Differential Localization of Human Pancreas Cancer-Associated Antigen and Carcinoembryonic Antigen in Homologous Pancreatic Tumoral Xenograft

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ABSTRACT — Tissue localization of a human pancreas cancer-associated antigen (PCAA) and carcinoembryonic antigen (CEA) was studied in a homologous pancreatic tumoral xenograft, a human pancreatic cancer line established from ascites (AsPC-1), with the use of the indirect immunofluorescence technique with specific anti-PCAA and anti-CEA antisera. Histologically, AsPC-1 xenograft was composed of mucinous adenocarcinoma of variable size and degree of glandular differentiation. PCAA was selectively associated with the columnar cytoplasm, was involved primarily in the epithelial proliferation, and originated at the basal aspect of the glands. In contrast, CEA appeared diffuse and was associated with the columnar cytoplasm, was involved primarily in the epithelial proliferation, and originated at the basal aspect of the glands. In contrast, CEA appeared diffuse and was associated with the columnar cytoplasm. In well-differentiated adenocarcinoma and degenerating adenocarcinoma, cells positive for PCAA were released into the lumen of the glands, whereas in poorly differentiated adenocarcinoma these cells were often scattered singly and/or in focal clusters. Furthermore, growth comparison of the AsPC-1 adenocarcinoma revealed that, except in the juvenile gland that was morphologically consistent with grade 1 (G1) carcinoma in situ, the number of PCAA-positively stained cells decreased from G1 to grade 4 epithelial differentiation. In well-differentiated adenocarcinoma and degenerating adenocarcinoma, cells positive for PCAA were released into the lumen of the glands, whereas in poorly differentiated adenocarcinoma these cells were often scattered singly and/or in focal clusters and disappeared in very poorly differentiated adenocarcinoma. This study showed that the PCAA was different from CEA in its immunologic reactivity and distribution in its homologous tumoral tissues, in which PCAA was predominantly associated with the proliferative phase of the malignant epithelium. These results also indicated the potential applicability of this antigenic expression in the etiology, disease grading, and staging of human pancreatic cancer.

MATERIALS AND METHODS

Tumor tissues.—Human pancreatic adenocarcinoma (AsPC-1) was established as xenografts in athymic nude mice in this laboratory; the original tumor cells were derived from the ascites of a patient with cancer of the pancreas. AsPC-1 xenografts 6–9 weeks old from passage generations 1–3 were used. After excision, tumors 1–2 cm in diameter were immediately immersed in 10% Formalin–PBS at room temperature. After 1–2 days' fixation, tissues were conventionally processed and embedded in paraffin. Serial thin sections 5–6 μm thick were prepared and deparaffinized in a conventional manner, dipped in PBS (pH 7.2), and preincubated with normal goat serum (1:5 dilution; GIBCO, Grand Island, N.Y.) to prevent nonspecific staining prior to incubation with the antiserum. Correspondingly, serial slides were prepared for conventional mucicarmine and/or hematoxylin and eosin stainings. Tumor gradings (G1–G4) were performed according to a standardized criterion (10).

ABBREVIATIONS USED: CEA = carcinoembryonic antigen; FITC = fluorescein isothiocyanate; PBS = phosphate-buffered saline; PCAA = human pancreas cancer-associated antigen.

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3 Research procedures were in accord with the ethical standards of the Human Experimentation Committee, Roswell Park Memorial Institute. Guidelines for the care and use of laboratory animals were followed as set forth by Roswell Park Memorial Institute.
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Antisera.—Rabbit anti-PCAA and anti-CEA antisera were prepared separately as previously described (7, 11). Purified PCAA (500 μg each) was used for immunization of male New Zealand White rabbits (Amcare Corp., Manhasset, N.Y.). After a booster immunization (250 μg/rabbit) on day 21, antiserum was collected 10 days later, heat inactivated (56°C, 30 min), absorbed extensively with CEA and glutaraldehyde cross-linked human plasma components (12), and stored at 4°C until staining. The anti-PCAA antiserum (TS-10) was used at an optimal dilution of 1:50.

