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Monoclonal antibodies against CEA-related components discriminate between pancreatic duct type carcinomas and nonneoplastic duct lesions as well as nonduct type neoplasias*, **

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Summary. The expression of CEA and related antigens in formalin-fixed paraffin-embedded tissues of normal pancreas and different pancreatic neoplasms was studied immunocytochemically using three monoclonal antibodies (MAbs) recognizing different epitopes on CEA and related antigens. Additionally, a number of extrapancreatic malignancies were tested. The epitope recognized by MAb 250 (present on CEA and NCA 95) was expressed in all but one pancreatic ductal adenocarcinoma and ampullary carcinoma (42/43). The MAb 431 defined epitope (present only on CEA) was less frequently found (27/43). MAb 374, defining an epitope on CEA, NCA 95 and NCA 55 proved to be nearly as sensitive as MAb 250, but also reacted with normal duct epithelium. In contrast, MAb 250 and MAb 431 discriminated clearly between reactive duct lesions and malignant duct changes. Moreover, these MAbs differentiated between pancreatic duct carcinomas and nonduct type carcinomas as well as benign pancreatic tumours. In duct type carcinomas, the strongest staining was observed in well differentiated tumours. No discrimination was possible between pancreatic carcinomas and other adenocarcinomas of the gastrointestinal tract nor between most of the lung carcinomas and some other malignancies, specified below. MAb 250 and MAb 431 failed to react with hepatocellular carcinomas, renal cell carcinomas, carcinoids, sarcomas and melanomas. The findings suggest that paraffin-embedded tissues of pancreatic duct type carcinomas, in contrast to nonduct type tumours and normal ducts, are distinguished by the presence of a CEA and NCA 95 related epitope.

Key words: Carcinoembryonic antigen – Monoclonal antibodies – Pancreatic tumours – Immunoreactivity

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Elevated serum levels of carcinoembryonic (CEA) antigen (Gold and Freedman 1965) in pancreatic cancer patients have been reported by several workers (Dilawari et al. 1975; Kalser et al. 1978; Zamcheck and Martin 1981; Okabe et al. 1983). At the cellular level CEA has been demonstrated in neoplastic pancreatic lesions by immunocytochemical methods using conventional polyclonal antisera (Denk et al. 1972; Horie et al. 1984; Tsutsumi et al. 1984). However, the same antisera were frequently found to stain the epithelium of normal pancreatic ducts as well. This variability in specificity is due to the fact that CEA belongs to a family of closely related glycoproteins which have several antigenic determinants in common and therefore show considerable cross-reactivity with conventional antisera (v. Kleist et al. 1972; Wagener and Breuer 1982; Rogers 1983). Among the CEA-related antigens presently known the nonspecific cross-reacting antigens (NCA), NCA-1 (55) and NCA-2 (95) (Buchegger et al. 1984), are most widely studied (Rogers 1983). Since the sub-sets of CEA and related substances may be best characterized by monoclonal antibodies and since those monoclonal antibodies may be more cancer-specific than conventional antisera, it is of interest to study the tissue distribution of monoclonal antibody (MAb) defined CEA and CEA-related antigens. Using such MAbs, varying specificities for normal gastrointestinal mucosa and colonic as well as gastric carcinomas have been reported (Primus et al. 1983; Wagener et al. 1983; Yachi et al. 1984). Moreover, Tsutsumi and associates (1984) recently observed that a MAb directed against a CEA sub-set was able to differentiate between neoplastic and non-neoplastic lesions of the pancreas.

In the present report we studied the immunocytochemical reactivity of three MAbs recognizing different epitopes on CEA and CEA-related antigens on paraffin-embedded tissues of a series of pancreatic tumours of various histogenetic origin and related the findings to the histological tumour type and differentiation. In addition, a number of extrapancreatic neoplasms were tested.

Materials and methods

Tissues. Paraffin-embedded tissue blocks of surgical specimens from 40 pancreatic tumour patients with ductal adenocarcinomas were studied. Most specimens were fixed in formalin, but few were fixed in Bouin's solution. An additional 13 pancreatic tumours (3 pleomorphic giant cell carcinomas, 4 acinar cell carcinomas, 3 serous cystic adenomas, 2 mucinous cystic tumours, and 1 pancreatoblastoma) were taken from autopsy cases or obtained by surgery. Normal or inflamed pancreatic tissues were collected from patients who underwent surgery for advanced gastric cancer or alcoholic chronic pancreatitis, respectively. Fetal tissues were obtained from 6 fetuses after spontaneous or therapeutic abortion between 5 and 26 weeks of gestation. All these tissues were fixed in formalin. For comparison with the results in pancreatic tissues surgical specimens or autopsy material were obtained from a variety of extrapancreatic malignancies (10 colonic carcinomas, 8 gastric carcinomas, 3 gallbladder carcinomas, 10 lung carcinomas, 10 breast carcinomas, 5 prostate carcinomas, 2 renal cell carcinomas, 5 melanomas, 2 carcinoids, 2 sarcomas).

The pancreatic tumours were classified into ductal adenocarcinoma, mucinous cystic tumour (mucinous cystadenocarcinoma), pleomorphic carcinoma of giant cell type, acinar cell carcinoma, pancreatoblastoma, solid-cystic (papillary-cystic) tumour, and pancreatic endocrine carcinoma (Morohoshi et al. 1983; Cubilla and Fitzgerald 1984; Klöppel 1984). In addition,

the ductal adenocarcinomas were graded according to our own system distinguishing three grades of malignancy (Klöppel et al. 1985).

