22. Sharma FD, Johnson AF, Whitton AC. Radiotherapy for jugulotympanic paragangliomas. *J Laryngol and Otol* 1984, **98**, 621–629.
23. Hudgkins PT. Radiotherapy for extensive glomus jugulare tumors. *Radiology* 1972, **103**, 427–429.
24. Kraus DH, Sterman BM, Hakaim AG, *et al.* Carotid body tumors. *Arch Otolaryngol Head and Neck Surg.* 1990, **116**, 1384–1387.
25. Wang LC. What is the optimum dose of radiation therapy for glomus tumours? *Int J Radiation Oncol Biol Phys* 1980, **6**, 945–946.
26. Tidwell TJ, Montague ED. Chemodectomas involving the temporal bone. *Radiology* 1975, **116**, 147–149.
27. Arthur K. Radiotherapy in chemodectoma of the glomus jugulare. *Clin Radiol* 1977, **28**, 415–417.
28. Gibbin KP, Henk JM. Glomus jugulare tumors in South Wales—a twenty year review. *Clin Radiol* 1978, **29**, 607–609.
29. Thomsen KA, Hansen HS. Therapie des glomus Jugulare—Tumors. *HNO* 1979, **27**, 189–191.
30. Kim JA, Elkon D, Lim ML. Constable WC. Optimum dose of radiotherapy for chemodectomas of the middle ear. *Int J Radiation Oncol Biol Phys* 1980, **6**, 815–819.
31. Lybeert MLM, Van Andel JG, Eijkenboom WMH, De Jong PC, Knegt P. Radiotherapy of paragangliomas. *Clin Otolaryngol* 1984, **9**, 105–109.
32. Konefal JB, Pilepich MV, Spector GJ, Perez CA. Radiation therapy in the treatment of chemodectomas. *Laryngoscope* 1987, **97**, 1331–1335.
33. Hansen HS, Thomsen KA. Radiotherapy in glomus tumours (paragangliomas). A 25 year review. *Acta Otolaryngeol* 1988, **449**, 151–154.
34. Friedland JL, Mendenhall WM, Parsons JT, Million RR, Cassisi NJ. Chemodectomas arising in temporal bone structures. *Head Neck Surg* 1988, **10**, supplement I, S52–S55.
35. Hoogenhout J, Ligthart JCP, Van den Brock P, *et al.* Surgery and radiotherapy in the management of glomus body tumours. Abstract paper. 9th Annual meeting ESTRO, Montecatini, Italy, 12–15 September 1990, 67.
36. Boyle JO, Shimm DS, Coulthard SW. Radiation therapy for paragangliomas of the temporal bone. *Larynscope* 1990, **100**, 896–901.
37. Verniers D, Van Limbergen E, Leysen J, Ostyn F, Segers A. Radiotherapie als voorkeursbehandeling voor chemodectoma? *Acta otorhino-laryngologica belg* 1990, **44**, 21–26.
38. Rosenwasser H. Current management: glomus jugulare tumors. *Ann Otol Rhinol Laryngol* 1967, **76**, 603–610.
39. Hatfield PN, James AE, Schultz MD. Chemodectomas of the glomus jugulare. *Cancer* 1972, **31**, 1164–1168.
40. Newman H, Rowe JF, Philips TL. Radiation therapy of the glomus jugulare tumour. *Am J Roentgenol* 1973, 663–669.
41. Spector GJ, Fierstein J, Ogura JH. A comparison of therapeutic modalities of glomus tumors in the temporal bone. *Laryngoscope* 1976, **88**, 690–696.

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Immunocytochemistry and *in situ* Hybridisation of Epidermal Growth Factor Receptor and Relation to Prognostic Factors in Breast Cancer

Michael Bilous, Jane Milliken and Jean-Marie Mathijs

The breast tumour distribution of epidermal growth factor receptor (EGFR) was studied in 193 patients with primary breast cancer by immunocytochemistry on frozen sections. EGFR was correlated ($P = 0.0009$) with growth fraction assessed by Ki-67, and negatively correlated with oestrogen receptor (ER, $P = 0.0001$) and progesterone receptor (PR, $P = 0.0001$) status. In 47 patients, in-situ hybridisation for EGFR mRNA showed good agreement with the immunocytochemically assessed EGFR protein. There were, however, several tumours in which EGFR mRNA could be detected in the absence of EGFR protein and there were differences between the ER and PR status of those tumours in which translation of EGFR mRNA was not seen. The cause of these differences is unclear, but these findings may represent a clue as to the differential control of breast cancer cell receptors.
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INTRODUCTION

