c-erbB-2 overexpression and histological type of in situ and invasive breast carcinoma

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Abstract

Aims: To assess *c-erbB-2* immunostaining in relation to morphological type of in situ and invasive breast carcinoma.

Methods: Formalin fixed, wax embedded archival tissue was used. Invasive carcinomas comprised 50 infiltrating ductal (NOS); seven medullary, 10 tubular, 15 mucinous and 24 classic invasive lobular. In situ carcinomas comprised 48 ductal (DCIS) and 10 cases of lobular (LCIS). The antibodies used were pAB1 (polyclonal) which stains cell lines that over express the *c-erbB-2* oncogene, and ICR 12 (monoclonal) which stains sections of breast carcinoma known to show *c-erbB-2* amplification.

Results: Immunostaining consistent with *c-erbB-2* overexpression was found in 10 out of 50 cases of infiltrating ductal carcinoma (NOS), one of 24 infiltrating lobular carcinomas and one of seven medullary carcinomas only. Seventy per cent of ICR 12 positive cases of infiltrating ductal carcinoma also had extratumoral DCIS. Forty six per cent of pure DCIS lesions also showed strong membrane staining for *c-erbB-2* protein, confined to large cell types.

Conclusions: Immunostaining for *c-erbB-2* oncoprotein occurs mainly in large cell DCIS and infiltrating ductal carcinoma NOS, especially those with an extratumoral DCIS component. There is a low incidence in other types of breast cancer, including those associated with a better prognosis. Different biological mechanisms may be responsible for histologically distinct types of breast carcinoma.

The *c-erbB-2* oncogene is homologous with, but distinct from, the *erbB* gene that encodes the epidermal growth factor receptor.¹ It codes for a membrane protein with a tyrosine kinase domain that is similar in structure (but not identical) with that of the epidermal growth factor receptor.^{2,3} This gene has been found to be amplified in some human adenocarcinomas⁴⁻⁷ and particular interest has been shown in its role in breast carcinoma, up to 30% of which have been described as showing amplification.^{7,8-12} Amplification of the gene results in overexpression of the *c-erbB-2* mRNA and protein product.^{13,14} This manifests

as strong membrane staining using an antibody to the *c-erbB-2* protein, and several studies have shown a good correlation between immunohistochemical membrane staining in formalin fixed, paraffin wax embedded tissue and gene amplification, using a variety of antibodies^{10,11,15-18} to study archival material.

Much of the interest in *c-erbB-2* amplification has been because of its possible prognostic implications, with many reports suggesting that it may be associated with a poorer prognosis.^{7,8,14,19} Most studies, however, have concentrated on infiltrating ductal carcinoma NOS, with only occasional examples of the less common types of breast cancer.^{15,20-22} More recent work by Soomro *et al*²³ suggests that the "better prognosis" types of breast carcinoma, such as medullary and tubular carcinoma, may not be associated with *c-erbB-2* immunostaining. Ductal carcinoma in situ (DCIS) shows an even higher incidence of *c-erbB-2* overexpression than invasive carcinomas, with 40% or more reported in most series,^{15,20,24,25} suggesting that gene amplification may occur as an early event, with a possible role in pathogenesis. A study by Ramachandra *et al*²⁴ has shown that this may not be the case for lobular carcinoma in situ (LCIS). It seems, therefore, that the histologically distinct types of breast cancer which are known to exhibit different behaviour and prognosis²⁶⁻³⁰ may have an underlying biological difference with respect to *c-erbB-2* overexpression. The aim of this study was to assess this possibility by looking at *c-erbB-2* immunostaining in relation to morphological type of both in situ and invasive breast carcinomas.

Methods

Archival material was used from the files of the Belfast City Hospital. All material was fixed in 10% formalin and embedded in paraffin wax. The invasive carcinomas comprised 50 consecutive infiltrating ductal carcinomas (NOS); seven medullary carcinomas; 10 tubular carcinomas; 15 mucinous carcinomas and 24 classic invasive lobular carcinomas. The in situ carcinomas comprised 48 cases of DCIS and 10 cases of LCIS, none of which had an invasive component. The cases of DCIS were further divided according to the predominant morphological pattern using standard criteria³¹—namely, comedo, micropapillary, cribriform and papillary (non-invasive). Micropapillary/cribriform lesions were further classified into

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large pleomorphic cell type or small regular cell type.

