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c-*erb*B-4 protein expression in human breast cancer

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Summary The Type 1 family of growth factor receptors includes epidermal growth factor receptor (EGFR), c-*erb*B-2, c-*erb*B-3 and c-*erb*B-4. Overexpression of the first two members is associated with poorer prognosis in patients with breast carcinoma. In this study we examined the expression of c-*erb*B-4 protein using the monoclonal antibody HFR-1. A total of 127 consecutive cases of primary operable invasive breast carcinoma presenting between 1975 and 1977 were studied. All patients were managed by simple mastectomy or conservation surgery with radiotherapy and no adjuvant therapy given. Long-term follow-up was maintained. Routine, formalin-fixed, paraffin-embedded tumour samples were used and sections were stained immunohistochemically using the Duet StreptABC method. Immunoreactivity was classified using a simple semi-quantitative scoring method. Protein expression was generally low but definite positive cytoplasmic, membranous and nuclear reactivity was identified in 58%, 41% and 25% of cases respectively. Expression at all three sites demonstrated significant inverse associations were histological grade. In addition, membrane accentuation correlated inversely with the Nottingham Prognostic Index (NPI), while cytoplasmic reactivity showed a positive association with c-*erb*B-3 expression. No significant associations were found with disease-free interval or survival. The results of this study demonstrate that higher levels of c-*erb*B-4 protein expression are associated with a more differentiated histological phenotype in contrast to the other members of the Type 1 family. Larger series with extended follow-up will be required to ascertain definitively the prognostic value of c-*erb*B-4 expression in breast carcinoma. © 2000 Cancer Research Campaign

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Four distinct transmembranous glycoprotein receptors have been identified as members of the Type 1 growth factor receptor family, a class of oncogenes prevalent in several solid tumours, especially those of the breast (reviewed in Mason and Gullick, 1995). They are the epidermal growth factor receptor (EGFR), c-*erb*B-2, c-*erb*B-3 and c-*erb*B-4 (alternatively, authors may use the HER terminology instead, e.g. HER1 etc). Receptor heterodimerization (Earp et al, 1995), a unique feature of the Type 1 receptors, enables multifunctional intracellular responses to be generated with differing strengths and specificities dependent on the ligand type, specific receptor combination or relative level of receptor expression. Previous studies have shown that overexpression of EGFR, c-*erb*B-2 and c-*erb*B-3 is associated with worse disease prognosis in breast cancer (Slamon et al, 1987; Lewis et al, 1990, 1996; Lovekin et al, 1991; Klijn et al, 1992; Lemoine et al, 1992; Travis et al, 1996). The most common causes of Type 1 receptor overexpression are gene amplification and/or an increased gene transcription rate. Much interest has been generated recently by the possibility of utilizing the Type 1 receptors as prognostic indicators in breast carcinomas and the development of treatments in the form of kinase inhibitors and monoclonal antibodies (Bridges, 1996).

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The most recently identified member of this family, c-*erb*B-4, was cloned in 1993 (Plowman et al, 1993). The c-*erb*B-4 gene is localized on chromosome 2q33.3–34 (Zimonjic et al, 1995) and encodes a protein of 1283 amino acids with a molecular weight of 180 kDa after post-translational glycosylation. Although EGFR, c-*erb*B-2 and c-*erb*B-3 are all expressed as soluble, truncated receptors as a result of mRNA splicing, c-*erb*B-4 exists as two full-length variants possessing alternative extracellular juxtamembrane sequences which are differentially sensitive to proteolysis (Elenius et al, 1997*b*). Protein kinase C activators (e.g. 12-*O*-tetradecanoylphorbol-13-acetate, platelet-derived growth factor) have been shown to induce rapid and extensive selective proteolytic cleavage of the c-*erb*B-4 receptor molecule, yielding a membrane-dependent 80-kDa fragment consisting of the entire cytoplasmic and transmembrane domain, and a soluble 120-kDa ectodomain fragment which is released into the extracellullar space (Vecchi et al, 1996; Vecchi and Carpenter, 1997).

