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Expression of epidermal growth factor receptor in benign and malignant primary tumours of the breast

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Summary. Using the monoclonal antibody EGF-R1, normal mammary gland and a series of 213 unselected primary breast tumours were investigated immunohistochemically for expression of epidermal growth factor receptor (EGFR). In normal breast EGFR was expressed in variable patterns in lobular, ductal, and myoepithelial cells. In fibroadenoma, EGFR was detectable in variable numbers of ductal and myoepithelial cells and in stromal fibroblasts. The myoepithelial compartment of 2 cystosarcomas phyllodes also expressed EGFR. Among the 197 carcinomas tested only 20.3% contained EGFR expressing tumour cells which represented a minority in 12.2%, the majority in 2.1%, and the entire neoplastic population in 6.1% of the cases. Again, non-neoplastic ductal remnants often contained EGFR positive myoepithelial and ductal cells whereas stromal fibroblasts expressed EGFR only occasionally. We conclude that in contrast to the normal state, EGFR-expression is a rather rare phenomenon in breast carcinoma cells, positively correlated with a declining grade of differentiation ($p < 0.025$) and at least occasionally associated with squamous metaplasia within the tumour, that EGFR expression is not exclusively restricted to cells of the epithelial lineage, and that EGFR may have other functions not related to proliferation, since it is commonly detectable in myoepithelial cells.

Key words: Epidermal growth factor receptor (EGFR) – Breast carcinoma – Fibroadenoma – Myoepithelial cells – Fibroblasts

Introduction

The epidermal growth factor receptor (EGFR) is a transmembrane surface glycoprotein of a molecular weight of 170 kDa, with intrinsic protein tyrosine kinase activity. Epidermal growth factor (EGF) binding induces ligand-induced endocytosis of the receptor (Glenney et al. 1988), its autophosphorylation and inositol phosphate formation, Ca^{2+} influx, activation of Na^+/H^+ exchange resulting in intracellular alkalization, and DNA synthesis (Moolenaar et al. 1988). Although there is a considerable amount of data on EGFR concentrations in benign and malignant breast tissue mainly based on radio-labelled EGF binding to cytomembrane preparations of tissue homogenates (e.g. Fitzpatrick et al. 1984; Sainsbury et al. 1985a; Skoog et al. 1986; Wright et al. 1987), immunohistochemical data on the receptor distribution within the tissue are still scarce. Waterfield et al. (1982) published a monoclonal antibody "EGF-R1" directed against EGFR. Using this antibody, Sainsbury et al. (1985b) reported on an inverse in situ correlation of EGFR and estrogen receptor in breast carcinoma, a finding that was confirmed by Cattoretti et al. (1988). According to previous biochemical data and to the latter two groups of authours, about 30 to 50% of breast carcinomas were EGFR-positive (Fitzpatrick et al. 1984; Pérez et al. 1984; Sainsbury et al. 1987; Wright et al. 1987; Cattoretti et al. 1988). Upon application of the same monoclonal antibody EGF-R1 on our own material, we were surprised by the obvious rareness of EGFR-positive breast carcinomas on the one hand, and by the expression of EGFR in myoepithelial and sometimes even in stromal cells, on the other.

We present here the EGFR distribution in a series of 213 unselected primary breast tumours including carcinomas, adenomas, fibroadenomas, and 2 cases of cystosarcoma phyllodes.

Materials and methods

A series of 213 unselected primary breast tumours was collected in the course of a clinico-pathological study on mammary carcinoma. This series of frozen tumours stored at −70° C com-

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prised 197 carcinomas (4 ductal, 138 ductal-invasive, 37 invasive-lobular, 7 medullary and 11 unclassified carcinomas), 2 adenomas (1 of them oxyphilic), 12 fibroadenomas and 2 cystosarcomas phyllodes of the breast. In addition, representative tissue specimens of 5 normal mammary glands were examined. From each specimen 4 serial frozen sections of about 1 cm^2 and a thickness of 4 to 6 μm were cut and air-dried overnight, fixed in acetone for 10 min at room temperature and immediately immunostained.

