Eur J Cancer Clin Oncol, Vol. 24, No. 11, pp. 1685–1690, 1988.
Printed in Great Britain

0277–5379/88$3.00 + 0.00

Epidermal Growth Factor Receptor in Human Breast Cancer: Correlation with Steroid Hormone Receptors and Axillary Lymph Node Involvement

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Abstract—*Epidermal growth factor (EGF-R) estrogen (ER) and progesterone (PR) receptors were evaluated in 89 primary breast cancers and 23 axillary lymph node metastases. About 57% of primary and 72.2% of metastatic tumors were EGF-R positive and median EGF-R levels were higher in metastatic deposits than in primary breast tumors* ($P < 0.05$). *An inverse distribution of EGF-R and steroid hormone receptor positive tumors was found* ($\chi^2 = 10.87$; $P < 0.001$ *for PR and* $\chi^2 = 5.01$; $P < 0.05$ *for ER) and an interesting correlation between EGF-R expression in primary tumor and axillary lymph node involvement was demonstrated* ($\chi^2 = 21.4$; $P < 0.001$).

Immunohistochemical studies with a monoclonal antibody against EGF-R revealed the presence of EGF-R only in malignant cells. Our data suggest that EGF-R could identify a class of more aggressive breast tumors endowed with a higher metastatic potential and may therefore represent an unfavorable prognostic parameter in breast cancer.

INTRODUCTION

THROUGH interaction with the specific transmembrane receptor, epidermal growth factor (EGF) seems to play an important role in regulating the growth of human breast cancer [1, 2]. Some authors have recently described the presence of EGF receptors (EGF-R) in primary and metastatic cancer but the clinical implications of these findings are still not clear [3]. It has been suggested that the presence of EGF-R may identify a group of more aggressive tumors endowed with higher metastatic potential [3–6].

In this report we have quantified the simultaneous binding of EGF in tissue specimens of breast cancer and in axillary lymph node metastases in relation to the presence of estrogen receptors (ER) and progesterone receptors (PR). We show that EGF-R are more abundant in metastatic foci than in primary tumors and correlated negatively with PR.

Accepted 18 May 1988.
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MATERIALS AND METHODS

This study included 89 patients (aged 40–79 years) with early breast cancer (stages I–II) admitted from January 1987 to February 1988 to the University Hospital 'A. Gemelli', Rome, for surgical treatment. Of these patients 54 were found to have regional axillary lymph node metastases (N+ patients) and 35 were free of lymph node metastases (N− patients).

Fresh tissue specimens from the primary tumor and metastatic lymph nodes were removed in the operating room, frozen immediately on dry ice, and stored at −80°C until assay. Representative tissue samples were taken and processed for histopathological evaluation as either frozen sections or formaldehyde-fixed paraffin-embedded sections. A procedure developed in our laboratory [7] which permits the simultaneous measurement of EGF-R, ER and PR in the same tissue specimen was used. Briefly, our specimens were finely chopped with a scalpel and homogenized in 5 volumes of ice-cold buffer consisting of 25 mM Tris, 1.5 mM EDTA, 5 mM NaN_3, 0.1% monothioglycerol and 20% glycerol (TENMG), by applying three to four 10-s bursts of an Ultra-Turrax homogenizer with inter-

mittent cooling. The crude homogenate was centrifuged at 7000 ***g*** for 20 min at 0°C and the supernatant was further centrifuged at 105,000 ***g*** for 75 min at 0°C. The subsequent supernatant was saved for ER and PR analysis and the pellet was used for EGF-R. ER and PR assay was performed by the dextran-coated charcoal procedure [8] using [^{3}H]estradiol and [^{3}H]ORG-2058 (both from Amersham Corp.) as radiolabelled ligands for ER and PR, respectively. DES (Merck) and ORG-2058 (Amersham Corp.) were used as competing unlabelled steroids. Tumors with 10 or more fmoles/mg protein of PR were considered to be positive. For EGF-R assay, membrane pellets were resuspended in 25 mM Tris, 1.5 mM EDTA, 5 mM NaN_3, 20% glycerol and 10 mM $MgCl_2$ (TENG + $MgCl_2$). Aliquots of the suspension (100 μl containing 300 to 500 μg protein) were incubated with [^{125}I]EGF (2.6 nM) in the presence or absence of unlabelled EGF (1 μg) for 12–16 h at room temperature in a final volume of 400 μl. Binding was blocked by addition of 3 ml of ice-cold 25 mM Tris, 20% glycerol, 5 mM NaN_3 and 0.1% BSA. After centrifugation at 2000 ***g*** for 20 min at 0°C the supernatant was carefully aspirated, and pellets counted in a γ-counter. Results were expressed as fmoles [^{125}I]EGF bound per mg membrane protein.