Expression of the c-*erb*B-4 receptor is found in several breast adenocarcinoma cell lines (e.g. MDA-MB-453, T47-D, BT-474 and H3396), and in a range of normal human fetal and adult tissues at the mRNA (Plowman et al, 1993*a*); Srinivasan et al, 1998) and protein levels (Srinivasan et al, 1998), notably in the brain and heart. More specifically, c-*erb*B-4 has also been found expressed at the post-synaptic membrane of neuromuscular synapses (Zhu et al, 1995). The c-*erb*B-4 receptor is activated by ligands known as heregulins or neuregulins (Plowman et al, 1993*b*). These are encoded by three separate genes, NRG-1 (the human homologue of the mouse NDF gene) found at chromosome 8p12–21 (Orr-Urteger et al, 1993), and two more recently described genes NRG-2 (also known as Don1 and NTAK) (Busfield et al, 1997; Carraway et al, 1997; Chang et al, 1997; Higashiyama et al, 1997)

and NRG-3 located on chromosome 10q22 (Zhang et al, 1997). The first two of these genes are known to produce multiple proteins possessing different structural domains as a consequence of mRNA splicing (Wen et al, 1994). In addition, several ligands originally described as activators of EGFR are now known also to bind directly to and to stimulate the activity of c-*erb*B-4. Examples of these include betacellulin (Riese et al, 1996), heparin-binding EGF (Elenius et al, 1997*a*) and epiregulin (Komurasaki et al, 1997).

Consistent with its pattern of expression, knock-out mice studies have suggested that c-*erb*B-4 plays an essential role as an in vivo regulator of both cardiac muscle differentiation and axonal guidance in the central nervous system (Gassmann et al, 1995; Zhu et al, 1995). Gassman et al reported that mice lacking c-*erb*B-4 died during mid-embryogenesis from the aborted development of myocardial trabeculae in the heart ventricle. In addition, alterations of the innervation of the hindbrain were also reported.

The relationship between c-*erb*B-4 expression and cancer prognosis is, at present, poorly understood. Srinivasan et al (1998) found that the immunohistochemical expression of c-*erb*B-4 tended to be higher in normal tissues than in most of the types of cancers studied. Similar results have been obtained by Lyne et al (1997) in prostate cancer and by Baccus et al (1996) breast carcinoma, the latter also demstrating divergent correlates between c-*erb*B-2 and c-*erb*B-4 (Baccus et al, 1996). In this study, we demonstrated the immunohistochemical expression of c-*erb*B-4 in breast cancer using the HFR-1 monoclonal antibody. We also examined for possible correlations between its expression and various indicators of clinical prognosis, with a view to assessing the possible value of c-*erb*B-4 expression as an independent prognostic indicator in breast carcinomas.

MATERIALS AND METHODS

Patients

A consecutive series of 133 patients presenting with primary operable invasive breast carcinoma to a single surgeon (RW Blamey) at the Nottingham City Hospital between 1975 and 1977 was examined. The treatment administered involved mastectomy (simple or subcutaneous) or breast conservation surgery with radiotherapy. During surgery itself, lymph nodes were sampled by the triple node biopsy technique (Todd et al, 1987) and the tumour subsequently staged. No systemic adjuvant therapy was administered. Long-term follow-up of patients was maintained after surgery by regular visits to the clinic. These were conducted at 3-monthly intervals for 18 months, and then every 6 months for 5 years, and then annually. Any major tumour recurrence was recorded as either loco-regional (recurrences requiring some form of major treatment, e.g. radiotherapy) or distant (confirmed radiologically by isotope scan or liver function tests). The disease-free interval was taken as the time (in months) from the date of primary treatment to the first loco-regional or distant recurrence. The overall survival was defined as the time (in months) from the date of primary treatment to the time of death.

Of the 133 patients entered into the study, six were removed because data on certain prognostic variables was not available from the computer database. This leaves a total of 127 patients. All patients were aged 70 years or below.

