tissue.

Local Growth. Extensive local growth was seen in all five cases transplanted. The range in local tumor size was large, even for a given case. For example, in tumor 1957, tumor size ranged from 2 × 2 to 20 × 30 mm >200 days after transplantation (Fig. 1*A*). For case 2008, local growth ranged from 2.5 × 3.9 to 20 × 30 mm >174 days after transplantation.

Regional Invasion. In case 2020, where local tumor growth ranged from 6.3 × 5 to 16 × 15 mm, extensive invasion of the stomach and duodenum occurred in one out of three mice. In case 2008, regional invasion of the stomach and duodenum was also observed (Fig. 1*D*).

Distant Metastasis to Liver. Metastases to the liver surface were seen in case 2020 (Fig. 1*B*).

Very Distant Metastases. Case number 2020 involved metastasis to mesenteric lymph nodes, and case number 2008 involved distant metastases to the diaphragm, adrenal glands (Figs. 1 *C*, *F*, and *G*), mesenteric lymph nodes, and mediastinal lymph nodes (Fig. 1*E*), and iliac lymph nodes (Fig. 1*C*). It should be emphasized that metastasis to distant sites such as mediastinal lymph nodes clearly demonstrates that actual metastasis can occur in the model, as opposed to just extension or seeding. The metastatic pattern seen in the model thus resembles, to a much greater degree, the clinical picture than subcutaneous transplant models, in which metastasis rarely occurs.

Maintenance of Expression of TAAs TAG-72 and CEA After Orthotopic Transplantation. Human pancreatic carcinoma cells express a variety of human TAAs including TAG-72, CA 19-9, DU-PAN-2, and CEA (37, 38). Numerous biochemical and immunohistochemical studies on human carcinoma biopsies or surgical samples have characterized the tissue distribution of these antigens and their biochemical characteristics (37, 39, 43). These human TAAs (i.e., CEA) are normally not expressed in rodent tumors (44). However, efforts have been made to transfect human CEA in murine carcinoma (45, 46). The expression of two different TAAs, TAG-72 and CEA, in the human pancreatic-tumor tissue specimens before and after orthotopic transplantation, growth and metastasis is similar, further suggesting a resemblance of clinical behavior of the human pancreatic cancer in the nude-mouse model described here.

Serial sections from formalin-fixed, paraffin-embedded human pancreatic adenocarcinomas either before or after orthotopical transplantation in the mouse model were treated with mAbs CC49 and COL-1 by using the ABC immunoperoxidase method to determine whether the expression of TAG-72 and CEA, respectively, was maintained after orthotopic transplantation of the human pancreatic tumor to the pancreas of the nude mice. Fig. 2 illustrates the immunohistochemical reactivity of mAbs CC49 with tissue sections of two different cases (2008 and 1957). Fig. 2*A* shows the mAb CC49 reactivity with the pretransplantation human post-surgical specimens of case 2008, and Fig. 2 *B* and *C* show the CC49 reactivity with two lymph-node metastases found in the nude mouse itself after orthotopic transplantation. Note the similarity of expression in the patient and in the metastases formed in the mouse after orthotopic transplantation of the specimens.

Fig. 2*D* represents the reactivity of mAb CC49 with the locally growing tumor in the nude mouse of case 1957. Fig.

