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Expression of epidermal growth factor receptor in normal colorectal mucosa, adenoma, and carcinoma

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Summary. Using the monoclonal antibody EGF-R1, the expression of epidermal growth factor receptor (EGFR) was investigated immunohistochemically in normal colonic mucosa distant from and adjacent to colonic neoplasms, in 25 adenomas and in 144 unselected colorectal carcinomas. EGFR expression was an inconsistant phenomenon in each of these conditions. It was not expressed in 23/44 non-neoplastic mucosa specimens distant from and in 26/44 mucosae adjacent to colon tumours; 15/25 adenomas and 71 (49.3%) of the carcinomas failed to contain detectable amounts of EGFR. In contrast, large amounts of EGFR were found in 4 non-neoplastic mucosae at both locations, in 3 adenomas and in 11 (7.6%) carcinomas. The remaining cases showed complex patterns of EGFR-expression. In comparing mucosae close to and distant from a colonic tumour, only minor differences in EGFR content were observed. The intra-individual comparison of the mode of EGFR expression in non-neoplastic and neoplastic epithelium revealed an overexpression of EGFR in carcinomas in about one third of the 44 cases examined. One third showed no obvious differences, and one third showed lower levels of EGFR expression within the tumour. We conclude that the mode of EGFR expression in normal and neoplastic colon epithelium is variable and reflective of inter-individual constitutive differences rather than of abnormalities in gene regulation. Statistical analysis failed to reveal correlations between the mode of EGFR expression and tumour grade, type or Dukes stage.

Key words: Epidermal growth factor receptor (EGFR) – Colorectal mucosa – Colorectal adenoma – Colorectal carcinoma

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Introduction

The epidermal growth factor receptor (EGFR), a transmembrane glycoprotein with intrinsic tyrosine kinase activity (Carpenter 1983), is located on the cell surface of various cell and tissue types. The 170 kD glycoprotein is encoded on the short arm of chromosome 7 (Shimizu et al. 1984) and reveals close similarity with the v-erb-B oncogene protein sequences (Downward et al. 1984). One ligand is the epidermal growth factor (EGF), which is thought to have regulatory functions concerning the proliferation of a variety of cells (Burgess 1989; Eker 1989; Squinto et al. 1989), including those of the gastrointestinal tract (Al-Nafussi and Wright 1982). EGF activates tyrosine phosphorylation of the phospholipase C (Wahl et al. 1989), ligand-receptor internalization (Glenney et al. 1988; van't Hof et al. 1989), ion influx and mRNA and DNA synthesis (Carpenter 1987; Moolenaar et al. 1988). EGFR was found expressed in various normal tissues, especially in squamous and some non-squamous epithelium and breast, however, the results with regard to the gastrointestinal tract are conflicting (Gusterson et al. 1984; Damjanov et al. 1986). In several malignant tumours an overexpression of the EGFR or an amplification of the EGFR gene has been described, especially in the group of squamous carcinomas (Hunts et al. 1985; Yamamoto et al. 1986; Berger et al. 1987b; Ishitoya et al. 1989), but also in non-epithelial malignant tumours in the nervous system (Reifenberger et al. 1989; Reubi et al. 1989), and in mesenchymal tumours (Gusterson et al. 1985; Perosio and Brooks 1989). With regard to the receptor density, as revealed by binding of mAb EGF-R1, squamous cell carcinomas were found to contain more EGFR than adenocarcinomas (Gullick et al. 1986). The correlation of EGFR expression and morpho-

logical and clinical parameters have been investigated in urothelial carcinomas (Neal et al. 1985; Berger et al. 1987a) and in breast tumours (Sainsbury et al. 1988; Möller et al. 1989; Toi et al. 1989). It was found that the EGFR status was associated with the stage and grade of malignancy in bladder tumours and the grade, recurrence rate and lymphatic invasion in breast tumours. In adenocarcinomas of the gastrointestinal tract the level of EGFR expression in gastric tumours was found to correlate with the extent of invasion (Yasui et al. 1988a), with staging (Yasui et al. 1988b) and the histological differentiation (Sugiyama et al. 1989). With regard to colonic carcinomas, no correlation was found between EGFR antigen expression and the histological type (Yasui et al. 1988b). Recently, EGFR content in colon carcinomas, adenomas and normal mucosa was investigated in radioligand-binding studies (Rothbauer et al. 1989): The carcinomas were found to contain either similar or decreased levels of EGFR in most cases; the adenomas had a similar content of EGFR when compared with normal mucosa; by contrast an overexpression of EGFR was a rare phenomenon in colon carcinomas.