Tissue preparation

The excised tumours were sliced and measured in three perpendicular planes. This was done immediately post-excision to reduce the possibility of autolytic artifacts. The largest of these three dimensions constituted the actual tumour size recorded. Fresh tumour blocks were snap-frozen or fixed in neutral buffered formalin and then embedded in paraffin wax for receptor assay, immunochemistry and archival storage. Histological grade (Elston and Ellis, 1991), tumour type (Ellis et al, 1992), menopausal status (Todd et al, 1987) and vascular invasion (Pinder et al, 1994) were recorded for each tumour sample. A Nottingham Prognostic Index (NPI) score was calculated for each patient based on the following equation (Haybittle et al, 1982):

$$I = 0.2 \times \text{size (in cm)} + \text{stage} + \text{grade}$$

Patients were then assigned into the good ($I \leq 3.4$), moderate ($3.4 < I \leq 5.4$) or poor ($I > 5.4$) prognostic groups according to the score obtained. In addition, assays to determine the oestrogen receptor (ER) status of each tumour section were conducted (at the Tenovus Institute, Cardiff) by the dextran-coated charcoal method. Tumours were classified ER-positive if they contained more than 5 femtomoles of specific oestradiol binding per mg of cytosol protein (Todd et al, 1987). Finally, tumour sections were divided into four different histological groups with different prognostic indications (Pereira et al, 1995):

Group 1: Tubular, tubulo-lobular, mucoid and invasive cribriform carcinoma.
Group 2: Tubular mixed, mixed ductal with special type, and alveolar lobular carcinoma.
Group 3: Classical lobular, medullary, atypical medullary and lobular mixed.
Group 4: Ductal NST, solid lobular, mixed ductal and lobular carcinoma.

Immunohistochemistry

The tumour samples were then stained immunohistochemically with the HFR-1 monoclonal antibody using the Duet StreptABC Method (A = streptavidin, B = biotinylated peroxidase, C = biotinylated goat anti-mouse/rabbit immunoglobulin) with DAB as the chromogen and haematoxylin as the counterstain. HFR-1 is a mouse monoclonal antibody raised against residues 1249–1264 of the c-*erb*B-4 cytoplasmic domain. It has been shown to recognize c-*erb*B-4 by immunoprecipitation, Western blotting and immunostaining of cytocentrifuge preparations of NIH3T3 cells transfected with c-*erb*B-4 (Srinivasan et al, 1998). Initially, the antibody was supplied at a concentration of 1.5 mg ml^{-1}. After repeated trials to find a suitable dilution concentration with the most appropriate balance of protein expression and background staining, a ratio of 1/700 was decided upon, giving a final antibody concentration of approximately 2.14 μg ml^{-1}. Sections of normal breast tissue were used as positive controls, and omission of the primary antibody and blockade of the primary antibody with peptide 96.4 were used as negative controls.

The final protein expression was classified using a simple semi-quantitative scoring method, based on three distinct sites of reactivity. To increase the objectivity and accuracy of the scoring process, the shade of brown reaction product expressed in the cytoplasm was compared to a colour chart available commercially as the ICI Dulux Colour Palette™. The scoring criteria used are as follows:

1. Nuclear reactivity
   0 = No reactivity
   1 = Pale brown and finely granular
   2 = Dark opaque brown
2. Cytoplasmic reactivity
   0 = Clear cytoplasm
   1 = First shade of colour chart
   2 = Second shade of colour chart
   3 = Third shade of colour chart
3. Membrane accentuation
   0 = No membrane accentuation
   1 = 0–10% of cells demonstrating membrane accentuation
   2 = >10% of cells demonstrating membrane accentuation.

To ensure consistency of scoring, pairs of sections with similar scores were randomly chosen for re-assessment. The observed reactivities were compared between the two sections, and scores were re-adjusted if necessary. Approximately 20 sections were scored blind by two pathologists (IOE and HD) using the same scoring criteria. Results from Spearman rank correlation analysis indicated that the inter-observer agreement was good for cytoplasmic (Rho = 0.647) and membrane (Rho = 0.510) reactivity.