Antisera. Monoclonal antibodies BW 374/14 (MAb 374)[1] and BW 431/31 (MAb 431)[1] were generated by immunizing Balb/c mice with cells mechanically removed from the tissue of a human adenocarcinoma of the lung as was previously described (Bosslet et al. 1985). The third monoclonal antibody, BW 250/183 (MAb 250) was raised against a purified CEA preparation isolated from a colonic tumour liver metastasis. The binding characteristics of the three MAbs are described elsewhere (Bosslet et al. 1985). Briefly MAb 374 detects an epitope being present on the CEA molecule and the nonspecific cross-reacting antigens (NCA) with molecular weights of 55 kD and 95 kD, respectively (NCA 55 and NCA 95; Buchegger et al. 1984). MAb 250 defines an epitope present on the CEA molecule and the NCA 95. MAb 431 only binds to an epitope on the CEA molecule. All antibody supernatants had the same approximate immunoglobulin content.

Immunostaining procedures. Four-micron thick sections were deparaffinized and treated with 1% methanolic hydrogen peroxide to eliminate endogenous peroxidase activity. After rinsing in PBS for 2×10 min the sections were covered for 15 min with normal horse serum (1:50) to reduce background staining due to nonspecific binding sites. The slides were then incubated for 24 h at $+4°$ C with 100 µl of the supernatant of the MAbs diluted 1:5 (MAb 374 and 250) or 1:3 (MAb 431) with BSA. After washing with PBS a second 30 min incubation was performed with a 1:200 dilution of a biotinylated horse anti-mouse serum followed by another wash in PBS. The sections were then incubated for 30 min with the ABC reagent (100 µl). After rinsing in PBS, DAB was added for 5 min. Positive sections showed an intense brown colour which was enhanced by adding OsO_4 (0.5%) for 2 min. During the whole procedure the slides were wiped after each washing and prior to the next immunoreagent.

Controls. To ascertain the specificity of the staining observed with the three MAbs unspecific myeloma cell supernatant was used as negative control. Sections of a well differentiated colonic carcinoma served as positive staining control. To demonstrate that the epitopes detected by our MAbs are also recognized by a conventional polyclonal CEA antiserum (DAKO), sections were incubated with the CEA antiserum prior to the staining with the MAb.

Scoring of immunoreactivity. To describe the various staining intensities observed, sections showing a very strong reaction product in the majority of cells were scored as 3+, a strong or moderate labelling in some cells was 2+, and a weak but definite staining of individual cells was graded as 1+. A staining indistinguishable from the background or a doubtful very weak staining were regarded as negative.

Results

Nonneoplastic pancreatic tissues

The four normal pancreatic tissues tested (two adults, two newborns) failed to react with MAb 250 and MAb 431 (Table 1). MAb 374, in contrast, stained the duct epithelium of the normal adult pancreas, while the newborn pancreas was negative. The tissues of cases with chronic pancreatitis were negative for MAb 250 (Fig. 1) and MAb 431, except in one instance showing minimal apical reactivity of single hypertrophic duct cells. MAb 374 reacted constantly with hyperplastic duct epithelium. None of the MAbs labelled acinar cells or endocrine cells. All fetal tissue specimens were unstained.

[1] MAbs 374/14, 250/183 and 431/31 are commercially available as BMA 130a, b, c from Behring Diagnostics, D-3550 Marburg, P.O. Box 1140, Federal Republic of Germany

Table 1. Immunocytochemical reactivity of formalin-fixed paraffin-embedded nonneoplastic and neoplastic pancreatic tissues with monoclonal antibodies recognizing different CEA related epitopes

Tissues	Monoclonal antibodies		
	250	431	374
	(no. positive/no. tested)		
Pancreatic ducts			
Adult	0/2	0/2	2/2
Newborn	0/2	0/2	0/2
Chronic pancreatitis	1/5	1/5	5/5
Fetal tissue	0/6	0/6	0/6
Malignant pancreatic tumours			
Duct type carcinoma	39/40	26/40	36/40
Ampullary carcinoma	3/3	1/3	3/3
Giant cell type carcinoma	1/3	1/3	1/3
Mucinous cystic tumour	1/2	1/2	1/2
Acinar cell carcinoma	0/4	0/4	0/4
Endocrine carcinoma	0/2	0/2	0/2
Pancreatoblastoma	0/1	0/1	0/1
Benign pancreatic tumours			
Solid-cystic tumour	0/3	0/3	0/3
Serous cystadenoma	0/3	0/3	1/3

Malignant and benign pancreatic tumours

Ductal adenocarcinomas. Among the 40 pancreatic ductal adenocarcinomas tested, 39 reacted definitely with MAb 250 and 36 with MAb 374, whereas only 26 tumours were positive with MAb 431 (Table 1).

Both MAb 250 and MAb 431 showed a characteristic staining pattern, positivity being restricted to the glandular structures of the adenocarcinoma and to the intralumenal content which consisted of mucus or cellular debris. In the neoplastic duct-type cells the strongest reaction was usually observed on the lumenal surface (Fig. 2). In general, MAb 431 showed a weaker staining than MAb 250 and MAb 374 (Fig. 2). While MAb 250 and MAb 431 failed to react with normal or hyperplastic pancreatic ducts in areas adjacent to tumours, they clearly stained intraductal branches (carcinoma in situ lesions) of ductal adenocarcinomas (Fig. 3).