In this study, we investigated the EGFR antigen expression in colorectal carcinomas, adenomas and non-neoplastic mucosa adjacent to and distant from the tumour by an immunohistological method. The aim was to reevaluate the conflicting published data on EGFR expression in the normal colorectal mucosa, and to study the relation of EGFR expression in tumours and normal mucosa.

Material and methods

A total of 213 colorectal tissue specimens comprising 44 normal mucosal tissues, 25 adenomas, and 144 adenocarcinomas were collected from surgically removed tumours. Out of the 144 adenocarcinomas, 82 (56.9%) were localized in the rectum, 20 (13.9%) in the sigmoid colon, and 42 (29.2%) in the residual colon. One hundred and three (71.5%) were of the non-mucinous type, 32 (22.2%) were of the mucinous type, and 9 carcinomas could not be classified. Seven carcinomas (4.9%) were grade I, 117 (81.3%) were grade II, 11 (7.6%) were grade III and 9 were not classified because of intratumoral heterogeneity. The Dukes classification revealed 35 tumours (24.3%) to belong to stage A, 31 (21.5%) to stage B, 46 (31.9%) to stage C and 28 (19.4%) to stage D; 4 tumours were not specified. In this study, the 25 adenomas were grouped together; no histological subtyping was performed. Forty-four out of the 144 adenocarcinoma specimens contained residues of tumour-adjacent mucosa. From these cases specimens of normal colorectal mucosa from the resection margins were used to investigate EGFR expression in mucosa distant from the tumour. Tissue samples were quick frozen in liquid nitrogen and stored at −70° C until sectioning. Sections of 4–6 μm thickness were cut, air-dried, and fixed in acetone for 10 min at room temperature.

The monoclonal antibody EGF-R1 (isotype: IgG2b; Waterfield et al. 1982) was obtained from Amersham, High Wycombe, Uk. A polyclonal biotinylated sheep antibody to mouse Ig and a streptavidin-biotinylated peroxidase complex, both obtained from Amersham, served as the detection system for the mouse monoclonal primary antibody. Dilutions were 1:10 for EGF-R1, 1:50 for the secondary anti-mouse Ig antibody, and 1:100 for the streptavidin-peroxidase complex. All dilutions and washing steps were carried out in phosphate-buffered saline solution (PBS). Incubation times were 1 h at room temperature for the primary antibody and 30 min for the second and third step reagents. Using 3-amino-9 ethylcarbazole (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 15 min), the peroxidase reaction resulted in an intensive red precipitate. The sections were rinsed in tapwater, counterstained with Harris' haematoxylin and mounted with glycerol gelatin.

A positive control for the immunoreactivity in each staining series was carried out by a parallel staining of esophageal squamous epithelium. An isotype-matched control was performed by CD20 (B1), which is a B cell-specific monoclonal antibody of the IgG2b class. From each tissue specimen, a negative control without the primary reagent was carried out: Staining was observed in granulocytes whose endogenous peroxidase was not blocked, and faintly in some epithelial areas due to endogenous biotin; this reactivity was disregarded during evaluation.

The evaluation of the staining intensity of the normal, adenoma and carcinoma epithelium was carried-out in comparison with esophageal squamous epithelium. Thus, three grades of intensity were formulated: strong staining "+", when the staining intensity was comparable with that of the squamous epithelium; weak staining "(+)", and no staining "−". For the evaluation of the amount of stained cells a semiquantitative score was set up: "A>B" meant that cells with staining intensity A clearly outnumbered those with intensity B; "A>>B" meant that only a small minority of cells revealed the staining intensity B. To permit a statistical evaluation, these combined semiqualitative/semiquantitative characterizations were compiled in a score ranging from 0 to 4 (Table 1). According to this score, the expression of EGFR in the tumour cohort was correlated with tumour grade, type, localization and the Dukes' stage. The Chi-square test was applied for statistical analysis.

