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THE EXPRESSION AND PROGNOSTIC SIGNIFICANCE OF c-erbB-2 MOLECULES IN PATIENTS WITH BREAST CANCER IN IRAN

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- **ABSTRACT**

Background: Epidermal growth factor receptors (EGFRs) are a group of molecules with structural and functional similarities, which are expressed in some breast cancers. In the present investigation, the presence of two members of the EGFR family namely *c-erbB-1* and *c-erbB-2* was assessed as a prognostic indicator in a series of breast tumors.
Objective: To study the over-expression of *c-erbB-1* and *c-erbB-2* in a series of primary breast cancer as a prognostic factor.
Methods: A series of 100 patients with primary operable breast cancers were investigated for expression of EGFRs in their tumors by immunocytochemical staining with the monoclonal antibodies against *erbB-1* and *erbB-2* molecules. An indirect immunoperoxidase method was used for determination of these receptors in formalin-fixed paraffin-embedded breast carcinoma tissues. This technique was also used to investigate the expression of *c-erbB-1* and 2 in 23 out of the 100 fresh frozen tissues of breast carcinoma.
Results: Our results indicate that 20 (20%) and 41 (41%) tumors showed positive immunoreactivity with *erbB-1* and *erbB-2* monoclonal antibodies, respectively. Results also indicated that in 100% of cases the pattern of immunoreactivity in fresh tissues were comparable with those of the formalin-fixed paraffin embedded tissues. Strong correlation between distinct c-erbB-2 expression and short disease-free interval was observed but no significant association was found with *c-erbB-1*.
Conclusion: The results show that the c-erbB-2 receptor status may be a useful prognostic marker in breast cancer.

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Key Words • Epidermal growth factor • c-erbB1• c-erbB2• breast cancer

Introduction

The receptor for epidermal growth factor (EGF) has been found in a wide range of normal epithelial tissue and tumors arising from these tissues. However, the level of expression of the receptor has been found to be higher in a number of human malignancies compared to their normal tissue counterparts.[1-4] Expression of the EGF receptor varies considerably in normal tissue, while some cells, such as keratinocytes, express relatively high levels, other cells such as the lymphoid series express none or very low levels of the receptor.[5] Biochemical and histological examination of human biopsies and cell lines established in vitro have shown that over-expression of normal or mutated forms of EGF receptor, and/or its ligands, is a characteristic feature of squamous cell carcinoma and is found in a number of human malignancies including those of the breast, bladder, ovary, lung, brain, pancreas and head and neck[5]. EGFR over-expression in human breast cancers was first reported by Sainsbury et al[6] and *c-erbB-2* receptor over-expression as a prognostic factor by Salmon et al in 1987.[7] *c-erbB-1* and *c-erbB-2* in breast carcinoma has been reported with a different pattern of significance.[2,8-11] In this investigation the expression of*c- erbB-1* and *2* in Iranian patients with breast carcinoma was investigated and results were compared with the Nottingham Prognostic Index.

Materials and Methods

Tumors:

One-hundred formalin-fixed paraffin-embedded samples of which 23 were biopsies taken from primary cancer patients during mastectomy at Nemazi and Faghihi Hospitals (University Hospitals, affiliated to

Shiraz University of Medical Sciences, Shiraz-Iran)) between 1995 and 1997 formed the study material for this project. The specimens were stored at –20 ?C. Pathological data including tumor size (TNM), modified Bloom and Richardson grading[12] and axillary lymph node involvement were recorded. Specimens from normal skin tissue were used as positive control for immunohistochemical analysis of EGFR's. Omission of the primary antibody was used as the negative control.

Antibodies and conjugates:

For determination of *c-erbB-1* and *c-erbB-2* receptors, ICR16 and ICR12 rat mAbs were used respectively (European Institute of Health and Medical Sciences, University of Surrey, England). Peroxidase-labelled sheep anti-rat Ig (Amersham Co., England) was used as the secondary antibody in immunocytochemical studies.

Immunocytochemistry:

Sections were cut from frozen or formalin fixed paraffin-embedded tissues. Frozen sections were dried at 37 ?C (30 minutes), fixed in formol-calcium for 5 mins, rinsed in acetone, and immersed in cold chloroform/acetone (1:1) for 5 mins. Sections were then washed three times with ohosphor buffered saline (PBS) and the endogenous peroxidase was blocked by immersing the sections for 10 minutes in PBS containing 3% H2O2. The sections were then incubated with about 100 ml of primary antibody (ICR12 or ICR16) for 90 minutes at room temperature. After three washes in PBS, the sections were incubated with about 100 ml of a 1/100 dilution of sheep conjugated anti-rat-IgG for 90 minutes at room temperature then washed twice with PBS and twice with distilled water (DW). The final reaction product was visualized using diaminobenzidine (DAB) [100 mg DAB in 100 ml of 0.1 M tris buffer(pH 7.2), 100ml H2O, 66 ml H2O2]. After5 mins, the sections were washed twice in DW and counter-stained in hematoxylin and mounted in Entellan. Indirect immunoperoxidase method was also performed on formalin fixed paraffin-embedded sections. After blocking of endogenous peroxidase, the slides were incubated with 200 ml of primary antibody overnight in 4 ?C. The slides were washed three times in PBS, the sections were incubated with 200 ml of a 1/50 dilution of sheep conjugated anti-rat Ig for 120 minutes at room temperature then washed with PBS and DW. The procedure was curried out as described for frozen sections.

