

Correlation between histological grade and c-erbB2 oncoprotein overexpression in infiltrating ductal carcinoma of breast

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Abstract

One hundred and twelve infiltrating ductal carcinoma of breast were studied by the standard avidin-biotin complex immunoperoxidase method on formalin-fixed, paraffin-embedded tissue sections, using a monoclonal antibody to c-erbB-2 oncoprotein. The same tumours were assessed and scored according to the Bloom and Richardson criteria into three histological grades. The distribution of tumours according to grade were: 8 Grade I, 34 Grade II and 70 Grade III. Forty-three (38.4%) tumours showed positive membrane staining for c-erbB-2 oncoprotein. These comprised 7 Grade II and 36 Grade III tumours with c-erbB-2 immunopositivity rates of 20.6% and 51.4% respectively. The oncoprotein was not expressed by Grade I tumours.

This study shows a good correlation between c-erbB-2 expression and histological grade, a known prognostic indicator of invasive breast carcinoma. Because the c-erbB-2 oncogene has extensive structural homology to the epidermal growth factor receptor gene, its overexpression can be expected to result in more aggressive tumour behaviour. While it may be regarded as another indicator of poor prognosis breast cancers, its value in the selection of carcinomas less responsive to hormonal therapy and those more suitable for immunotherapy than chemotherapy has been mooted but remains to be clarified.

Key words: Malignancy, prognostic factors, immunoperoxidase, cancer therapy, breast, c-erbB-2

INTRODUCTION

Breast carcinoma ranks as the most common malignancy in Malaysian females. Assessment for tumour parameters that may relate to prognosis and selection of patients for various therapeutic options has become an important activity in diagnostic pathology laboratories. In the evaluation of such indices, consideration has to be given to their clinical utility so that cost and wastage of laboratory resources are minimised.

Expression of the c-erbB-2 oncoprotein is one of many such parameters currently being investigated for adoption for use in Malaysian laboratories. The c-erbB-2 (neu or HER-2) oncogene has been shown to have extensive structural homology to the epidermal growth factor receptor gene.¹ Various antibodies have been raised to the extra- and intracellular domains of its gene product which is localised to the cell membrane. These have been successfully applied in immunohistochemical detection of the oncogene product in tumour cells and results have shown good correlation between membrane

staining and c-erbB-2 gene amplification.^{2,3,4}

An overexpression of c-erbB-2 oncoprotein has been reported in 15% to 30% of invasive breast carcinomas,^{2,5} however its role in the pathophysiology of breast cancer remains controversial.^{6,7} While generally considered to be an indicator of poor survival:⁹ its predictive value in node-negative breast cancer patients is unclear.^{10,11} We endeavoured to investigate its association with tumour aggressiveness by a comparative histomorphological study between c-erbB-2 oncoprotein expression and histological grade, a universally accepted histological prognostic indicator.¹²

MATERIALS AND METHODS

All cases of infiltrating ductal carcinoma of breast diagnosed histologically at the Department of Pathology, University of Malaya from patients of the University Hospital over a 5 year period were retrieved from the Department files. All histological sections from these cases, whether from biopsies or mastectomies, were reviewed. Patient data were analysed to eliminate

duplication of cases due to repeated biopsies and subsequent mastectomies. One hundred and twelve cases reconfirmed histologically as **infiltrating** ductal carcinoma were included into this study after the deletion of one case because of insufficient tissue for further immunohistochemical study. The tumours were graded histologically and scored according to the Bloom and **Richardson** criteria into Grade I (well-differentiated), Grade II (**moderately**-differentiated) and Grade III (**poorly**-differentiated) **categories**.¹²

Further histological sections, cut at 4µm, were made from the most representative paraffin-embedded tumour block. These were mounted on **silane-coated glass** slides and examined for tumour immunoreactivity against a monoclonal antibody to **c-erbB-2** protein (1 : 30 dilution) obtained commercially from Triton Biosciences Inc., using the standard avidin-biotin complex immunoperoxidase (IP) method with overnight incubation. In accordance with the criterion used in other studies?¹³ only tumours with at least 5% of neoplastic cells exhibiting membrane immunoreactivity were regarded as positive for **c-erbB-2** expression. Cytoplasmic or nuclear positivity were not accepted as positive expressions.

