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Epidermal Growth Factor Receptor Expression as a Prognostic Indicator in Breast Cancer

Masakazu Toi, Akihiko Osaki, Hirofumi Yamada and Tetsuya Toge

The significance of epidermal growth factor receptor (EGFR) status as a prognostic indicator was investigated by a competitive binding assay in 135 primary breast cancer patients. 55 patients (41%) were EGFR positive and EGFR status was negatively correlated with oestrogen receptor (ER) status ($P < 0.01$). 5-year postoperative follow-up showed that relapse-free survival for EGFR positive patients was significantly worse than that for EGFR negative patients ($P < 0.05$). There was no difference between the two groups in tumour size, axillary node involvement, age and menopausal status. Analysis by axillary node status demonstrated the poor prognosis of the EGFR positive group in node positive patients. As yet, no difference in prognosis has been seen in node negative patients. A higher frequency of haematopoietic relapse was observed in EGFR positive patients. Simulataneous or sequential EGFR measurements in primary tumour and metastatic sites of 34 patients showed that expression of EGFR was more enhanced in metastatic sites.
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INTRODUCTION

ENHANCED EXPRESSION of growth factors and growth factor receptors is a possible prognostic indicator for various human tumours [1-3]. In breast cancer the expression of epidermal growth factor (EGF), transforming growth factor alpha (TGF-α) and EGF receptor (EGFR) is important in the growth of cancer cells. In addition, there is a strong inverse correlation between EGFR and oestrogen receptor (ER) status [4, 5]. However, patients with ER negative tumours have a worse prognosis than those with ER positive tumours [6]. Therefore, EGFR status may become an independent prognostic factor for primary breast cancer patients.

Sainsbury *et al.* first described the poor prognosis for patients with EGFR positive breast cancer compared with those who were EGFR negative [2]. However, a controversial report failed to demonstrate the prognostic significance of EGFR, regardless

of it being a large-scale study with a competitive binding assay [7]. Foekens *et al.* indicated several points of contrast including methodological differences: in Sainsbury *et al.*'s report, less than 10% of the tumours were analysed by Scatchard analysis, and the patients had a different background. We have also measured EGFR levels by a competitive binding assay, with Scatchard analysis, and have investigated the prognostic importance of EGFR in a prospective study. Here we present our 5-year results, as well as the relation between EGFR expression and tumour characteristics.

PATIENTS AND METHODS

135 primary breast cancer patients who had undergone either standard radical mastectomy or modified radical mastectomy with full dissection of axillary lymph-nodes were included in this study. Adjuvant therapy was as follows. Patients with stage I cancer (UICC criteria) without nodal metastasis received no therapy. Stage I patients with nodal metastasis, stage II and stage III patients were involved in a prospective randomised adjuvant trial and received each of two types of an adjuvant chemo-endocrine regimen for 2 years. In the trial all ER positive patients were given tamoxifen for 2 years.

The EGFR level in metastatic lesions was measured in 34 out of 135 patients, simultaneously or sequentially. The metastatic sites included 27 axillary nodal metastases, 5 soft tissue recurrence sites and 2 liver metastases. Liver metastastic samples were obtained by transabdominal operation for the purpose of insertion of a special tube into the hepatic artery for immunochemotherapy. Histological examination confirmed the malignancy before EGFR assay.

Tumour samples were immediately frozen in liquid nitrogen after surgical removal and stored at −80°C until assays within 2 weeks after removal. A competitive binding assay with ^{125}I-EGF (specific binding 1.85-4.44 MBq/μg) was used [5]. Crude membrane fraction was incubated with 3–7 concentrations of ^{125}I-EGF ranging from 0.05-5 nmol/l and a 200-fold excess of unlabelled EGF for blocking non-specific binding. Specific high affinity EGFR was calculated by Scatchard plot. Tumours were designated EGFR positive when the specific binding was greater than 1 fmol/mg protein. ERs were measured by a dextran-coated charcoal method and specimens with ER greater than 5 fmol/mg protein were designated positive.