Monoclonal antibody HEA125 recognizing the broadly distributed epithelium specific membrane glycoprotein Egp34, which is not expressed on myoepithelial cells, was raised and characterized in our laboratories (Moldenhauer et al. 1987; Momburg et al. 1987). CD10(J5) (Ritz et al. 1980) reacting with mammary myoepithelial cells (but not with ductal or lobular epithelium) (Metzgar et al. 1984; Gusterson et al. 1986) is distributed by Coulter Immunology, Hialeah, FA, USA. EGF-R1 (Waterfield et al. 1982) was obtained from Amersham, High Wycombe, U.K. A polyclonal biotinylated sheep antibody to mouse Ig (reactive with all mouse isotypes) and a streptavidin-biotinylated peroxidase complex, both obtained from Amersham, served as detection system for the mouse monoclonal primary antibodies.

The mAb preparations were diluted as follows: HEA125 10 μg/ml, EGF-R1 1:10 and J5 1:100. The secondary anti-mouse Ig antibody was diluted 1:50, and the streptavidin-peroxidase complex was applied 1:100. All dilutions and washing steps were carried out in phosphate-buffered saline solution (PBS). Incubation times of tissue were 1 h at room temperature for the primary antibody and 30 min for the second- and third-step reagents. Using aminoethyl-carbazole (AEC) as the chromogen (0.4 mg/ml in 0.1 mol/l of acetate buffer pH 5.0 with 5% dimethylformamide (DMF) and 0.01% H_2O_2 for 10 min), the peroxidase reaction resulted in an intense red precipitate. The sections were counterstained with Harris' haematoxylin and mounted with glycerol gelatin.

In order to assess the reactivity of EGF-R1 and to achieve the optimal dilution for this mAb, cell line A431, originally used as immunogen to raise this antibody (Waterfield et al. 1982), served as control. To demonstrate the total myoepithelial cell compartment of fibroadenomas and cystosarcomas, one of the serial sections of each tissue block was incubated with CD10(J5) which regularly stains the entire population of normal myoepithelial cells in mammary gland (Metzgar et al. 1981; Gusterson et al. 1986). Negative controls were performed in each case without the primary antibody; no staining was observed except for scattered granulocytes and a faint staining of the luminal border of the duct epithelium. This staining was caused by endogenous peroxidase which was not blocked for the benefit of optimal antigenicity.

Evaluation of EGFR expression was carried out by two independent observers. The serial sections stained with CD10(J5) and HEA125 facilitated the distinction of ductal, myoepithelial and stromal cells in fibroadenoma and cystosarcoma phyllodes. The evaluation of EGFR expression in the carcinoma series was performed against the background of the overall HEA125-positivity of every carcinoma serving as reference in each individual case. Differences in staining intensity of the EGFR mAb-reaction were disregarded. For the evaluation of the amount of stained cells a simple semiquantitative score was set up (see footnotes of Table 1 and 2): a tumour was considered negative, −, when all tumour cells were clearly negative. A mixed pattern of EGFR positive and negative tumour cells was subdivided into $-{>}+$ and $+{>}-$. However, for statistical analysis these two groups were combined. A tumour was considered positive, +, when the whole neoplastic population expressed EGFR. The Chi^2 test was applied for statistical analysis.

Results

In addition to the 5 normal glands, 26 of the tumour samples contained non-neoplastic glandular remnants, either normal or exhibiting various aspects of fibrocystic mastopathy. Both normal mammary gland and fibrocystic mastopathy revealed very variable patterns of EGFR expression. Lobular epithelium was negative in most areas examined. However, single EGFR-positive lobules were also detected (Fig. 1). The ductal epithelium was mixed, negative and faintly positive in some cases and was entirely but faintly positive in others. In the close vicinity of 18 carcinomas EGFR expression was found in duct epithelium of non-neoplastic ductal remnants (Fig. 3); it was strong in 11 and faint in 7 cases. Periductal myoepithelial cells were EGFR-positive in about half of the non-neoplastic cases examined (Fig. 1); those areas containing strongly positive myoepithelial cells were often characterized by pronounced periductal fibrosis. In the 5 normal glands there were no EGFR-positive stromal cells whatsoever.

The benign tumours examined included an intraductal papilloma which was EGFR-negative.

