Bax Protein Expression in DCIS of the Breast in Relation to Invasive Ductal Carcinoma and Other Molecular Markers

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This study describes the incidence of Bax protein expression in a series of 106 cases of breast cancer including 56 cases of ductal carcinoma in situ (DCIS) and 50 cases of invasive ductal carcinoma (IDC). Relationships of Bax expression to the histological grades of DCIS & IDC, and to the expression of Ki67, ER, p53, cerbB2 & Bcl2 are described. The expression of Bax, Ki67, ER, p53, cerbB2 and Bcl2 proteins is determined immunohistochemically. Cases were regarded positive for Bax, Bcl2 and cerbB2 when they showed either moderate or strong staining for these markers. The nuclear stains (Ki67, ER, and p53) were quantified in terms of percentage positive cells and cases for ER and p53 were considered positive when more than 10% cells were labelled. DCIS were graded histologically as well (n=18), moderately (n=18), and poorly differentiated (n=20) Invasive ductal carcinoma was graded as grade I (well-differentiated) n=17, grade II (intermediate) n=24 and grade III (poorly differentiated) n=19. 65/106 cases (61%) were Bax positive including 37/56 (66%) of DCIS and 28/50 (56%) of IDC. Bax expression did not correlate to increasing histological grades of either DCIS or IDC. It did not correlate to Ki67, ER, p53 or cebB2 but positive correlation was seen with Bcl2 (p=0.003). Bcl2 immunostaining displayed a negative correlation with increasing histological grades both of DCIS and IDC (p=0.026), (p=0.041) respectively. There was a trend of negative correlation of Bcl2 with Ki67 (p=0.062). It correlated positively with Bax (p=0.003) and ER (p<0.0001). Results suggest that the regulation of apoptosis is important in ductal carcinoma in situ of the breast as well as invasive ductal carcinomas. Bcl2 is associated with good prognostic markers in both DCIS and IDC, whereas the regulation of Bax is complex and does not necessarily correlate with mutant p53. (Pathology Oncology Research Vol 6, No 4, 256–263, 2000)

Keywords: breast cancer, ductal carcinoma in situ, Bax, Ki67, oestrogen receptor, p53, cerbB2, Bcl2

Introduction

Bax is a 21 kD protein with extensive amino acid homology with Bcl2.1,2 The protein is encoded by six exons and has been shown to undergo alternative splicing leading to at least two cytoplasmic forms.3,4 Bax has been shown to form heterodimers with Bcl2 and the ratio of Bcl2 to Bax determines the survival or death of cells following an apoptotic stimulus such as removal of growth factor.5,6 Stimulation of Bax synthesis also appears to be a result of wild type but not mutant p53 activity.5,6

Recently, it has been suggested that dysregulation of apoptosis due to imbalances in Bax/Bcl2 levels may contribute to the pathogenesis of breast cancer.3 Bax has been suggested as a good prognostic marker in node negative breast cancer.4 There is little information available on the significance of Bax expression in DCIS and its various histological grades in comparison to IDC and its histological grades.

The Bcl2 gene, located on chromosome 18 (18q21), encodes a 26kD protein, which appears to play a key role in cell regulation by inhibiting apoptosis. Abnormalities of the Bcl2 gene were first discovered in human follicular lymphoma in which the gene is translocated to the immunoglobulin heavy chain locus of chromosome 14.7 Expression of the Bcl2 protein product has been documented in a variety of normal human tissues including breast epithelium.8 Bcl-2 protein is also expressed in invasive...
breast carcinoma and is associated with well differentiated tumors and positive oestrogen receptor (ER) status. Abnormalities of the p53 tumor suppressor gene, which also plays a role in cell regulation are common in all forms of cancer. Recent studies have demonstrated an inverse relationship between p53 and Bcl2 protein expression in breast cancer and other solid tumors. The altered expression of p53 in breast carcinomas is associated with high grade, ER negative tumors and been reported to be prognostically significant in breast carcinomas.