Results

The unaffected mucosa, adenomas and the carcinomas revealed variable patterns of EGFR expression (Table 2). The colorectal mucosa was investigated twice, once from a resection margin distant from the tumour, and once from an area adjacent to the tumour. Out of the 44 mucosa specimens distant from the tumour, 23 cases were negative for EGFR, 4 cases showed an extensive antigen expression (Fig. 1), 3 specimens had a moderate staining intensity, and 7 cases revealed only sparse EGFR-positive cells or minimal overall epithelial antigen expression. The receptor density was usually weaker than in the control esophagus, and the immunoprecipitate was located intracytoplasmatically in a fine granular form. The gradual differ-

Table 1. Score of the evaluation system for EGFR expression in non-neoplastic colorectal mucosa distant from and adjacent to carcinomas, in adenomas, and in carcinomas

Semiquantitative*/Semiqualitative** characterization		Score	Verbal characterization of antigen expression***
+	(+)	4	extensive
+>−	(+)>−	3	moderate
−>+	−>(+)	2	sparse
−≫+	−≫(+)	1	minimal
−		0	no

* Quantitative statements: "A>B", pattern A clearly dominates pattern B; "A≫B", pattern B only rarely observed
** Staining intensity: " + " strong staining; "(+)" weak staining; " − " no staining
*** The verbal characterization reflects the number of epithelial cells expressing the antigen in the tissue section examined

Table 2. Expression of EGFR in non-neoplastic colorectal mucosa distant from and adjacent to carcinomas (Ca. dist; Ca. adj.), in adenomas, and in carcinomas

EGFR (Score)	Mucosa				Adenoma		Carcinoma	
	Ca. dist.		Ca. adj.					
	$n=44$	%	$n=44$	%	$n=25$	%	$n=144$	%
4	4	9.1	4	9.1	3	12.0	11	7.6
3	3	6.8	6	13.6	3	12.0	19	13.2
2	7	15.9	5	11.4	2	8.0	18	12.5
1	7	15.9	3	6.8	2	8.0	25	17.4
0	23	52.3	26	59.1	15	60.0	71	49.3

ences in antigen expression were not only restricted to a patchy distribution of the antigen in the mucosal epithelium but also had a tendency to be localized in apical parts of the crypts. The tumour-adjacent mucosa was either hyperplastic or revealed an inconspicuous morphology. Out of 44 tumour specimens, the adjacent mucosa was negative in 26 cases, extensively positive in 4 cases, moderately positive in 6 cases, sparsely positive in 5 specimens, and minimally induced in 3 cases. There was no difference whatsoever in EGFR expression or non-expression in hyperplastic or non-hyperplastic epithelium. The staining intensity was weaker than in the esophageal epithelium, and the staining pattern appeared to be exclusively intracytoplasmatic.

Of the 25 colorectal adenomas, 15 cases expressed no EGFR, 3 showed extensive expression, 3 had a moderate, 2 revealed a sparse, and 2 had a minimal expression. The staining intensity was weak when compared with the esophageal epithelium; the staining pattern was either cytoplasmatic or superficial (Fig. 2). Comparing EGFR in adenomas and carcinomas, the receptor expression appeared to be higher in carcinomas, but when the gradual differences were taken into account, the EGFR-induction was largely due to an increase in the lower score category. Thus, no significant differences between carcinomas and adenomas were evident concerning the EGFR content.

Out of the 144 carcinomas, only 11 cases showed an extensive receptor expression (Fig. 3), that is to say an almost complete staining of the tumour cell population by the mAb EGF-R1. Nineteen tumours revealed a moderate, 18 a sparse, and 25 a minimal EGFR expression. Seventy-one cases revealed no EGFR detectable by our immunohistochemical method. As a general feature, the staining intensity varied either intra- or interpersonally but it was a rare phenomenon to detect an antigenic density comparable with that of the esophageal epithelium. The staining pattern of the neoplastic cells suggested two forms of EGFR localization: one form consisted of an intense staining of the cell surface membrane (Fig. 4), resembling the staining pattern of A 431 cells (Waterfield et al. 1982) and the esophageal epithelium. The other form was a more diffuse cytoplasmatic localization beneath the luminal cell surface. The EGFR expression did not correlate statistically with grade of differentiation, type, or

