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Epidermal growth factor receptor expression in colorectal cancer

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Epidermal growth factor (EGF) receptor expression was estimated in 50 invasive human colorectal cancers using immunohistochemistry and the degree of expression was quantified from integrated optical density measurements on the stained sections. All tumours stained positively, but Dukes' C tumours exhibited significantly higher levels of receptor than either Dukes' A or B tumours. In addition, histologically high grade cancers expressed receptors more strongly than those of low grade. It is concluded that a high EGF receptor concentration is associated with poor prognostic factors in colorectal malignancy.

Keywords: Colorectal carcinoma, epidermal growth factor receptor

Epidermal growth factor (EGF) is a polypeptide which regulates cellular proliferation in a wide variety of tissues[1–3], but its precise role is not understood. The receptor for EGF is a 170 kDa phosphoglycoprotein located across the cell membrane[1], and the internal portion of this molecule bears a close relationship to the *v-erb-B* oncogene product[4]. It is thought that growth factors may be important in the proliferation of neoplastic cells and a number of human tumours have been shown to express the EGF receptor to a variable degree[5].

Colorectal carcinoma has been studied very little in this respect; EGF receptors have been demonstrated on neoplastic cells in this tumour[6,7] but their significance is unclear. In this study, using a mouse monoclonal antibody raised against the human EGF receptor, we have investigated the relationship between the degree of receptor expression and two well recognized prognostic factors in colorectal cancer: tumour grade and histological differentiation.

Patients and methods

Fifty patients with histologically proven invasive colorectal carcinoma were studied. There were 26 women and 24 men whose ages ranged from 49 to 96 years. Immediately after resection, a portion was cut from the edge of each tumour and placed into liquid nitrogen for transport to the laboratory. Frozen sections (6 μm thick) were cut, air dried overnight, wrapped in aluminium foil and stored at -20°C.

The primary antibody used to identify the EGF receptor on the sections was a mouse monoclonal immunoglobulin G2b raised against the human EGF receptor (Amersham International, Amersham, UK) using the receptor-rich vulval carcinoma cell line A431 as the immunogen[8]. The method used to visualize the bound antibody was the streptavidin–biotin system[9] as this has been previously shown to be the most sensitive technique for detection of EGF receptor[10]. The primary antibody was diluted 1:100 and the sections were incubated at room temperature for 60 min. This was followed by biotinylated rabbit antimouse immunoglobulin at 1:200, which was absorbed with 10 per cent human AB serum. Streptavidin complexed to biotinylated peroxidase was then used and the bound peroxidase was visualized using the diaminobenzamine/H_2O_2 reaction. Endogenous peroxidase was not blocked, as preliminary investigations revealed this to be confined to granulocytes, which did not interfere with the interpretation of the tissue sections. All stages of the staining routine were timed exactly to ensure uniformity throughout the series of tumours.

Frozen sections of human placental tissue were used as positive controls and cytocentrifuged preparations of human lymphocytes served as negative controls[5,11]. Each stained slide of colorectal tumour was accompanied by an identical substitution control in which the primary antibody had been replaced by non-immune mouse immunoglobulin G (Sigma Chemical Company, Poole, UK).

Intensity of staining in the study sections was estimated using a flying-spot scanning microdensitometer (Vickers 85a, Vickers Instruments, York, UK) which provides integrated optical density measurements by automatically integrating absorbance measurements from a fine beam of monochromatic light (wavelength 466 nm) which rapidly scans a masked area of the specimen. Each slide was studied with a mask area of 78·5 μm^2, chosen so that it could be easily located over a single cell. Random readings were obtained from individual cells on each section, subtracted from the background reading and the mean value was taken as the integrated optical density value for the tumour. The reproducibility of this technique was tested by obtaining 25, 50 and 100 readings from a single tumour and repeating this on three separate occasions. The mean values did not differ significantly between sessions when 50 or 100 readings were taken. Fifty readings were therefore used routinely. In addition to integrated optical density measurements the immunohistochemical staining was graded as 0, + or ++ on all sections.

