Spectrum of carcinoembryonic antigen immunoreactivity from isolated ductal hyperplasias to atypical hyperplasias associated with infiltrating ductal breast cancer

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Abstract

Aims—To study the immunohistochemical expression of carcinoembryonic antigen (CEA) in ductal hyperplasia of the breast and to investigate its putative relation with atypia and co-existing infiltrating ductal carcinoma.

Methods—Paraffin wax embedded tissue from 37 cases of isolated ductal hyperplasia (five with atypia and 32 without atypia) and 25 cases of ductal hyperplasia associated infiltrating ductal carcinoma (IDC) (seven with atypia and 18 without atypia) was stained with a monoclonal anti-CEA antibody using a standard avidin biotin immunoperoxidase method.

Results—CEA immunoreactivity was observed in eight (12.8%) ductal hyperplasia cases. The percentage of CEA positivity in ductal hyperplasia cases with atypia (33.3%) was substantially higher than that observed in cases of ductal hyperplasia without atypia (8.0%). Six cases of ductal hyperplasia associated IDC reacted with CEA; in these six cases the neoplastic cells of the co-existing carcinoma were also CEA positive. The percentage of CEA immunoreactivity in ductal hyperplasia associated IDC was higher than that observed in isolated ductal hyperplasia (24.0 v 5.4%). The percentage of CEA immunoreactivity in atypical ductal hyperplasia associated IDC was similar to that observed in IDC alone (42.9 v 40.0%).

Conclusions—The presence of CEA immunoreactivity has been confirmed in benign proliferative breast lesions. The prevalence of such immunoreactivity increases from 3.1% in isolated, non-atypical ductal hyperplasia to 42.9% in atypical ductal hyperplasia associated IDC. This finding and the similarity of the frequency of CEA positivity in atypical ductal hyperplasia associated IDC and in IDC alone suggests that there is a pathogenetic link between ductal hyperplasia and some types of breast cancer.

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Women with a history of benign breast disease have an increased risk of subsequent breast cancer.¹ This increased risk is more evident among women with a proliferative epithelial component in their disease.² At present, the

relation between benign and malignant breast disease is unclear. Benign lesions may either be an early stage in the progression to malignant disease or completely independent.³

Ductal hyperplasia is a form of benign breast disease characterised by proliferation of the epithelium into the lumen.⁴ The phenotypic and genotypic patterns of these lesions have not been extensively studied. Recently, similarities were found between ductal hyperplasia and breast carcinoma cells with regard to DNA content and expression of some immunohistochemical markers.⁵⁻⁸

Carcinoembryonic antigen (CEA) is a member of the immunoglobulin supergene family and probably acts as an accessory molecule to cell adhesion, and is often used as a marker of malignancy.⁹⁻¹¹ The association between CEA immunoreactivity and breast cancer is well established despite the quite different figures in the literature, ranging from a minimum of 42.8% to a maximum of 90.0%, reported, respectively, by Nap *et al*¹² and Alexiev *et al*.¹³

Data on the putative association between CEA immunoreactivity and benign breast lesions are scarce and controversial. Most authors did not find any immunoreactivity at all,¹²⁻¹⁴ whereas others found immunoreactivity ranging from a minimum of 5.2%¹⁵ to a maximum of 42.1%.¹⁶ This controversy partly reflects the well known variations in the specificity and sensitivity of anti-CEA sera,^{12 17 18} and may also depend on the types of lesions classified as benign.

In a previous study we found CEA immunoreactivity in one of 18 cases of ductal hyperplasia.¹⁹ We aimed to expand the aforementioned series in an attempt to determine whether this immunoreactivity is related to the presence of atypia and co-existing invasive ductal carcinoma.