THERE IS a need to distinguish between patients with breast cancer whose disease is localised to the breast and who will be cured by tumour removal and axillary lymph node clearance, and those whose tumour has spread further and who need additional systemic therapy. At present, axillary lymph node status, tumour grade and oestrogen receptor (ER) status are three of the selection criteria used. Relapse, however, is seen in 20–30% of axillary lymph node negative patients, and response to endocrine therapy is not strictly linked to ER status [1]. This has led to the search for alternative new prognostic indicators such as growth factors and their receptors, and to the measurement of tumour proliferation rates using techniques such as monoclonal antibodies and flow cytometry.

Epidermal growth factor (EGF) is a 6045 molecular weight polypeptide which is known to mediate cell proliferation in a range of tissues including human breast epithelium *in vivo* and *in vitro* [2]. The action of EGF is mediated via the EGF receptor

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(EGFR). The presence of EGFR has been described in a number of breast cancer cell lines and in biopsies of benign and malignant breast lesions, as well as in other cell types [3–5]. EGFR is a transmembrane growth regulating glycoprotein with an extracellular ligand-binding domain and an intracellular tyrosine kinase domain and is a member of the c-*erb*B oncogene family [6]. It has been shown that the presence of EGFR is an important predictor of survival in patients with breast cancer [7, 8].

In this study we have examined by immunocytochemistry, the distribution of EGFR in 193 breast cancers. The results have been correlated with a variety of clinical and pathological variables in the patients. In addition, 47 of the tumours have been assessed for EGFR mRNA expression by *in situ* hybridisation, to further our knowledge concerning the expression of this receptor gene in relation to the presence of ER and progesterone receptor (PR).

MATERIALS AND METHODS

Patients and histopathological assessment

Frozen specimens of 193 primary breast cancers were received in the laboratory for assessment. The age range of the patients from which they were derived was 24 to 90 years. 81 were premenopausal and 112 were postmenopausal. The frozen specimens were stored at −70°C prior to cutting 6 μm frozen sections for immunocytochemical staining. In addition, 6 μm paraffin sections of the tumours were also prepared from separately received formalin-fixed specimens of the tumours. These were assessed for mitotic rate, expressed as the number per 10 fields (400×), and for histopathological grade using the method employed by Contesso *et al.* [9], which is a modification of the Bloom and Richardson grading system [10]. Tumour size and lymph node status where known, were subsequently derived from the macroscopic description on the completed histopathology reports of the patients.

Immunocytochemistry

4–6 μm frozen sections were cut and stained by standard immunoperoxidase techniques using the following antisera:

(a) Ki-67 (DAKO-PC).
(b) EGFR (Amersham). This antibody recognises an antigen on the extracellular domain of the receptor and has been prepared from A431 cells.
(c) Oestrogen receptor immunocytochemical assay (ERICA, Abbott Diagnostics). This antibody was prepared from MCF-7 human breast cancer cells.
(d) A monoclonal antibody to human PR. This antibody was prepared from a human endometrial carcinoma cell line grown in nude mice, in which two progesterone hormone binding proteins were present. The antibody recognises both receptor proteins and has been shown to be suitable for study by both biochemical and immunocytochemical methods. Details of antibody preparation and evaluation are published elsewhere [11].

Ki-67 staining was assessed by counting 500 tumour cell nuclei and expressing the result as the percentage of nuclei that were stained.

EGFR staining was assessed as grade 0–3 staining of tumour cell membranes and peripheral cytoplasm. Grade 0 represents no staining; 1, feint focal tumour cell staining; 2, moderate staining of clusters of tumour cells and 3, heavy staining of sheets of tumour cells.

ER and PR antibody staining results were expressed as a staining index 0–6, being the sum of the intensity of nuclear staining (0–3), and the percentage of tumour cell nuclei stained (0–3), thus 5–30% = 1, 31–70% = 2, 71–100% = 3. A tumour with a staining index of 2 or more was considered to be ER/PR positive after counting 500 tumour cells [12]. Negative control sections for immunocytochemistry were those in which the primary antibody was omitted from the procedure. Positive control sections were derived from those cases of breast cancer that were known to be positive for ER and PR, as assessed by enzyme immunoassay technique on cytosol preparations. Positive control sections for Ki-67 and EGFR immunocytochemical staining were derived from those tumours which had consistently given strong positive results on their tumour cells.