The 50 cases of infiltrating ductal carcinoma NOS were graded I (low grade) to III (high grade) using Elston's modification of the Bloom and Richardson method.³² This assesses tubule formation, nuclear pleomorphism, and mitotic count. The presence of an in situ component for these 50 cases was assessed both as the approximate percentage and as the presence or absence of an extra-tumoral intraductal component, and this component was classified according to predominant histological type. Tumours in which 20% or more of the main mass was composed of DCIS were judged to have a "significant" in situ component. Extra-tumoral intraduct carcinoma was taken to be in situ carcinoma outside the invasive tumour edge; this did not include cases with occasional affected ducts just at the tumour edge.

Two antibodies were used: pAb1 (Triton Biosciences Inc), a polyclonal antibody generated against the c-terminal amino acid sequence of the *c-erbB-2* receptor. This immunochemically stains cell lines which overexpresses the *c-erbB-2* oncogene, and ICR12 (kindly provided by Dr C J Dean, Institute of Cancer Research, Royal Cancer Hospital, Belmont, Sutton Surrey), a rat monoclonal antibody¹⁶ directed against the external domain of the *c-erbB-2* gene product, and which stains sections of breast carcinoma known to show *c-erbB-2* amplification.

Sections 4 μ m thick were cut from paraffin wax embedded tissue blocks. After dewaxing, endogenous peroxidase was blocked with 1.5% H₂O₂ in methanol, and sections were then incubated with either antibody for one hour at 37°C. pAb1 was used at a dilution of 1 in 15 in 1% bovine serum albumin (BSA) and was followed by 30 minutes incubation with biotinylated swine anti-rabbit immunoglobulin (Dakopatts) diluted 1 in 200. ICR12 was diluted to about 1 μ g/ml in 1% BSA. This dilution is that used by Styles *et al.*¹⁶ but was also titred in this laboratory and was followed by 30 minutes of incubation with goat anti-rat IgG biotin conjugate (Sigma) at a 1 in 100 dilution in phosphate buffered saline (PBS).

For both antibodies, the final step was treatment with the avidin-biotin peroxidase complex (Dakopatts ABCComplex-HRP) for 30 minutes, colour development with diaminobenzidine (Sigma) and counterstaining of nuclei with haematoxylin. PBS washes were used between each step. Positive controls were cases that were consistently strongly positive with both antibodies; for negative controls the primary antibody was omitted.

Cases which showed strong membrane staining (either focal, only affecting some tumour cells, or diffuse affecting all or most tumour cells) were classed as positive.

Fisher's exact test was performed to assess the significance of the relation between *c-erbB-2* immunostaining and histological tumour type, nodal state, and extra-tumoral in situ component. The χ^2 test was performed to assess the significance of tumour grade; and mean age and tumour size for ICR12 positive and negative cases were compared using a Mann-Whitney non-parametric test.

Results

INVASIVE CARCINOMA

Correlation with histological tumour type

Results are summarised in table 1. Using the pAb1 antibody, eight out of 50 cases of infiltrating ductal carcinoma NOS showed strong membrane staining, compared with 10 out of 50 with ICR12 (Fig 1). The 8 cases which were positive with pAb1 were all positive with ICR12; an extra two cases were also positive with ICR12 which were negative with pAb1. ICR12 staining was stronger, more diffuse—that is, affecting most or all tumour cells—and easier to interpret than pAb1 staining. With ICR12, staining was diffuse in six cases and focal in four; with pAb1, only two cases showed diffuse staining and six cases focal staining. Staining consistent with *c-erbB-2* overexpression was only seen in one out of seven medullary carcinomas and focally in one out of 24 invasive lobular carcinomas. The positive case of infiltrating lobular carcinoma was of classic type. Membrane staining, although focal, was present on tumour cells showing an Indian filing pattern and with intracytoplasmic lumina. No mucinous or tubular neoplasms were positive. Two of the positive cases were recurrences. In one of these the original carcinoma also showed positive membrane staining. In the other the primary tumour did not stain positively.