Fig. 1. EGFR expression in normal mammary gland (×90). In this area lobular epithelial cells are strongly positive while ductal myoepithelial cells are only weakly stained. Ductal epithelial cells are negative. (Immunoperoxidase staining of frozen sections, using aminoethylcarbazole as chromogene, faint haematoxylin counterstain; same technique for all photomicrographs)

Fig. 2. EGFR expression in a fibroadenoma; case No. 2 in Table 1 (×180). The myoepithelial cells are weakly positive while the duct epithelium is negative. The majority of fibroblasts express considerable amounts of EGFR

Fig. 3. EGFR expression in a lobular-invasive carcinoma (×180). Ductal remnants are positive in both myoepithelial and ductal cells. Tumour cells barely visible within the stroma are EGFR-negative. The stromal immunoreactivity is restricted to fibroblasts

Fig. 4. EGFR expression in a ductal-invasive carcinoma (×90). This case shows marked heterogeneity of immunoreactivity of the neoplastic cells; the intraductal component is clearly negative whereas the invasive component shows variable degrees of positivity. The myoepithelial remnants of the duct are the cells that express the highest amount of antigen

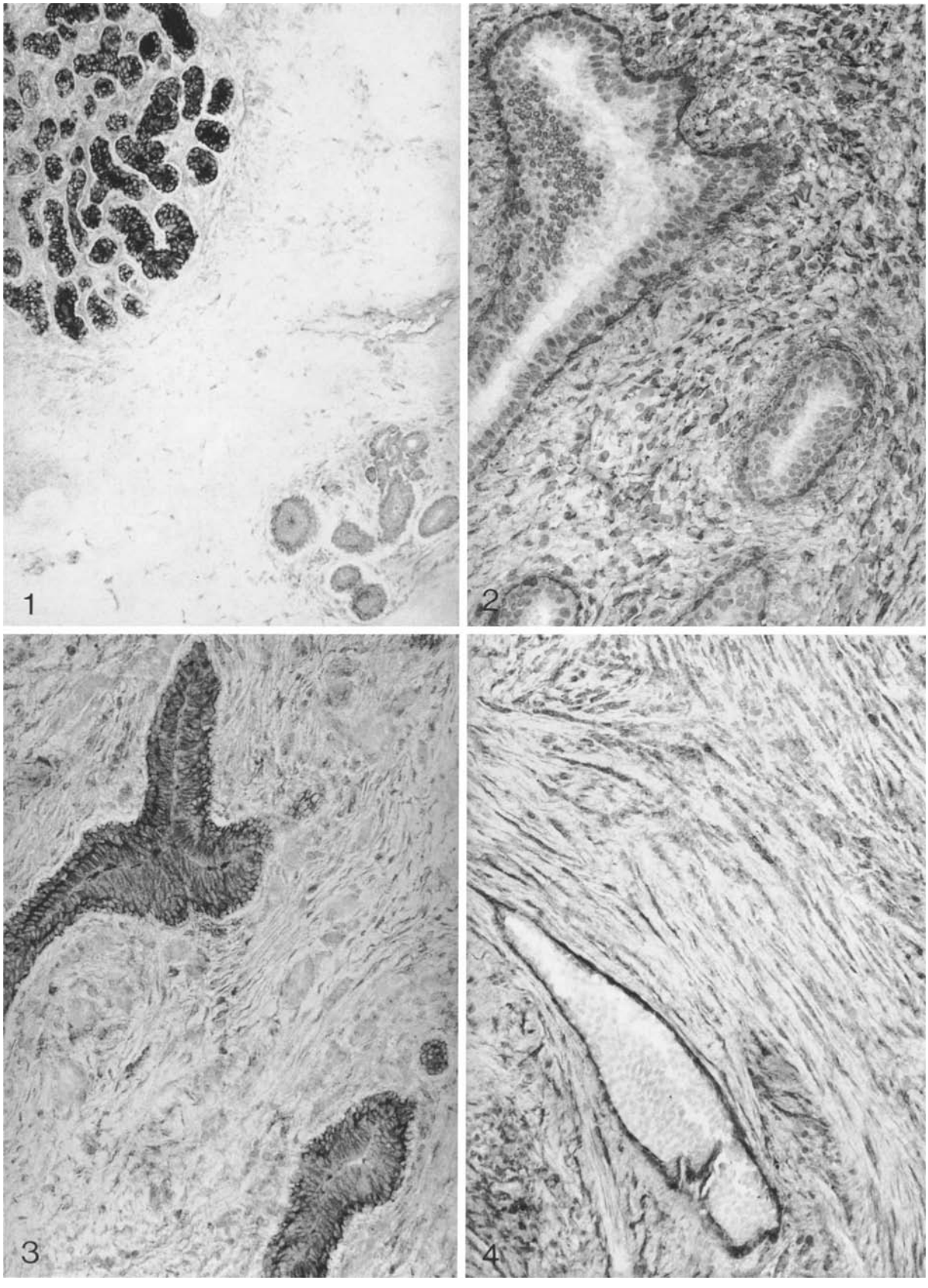
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Table 1. EGFR expression in 12 fibroadenomas and 2 phyllodes cystosarcoma