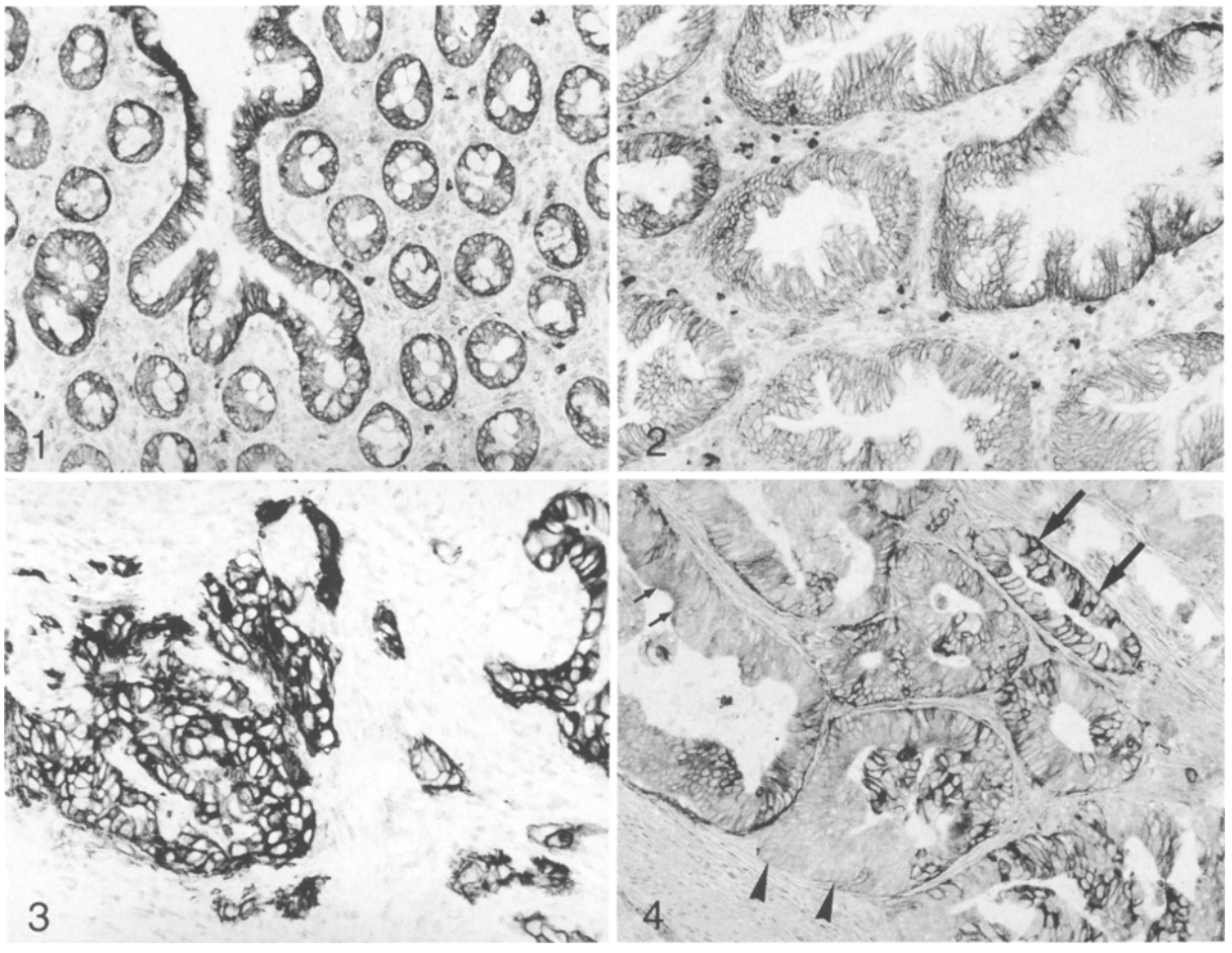

Fig. 1. EGFR expression in normal colorectal mucosa distant from a colorectal carcinoma, i.e. from the cut border of a tumour resection (×125). The crypts react strongly with mAb EGF-R1. The antigen is located in the cytoplasm and in equal density in the basic and apical parts of the crypt. Some interstitial granulocytes are strongly stained because of their endogenous peroxidase activity