Statistical analysis

The semi-quantitative scores assigned to each patient case were entered into a statistical software program which contained information on a variety of factors recorded routinely (see Table 1). This is the fourth in a series of similarly designed studies investigating the individual expression of Type 1 receptors in breast carcinoma patients, enabling us to utilize data on the expression of EGFR (Lewis et al, 1990), c-*erb*B-2 (Lovekin et al, 1991) and c-*erb*B-3 (Travis et al, 1996) for statistical analyses. Univariate χ^2 analyses were conducted for each of the sites of reactivity mentioned and several known prognostic factors in breast cancer. Survival data were examined using the Kaplan–Meier method and the log-rank (Mantel–Cox) test. All analyses were carried out using standard commercial statistical computer software (Stat View 4.1).

RESULTS

In this study, c-*erb*B-4 immunoreactivity using HFR-1 antibody was localized to ductal and lobular units (both normal and malignant) and accompanied by an acceptable level of background staining. Within malignant cells, immunoreactivity was predominantly cytoplasmic, although nuclear and membrane reactivity were also present. The level of c-*erb*B-4 protein expression in tumour cell populations was generally low (as such the scoring process more difficult than initially envisaged), but definite cytoplasmic, membrane and nuclear reactivity (score 2 or above) was identified in 58%, 41% and 25% of cases respectively. Although statistically significant correlations were demonstrated between these three sites of expression, careful analysis of the contingency tables revealed no definite pattern of interaction.

Chi-square tests

Statistically significant associations were demonstrated between histological grade and c-*erb*B-4 nuclear (Table 1 and 4; Figure 4), cytoplasmic (Tables 2 and 5; Figure 5) and membrane expression (Tables 3 and 6; Figure 6). In contrast to the other Type 1

Table 1 Results of chi square tests for c-erbB-4 nuclear reactivity

Prognostic factor	Cut-off points	χ^2	χ^2 *P*-value
Grade	1, 2, or 3	10.781	**0.0291**[a]
Lymph node stage	1, 2, or 3	5.888	0.2077
Local recurrence	Absent or present	0.048	0.9761
Distant metastases	Absent or present	0.245	0.8845
Vascular invasion	None or definite	0.135	0.9346
Tumour size	≤ 1.5 or > 1.5 cm	1.174	0.5559
NPI groups	Good, moderate, or poor prognostic group	3.112	0.5393
Histological type	1, 2, 3, 4 or 5	6.814	0.3384
Age	< 40, 40–49, 50–59, 60–69 or > 70 years	5.919	0.6563
Menopausal status	Pre- or post-menopausal	2.324	0.3128
ER status	Negative or positive	2.486	0.2886
EGFR	Negative, mild, moderate or strong immunoreactivity[b]	6.944	0.3260
C-erbB-2	Negative or positive[b]	1.681	0.4314
C-erbB-3	Negative, mild, moderate or strong immunoreactivity[b]	12.078	0.0602

See under Materials and Methods for detailed description of criteria for cut-off points. [a]Figures highlighted indicate statistically significant *P*-values ($P < 0.05$). [b]For further information on EGFR, c-*erb*B-2 and c-*erb*B-3 scoring criteria, refer to Lewis et al (1990), Lovekin et al (1991) and Travis et al (1996) respectively.

Table 2 Results of chi square tests for c-erbB-4 cytoplasmic reactivity

Prognostic factor	χ^2	χ^2 *P*-value
Grade	13.141	**0.0408**
Lymph node stage	13.554	**0.0350**
Local recurrence	0.566	0.9042
Distant metastases	0.662	0.8822
Vascular invasion	1.195	0.7541
Tumour size	4.767	0.1897
NPI groups	6.567	0.3628
Histological type	9.456	0.3963
Age	16.303	0.1778
Menopausal status	1.906	0.5922
ER status	1.132	0.7694
EGFR	15.012	0.0906
C-*erb*B-2	2.514	0.4727
C-*erb*B-3	23.913	**0.0044**

