Malaysian J Pathol 1998; 20(1): 19 - 23

C-erbB-2 oncoprotein amplification in infiltrating ductal carcinoma of breast relates to high histological grade and loss of oestrogen receptor protein

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Abstract

Eighty-six infiltrating ductal carcinoma of breast were studied by the standard avidin-biotin complex imrnunoperoxidase method on formalin-fixed, paraffin-embedded tissue sections, for oestrogen receptor (ER) protein and c-erbB-2 oncoprotein expression. They were categorized according to the modified Bloom and Richardson criteria into three histological grades. 21% tumours were ER positive while 44% were c-erbB-2 positive. Of ER positive tumours, 33.3% were c-erbB-2 positive whereas the c-erbB-2 positivity rate was much higher (47.1%) in ER negative tumours. Only 16% of c-erbB-2 positive tumours were ER positive while 25% of c-erbB-2 negative tumours were ER positive. This negative relationship between ER and c-erbB-2 expression was statistically significant (Mc Nemar's test, $p<0.005$). The ER positivity rate did not vary significantly with histological grade. However, c-erbB-2 overexpression was significantly more prevalent in grade III tumours compared with grade I and II tumours (Chi-square test, $p<0.005$).

Since the c-erbB-2 oncogene has extensive structural homology to the epidermal growth factor receptor (EGFR) gene, we expect that c-erbB-2 oncoprotein would share functional similarities with EGFR leading to both loss of oestrogen receptor and poor prognosis in breast cancer. Its overexpression can be expected to relate to more aggressive tumour proliferation and may explain its correlation with high histological grade, a known indicator of aggressive cancer behaviour. As there is no indication that ER protein activity contributes to advancement in histological grade, it would appear that cellular dedifferentiation precedes ER loss during malignant transformation.

It has been mooted that ER positive breast cancers which also show c-erbB-2 oncoprotein overexpression have a poorer response to hormonal therapy. The use of this parameter in the routine assessment of breast cancer patients may identify subsets of patients for more aggressive therapy.

Key words: Malignancy, prognostic factors, immunoperoxidase, cancer therapy.

INTRODUCTION

In most communities, breast carcinoma has emerged as one of the most common malignancies in females. Hence, assessment for tumour parameters that may relate to prognosis and selection of breast cancer patients for various therapeutic options has become an important activity in diagnostic pathology laboratories. Determination of oestrogen receptor (ER) expression is now a routine assessment that aids in selection of patients who may benefit from hormonal therapy.[1] On the other hand, amplification of the c-erbB-2 oncoprotein is a parameter being mooted as an indicator of poor survival.[2] Overexpression of the c-erbB-2 oncoprotein has been reported in 15% to 40% of invasive breast carcinomas,[3-6] and may modify the responsiveness of oestrogen receptor positive breast cancers to antioestrogen therapy, while requiring more aggressive chemotherapy.[5,7] Studies have shown that both oestrogen receptor protein and c-erbB-2 oncogene product can be detected irnmunohistochemically on formalin-fixed, paraffin-embedded tumom issue.[6,8] Previous studies from this centre have also validated that immunoperoxidase staining for oestrogen receptor protein correlates well with ER status as determined by cytosolic biochemical assay? Also, immunoperoxidase staining for c-erbB-2 oncogene product has been shown to correlate well with c-erbB-2 gene amplification.[9] We have endeavoured to investigate the association between oestrogen receptor protein status and c-erbB-2 oncoprotein overexpression in an attempt to better understand the interaction of these two parameters in breast cancer pathobiology.

