SHORT COMMUNICATION

ErbB-2 protein levels in healthy, asymptomatic women

Brenda Breuer, Steven Smith, Michael P. Osborne, Rache M. Simmons, Walter P. Carney and Paul W. Brandt-Rauf

Recently, a number of studies have found a relationship between having breast cancer and elevated serological levels of the extracellular domain (ECD) of the erbB-2 protein. This study focuses on healthy, asymptomatic women, and evaluates the relationship between serological concentration of the ECD of erbB-2 protein and the following parameters: age, ethnicity, smoking status, age at menarche, age at first live birth, menopausal status, whether surgery had been performed within the prior year, history of breast cancer, history of any other cancer, family history of breast cancer, history of other cancers in first degree relatives, and number of prior benign breast biopsies. Blood samples were stored at –70 °C and analysed within 3 weeks of phlebotomy. Statistical analysis indicates that in healthy women, the level of erbB-2 protein in the blood is directly related to age ($p = 0.0002$) and inversely related to having had a live birth ($p = 0.018$). The relationship to age is independent of the association between the oncoprotein level and menopausal status. The data indicate that rather than having only one threshold value for serological erbB-2 positivity, it may be necessary to have values that reflect age and nulliparity status.

Keywords: *erbB-2, protein, serum, healthy, women.*

Introduction

Numerous recent investigations have found an association between increased serological levels of the extracellular domain (ECD) of the erbB-2 protein and breast cancer (Leitzel *et al.* 1991, Breuer *et al.* 1994, Kandl *et al.* 1994), especially recurrent breast cancer (Sugano *et al.* 1994a, b, Watanabe *et al.* 1994, Andersen *et al.* 1995); metastatic disease (Kynast *et al.* 1993, Sugano *et al.* 1994a, b, Andersen *et al.* 1995), especially distant metastasis (Narita *et al.* 1992, Isola *et al.* 1994); and disease that is not responsive to treatment (Isola *et al.* 1994, Leitzel *et al.* 1995). While some have found an association between elevated serological levels of erbB-2 and shorter survival (Leitzel *et al.* 1995), we have previously reported an increased prevalence of elevated levels among women with *in situ* carcinoma (Breuer *et al.* 1994). As a logical extension of this finding in early stages of breast cancer, we decided to investigate the levels of this oncoprotein in the blood of healthy, asymptomatic women.

***Brenda Breuer** (author for correspondence), **Michael P. Osborne**, **Rache M. Simmons** are at the Strang Cancer Prevention Center, New York, New York 10021, USA and the New York Hospital-Cornell Medical Center, USA; **Steven Smith**, **Paul W. Brandt-Rauf** are in the Division of Environmental Health Sciences, Columbia University, New York, New York 10032, USA; **Walter P. Carney** is at Oncogene Sciences, Cambridge, Massachusetts, USA; and **Brenda Breuer** is also at the Jewish Home & Hospital for Aged, 120 West 106th Street, New York, New York 10025, USA and the Department of Geriatrics and Adult Development, Mount Sinai School of Medicine, New York 10029, USA.*

MATERIALS AND METHODS

Participants

Sixty healthy, asymptomatic women were recruited from patients who came to the Strang Cancer Prevention Center (hereafter called Strang) for routine, physical examinations. They were not members of Strang's 'high-risk' group, which is composed of women with strong family histories of breast cancer (e.g., those with at least two affected first-degree relatives). For each woman we collected the following data: age, ethnicity, smoking status, age at menarche, age at first live birth, menopausal status, whether surgery had been performed within the prior year, history of breast cancer, history of any other cancer, family history of breast cancer, history of other cancers in first degree relatives, and number of prior benign breast biopsies. The institutional review boards of Strang and the New York Hospital-Cornell Medical Center approved this project.

Blood specimens

Blood specimens were collected by routine venipuncture technique. The samples were placed on ice immediately after drawing; the serum was separated by centrifugation within 1 h of collection. Duplicate aliquots of each serum specimen were transferred into two 2-ml cryogenic test tubes, respectively, each containing 0.5 units aprotinin, 0.2 microgram Na_2EDTA, and 0.25 microgram leupeptin as protease inhibitors, and stored frozen at –70 °C until the time of analysis. All samples were analysed within 3 weeks of phlebotomy.

Serum analysis

Duplicate samples were analysed blindly (i.e. blind to which two samples came from the same participant) for levels of the ECD of erbB-2 p 185. Samples were assayed via a modification of the sandwich enzyme-linked immunosorbent assay (ELISA; Oncogene Sciences, Cambridge, MA), described previously (Carney *et al.* 1991, Luo *et al.* 1993). The modified assay utilizes a rabbit polyclonal antibody against the ECD of the erbB-2 protein for detection, rather than the previously used monoclonal antibody.

Preliminary serum analysis

The short-term stability of the ECD of the erbB-2 protein in serum was evaluated by analysing aliquots from freshly drawn blood samples from 10 individuals immediately after collection and again after 3 weeks' storage at –70 °C.

Statistical analysis

All analyses were conducted with SAS procedures (1990). The distribution of serological erbB-2 levels was evaluated, as was the relationship between these levels and each of the parameters for which we had collected data about the participants. Univariate tests included evaluation of Pearson correlation coefficients and the Wilcoxon rank sums test. Multivariate procedures included the General Linear Models procedure, as well as the Regression procedure with backward elimination. To avoid a potential bias that may stem from ethnic-related differences in levels of protein expression, data

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from the three non-white participants were excluded from statistical analyses related to erbB-2 protein levels.