In situ *hybridisation*

The oligonucleotides used for *in situ* hybridisation (ISH), were synthesised on a DNA synthesiser (model 380B; Applied Biosystems). The sequences for EGFR were complimentary to sequences at position 3118-31 and were ATGGCGGGCGTGGACGCCGACGAG and CTCCTCATGGACTAGGGGGTCGTCCCGAAG [13]. Specificity of the oligonucleotide probes was checked by reference to Genebank and EMBL sequence databank. The oligonucleotides were linked at the 5′ end with aminolink (ABI) dissolved in coupling buffer, incubated for 6 h with a 50 molar excess of alkaline phosphatase (enzyme grade, Boehringer), and purified on a G-50 column in phosphate buffered saline (PBS) containing 0.1% bovine serum albumin.

6 μm paraffin sections of the tumours were dewaxed and rehydrated before being fixed in 4% paraformaldehyde for 10 min. The sections were stored in 70% ethanol at 4°C until used, and then rehydrated in 5 mmol/l $MgCl_2$ in PBS for 10 min and prehybridised in 70% formamide/0.2 × sodium saline citrate (SSC) (20 × SSC: sodium citrate 0.3 mol/l, sodium chloride 3 mol/l, pH 7), at 70°C for 10 min. They were washed in 5 × SSC at 55°C and hybridised for 45 to 60 min at 55°C. The sections were washed in 1 × SSC and incubated with Vector Red or Vector Blue (Vector Laboratories, Burlingane, California). Sections were counterstained with either haematoxylin or fast red before being mounted in depex. Negative controls involved the omission of the hybridisation step of the procedure. In addition, RNase pretreatment of the slides eliminated the signal. As a positive control, a tumour known to be strongly positive for EGFR by immunocytochemistry was employed.

Tumours were arbitrarily assessed as positive for EGFR with this technique when at least one positively staining nucleus was seen in each of five high power (×400) microscope fields.

Statistics

Kendall's rank correlation test was used to analyse the continuous variables. P values of < 0.05 were considered significant.

RESULTS

Table 1 summarises some of the data obtained from the 193 cases studied. Immunocytochemical staining for EGFR was predominantly localised to the cell membrane, but feint cytoplasmic staining was also seen in some tumour cells and this was frequently accentuated at the periphery of the cytoplasm. The distribution of staining was heterogeneous but among groups of tumour cells rather than between individual cells. Some tumours showed heavy staining of most of the malignant cells, but in other cases only small groups of tumour cells stained feintly. Fig. 1a and b shows a tumour stained by haemotoxylin and eosin and with EGFR antibody, respectively. EGFR staining

Table 1. Pathological data

EGFR IC staining	59/193 (30.5%)	Grade 1 29 Grade 2 18 Grade 3 12
Ki-67 staining	Range 1–57% Average 14.9%	91 cases assessed
ER and PR staining	ER+ PR+ 94 ER+ PR− 33	ER− PR− 58 ER− PR+ 8
Lymph node status	148 cases where lymph node dissection performed 83 lymph node + 65 lymph node −	

Table 2. Statistical analysis

	Kendall correlation coefficient	*P* value
EGFR IC vs.		
Ki-67	3.325	0.0009
ER	−6.934	0.0001
PR	−4.883	0.0001
Lymph node status	N.S.	N.S.
Tumour size	N.S.	N.S.
Ki-67 vs.		
Mitotic rate	5.801	0.0001
ER	−4.686	0.0001
PR	−4.392	0.0001

N.S. = not significant.

was seen in some benign duct epithelial and myoepithelial cells, but in general, there was negligible staining of the stromal connective tissue cells. A tumour was considered to be 'EGFR positive' if it showed ≥ grade 1 cytoplasmic staining. Table 2 summarises the statistical correlations found with EGFR.

Ki-67 staining was confined to cell nuclei. In addition to tumour cells, the nuclei of occasional duct epithelial cells, lymphocytes, and some stromal cells were also stained. Although the intensity of staining was variable among tumour cell nuclei, there was usually most intense staining of the nucleoli (Fig. 1c).