The antigen detected by MAb 374 exhibited a diffuse cytoplasmic distribution. Moreover, this MAb did not discriminate between neoplastic glandular structures and normal or hyperplastic ducts. When the tumour sections were preincubated with polyclonal CEA antiserum in excess in order to cover all binding sites, the subsequent staining with MAb 374 was not completely abolished, indicating that the epitopes defined by this MAb were not totally blocked by polyclonal CEA antiserum. In contrast, reactivity

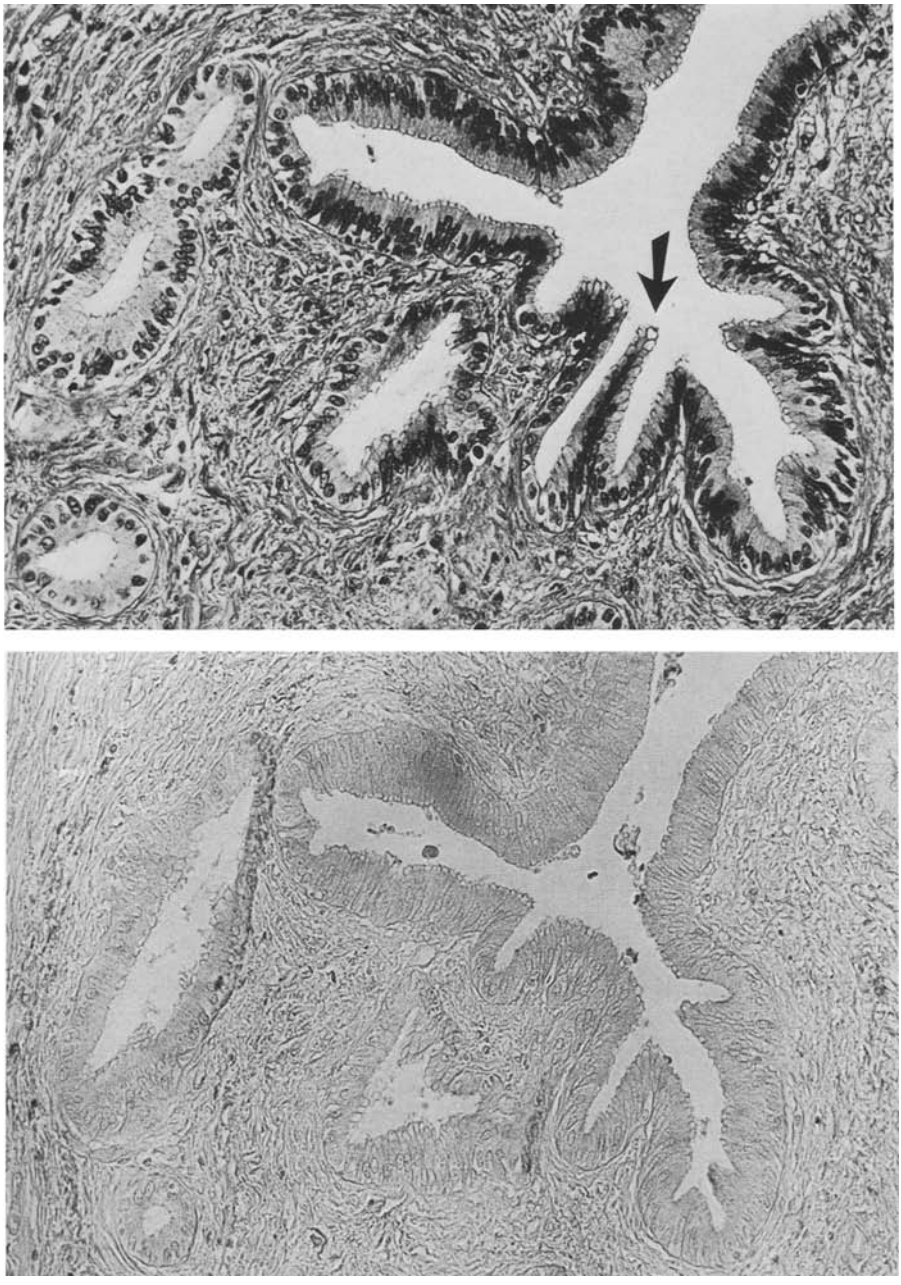
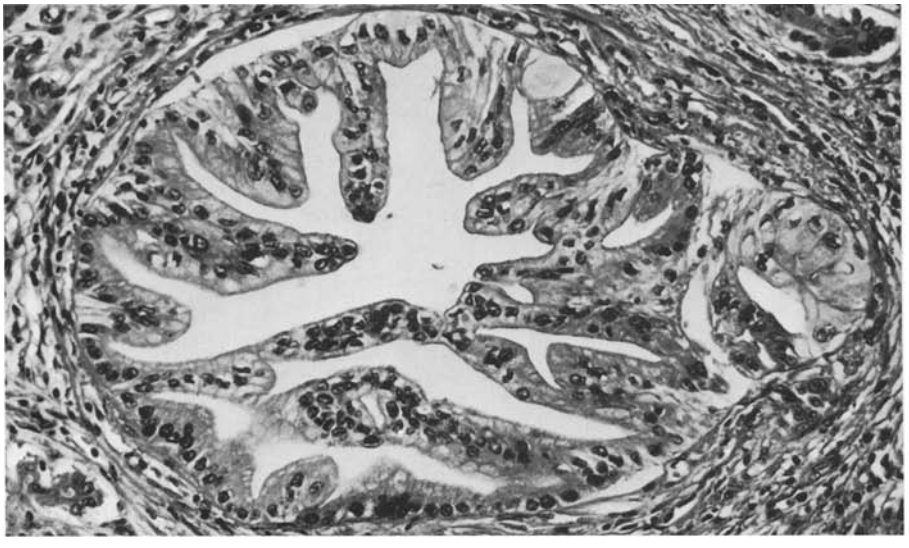


Fig. 1. Non-neoplastic pancreatic ducts embedded in fibrous tissue. **a** Duct epithelium shows no atypia but papillary hyperplasia (*arrow*). HE ×250. **b** Adjacent section demonstrates no immunostaining with MAb 250