Nottingham Prognostic Index:

The Nottingham Prognostic Index score is calculated by combining the results of the lymph node stage, histological grade, and tumor size in the following manner.[13]

Index= stage (1-3) + grade (1-3) + (size x 0.2). Therefore, an index bellow 3.4 (index ? 3.4) was assessed as good and an index above 3.4 (index>3.4) was considered as moderate or poor, prognosticly Positive reactions were graded on a +++,++, and + basis according to the intensity of staining. Result of immunohistochemistry were analyzed according to degree of positive membrane staining and total amount of staining by allocating grades 0,1,2,3, in which 0 = no detectable staining, 1 = very faintly visible only by power 400, 2 = strongly visible by power 400 and weakly by power 100 and 3 = strongly visible by power 100 microscopy.

Statistical analysis:

Microsoft EPI-5 was used for statistical analysis. P values were calculated by using Chi-square or Fisher exact tests.

Results

Immunocytochemical analysis of 100 formalin-fixed and paraffin-embedded breast cancer tissues revealed that 20% and 41% of the cases were positive for *c-erbB-1* and *c-erbB-2* receptors respectively (Figure 1). Statistical analysis indicated that lymph node status, histological grade and tumor size were not related to EGFR (*c-erbB-1*) expression. There was no relation between the Nottingham Prognostic Index score and *c-erbB-1* status (Table 1). There was, however, a strong correlation between histological grade ($P<0.02$), lymph node status ($P<0.02$) and *c-erbB-2* receptor expression and none with tumor size. A strong correlation ($P<0.005$) between Nottingham Index score and *c-erbB-2* expression was also observed (Table 2). For comparison between Immunocytochemical staining on formalin-fixed paraffin embedded and fresh frozen tissues, indirect immunoperoxidase staining was used to investigate the expression of*c- erbB-1* and *c-erbB-2* in 23 out of the 100 breast carcinoma fresh frozen tissue samples (Figure 2). Results indicated that *c-erbB-1* and *c-erbB-2* expression on fresh frozen samples is the same as formalin-fixed paraffin-embedded tissue, though with a lower intensity in some cases using formalin-fixed sections.

Discussion

In the current study the prognostic potential of *c-erbB-1* and *c-erbB-2* molecules (two members of EGFR family) in primary breast cancer was assessed. There are several factors that might be associated with the rate of positive cases. For example, in patients with a high incidence of lymph node involvement the expression of *c-erbB-2* receptor may reach a mean of 44%.[14] Other parameters such as histological grade may also influence the receptor up-regulation.[15, 17] Contradictions concerning the significance of *c-erbB-2* up-regulation may be explained by the clinical status of the patients or by differences in the methodology. In our cases, only patients with primary operable breast cancer were studied and only tumors with unequivocal distinct cytoplasmic or membrane reactivity were regarded as positive. Contradiction also exists on the prognostic significance of *c-erbB-2* in breast cancer, with some authors claiming presence of correlation[16,18,19] and others reporting no correlation.[20-23]

We have shown that there is a strong relationship between expression of *c-erbB-2* receptor and Nottingham Prognostic Index. Our findings suggest that patients with index gradings above 3.4 (especially above 5.4) have a poor prognosis compared to those with index gradings below 3.4. In this investigation no correlation was found between *c-erbB-1* receptor expression and tumor size, lymph node status or histological grade. These findings are consistent with the majority of previously published works.[24-27]

There is an increasing awareness that polypeptide growth factors, such as EGFR and *c-erbB-2* receptor are involved not only in the control of normal cellular growth and proliferation, but also in as yet poorly understood mechanisms of cellular transformation. The investigation of up- and down-regulation of tumor markers such as *c- cerbB-2* and other related members of EGFR has two important clinical advantages. Firstly, the prognostic value, which has been previously reported for this molecule in breast carcinoma the *c-rebB-2* level is a strong prognostic factors in primary breast carcinoma in its own right. Thus, in the absence of knowledge of histological grade, as shown in the multivariate analysis, *c-erbB- 2* is a particularly useful indicator. Secondly, it should be noted that over-expression of*c- erbB-2* has made this receptor an attractive target for antibody-directed therapy.[2]

Chemotherapy is more effective in patients who do not have *c-erbB-2* over expression. Conversely, patients with *c-erbB-2* over expression showed virtually no improvement on the chemotherapy regimen.

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