The anti-CEA antiserum was prepared as follows. Rabbits were immunized with purified CEA preparations (100–200 μg each). After 4 weeks of initial injection, a booster immunization was given, and the antibody response was monitored by radioimmunoassay, double diffusion, and immunoelectrophoresis at 1–2 weeks later. Following absorption with PCAA, the anti-CEA antiserum (R-11) was used at an optimal dilution of 1:500.

FITC-conjugated goat antirabbit IgG antiserum was obtained from Miles Laboratories, Inc., Elkhart, Ind. The FITC-labeled antiserum was used at a dilution of 1:20.

Staining procedure.—Prior to incubation, the specificity of anti-PCAA and anti-CEA antisera was tested by double immunodiffusion with purified PCAA and CEA. After preincubation with normal goat serum and repeated washing (three times) with PBS, tissue sections were separately incubated with specific anti-PCAA and anti-CEA antisera. After washings with PBS, the specimens were treated with FITC-labeled goat antirabbit IgG antiserum. The slides were again repeatedly washed with PBS, mounted with 50% glycerine–PBS and a cover slip, and stored at 4°C or followed immediately by fluorescence microscopy. Throughout the experiment, PBS and normal rabbit serum (1:5 dilution) and antiserum preabsorbed with CEA or PCAA were used as controls; each serum was incubated for 40 minutes at room temperature.

Photomicroscopy.—The specimens were examined on an Olympus BHA microscope mounted with a BH-RFL fluorescence vertical illuminator. FITC excitor filter and DM 500 dichroic mirror were used in combination with either #0515 or #0530 barrier filter. Aligned light and fluorescence photomicrographs were recorded on Kodak Plus-X panchromatic film with a PM-10-35-M system camera (Upstate Technological Equipment Co., Syracuse, N.Y.).

RESULTS

Prior to antigenic localization, the anti-PCAA and anti-CEA antisera were extensively cross-absorbed with opponent antisera, although the specificity of these antisera has been previously established (7, 11). Figures 1A–1C show that both the treated and untreated anti-PCAA and anti-CEA antisera were of defined specificity to their corresponding purified antisera. No immunologic cross-reactivity was evident.

Tumor Morphology

As documented in figures 2A, 3A, 4A, and 5A, AsPC-1 tumor was composed of mucin-producing adenocarcinoma with a broad spectrum of glandular differentiation ranging from well differentiated to very poorly differentiated adenocarcinoma (G1–G4). Generally, juvenile glands were located at the tumor periphery (fig. 2A), and poorly differentiated to very poorly differentiated adenocarcinomas (G3–G4) were found around the tumor core (fig. 5A). Among these structures, mucin-distended glands of variable size and degree of differentiation were observed (fig. 3A).

In well-differentiated glands, papillary formation and glandular formation of the epithelial component were evident (figs. 3A, 4A). Also, deteriorating glands could be observed near the tumor core.

Distribution of Tumor-Associated Antigens

Figures 2B, 2C, 3B, 3C, 4B, 4C, 5B, and 5C show that the immunofluorescence was more localized for PCAA than for CEA and was found in cytoplasm. PCAA was selectively associated with the columnar epithelial component of the glands, whereas CEA appeared diffuse and was predominantly found in the cuboidal cells with higher intensity at the luminal border and mucin. The number of PCAA-positive cells decreased with increased glandular differentiation and degeneration as a result of cellular maturation and release of epithelium into the lumen (fig. 3B). Furthermore, cells positive for PCAA were found scattered singly and/or in small clusters among poorly differentiated adenocarcinomas (G3) and almost disappeared in very poorly differentiated adenocarcinoma (G4) (fig. 5B). Therefore, although variation in the intensity of PCAA staining was observed in morphologically similar glands, PCAA staining was most evident in well-differentiated adenocarcinoma (G1) (fig. 4B) and was moderate in mixed moderately well-differentiated adenocarcinoma (G2) and G3 or G4 alone (fig. 5B), whereas juvenile gland, morphologically as carcinoma in situ G1 (fig. 2B) and very poorly differentiated adenocarcinoma G4 (fig. 5B), expressed less and almost no PCAA. In contrast, CEA was present in all tumor grades with strongest intensity in well-differentiated adenocarcinoma (G1) (fig. 4C).