Table 3 Results for c-*erb*B-4 membrane accentuation

Prognostic factor	χ^2	χ^2 *P*-value
Grade	9.935	**0.0415**
Lymph nodes stage	3.016	0.5552
Local recurrence	3.500	0.1738
Distant metastases	0.094	0.9543
Vascular invasion	0.785	0.6753
Tumour size	1.999	0.3681
NPI groups	10.460	**0.0334**
Histological type	6.345	0.3857
Age	8.900	0.3508
Menopausal status	2.166	0.3385
ER status	1.287	0.5255
EGFR	8.717	0.1901
C-*erb*B-2	0.761	0.6835
C-*erb*B-3	7.012	0.3197

Table 4 C-*erb*B-4 nuclear reactivity vs grade (*P* = 0.0291)

	1	(%)	2	(%)	3	(%)	Row Total
0	2	(15)	12	(24)	26	(41)	40
1	4	(31)	27	(52)	24	(38)	55
2	7	(54)	12	(24)	13	(21)	32
Column total	13	(100)	51	(100)	63	(100)	127

Table 5 C-*erb*B-4 cytoplasmic reactivity vs grade (*P* = 0.0408)

	1	(%)	2	(%)	3	(%)	Row total
0	0	(0)	4	(8)	7	(11)	11
1	1	(8)	22	(43)	19	(30)	42
2	9	(69)	16	(31)	32	(51)	57
3	3	(23)	9	(18)	5	(8)	17
Column total	13	(100)	51	(100)	63	(100)	127

Table 6 C-erbB-4 membrane accentuation vs grade (*P* = 0.0415)

	1	(%)	2	(%)	3	(%)	Row total
0	5	(38)	12	(24)	27	(43)	44
1	0	(0)	15	(29)	16	(25)	31
2	8	(62)	24	(47)	20	(32)	52
Column total	13	(100)	51	(100)	63	(100)	127

receptors (Lewis et al, 1990; Lovekin et al, 1991; Travis et al, 1996), the relationship is an inverse one, i.e. c-*erb*B-4 expression tended to favour well differentiated tumours. In addition, c-*erb*B-4 membrane accentuation demonstrated an inverse association with the NPI: fewer tumours expressed c-*erb*B-4 with worsening prognostic grouping (Tables 3 and 7; Figure 7). Statistically significant associations were also seen between c-*erb*B-4 cytoplasmic expression and both c-erbB-3 expression and tumour stage (Table 2), although no distinct trend was identifiable from contingency tables (not shown). No correlations were found between the three sites of immunoreactivity for c-*erb*B-4 and the prognostic factors and follow-up events viz. overall survival or disease-free interval (DFI), local recurrence, distant metastasis, vascular invasion, tumour size, histological type, age, menopausal status and ER status.

DISCUSSION

This study has demonstrated that c-*erb*B-4 protein expression can be identified in both normal and malignant breast epithelium. Expression in adenocarcinoma is most frequently cytoplasmic, with nuclear and membrane localization seen in a small proportion of cases. Other studies on the immunohistochemical expression of c-*erb*B-4 in tumours of the prostate (Lyne et al, 1997), thyroid (Faksvag Haugen et al, 1996) and in medulloblastoma (Gilbertson et al, 1997) also demonstrated predominantly cytoplasmic reactivity. Srinivasan et al (1998) however showed occasional nuclear staining in paraffin-embedded and in frozen sections of some

Table 7 C-*erb*B-4 membrane accentuation vs NPI (*P* = 0.0334)

	Good prognostic group	(%)	Average prognostic group	(%)	Poor prognostic group	(%)	Row Total
0	10	(34)	19	(28)	15	(50)	44
1	3	(10)	23	(34)	5	(17)	31
2	16	(55)	26	(38)	10	(33)	52
Column total	29	(100)	68	(100)	30	(100)	127