RESULTS

The distribution of tumours according to histological grade were: 8 (7.1%) Grade I, 34 (30.4%) Grade II and 70 (62.5%) Grade III (Fig. 1). Forty-three (38.4%) tumours showed positive membrane staining for **c-erbB-2** oncoprotein. These comprised 7 Grade II and 36 Grade III tumours with **c-erbB-2 immunopositivity** rates of 20.6% and 51.4% respectively. The oncoprotein was not expressed by Grade I tumours. All instances of positive expression exhibited membrane positivity of moderate to severe intensity (Fig. 2). The correlation between histological grade and **c-erbB-2** expression by these tumours is summarised in Table 1. The chi-square test showed a statistically significant correlation between grade and **c-erbB-2** positivity ($p < 0.01$).

DISCUSSION

This study has shown that a high proportion (38.4%) of infiltrating ductal carcinoma of the breast overexpress the **c-erbB-2** oncogene product. This proportion is higher than most earlier reports based on Western populations

TABLE 1: Correlation between histological grade and c-erbB-2 expression

Grade	c-erbB-2 expression	
	Positive No. (%)	Total tested No.
I	0	8
II	7 (20.6)	34
III	36 (51.4)	70
Total	43 (38.4)	112

and may be related to the larger proportion of high grade (grade III) tumours in this study.^{8,9,13} There appears to be good **correlation** between histological grade and **c-erbB-2** expression. This observation is in agreement with other studies which have shown that histological grade of infiltrating ductal breast carcinoma can be positively correlated with aggressive tumour behaviour and is an important prognostic factor in the assessment of patients with breast **cancer**.¹² The finding that higher grade tumours express the **c-erbB-2** oncoprotein more frequently than lower grade tumours may have identified one of the parameters or mechanisms whereby tumour aggressiveness is based. Since the **c-erbB-2** oncogene has extensive structural homology to the epidermal growth factor receptor gene, a well-characterized receptor for mitogenesis, its overexpression is likely to have a stimulatory effect on cell growth and proliferation. In this respect its overexpression is not an unexpected **indicator** of aggressive biological behaviour of tumour cells.

Immunohistochemistry is a relatively simple technique available in most histopathology laboratories. It does not involve costly equipment and the antibodies required are relatively inexpensive. As has been shown in this study, it can be easily applied for the detection of **c-erbB-2** oncoprotein overexpression in breast carcinoma tissue. Because it can be performed on **formalin-fixed** paraffin-embedded material, **c-erbB-2** expression can also be studied retrospectively on archival cancer tissue and can easily be included as one of the parameters in the histological assessment of breast cancer patients. However, whether it is justifiable to include it as a routine parameter relates to its clinical utility. Since there is a close correlation between its expression and histological grade, it can be argued that it **confers** no additional information