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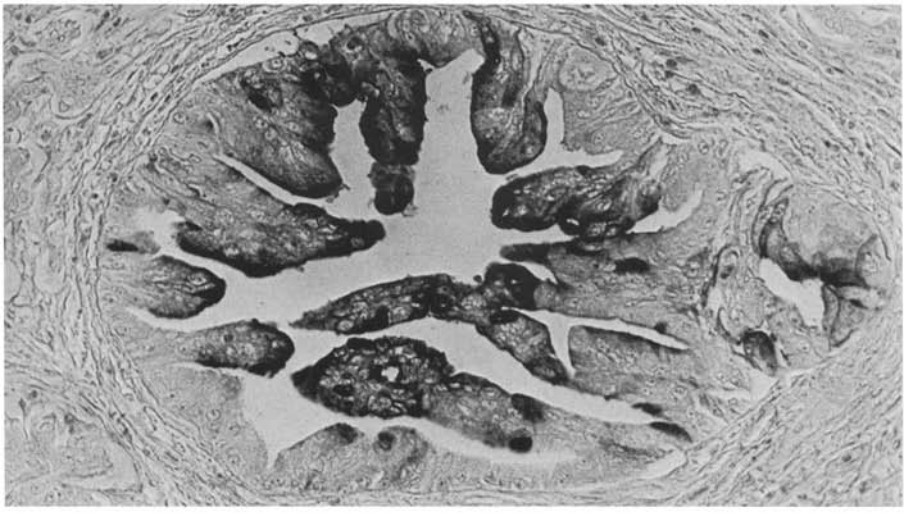

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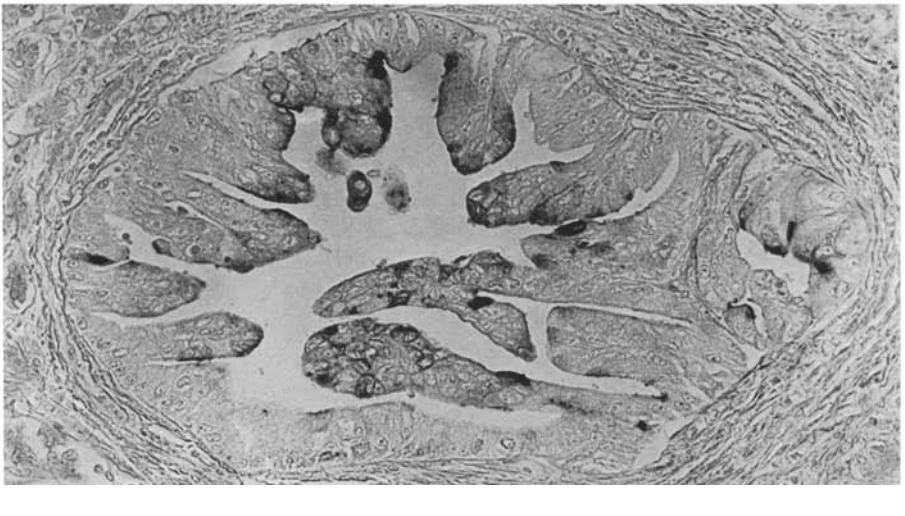

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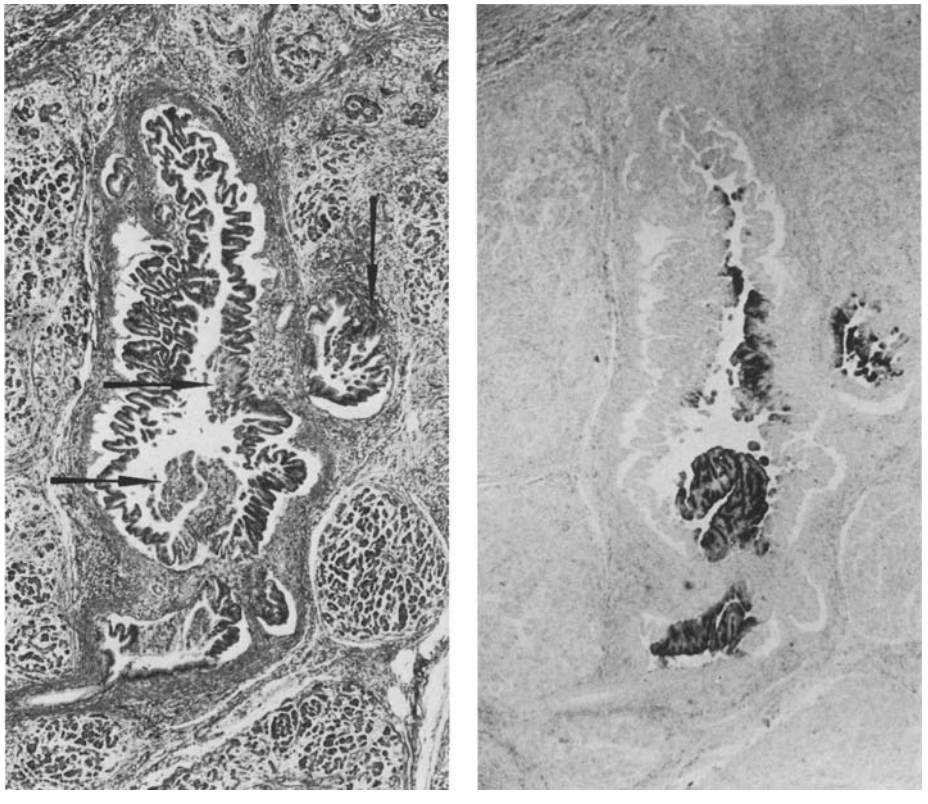

Fig. 3. Pancreatic ductal adenocarcinoma: **a** Intraductal tumour branch distant from the primary tumour with foci of papillary carcinoma in situ (*arrows*). HE ×40. **b** The carcinoma in situ foci stain strongly positive with MAb 250

of MAb 250 and MAb 431 was completely inhibited by preincubation with the polyclonal CEA antiserum.

The immunoreactivity of MAb 250 and MAb 431 appeared to be less intense in adenocarcinomas showing a lower degree of differentiation (G 3) than in well-differentiated tumours (G 1–2; Table 2). The cells of G 3 tumours were only sporadically stained (Fig. 4).