Fig. 2. EGFR expression in colorectal adenoma (×125). A group of adenomatous crypts reveal a rather faint receptor expression which was mostly confined to the latero-basal cell surface. A cytoplasmatic receptor expression is hardly visible. Interstitial granulocytes are strongly stained because of their endogenous peroxidase activity

Fig. 3. EGFR expression in a colorectal carcinoma (×125). The tumour contains the antigen in high amounts throughout

Fig. 4. EGFR expression in a colorectal carcinoma (×125). The tumour reveals a patchy antigen expression. EGFR is confined to the cytomembrane (*large arrows*), cytoplasmatically beneath the luminal cell surface (small arrows), or EGFR is missing (*arrow heads*)

localization of the tumour and Dukes' stage. However, there was a trend that, in the rectum, more carcinomas were EGFR-negative when compared with the colon/sigmoid tumours, and, more EGFR positive tumours were located in the colon/sigmoid.

In order to investigate individual EGFR expression in colorectal carcinomas in correlation with their adjacent non-neoplastic mucosa as well as distant normal mucosa, we analyzed 44 cases. Regarding the distant mucosa in comparison to the tumour, 13 tumours showed a higher degree of EGFR expression than the non-neoplastic mucosa, 17 tumours revealed similar amounts of EGFR and 14 tumours showed a lower antigen content. With regard to the adjacent mucosa, 15 tumours out of 44 showed a higher EGFR antigen expression, 16 tumours revealed a similar pattern of antigen expression/non-expression, and 13 tumours showed a lower antigen content than the adjacent mucosa. Thus, about one third of carcinomas showed a similar antigen expression when

compared to the adjacent and distant mucosa. Another third of the carcinomas revealed an increase in EGFR expression, whereas the remaining third had an even lower EGFR antigen content.

Discussion

In the present study, we have investigated the expression of the epidermal growth factor receptor (EGFR) in non-neoplastic colonic mucosa distant from and adjacent to colonic carcinomas, adenomas and colorectal carcinomas. EGFR expression was an inconsistant phenomenon in all tissues examined. We have used a five-step-score to reveal graded differences in antigen expression and a semiqualitative score for a rough estimation of the antigen density in comparison with the esophageal squamous epithelium, known to contain high amounts of cell surface EGFR antigen in the basal cell layers (Gusterson et al. 1984). To address the question of whether a relative hyper- or hypoexpression of EGFR exists in colorectal carcinomas, we investigated adjacent mucosa and unaffected mucosa from the resection margin. All three compartments revealed an heterogeneous antigen content. Gusterson et al. (1984) examined several normal tissues by an immunohistological method and found no EGFR antigen expression in the colon mucosa; they assumed that there was a level of EGFR in gut epithelium too low to be detectable by standard immunohistology (Forgue-Lafitte et al. 1980), but it is noteworthy that only two cases were examined. In contrast, Damjanov and collegues (1986) detected a total induction of EGFR in all parts of the crypts, but they too only investigated a small number of cases. In our cohort, a total expression of EGFR was detected only in a minority of cases. However, a partial induction of the crypt epithelium, either in a patchy pattern or predominantly in the apical parts of the crypts, in more differentiated cells, was found frequently. Concerning the localization of EGFR in positive cells, two patterns are described in the literature: strict cell surface expression as described by Waterfield et al. (1982) for the epidermoid carcinoma line A431, and diffuse intracytoplasmatic antigen deposits as observed in several normal tissues (Damjanov et al. 1986) and tumours (Gusterson et al. 1985; Sugiyama et al. 1989). The latter form was suspected to involve intracellular receptor production, receptor internalization, or intracytoplasmic receptor recycling (Damjanov et al. 1986). Furthermore, Damjanov and collegues observed that in the normal colon epithelium, the antigen deposits were located intracytoplasmatically, whereas cell surface expression was not observed. This finding is in line with our own results in that inconspicuous and hyperplastic mucosa revealed an intracellular, diffuse, and micro-granular antigen pattern. In addition, this antigen pattern was not only restricted to unaffected mucosa, but was also be detected in some adenomas and carcinomas in our study. The intracellular localization of EGFR as the result of an internalization process of the EGF-EGFR complex has been discussed by several authors (Cohen and Fava 1985; Glenney et al. 1988; van't Hof et al. 1989). The receptor-ligand complex has been shown to be located first on the cell surface but than rapidly internalized into endosomal compartments and finally degraded in lysosomes (Carpenter and Cohen 1976). This pathway has been recently reviewed by van't Hof et al. (1989). In this study van't Hof and coworkers were able to demonstrate EGFR aggregates in the perinuclear area by Nanovid light microscopy and electron microscopy. Gusterson et al. (1984) observed weak intranuclear staining in the ducts of salivary glands by an immunohistochemical method. This receptor internalization is induced by the physiological ligand EGF, a rather stable peptide (Cohen and Taylor 1974) which has not yet been found in the colonic epithelium but has been detected in salivary glands, gastric and pyloric cells of the stomach, Brunner's duodenal glands, Paneth cells, and pancreatic islet cells (Kasselberg et al. 1985; Poulsen et al. 1986; Joh et al. 1987). EGF is secreted into the gut, and because of its resistance to proteolytic enzymes and acid (Carpenter and Cohen 1979; Rall et al. 1985), the growth factor has been found even in the colonic lumen (Joh et al. 1987). EGF stimulated intestinal crypt cell proliferation on the one hand (Al-Nafussi and Wright 1982), and reduced EGFR on the cell surface of an intestinal cell line (Blay and Brown 1985) on the other; this indicated a regulatory function of EGF in the intestinal cell proliferation pathway. With regard to the epithelial receptor localization, Damjanov and coworkers (1986) argued that proliferating cells reveal strong reactivity with the EGFR antibody on the cell surface membrane, and postmitotic cells showed intracellular localization; the latter might explain the expression of intracytoplasmatically located EGFR in the apical parts of crypts in some normal mucosal tissues, in some well-differentiated carcinomas and in some adenomas in our study. With regard to the EGFR content in malignant tumours high receptor levels could be found in both, poorly differentiated tumours (Möller et al. 1989; Reifenberger et al.

