Pergamon

European Journal of Cancer, Vol. 33, No. 11, pp. 1846–1850, 1997

Printed in Great Britain
0959-8049/97 $17.00+0.00

PII: S0959-8049(97)00244-X

Original Paper

c-erbB-3 Protein Expression in Ductal Carcinoma *In Situ* of the Breast

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c-erbB-3, A recently identified member of the type I tyrosine kinase receptor family, has been shown to be overexpressed in invasive ductal carcinoma of breast. In this study, expression of the c-erbB-3 protein was examined in 57 cases of pure ductal carcinoma *in situ* of the breast (DCIS) by immunocytochemical methods. Staining was either absent (17 cases), present at levels equivalent to that found in adjacent normal tissue (20) or greater than in normal tissue (20). In most cases the pattern of staining was cytoplasmic, but in 4 cases with the most intense reaction there was also focal membrane staining. In the same series of cases, c-erbB-2 protein had previously been shown to be overexpressed in 28 of 57 cases. c-erbB-2 overexpression was correlated with normal levels of c-erbB-3, and lack of c-erbB-2 expression was correlated with c-erbB-3 overexpression. © 1997 Elsevier Science Ltd.

Key words: breast carcinoma, c-erbB-3, ductal carcinoma *in situ*, tyrosine kinase receptors
Eur J Cancer, Vol. 33, No. 11, pp. 1846–1850, 1997

INTRODUCTION

c-ERBB-3 IS a member of the type I growth factor receptor family [1]. These are an important group of tyrosine kinase receptors which also include epidermal growth factor (EGF), c-erbB-2 and c-erbB-4 [2]. The former two proteins which have been well characterised are expressed at low levels in normal breast epithelium and are expressed or overexpressed in some breast carcinomas [3–6]. c-erbB-2 expression in breast carcinomas has been extensively studied. It is overexpressed in approximately 12–25% of infiltrating carcinomas and in 40–60% of *in situ* carcinomas. [5,7–10]. In invasive carcinoma, expression of EGF receptor and overexpression of c-erbB-2 is associated with a poor prognosis [6–8]. The true significance of c-erbB-2 overexpression in *in situ* carcinoma is less well defined, but it is usually found in poorly differentiated high-grade DCIS which is thought to be associated with an increased risk of recurrence and of progression to invasive carcinoma [9,10]. Immunohistochemical demonstration of membrane staining with EGFR and c-erbB-2 antibodies has been found to correlate with overexpression of these proteins and in the case of c-erbB-2, with gene amplification [11].

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Received 22 Oct. 1996; revised 25 Apr. 1997; accepted 10 May 1997.

c-erbB-3, Which was first identified in 1989 was initially detected at the mRNA level in a number of tumour cell lines. The c-erbB-3 protein is a 160–180 kDa membrane-bound product which has now also been demonstrated immunohistochemically. In contrast to EGF receptor and the c-erbB-2 protein which are usually detected on cell membranes, immunohistochemical staining of c-erbB-3 is predominantly cytoplasmic. c-erbB-3 is normally present in breast epithelium, and in breast carcinomas overexpression has been reported in four studies [12–15] in 22, 28.8, 65 and 15% of cases, respectively. In two of the studies c-erbB-3 expression was associated with the presence of lymph node metastases, one showed a positive [12] and one an inverse [13] correlation. In the study showing an inverse correlation between the two, there was also a relationship with improved patient survival.

The third study was carried out on a node negative series of breast cancers and no significant associations were found. [14]. In the study by Travis and associates, strong c-erbB-3 staining was associated with large tumour size and aggressive tumour type [15]. In one of these studies [12], although expression of c-erbB-3 was greater in the tumour cells than in surrounding normal epithelium in a proportion of cases, gene amplification was not identified in these cases and it was suggested that overexpression resulted from increased levels of gene transcription.

Recent work strongly suggests that c-erbB-2 and c-erbB-3 function in a heterodimeric manner in normal tissues [16]. Because of the increased incidence of c-erbB-2 oncoprotein in ductal carcinoma *in situ*, as compared with infiltrating mammary carcinoma, and because it appears that these receptors may act in a heterodimeric manner, we thought it would be of interest to examine the expression of c-erbB-3 in a range of cases of ductal carcinoma *in situ* and to examine the possible relationship between c-erbB-2 and c-erbB-3 expression in these cases. We also examined the relationship between c-erbB-3, p53, hormone receptors and proliferation factors known to be interrelated with c-erbB-2 and EGFR.