Tissue weight permitting, Scatchard analysis was carried out with concentrations of [^{125}I]EGF ranging from 0.4 to 2.6 nM either alone or in the presence of unlabelled EGF (1 μg). The analysis showed a single class of high affinity limited capacity binding sites with a dissociation constant of 1.02 nM (mean of 14 different determinations). A value of 1.5 fmoles/mg protein was arbitrarily chosen as the cut-off to discriminate between EGFR+ and EGFR− tumors. EGF binding was verified by using a monoclonal antibody against the external domain of the EGF-R (MAb 108, courtesy of Dr. J. Schlessinger, Rohrer Biotechnology Inc., Rockville, MD, U.S.A.).

An indirect immunoperoxidase technique was used on frozen sections that were briefly fixed in 5% formaldehyde in phosphate buffer solution (PBS), pH 7.4. Slides were coded and read by two independent pathologists, and data obtained were compared to the results of radiometric assay.

The χ^2 test and the Wilcoxon rank sum test were used for statistical analysis.

RESULTS

Of 89 primary breast cancer specimens examined, 51 (57.3%) were EGF-R positive and 38 (42.6%) EGF-R negative. For positive specimens (containing more than 1.5 fmoles/mg protein), receptor values ranged from 1.5 fmoles/mg protein to 25.5 fmoles/mg protein, with a median value of 3.7 fmoles/mg protein. To verify that EGF-R measured by the radiometric assay was indeed expressed by tumor cells themselves and correlated with receptor positivity, an indirect immunoperoxidase staining procedure with a monoclonal antibody (MAb) was used. As shown in Fig. 1, only malignant cells reacted to the MAb while the stroma was completely unreactive. Moreover, a correlation was observed between positivity in immunoperoxidase staining and positivity as measured by the radiometric assay (data not shown). Thirty-six (40.4%) tumors were found to be ER positive and 21 (23.59%) PR positive. Table 1 shows the relationship between ER, PR and EGF-R. A negative correlation between the presence of EGFR and PR ($\chi^2 = 10.87$; $P < 0.001$) was observed, with 46/51 (90.1%) EGFR positive tumors being PR negative. A similar correlation was found between EGF-R and ER ($\chi^2 = 5.01$, $P < 0.05$), the percentage of EGFR+ tumors being higher in ER− than in ER+ tumors (36/53, 67.9% vs. 15/36, 41.6%). As expected, a positive correlation between ER and PR expression was found ($\chi^2 = 25.9$; $P < 0.001$).

Table 2 shows the relationship between the expression of EGF-R, ER and PR in the primary tumor as a function of axillary lymph node involvement. In brief: (i) 77.7% of N+ patients and only 25.7% of N− patients were EGFR+, this difference being statistically significant ($\chi^2 = 21.4$; $P < 0.001$); (ii) PR were less frequently expressed in N+ than in N− tumors ($\chi^2 = 10.17$; $P < 0.001$);

