Demonstration of carcinoembryonic antigen (CEA) expression in normal, chronically inflamed, and malignant pancreatic tissue by immunohistochemistry

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SUMMARY The expression of carcinoembryonic antigen (CEA) was evaluated by immunoperoxidase staining with two anti-CEA monoclonal antibodies in normal, chronically inflamed, and malignant pancreatic tissue. Positive staining was not observed in normal specimens. In pancreatic cancer the expression of CEA was related to the degree of differentiation of the tumour. Positive staining was also observed in chronic pancreatitis.

Carcinoma of the pancreas is often diagnosed by exclusion despite advances in computed tomography and ultrasonography. The early results of tumour localisation with radiolabelled antibodies to tumour associated antigens suggest that this technique may be complementary to the established methods of investigation.^{1,2} Indeed, it is in conditions such as carcinoma of the pancreas that immunolocalisation may realise its full potential.

The well established association of carcinoembryonic antigen (CEA) with carcinoma of the pancreas suggests that it may be a suitable target for radiolabelled antibodies. Reports have described high values both in the sera^{3,4} and the pancreatic juice of patients with pancreatic cancer.^{5,6} Few studies, however have described the tissue distribution of CEA,^{7,8} which is of great importance if it is to be regarded as a target for immunolocalisation. Studies have also described high values both in the sera and the juice of patients with chronic pancreatitis.^{5,9,10} This apparent lack of specificity of CEA for malignancy has resulted in its limited clinical role as a marker for pancreatic cancer. The development of monoclonal antibodies¹¹ has produced agents that have the potential to identify specific tumour antigens or antigenic determinants and so differentiate malignant from benign tissues.

In this study two monoclonal anti-CEA antibodies were evaluated immunohistochemically to determine whether the staining pattern for CEA could differentiate normal, chronically inflamed, and malignant

pancreatic tissue and to investigate antigenic heterogeneity in pancreatic tumours.

Material and methods

TISSUE SECTIONS

Serial tissue sections 5 μ m thick were cut from formalin fixed paraffin embedded specimens of chronic pancreatitis (n = 10) and carcinoma of the pancreas (n = 30). Most of these specimens were obtained as biopsies at operation and had been stored as paraffin embedded blocks for up to 12 months. The degree of

Table 1 Tissue reactivity of anti-CEA monoclonal antibodies 11-285-14 and 11-359-6

Tissues	11-285-14 (No positive/No tested)	11-359-6 (No positive/No tested)
Skin	0/1	
Breast	0/1	
Thyroid	0/2	0/2
Brain	0/1	
Lung	0/6	1/6
Stomach	2/5	1/2
Colon	3/4	3/4
Rectum	1/1	1/1
Appendix	4/4	4/4
Gall bladder	3/5	2/4
Liver	0/6	0/6
Spleen	0/7	1/7
Lymph node	0/6	0/6
Tonsil	6/6	6/6
Lymphocyte	0/3	0/3
Erythrocyte	0/3	0/3
Kidney	0/3	0/3
Prostate	0/6	0/6
Testis	0/3	0/3
Uterus	0/2	0/2
Gastic cancer	208/226	7/10
Colorectal cancer	49/49	25/25
Ovarian cancer	4/19	4/19

Figure Microphotographs of consecutive sections of specimen of well differentiated pancreatic carcinoma with (a) 11-285-14 and (b) 11-359-6 showing differential staining of tumour cells.

differentiation of the primary tumours was determined by pathological examination of routine haematoxylin and eosin stained sections.

Specimens of normal pancreas were also obtained either at necropsy or from parts of glands removed incidentally at other surgery ($n = 10$).

MONOCLONAL ANTIBODIES

Monoclonal anti-CEA antibodies 11-285-14 and 11-359-6 were developed by standard methods using CEA extracted from hepatic metastases of a colonic

adenocarcinoma. The antibodies have been well characterised and recognise different epitopes on the CEA molecule.¹² Both belong to the IgG1 subclass. In immunohistochemical studies neither 11-285-14 nor 11-359-6 bound to normal tissues except some gastrointestinal tract epithelium and tonsillar epithelium (Table 1). The tonsillar epithelial binding probably reflected the presence of CEA in overlying pharyngeal mucosa rather than in the actual tonsillar tissue. Cross reactivity with non-specific cross reacting antigen (purified from lung) varied, as shown

Table 2 Proportion of cells staining for CEA in sections of normal pancreas, chronic pancreatitis, and pancreatic adenocarcinoma

Total		Percentage staining				Total
		0	0-5	5-50	> 50	
Normal	11-285-14	10				10
Pancreas	11-359-6	10				10
Chronic	11-285-14	4	2	4	0	10
Pancreatitis	11-359-6	4	5	1	0	10
Pancreatic	11-285-14	7	5	9	9	30
Adenocarcinoma	11-359-6	9	7	9	4	29

by enzyme linked immunosorbent assay, in which 11-285-14 showed little or no activity, whereas 11-359-6 reacted positively. Both antibodies bound to 100% of the colorectal adenocarcinomas examined¹³ and to 94% (11-285-14) and 70% (11-359-6) of gastric cancers.¹⁴

HISTOCHEMICAL STAINING

The staining of tissue sections for CEA was carried out using a modified indirect immunoperoxidase technique.¹⁵ The antibodies at a dilution of 1/1000 (protein concentration, 7 3 µg/ml) were used as the first antibody and rabbit antimouse antibody conjugated to horseradish peroxidase (Dako) was used as the second antibody at a 1/50 dilution. A 1/1000 dilution of P3-X63-Ag8 ascitic fluid (Bethesda Research Laboratories) was used as a negative control for each batch of sections. In addition, a positive control of primary adenocarcinoma of the colon known to express CEA was included. The tissue sections were examined using light microscopy to determine the number of cells showing a positive reaction. Sections were classified as (–) in which all cells were negative, (+) in which <5% of cells were positive, (++) in which 5–50% were positive, and (+++) in which more than 50% were positive. This was a subjective assessment made independently by two observers.