MATERIALS AND METHODS

Eighty-six (86) cases of infiltrating ductal carcinoma of breast diagnosed histologically at the

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Department of Pathology, University of Malaya were retrieved from the Department files. Histological sections from these cases, whether from biopsies or mastectomies, were reviewed and reconfirmed as showing infiltrating ductal carcinoma of no special type, while patient data was analysed to eliminate duplication of cases due to repeated biopsies and subsequent mastectomies. Histological sections were reviewed and the tumours scored and graded into three categories namely Grade I (well-differentiated), Grade II (moderately-differentiated) and Grade III (poorly-differentiated), according to the modified Bloom and Richardson criteria.[7]

Further histological sections, cut at 4μm, were made from the most representative paraffin-embedded tumour block. Consecutive sections were mounted on silane-coated glass slides and stained, using the standard avidin-biotin complex immunoperoxidase (IP) method, for tumour immunoreactivity against oestrogen receptor (ER) protein and c-erbB-2 oncoprotein according to the following specifications: for ER protein, the primary antibody was a monoclonal antibody (DAKO-ER 1D5) used at 1:80 dilution with microwave antigen retrieval; for c-erbB-2 protein, the primary antibody was a monoclonal antibody to c-erbB-2 protein (1:30 dilution) obtained commercially from Triton Biosciences Inc., staining with overnight incubation. The staining methods for these parameters have been previously described.[6,8]

In accordance with the criterion used in other studies,[6,8] a amour was considered ER positive by IP when more than 10% of tumour nuclei showed positive staining for ER (Fig. 1). Cytoplasmic positivity was not regarded as a true positive expression of ER. Tumours with at least 5% of neoplastic cells exhibiting membrane immunoreactivity for c-erbB-2 were regarded as positive for c-erbB-2 expression (Fig. 2). Cytoplasmic or nuclear positivity were not accepted as positive expressions.

RESULTS

Table 1 summarises the correlation between ER and c-erbB-2 expression in the 86 infiltrating ductal carcinoma tested. 21% of cases were ER positive while 44% were c-erbB-2 positive. Of ER positive tumours, 33.3% were c-erbB-2 positive whereas the c-erbB-2 positivity rate was much higher (47.1%) in ER negative tumours. Conversely, only 16% of c-erbB-2 positive tumours were ER positive while 25% of c-erbB-

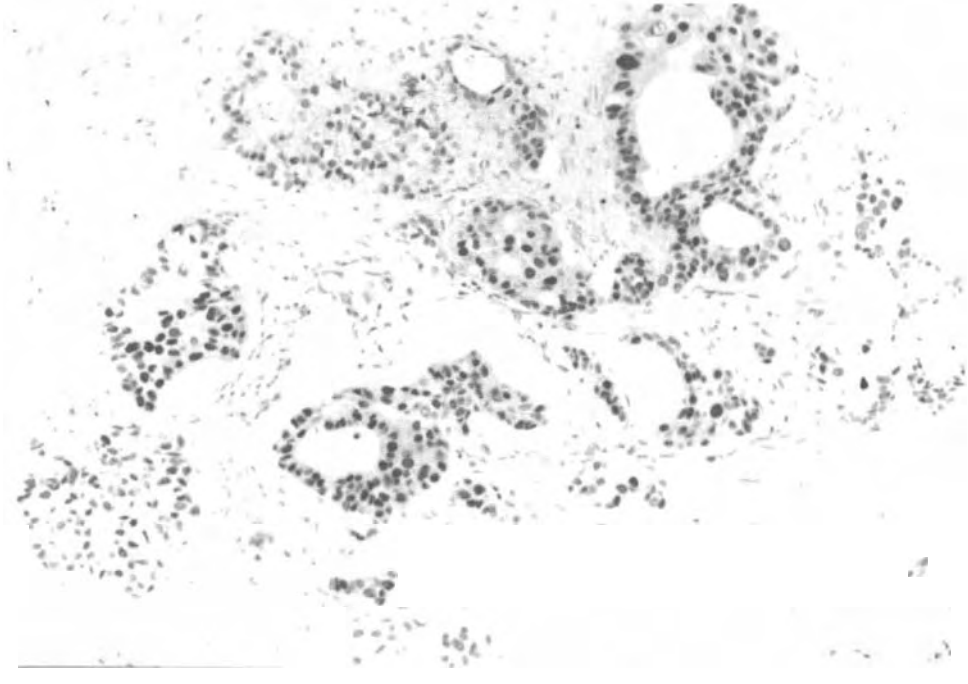

FIG. 1: Photomicrograph of infiltrating ductal carcinoma of breast showing strong nuclear positive staining for oestrogen receptor protein. Avidin-biotin-complex immunoperoxidase staining using monoclonal antibody against human ER protein X 150.

