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Demonstration of carcinoembryonic antigen in human breast carcinomas by the immunoperoxidase technique

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SUMMARY Using an antiserum against carcinoembryonic antigen, which was free from non-specific cross-reacting antigen activity, carcinoembryonic antigen has been demonstrated in 45 out of 90 breast carcinomas by an indirect three-stage immunoperoxidase method. The presence of carcinoembryonic antigen appears to be related to good histological differentiation but not to histological type, lymph node metastasis, or recurrence within two years of primary diagnosis. It is suggested that the varying results obtained by different workers may be due to the differing characteristics of the anti-carcinoembryonic antigen serum used.

The immunoperoxidase technique is now recognised as a useful method for demonstrating various proteins within tumours,[1 2] and one of its advantages is that it can be used retrospectively. However, interpretation of results is dependent upon the specificity of the primary specific antiserum and the use of proper controls.

Carcinoembryonic antigen (CEA) is an oncofetal antigen, which has been demonstrated in human breast carcinomas, but the incidence of detection when the immunoperoxidase technique has been used has ranged from one positive out of 62,[3] to 66%,[4] to 10 positive out of 12 carcinomas.[1]

Shousha *et al.*[4 5] have suggested that there is a significant relationship between the demonstration of CEA in breast carcinomas and the presence of lymph node metastasis and five- and 10-year survival rates.

In this study an indirect three-stage immunoperoxidase technique was used to examine a series of breast carcinomas retrospectively to assess the incidence of detection of CEA, and to relate its presence to histological type and differentiation, lymph node status, and recurrence within two years of presentation.

Patients, materials and methods

Ninety carcinomas surgically removed from 90 patients between February 1976 and March 1977 were examined retrospectively. Forty-eight patients had histological evidence of axillary lymph node metastasis at the time of mastectomy, and 23 have since developed recurrent disease. Forty-two patients had no evidence of lymph node metastases, and 13 of these have developed recurrent disease. Sixteen of the patients with lymph node metastases received intravenous chemotherapy after mastectomy (9 of whom subsequently have had recurrences), and 12 patients without lymph node metastases received oral chemotherapy (one has since had a recurrence).

All tissues were fixed in either 4% formaldehyde in 0·15M sodium chloride solution or 4% formaldehyde in Sorensen's buffer pH 7·0, routinely processed, and embedded in paraffin wax. The method was similar to that described by Walker.[2] Sections were dewaxed, taken to water, and treated with a 0·1% solution of Trypsin (Difco 1:250) pH 7·8 at 37°C for 20-25 minutes. After thorough washing in running water endogenous peroxidase was blocked by immersion of sections in 0·2% HCl in methanol for 30 minutes. Non-specific background staining was reduced by treatment of sections with normal swine serum at a dilution of 1 in 5 for 10 minutes. Rabbit anti carcinoembryonic antigen (CEA) serum (Dakoimmunoglobulins A115) was applied to the sections at a dilution of 1 in 50. Because this antiserum contains antibody directed against non-specific cross-reacting antigen (NCA) it was absorbed, before application, with a perchloric acid treated spleen extract (a source of NCA). The sections were then treated with swine anti rabbit

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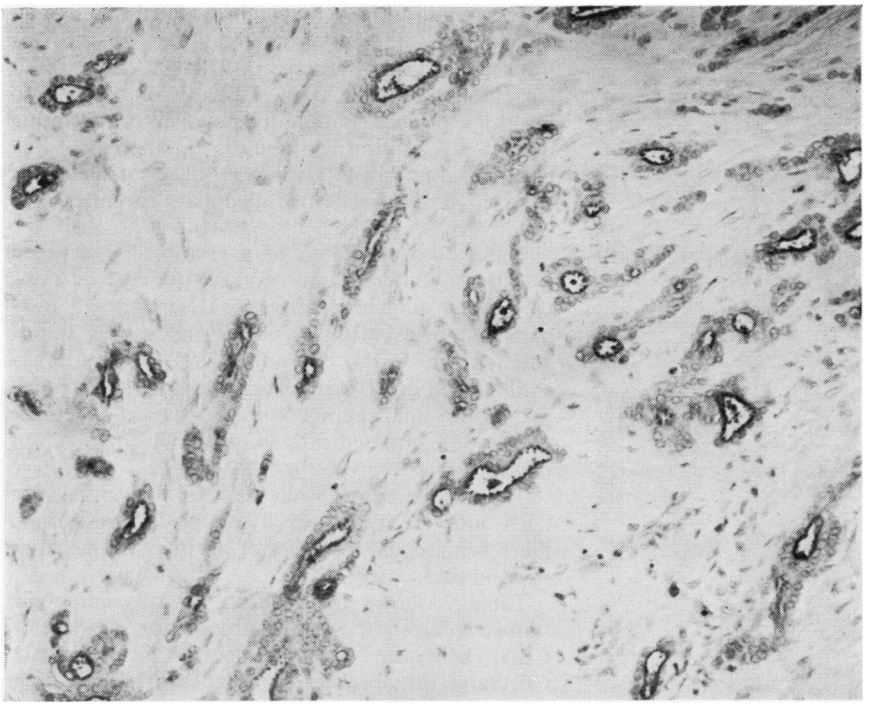

Fig. 1 *Low-power view of a well-differentiated carcinoma showing positive staining within the lumens of some tubules and at the periphery of cells forming the lumens. Immunoperoxidase/haematoxylin. × 45.*

gammaglobulin serum and peroxidase-antiperoxidase complex (both from Dakoimmunoglobulins) with washing in TRIS buffer pH 7·6 between each stage. Peroxidase was localised by the diaminobenzidine-hydrogen peroxide reaction with a resultant brown colour. The sections were counterstained with Mayer's haemalum, dehydrated, cleared, and mounted in Canada balsam.