Methods

Breast tissue was obtained from 62 patients following biopsy, plastic surgery or mastectomy. In all cases histological slides from paraffin wax embedded material were critically reviewed and classified according to the criteria of Page and Anderson⁴: ductal hyperplasia without atypia was defined as swirling patterns of cells with intercellular borders, usually ill defined, with an irregular nuclear shape, chromasia and position, and irregular, often ragged, serpiginous slit-like secondary spaces. Atypical ductal hyperplasia was defined as partial involvement of the basement

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Table 1 CEA immunoreactivity in isolated ductal hyperplasia (DH) with and without atypia, and in ductal hyperplasia associated IDC with and without atypia

	CEA		
	Positive n (%)	Negative n (%)	Total n
Isolated DH	2 (5.4)	35 (94.6)	37
with atypia	1 (20.0)	4 (80.0)	5
without atypia	1 (3.1)	31 (96.9)	32
DH associated IDC	6 (24.0)	19 (76.0)	25
with atypia	3 (42.9)	4 (57.1)	7
without atypia	3 (16.7)	15 (83.3)	18

membrane bound space by evenly spaced uniform cells with uniformly oval to rounded nuclear features and distinct intercellular borders. Non-atypical cells were frequently columnar, polarised cells of the type seen in the ducts immediately above the basement membrane. Following these criteria, the 37 cases of isolated ductal hyperplasia were divided into those with ($n = 5$) and without atypia ($n = 32$). The 25 cases of ductal hyperplasia associated infiltrating ductal carcinoma (IDC) were also divided into those with ($n = 7$) and without atypia ($n = 18$). In all cases of isolated ductal hyperplasia the patients were followed for a minimum of five years with no signs of breast cancer.

Formalin fixed, paraffin wax embedded tissue sections, 4 μ m thick, were stained with the CEA monoclonal antibody (Boehringer, Indianapolis, Indiana, USA) the epitope reactivity of which has not been characterised,¹⁷ using an avidin biotin complex immunoperoxidase method. All sections were predigested with 0.1% trypsin (Sigma, St Louis, Missouri, USA) for 20 minutes at room temperature to reveal the immunological binding sites before the immunoperoxidase reactions. Slides with primary antibody were incubated for two hours at room temperature. A standard avidin biotin complex method (Vector Labs, Burlingame, California, USA) was used with 3,3'-diaminobenzidine (Sigma) as the chro-

mogen and haematoxylin as the counterstain. Sections of colonic adenocarcinoma were used as positive controls. Specific antisera were replaced by normal sera and used as negative controls.

CEA positivity was evaluated immunohistochemically by estimating the percentage of immunostained cells (at least 100 cells were counted in each case) and by assessing the intensity—graded as follows: weak (+); strong (++); and very strong (+++)—and the location of staining within each cell. Cases with no or weak staining in less than 5% of cells were regarded as negative. Cases in which only apical or luminal border staining was observed were also regarded as negative because such immunoreactivity has been found in normal breast tissue and may represent cross-reactivity with proteins from the glycocalyx.¹⁴ Cases were regarded as “positive” when more than 1% of the cells displayed either strong or very strong cytoplasmic immunoreactivity.

Borderline cases—that is, cases in which a high percentage of cells displayed weak immunostaining and cases in which a small percentage of cells displayed strong or very strong immunoreactivity, were not found in our series.

Sections of colonic adenocarcinoma were always positive for CEA, while omission of primary antisera resulted in the complete loss of immunoreactivity.

Results

The results are summarised in table 1. The mean age of those patients with isolated ductal hyperplasia was 40 years (range 17–60 years) and of those with ductal hyperplasia associated IDC was 54 years (range 31–75 years). The percentage of atypia in cases of isolated ductal hyperplasia was similar to that in cases of ductal hyperplasia associated IDC (21.6 *v* 28.0%).

CEA immunoreactivity was observed in eight (12.8%) cases of ductal hyperplasia. The hyperplastic cells showed strong cytoplasmic positivity (fig 1). Myoepithelial cells were consistently CEA negative. The percentage of CEA positivity in cases of ductal hyperplasia with atypia (33.3%) was significantly higher ($p < 0.05$) than that in those without atypia (8.0%).