Correlation of ICR12 staining with clinicopathological and morphological variables

These were assessed for the 50 cases of ductal carcinomas NOS, and the results are summarised in table 2. There was no significant correlation with age, tumour size, or nodal state. Most of the ICR12 positive tumours (90%) were Bloom and Richardson grade II ($p < 0.05$). There was a significant tendency for *c-erbB-2* positive tumours to have an extratumoral intraductal component ($p = 0.035$). Five out of the 50 tumours had an intraductal component comprising at least 20% of the tumour mass. Eighteen cases had an extra-tumoral intraduct component—these 18 cases included the former five cases. Of these 18 cases, seven (39%) were ICR12 positive. Put another way, 70% of ICR12 positive cases had an extra-tumoral intraduct component compared with only 27% of ICR12 negative cases. In all cases the staining of the in situ component paralleled that of the invasive component, regardless of which antibody was used. Hence the two pAb1

Table 1 *c-erbB-2* oncoprotein in relation to histological type of infiltrating ductal carcinoma

Tumour type	No of cases	No positive pAb1 (%)	No positive ICR12 (%)
Infiltrating ductal NOS	50	8 (16)	10 (20)
Medullary	7	1 (14)	1 (14)
Tubular	10	0 (0)	0 (0)
Mucinous	15	0 (0)	0 (0)
Infiltrating lobular	24	1 (4)	1 (4)

Figure 1 Infiltrating ductal carcinoma NOS with strong ICR12 membrane staining.

negative cases positive for ICR12 also showed no pAb1 positivity of their in situ component.

IN SITU CARCINOMA
Results are summarised in table 3. Overall, 46% of intraductal carcinomas stained positively with pAb1 antibody and 44% with ICR12. (Fig 2). None of 10 LCIS showed positive staining. The difference between staining of DCIS and LCIS was significant ($p=0.01$). In these cases staining was generally stronger and more diffuse with pAb1. One case which was negative with ICR12 showed very

strong membrane staining of most tumour cells with pAb1; five cases showing diffuse staining with pAb1 also showed only focal staining with ICR12. The breakdown of types of intraductal lesion showed that staining was confined to comedo and micropapillary/cribriform large cell types (table 3). Of the 33 cases which contained comedo DCIS, alone or in combination, 21 (63%) stained positively. None of the cribriform/micropapillary intraduct lesions with the small regular cell type showed positive staining ($p<0.05$).

Assessment of the DCIS component in the 18 cases of infiltrating ductal carcinoma, where this was present, was also carried out, and the results summarised in table 4. Again, in all pAb1/ICR12 positive cases the in situ component comprised comedo DCIS either alone or in combination with large cell cribriform/micropapillary DCIS. The two cases with small cell cribriform DCIS showed no immunostaining with either antibody.

Discussion
The aim of this study was to investigate expression of the *c-erbB-2* oncoprotein in breast carcinoma in relation to histological tumour type. Staining consistent with *c-erbB-2* overexpression was mainly displayed by infiltrating ductal carcinoma NOS, 20% of which were positive, with only one out of 24 invasive lobular carcinomas showing membrane staining of tumour cells. Although the numbers of cases used meant that this did not achieve significance ($p=0.14$), the results nevertheless suggest a biological difference in relation to oncoprotein expression between invasive ductal and lobular tumours. This situation was mirrored by the in situ lesions, with 46% of cases of DCIS showing *c-erbB-2* protein

Table 2 Association of *c-erbB-2* staining with clinicopathological variables (infiltrating ductal carcinoma NOS)

Variable	ICR12 positive (%) (n = 10)	ICR12 negative (%) (n = 40)	
Mean age	57.1	59.9	
Mean tumour size (cm)	2.95	2.2	
Nodes: Positive	7 (70)	14 (35)	p calculated excluding unknown cases
Negative	2 (20)	9 (22.5)	
Unknown	1 (10)	17 (42.5)	
Grading: I	1 (10)	17 (42.5)	p < 0.05
II	9 (90)	14 (35)	
III	0 (0)	9 (22.5)	
Significant in situ component in tumour	1 (10)	4 (10)	p = 0.035
Extra-tumoral in situ component	7 (70)	11 (27.5)	