Controls were the substitution of the rabbit anti CEA serum by normal rabbit serum, and the use of rabbit anti CEA serum which had been absorbed with CEA (kindly donated by Professor A Munro Neville). Normal breast tissue (which had been processed in the same way as the breast carcinomas) from 20 patients was also examined.

The staining in those carcinomas that gave a positive reaction was assessed as: +, a small number of positive cells; ++, a moderate number of positive cells; and +++, many positive cells. Intensity of staining was not assessed.

Haematoxylin and eosin stained sections of all carcinomas were studied. They were classified as infiltrating duct, infiltrating lobular, medullary, mucinous, and papillary. Histological differentiation was assessed using a modification of the grading criteria of Bloom and Richardson[6] in that only the numbers of mitotic figures, and not mitotic figures and hyperchromatic nuclei, were counted per high-power field. The degree of lymphocytic infiltration was assessed as absent, mild, moderate, or marked and divided into two groups, depending on whether the infiltrate was throughout the tumour or confined to the periphery.

Results

Forty-five of the 90 carcinomas (50%) gave a positive reaction by the immunoperoxidase method (Fig. 1). There was no staining in control sections with normal rabbit serum, and none after absorption of the specific antiserum with purified CEA, 0·05 μg being required to absorb 2·5 ml of 1:50 anti CEA. There was no staining of polymorphs or macrophages, cells which contain NCA, or of red blood cells and vascular endothelium. All sections of normal breast tissue gave a negative reaction. However, some positive matter could be seen in normal breast ducts adjacent to breast carcinomas in which there were many positive cells. It was not seen in normal breast tissue around carcinomas that were negative or had only few to moderate numbers of positive cells.

Nineteen of the carcinomas that gave a positive reaction had only a small number of positive cells, 19 a moderate number of positive cells, and seven many positive cells. The site of staining within a cell appeared to depend on the structure of the tumour. The positive reaction was at the luminal periphery of cells forming tubules (Fig. 2) and in some areas of

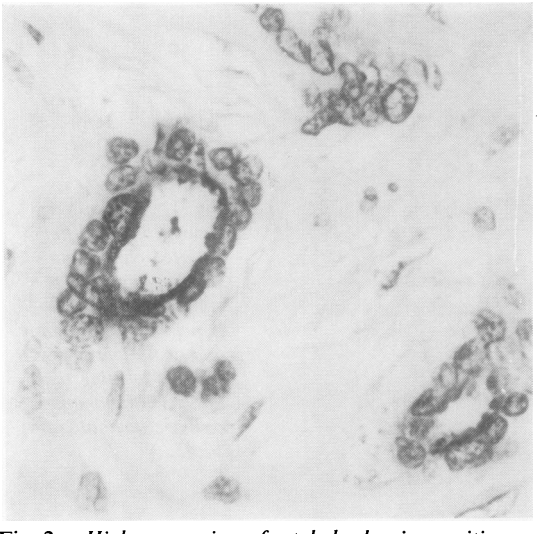

Fig. 2 *High-power view of a tubule showing positive staining at the periphery of the cells. Immunoperoxidase/haematoxylin.* × *190.*

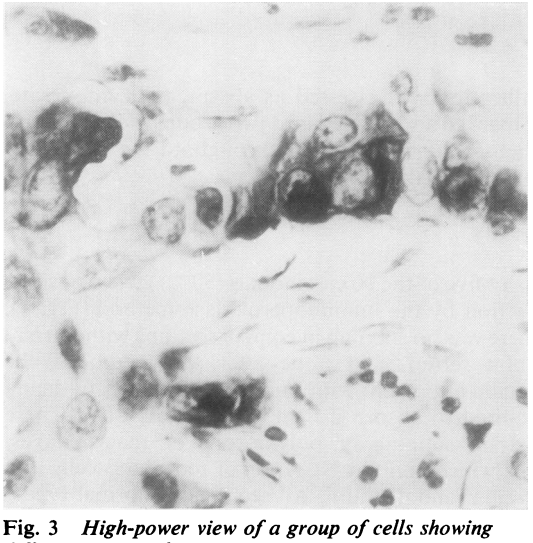

Fig. 3 *High-power view of a group of cells showing diffuse intracytoplasmic positive staining. Immunoperoxidase/haematoxylin.* × *190.*

intraduct carcinomas. Positive matter was present in the lumens of tubules and intraduct carcinoma whose cells gave a positive reaction. In areas where the cells formed solid groups, the staining was diffusely intracytoplasmic, with a focal accentuation in some (Fig. 3). The pattern of staining in cells of infiltrating lobular carcinomas was both diffusely intracytoplasmic and at the periphery.