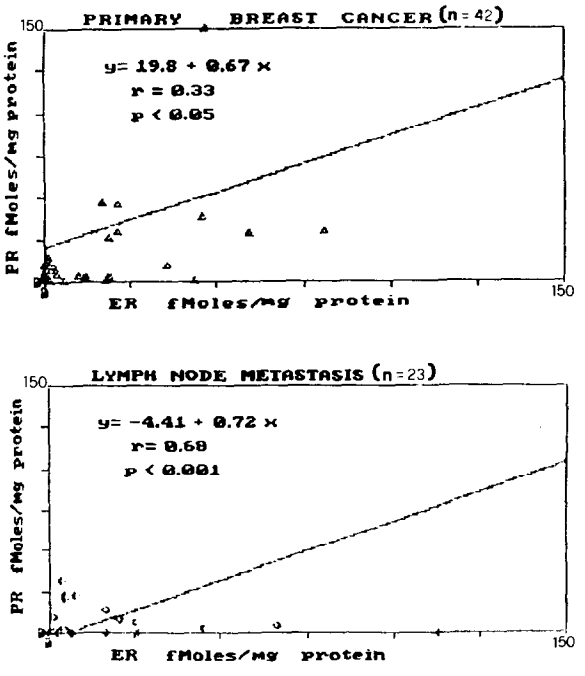

Fig. 2. EGF-R levels in primary human breast cancer and simultaneous lymph node metastases. The Wilcoxon test shows a statistically significant difference. ----- Median.

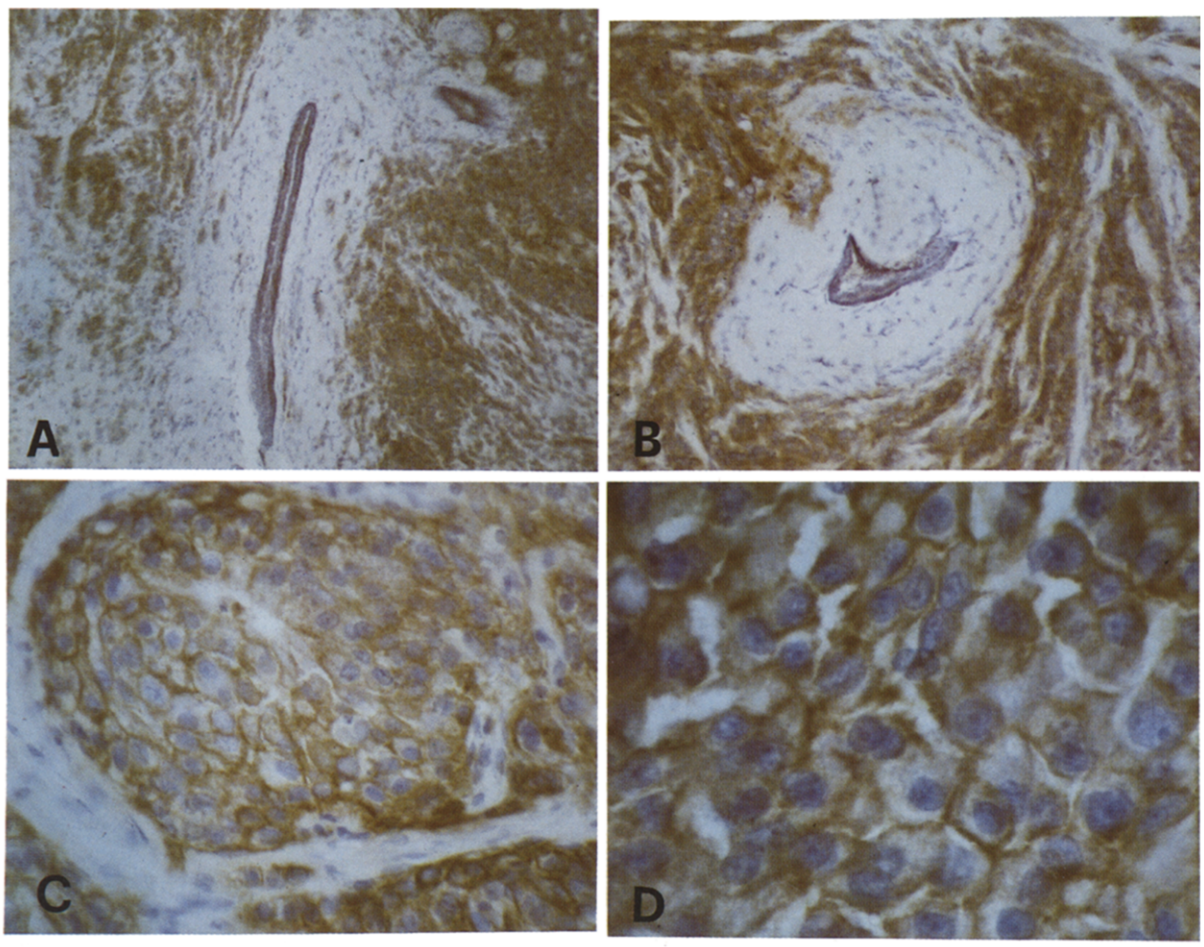

Fig. 1. *Immunohistochemical localization of EGF-R in primary breast tumor. Only malignant cells were reactive with MAb* 108, *while the stroma and normal ductal cells were completely unreactive* (*magnifications*: *A*, *B*, 10×; *C*, 16×; *D*, 40×).