Ampullary carcinomas. The histology of the ampullary carcinomas resembled that of well-differentiated ductal adenocarcinomas of the pancreas. All three cases examined reacted with both MAb 250 and MAb 374, whereas the MAb 431 defined antigen was only present in one tumour (Table 1).

Fig. 2. Pancreatic ductal adenocarcinoma, well differentiated: **a** Neoplastic duct with irregular papillae of atypical epithelium. HE ×250. **b** and **c** Consecutive sections with positive immunostaining for MAb 250 **b** and MAb 431 **c**

Table 2. Intensity of immunocytochemical reactivity of duct type pancreatic adenocarcinomas to MAb 250 in relation to tumour grade

Tumour grade	Staining intensity		
	3+	2+	1+
I	4/19	6/19	9/19
II	1/15	7/15	7/15
III	1/ 6	1/ 6	4/ 6

Giant cell type carcinomas. The three pleomorphic carcinomas of the giant cell type tested showed different results. One case failed to react with any of the MAbs. In the second one positivity was restricted to a small area still showing glandular differentiation. In the third case a very faint staining was observed in individual cells.

Mucinous cystic tumours. (Mucinous cystadenocarcinoma): Among the two mucinous cystic tumours, one showed an excessive mucus production and reacted strongly with each MAb. The staining appeared to be restricted to the mucus and to those epithelial cells of the cysts, which showed a high degree of atypia. The other tumour producing smaller amounts of mucus and disclosing well-differentiated epithelial cells reacted faintly with MAb 374 only.

Extrapancreatic malignancies

Within the test panel of the extrapancreatic malignancies all (or almost all) carcinomas from colon, stomach, gallbladder and lung were positive for MAb 250 and MAb 374 (Table 3; Fig. 5). The MAb 431 showed a somewhat reduced reactivity. Within the group of the breast carcinomas only about half of the cases were stained. All but one of the prostate carcinomas were negative (Fig. 6). Negative results were also obtained with MAb 250 and MAb 431 in hepatocellular carcinomas, soft tissue sarcomas and melanomas. MAb 374 stained 3/5 hepatocellular carcinomas and 1/5 melanomas.

Discussion

CEA is mainly characterized by its immunological properties. Monoclonal antibodies have therefore been generated by a number of workers to further define this molecule and to differentiate it from a group of closely related glycoproteins (Buchegger et al. 1982; Rogers 1983). In this report we used three recently characterized MAbs directed against different epitopes on CEA and related glycoproteins (Bosslet et al. 1985). MAb 431 was found to react with CEA only and did not bind to cells known to be rich in NCA such as granulocytes and macrophages. The epitope recognized by the second antibody, MAb 250, was located on CEA and NCA 95 (NCA-2;