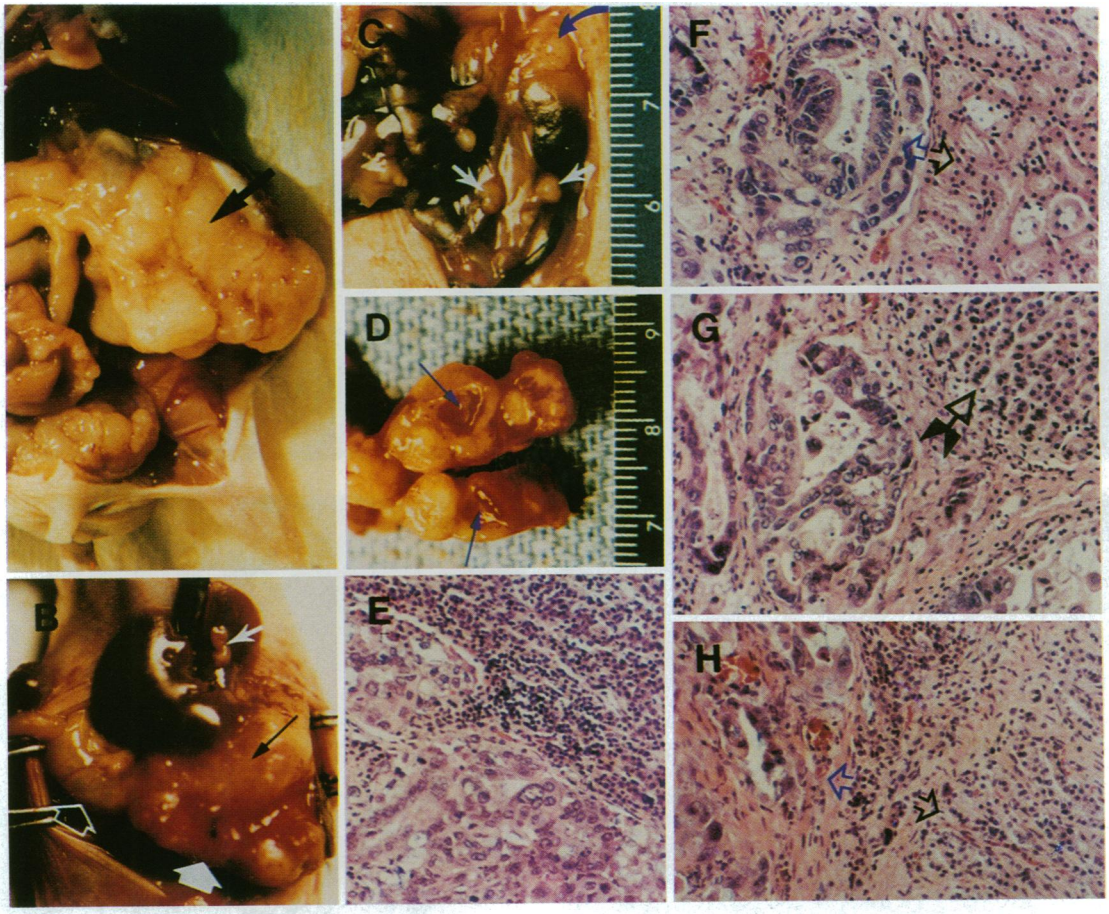

FIG. 1. (*A*) Nude mouse bearing human pancreatic cancer. Arrow indicates extensive tumor growth on nude-mouse pancreas. (*B*) Human pancreatic cancer growing in nude-mouse orthotopically (wide white arrow) and metastasized to the liver (small white arrow). Black arrow indicates that tumor invaded the stomach. White hollow arrow indicates nude-mouse duodenum. (*C*) Human pancreatic cancer metastasis to nude-mouse iliac lymph nodes (white arrows) and to the nude-mouse adrenal gland (blue curved arrow). (*D*) Section of the nude-mouse stomach invaded by human pancreatic cancer grown on nude-mouse pancreas. Arrows indicate the stomach lumen of nude-mouse. Note the stomach wall is thickened as a result of tumor invasion. (*E*) Histopathology of nude-mouse mediastinal lymph-node metastases after orthotopic transplantation of human pancreatic cancer. (×115.) (*F*) Histopathology of human pancreatic-cancer metastasis to the nude-mouse adrenal gland. Arrow pointing left indicates tumor growth. Arrow pointing right indicates nude-mouse renal tubules. (×115.) (*G*) Histopathology of human pancreatic-cancer metastasis to the nude-mouse adrenal gland after orthotopic transplantation of human pancreatic cancer. Arrow indicates nude-mouse adrenal gland. (×115.) (*H*) Histopathology of human pancreatic-cancer invasion of nude-mouse duodenum. Arrow pointing left indicates tumor invasion; arrow pointing right indicates normal mucosa. (×115.)

