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Immunochemical and Immunohistological Analyses of Tumor-associated Antigens Defined by Murine Monoclonal Antibodies against Human Pancreatic Carcinoma Cells

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Two murine monoclonal antibodies, SK-930 (isotype IgG2a) and SK-117 (isotype IgG1), were produced from spleen cells of mice immunized against human pancreatic carcinoma cell lines, MIA-PaCa 2 and Panc-1. With the use of the avidin-biotin-immunoperoxidase technique, the SK-930 and SK-117 antibodies detected an antigen found in 24 and 23 formalin-fixed tissue sections, respectively, of tumors obtained from 30 different patients with pancreatic carcinoma. Reactivity was also frequently found with tumors of the gallbladder, bile duct, stomach, colon and esophagus, while a large panel of normal human tissues, including normal pancreatic tissues, displayed little reactivity. These observations suggest that SK-930 and SK-117 are of value in identifying tumor-associated antigen (TAA) expressed in pancreatic carcinoma and other carcinomas of the digestive system. SK-930 antibody immunoprecipitated a 134 kilodalton molecule from extracts of ^{125}I- or [^{35}S]methionine- or [^{3}H]glucosamine-labeled tumor cells. The SK-117-defined antigen corresponds to 152/137 kilodalton molecules. Moreover, cytofluorometric analyses showed that cells treated with periodic acid exhibited greatly decreased reactivity to the two antibodies, but cells treated with neuraminidase, trypsin or pronase showed unchanged reactivity. The findings suggest that the epitopes of the novel TAA expressed on pancreatic carcinoma cells are carbohydrate moieties.

Key words: Pancreatic carcinoma — Monoclonal antibodies — Tumor-associated antigen

Pancreatic cancer in man is commonly a fatal disease due in part to the lack of a reliable means for early detection. To facilitate early diagnosis of pancreatic carcinoma, intensive efforts to develop immunologic assays have been made during the last few years and a number of pancreatic carcinoma-associated antigens have been described. Pancreatic oncofetal antigens (POA)*[4] specific to pancreatic cancer[1-3] and tumor-associated antigen (TAA)[4-6] have been reported. With the recent advent of the somatic hybridization technique,[7] antigens preferentially expressed in human pancreatic carcinoma can be identified and characterized by using monoclonal antibodies (MoAbs).

One TAA, CA 19-9, was originally found in human colon cancer cells,[8] but was also detected in a number of gastric and pancreatic cancer tissues[9, 10] and other cell lines[11] as well in sera of patients with gastric and pancreatic cancer.[12] Reactivity with normal pancreas has also been reported.[9, 10] The first demonstration of pancreatic carcinoma-associated antigen using MoAbs raised against human pancreatic carcinoma cells was made by Metzgar *et al.*[13] They developed MoAbs Du-Pan-1, 2, 3, 4 and 5 against the human pancreatic carcinoma cell line HPAF, and these MoAbs were found to encompass a wide range of specificities against other pancreatic cell lines, other tumors, and normal tissues.[13, 14] Several studies concerning production of MoAbs against human pancreatic carcinoma cells have followed.[15-19]

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*[4] Abbreviations used are: POA, pancreatic oncofetal antigen; TAA, tumor-associated antigen; MoAb(s), monoclonal antibody(ies); PBL, peripheral blood lymphocytes; PBS, Dulbecco's modified phosphate-buffered saline; FCS, fetal calf serum; ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence-activated cell sorter; NP-40, Nonidet P-40; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; ABC, avidin-biotin-peroxidase complex; CEA, carcinoembryonic antigen.

In this report, we describe the tissue distribution and immunological characterization of novel tumor-associated antigens defined by MoAbs SK-930 and SK-117 raised against human pancreatic adenocarcinoma cell lines MIA-PaCa 2[20] and Panc-1.[21]

MATERIALS AND METHODS

Tissue Culture Cell Lines The pancreatic ductal cell adenocarcinoma cell lines MIA-PaCa2[20] and Panc-1[21] were used for the immunization of Balb/c mice for the production of the MoAbs. The cell lines were obtained from the Laboratory Products Division of Dainippon Pharmaceutical Co., Ltd., Osaka. Gallbladder carcinoma cell line G-415,[22] gastric cancer cell line SC-1 and pancreatic carcinoma cell line Pl119 were established in our laboratory. Hepatocellular carcinoma cell line Alexander,[23] and uterus carcinoma cell line HeLa[24] were kindly provided by Dr. K. Mitamura (University of Tsukuba) and large cell lung carcinoma cell line TKB-1 by Dr. K. Endo (University of Tsukuba). Other cell lines used in this study were obtained from the Laboratory Products Division of Dainippon Pharmaceutical Co., Lt., Osaka. All cell lines were maintained in DMEM (Flow Laboratory, McLean, VA) or RPMI medium 1640 (GIBCO, Grand Island, NY) supplemented with 10% fetal calf serum (FCS, Flow Lab., McLean, VA) and 100 μg/ml of kanamycin (Meiji Seika Co., Tokyo).