1989) and well-differentiated tumours (Yasui et al. 1988a, b; Ishitoya et al. 1989). In our study, the mode of EGFR expression in colorectal carcinomas failed to correlate with grade of differentiation, tumour type, localization or Dukes stage. Concerning tumour localization, EGFR tended to be expressed more frequently in carcinomas of the proximal colon and the sigmoid rather than in the rectum. This is interesting since in unaffected mucosa of the rectum, proximal colon and sigmoid both presence and absence of EGFR could be found. Thus, interindividual differences in epithelial EGFR content characterize the normal colon mucosa. Therefore the mode of EGFR expression in carcinomas may also reflect an inherent individual characteristic. The adenoma-carcinoma-sequence seems not to be influenced by this growth factor system because antigen contents in adenomas and accompanying normal mucosa are similar; in this respect, our results support those of Rothbauer et al. (1989). In sum, EGFR expression in colorectal carcinomas is not correlated with tumour type, grade, and stage but is an inconsistant phenomenon in colorectal mucosa, adenoma and carcinoma.

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References

Al-Nafussi AI, Wright NA (1982) The effect of epidermal growth factor (EGF) on cell proliferation of the gastrointestinal mucosa in rodents. Virchows Arch [A] 40:63–69

Berger MS, Greenfield C, Gullick WJ, Haley J, Downward J, Neal DE, Harris AL, Waterfield MD (1987a) Evaluation of epidermal growth factor receptors in bladder tumours. Br J Cancer 56:533–537

Berger MS, Gullick WJ, Greenfield C, Evans S, Addis BJ, Waterfielde MD (1987b) Epidermal growth factor receptors in lung tumours. J Pathol 152:297–307

Blay J, Brown KD (1985) Functional receptors for epidermal growth factor in an epithelial-cell line derived from the rat small intestine. Biochem J 225:85–94

Burgess AW (1989) Epidermal growth factor and transforming growth factor α. Br Med Bull 45:401–424

Carpenter G (1983) The biochemistry and physiology of the receptor-kinase for epidermal growth factor. Mol Cell Endocrinol 31:1–19