To evaluate tumour characteristics in relation to growth fractions, histological grading [8] and immunocytochemical assay [9] with monoclonal antibody Ki-67, which recognises nuclear protein of proliferating cells, were used. The KI-67 positive cell rate per certain numbers of tumour cells was used for the evaluation. All patients were followed up every 3 months and no patient was lost from this survey. Bone scintigraphy was done every year. Chest X-ray and abdominal ultrasound examination for the detection of lung and liver metastasis were done every 6 months after surgery. The mean follow-up period was 31 months.

The association between EGFR and ER was evaluated by Spearman's rank correlation. Other associations between EGFR and background factors were studied by χ^2 and t tests. Relapse-free survival probabilities were calculated with the Kaplan–Meier method and the logrank test for a difference between the groups. Cox's model was used for multivariate analysis.

Table 1. Patients' characteristics

	EGFR	
	Positive	Negative
No.	55	80
Menopausal status		
Premenopausal	28 (51)	44 (55)
Postmenopausal	27 (49)	36 (45)
Tumour size (cm)		
≤2	11 (20)	18 (23)
2–5	36 (65)	49 (61)
>5	8 (15)	13 (16)
Axillary metastases		
0	25 (45)	51 (64)
1–3	17 (31)	16 (20)
≥4	13 (24)	13 (16)
ER*		
Positive	21 (38)	54 (68)
Negative	34 (62)	26 (33)
Histological grade †*		
I	10 (22)	35 (54)
II	17 (38)	21 (32)
III	18 (40)	9 (14)
Ki-67 positive cell rate*	21%	7%

No. (%)
$P < 0.01$, EGFR positive vs. EGFR negative.
†% of 110.

RESULTS

55 patients (40.7%) were EGFR positive. An inverse relation between EGFR and ER status was seen by Spearman's test ($P < 0.01$). No significant correlation was seen in age, menopausal status, axillary nodal involvement and tumour size (Table 1). However, significant correlations with histological grade and with Ki-67 positive cell rate were observed. The average Ki-67 positive cell rate of 40 EGFR negative patients was 7% compared with 21% in 28 EGFR positive patients.

The 5-year postoperative follow-up showed that EGFR positive patients had a worse prognosis than EGFR negative patients.

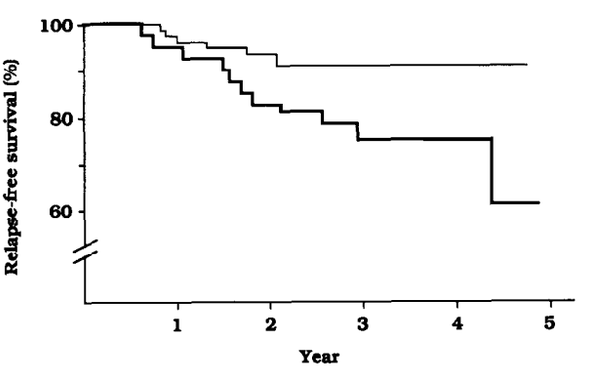

Fig. 1. EGFR status and postoperative relapse-free survival. Median follow-up 31 months. ——— = EGFR positive, ——— = EGFR negative.

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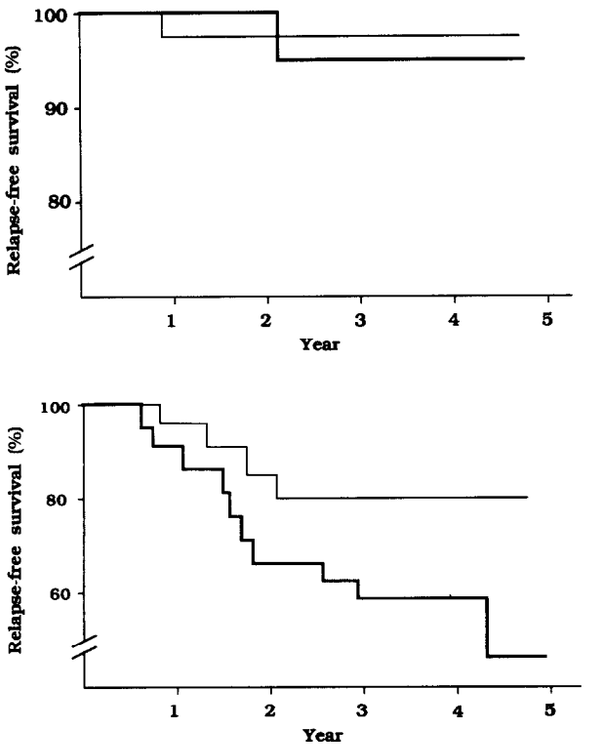