No	Type	Stroma	Egp34[a]			CD10[b]			EGFR		
			DE	ME	SC	DE	ME	SC	DE	ME	SC
1	PC	M/F–C	+	–	–	–	+	–	–	(+)	–
2	PC	M	+	–	–	–	+	–>+	–	(+)	(+)
3	PC	F–C	+	–	–	–	+	–≫+	(+)	(+)	(+)
4	IC	S	+	–	–	–	+	–≫(+)	(+)/–	(+)/–	–
5	PC	M/F–C	+	–	–	–	+	–≫(+)	–	(+)	+
6	PC	M/F–C	+	–	–	–	+	–	–>(+)	(+)	–
7	IC	S	+	–	–	–	+	–	–	(+)	–
8	PC	M	+	–	–	–	+	–>(+)	–	(+)/–	–
9	IC	S	+	–	–	–	+	–	(+)/+	(+)	–
10	PC	S	+	–	–	–	+	–>+	–	(+)	–
11	PC	F–C	+	–	–	–	+	–	–	–	(+)/–
12	PC	M/F–C	+	–	–	–	+	–>(+)	–	–	–
CSP1			+	–	–	–	+	+	–>(+)	+	–
CSP2			+	–	–	–	+	–≫+	–	+	+

[a] Epithelium specific 34 kDa antigen
[b] common acute lymphoblastic leukaemia antigen (CALLA); DE: ductal epithelium; ME: myoepithelial cells; SC: stromal cells; PC: pericanalicular; IC: intracanalicular; M: myxoid; F–C: fibro-cellular; CSP: cystosarcoma phyllodes; +: strongly positive; (+): weakly positive; –: negative immunoreactivity; x/y: two modalities of reactivity in about equal proportion; x>y: pattern x in the majority of cells; x≫y: pattern y found but very rarely

The single case of oxyphilic adenoma was composed of EGFR-positive cells while a tubular adenoma was EGFR-negative. The results of the series of fibroadenomas are listed in Table 1. EGFR was detectable in ductal epithelium, myoepithelial cells and stromal cells but in very variable patterns of expression. Only 4/12 fibroadenomas contained subsets of EGFR-positive duct cells, and most of them were stained only weakly. The myoepithelial compartment expressed EGFR in 10/12 cases (Fig. 2), staining intensity was relatively low; two cases contained both EGFR-positive and -negative myoepithelial cells. There was also a definite EGFR-positivity of (a subset) of stromal cells in 2 (3) cases (Fig. 2).