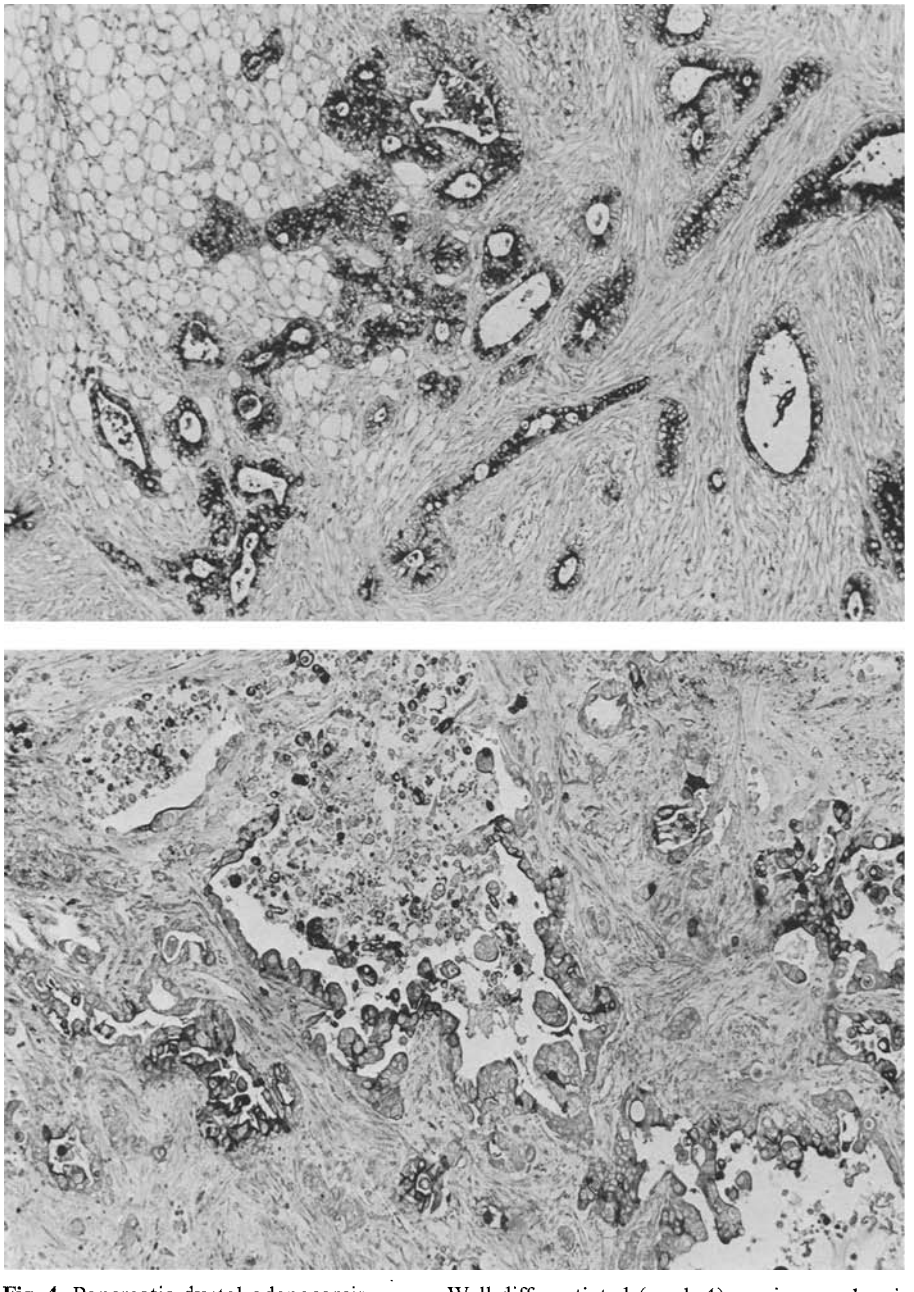

Fig. 4. Pancreatic ductal adenocarcinoma. **a** Well-differentiated (grade 1) carcinoma showing strong immunostaining with MAb 250. **b** Poorly differentiated (grade 3) carcinoma displaying less intense staining with the same MAb, ×250

Table 3. Immunocytochemical reactivity of formalin-fixed paraffin-embedded tissues of extrapancreatic malignancies to monoclonal antibodies recognizing different CEA-related epitopes

Tissues	Monoclonal antibodies		
	250	431	374
	(no. positive/no. tested)		
Extrapancreatic malignancies			
Colon	10/10	10/10	10/10
Stomach	8/8	7/8	8/8
Gallbladder	3/3	1/3	3/3
Hepatocellular	0/5	0/5	3/5
Lung: Adeno	3/4	2/4	4/4
Squamous	3/3	3/3	3/3
Small Cell	2/3	2/3	2/3
Breast	4/10	4/10	7/10
Prostate	1/5	1/5	1/5
Kidney	0/2	0/2	0/2
Carcinoid	0/2	0/2	0/2
Sarcoma	0/2	0/2	0/2
Melanoma	0/5	0/5	1/5

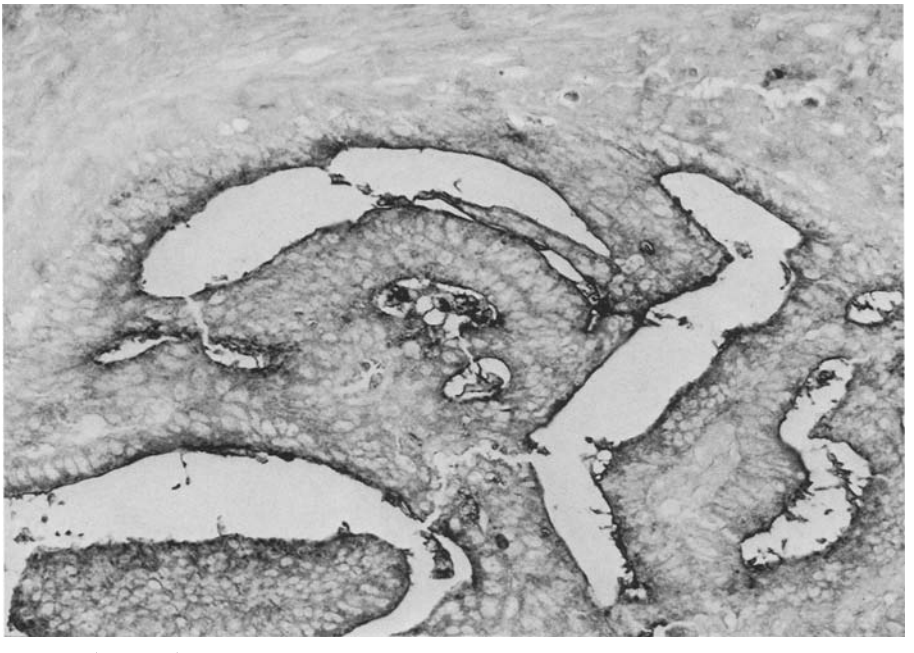

Fig. 5. Adenocarcinoma, colon: Apical immunostaining of neoplastic epithelium with MAb 431. ×250

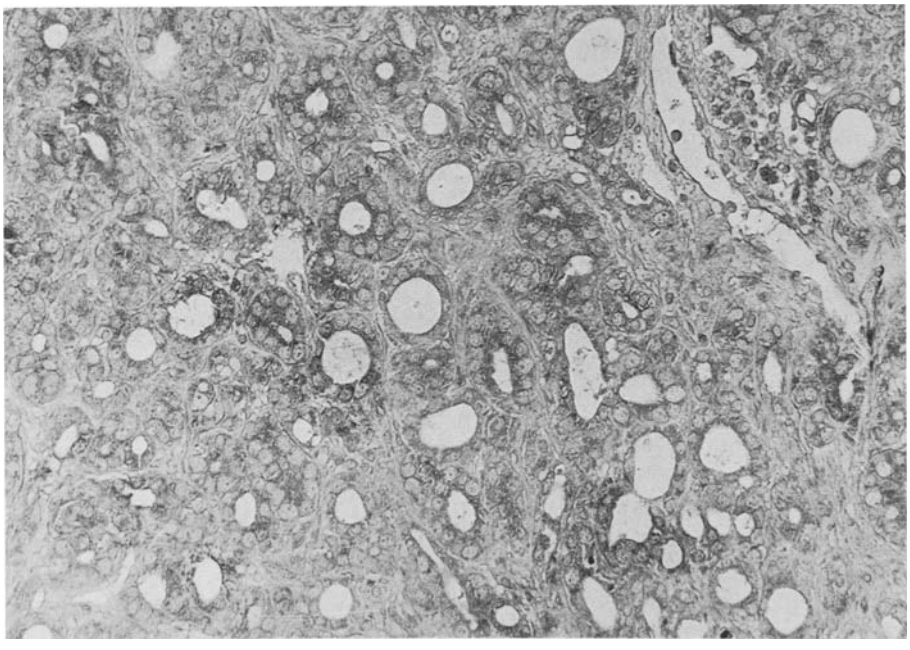

Fig. 6. Adenocarcinoma, prostate: Negative immunostaining with MAb 250. × 250

Burtin et al. 1973; Buchegger et al. 1984). MAb 374, finally, bound to an epitope present on CEA, NCA 95 and NCA 55 (NCA-1; v. Kleist et al. 1972; Buchegger et al. 1984).