Six ductal hyperplasia associated IDC cases were positive for CEA (table 1). In these six cases the neoplastic cells of the co-existing carcinoma were also CEA positive. CEA immunoreactivity was observed in 10 of the 25 (40.0%) IDC cases (fig 2), including four cases in which adjacent ductal hyperplasia cells were negative. CEA expression was not observed in ductal hyperplasia associated non-immunoreactive IDC. Only two (5.4%) cases of isolated ductal hyperplasia exhibited CEA positivity. The percentage of CEA immunoreactivity in ductal hyperplasia associated IDC was higher, although not significantly ($p < 0.20$), than that in isolated ductal hyperplasia (24.0 *v* 5.4%).

Figure 1 CEA immunostaining in a case of isolated ductal hyperplasia.

Figure 2 CEA immunostaining in a case of infiltrating ductal carcinoma. Note the immunoreactivity within the intraductal component of the tumour.

Polymorphonuclear leucocytes in breast epithelial tissue sections were always negative for CEA, excluding a putative cross-reaction with non-specific cross-reacting antigen.

Discussion

The major concern when using CEA as a putative marker of malignant transformation is to choose an antibody with appropriate specificity and sensitivity,¹⁷ as the different preparations of anti-CEA sera produce varying results.¹² The large CEA molecule may also cross-react with several normal antigens present in some tissues.²⁰ Despite these drawbacks, monoclonal and well absorbed polyclonal anti-CEA sera have unequivocally reacted with non-malignant epithelia.^{19 21 22} In these cases CEA expression was related to disorders of cell growth and differentiation. Hasleton *et al*²³ reported expression of CEA in rectal polyps which progressed to cancer. Greaves *et al*²¹ observed CEA positivity in adenomas and metaplastic polyps of the large

bowel, while Maxwell *et al*²¹ observed CEA positive staining in the dysplastic epithelium of the biliary tract.

The present study confirms our previous finding of CEA positivity in a single proliferative benign breast lesion.¹⁹ It shows, moreover, that the frequency of immunoreactivity differs between isolated ductal hyperplasia and ductal hyperplasia associated IDC. The frequency of CEA expression in benign breast lesions also varies (table 2). Several authors did not observe positive staining for CEA in these lesions,¹²⁻¹⁴ whereas others reported CEA positivity in a large number of non-malignant breast lesions.^{12 15 16 18 24} These discrepant results are difficult to evaluate from a critical point of view because the antibodies as well as the morphological criteria and the immunohistochemical methods vary from series to series (table 2).

In material obtained using fine needle aspiration Kandaraki *et al*¹⁶ reported a high frequency of CEA immunoreactivity in benign breast lesions (42.1%), but these authors did not classify the lesions studied in their series. Our data, showing CEA immunoreactivity in 5.4% cases of isolated ductal hyperplasia, support the hypothesis that CEA positivity is not restricted to malignant cells in the breast. As CEA expression is not restricted to malignant cells in other tissues,^{21 22} it is not surprising that some cases of benign breast disease are CEA positive.

Papotti *et al*²⁴ and Kuhajda *et al*¹⁵ observed CEA positive staining in papillomas (table 2). Furthermore, Papotti *et al*²⁴ suggested that the progression from multiple intraductal papillomas to breast cancer was related to CEA expression. Lee *et al*¹⁸ observed CEA positivity in atypical ductal hyperplasia associated IDC without mentioning the frequency of this observation. In the present study we observed three distinct levels of CEA immunoreactivity: 3.1% in isolated, non-atypical ductal hyperplasia; 16.7 and 20.0%, respectively, in non-atypical ductal hyperplasia associated IDC and isolated atypical ductal hyperplasia; and 42.9% in atypical ductal hyperplasia associated IDC. The important

Table 2 Summary of the prevalence of CEA immunoreactivity in benign breast lesions