The oncogene cerbB2 (HER2/neu) is located on chromosome 17q21 and encodes for a 185 kD membrane protein with tyrosine kinase activity, which has a certain homology to epidermal growth factor receptor (EGF R). Amplification and over expression is found in 20-30% of breast carcinoma cases and is associated with worse prognosis, low ER content, high histopathological grade and shortened survival.

Ki67 is a cell cycle associated antigen expressed in all phases of the cell cycle except G0. The monoclonal antibody Ki67 was first described in 1983 by Johannes Gerdes and colleagues, who suggested that it might be used as a marker for proliferating cells. It is useful in the identification of hormone insensitivity in breast cancer and in the prediction of tumour growth rates and patient survival, therefore it is useful prognostically. It has been shown that elevated levels of this antigen are associated with earlier breast cancer recurrence, shorter survival time and disease free interval as well as a poorer response to therapy.

Determination of ER status is an important parameter in the clinical management of breast cancer. Expression of Bcl2 in breast cancer in vivo is strongly correlated to that of ER and both are predictive for response to endocrine therapy. This study investigates the incidence of Bax expression in DCIS and IDC relative to their histological grades and also the relationship between Bax protein and the expression of other biological markers including Ki67, ER, p53, cerbB2 and Bcl2.

Materials and Methods

Case Selection

The breast cancer specimens were retrieved from the Histopathology Department Archives between 1985 and 1995. The 106 cases comprised 56 DCIS and 50 IDC. Of DCIS cases, 30 were pure DCIS as there was absence of any associated invasive component and no past history of breast cancer in either ipsilateral or contralateral breast, whereas 26 had associated invasive carcinoma. Within the invasive ductal carcinoma group, 37 cases were histologically proven lymph node negative and 13 were lymph node positive. All patients were diagnosed and treated at the Royal Free Hospital, London and were identified initially using the SNOMED Diagnostic Retrieval System.

Age at presentation ranged from 40 to 70 years (median 56 years). All patients were treated by mastectomy or local excision with or without radiotherapy.

Histological Grading of DCIS and IDC

DCIS was classified as well differentiated (n=18), immediately differentiated (n=18) and poorly differentiated (n=20) according to the published criteria of Holland et al. In cases in which more than one histological grade was identified, DCIS was graded according to the highest nuclear grade.

Invasive ductal carcinomas were classified according to the Elston and Ellis grading system, as grade I (n=7), grade II (n=24) and grade III (n=19).

Immunohistochemistry

Three µ thick, formalin fixed, paraffin wax embedded sections were immunostained using primary antibodies to Bax, Ki67, ER, p53, cerbB2 and Bcl2 as described in Table 1.

Sections were dewaxed in xylene and rinsed in graded alcohols. Endogenous peroxidase was blocked by incubation in 1% hydrogen peroxide for 15 minutes followed by rinsing in distilled water. Subsequently sections were subjected to antigen retrieval by heating in a microwave oven in (10mmol/litre) citrate buffer (pH6) for all antibodies except Bax which was pressure cooked, then washed in tris buffered saline (TBS). Non specific staining was blocked by treating with (10%) normal goat serum for 15 minutes. Sections were incubated with primary antibodies for 60 minutes each followed by washing with TBS and application of secondary biotinylated antimouse/rabbit antibody (DAKO) as appropriate at a dilution of 1:100 for 30 minutes. Once again, sections were washed in TBS and finally incubated with Streptavidin biotin complex reagent (Strept ABC Complex/HRP Duet, DAKO) for 30 minutes. The Immunoprecipitate was visualised by treating with diaminobenzidine tetrahydrochloride (Sigma Chemicals Co) and counterstaining with haematoxylin. Negative controls were run with each batch by replacing the primary antibody with TBS. Positive controls for each antibody (see Table 1) were included on each occasion that staining was performed.

Tonsil was used as a positive control for Ki67 and Bcl2, colon for Bax and known cerbB2, ER and p53 positive cases of breast and prostate cancer were used as positive controls for cerbB2, ER and p53 respectively.

Staining Characteristics and Assessment of Staining

Ki67, ER and p53 staining was nuclear and the percentage of positive tumor cells with these markers were determined by counting 1000 cells per case. Cases for