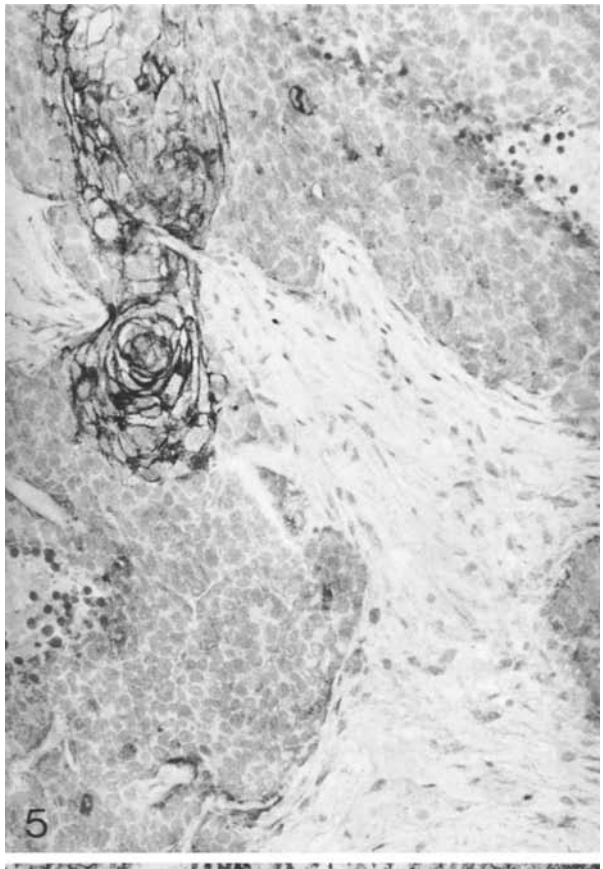
The 2 cystosarcomas phyllodes expressed EGFR in the entire myoepithelial population (Fig. 6) while the stromal cells were devoid of EGFR in case No. 1 and clearly EGFR-positive in case No. 2 (Fig. 7). The pattern within the ductal compartment was very complex. Although most of the EGFR-positive cells were of myoepithelial nature (since most of them co-expressed CD10), it was, as a consequence of a peculiar inversion of duct cells and myoepithelial cells, likely that a minority of duct cells also expressed EGFR.

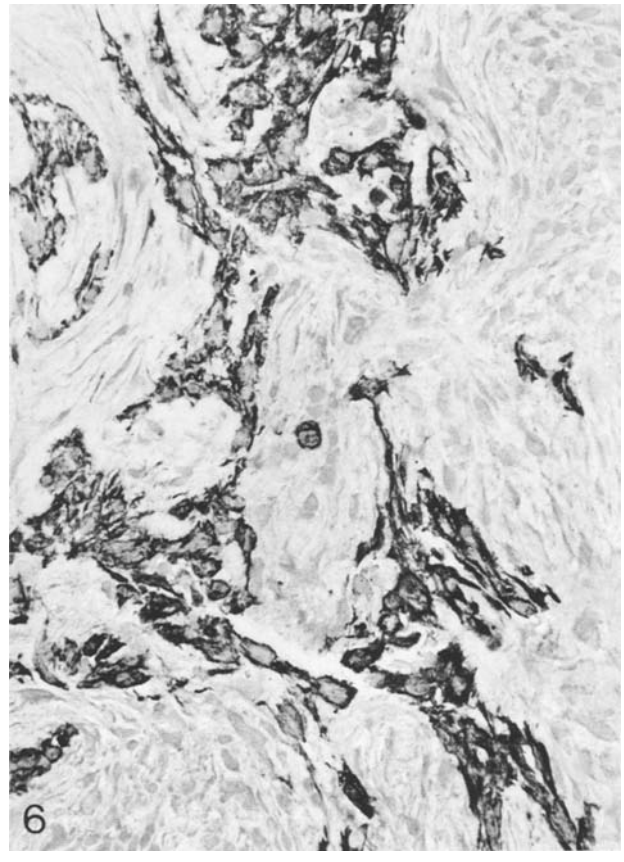
For carcinoma the results of the immunostainings are listed in Table 2. One hundred and fifty seven (79.7%) carcinomas were completely EGFR-negative (Fig. 3). Twenty four (12.1%) contained minor subsets and 4 (2.1%) major subsets of mostly faintly stained tumor cells (Fig. 4). Only 12 carcinomas (6.1%), one of them displaying an adenosquamous differentiation, were composed entirely of EGFR-positive neoplastic cells. Six tumours expressed EGFR exclusively in focal areas

Table 2. EGFR expression in 197 primary breast carcinomas

Type	Grade	–[a]	–>+	+>–	+	
intraductal	I	0[b]	0	0	0	
	II	2	1	0	0	
	III	1	0	0	0	
invasive ductal	I	2	0	0	0	
	II	73	8	1	5	
	III	31	10	2	6	
invasive lobular	I	0	0	0	0	
	II	22	1	0	0	
	III	10	3	1	0	
medullary	I	0	0	0	0	
	II	4	0	0	0	
	III	2	1	0	0	
n.f.sp.[c]	I	0	0	0	0	
	II	6	0	0	0	
	III	4	0	0	1	
		157	24	4	12	$N=197$