Reference	Source of CEA	Staining method	Papilloma (%)	Fibro-adenoma (%)	Isolated DH without atypia (%)	DH associated IDC without atypia (%)	Isolated DH with atypia (%)	DH associated IDC with atypia (%)	Total benign lesions (%)
Nap, <i>et al</i> ¹²	Hoffman-La Roche (Basel, Switzerland)	PAP	-	0	0	0	-	-	0
Alexiev, <i>et al</i> ¹³	Biogenex (Dublin, Ireland) (Clone SP651)	Streptavidin	-	0	0	-	-	-	0
De Potter, <i>et al</i> ¹⁴	Amersham (Amersham, UK) (ac RNN8)	PAP	-	-	0	0	0	0	0
Kuhajda, <i>et al</i> ¹⁵	Dako (Santa Barbara, USA)	PAP	25.0	-	-	-	-	0	5.2
Kandaraki, <i>et al</i> ¹⁶	Not given	Not given	-	-	-	-	-	-	42.1
Lee, <i>et al</i> ¹⁸	Dako	ABC	-	-	-	-	-	Some cases	-
Schmitt, <i>et al</i> ¹⁹	Boehringer (Indianapolis, USA)	ABC	-	-	0	-	5.5	-	5.5
Papotti, <i>et al</i> ²⁴	Hybritech (La Jolla, USA)	Not given	Some cases	-	-	-	-	-	-
Present study	Boehringer	ABC	-	-	3.1	16.7	20.0	42.9	12.9

DH = ductal hyperplasia; IDC = infiltrating ductal carcinoma; PAP = peroxidase-antiperoxidase method; ABC = avidin biotin peroxidase complex.

question to be answered from the oncobiological point of view is whether the abnormal expression of CEA in hyperplastic breast lesions is related to the progression to malignancy. In the colorectum the adenoma-carcinoma sequence is strongly supported by epidemiological, pathological, and genetic data.²⁵ In these cases the CEA content of the adenomas increased gradually with increasing dysplasia.²² It is tempting to hypothesise that the ductal hyperplasia-carcinoma spectrum in the breast could be analogous. There is epidemiological, cytometric, and immunohistochemical evidence linking atypical proliferative epithelial breast lesions with breast cancer.^{25,6} Our finding of a similar prevalence of CEA expression in atypical ductal hyperplasia associated IDC (42.9%) and in IDC (40.0%) is suggestive of a pathogenetic link between ductal hyperplasia and some types of breast cancer.

We did not study a sufficient number of intraductal carcinoma cases to draw definitive conclusions about the biological role of this lesion in the progression to invasive cancer. Other series, however, have demonstrated a similar incidence of CEA immunoreactivity in intraductal and invasive carcinomas.^{14,15} In some of our cases we detected CEA positivity in intraductal carcinomas close to infiltrative areas (data not shown), which is suggestive of a pathogenetic link between these types of carcinoma. On the other hand, we observed four IDC cases which expressed CEA immunoreactivity without CEA positivity in the neighbouring ductal hyperplasia (including one case with atypia). Whether or not this finding represents an alternative route for breast carcinogenesis, as suggested by some authors,^{3,26,27} has yet to be clarified.

Despite having demonstrated that the CEA expression is more frequently seen in atypical ductal hyperplasia associated with invasive cancer, we do not know if CEA expression by hyperplastic cells represents a new antigenic determinant associated with malignant transformation, as CEA messenger RNA (mRNA) has been detected in apparently normal mucosas.²⁸ To the best of our knowledge, analysis of CEA mRNA using northern blotting or in situ hybridisation of normal or hyperplastic breast epithelia has not been performed to date.

Bearing these observations in mind, prospective studies, including follow up and molecular biology studies of isolated ductal hyperplasia, mainly in CEA positive cases, are required to assess whether such immunoreactivity is indicative of an increased risk for malignant transformation.

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