Dukes' classification and histological grading was carried out by a pathologist (B.E.) who was not aware of the immunohistochemical assay results. A two-stage system of grading was used: well differentiated and moderately differentiated tumours were classified as low grade, and poorly differentiated or anaplastic tumours as high grade. This approach is more reproducible than more complex systems and it is known that well and moderately differentiated tumours behave in a similar way[12].

Results

Of the 50 tumours, eight were Dukes' A, 22 were Dukes' B and 20 were Dukes' C. In all cases, background and non-specific staining was minimal and positive staining for EGF receptor was confined to the neoplastic cells within the tumour. Integrated optical density measurements showed that all tumours exhibited EGF receptor expression although in five cases this was barely discernible by eye.

When the tumours were grouped according to Dukes' classification the more prognostically favourable tumours were associated with low integrated optical density values for the EGF receptor concentration (*Figure 1*). The mean(s.d.) value for Dukes' A tumours was 6·06(2·64); for Dukes' B tumours, 8·56(3·04); and for Dukes' C tumours, 14·15(4·36). All the differences between these means were statistically significant (*Table 1*). The tumours of low histological grade had a significantly lower mean EGF receptor concentration than high grade tumours (*Table 2*). Subjective grading of the EGF receptor staining gave similar results (*Table 3*).

Discussion

Quantification of antigen by immunohistochemistry is normally done using subjective histological grading systems, and is accordingly subject to interobserver and intraobserver variations[13]. Intensity of immunoperoxidase staining, however, can be measured by photometric techniques as the absorbance of light is directly proportional to the amount of the peroxidase reaction product. If monoclonal antibodies are used and assay conditions are held constant it is then justifiable to extrapolate

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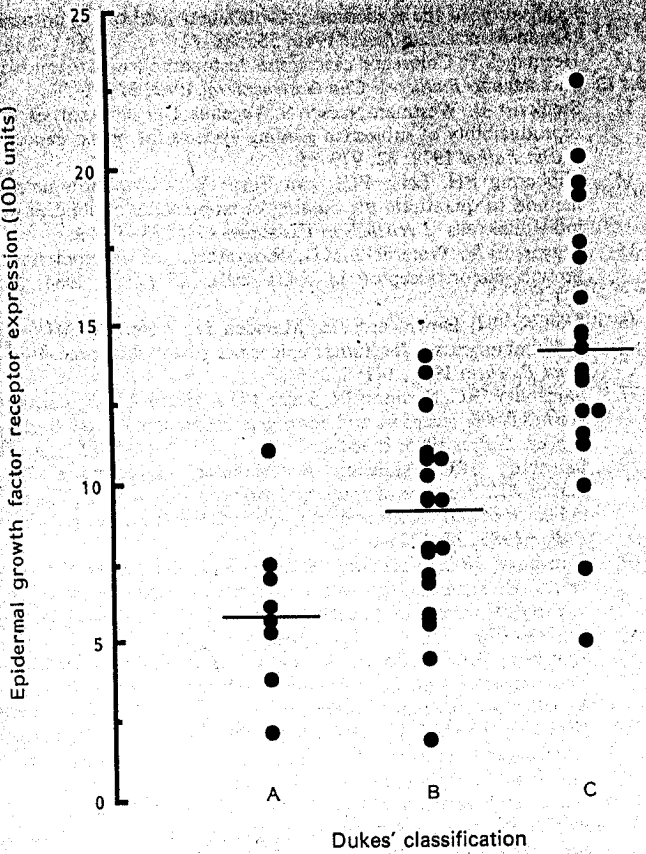

Figure 1 *Integrated optical density (IOD) values for epidermal growth factor receptor expression by human colorectal carcinoma as estimated by immunohistochemical staining. Values grouped according to Dukes' classification*

Table 1 *Differences between mean integrated optical density values for epidermal growth factor receptor expression by colorectal carcinoma according to Dukes' classification*

Dukes' classification	Difference between means	95% confidence interval	*P**
A *versus* B	2·50	0·02–5·0	<0·05 >0·01
B *versus* C	5·58	3·3–7·9	<0·001
A *versus* C	8·08	4·7–11·6	<0·001

* Values calculated using Student's *t* test

from optical density measurements to the concentration of antigen recognized by the antibody[14].