Table 1. Relationship between EGF-R, ER and PR in 89 primary breast cancers

	PR+	PR−		
EGFR+	5	46	51	$\chi^2 = 10.87$
EGFR−	16	22	38	$P < 0.001$
	21	68	89	
	ER+	**ER−**		
EGFR+	15	36	51	$\chi^2 = 5.01$
EGFR−	21	17	38	$P < 0.025$
	36	53	89	
	PR+	**PR−**		
ER+	19	17	36	$\chi^2 = 25.9$
ER−	2	51	53	$P < 0.001$
	21	68	89	

Table 2. Relationship between EGF-R, ER, PR in primary tumor and axillary lymph node metastases

	N+	N−		
EGFR+	42	9	51	$\chi^2 = 21.44$
EGFR−	12	26	38	$P < 0.001$
	54	35	89	
PR+	6	15	21	$\chi^2 = 10.17$
PR−	48	20	68	$P < 0.01$
	54	35	89	
ER+	15	21	36	$\chi^2 = 7.86$
ER−	39	14	53	$P < 0.01$
	54	35	89	

(iii) an inverse correlation of lymph node involvement and ER status was also observed ($\chi^2 = 7.86$; $P < 0.01$). In 23 patients it was possible to measure EGF-R simultaneously in the primary tumor and in axillary lymph node metastases (Fig. 2). In 17 of these cases higher levels of EGF-R were measured in metastases than in respective primary tumors, with a median increase of 157% (range 27–590%). Overall, EGF-R concentrations were significantly higher in metastases (median 3.72; range 0–44.2 fmoles/mg protein) than in primary tumors (median 2.2; range 0–8.5 fmoles/mg protein) ($P < 0.05$). No significant difference in the concentrations of ER or PR in primary vs. metastatic specimens was observed (data not shown).

DISCUSSION

In this study we have observed that EGF-R is expressed in approximately one out of two primary breast cancers and two out of three lymph node metastases. EGF-R content was higher in metastatic than in primary breast tumors. Similar results have been reported by other authors [3, 4]. In our series most of the EGFR+ tumors were also N+ (82.3%). It is well known that lymph node status at the time of primary surgery is the most important prognostic factor in breast cancer [9]. Therefore the finding that patients with EGF-R positive primary tumors have a greater likelihood of axillary lymph node involvement suggests that the receptor for this growth factor may identify tumors with a higher metastatic potential. A recent report [10] has shown that EGF-R expression in primary breast cancer is the most important variable for predicting relapse-free and overall survival. Macias *et al.* [3] have hypothesized that the expression of EGF-R in breast cancer cells is a necessary, although not ultimate, requirement for metastatic potential. Also in support of this hypothesis our finding of a negative correlation between EGF-R and steroid hormone receptors (these latter being markers of favorable prognostic outcome in breast cancer [9, 11]) and the finding that a large proportion of breast cancer steroid hormone-negative patients have metastatic deposits in their axillary lymph nodes. Moreover, since steroid hormone receptors are indicators of estrogen regulated cell proliferation [12], it can be also hypothesized that the growth of steroid-independent breast cancer is regulated by peptide factors such as EGF or EGF-like molecules [8, 13]. In this respect it is noteworthy that Roos *et al.* [14] have shown significantly higher levels of EGF-R in hormone-independent than in hormone-dependent human breast cancer cells. Other investigators have either failed to show any correlation between EGF-R and PR or have found an inverse relationship between EGF-R and ER [3, 4, 10]. The reason for these discrepancies is not clear.

A recent report has shown that monoclonal antibodies against EGF-R are able to inhibit the proliferation of human cancer cells *in vitro* as well as the growth of human mammary tumor xenografts transplanted into nude mice [15]. Thus the possibility of a new therapeutic approach in breast cancer based on interference with EGFR binding can be envisaged, particularly in the case of hormone-independent tumors no longer amenable to endocrine treatment.

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