[a] semiquantitative score: –: all tumour cells negative; –>+: more negative than positive tumour cells; +>–: more positive than negative tumour cells; +: all tumour cells positive (irrespective of staining intensity),
[b] number of cases,
[c] not further specified





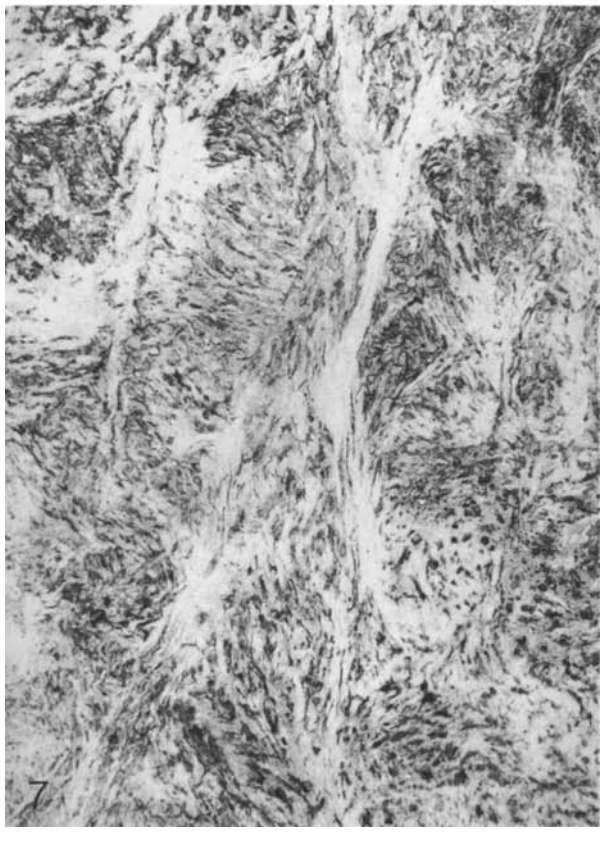


Fig. 5. EGFR expression in a ductal invasive carcinoma with focal squamous differentiation (×180). EGFR is in this case restricted to the squamous parts

Fig. 6. EGFR expression in the cystosarcoma (No. 1 in Table 1) (×225). The staining pattern indicates that EGFR is in this special case restricted to the myoepithelial compartment of the tumour

Fig. 7. EGFR expression in a cystosarcoma (No. 2 in Table 1) (×90). In this case the antibody EGF-R1 stains both myoepithelial and fibroblastic cells of the lesion

of squamous differentiation (Fig. 5). With regard to the tumour grading carried out according to Bloom and Richardson (1957), 0/2 of grade I, 16/123 of grade II, and 24/72 of grade III carcinomas contained EGFR-positive cells in various proportions. Statistical analysis revealed a significant correlation between EGRF expression and a declining degree of differentiation ($p < 0.025$). The myoepithelial compartment, forming intact or destroyed ductal structures or remnants in many cases, was strongly positive in 43 and weakly positive in 37 cases. Hence, there were more carcinomas with EGFR-positive myoepithelial cells than EGFR-positive neoplastic cells. Ductal epithelium of normal ducts or ductal remnants within the carcinoma were found to express EGFR strongly in 11 and weakly in 7 cases. EGFR expression in the fibrocytic stromal cell compartment was a rare but sometimes impressive feature.

In sum, EGFR expression in normal and neoplastic mammary gland is not restricted to the lobular or ductal epithelium but can also be observed in the myoepithelial compartment and – occasionally – even in stromal cells. It represents an inconstant, however frequently observed, phenomenon within the myoepithelial compartment but is relatively rare in carcinoma cells and only occasionally detectable in (myo-?)fibroblasts.