In this study the technique has been used to quantify EGF receptor concentration in human colonic cancer. The monoclonal antibody used is a well characterized immunoglobulin which recognizes an epitope on the extracellular domain of the receptor[15], but does not compete with EGF for its binding site[16]. It is therefore unlikely that the antibody is detecting an oncogene product, as *v-erb-B* encodes a truncated EGF receptor which lacks the external domain[4]. On the other hand, it is not clear whether the receptor identified by this means is functional, as the antibody gives no indication of ligand binding.

Accepting this limitation, the study shows that the concentration of EGF receptor tends to be higher in those colorectal tumours that are locally advanced and poorly differentiated. This is in keeping with findings in other human tumours. Sainsbury *et al.* have established that, in breast cancer, EGF receptor expression is associated with poor differentiation, lack of oestrogen receptors and early disease recurrence and death[17–20]. Similarly, EGF receptor-positive bladder cancers tend to be invasive and poorly differentiated[21] and advanced gastric cancers are more likely to have receptors than are early tumours[7].

A major discrepancy between these results and those of others is the frequency of EGF receptor-positive cancers. All the colorectal cancers in this series had detectable receptor, albeit to a variable degree, whereas Sainsbury *et al.* found EGF receptors in only 32 per cent of breast cancers using both ligand binding and immunohistochemistry[17]. In bladder cancer, 58 per cent of the specimens studied by Neal *et al.* were found to be EGF receptor positive[21] and Yasui *et al.* detected receptor in 72 per cent of colorectal cancers[7]. This may be related to the sensitivity of the streptavidin-biotin method[10] and to the ability of the scanning microdensitometer to detect low levels of staining which are difficult to appreciate without objective subtraction measurements of optical density. Simple subjective visual grading correlated well with prognostic factors and this may be of more practical value in view of the complex nature of the objective technique. EGF receptor expression appears to be a continuum rather than an 'all or none' phenomenon in colorectal cancer and this was only demonstrated by a sensitive, precise method.

The role of the EGF receptor in the behaviour of cancer is still obscure. Ligand binding studies in human tumours indicate that the receptor is capable of binding EGF, and its increased expression may be partly responsible for the rapid growth and consequent poor prognosis associated with locally advanced and poorly differentiated tumours. The EGF receptor, therefore, may have prognostic significance which could be used to supplement other factors in order to obtain a more precise prediction of outcome[22]. More exciting, however, is its possible influence over tumour growth. A fuller understanding of the interactions between the receptor, EGF and tumour growth factor-α might have important therapeutic implications in the future.

Table 2 *Differences between integrated optical density values for EGF receptor expression by colorectal carcinomas according to histological grade*

	Histological grading of colorectal tumour	
	Low grade	High grade
Number	39	11
Mean EGF receptor concentration (integrated optical density units)	9·2	14·5
Standard deviation	4·0	5·2
Difference between means	5·3	
95% confidence interval	2·4–8·2	
*P**	<0·001	

* Value calculated using Student's *t* test; EGF, epidermal growth factor

Table 3 *Relationship between subjective grading of epidermal growth factor receptor staining intensity and both Dukes' classification and histological grade*

	Grading of staining intensity	
	0/+	++
Dukes' classification		
A	8	0
B	19	3
C	5	15
	$\chi^2 = 22{\cdot}48$ $P < 0{\cdot}001$	
Histological grade*		
High grade	3	8
Low grade	29	10

* High grade *versus* low grade; $\chi^2 = 8{\cdot}26$, $P < 0{\cdot}01$