Results

Table 2 shows the pattern of expression of CEA. All of the sections from specimens of normal pancreas were negative for CEA with both antibodies. In addition, normal acini present in sections from malignant

or chronically inflamed specimens were similarly negative. Positive staining was present in 23 of 30 (77%) of the specimens of pancreatic adenocarcinoma (11-285-14) and in 21 of 29 (70%) (11-359-6). CEA was detected by each antibody on the luminal surface of the neoplastic glands. In most specimens, however, the cells with positive staining varied according to the antibody used. Thus in one specimen some cells were positive with 11-285-14, and a different population was positive with 11-359-6 (Figure).

There was also considerable variation between the different specimens in the number of tumour cells expressing CEA. In none of the tumours was there positive staining of all the tumour cells. Staining of more than 50% of the cells was observed in nine of 23 (39%) of the tumours positive with 11-285-14 and in four of 20 (20%) with those positive for 11-359-6. Comparison of the percentage of positive cells with the degree of differentiation indicated that those tumours with larger numbers of positive cells were the most differentiated, particularly when stained with 11-285-14 (Table 3). This did not seem to be an absolute relation, however, as negative staining with 11-285-14 was also detected in one of the well differentiated tumours, as was positive staining in one of the undifferentiated growths. Nevertheless, with both antibodies negative staining was most common in either anaplastic or poorly differentiated tumours, reflecting the correspondingly low potential for glandular formation and CEA expression.

Positive staining with either antibody was observed in six of 10 (60%) specimens of chronic pancreatitis. The actual number of cells that stained, however, was

Table 3 Proportion of cells stained for CEA according to degree of tumour differentiation

		Percentage staining			
		0	0-5	5-50	> 50
Well differentiated (n = 8)	11-285-14	1	1	2	4
	11-359-6	0	2	3	3
Moderately well differentiated (n = 14)	11-285-14	2	3	4	4
	11-359-6	3	3	6	1
Poorly differentiated (n = 8)	11-285-14	4	1	2	1
	11-359-6	6	2	0	0

CEA expression in pancreatic tissue

small. As in the malignant specimens the luminal surfaces of the glandular cells were the principal sites of CEA binding. There was also evidence of heterogeneity of antigenic expression, with some cells positive with one antibody and different cells binding the other antibody.

Discussion

The role of CEA in the management of patients with pancreatic cancer is limited. Studies of early diagnosis have described low serum values associated with resectable lesions and high serum values associated with invasive metastatic tumours.¹⁶ Concentrations within normal limits, however, have also been described in patients with advanced disease.¹⁷ Similarly, CEA values in pancreatic juice have been reported to show a wide variation, irrespective of the presence of pancreatic cancer.⁵ Goldenberg *et al*⁸ suggested that the identification by immunohistochemistry of tumours that express CEA could be used to determine which tumours should be monitored by serial serum CEA estimation. Their study of pancreatic tumours, however, showed positive staining in only three of 11 specimens.

These studies of serum and tissue expression of CEA used polyclonal antisera to CEA. As CEA is physically, chemically, and immunologically a heterogeneous entity,¹⁸ polyclonal antibodies have failed to identify a truly tumour specific epitope. The development of monoclonal antibodies,¹¹ however, may allow more specific subpopulations of CEA to be identified, which would improve the detection of tumour associated CEA. In this study evaluation of two antibodies, which have different binding characteristics for CEA, showed heterogeneity of CEA expression in pancreatic tumours. Both bound to higher percentages of tumour cells than has been previously reported, suggesting that these antibodies have a higher affinity for their respective epitopes of CEA than conventional antisera, or recognise epitopes not identified by the other antibodies.

In a recent study of several different monoclonal anti-CEA and anti-NCA antibodies in pancreatic cancer Tsutsumi *et al*¹⁹ showed that staining patterns vary in frozen and paraffin embedded tissues. In particular, NCA, or CEA related substances with antigenic determinants common to NCA, were more readily identifiable in frozen sections of normal tissues. In this study we examined only paraffin embedded sections. Although formalin fixation and paraffin embedding may have interfered with the binding of 111-359-6 to NCA in normal pancreatic cells it is more likely that 11-359-6 recognises a different epitope to that detected by the antibodies reported by Tsutsumi *et al*.

These results have shown that the epitope recognised by 11-359-6 is less often expressed by pancreatic cancer cells than that recognised by 11-285-14. Nevertheless, neither antibody can be claimed to be specific for CEA in pancreatic cancer, as each reacted positively with cells in 60% of samples of chronic pancreatitis. This cross reactivity suggests that these antibodies would not be of value in discriminating between chronic pancreatitis and carcinoma in *in vivo* localisation studies. Such studies would also be limited because of the small number of cells in most tumours that express the antigens.

This study has shown a higher rate of CEA expression in pancreatic cancer cells than has been previously reported. The epitopes of CEA that were detected, however, were also present in chronic pancreatitis cells, indicating that they are not tumour specific. Further monoclonal antibodies to CEA or to other tumour associated antigens, such as pancreatic oncofetal antigen,²⁰ should be evaluated in pancreatic cancer to determine whether a tumour specific agent can be identified which differentiates cancer from chronic pancreatitis.

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