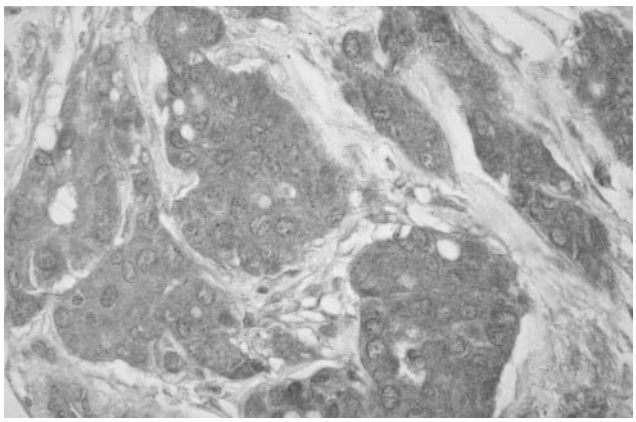

Figure 1 C-*erb*B-4 cytoplasmic expression: the cytosol of this invasive carcinoma shows dense immunoreactivity (× 1600 original magnification)

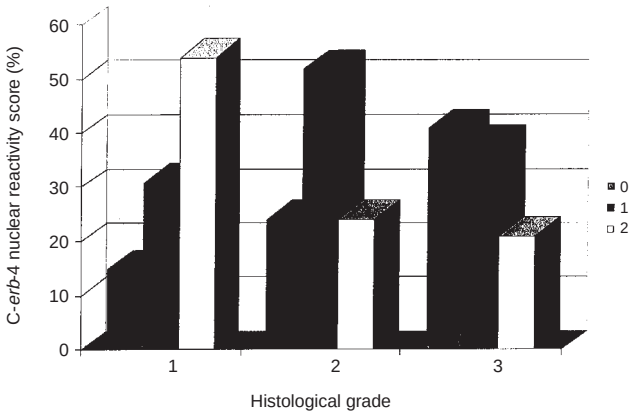

Figure 4 With increasing histological grade, the percentage of tumour samples demonstrating score 0 (absent) nuclear reactivity increases, while that of tumours demonstrating score 2 (high) nuclear reactivity decreases

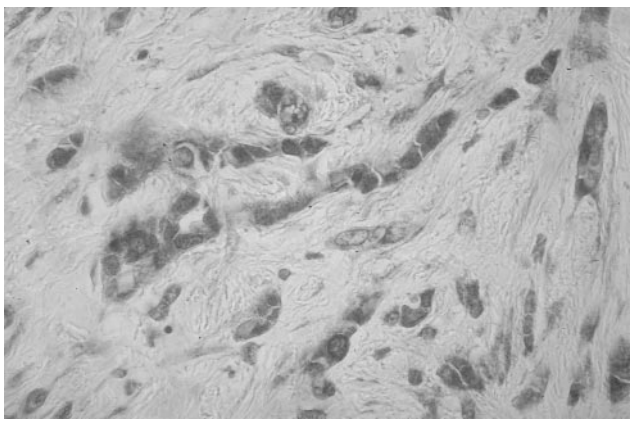

Figure 2 C-*erb*B-4 nuclear expression: this invasive carcinoma shows some cytoplasmic reactivity but distinct nuclear reactivity is also visible (× 1600 original magnification)

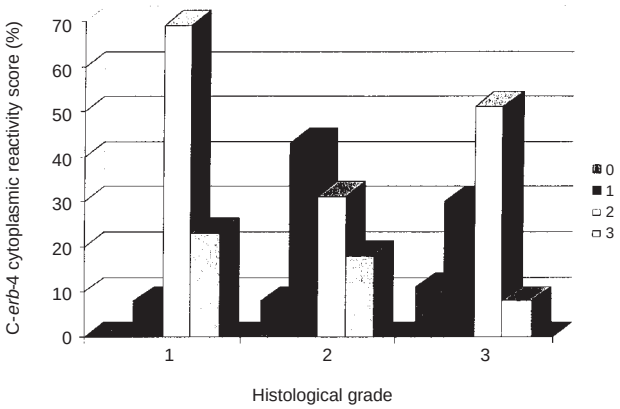

Figure 5 Increasing percentage of tumour samples demonstrating score 0 cytoplasmic reactivity with increasing histological grade, and vice versa for score 3 cytoplasmic reactivity