Production of Monoclonal Antibodies Balb/c mice were given injections of 1×10^7 MIA-PaCa2 or Panc-1 cells. Then, the mice were given 3 injections at 2-week intervals and a final injection 3 days prior to fusion. Cell fusion was performed according to the method of Köhler and Milstein.[7] Spleen cells from immunized mice were fused with SP2/0 Balb /c myeloma cells[25] at a ratio of 10:1 (splenocytes: myeloma). The fused cells were distributed in 96-well microtiter plates (Nunc, No. 167008, Denmark) containing hybridoma medium with hypoxanthine-aminopterin-thymidine (Sigma Chemical Co., St. Louis, MO), and incubated at 37° with 5% CO_2. Two weeks later, hybridoma cultures were screened against MIA-PaCa2 or Panc-1 and T cell leukemia cell line, CCRF-CEM[26] cells in a whole-cell preparation ELISA described previously.[27] The culture cells containing antibodies reactive with immunizing tumors but unreactive with CCRF-CEM cells were selected and cloned three times by the limiting dilution method. Finally, supernatants obtained from two of these clones were unreactive to CCRF-CEM cells and were highly reactive with MIA-PaCa2 or Panc-1 cells. These hybridomas were passed into Balb/c mice to produce malignant ascites. Monoclonal antibodies against MIA-PaCa2 and Panc-1 were designated as SK-930 and SK-117, respectively.

Isotyping of Monoclonal Antibodies Immunoglobulin isotypes of supernatants produced in hybridoma were determined by immunodiffusion[28] in 1% agarose gel (Pharmacia Fine Chemicals, Uppsala, Sweden) with class- or subclass-specific rabbit anti-mouse antisera (Miles Scientific, Naperville, IL).

Microcytotoxicity Test The complement-dependent microcytotoxicity test was performed according to the standard microtechniques.[29]

Immunofluorescence Assay Immunofluorescence staining of various human cancer cell lines, 10 samples of peripheral blood lymphocytes (PBL), and a panel of RBC (A, B and O blood types) was performed with an indirect system using SK-930 or SK-117-containing ascites (dilution of 1/100) in the first step and FITC-conjugated affinity column-purified $F(ab')_2$ goat anti-mouse immunoglobulin antibody (Cappel Lab., Cochraneville, PA), as described previously.[27] Frequency of antibody-reactive cells was analyzed by using a fluorescence-activated cell sorter (FACS-IV, Becton Dickinson, Mountain View, CA). Background fluorescence was determined using the same cell population incubated with FITC-conjugated $F(ab')_2$ goat anti-mouse immunoglobulin antibody. The results were expressed as the net percent positive cells in the total number of the cells counted, calculated as follows;

$$\text{Percent positive cells} = \frac{\text{positive cell counts of samples} - \text{background}}{\text{total cell counts of sample} - \text{background}} \times 100.$$

Immunoprecipitation Procedures Monoclonal antibodies were tested for precipitating activity by using radiolabeled antigen from detergent-solubilized extracts of the immunizing cells and other cell lines. Labeling with ^{125}I (1.7–1.9 Ci/μmol, Amersham Japan Ltd., Tokyo) by using iodogen (Pierce Chemical Co., IL) or with [^{35}S]methionine (1.04 mCi/μmol, Amersham Japan Ltd.) or with [^{3}H]glucosamine (22 Ci/mmol, Amersham Japan Ltd.) and extraction with Nonidet P-40 (NP-40) buffer were carried out as described.[27] Radioimmunoprecipitation procedures with these labeled samples were performed as described.[27] The radiolabeled antigen was analyzed by 9% SDS-PAGE. Autoradiography was done on Fuji X-ray films with an intensifying screen (Kasei Optonix Ltd., Tokyo) at −80° during a week for ^{125}I-labeled samples and during a month for [^{35}S]methionine or [^{3}H]glucosamine-labeled samples. For determining protein molecular weight, low-molecular-weight calibration kits (Pharmacia Japan Ltd., Tokyo),

pancreatic DNase 1 (Worthington Biochemical Co., NJ), myosin and vinculin were used, and visualized by Coomassie brilliant blue staining.

Enzymatic Treatment of the Cells This was described previously in detail.[27] Briefly, MIA-PaCa2 or Panc-1 cells were washed with Hanks' solution and adjusted to 1×10^6/ml. Trypsin (0.25%) (1:250, Difco Lab., Detroit, MI) or 0.25% protease (type IV, Sigma Chemical Co.) or neuraminidase (1.0 μ/ml, Behlingen Inst.) digestion was performed for 30 min at 30°. Periodic acid (0.25%) oxidation of the cells was carried out for 1 or 5 min at room temperature. After these treatments, bindings of the monoclonal antibodies were examined by FACS as described above.