Table 3 *c-erbB-2* protein staining in in situ carcinoma

Tumour type	No of cases	No positive pAb1 (%)	No positive ICR12 (%)	
DCIS	48	22 (46)	21 (44)	p = 0.01
LCIS	10	0 (0)	0 (0)	
Histological type of DCIS: comedo	25	18 (72)	18 (72)	p < 0.05
Micropapillary/ci	7	1 (14)	1 (14)	
briform (large cell)	8	3 (37)	2 (25)	
Comedo and micropapillary/ci	7	0 (0)	0 (0)	
briform (small cell)	1	0 (0)	0 (0)	
Papillary				

Figure 2 Micropapillary intraduct carcinoma stained with pAb1.

expression, but no positively staining cases of LCIS ($p=0.01$). In most previous papers the samples used have been composed mainly of cases of infiltrating ductal carcinoma NOS, but occasional cases of lobular carcinoma showing *c-erbB-2* over-expression have been described,^{15 22} and it seems that although much commoner in ductal than lobular tumours, overexpression of *c-erbB-2* is not confined to the former group.

We were particularly interested in the incidence of *c-erbB-2* overexpression in the "good prognosis" types of breast cancer, given the possible prognostic implications of amplification of the *c-erbB-2* gene. Our results agree with those of Soomro *et al*²³ and suggest that the "better prognosis" types of breast carcinoma may have a lower incidence of *c-erbB-2* overexpression.

One case out of seven medullar carcinomas showed *c-erbB-2* membrane staining, but we found no positive staining in any of our mucinous or tubular carcinomas. Overexpression of the *c-erbB-2* gene is thought to be an early event in tumour development,^{10 15 20 22} and it may be that the histologically distinct types of breast carcinomas arise by different mechanisms. The significance of finding *c-erbB-2* immunostaining in a good prognosis type of tumour, such as our case of medullar carcinoma, is unknown. Clinical follow up would be needed to see if this

implies a subgroup of such tumours which will behave more aggressively than would be predicted from the tumour's histological type.

c-erbB-2 protein staining in infiltration ductal carcinoma NOS was also assessed in relation to various other variables. Of interest was the observation that a high proportion of *c-erbB-2* positive cases had an extra-tumoral intraductal component. Seventy per cent of ICR12 positive tumours had an intraductal component outside the tumour, compared with only 27% of ICR12 negative cases ($p=0.035$). In most studies (including this one) DCIS shows a much higher incidence of *c-erbB-2* overexpression than infiltrating ductal carcinoma.^{15 20 24 25} Our results, however, suggest that those invasive tumours with a significant intraductal component show a similar rate of *c-erbB-2* oncoprotein staining as DCIS alone. In this study seven out of 18 cases (39%) with extra-tumoral intraductal carcinoma stained for *c-erbB-2*, a figure comparable with that for pure DCIS (46%).

In both the pure DCIS and the DCIS component of the invasive carcinomas, most of the positively stained cases were of comedo type, in keeping with the studies of Van de Vijver *et al*,¹⁵ who found all positive DCIS cases to show a large cell comedo pattern. Several cases with a mixed comedo/cribriform pattern and one case of pure micropapillary DCIS also showed strong membrane staining. These were of a large pleomorphic cell type, however, more in keeping with the comedo group. Ramachandra *et al* found a high proportion of positive cases to be micropapillary in type,²⁴ but these again were of a large cell type with necrosis, rather than the conventional small cell pattern. We agree with their sugges-

Table 4 *c-erbB-2* protein staining in DCIS component of infiltrating ductal carcinoma

Histological type of DCIS	No of cases (n = 18)	No positive pAb1 (%)	No positive ICR12 (%)
Comedo	10	2 (20)	3 (30)
Comedo and cribriform (large cell)	6	3 (50)	4 (66)
Cribriform/micropapillary (small cell)	2	0 (0)	0 (0)

tion that the micropapillary/cribriform pattern should perhaps be subdivided into large/pleomorphic and small/regular cell types. None of the cases of micropapillary/cribriform histology with a small cell type showed *c-erbB-2* overexpression, thus forming two biologically distinct subgroups of DCIS. Further work is needed to assess whether *c-erbB-2* overexpression is of prognostic value in these non-invasive lesions.

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