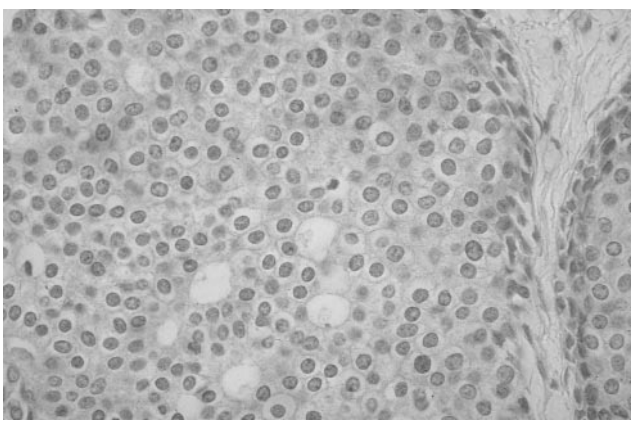

Figure 3 C-*erb*B-4 membrane accentuation: the cellular membranes show light but distinct reactivity in this case of low grade cribriform DCIS (× 1600 original magnification)

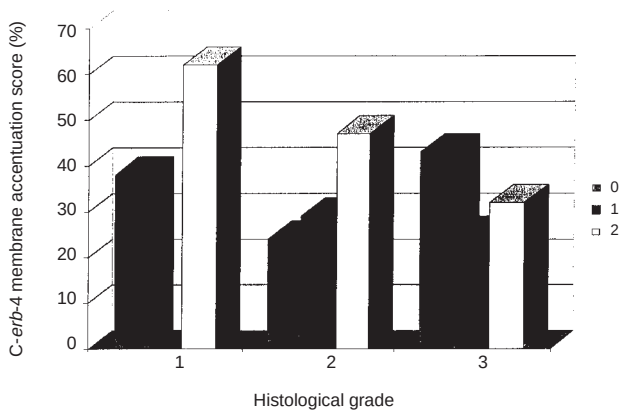

Figure 6 Decreasing percentage of tumour samples demonstrating score 2 membrane accentuation with increasing histological grade

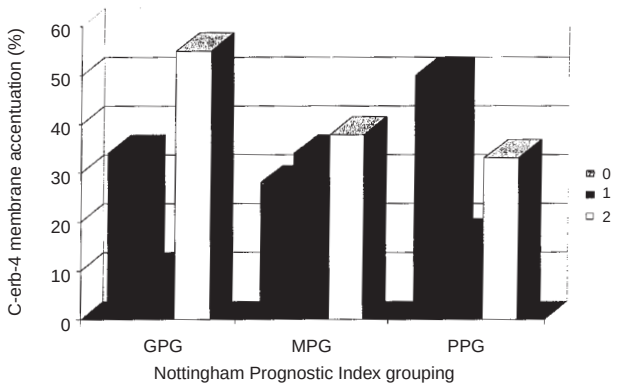

Figure 7 Decreasing percentage of tumour samples demonstrating score 2 membrane accentuation with worsening prognostic grouping. NPI, Nottingham Prognostic Group; GPG, good prognostic group; MPG, moderate prognostic group; PPG, poor prognostic group

normal tissues, including the breast. In this study, tumours with little or no expression of c-*erb*B-4 tended to possess characteristics associated with poorer prognosis in breast cancer i.e. histological grade 3 and the NPI poor prognosis grouping (PPG). These results suggest that an underlying mechanism of underexpression is present, which is a significantly divergent finding. Studies on the other members of the Type 1 family have shown repeatedly that overexpression of the receptor is correlated with poorer prognosis and shorter survival (Slamon et al, 1987; Lewis et al, 1990, 1996; Klijn et al, 1992; Lemoine et al, 1992).

Srinivasan et al (1998) described a similar pattern of receptor underexpression in their study on the expression of c-*erb*B-4 in nine common human malignancies using the same monoclonal antibody, in which they found less than normal expression of c-*erb*B-4 in 40–80% of these malignancies and in 100% of squamous cell carcinomas of the head and neck. Another study reported that loss of c-*erb*B-4 expression also occurs in prostatic cancers as compared to benign lesions and normal prostatic tissue (Lyne et al, 1997). However, a study on papillary thyroid carcinomas found overexpression of c-*erb*B-4 in 64% of cases (Faksvag Haugen et al, 1996). Knowlden et al (1998) examined c-*erb*B-3 and c-*erb*B-4 mRNA expression in human breast carcinoma and found increased expression of both to be associated with the prognostically favourable ER phenotype.