Immunohistochemical Methods All tumor and adult normal tissues consisted of surgically resected specimens obtained at operation in the University Hospital of Tsukuba. Normal embryonal tissues were obtained at autopsy from the Department of Pathology, University of Tsukuba. Formalin-fixed, paraffin-embedded human tumors or normal tissues were assayed for antigen expression with the use of a slight modification of the avidin-biotin-peroxidase complex (ABC) immunohistochemical method of Hsu *et al.*[30] The methods used have been described in detail.[27] Briefly, tissue sections mounted on gelatinized slides were deparaffinized in xylene and rehydrated in graded alcohols, and the endogenous peroxidase activity was blocked for 15 min (2 times) at room temperature with methanol containing 0.3% H_2O_2. After rinsing in PBS, the sections were incubated in normal horse serum. The pretreatment serum was removed and primary monoclonal antibody was added (SK-930- or SK-117-containing ascites; dilution of 1/100) for 60 min at room temperature. PBS instead of primary antibody was used on duplicate slides (from serial sections) as a control in all experiments. Also, ascites containing MoAb raised against HBe antigen described previously[27] were used as an unrelated ascites as a negative control. The primary monoclonal antibodies were removed, and the sections were rinsed in PBS, followed by a 30-min incubation in biotinylated horse anti-mouse immunoglobulin (Vector Laboratories, Inc., Burlingame, CA). The slides were again rinsed in PBS and then incubated for 30 min with ABC (Vector Laboratories, Inc.)-peroxidase at room temperature. After another PBS rinse, 0.05% 3,3'-diaminobenzidine tetrachloride with 0.03% H_2O_2 was added. The sections were rinsed in H_2O_2 briefly

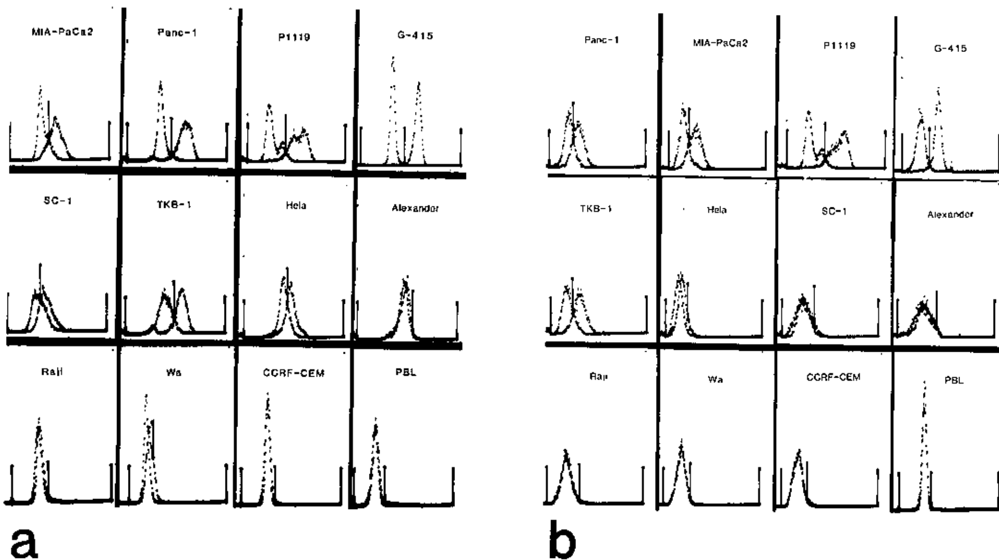

Fig. 1. Reactivity of MoAbs SK-930 (a) and SK-117 (b) with various cultured cell line and PBL by FACS analyses. (a) SK-930 was reactive with MIA-PaCa2, Panc-1, Pl119, G-415, SC-1, TKB-1, HeLa but not with Alexander, Raji, Wa, CCRF-CEM or PBL. (b) SK-117 was reactive with Panc-1, MIA-PaCa2, Pl119, G-415, TKB-1, HeLa but not with SC-1, Alexander, Raji, Wa, CCRF-CEM or PBL. Background (second step alone) control values are superimposed as dotted lines in each panel. Each histogram displays the number of cells (ordinate) versus the intensity of fluorescence (abscissa) expressed on a logarithmic scale.

counterstained with hematoxylin, dehydrated in graded alcohols, cleared in xylene, mounted under a cover slip, and examined with a light microscope. Semiquantitative scores (+, ++, +++) for cells positive for monoclonal antibodies were judged by observing tumor areas of at least 10 low-power fields.[13, 17, 27]

Results

Production of Monoclonal Antibodies Splenic lymphocytes were fused with the non-secreting mouse myeloma cell line Sp2/0 to generate primary hybridoma cultures (72% of the wells). Tissue culture supernatants from all cultures were tested in ELISA for the presence of immunoglobulins reactive with MIA-PaCa2 or Panc-1 used as the immunogen. After cloning 3 times by limiting dilution, tissue-culture supernatants from the cloned cell lines were then tested in ELISA for immunoreactivity with MIA-PaCa2 or Panc-1 and CCRF-CEM. Finally, two MoAbs (subsequently designated as SK-930 against MIA-PaCa2, and as SK-117 against Panc-1) showed reactivity only with pancreatic cancer cell lines but not with CCRF-CEM cells.