Carpenter G (1987) Receptors for epidermal growth factor and other polypeptide mitogens. Annu Rev Biochem 56:881–914

Carpenter G, Cohen S (1976) ^{125}I-labeled human epidermal growth factor: binding, internalization and degradation in human fibroblasts. J Cell Biol 71:159–171

Carpenter G, Cohen S (1979) Epidermal growth factor. Annu Rev Biochem 48:193–216

Cohen S, Fava RA (1985) Internalization of functional epidermal growth factor: receptor/kinase complexes in A431-cells. J Biol Chem 260:12351–12358

Cohen S, Taylor JM (1974) Epidermal growth factor: chemical and biological characterization. Recent Prog Horm Res 30:533–550

Damjanov I, Mildner B, Knowles BB (1986) Immunohistochemical localization of the epidermal growth factor receptor in normal human tissues. Lab Invest 55:588–592

Downward J, Yarden Y, Mayes E, Scrace G, Totty N, Stockwell P, Ullrich A, Schlessinger J, Waterfield MD (1984) Close similarity of epidermal growth factor receptor and v-erb-B oncogene protein sequences. Nature 307:521–527

Eker P (1989) Effects of epidermal growth factor, fibroblast growth factor, retinoic acid and serum on anchorage-dependent and anchorage-independent growth of HRRT cells. Eur J Cancer Clin Oncol 25:499–504

Forgue-Lafitte M-E, Laburthe M, Chamblier M-C, Moody A, Rosselin G (1980) Demonstration of specific receptors for EGF-Urogastrone in isolated rat intestinal epithelial cells. FEBS Lett 114:243–246

Glenney JR, Chen WS, Lazar CS, Walton GM, Zokas LM, Rosenfeld MG, Gill GN (1988) Ligand-induced endocytosis of the EGF receptor is blocked by mutational inactivation and by microinjection of anti-phosphotyrosine antibodies. Cell 52:675–684

Gullick WJ, Marsden JJ, Whittle N, Ward B, Bobrow L, Waterfield MD (1986) Expression of epidermal growth factor receptors on human cervical, ovarian, and vulval carcinomas. Cancer Res 46:285–292

Gusterson B, Cowley G, Smith JA, Ozanne B (1984) Cellular localization of human epidermal growth factor receptor. Cell Biol Int Rep 8:649–658

Gusterson B, Cowley G, McIlhinney J, Ozanne B, Fisher C, Reeves B (1985) Evidence for increased epidermal growth factor receptors in human sarcomas. Int J Cancer 36:689–693

Hunts J, Ueda M, Ozawa S, Abe O, Pastan I, Shimizu N (1985) Hyperproduction and gene amplification of the epidermal growth factor receptor in squamous cell carcinomas. Jpn J Cancer Res [Gann] 76:663–666

Ishitoya J, Toriyama M, Oguchi N, Kitamura K, Ohshima M, Asano K, Yamamoto T (1989) Gene amplification and overexpression of EGF receptor in squamous cell carcinomas of the head and neck. Br J Cancer 59:559–562

Joh T, Itoh M, Katsumi K, Yokoyama T, Miyamoto T, Imai S, Ikeda K, Matsusako K, Takeuchi T, Kato T, Tanaka R (1987) Immunoreactive epidermal growth factor in mouse digestive organs. Use of a sensitive enzyme immunoassay. Acta Endocrinol (Copenh) 115:203–210

Kasselberg AG, Orth DN, Gray ME, Stahlman MT (1985) Immunocytochemical localization of human epidermal growth factor/urogastrone in several human tissues. J Histochem Cytochem 33:315–322

Möller P, Mechtersheimer G, Kaufmann M, Momburg F, Mattfeldt T, Otto HF (1989) Expression of epidermal growth factor receptor in benign and malignant tumours of the breast. Virchows Arch [A] 414:157–164

Moolenaar WH, Bierman AJ, Tilly BC, Verlaan I, Defize LHK, Honegger AM, Ullrich A, Schlessinger J (1988) A point mutation at the ATP-binding site of the EGF-receptor abolishes signal transduction. EMBO J 7:707–710