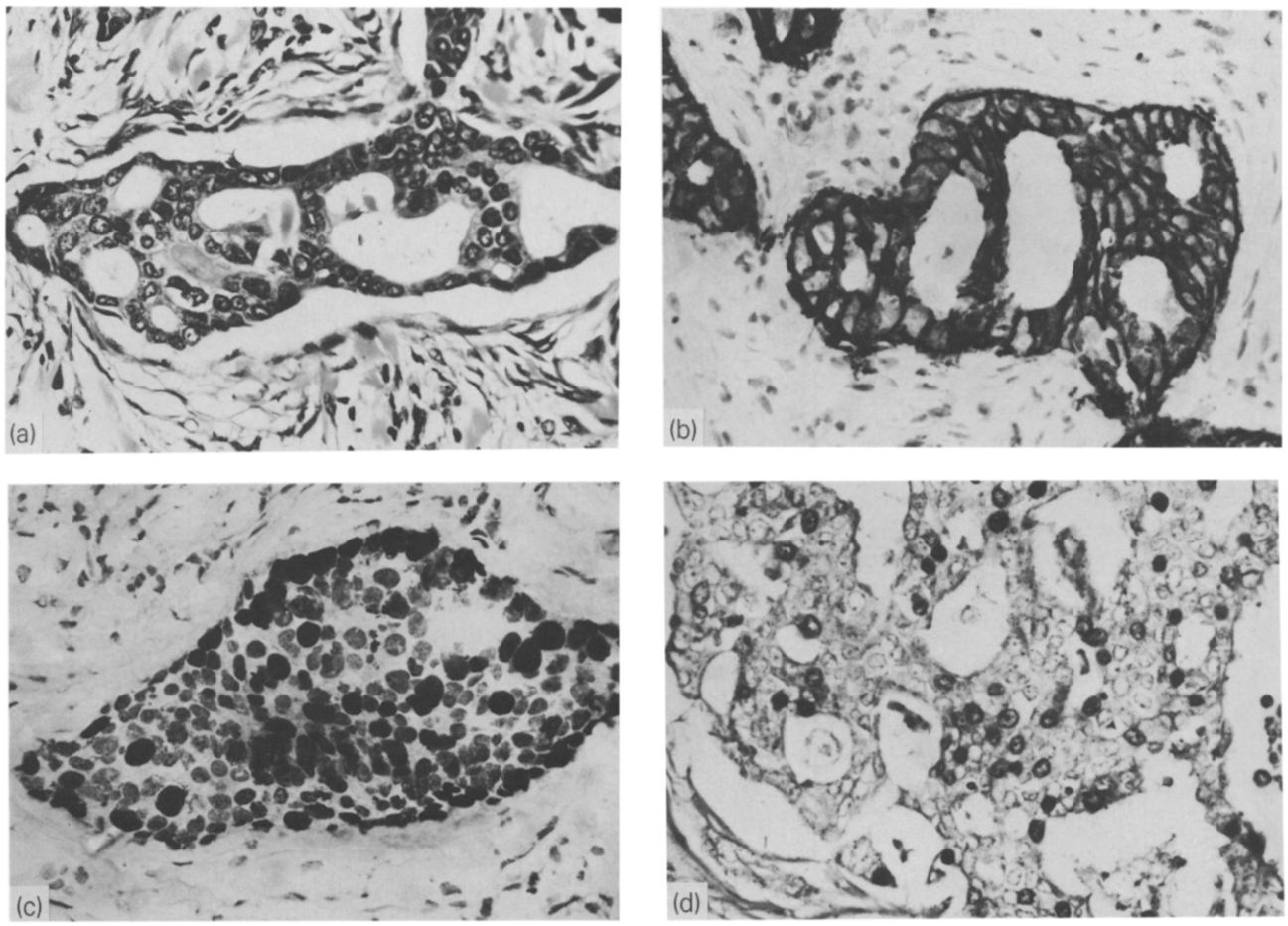

Fig. 1. (a) Infiltrating duct carcinoma. Group of carcinoma cells with surrounding fibrovascular stroma (haemotoxylin and eosin × 350). (b) Infiltrating duct carcinoma. Same case as (a), immunocytochemically stained for EGFR. Note: heavy positive staining of carcinoma cell membranes and peripheral cytoplasm (anti EGFR × 350). (c) Infiltrating duct carcinoma. Ki-67 stained nuclei appear black in a group of carcinoma cells (anti Ki-67 × 350). (d) Infiltrating duct carcinoma. Nuclei stained in paraffin section using *in situ* hybridisation for EGFR oligonucleotide. Note: numerous positive carcinoma cell nuclei and negative (counter stained) nuclei (EGFR *in situ* hybridisation × 275).