Characterization of Monoclonal Antibodies MoAbs SK-930 and SK-117 were of IgG2a and IgG1 murine isotypes, respectively. These antibodies did not have complement-dependent cytotoxicity against the target-MIA-PaCa2 and Panc-1.

Reactivities of Monoclonal Antibodies with Various Cancer Cell Lines and PBL To further characterize the reactivities of MoAbs with different types of carcinoma cells, various cancer cell lines, PBL and red blood cells were tested in FACS analyses. As shown in Fig. 1a, SK-930 reacted with MIA-PaCa2, Panc-1, Pl119, G-415, SC-1, TKB-1 and HeLa cells (net percentages of positive cells were 72.3%, 86.9%, 85.4%, 97.7%, 6.4%, 88.2% and 14.4%, respectively). However, none of the other cells tested (Alexander, Raji, Wa, CCRF-CEM and PBL) showed any significant binding. On the other hand, SK-117 reacted with Panc-1, MIA-PaCa2, Pl119, G-415, TKB-1 and HeLa cells, and failed to react to SC-1, Alexander, Burkitt's lymphoma cell line, Raji,[31] B lymphoblastoid cell line, Wa,[32] CCRF-CEM and PBL (Fig. 1b). Although the results are not shown, SK-930 and SK-117 did not bind to a panel of RBC.

Immunochemical Characterization of the Cell Surface Antigens Defined by Monoclonal Antibodies SK-930 and SK-117 When ^{125}I-labeled MIA-PaCa2 cell lysates were used as the antigen source, SK-930 antibody immunoprecipitated a broad single band with an apparent molecular weight of 134,000 in 9% SDS-PAGE under reducing conditions (Fig. 2). When G-415 cells were used, an identical 134 kilodalton molecule was precipitated. However, there was no corresponding band in Raji, Alexander, or CCRF-CEM cells. When

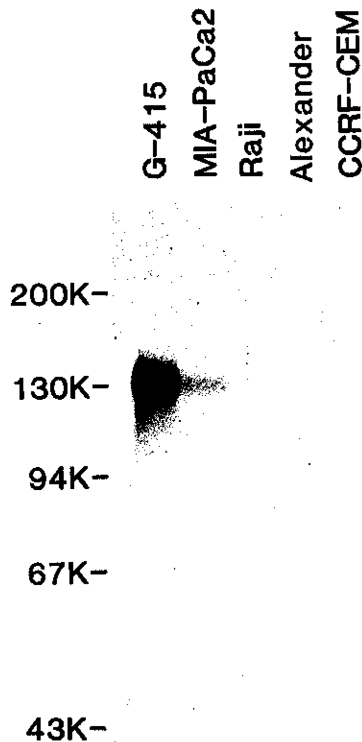

Fig. 2. Autoradiograph of SK-930-defined antigen. After ^{125}I-labeling of tumor cells, NP-40 cell lysates were subjected to immunoprecipitation followed by SDS-PAGE (reducing conditions). SK-930-defined antigen with a molecular weight of 134,000 was immunoprecipitated from G-415 and MIA-PaCa2 cell lysates, but not from Raji, Alexander or CCRF-CEM cell lysates.

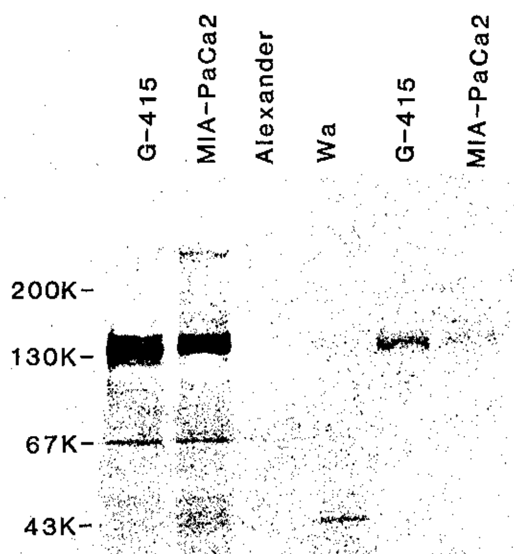

Fig. 3. SDS-PAGE analyses (reducing conditions) of immunoprecipitates obtained by reacting the indicated MoAbs immunosorbents with detergent extracts of tumor cells labeled with either [^{35}S]-methionine or [^{3}H]glucosamine. SK-930 precipitated a 134 kilodalton (134K) molecule in the 1st and 2nd lanes (from left to right) from [^{35}S]-methionine-labeled extracts of G-415, MIA-PaCa2 cells. No band of 134K could be detected in the extracts of Alexander and Wa cells. A single band of 134K appeared in extracts of [^{3}H]glucosamine-labeled G-415 and MIA-PaCa2 in the 5th and 6th lanes (from left to right).