MATERIALS AND METHODS

64 cases of pure DCIS were extracted from the files of the ICRF Clinical Oncology Unit at Guy's Hospital. These cases had all previously been the subject of an immunohistochemical study in which c-erbB-2, p53, progesterone receptor and Ki S1 (a marker of proliferation) were assessed [17] (Table 1). The results from this previous study are included in this study. All cases had been classified according to a recently proposed system based on cytonuclear and architectural differentiation [18]. This classification divides DCIS into three groups, poorly, intermediately and well differentiated. Poorly differentiated DCIS is composed of cells with very pleomorphic nuclei and little or no evidence of polarisation (architectural differentiation) around intercellular spaces or over papillae. Intermediately differentiated DCIS consist of cells with moderately pleomorphic nuclei and definite but usually inconspicuous polarisation of cells. The cells of well-differentiated DCIS have monomorphic nuclei and polarisation is conspicuous. Previously, the majority of cases of poorly differentiated DCIS were classified as comedo or mixed DCIS and well and intermediately differentiated as non-comedo or mixed DCIS.

The relationships between different tumour variables were calculated using the chi-squared test.

Immunohistochemistry

c-erbB-3 expression was assessed using the antibody RTJ2, a monoclonal antibody raised against a synthetic peptide from the cytoplasmic domain of c-erbB-3. It is of the IgG class and is able to be blocked by its immunising peptide [19]. Sections were dewaxed in xylene and rinsed in graded alcohols. Endogenous peroxidase was blocked by incubation in 1% hydrogen peroxide in methanol and then rinsed in tap water. After blocking with 5% normal rabbit serum, primary antibody at a dilution of 1 in 50 was applied for 30 min. The bound RTJ2 was then visualised using a standard ABC complex and DAB. c-erbB-3 staining was predominantly cytoplasmic and was generally homogeneous throughout a given lesion. Negative or equivocal staining was regarded as negative; weak but definite staining was considered to represent normal expression and strong staining was regarded as indicative of overexpression. This method of scoring was adopted as being consistent with that used previously [12, 13]. RTJ2 was omitted in negative control sections, and a known c-erbB-3 expressing invasive mammary carcinoma was included in each run as a positive control.

All cases were scored independently by two pathologists (LGB and RRM) and differences were resolved jointly over a double-headed microscope.

RESULTS

The 64 cases initially selected were chosen to include a range of types of DCIS. Insufficient material for adequate assessment was present in 7 cases, leaving 57 cases for the study. There were 32 poorly differentiated, 13 intermediately differentiated and 12 well differentiated cases.

c-erbB-3 Staining

Table 1 shows the results of c-erbB-3 staining. Positive staining with the c-erbB-3 antibody was seen in 40 of the 57 cases of DCIS (Table 1), of which 20 had strong staining and 20 weak staining (comparable to staining in normal tissue). Staining was cytoplasmic and generally homogeneous throughout the lesion in all cases except 4, where additional focal membrane staining was seen—all 4 cases were poorly differentiated and showed strong cytoplasmic staining. The membrane staining was only positive in the *in situ* lesion and not in adjacent normal tissue. Positive staining was present in 24 poorly differentiated (Figure 1), 8 intermediately differentiated and 8 well differentiated lesions.

Normal tissue was present in 49 of the cases and, in the majority, the staining in the normal tissue was equal to or less than in the *in situ* lesion (Figure 2). In 4 cases, however, the staining was stronger in the normal tissue. This occurred in 3 poorly and one well differentiated case. The staining was stronger in the DCIS than in adjacent normal tissue in 12 cases (7 poorly differentiated, one intermediately and 4 well differentiated). Equal staining was seen in the DCIS and adjacent normal tissue in 20 cases and both were negative in 13. No correlation was noted between DCIS type and pattern of staining.

Table 1. Staining patterns of different grades of DCIS

Type of DCIS	c-erbB-3			c-erbB-2*			p53*			PR*			Ki S1*		
	+	N	−	+	−	(NA)	+	−	(NA)	+	−	(NA)	H	L	(NA)
Differentiation															
Poorly $n=32$	12	12	8	22	7	(1)	17	4	(1)	3	24	(4)	29	2	(1)
Intermediate $n=13$	2	6	5	5	8		0	13		6	5	(2)	4	8	(1)
Well $n=12$	6	2	4	0	11	(1)	1	10	(1)	10	20		0	11	(1)

NA, not assessed (either insufficient material or technically unsatisfactory); N, expression levels equivalent to normal tissue; H, high proliferation rate; L, low proliferation rate.

*c-erbB-2, p53, progesterone receptor and Ki S1 results are taken from a previous publication [17]. Briefly, any definite membrane staining was scored positive with c-erbB-2 and likewise any nuclear staining was scored positive with p53 and progesterone receptor. For Ki S1, a cut-off point of 10% tumour nuclei positive was used to distinguish between low and high proliferation.