DISCUSSION

Both aligned immunofluorescence and light microscopic data presented in this study have elucidated the cytoplasmic distribution of a PCAA in its homologous pancreatic tumoral xenograft (AsPC-1). It is imperative to study the histologic localization of this newly identified PCAA to define its origin and establish its pathologic relationship to the pancreatic neoplasm. PCAA has been recently reported from this laboratory; this antigen is initially isolated from the malignant ascites of patients with cancer of the pancreas; the reactive antigen in serum of this cancer is most elevated when compared to those of lung, colon, and
breast cancers; and its levels in serum increases with the degree of pancreatic tumor involvement in patients (7). With the use of specific rabbit anti-PCAA antiserum, this antigen has been detected in the homologous AsPC-1 tumoral xenografts.

As shown in the light microscopic data (figs. 2A, 3A, 4A, 5A), AsPC-1 xenograft is composed of mucinous adenocarcinoma of variable size and degree of glandular differentiation. An attempt has been made to define the heterogeneity of glandular development in this tumor. Juvenile glands are predominantly observed at the tumor periphery (fig. 2A); poorly to very poorly differentiated adenocarcinomas are often found around the necrotizing tumor core (fig. 5A). Among these structures, mucinous adenocarcinoma ranging from well to moderately well differentiated in glands of variable size were seen (figs. 3A, 4A). Furthermore, in comparison of the degree of cellular differentiation among these tumor grades, in poorly and very poorly differentiated adenocarcinomas, tissues are mostly composed of more mature cuboidal epithelia which often arrange in cords (fig. 5A). These observations suggest that the differentiation of G3-G4 in AsPC-1 xenograft may be due to the collapse of more differentiated adenocarcinoma as the intratumoral pressure increases. In humans, it is difficult to obtain fresh pancreatic tumor tissues for the laboratory studies and is even harder to find various degree of tumor differentiation in a single specimen. Thus the use of AsPC-1 xenograft as reported here may represent a unique approach to the study of tumor differentiation in human pancreatic cancer.

CEA is the most widely studied tumor-associated antigen and has been extensively investigated in pancreatic cancer (13). By the indirect immunofluorescence method, a comparative study has been performed to determine the site and source of PCAA and CEA in AsPC-1 xenograft. Specific anti-PCAA and anti-CEA antisera were used as reagents to localize these antigens. Morphologically, the tissue immunofluorescence is more localized for PCAA than for CEA and is found in cytoplasm; in contrast, localization of CEA appears diffuse and is predominantly found in the cuboidal cells, with higher intensity at the luminal border and in mucin (figs. 2B, 2C, 3B, 3C, 4B, 4C, 5B, 5C). These CEA findings are consistent with the published data on CEA localization in pancreatic cancers (14), indicating that CEA, not PCAA, is related to the secretory glycoprotein. On the contrary, PCAA is predominantly localized in cytoplasm, is selectively associated with the columnar epithelium, and arises from the basal aspects of the glands.

Furthermore, a growth comparison of AsPC-1 adenocarcinoma has been made. The number of PCAA-positive cells decreased from well to less glandular in differentiation, and degeneration was primarily due to cellular maturation and release of epithelia into the lumen (fig. 3B). In poorly differentiated adenocarcinomas, cells related to PCAA are found scattered singly and/or in small clusters and almost absent in very poorly differentiated adenocarcinoma (fig. 5B). In contrast, CEA is present in all tumor grades with higher intensity in well-differentiated adenocarcinoma (fig. 4C). These observations indicate that PCAA may be a differentiation antigen that is selectively associated with the proliferative phase of malignant epithelium in AsPC-1 xenografts (fig. 4B).