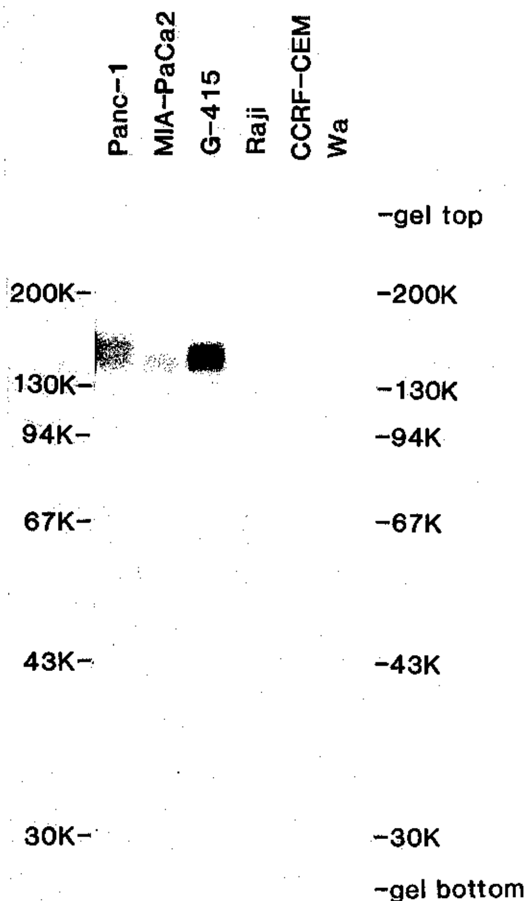

Fig. 4. Autoradiograph of SK-117-defined antigen. After ^{125}I-labeling of tumor cells, NP-40-solubilized cell lysates were used for immunoprecipitation and subjected to SDS-PAGE. Two ^{125}I-labeled bands migrating to positions corresponding to molecular weights of 152,000 and 137,000 appeared in the extracts from Panc-1 cells, and one band at the position of molecular weight 137,000 from MIA-PaCa2 and G-415 cells, but no band was detected from Raji, CCRF-CEM or Wa cells.

the immunoprecipitates were analyzed under nonreducing conditions, a similar band was identified in G-415 and MIA-PaCa2, but not in Raji, Alexander or CCRF-CEM (data not shown). To further characterize the 134 kilodalton molecule, tumor cells were metabolically labeled with [^{35}S]methionine or [^{3}H]-glucosamine. Cell lysates were collected as labeled antigen sources for immunoprecipitation. Molecules precipitated specifically with SK-930 antibody were run on 9% acrylamide gels in the presence of SDS and 2-mercaptho-ethanol. As shown in Fig. 3, the 134 kilodalton molecule was precipitated as a major band from only G-415 and MIA-PaCa2 cell lysates (1st and 2nd lanes from left to right). However, SK-930-defined antigen could not be detected in the extracts from Alexander and Wa cells. The data of radioimmunoprecipitation with ^{125}I-labeled tumor cell lysates are consistent with the results of FACS analyses, as described in Fig. 1a. In addition, SDS-PAGE analyses of immunoprecipitates from [^{3}H]glucosamine-labeled G-415 and MIA-PaCa2 cell lysates revealed the presence of a definite 134 kilodalton band. The results suggested that the 134 kilodalton molecule consisted of glycoprotein.

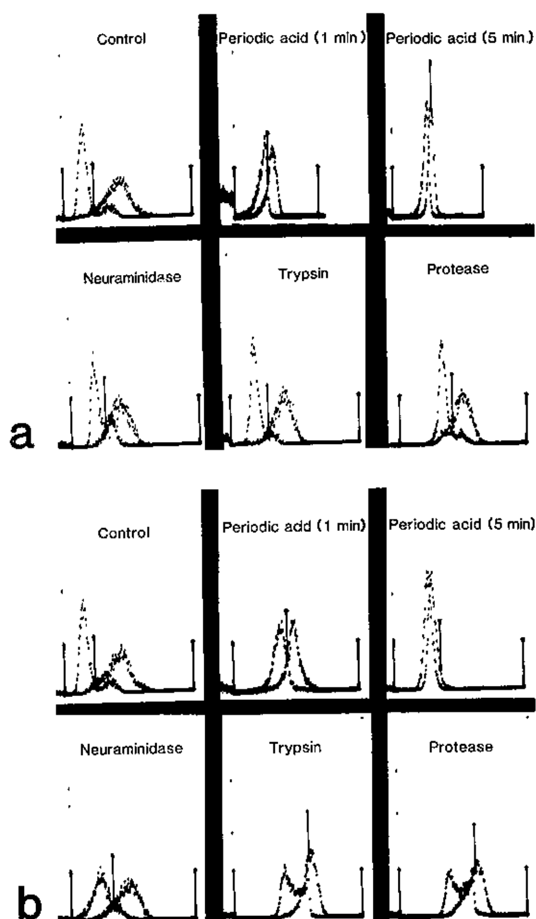

Fig. 5. Reactivity of MoAbs SK-930 (a) and SK-117 (b) to tumor cells treated with periodic acid and several kinds of enzymes analyzed by FACS. (a) MIA-PaCa2 cells were used. Values of net percent positive cells were 86.1% for the control, 39.0% for periodic acid (1 min), 5.5% for periodic acid (5 min), 83.8% for neuraminidase, 85.4% for trypsin and 84.6% for protease. (b) Panc-1 cells were used. Values of net percent positive cells were 81.7% for the control, 41.5% for periodic acid (1 min), 1.2% for periodic acid (5 min), 83.1% for neuraminidase, 79.5% for trypsin and 78.2% for protease. Background (second step alone) control is superimposed as a dotted line in each panel. Each histogram displays the number of cells (ordinate) versus the intensity of fluorescence (abscissa) expressed on a logarithmic scale.