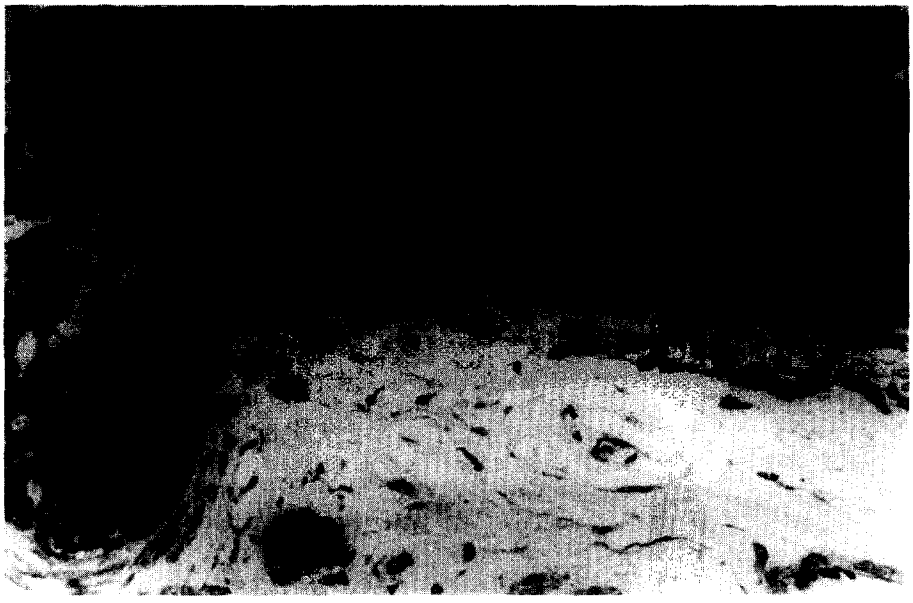

Figure 1. A section of poorly differentiated DCIS in which there is strong cytoplasmic staining and focal membrane staining (arrowhead) with RTJ2. Magnification × 400.

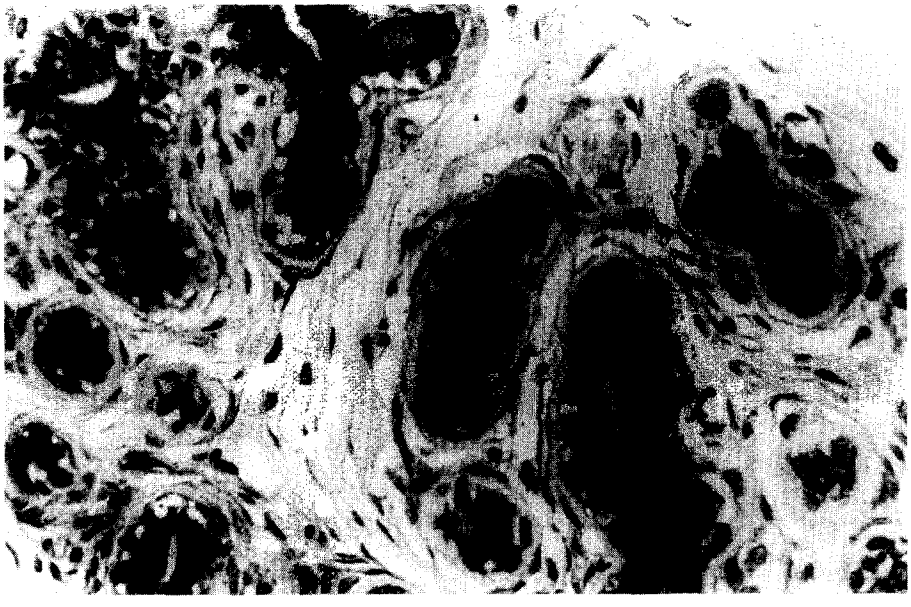

Figure 2. A section of normal breast tissue which was adjacent to an area of poorly differentiated DCIS. There is definite cytoplasmic staining with RTJ2 in the normal breast epithelium which was scored as normal expression of c-erbB-3. Magnification × 400.

Correlation of c-erbB-3 with c-erbB-2

Normal expression of c-erbB-3 (weak staining) was significantly associated with c-erbB-2 positivity, whereas overexpression of c-erbB-3 (strong staining) was associated with negative c-erbB-2 staining (Table 2; c-erbB-2 results obtained in a previous study [17]). Previously, a strong correlation between DCIS type and c-erbB-2 expression had been found [17]. No significant associations were observed between c-erbB-3 and expression of p53, PR and Ki S1.