Applying these MAbs on formalin-fixed paraffin-embedded pancreatic tissues, we observed a high sensitivity (% positive tumours) for ductal adenocarcinomas with MAb 250 (98%) and MAb 374 (91%), whereas MAb 431 was less reactive (65%). However, while MAb 250 and, to a lesser extent, also MAb 431 discriminated well between nonneoplastic duct epithelium and neoplastic duct lesions, heterogeneous specificity, with positive immunoreactivity of both normal and neoplastic epithelium, was observed with MAb 374. This MAb therefore proved to be of no help in the detection of intraductal branches of ductal adenocarcinomas.

In contrast, MAb 431 and, especially, MAb 250 clearly labelled all carcinoma in situ lesions in the pancreases examined and were found to be excellent markers for residual tumour at the resection line of surgical specimens of pancreatic carcinomas. The only exception from this rule was seen in a case of chronic pancreatitis where an individual duct showed a faint epithelial staining with both MAb 250 and MAb 431. Excellent discrimination of noncancerous pancreatic tissue from adenocarcinomas in paraffin sections was also reported by Tsutsumi and associates (1984) using a MAb probably recognizing a non-NCA 55 related epitope on the CEA molecule.

In addition, these authors showed that the staining intensity of CEA antisera is generally weaker in paraffin sections than in comparable frozen sections, – a finding which we can confirm (data not shown).

Another point of interest of our study was that MAb 250 and MAb 431 only stained those pancreatic tumours which were clearly malignant, belonged to the duct type category and showed a certain degree of differentiation. Among the two mucinous cystic tumours tested, which despite their benign appearance are known for their inherent latent or overt malignant potential (Compagno and Oertel 1978b) and are therefore also called mucinous cystadenocarcinomas, only the tumour displaying most marked atypia of the columnar epithelium was positive while the other tumour, with minimal atypia remained unlabelled. The fact that mucinous cystic tumours may produce CEA is also underlined by the report of Ferrer and associates (1978) who found a high CEA content in the cyst fluid of such a tumour. The pleomorphic carcinomas of the giant (large) cell type, on the other hand, were negative in the anaplastic regions, but showed a slight positivity within a small area exhibiting residual ductal differentiation. Tumours which are known to behave benign, such as the serous cystadenoma (Compagno and Oertel 1978a) and the solid and cystic (papillary-cystic) tumour (Klöppel et al. 1981; Klöppel 1984), remained unstained. This was also true for malignant tumours of endocrine or acinar origin, including a pancreatoblastoma (Cubilla and Fitzgerald 1984). Regarding the acinar carcinomas, our findings are at variance with others (Horie et al. 1984) who reported a positive CEA-staining and a high plasma CEA level in an acinar carcinoma. However, since the classification of this tumour was solely based on its histological appearance, while all our acinar carcinomas were characterized by unequivocal immunocytochemical demonstration of pancreatic enzymes such as lipase and trypsinogen (Klöppel et al. 1984), we assume that the acinar carcinoma of the Japanese authors represented a low grade ductal adenocarcinoma.

Within the group of ductal adenocarcinomas, the immunoreactivity of MAb 250 tended to decline with tumour dedifferentiation. Tumours which were classified according to our grading system (Klöppel et al. 1985) as grade 1 and grade 2 were found to show a more intense staining than poorly differentiated grade 3 carcinomas. This finding somewhat contradicts the observation by Kalser et al. (1978) that well-differentiated pancreatic cancers were associated with lower serum CEA levels than poorly differentiated tumours. The most plausible explanation for the discrepancy of these data with our results is that staining intensity of tumour CEA and serum CEA levels can hardly be correlated. It is known that serum CEA levels in pancreatic carcinoma patients depend not only on the amount of CEA produced by the tumour but also on such variables as the inability of the liver to clear the circulation of CEA (Zamcheck and Martin 1981) or to the preferable excretion of CEA into the pancreatic juice by some tumours (DiMagno et al. 1977). It is therefore conceivable that a poorly differentiated carcinoma rapidly causing obstructive jaundice is associated with a high serum CEA level though the CEA immunoreactivity of this tumour may be low.

As was to be expected, all our MAbs against CEA-related epitopes failed to discriminate between pancreatic ductal adenocarcinomas and other adenocarcinomas of the gastrointestinal tract as well as most of the lung carcinomas and some other malignancies. With the exception of MAb 374 they were, however, completely negative for all hepatocellular carcinomas, renal cell carcinomas, intestinal carcinoids, melanomas and sarcomas so far tested.

In summary, our study showed that almost all paraffin-embedded ductal adenocarcinomas of the pancreas react with monospecific antisera directed against epitopes present on the CEA and NCA 95 molecule. Since the same epitope, as a rule, could not be detected in non-neoplastic duct epithelium, these MAbs can be used for the exact discrimination of hyperplastic duct changes from intraductal carcinoma in situ lesions. In addition, the same MAbs render a clear distinction of pancreatic carcinomas of duct type from nonduct type malignancies and benign pancreatic tumours possible.