To analyze the molecules recognized by SK-117, Panc-1, MIA-PaCa2, G-415, Raji, CCRF-CEM and Wa cells were labeled with ^{125}I and were lyzed in the lysis buffer with 0.5% NP-40. Figure 4 shows the SDS-PAGE pattern of immunoprecipitated antigen detected by SK-117. The antigens were resolved into two bands at 152K and 137K in the case of Panc-1 cells and one band at 137K in the cases of MIA-PaCa2 and G-415 cells under reducing conditions. The antigen bands were not found in Raji, CCRF-CEM and Wa cells used as negative controls. This is in agreement with the FACS analyses described above (Fig. 1b).

To further characterize SK-930- and SK-117-defined antigens on the tumor cells, the cultured tumor cells were treated with periodic acid or several kinds of enzymes. Figure 5 (a and b) shows that periodic acid oxidation of the cells markedly decreased the reactivity with either SK-930 or SK-117, in a time-dependent manner. However, neuraminidase, trypsin and protease treatments did not affect the reactivities of SK-930 or SK-117 with the cells. Thus, SK-930 or SK-117 reactivities to the cells were periodic acid-sensitive but neuraminidase-, trypsin- and protease-insensitive. These facts suggest that both the monoclonal antibodies may recognize carbohydrate moieties.

Immunohistological Studies The reactivity of MoAbs SK-930 and SK-117 with neoplastic and normal tissues from pancreas and other various site is summarized in Tables I and II. Both SK-930 and SK-117 antibodies preferentially reacted with pancreatic carcinoma tissues, showing a range of reactivity from weak to strong. Figure 6 (a and c) shows the staining of pancreatic carcinoma for SK-930 and SK-117 antigens. High frequencies of the antigens defined by these antibodies could be found on certain other carcinomas, such as gallbladder, bile duct, stomach, colon and esophagus carcinomas. Small numbers of tumor tissues from lung, breast or salivary gland were positive for SK-930 and/or SK-117. We found no strict correlation between the percent of cellular reactivity with MoAbs SK-930 or SK-117 and the degree of histologic differentiation. No reactivity was noted with either SK-930 or SK-117 in samples from the following types of carcinoma: liver, kidney, thymus, brain, thyroid, uterus, ovary, testis, adrenal, prostate, tongue and maxillary.

Table I. Immunoperoxidase Reactivity of Monoclonal Antibodies with Human Cancer Tissues

Tissues	Total No.	SK-930					SK-117				
		0	+[a]	++	+++	% Positive	0	+	++	+++	% Positive
Pancreas	30	6	5	12	7	80	7	4	13	6	77
Gallbladder	10	4	2	3	1	60	3	3	2	2	70
Bile duct	25	9	5	10	1	64	6	4	12	3	76
Stomach	22	7	5	8	2	68	5	4	8	5	77
Colon	10	3	1	4	2	70	3	2	4	1	70
Esophagus	8	2	2	2	2	75	1	1	5	1	88
Liver	8	8	0	0	0	0	8	0	0	0	0
Lung	5	4	1	0	0	20	3	1	1	0	40
Breast	4	4	0	0	0	0	3	1	0	0	25
Kidney	3	3	0	0	0	0	3	0	0	0	0
Thymus	3	3	0	0	0	0	3	0	0	0	0
Brain	3	3	0	0	0	0	3	0	0	0	0
Thyroid	3	3	0	0	0	0	3	0	0	0	0
Uterus	3	3	0	0	0	0	3	0	0	0	0
Ovary	2	2	0	0	0	0	2	0	0	0	0
Salivary gland	1	0	1	0	0	100	1	0	0	0	0
Testis	1	1	0	0	0	0	1	0	0	0	0
Adrenal	1	1	0	0	0	0	1	0	0	0	0
Prostate	1	1	0	0	0	0	1	0	0	0	0
Tongue	1	1	0	0	0	0	1	0	0	0	0
Maxillary	1	1	0	0	0	0	1	0	0	0	0

a) Reactivity: +, weak; ++, intermediate; +++, strong; and 0, none.