Fig. 2. Stratification by axillary nodal status. Upper = node negative and lower = node positive patients.

At 3 years after operation, a difference of about 20% in the relapse-free survival rate was seen in favour of patients with a EGFR negative tumour (Fig. 1). A statistically significant difference was observed from 22 months after surgery ($P < 0.05$). Figure 2 shows a breakdown according to axillary nodal involvement. The prognostic importance of EGFR expression was seen in node positive patients, who had significantly poorer prognosis if EGFR positive ($P < 0.05$). No difference was seen in node negative patients; however, the follow-up period may not have been long enough to draw any conclusions.

After stratification by ER status (Table 2), the relapse rate of patients who were EGFR positive and ER negative was higher than other subgroups, including either the EGFR or the ER positive group and both receptor negative groups.

Univariate analysis according to different tumour and patients' characteristics showed the following results. Axillary lymph-node involvement was strongly associated with a high relapse rate ($P < 0.01$). Tumour size, ER status and histological grading were also significantly associated ($P < 0.05$). However, for other factors including age and menopausal status, no statistical difference was seen. Multivariate analysis with Cox's model showed that axillary nodal status, ER status and histological grading were significantly associated with relapse rate.

Table 3. Relation between EGFR content and relapse rate

EGFR content (fmol/mg protein)	Relapse rate
<1	5/80 (6%)
1–5	1/14 (7%)
5–10	5/21 (24%)
≥10	5/20 (25%)

When relapse rate was analysed according to EGFR level, patients with more than 5 fmol/mg protein tended to have a worse prognosis compared with those with under 5 fmol/mg protein (Table 3). No difference was seen at higher EGFR levels. Haematopoietic recurrences tended to be more frequent in EGFR positive patients than in EGFR negative patients. Out of 11 relapses, 7 were haematopoietic recurrences in EGFR positive patients, compared with 1 out of 5 relapses in EGFR negative patients (Table 4). Prognosis after relapse was worse in EGFR positive patients than in EGFR negative patients (data not shown).

Table 5 shows the variation of EGFR status between primary tumour and metastatic site with in patients. The EGFR positive rate of primary tumour was 38%. However, that of secondary lesions was 50%, indicating that EGFR expression was enhanced in metastatic lesions.

DISCUSSION

The relapse-free survival rate of EGFR positive patients was worse than that of EGFR negative patients, particularly in node positive cases. Although it is difficult to assess the prognostic value in node negative patients because of the biological characteristics of breast cancer in Japanese women, whose prognosis is better than those from western countries [10], our results concur with those of Sainsbury *et al.* [4]. We also found a significant correlation between EGFR expression and histological grading. A correlation with monoclonal antibody Ki-67 positive cell rate, which represents the proportion of cycling cells, suggests that EGFR expression is closely linked to the cell proliferation activity of breast tumours. Thus the EGFR phenotype appears to be a poor prognostic indicator. Grimaux *et al.* reported the association between EGFR phenotype and poor prognosis in node positive patients, and also indicated a significant correlation with histological grading [11]. However, Foekens *et al.* failed to demonstrate the prognostic importance of EGFR, with a lack of association between EGFR and histological grading, in a 5-year follow-up [7].

Table 2. Relapse rate stratified by ER status

	EGFR		
	Positive	Negative	Total
ER+	3/21 (14)	3/54 (6)	6/75 (8)
ER−	8/34 (24)	2/26 (8)	10/60 (17)
Total	11/55 (20)	5/80 (6)	16/135 (12)

No. (%).