With regard to the other members of the Type 1 family, gene amplification and/or an increase in gene transcription have been shown to be responsible for protein overexpression in tumour samples (Kageyama et al, 1988; Hollywood and Hurst, 1993). The exact role of these receptors in the pathogenesis of breast cancer is still being elucidated, but we can speculate that overexpression of an oncogene encoding a putative growth factor receptor would give a growth advantage to the cells expressing it. In the case of c-*erb*B-4, however, underexpression of the receptor seems to be correlated with poorer clinical prognosis of breast cancer, possibly suggesting a mechanism akin to that of tumour suppressor genes, whereby normal suppression of mitogenesis is obliterated by loss of function mutations.

The nuclear and cytoplasmic expression of c-*erb*B-4 raises some intriguing issues, since members of the Type 1 family have long been shown to be transmembranous glycoprotein receptors. The cytoplasmic reactivity observed in this study may represent the presence of the c-*erb*B-4 glycoprotein in the cytoplasm due to reduced ligand-induced metabolic turnover and down-regulation of the receptor. While binding of EGF to the EGFR rapidly induces the clustering of ligand-receptor complexes in clathrin-coated pits, internalization of the complexes, and finally lysosomal degradation of both EGF and its receptor, the other Type 1 members, including c-*erb*B-4, are not subject to rapid internalization and down-regulation (Baulida et al, 1996). Putative internalization codes (e.g. sequences ^{996}QQGFF and ^{973}FYRAL) which have been discovered within the cytoplasmic domain of EGFR are not preserved in the cytoplasmic domain of c-*erb*B-4 (Chang et al, 1993). Experiments are underway to address this issue using GFP-tagged receptors and digital microscopy.

The nuclear expression, on the other hand, is something of an enigma, although it has been demonstrated that several of the neuregulin isoforms do contain putative nuclear targeting sequences near their amino termini (Holmes et al, 1992). NRG1-β has also been reported to be internalized and efficiently transported to the nucleus in breast cancer cells (Li et al, 1996). Further elucidation of the complex signal transduction cascades elicited by the Type 1 receptors may explain the nuclear expression of a receptor that at present has not been shown to be translocated to the nucleus.

The value of a c-erbB-4 immunohistochemical assay as a prognostic indicator in breast cancer is doubtful. As the spectrum of expression of c-*erb*B-4 in this study was shown to be relatively limited, semi-quantitative scoring would present difficulties with inter-observer variability. Although distinguishing between negative and positive expression may seem more easily reproducible, our analyses demonstrate that there is no significant correlation with patient prognosis even with this method (data not shown). Furthermore, c-*erb*B-4 expression demonstrated no significant association with either patient survival or disease-free interval.

In conclusion, the results of this study indicate that higher levels of c-*erb*B-4 protein expression are associated with a more differentiated histological phenotype. Immunoreactivity with the use of HFR-1 is generally low, although it does appear to have three separate components: cytoplasmic (which is predominant), nuclear and membranous. However, the immunoreactive expression of c-*erb*B-4 appears to be of limited prognostic value in breast carcinoma, although it may well prove to be of greater prognostic value in human malignancies other than the breast. Further studies may examine this possibility as well as the role of receptor heterodimerization in cancer progression and development. Indeed, a recent study on childhood medulloblastoma reported that the presence of c-*erb*B-4 expression with c-*erb*B-2 was indicative of tumour aggressiveness and poor prognosis (Gilbertson et al, 1997). We may discover that receptor heterodimerization among the Type 1 family of receptors plays a far more important role in human oncogenesis than individual receptors themselves (Earp et al, 1995). Further studies will be enhanced by our ability to measure all four Type 1 receptors in parallel with their known ligands and ligand variants.

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