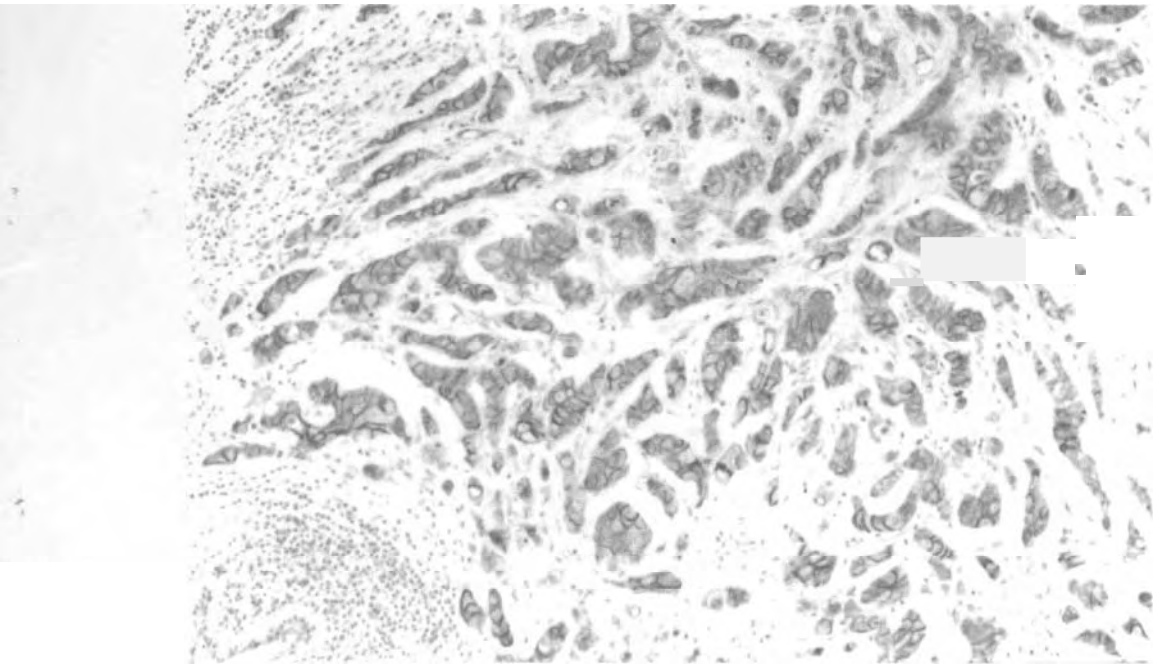

FIG. 2: Infiltrating ductal carcinoma of breast showing strong cytoplasmic membrane positivity for c-erbB-2 oncoprotein. Avidin-biotin-complex immunoperoxidase staining X 150.

2 negative tumours were ER positive. This negative relationship between ER and c-erbB-2 expression was statistically significant (Mc Nemar's test, $p<0.005$).

The correlation between ER and c-erbB-2 expression and histological grade are presented in Tables 2 and 3. The ER positivity rate did not vary significantly with histological grade. However, c-erbB-2 overexpression was significantly more prevalent in grade III tumours compared with grade I and II tumours (Chi-square test, $p<0.005$).

DISCUSSION

The relatively high proportion (44%) of infiltrating ductal carcinoma of the breast expressing c-erbB-2 positivity in this study compared to findings from Western populations has been noted in a previous study.[6] This may be related to the larger proportion of histological high grade tumours encountered in the Malaysian population. Since the c-erbB-2 (neu or HER-2) oncogene has extensive structural homology to the epidermal growth factor receptor gene," its overexpression can be expected to relate to more aggressive tumour proliferation. Our observation that high histological grade tumours express the c-erbB-2 oncoprotein more frequently than lower grade tumours is therefore not unexpected. The oestrogen receptor protein, however, does not directly contribute to cellular proliferation or differentiation but affects the behaviour of cancer cells through modulation by steroid hormones. Hence, it is also not surprising that there is no clear relationship between ER status and histological grade.