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References

Bosslet K, Lüben G, Schwarz A, Hundt E, Harthus HP, Seiler FR, Muhrer C, Klöppel G, Kayser K, Sedlacek HH (1985) Immunohistochemical localization and molecular characteristics of three monoclonal antibody-defined epitopes detectable on carcinoembryonic antigen (CEA). Inter J Cancer 36:75–84

Buchegger F, Schreyer M, Carrel S, Mach JP (1984) Monoclonal antibodies identify a CEA cross reacting antigen of 95 kD (NCA 95) distinct in antigenicity and tissue distribution from the previously described NCA of 55 kD. Inter J Cancer 33:643–649

Burtin P, Chavanel G, Hirsch-Marie H (1973) Characterization of a second normal antigen that cross-reacts with CEA. J Immunol 111:1926–1928

Compagno J, Oertel JE (1978a) Microcystic adenomas of the pancreas (glycogen-rich cystadenomas). A clinicopathologic study of 34 cases. Am J Clin Pathol 69:289–298

Compagno J, Oertel JE (1978b) Mucinous cystic neoplasms of the pancreas with overt and latent malignancy (cystadenocarcinoma and cystadenoma). A clinicopathologic study of 41 cases. Am J Clin Pathol 69:573–580

Cubilla AL, Fitzgerald PJ (1984) Tumors of the exocrine pancreas. In: Atlas of tumor pathology. Second series, fasc. 19. Armed Forces Institute of Pathology, Washington

Denk H, Tappeiner G, Eckerstorfer R, Holzner JH (1972) Carcinoembryonic antigen (CEA) in gastrointestinal and extragastrointestinal tumors and its relationship to tumor-cell differentiation. Int J Cancer 10:262–272

Dilawari JB, Philippakos D, Blendis LM, Wallar SL (1975) Carcinoembryonic antigen in differential diagnosis of carcinoma of pancreas from chronic pancreatitis. Br Med J 2:668

DiMagno EP, Malagelada JR, Moertel CG, Go VLW (1977) Prospective evaluation of the pancreatic secretion of immunoreactive carcinoembryonic antigen, enzyme, and bicarbonate in patients suspected of having pancreatic cancer. Gastroenterology 73:457–461

Ferrer JP, Hensley G, Kalser MH, Zeppa R (1978) Cystadenocarcinoma and carcinoembryonic antigen (CEA). Cancer 42:632–634

Gold P, Freedman SO (1965) Demonstration of tumor-specific antigens in the human colonic carcinoma by immunological tolerance and absorption techniques. J Exp Med 121:439–462

Horie Y, Gomyoda M, Kishimoto Y, Ueki J, Ikeda F, Murawaki Y, Kawamura M, Hirayama C (1984) Plasma carcinoembryonic antigen and acinar cell carcinoma of the pancreas. Cancer 53:1137–1142

Kalser MH, Barkin JS, Redlhammer D, Heal A (1978) Circulating carcinoembryonic antigen in pancreatic cancer. Cancer 42:1468–1471

von Kleist S, Chavanel G, Burtin P (1972) Identification of an antigen from normal human tissue that cross reacts with the carcinoembryonic antigen. Proc Natl Acad Sci USA 69:2492–2494

Klöppel G (1984) Pancreatic, non-endocrine tumours. In: Klöppel G, Heitz PU (eds) Pancreatic Pathology, p 79-113. Churchill Livingstone, Edingburgh London Melbourne New York

Klöppel G, Dreyer T, Lampe V, Kalthoff H, Schmiegel WH, von Bülow M, Kern HF, Heitz PU (1984) Immunzytochemische Typisierung von exokrinen Pankreastumoren and ihren Metastasen. Verh Dtsch Ges Pathol 68:104–107

Klöppel G, Lingenthal G, von Bülow M, Kern HF (1985) Histological and fine structural features of pancreatic ductal adenocarcinomas in relation to growth and prognosis: Studies in xenografted tumours and clinicohistopathologic correlation in a series of 75 cases. Histopathology 9:841–856

Morohoshi T, Held G, Klöppel G (1983) Exocrine pancreatic tumours and their histological classification. A study based on 167 autopsy and 97 surgical cases. Histopathology 7:645–661

Okabe T, Yamaguchi N, Ohsawa N (1983) Establishment and characterization of a carcinoembryonic antigen (CEA)-producing cell line from a human carcinoma of the exocrine pancreas. Cancer 51:662–668

Primus FJ, Kuhns WJ, Goldenberg DM (1983) Immunological heterogeneity of carcinoembryonic antigen: Immunohistochemical detection of carcinoembryonic antigen determinants in colonic tumors with monoclonal antibodies. Cancer Res 43:693

Rogers GT (1983) Carcinoembryonic antigens and related glycoproteins. Molecular aspects and specificity. Biochim Biophys Acta 695:227–249

Tsutsumi Y, Nagura H, Watanabe K (1984) Immunohistochemical observations of carcinoembryonic antigen (CEA) and CEA-related substances in normal and neoplastic pancreas. Am J Clin Pathol 82:535–542

Wagener C, Breuer H (1982) Biochemical and immunochemical aspects of carcinoembryonic antigen (CEA) and CEA-related antigens: current status and further perspectives. J Clin Chem Clin Biochem 20:705–712

Wagener C, Yang YHJ, Grawford FG, Shively JE (1983) Monoclonal antibodies for carcinoembryonic antigen and related antigens as a model system: a systematic approach for the determination of epitope specificities of monoclonal antibodies. J Immunol 130:2308

Yachi A, Imai K, Fujita H, Moriya Y, Tanda M, Endo T, Tsujisaki M, Kawaharada M (1984) Immunohistochemical distribution of the antigenic determinants detected by monoclonal antibodies to carcinoembryonic antigen. J Immunol 132:2998–3004

Zamcheck N, Martin EW (1981) Factors controlling the circulating CEA levels in pancreatic cancer: some clinical correlations. Cancer 47:1620–1627

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