Table 4. First relapse site and EGFR status

	EGFR	
Relapse sites	Positive	Negative
Soft tissue	4 (36%)	3 (60%)
Bone	2 (18%)	1
Viscera	5 (46%)	1
Total	11	5

*Table 5. EGFR status in primary tumours and metastatic sites**

Primary tumour	Metastatic site EGFR+	EGFR−	Total
EGFR+	12 (35%)†	1 (3%)	13 (38%)
EGFR−	5 (15%)	16 (47%)	21 (62%)
Total	17	17	34

*EGFR status was examined simultaneously or sequentially in 34 patients.
† % of 34.

Methodological differences, including the cut-off point, should be noted. Most previous reports have used competitive binding assays. However, membrane preparation, free ligand separation method and cut-off were different between studies. The cut-off of EGFR varied between 0 and 10 fmol/mg protein. Sainsbury *et al.* chose 10, Grimaux *et al.* used 5 [11], and Fitzpatrick *et al.* used 1 fmol/mg protein [12]. Foekens *et al.* divided the patients into three groups by EGFR level: under 0.5, 0.5-2.0 and over 2.0 fmol mg/protein [7]. Although an inverse correlation between EGFR and ER was demonstrated in all previous studies, the proportion of EGFR positive patients differed, ranging from 20% to 52% [11]. It is therefore to be expected that the cut-off may influence clinical assessment on EGFR status, Foekens *et al.* found no significant difference among three different EGFR groups. However, in our preliminary analysis, when the cut-off was changed from 1 to 5 fmol/mg protein, the prognostic value of EGFR tended to be increased. Because no clinically relevant cut-off is available, it is difficult to discuss this point. We chose the cut-off with reference to a combination of a biochemical and an immunocytochemical assay (which can distinguish normal epithelial EGFR expression from that in tumour cells). It may also be advisable to determine the cut-off, due to its prognostic importance in view of application for treatment. Interlaboratory collaboration should begin to establish a standard method.

For patients' characteristics, especially pretreatment, differences between studies should be considered because, recently, a remarkable survival advantage by tamoxifen and multidrug chemotherapy has been demonstrated [13]. All our patients had undergone mastectomy with full dissection of axillary lymph-nodes without irradiation and received each of two types of chemo-endocrine adjuvant regimens for 2 years. Tamoxifen was given to most of ER positive patients. We have found no prognostic difference between the adjuvant regimens included in this study. However, tamoxifen may contribute to the better prognosis of EGFR negative patients because of 68% of EGFR negative patients were ER positive, which is a reliable indicator for the success of hormone therapy [14]. Interestingly, in Sainsbury *et al.*'s study, no patient received adjuvant hormone therapy or chemotherapy [4] but, in Foekens *et al.*'s study 19% of the patients received adjuvant chemotherapy and 5% patients received hormone treatment [7]. In our study EGFR status seemed to divide hormone independent patients, who are known to be in a poor prognostic group, into two subgroups: EGFR negative, good prognosis and EGFR positive, poor prognosis. This finding will be useful not only for studying the characteristics of ER negative tumour but also for planning the treatment strategy in ER negative patients.

The relation between EGFR expression and tumour characteristics needs to be better clarified. EGFR expression is related to histologically undifferentiated markers and high proportion of growth fractions [4, 9, 15]. Little is known about the association with other characteristics, such as metastatic potential and angiogenic activity. c-*erb*B-2 overexpression is not strongly associated with histological undifferentiation but has a remarkable prognostic importance [16, 17]. We have found a close correlation between EGFR expression and lymphatic invasion [9] and furthermore, in this study, we have shown the tendency for EGFR expression to be more likely enhanced in metastatic sites than in primary sites. This suggests an important role for EGFR expression, not only for the growth of tumour cells in the secondary lesion but also for the process of metastasis. Our data may show the importance of measuring EGFR status for the design of adjuvant therapy regimens.

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