Table 1: Comparison of ER and c-erbB-2 expression by infiltrating ductal carcinoma of breast

	c-erbB-2 positive No. (%)	c-erbB-2 negative No. (%)	Total No. (%)
ER positive	6 (33.3)	12 (66.7)	18 (100)
ER negative	32 (47.1)	36 (52.9)	68 (100)
Total	38 (44.2)	48 (55.8)	86 (100)

Mc Nemar's test: $p<0.005$

Table 2: Correlation between histological grade and ER expression in infiltrating ductal carcinoma of breast

	ER positive No. (%)	ER negative No.	Total No.
Grade I	1 (33.3)	2	3
Grade II	6 (21.4)	22	28
Grade III	11 (20.0)	44	55
Total	18 (20.9)	68	86

Chi-square test: $p>0.05$

Table 3: Correlation between histological grade and c-erbB-2 overexpression in infiltrating ductal carcinoma of breast

	c-erbB-2 positive No. (%)	c-erbB-2 negative No.	Total No.
Grade I	0	3	3
Grade II	6 (21.4)	22	28
Grade III	32 (58.2)	23	55
Total	38 (44.2)	68	86

Chi-square test: $p=0.003$

Although several studies have indicated that c-erbB-2 amplication in breast cancer patients relates to poorer survival,[2,12] its relationship with ER protein expression has been unclear. Many studies have failed to demonstrate a correlation[13] and regard c-erbB-2 oncoprotein overexpression as an independent poor prognostic indicator in breast cancer.[2,12,14,15] Our study has, however, suggested a negative relationship between these two parameters. This raises several interesting questions on the pathobiology of breast carcinoma. Since benign breast epithelium in its normal physiologicalstate is oestrogen sensitive, the loss of oestrogen receptor protein in some breast carcinomas can be regarded as a regressive phenomenon occurring during cellular dedifferentiation and malignant transformation. Recent studies have shown that epidermal growth factor receptor (EGFR) overexpression correlates with both loss of oestrogen receptor and poor prognosis in breast cancer.[16] Recognising that the c-erbB-2 oncogene has extensive structural homology with the EGFR gene, we expect that the c-erbB-2 oncoprotein would have functional interactions with EGFR and lead to similar effects on breast cancer cells. That c-erbB-2 may play a role in cellular dedifferentiation is supported by cell culture studies which show that antibodies to c-erbB-2 can mediate an inhibitory effect on cell growth and induce cellular differentiation.[17,18] The mechanism whereby c-erbB-2 mediates ER loss, however, remains unclear. Since there is no evidence that ER protein activity contributes to advancement in histological grade, it would appear that histological dedifferentiation precedes ER loss during malignant transformation. We note that the ER positivity rate of low grade tumours was greater than high grade tumours in our study, although the difference did not reach statistical significance. This observation agrees with the notion that with greater cellular dedifferentiation, there is an increased likelihood of ER loss, supporting the postulation that histological grade contributes to ER loss rather than ER loss to worsening of histological grade. Hence, it may be speculated that the c-erbB-2 oncogene plays a role in ER loss through its effect on cellular dedifferentiation.

Recent studies suggest that ER positive breast cancers which also show c-erbB-2 oncoprotein overexpression have a poorer response to hormonal therapy.[19,20,21] Since adjuvant tarnoxifen may be insufficient in the presence of c-erbB-2 overexpression,[22] the use of this parameter in the routine assessment of breast cancer patients can have important clinical utility in identifying subsets of patients for more aggressive therapy.

ACKNOWLEDGEMENT

This study was funded by'the Research and Development Grant "IRPA 06-02-03-0181 University of Malaya," from the Ministry of Science, Technology and the Environment, Malaysia. We are grateful to Mr. E.K. Lee, Department of Pathology, University of Malaya for technical assistance.

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