Results

Preliminary serum analysis

This showed that the average of the 10 samples initially was 3161 HNU ml^{-1} (range: 2413–4083 HNU ml^{-1}), and the average after 3 weeks was 3115 HNU ml^{-1} (range: 2115–3828 HNU ml^{-1}). Thus, on average, the sera levels after 3 weeks were greater than 98% of the levels detected immediately after collection.

Reproducibility of erbB-2 readings

The intra-assay variability, i.e. the mean of the absolute difference between duplicate samples/mean of duplicate samples, was 9.6% (standard deviation: 7.6%).

The study group

The participants were predominantly white, and their median age was 63.5 years. The descriptive data of the group are presented in Table 1.

Univariate analysis

This indicated that neither the level of erbB-2, nor its logarithm, was normally distributed. Further statistical tests showed that the serological concentration of erbB-2 was significantly associated only with age ($r = 0.42$, $p = 0.001$) and menopausal status ($p = 0.049$).

Multivariate analysis

We included in our multivariate analysis any variable that, upon univariate analysis, was associated with the level of erbB-2 at $p < 0.15$; i.e. age, menopausal status, having had a live birth, and having a grandmother who had breast cancer. The analysis indicated that two characteristics contribute significantly and independently to the serological level of erbB-2 protein; i.e. age ($\beta = 29.39$, $p = 0.0002$), and having had a live birth ($\beta = -491.48$, $p = 0.018$). When the analysis was stratified by menopausal status, we found that among post-menopausal white women ($n = 47$), age and having had a live birth remained significantly related to erbB-2 level ($p = 0.004$ and 0.019, respectively); among pre-menopausal white women ($n = 10$), there was a statistical trend with age ($p = 0.095$), but having had a live birth was no longer significant ($p = 0.47$).

Factor	Value
Age, years; median (range)	63.5 (37–83)
Pre-menopausal; number (%)	10 (16.7)
Post-menopausal; number (%)	50 (83.3)
Nulliparous; number (%)	23 (38.3)
Age at first live birth, years; median (range)	26 (18–41)
Age at menarche, years; median (range)	13 (11–14)
Ethnicity; number (%):	
White	57 (95.0)
Black	1 (1.7)
Asian	2 (3.3)
Current smoker; number (%)	6 (10.0)
Number of previous benign breast biopsies; number (%)	
0	49 (81.7)
1	7 (11.7)
2	3 (5.0)
3	1 (1.7)
Prior history of breast cancer	1 (1.7%)
Prior history of other cancer	1 (1.7)
Have a first degree relative with breast cancer; number (%)	18 (30.0)
Number first degree relatives with cancer at any site; number (%)	
0	26 (43.3)
1	27 (45.0)
2	5 (8.3)
3	2 (3.3)
Level of erbB-2 (HNU ml^{-1}); median (range)	3170 (2132-6959)

Table 1. Descriptive data of the study group; n = 60.

Discussion

While numerous studies have investigated the relationship between levels of serological erbB-2 protein and breast cancer, none have focused on the levels of this protein in healthy women. In studies that did include well women, the purpose of their participation was usually to calculate the threshold of serological positivity for erbB-2 protein (e.g. their mean value plus 2 standard deviations (Breuer *et al.* 1994, Watanabe *et al.* 1994)). One isolated study (Watanabe *et al.* 1994) reported that there was no age-dependency of erbB-2 concentration in healthy controls. It may be that the difference between the results of that study and ours is attributable to the different antibodies used in the two studies and their different epitopes. A second possibility relates to the possible decay of erbB-2 over time, even when kept at –70 °C. While this explanation is speculative at the present time, our finding that myc protein decays over time, even at very low temperatures (DeVivo *et al.* 1993), as well as recent findings of the instability of other oncoproteins (R. Puntoni, personal communication), even at very low temperatures, indicate that we cannot assume that the decay of a protein is prevented by storage at low temperatures. Therefore, in the present study, levels of erbB-2 in the blood were measured within a period of time where the decay of the protein was shown to be negligible; i.e. within 3 weeks. Watanabe *et al.* did not indicate what their interval was, nor whether they had demonstrated the stability of the protein over the time that the samples had been stored.

Multivariate analyses indicate that the relationship between age and the serological levels of erbB-2 was independent of menopausal status. The direct relationship between the level of erbB-2 protein in the serum and the age of a woman, as well as its relationship to nulliparity, parallels the association of age and nulliparity with the risk of breast cancer (the lack of an association in *pre*-menopausal women between serological

erbB-2 levels and nulliparity may be attributable to the low power associated with the small sample size of 10). Thus, erbB-2 concentration may not only serve as a prognostic tool for women with diagnosed breast cancer; an elevated serological level may also be a risk factor for this disease. One implication of such a finding is that it may not be appropriate to simply calculate one threshold value of this protein to predict whether a woman is likely to have a recurrence of breast cancer. Rather, these values may vary by age and by whether the patient has had a live birth. Another implication of the relationship between erbB-2 and breast cancer risk factors in healthy, asymptomatic women is that the serological concentration of this oncoprotein may prove to be valuable as part of a screening instrument for breast cancer.

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