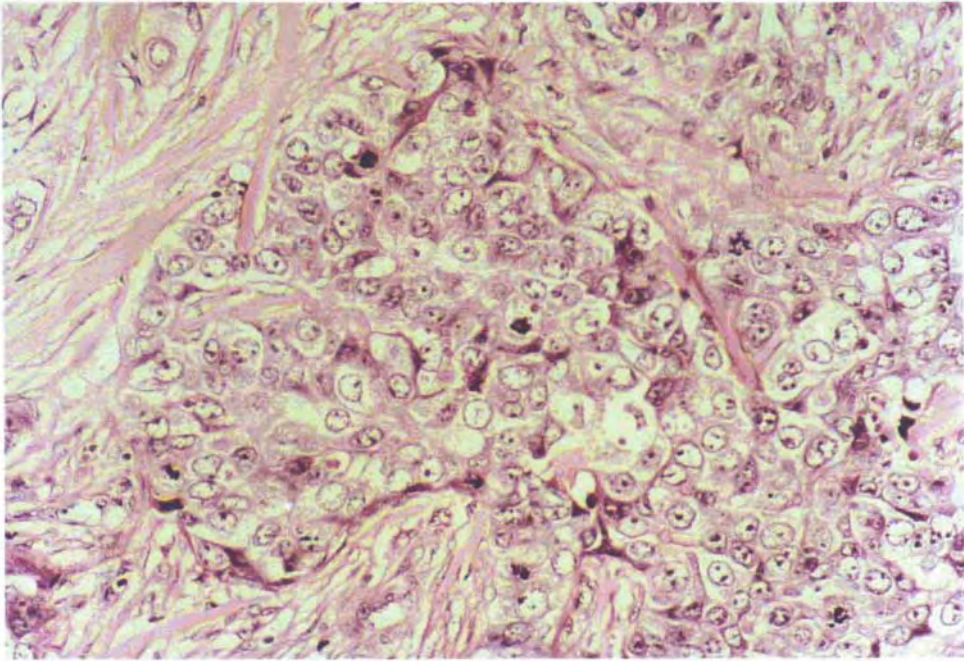


FIG. 1: Photomicrograph of a grade III infiltrating ductal carcinoma of breast H&E X 300.

as a prognostic marker.^{6, 14, 15} However, studies are emerging to support its utility as an independent prognostic factor in breast carcinoma.^{16, 17, 18} Furthermore, recent observations suggest that **c-erbB-2** expression

by tumour cells may impose a **poorer** response to hormonal therapy, even in oestrogen-receptor positive breast cancer patients.^{19, 20, 21}

Notwithstanding the above, **c-erbB-2** expression may also provide further information

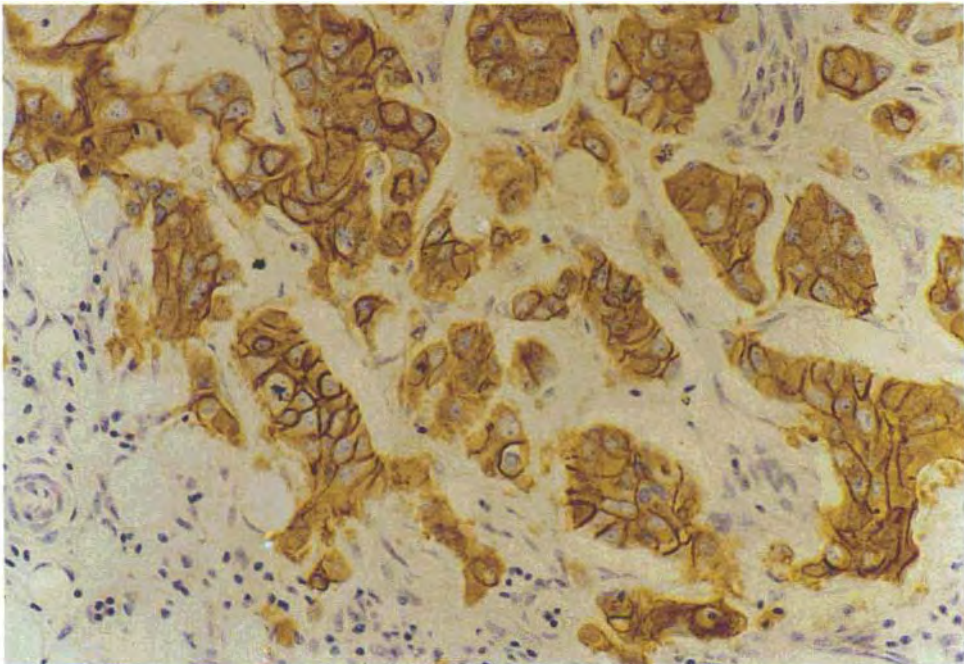


FIG. 2: Infiltrating ductal carcinoma cells with positive membrane expression for **c-erbB-2** oncoprotein. Immunoperoxidase staining for **c-erbB-2** X 300.

in the selection of patients for different therapeutic options. Cell culture studies have shown that antibodies to **c-erbB-2** can mediate an inhibitory effect on cell growth and induce cell **differentiation**,^{22,23} suggesting that **c-erbB-2** positive carcinomas may respond better to **immunotherapy** than chemotherapy."²⁴

In view of the above, the immunohistochemical **determination** of **c-erbB-2** expression has potential as a breast cancer marker with useful clinical utility. Further multicentre trials should be encouraged to **clarify** the algorithm of its estimation in breast cancer patients.

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