Table 3. EGFR measured by immunocytochemistry and in situ *hybridisation*

		EGFR (ISH)	
		+	−
	+	17	0
EGFR (IC)	−	17	13

IC = Immunocytochemical method.
ISH = *In situ* hybridisation method.

ER and PR staining was confined to the nuclei of tumour cells and occasional benign duct epithelial cells. Heterogeneity of tumour cell staining was present but less pronounced than that seen with EGFR and tended to be seen among individual cells rather than cell groups.

The 47 tumours stained for EGFR using *in situ* hybridisation showed specific staining of tumour cell nuclei with no cytoplasmic staining. There was very little variation in intensity of staining of the nuclei of individual tumours and, where present, the variation tended to be among individual tumour cells rather than groups of cells (Fig. 1d). In addition, there were only two tumours which were considered borderline in that they had very few positive nuclei. The remainder were either unstained or strongly positive. In some cases there was staining of benign duct epithelial cell nuclei and in one or two cases only, occasional stromal fibroblast nuclei were stained. Table 3 summarises the results of ISH and IC staining for EGFR. EGFR mRNA was detected by ISH in 34 of the 47 cases assessed (72%), while IC showed the receptor protein in only 50% of these. Table 4 shows the relationship between EGFR results measured by both techniques, and the different combinations of ER and PR. Of the 17 cases that were ISH and IC positive for EGFR, 15 were ER negative and 16 were PR negative. Of the 17 cases that were ISH positive but IC negative, 16 were ER positive and 11 were PR positive.

DISCUSSION

In this study, we have attempted to further categorise EGFR regarding its relationship to other prognostic factors in breast cancer. EGFR was demonstrated by immunocytochemistry in 59 (30%) of the 193 cases of primary breast cancer that were assessed. This result is in keeping with a number of other studies which have used either immunocytochemistry or radioligand binding techniques to give EGFR-positive rates ranging from 14 to 64% with a mean of 34% [14–21].

Table 4. A comparison between EGF R and ER and PR results

	ER+PR+	ER+PR−	ER−PR−	ER−PR+
EGFR				
ISH+ IC+	1	1	15	0
ISH+ IC−	11	5	1	0
ISH− IC−	11	2	0	0

IC = Immunocytochemical method.
ISH = *In situ* hybridisation method.

The heterogeneity of tumour cell staining was a striking feature in most tumours. As nearly all our EGFR positive tumours were ER negative (15 out of 17, Table 4), we were unable to demonstrate convincingly the reciprocal staining of individual tumour cells that has been reported by others [16]. A similar degree of heterogeneity, however, was seen with both ER and PR staining, which supports the belief that subsets of tumour cells may exist and be responsible for more aggressive behaviour [22].

The use of immunocytochemistry for the determination of ER and PR status is now well established and good correlations have been shown between the results using this technique and those involving cytosol preparation [23]. We have demonstrated a strong negative correlation between EGFR and both ER and PR in our patients. A similar relationship between ER and EGFR has been shown in other published studies [14, 16–18, 20, 21]. However, no statistically significant relationship was demonstrated by Bevilacqua *et al.* [15] or by Kommos *et al.* [25]. In addition, we have shown that those tumours having EGFR have a higher proliferation rate than those lacking EGFR, as measured using Ki-67 monoclonal antibody. This antibody reacts with cells in all phases of the growth cycle except Go [24], and our results therefore suggest that EGFR positivity is associated with more active cellular proliferation. In addition, a significant inverse relationship is present between Ki-67 staining and both ER and PR status. We have been unable to show a significant correlation between EGFR and tumour size or lymph node status. Variable results have been found with other pathological correlation studies. While some have found significant correlation with nuclear size [14], tumour grade [26], lymphatic invasion [26] and axillary lymph node metastasis [21], others have shown no correlation between EGFR and these features [15–17].