Table II. Immunoperoxidase Reactivity of Monoclonal Antibodies with Human Normal Tissues

Tissues	Total No.	SK-930					SK-117				
		0	+[a]	++	+++	% Positive	0	+	++	+++	% Positive
Pancreas	20										
acinar		20	0	0	0	0	18	2	0	0	10
islets		17	3	0	0	15	16	3	1	0	20
duct		18	2	0	0	10	17	3	0	0	15
Bile duct	5	4	1	0	0	20	4	1	0	0	20
Stomach	15	11	3	1	0	27	10	3	2	0	33
Esophagus	5	3	2	0	0	40	1	3	1	0	80
Duodenum	10	9	1	0	0	10	8	2	0	0	20
Colon	7	5	2	0	0	29	5	1	1	0	29
Kidney	10	8	2	0	0	20	10	3	0	0	30
Lung	5	0	0	0	0	0	4	1	0	0	20

a) Reactivity: +, weak; ++, intermediate; +++, strong; and 0, none. The following normal tissues were negative with both SK-930 and SK-117: liver (7); gallbladder (3), heart (3), spleen (3), brain (3), skeletal muscle (3), thymus (3), lymph node (7), thyroid (3), uterus (3), ovary (3), adrenal (2), testis (2).

The reactivity of the antibodies with normal adult tissues is summarized in Table II. Though normal pancreatic tissue (Fig. 6b) generally lacked any immunoreactivity to SK-930, a small number of normal pancreatic tissues were weakly positive to SK-930 or SK-117. The antibodies were further screened against a variety of higtologically normal

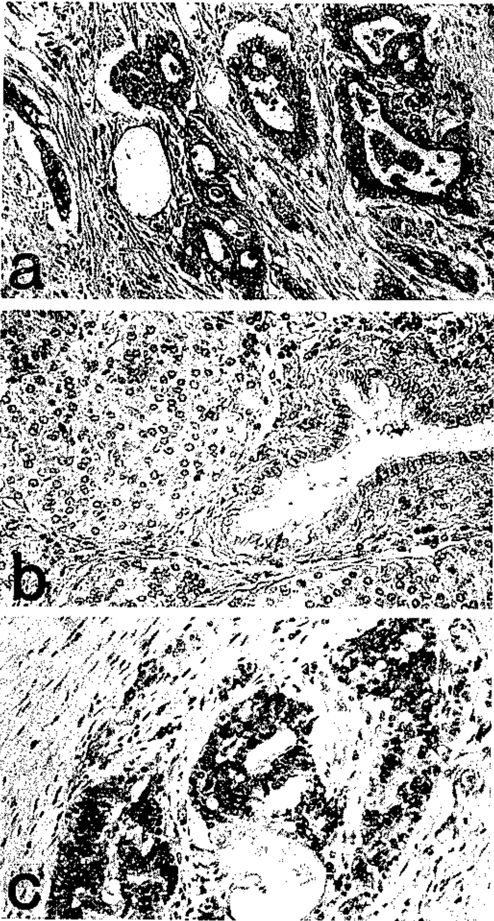

Fig. 6. Paraffin sections of pancreatic adenocarcinoma (a) and normal pancreatic tissue (b) immunostained with SK-930, and of pancreatic adenocarcinoma (c) immunostained with SK-117. (a) Positive staining of tumor glands of a pancreatic adenocarcinoma invading the nonstaining pancreatic parenchyma, ×50. No staining occurred in the absence of antibody or in the presence of MoAb against HBeAg (not shown). (b) Normal pancreatic duct, and adjacent acinar and islets cells demonstrating no detectable staining, ×50. (c) Positive staining of pancreatic adenocarcinoma, ×50.

tissues. Twenty to 30% cellular reactivity for bile duct, stomach, duodenum, colon, kidney and lung was seen. However, the reactivity of the tissues was weak. Esophagus tissues revealed higher reactivity to the two MoAbs. The two MoAbs-defined epitopes were weakly expressed in the absorptive or goblet cells of intestinalized stomach mucosa and of normal colonic mucosa. Some cytoplasmic reactivity was noted in kidney tubules. Little or no cellular reactivity to kidney glomerulus was observed. None of the other normal human tissues (liver, gallbladder, heart, spleen, brain, skeletal muscle, thymus, lymph node, thyroid, uterus, ovary, adrenal and testis) showed any significant binding. Fetal tissues were negative with both SK-930 and SK-117: pancreas (4), liver (6), spleen (4), G.I. tract (4), lung (6), heart (6), kidney (5), brain (2), thymus (5).

Discussion

The experimental data presented here reveal some characteristics of the two newly established MoAbs SK-930 and SK-117 against human pancreatic carcinoma cell lines. For the molecular characterization of the antigenic determinants detected by the MoAbs, we chose extracts from *in vitro* cultured tumor cell lines. Using radioimmunoprecipitation combined with SDS-PAGE and autoradiography, we showed that the epitopes defined by SK-930 and SK-117 on a human pancreatic carcinoma cell line were located on 134 and 152/137 kilodalton molecules, respectively. Thus, the SK-930- and SK-117-defined antigens are not identical. Though SK-930-defined antigen showed a single band, SK-117-defined antigens were resolved into two bands of 152 and 137 kilodaltons in Panc-1 cells (Fig. 4). These two bands may represent identical molecules with different glycosylation, resulting in the difference in molecular weight as observed in IL 2 receptor,[33] or two different precursor polypeptides.[34] To test these two possibilities, further experimentation is required to identify the antigen structures on tumor cells.