DISCUSSION

In this study we have shown that in DCIS, c-erbB-3 is expressed in 20 out of 57 (35%) cases at levels regarded as normal expression and similar to that seen in the adjacent normal breast epithelium. Overexpression, as judged by increased intensity of staining, was present in another 20 (35%) of cases. In the remaining 17 (30%), c-erbB-3 was not detectable in the DCIS. These findings are very like those

Table 2. Relationship between c-erbB-3 and c-erbB-2 expression

c-erbB-3	c-erbB-2	
	−ve	+ve
−	11	5
N	3	15
+	12	7

$\chi^2 = 10.4$ (with Yates' correction for small numbers), df = 2, $P = 0.006$.

reported in infiltrating breast carcinomas in two earlier studies employing scoring methods similar to ours. Previously, Lemoine and associates [12] demonstrated overexpression in 22%, normal expression in 65% and absence of expression in 13% of cases, whereas Quinn and associates [13] found overexpression in 28.8%, normal expression in 32% and no expression in 39%. Travis and associates [15] found that only 15% of cases had strong staining of tumour cells, but they subdivided their cases into four categories, whereas the other groups used only three. The results of Gasparini and associates [14] were, however, different from the other three groups and from those reported here as they describe a much higher incidence of membrane staining with 65% of cases showing this pattern. Lemoine and associates [12] observed only one case with focal membrane staining, Travis and associates [15] recorded it in less than 1% of their cases and Quinn and associates [13] reported no membrane staining at all, whereas we saw focal membrane staining in four cases. This difference cannot be accounted for by the use of a different antibody as Gasparini and associates [14] used RTJ_1, the same antibody used by Travis and associates [15] and Quinn and associates [13] who used the antibody at a higher concentration. The cases of Gasparini and associates [14] were all node-negative and, since Quinn and associates [13] demonstrated a correlation between c-erbB-3 overexpression and lymph node-negative status, this may explain some of the difference in numbers of positive cases between the two series. It does not, however, explain the difference in cellular distribution of staining.

The predominantly cytoplasmic distribution of c-erbB-3 protein is well described in previous studies both in normal tissue [2] and in breast cancer [12, 13]. This pattern of staining is interesting in that it differs from that seen with antibodies to c-erbB-2 and EGFR. It is, however, consistent with the observation that each member of the type I growth factor receptor family has a membrane and a cytoplasmic component [19]. It is also consistent with the observation that only a minority of c-erbB-2 protein expressed by the c-erbB-2 overexpressing breast cancer cell line S-kBR-3 is present on the cell surface [20]. Membrane staining usually indicates very high levels of protein that are associated with gene amplification. c-erbB-3 Gene amplification has not been demonstrated in breast cancers.

We found no correlation between c-erbB-3 positivity and type of DCIS, p53 expression, progesterone receptor expression or proliferative activity. We did, however, find a significant correlation between c-erbB-3 and c-erbB-2 expression. c-erbB-2 positivity was correlated with normal c-erbB-3 expression and c-erbB-2 negativity correlated with c-erbB-3 overexpression. This inverse correlation is interesting in the light of Quinn and associates' [13] observation of an association between c-erbB-3 overexpression and lymph node-negative status and increased overall survival. It does not, however, fit with the findings of Lemoine and associates [12] who noted a correlation between c-erbB-2 positivity and c-erbB-3 positivity. These differences require further study and are perhaps not so surprising when one considers the apparently paradoxical difference in incidence of c-erbB-2 membrane positivity between *in situ* and infiltrating breast cancer.

The relationship between c-erbB-3 expression and histological grade was examined in the studies of Gasparini and associates [14], Lemoine and associates [12] and Travis and associates [15] and no association was observed by any group, which is consistent with our findings in DCIS, as is the lack of any association of c-erbB-3 expression with hormone receptor status also demonstrated by the former two groups [12, 14], and the lack of association with p53 expression in infiltrating ductal carcinoma, as observed by Lemoine and associates [12].

Further study is needed to elucidate c-erbB-3's precise role in normal growth and neoplasia in the breast epithelium. If Quinn and associates' [13] finding of an association between c-erbB-3 overexpression in infiltrating ductal carcinoma and lymph node-negative status and increased overall survival can be corroborated, then our findings of an inverse association between c-erbB-3 and c-erbB-2 overexpression would further support this evidence that overexpression of c-erbB-3 may identify a better prognostic group of cases in breast cancer. This hypothesis is further supported by the findings of Binder and associates [21] that c-erbB-3 expression correlates closely with that of BCL2 in breast cancer. They and others have found that in infiltrating breast cancer, BCL2 is a marker of low proliferation, good differentiation and favourable prognosis [21, 22].

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Acknowledgements—The authors thank Paul Smith for statistical assistance and Lindsey Hinds for preparing the manuscript.