Clinical studies by Nicholson *et al.* in Newcastle [20], have demonstrated the value of EGFR assessment as a prognostic factor. In 231 patients with operable breast cancer, EGFR measurement proved to be second only to axillary node status as a prognostic marker for relapse-free and overall survival over a median follow-up period of 45 months. Furthermore, for axillary lymph node negative patients, EGFR was better than ER, size and tumour grade in predicting relapse and survival. EGFR also proved to be a better predictor of response to tamoxifen in 72 patients with recurrent disease [27]. More importantly, EGFR status divided the ER negative patients into responders and non-responders to this therapy.

In addition to acting as a prognostic factor, EGFR status may offer a mechanism to control breast cancer cell growth. Monoclonal antibodies to EGFR have been shown to inhibit the proliferation of human cancer cells *in vitro*, as well as the growth of human breast cancer xenografts in nude mice [28]. Also, in a study of the effect of antineoplastic agents on the binding of EGF to MCF-7 human breast cancer cells, Hanauske *et al.* [29] showed that cisplatin and vinblastine were able to inhibit the binding of EGF to these cells, which could explain their anti-proliferative effect.

Our finding of a marked discrepancy between *in-situ* hybridisation and immunocytochemically-assessed EGFR mRNA and receptor protein when compared with ER and PR status, raises some important questions. Approximately twice as many tumours were EGFR positive by ISH as by IC, suggesting that although EGFR mRNA was expressed in these cells, in a high proportion no receptor protein was detectable. The reasons for this are unclear but could be due to differences in the sensitivities

of the two methods. Given the different pattern of variation of staining intensity seen with the two methods however, this would seem unlikely. Other possibilities include EGFR protein instability in a subset of tumours and failure of EGFR mRNA to be translated. It is, of course, also possible that the probes used have cross-hybridised with an unrelated mRNA not listed in the Genebank or EMBL sequence databank. These possibilities are being investigated using larger numbers of tumours. Furthermore, the clinical course of all these patients will need to be assessed in order to determine the prognostic significance of the results.