2 *E* and *F* represent reactivity of CC49 with lymph-node metastases in the nude mouse.

As can be seen from these figures, the expression of human TAA TAG-72 is well maintained in the locally growing transplanted tumor and in the metastatic lymph nodes, further suggesting that the human pancreatic carcinomas growing and metastasizing in the nude mice resemble the original tumors removed from the patient. Similar results were seen for the human-TAA CEA (data not shown). Our findings indicate that this mouse model maintains the native structure of the human tumor and its original antigenic phenotype, further suggesting that this model is, indeed, resembling the natural biological behavior of the human tumor.

Significance of the Findings. In this report we demonstrate an animal model for the pancreatic-cancer patient, in that histologically intact pancreatic-cancer specimens can be taken directly from surgery and transplanted to the nude-mouse pancreas with resulting primary growth, extension, and metastases. As mentioned above, of the 12 sites most frequently involved with pancreatic-cancer metastases clinically, 8 are represented in our model developed from only five cases. Thus, the models resemble the clinical picture. Because distant sites, such as the nude-mouse mediastinal lymph nodes, can be involved by the human pancreatic tumors, it is strongly indicated that such metastases are specific and not from seeding.

Although Marincola and coworkers (27, 28), Pan and Chu (29), and Vezerides *et al.* (30) have established orthotopically growing pancreatic tumors in the nude-mouse pancreas, they all used human pancreatic-cancer cell lines and not patient specimens. In this study we report the orthotopic transplantation of intact human pancreatic-carcinoma biopsies, which now offers the possibility of evaluating treatment modalities *in vivo*, such as drug responses for individual cancer patients and prediction of their clinical course.

Although the use of nude mice for modeling human pancreatic tumors will differ somewhat from the patient in a number of areas—in particular, the immunological environment—it should be noted that we have successfully applied the orthotopic-transplant method described here to severe-combined immunodeficient (SCID) mice (data not shown), in which others have shown that human T cells and B cells can be engrafted (47). Orthotopically engrafting both the histologically intact tumor and immune elements from the same patient into SCID mice would further imitate the clinical situation.

Significance of the Approach. The key aspects of the models are the orthotopic transplantation of histologically intact