The relationship between the type of carcinoma, histological differentiation, and the absence or presence of CEA is shown in Table 1. There is no correlation between the presence of CEA and histological type, equal numbers of both infiltrating duct and infiltrating lobular carcinomas being negative and positive. There was only a small number of positive cells in the two medullary carcinomas in which CEA could be demonstrated.

There does appear to be a relationship between histological differentiation and the presence of CEA. Of the well differentiated (grade I) carcinomas, 69% had demonstrable CEA, 60·5% moderately differentiated (grade II), but only 32·5% poorly differentiated (grade III) carcinomas. Also, there tended to be only small numbers of positive cells in those poorly differentiated carcinomas in which CEA was present.

There is no relationship between the presence of CEA and the degree of lymphocytic infiltration, there being equal numbers of positive and negative cases in each category.

Table 2 shows the relationship between nodal status, recurrence, and the absence or presence of CEA. There is no correlation between the presence of CEA and the absence or presence of lymph node metastasis, or recurrence, there being equal numbers positive and negative.

Discussion

In this study CEA has been demonstrated in 50% of human breast carcinomas, which is similar to the findings of Wahren *et al.*,[7] markedly higher than that of Goldenberg *et al.*[3] but lower than those of Shousha and Lyssiotis[4] and Heyderman and Neville.[1] There is no relationship to histological type as has been found before,[4 7] but there is to histological differentiation, as has been found for colonic carcinoma.[8] The pattern of staining varied with localised differentiation within a carcinoma, being at the periphery of cells in areas of tubule formation but throughout the cell in solid areas. Unlike the findings of Shousha and Lyssiotis,[4] no relationship was found between the presence of CEA in the carcinomas and the presence of lymph node metastasis. Also, there is no correlation between recurrence within two years of surgery and the presence of CEA, which differs from the findings of Shousha *et al.*[5] for five- to 10-year survival rates.

The antiserum that was used in this study was one that was known to contain activity against non-specific cross-reacting antigen, a glycoprotein that cross-reacts with CEA,[9] and therefore the antiserum was absorbed with a perchloric acid spleen extract (a source of NCA). There was no staining of poly-

Table 1 *Relation between carcinoma type, histological differentiation, and CEA*

Classification	*CEA*					*All carcinomas*
	Negative	*Positive*				
		Total	+	++	+++	
Infiltrating duct	36	38	18	15	5	74
Infiltrating lobular	6	5	1	2	2	11
Medullary	2	2	2	0	0	4
Mucinous	0	0	0	0	0	0
Papillary	0	1	0	1	0	1
Grade						
I	4 (31%)	9 (69%)	2	4	3	13
II	17 (39·5%)	26 (60·5%)	10	12	4	43
III	23 (67·5%)	11 (32·5%)	8	3	0	34

morphs or macrophages, cells that contain NCA. Isaacson and Judd[10] noted that in one of their studies there was staining of red cells and vascular endothelium (after blocking of endogenous peroxidase), but none was seen in this study, and this may be due to the use of an anti-CEA serum from another source. There was no staining of normal breast tissue, which included occasional breast cysts, although Fleisher *et al.*[11] found that there was a glycoprotein present in breast cyst fluid which cross-reacted with CEA. The presence of positive matter in normal breast ducts adjacent to carcinomas with many positive cells can be explained by secretion from the tumour into the ducts. There was total abolition of staining after absorption of the antiserum with purified CEA from a different source from that used to raise the antiserum.

CEA consists of a heterogenous group of related glycoproteins,[12] and the various antisera available may well have differing characteristics. The variation between the results of this study and those of Shousha *et al.*[4 5] may be due to differences in the antiserum. Although Dakoimmunoglobulins antiserum was used in both cases, in this study the anti-NCA activity was absorbed out. However, even though the lack of staining in histological structures known to contain glycoproteins with immunological cross-reactivity to CEA suggests that the protein demonstrated is CEA, it is probably best to describe it as a CEA-like protein.

Table 2 *Relation between nodal status, recurrence, and CEA*

	CEA	
	Positive	*Negative*
Lymph node metastasis	25	23
Non recurrent	14	11
Recurrent	11	12
Lymph node without metastasis	22	20
Non recurrent	15	16
Recurrent	7	4
Total recurrences	18	16

The value of the immunoperoxidase technique is that it can be used to select those patients who should have monitoring of their plasma for CEA. In a recent study, Tormey and Waalkes[13] showed that CEA could be detected in the plasma of 14·2% of patients with primary breast carcinoma but in 70·9% of patients with metastatic disease, which suggests that this parameter is either related to tumour load, or that CEA is not released into the circulation until there is spread from the breast.

In conclusion, a CEA-like protein has been demonstrated in 50% of human breast carcinomas, and its presence appears to be related to histological differentiation. There appears to be no correlation between its presence and other prognostic factors such as lymph node metastasis and recurrent disease.

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