Molecular characteristics of the antigens defined by MoAbs against pancreatic carcinoma cells have been studied by several investigators.[15, 16, 19, 35] Lan *et al.*[35] reported that a mucin-like molecule showing two distinct broad antigen bands was detected by DU-PAN-2 antibody. However, the molecular weight of the antigen has not been clarified. It was also found that the C54-0 antibody obtained by Schmiegel *et al.*[15] defined a tumor-associated 122 kilodalton molecule. Chin and Miller[16] reported that MoAb against pancreatic carcinoma cell lines, RWP-1 or RPW-2,

recognized a 190 kilodalton molecule. Bosslet *et al.*[19] showed that MoAb against pancreatic carcinoma cells recognized a carbohydrate epitope located on a 200 kilodalton glycoprotein. Thus, the 134 and 152/137 kilodalton molecules defined by the MoAbs presented here are distinctly different from those mentioned above, and seem to be novel antigens on pancreatic carcinoma cells. Some other antigens associated with pancreatic carcinoma have been described. These include POA[2,3] and carcinoembryonic antigen (CEA).[36] The POA was shown be a glycoprotein with a molecular weight between 800 and 900 kilodaltons by Gelder *et al.*,[2] while Hobbs *et al.*[3] reported a molecular weight of 40 kilodaltons. Imai *et al.*[34] reported that antigens of 200 and 180 kilodaltons from iodinated CEA were detected by MoAbs. Thus, the antigens defined by SK-930 and SK-117 appear to be different molecules from CEA and POA.

The SK-930 and SK-117 epitopes are destroyed by oxidation with periodic acid, but are not affected by digestion with neuraminidase, trypsin or pronase. Our previous work[27] showed that the MoAb HI-531 against gallbladder carcinoma cells recognized a 43 kilodalton protein on the cell surface. It was observed that HI-531 antigen could withstand periodic acid oxidation, but not treatment with pronase or trypsin. The same experimental procedures and conditions were employed. Therefore, the data presented here strongly suggest that the epitopes defined by SK-930 and SK-117 are carbohydrate moieties of glycoprotein(s) expressed on the cell surface of the tumors.

FACS analyses showed that the 134 and 152/137 kilodalton molecules defined by our MoAbs were expressed on the cell surface of pancretic carcinoma cell lines, as well as certain other carcinoma cell lines. Thus, the antigens may be cell type-restricted antigens, reflecting malignancy. In addition, neither SK-930 nor SK-117 reacted with PBL or various types of RBC. This indicates that the SK-930 and SK-117 antigens are neither encoded by the major histocompatibility complex nor belong to defined major or minor blood group substances.

The tissue distribution of the antigens defined by the two MoAbs, SK-930 and SK-117, was studied by immunoperoxidase staining of surgically resected formalin-fixed sections (Tables I and II). Although sections of pancreatic carcinoma tissues obtained from 30 different patients that we tested were all of ductal origin, positive staining in 24 and 23 of the 30 tumor specimens from the patients suggests that SK-930- and SK-117-defined antigens may serve as useful markers for pancreatic adenocarcinoma. In addition to pancreatic carcinoma, other reactive digestive system carcinoma types included neoplasms of gallbladder, bile duct, stomach, colon and esophagus. However, hepatocellular carcinoma tissues was not reactive. Furthermore, faint reactivities were occasionally observed with defined areas of normal adults tissues. Judging from the intensity of immunoperoxidase staining, SK-930 and SK-117 antigens seem to exist in higher concentrations in tumor tissues than in their normal counterparts. Thus, the preferential expression of the antigens in tumor tissues reflects a malignant cellular phenotype of epithelial cells. Nevertheless, the antigens detected in this study were not specific for pancreatic carcinoma. This is in accordance with data presented for various tumor systems.[37–39] Taken together, the data suggest that 134 and 152/137 kilodalton molecules defined by SK-930 and SK-117 belong to TAA expressed on pancreatic carcinoma cells. Their distributions on neoplastic and normal human tissues, however, are different from those of MoAbs reported by other investigators.[13–19] Overall, on the basis of their tissue distribution and molecular weights, the pancreatic carcinoma-associated antigens described here do not show any obvious resemblance to any others reported in the literature.[13, 15–19]

In conclusion, two murine MoAbs have been prepared against human pancreatic ductal cell adenocarcinoma cell lines, and they define antigens with interesting distributions among different tumors and normal tissues. The epitopes recognized by these MoAbs were carried by 134 and 152/137 kilodalton glycoprotein(s). The MoAbs described herein may prove useful in studies of the biology of human pancreatic carcinoma-associated antigens and improve the diagnostic and therapeutic strategies for this disease.

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