Discussion

The normal state is characterized by an inconstant EGFR-expression in lobular and ductal mammary epithelium. Most breast cancer cells seem to lose this capacity. Indeed, the main finding of our study is that only 20% of mammary carcinomas contain EGFR-expressing carcinoma cells. Moreover, in about half of the "positive" cases EGFR-positive cells represented only a minority of the neoplastic population. Compared with the incidence of EGFR-expression in squamous cell carcinomas (Hendler and Ozanne 1984) its expression in breast carcinoma is a rather rare event. Interestingly in this respect, we observed, at least in some cases, EGFR-expression associated with local squamous differentiation. Our data on the frequency of EGFR-positivy are clearly in disagreement with the current view: Using biochemical binding assays of radio-labelled EGF to cytomembrane preparations of mammary carcinomas, Pérez et al. (1984) detected EGF binding in 42% of cases and a high binding capacity in 21%. Sainsbury et al. (1985a), applying a similar method, found that EGFR was detectable in 35% of human breast cancer specimens. Skoog et al. (1986), using the same methodological approach, reported EGF binding in 36% of ductal, in 100% of medullary but no binding in lobular carcinoma. Sainsbury et al. (1987) were the first to apply the mAb EGF-R1 generated by Waterfield et al. (1982) on breast carcinoma tissue; however, no detailed results on tissue distribution are given in their statement that 34.8% of the 135 primary tumours tested expressed EGFR. In collaboration with these authors, Wright et al. (1987) reported 38/97 EGFR-positive cases on the basis of a competitive EGF binding assay.

The first immunohistochemical data on EGFR expression in breast carcinoma were provided by Walker and Camplejohn (1986). Among their 48 carcinomas 17 were completely negative, 11 had "a very occasional cell reacting" and 20 carcinomas had "a more significant staining"; 8 of them were almost entirely positive. Cattoretti et al. (1988), regarding a tumour as positive whenever more than 10% of cells were stained, found 67/196 breast carcinomas expressing EGFR. Horne et al. (1988) detected EGFR immunohistochemically via the mAb EGF-R1 in 19/49 breast carcinomas, corresponding to 38.7%. In the series of Wrba et al. (1988) 60.2% of breast carcinomas were regarded as EGFR-positive. We cannot explain these high incidences of immunohistochemical EGFR-positive breast carcinomas on the basis of our data, unless selection has occurred. Although our cohort of tumours is not balanced with regard to tumour type and grade, it is nevertheless an unselected series of compiled cases reflecting the actual incidence of the tumour variants. The data most similar to ours are those presented by Pekonen et al. (1988) who found a high EGFR content in 26% of the poorly and in 13% of the well differentiated ductal carcinomas. The relationship between EGFR expression and grade of tumour differentiation has been addressed in several studies: On the basis of 108 tumours Sainsbury et al. (1985b) found a statistically significant correlation of EGFR-positivity and grade III, a correlation Walker and Camplejohn (1986) could not observe, which might be explained by their series of 48 breast carcinomas lacking grade III tumours. These authours, however, report a close positive correlation to the high S-phase content as determined by flow cytometry. Wrba et al. (1988) could not detect this correlation either although 89 cases had been examined. The examination of 171 tumours by Pekonen et al. (1988) once again revealed a statistically significant correlation between the declining degree of differentiation and EGFR ex-

pression. Our results, based upon the largest tumour group investigated so far, confirm this correlation although our series contains the smallest percentage of EGFR-positive carcinomas. In this context the recent survival analysis given by Sainsbury et al. (1987) is noteworthy in that it states that EGFR-positivity divides the estrogen receptor negative tumour group into a good ($EGFR^-$) and a poor ($EGFR^+$) prognosis subgroup. Their series, however, contained 14 grade I, 42 grade II, and 68 grade III tumours, strongly suggesting selection or overdiagnosis of grade III.

Biochemical data on tissue concentration of EGFR need to be reinterpreted against the background of our finding that the myoepithelial compartment frequently and the fibroblastic compartment occasionally, expresses EGFR. EGFR expression by fibroblasts has already been noted by Walker and Camplejohn (1986); however, this finding was not further considered by the authours.

Tissue distribution of EGFR leads us to conclude that this receptor is not restricted to the epithelial cell lineage. This was already suggested by Real et al. (1986) who detected EGFR expression in 5/6 sarcoma cell lines tested and by Gusterson et al. (1985) who found EGFR expressed in a substantial number of sarcomas. Moreover, EGFR expression may have other functions unrelated to cell cycle progression. It is a common feature in myoepithelial cells that normally lack the transferrin receptor (own unpublished data) and that are therefore not likely to proliferate.

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