Report

Prognostic significance of serum c-erbB-2 protein in breast cancer patients

Peter C. Willsher¹, Jayne Beaver², Sarah Pinder², Jane A. Bell², Ian O. Ellis², Roger W. Blamey¹, and John F.R. Robertson¹

¹ Professorial Department of Surgery, ² Department of Histopathology, City Hospital, Nottingham, UK

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Summary

The tissue expression of c-erbB-2 protein in breast cancer is a marker of poor prognosis in a number of studies. More recently it has also been suggested that c-erbB-2 expression may predict response to systemic therapy in patients with advanced breast cancer. The measurement of c-erbB-2 protein in the serum of breast cancer patients has now been reported, but the significance of this finding is not clear. In this study an ELISA assay was used to measure c-erbB-2 in the sera of 23 normal controls, 46 benign breast disease patients, and 119 breast cancer patients. Elevated serum c-erbB-2 protein levels were detected in 13% (3/23) of normal controls, 15% (7/46) of benign disease patients, 15% (7/46) of Stage I/II patients, 26% (9/35) of Stage III patients, and 21% (8/38) of Stage IV patients. The tissue expression of the c-erbB-2 protein showed no association with detection of the serum c-erbB-2 protein (p = 0.31). In the 67 Stage III and IV patients who had assessable disease the presence of the c-erbB-2 protein in the serum bore no relationship to response to hormonal therapy (p = 0.71). Serum detection of the c-erbB-2 protein in Stage I/II patients predicted for a worsening of both survival outcome (p = 0.002) and disease free interval (p = 0.002). A worse outcome was also seen for the Stage III patients (p = 0.04) and Stage IV patients, although the latter did not reach statistical significance (p = 0.27).

This study found that the presence of c-erbB-2 in the serum of breast cancer patients was of prognostic significance for all stages of disease.

Introduction

Detection of the c-erbB-2 protein in breast cancer tissue using immunohistochemical techniques has shown a correlation with prognosis. Specifically, a number of studies have shown an association between the tissue expression of c-erbB-2 and both the presence of lymph node metastases and poor clinical outcome [1 (review)]. Thus far detection of the c-erbB-2 protein in the serum of breast cancer patients has received less attention [2–4]. We have investigated the clinical significance of the c-erbB-2 protein in the serum of patients from all stages of breast cancer, using an ELISA assay. Control groups of normal females and patients with benign breast disease were assayed for comparison.

Methods

Patients

A total of 188 females were included in the study. They comprised 23 normal controls, 46 patients...
with benign breast disease, and 119 breast cancer patients (Stage I n = 15, Stage II n = 31, Stage III n = 35, and Stage IV n = 38). All the patients with primary breast cancer (Stage I-III) initially presented to the City Hospital Breast Unit during 1986–1987. Patients with Stage IV disease presented with their symptomatic metastases during the same period. The majority of these patients had previously presented with primary breast cancer and had subsequently developed distant metastases. Normal controls and benign disease patients were accrued from the symptomatic breast clinic and the breast cancer screening program during the same time period. All patients gave informed consent.

This study was performed on a bank of pre-treatment serum stored at minus 70 degrees. In the patients with breast cancer, fresh tumour tissue was obtained either by Tru-cut biopsy or at open surgery and fixed in neutral buffered formalin and processed to paraffin blocks. Tumour tissue was available for study for 57 of the Stage III/IV patients. Sixty-seven of the Stage III/IV patients were treated with endocrine therapy and had assessable disease using UICC criteria [5]. Oestrogen receptor status was known on 52 of these patients. Assessment of response was independently confirmed by an external assessor (see Acknowledgements). All these patients received first line hormone therapy in the form of tamoxifen 20 mg daily. Eight premenopausal women also received goserelin 3.6 mg by monthly subcutaneous injection. Thirty-one patients received hormone therapy for locally advanced disease while 36 were treated for metastatic disease (bone = 14, lung = 11, liver = 4, lung and soft tissue = 4, bone and lung = 2, soft tissue = 1).

**Laboratory techniques**

Detection of the c-erbB-2 protein was carried out using a commercially available ELISA kit (spl85HER-2 Bender MedSystems, Vienna, Austria). The assay detects the extracellular, soluble receptor portion of the c-erbB-2 protein. Assays were carried out in duplicate, according to the manufacturer's instructions, with appropriate standards and controls [6]. In brief, the assay is a sandwich ELISA. Sera were incubated on c-erbB-2 monoclonal antibody coated microwells to bind any c-erbB-2 protein in the specimen. A horse radish peroxidase (HRP) conjugated antibody was introduced to bind the captured antigen and the plates were incubated for two hours at room temperature. After washing, a HRP reactive substrate was added and after 15 minutes the absorbance was read at 450 nm on an automated ELISA plate reader. Samples were assayed in duplicate and were repeated where the variation was greater than 10%. Marker levels in the sample were determined from a linear regression constructed from recombinant protein control sera. Positive assays were defined above the serum cut-off determined by the manufacturer [6], as the mean value of the normal controls plus two standard deviations (20 ng/ml). The intra-assay and inter-assay coefficients of variation are less than 1.9% and 5.8% respectively.

Tissue staining for c-erbB-2 protein was performed using the AP2IN antibody (provided by W. Gullick) using a standard avidin-biotin peroxidase method. Tumours showing any membrane staining were classified as positive. ER status was determined using the enzyme immunoassay (Abbott) as described [7]. Tumours with greater than 5 femtomoles per milligram of cytosolic protein were considered ER positive.

**Statistical analyses**

All analyses were carried out using SPSS-X Data Analysis Program (SPSS UK Ltd.). A Students T test was used to assess for differences in serum levels between the groups. Chi-squared contingency