Isolation, characterization, and initial clinical evaluation of PCAA have been described (7). Other antigens characterized and reported to be associated with human pancreas cancer are pancreatic oncofetal antigen reported by Hobbs et al. (1, 15), Arndt et al. (16), and Gelder et al. (4); pancreatic oncofetal protein reported by Mihas (3); pancreas tumor-associated antigen reported by Schultz and Yunis (5); and pancreatic ascitic fluid glycoprotein of Chu et al. (2). Biochemical and physicochemical properties of PCAA differentiate it from other pancreas cancer-associated antigens (7), although immunologic cross-reactivity between our anti-PCAA and antipancreatic oncofetal antigen of Gelder et al. (4) has been observed (Gelder FB, Shimano T, Chu TM: Unpublished observations).

In conclusion, this study shows that the PCAA is different from CEA in its immunologic reactivity and distribution in its homologous tumor tissues, where it is closely associated with the cytoplasm of proliferative columnar epithelium. Furthermore, selective population of cells producing PCAA in AsPC-1 xenograft as a model may provide a means of examining the neogenesis and release of this antigen as an aid to detection and diagnosis, as well as a monitor of disease dissemination in pancreatic tumor. The findings also suggest the usefulness of this antigenic expression in the etiology, disease grading, and staging of human pancreatic cancer.

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FIGURE 1.—Double immunodiffusion [20 μl reagent(s)/well; PCAA = 50 μg/ml, CEA = 100 μg/ml] showing specific activity of: A) anti-PCAA (well 1) and anti-CEA (well 3) antisera to their respective antigens; B) PCAA-absorbed anti-CEA antiserum (1:1 vol/vol; well 3) to purified CEA (well 2), control = anti-PCAA antiserum (well 1); and C) CEA-absorbed anti-PCAA antiserum (1:1 vol/vol; well 1) to purified PCAA (well 3), control = anti-CEA antiserum (well 2).
FIGURE 2.—Aligned light and FITC-fluorescence photomicrographs of a juvenile adenocarcinoma found at the AsPC-1 tumor periphery. A) Premature glandular structure with traceable luminal mucin. Mucicarmine. B) Selective localization of PCAA in cytoplasms. C) Predominant deposition of CEA at the luminal borders. A-C) Bar=50 μm. × 240

FIGURE 3.—Aligned light and FITC-fluorescence photomicrographs of a moderately well-differentiated adenocarcinoma of the AsPC-1 tumor. A) G2-G3 adenocarcinoma. Hematoxylin and eosin. B) Sloughing of PCAA positive cells into the lumen, where cellular debris containing PCAA can still be noted. C) Profound CEA localization at the apical aspects of the epithelium and in mucin (bottom left corner). A-C) Bar=50 μm. × 240
FIGURE 4.—Aligned light and FITC-fluorescence photomicrographs of papillary formation due to epithelial regeneration in the well-differentiated AsPC-1 adenocarcinoma. A) Active proliferation of the epithelial component. Hematoxylin and eosin. B) Predominant localization of PCAA in the columnar cytoplasm. C) Diffused deposition of CEA in columnar as well as cuboidal cytoplasm and in lumen (top right corner). A–C) Bar=50 μm. × 240

FIGURE 5.—Aligned light and FITC-fluorescence photomicrographs of very poorly differentiated adenocarcinomas found around the AsPC-1 tumor core. A) G4 adenocarcinoma. Mucicarmine. B) Near-disappearance of PCAA-positive cells. C) Interstitial localization of CEA along the cellular cords. A–C) Bar=50 μm. × 240