1. Vogel CL, East D, Voigt W, Thomsen S. Response to Tamoxifen in estrogen receptor-poor metastatic breast cancer. *Cancer* 1987, **60**, 1184–1189.
2. Carpenter G, Cohen S. Epidermal growth factor. *Ann Rev Biochem* 1979, **48**, 193–216.
3. Barker S, Panahy C, Puddefoot JR, Goode AW, Vinson GP. Epidermal growth factor receptor and oestrogen receptors in the non-malignant part of the cancerous breast. *Br J Cancer* 1989, **60**, 673–677.
4. Tauchi K, Hori S, Itoh H, Osamura RY, Tokuda Y, Tajima T. Immunohistochemical studies on oncogene products (c-*erb*B-2, EGFR, c-*myc*) and estrogen receptor in benign and malignant breast lesions. *Virchows Archiv Pathol Anat* 1989, **416**, 65–73.
5. Werner MH, Nanney LB, Stoseheck CM, King LE. Localisation of immunoreactive epidermal growth factor receptors in the human nervous system. *J Histochem Cytochem* 1988, **36**, 81–86.
6. Downward J, Yarden Y, Scrace G, *et al.* Close similarity of epidermal growth factor receptor and v-*erb*B oncogene protein sequences. *Nature* 1984, **307**, 521–527.
7. Nicholson S, Richard J, Sainsbury C, *et al.* Epidermal growth factor receptor (EGFR) results of a 6 year follow-up study in operable breast cancer with emphasis on the node negative subgroup. *Br J Cancer* 1991, **63**, 146–150.
8. Grimaux M, Romain S, Remvikos Y, Martin PM, Magdelenat H. Prognostic value of epidermal growth factor receptor in node positive breast cancer. *Br Cancer Res Treat* 1989, **14**, 77–90.
9. Contesso G, Mouriesse H, Friedman S, *et al.* The importance of histological grade in long term prognosis of breast cancer: a study of 1010 patients uniformly treated at Institute Gustave-Roussy. *J Clin Oncol* 1987, 1378–1386.
10. Bloom HJG, Richardson WW. Histologic grading and prognosis in breast cancer. *Br J Cancer* 1957, **11**, 359–377.
11. Clarke C, Zaino RJ, Feil PD, *et al.* Monoclonal antibodies to human progesterone receptor: characterisation by biochemical and immunohistochemical technique. *Endocrinology* 1987, **121**, 1123–1132.
12. Bilous AM, Tyler JPP, Toppila M, Milliken J. Whither cytosolic estrogen receptor assays? A comparison of commercially available kits for estrogen receptor assay. *Pathology* 1987, **19**, 223–228.
13. Yamamoto T, Ikawa S, Akiyama T, *et al.* Similarity of protein encoded by the human *erb*-2 gene to epidermal growth factor receptor. *Nature* 1986, **319**, 230–234.
14. Lewis S, Locker A, Todd JH, *et al.* Expression of epidermal growth factor receptor in breast carcinoma. *J Clin Pathol* 1990, **43**, 385–389.
15. Bevilacqua P, Gasparini G, Dal-Fior S, Corrodi G. Immunocytochemical determination of epidermal growth factor receptor with monoclonal EGFR1 antibody in primary breast cancer patients. *Oncology* 1990, **47**, 313–317.
16. Toi M, Hamada Y, Nakamura T, *et al.* Immunocytochemical and biochemical analysis of epidermal growth factor receptor expression in human breast cancer tissues: relationship to estrogen receptor and lymphatic invasion. *Int J Cancer* 1989, **43**, 220–225.
17. Betta PG, Robutti F, Spinoglio G, Bottero G. Expression of epidermal growth factor receptor in human breast carcinoma and its correlation with morphological features of tumour aggressiveness. *Pathologica* 1989, **81**, 425–431.
18. LLorens MA, Bermejo MJ, Salcedo MC, Charro AL, Pirente M. Epidermal growth factor receptor in human breast and endometrial carcinomas. *J Steroid Biochem* 1989, **34**, 505–509.
19. Reubi JC, Torhorst J. The relationship between somatostatin epidermal growth factor and steroid hormone receptor in breast cancer. *Cancer* 1989, **64**, 1254–1260.
20. Nicholson S, Wright C, Sainsbury JR, *et al.* Epidermal growth factor receptor (EGFr) as a marker of poor prognosis in node negative breast cancer patients: neu and tamoxifen failure. *J Steroid Biochem Mol Biol* 1990, **37**, 811–814.
21. Hainsworth PJ, Henderson MA, Stillwell RG, Bennett RC. Comparison of *EGF-R*, *c-erbB*-2 product and rasp 21 immunohistochemistry as prognostic markers in primary breast cancer. *Eur J Surg Oncol* 1991, **17**, 9–15.
22. Clarke R, Brunner N, Katzenellenbogen BS, *et al.* Progression of human breast cancer cells from hormone dependant to hormone independant growth both *in vitro* and *in vivo*. *Proc Natl Acad Sci U.S.A.* 1989, **86**, 3649–3653.
23. Anderson J, Thorpe SM, King WJ, *et al.* The prognostic value of immunohistochemical estrogen receptor analysis in paraffin embedded and frozen sections versus that of steroid binding assays. *Eur J Cancer* 1990, **26**, 442–449.
24. Gerdes J, Schwab U, Lemke H, *et al.* Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. *Int J Cancer* 1983, **31**, 13–20.
25. Kommos F, Colley M, Hart C, Franklin WA. *In situ* distribution of oncogene products and growth factor receptors in breast carcinoma: *c-erbB*-2 oncoprotein, *EGF-R* and *PDGF-R-B*-subunit. *Mol and Cell Probes* 1990, **4**, 11–23.
26. Toi M, Naakamura T, Mukaida H, *et al.* Relationship between epidermal growth factor status and various prognostic factors in human breast cancer. *Cancer* 1990, **65**, 1980–1984.
27. Harris AL, Nicholson S, Sainsbury JR, *et al.* Epidermal growth factors in breast cancer: association with early relapse and death, poor response to hormones and interactions with neu. *J Steroid Biochem* 1989, **34**, 123–131.
28. Masui H, Kawamoto T, Sato JD, *et al.* Growth inhibition of human tumour cells in athymic mice by anti epidermal growth factor receptor monoclonal antibodies. *Cancer Res* 1984, **44**, 1002–1007.
29. Hanauske AR, Osborne CK, Chamness GC, *et al.* Alteration of EGFR binding in human breast cancer cells by anti-neoplastic agents. *Eur J Cancer Clin Oncol* 1987, **23**, 545–551.

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