Neal DE, Marsh C, Benett MK, Abel PD, Hall RR, Sainsbury JRC, Harris AL (1985) Epidermal-growth-factor receptors in human bladder cancer: comparison of invasive and superficial tumours. Lancet ii:366–368

Perosio PM, Brooks JJ (1989) Expression of growth factors

and growth factor receptors in soft tissue tumors. Implications for the autocrine hypothesis. Lab Invest 60:245–253
Poulsen SS, Nexo E, Olsen PS, Hess J, Kirkegaard P (1986) Immunohistochemical localization of epidermal growth factor in rat and man. Histochemistry 85:389–394
Rall LB, Scott J, Bell GJ, Niall H, Coghlan JC (1985) Mouse prepro-epidermal growth factor synthesis by the kidney and other tissues. Nature 313:228–231
Reifenberger G, Prior R, Deckert M, Wechsler W (1989) Epidermal growth factor receptor expression and growth fraction in human tumours of the nervous system. Virchows Arch A 414:147–155
Reubi JC, Horisberger U, Lang W, Koper JW, Braakman R, Lamberts SWJ (1989) Coincidence of EGF receptors and somatostatin receptors in meningeomas but inverse, differentiation-dependent relationship in glial tumors. Am J Pathol 134:337–344
Rothbauer E, Mann K, Wiebecke B, Borlinghaus P, Lamerz R, Pratschke E, Krämling H-J, Pfeiffer A (1989) Epidermal growth factor receptors and epidermal growth factor-like activity in colorectal mucosa, adenomas and carcinomas. Klin Wochenschr 67:518–523
Sainsbury JRC, Nicholson S, Angus B, Farndon JR, Malcolm AJ, Harris AL (1988) Epidermal growth factor receptor status of histological sub-types of breast cancer. Br J Cancer 58:458–460
Shimizu N, Kondo I, Gamou S, Belizadin MA, Shimizu Y (1984) Genetic analysis of hyperproduction of epidermal growth factor receptors in human epidermoid carcinoma A431 cells. Somatic Cell Mol Genet 10:45–49
Squinto SP, Block AL, Doucet JP (1989) Epidermal growth factor induction of cellular proliferation and protooncogene expression in growth-arrested rat H4IIE hepatoma cells: role of cyclic adenosine monophosphate. Mol Endocrinol 3:433–446
Sugiyama K, Yonemura Y, Miyazaki I (1989) Immunohistochemical study of epidermal growth factor and epidermal growth factor receptor in gastric carcinoma. Cancer 63:1557–1561
Toi M, Hamada Y, Nakamura T, Mukeida H, Suehiro S, Wada T, Toge T, Niimoto M, Hattori T (1989) 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 43:220–225
van't Hof RJ, Defize LHK, Nuijdens R, de Brabander M, Verklej AJ, Boonstra J (1989) Dynamics of epidermal growth factor receptor internalization studies by Nanovid light microscopy and electron microscopy in combination with immunogold labeling. Eur J Cell Biol 48:5–13
Wahl MT, Nishibe S, Suh P-G, Rhee SG, Carpenter G (1989) Epidermal growth factor stimulates tyrosine phosphorylation of phospholipase C-II independently of receptor internalization and extracellular calcium. Proc Natl Acad Sci USA 86:1568–1572
Waterfield MD, Mayes ELV, Stroobant P, Bennet PLP, Young S, Goodfellow PN, Banting GS, Ozanne B (1982) A monoclonal antibody of the human epidermal growth factor receptor. J Cell Biochem 20:149–161
Yamamoto T, Kamata N, Kawano H, Shimizu S, Kurochi T, Toyoshima K, Rikimaru K, Nomura N, Ishizaki R, Pastan I, Gamou S, Shimizu N (1986) High incidence of amplification of the epidermal growth factor receptor gene in human squamous carcinoma cell lines. Cancer Res 46:414–416
Yasui W, Hata J, Yokozaki H, Nakatani H, Ochiai A, Ito H, Tahara E (1988a) Interaction between epidermal growth factor and its receptor in progression of human gastric carcinoma. Int J Cancer 41:211–217
Yasui W, Sumiyoshi H, Hata J, Kameda T, Ochiai A, Tahara E (1988b) Expression of epidermal growth factor receptor in human gastric and colonic carcinomas. Cancer Res 48:137–141

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