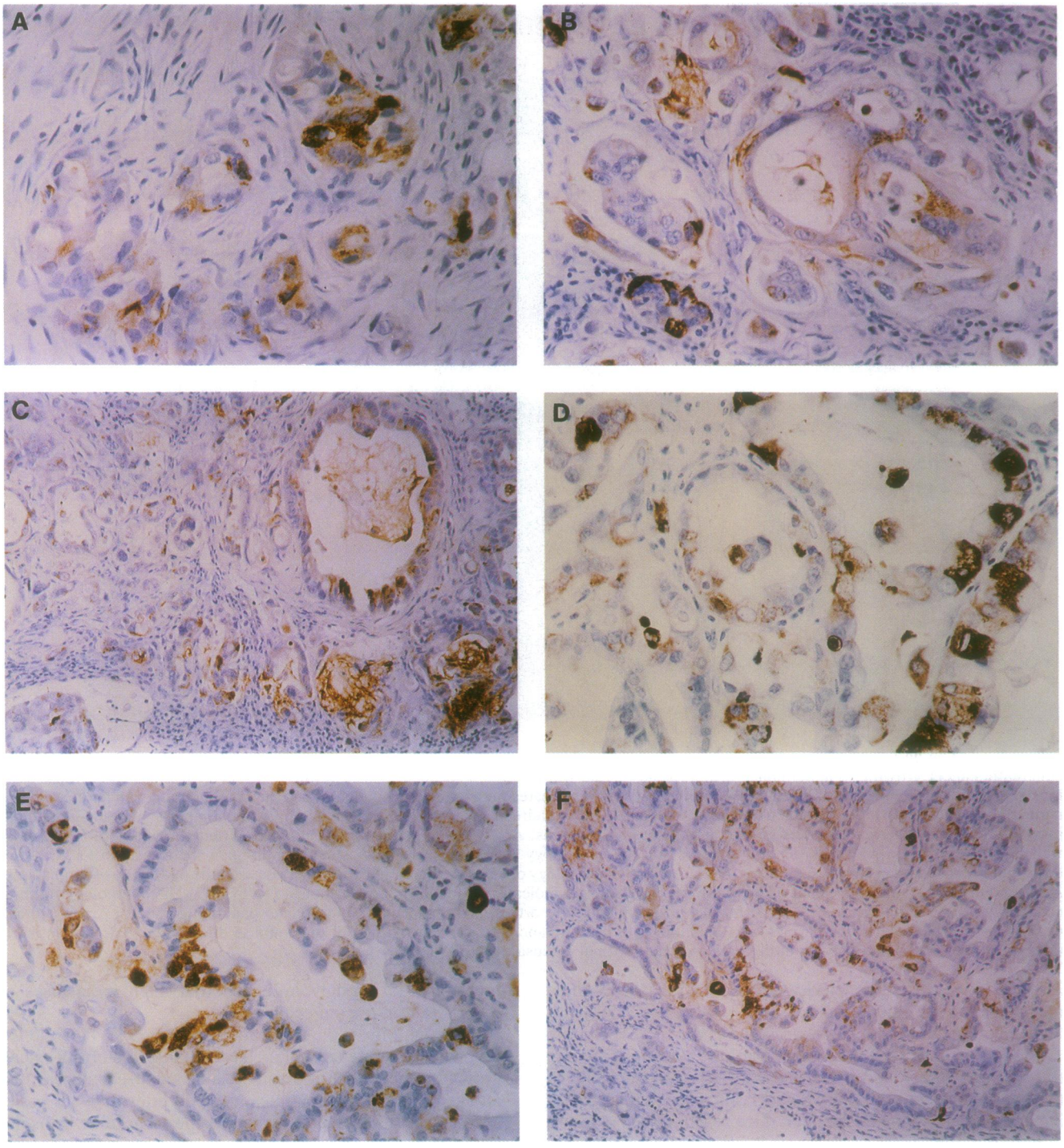

FIG. 2. Immunoperoxidase staining of human pancreatic adenocarcinoma 2008. Pretransplanted tumor (*A*) and mesenteric lymph-node metastases from the nude mouse found after orthotopic transplantation of the human pancreatic tumor (*B* and *C*), stained with mAb CC49. Immunoperoxidase staining of human pancreatic adenocarcinoma 1957, locally growing in nude mouse after orthotopic transplantation (*D*) and lymph-node metastases in the mouse found after orthotopic transplantation of the human pancreatic tumor (*E* and *F*), stained with mAb CC49.

patient tissue to the nude mice, as opposed to the use of tumor cell lines, which may greatly deviate from the original tumor. In our previous study of bladder tumors, orthotopic transplantation of intact tissue to nude mice (32) led to much more extensive metastasis than orthotopic injection of disaggregated cells (48). We also noted that the orthotopically growing and metastasizing human tumors in the nude mice can be passaged to other nude mice and maintain their characteristics (data not shown). This procedure, thus, suggests the creation of a library of specific types and subtypes, according to stage, grade, and drug-response spectrum, of patient tumors that can be indefinitely propagated and cataloged for use in research and treatment of human cancer. Such models should significantly enhance our understanding of human cancer and its treatment because the transplantation of a patient's intact tissue orthotopically potentially allows almost every patient to have his or her own tumor modeled in a system similar to that described here.

This paper is dedicated to Professor Konrad Bloch on his 80th birthday. We are grateful to Polly Jayne Pomeroy for word processing. This work was supported by National Cancer Institute Small

Business Innovation Research Grant 43 CA53963 and partially supported by the Consiglio Nazionale delle Ricerce (Italy).

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