

Prognostic value of epidermal growth factor receptor expression in cervical carcinoma

R J Hale, C H Buckley, W J Gullick, H Fox, J Williams, F L Wilcox

Abstract

Aims: To investigate the pattern of epidermal growth factor receptor expression and its prognostic value in the three main types of cervical carcinoma.

Methods: 62 cases of stage IB/IIA cervical carcinoma, all with a minimum of five years of follow up, were studied. Representative sections were stained for mucin to permit accurate tumour typing and a standard avidin-biotin immunoperoxidase technique using the polyclonal antibody 12E was used to demonstrate the presence of epidermal growth factor receptor.

Results: A proportion of all three tumour types expressed epidermal growth factor receptor, it being most common in squamous cell carcinomas (50%). Overall, there was a correlation between epidermal growth factor expression and mortality. This was particularly obvious in the absence of lymph node metastases. When the individual tumour types were considered this association with prognosis was not demonstrable for squamous cell carcinomas or adenocarcinomas but was a very prominent feature of adenocarcinomas.

Conclusions: Immunohistochemical demonstration of epidermal growth factor receptor expression may be useful in identifying those patients with a poor prognosis, particularly those with adenocarcinomas which have not metastasised to the regional lymph nodes.

membrane glycoprotein encoded for by the *c-erbB-1* oncogene. It has three components, an extracellular domain capable of binding the ligand, a transmembrane portion, and an intracellular domain facing the cytoplasm. The receptor is capable of binding EGF, transforming growth factor (TGF) α ,³ amphiregulin,⁶ and heparin-binding EGF.⁷ The function of EGFR is to bind the mitogen EGF or TGF α and to transduce the signal across the cell membrane to the cytoplasm. The intracellular component exhibits tyrosine kinase activity and has binding sites for ATP.³ This results in the autophosphorylation of the EGFR and phosphorylation of several target proteins.^{8,9}

EGFR is found on the surface of many cells, including normal and malignant cells of epidermal and mesenchymal origin but not in cells of the haemopoietic system.¹⁰⁻¹³ Different cell types express different numbers of cell surface EGF receptors, from none on lymphoid cells, to 250 000 per cell on keratinocytes.¹¹⁻¹⁴ This high level of receptors on keratinocytes reflects the important role EGF has in regulating the growth and differentiation of epidermal cells.

It has been suggested that overexpression of the EGFR is a common, if not constant, step in the malignant transformation of squamous cells, and that there is a close correlation between EGFR overexpression, EGF mRNA, and EGF gene amplification.⁵

EGF has been identified in breast carcinoma¹⁵⁻¹⁷ and has been shown to play an important part in regulating proliferation of both normal and neoplastic mammary epithelial cells.^{15,18,19} It has been suggested that expression of EGFR in breast carcinoma is related to aggressive clinical behaviour,²⁰⁻²² although this has not been demonstrated in other studies.^{23,24} It is also known that EGFR and oestrogen receptor status in breast carcinomas are inversely correlated.^{16,20,21,23,24}

There is a need to identify markers of tumour aggressiveness in patients with cervical carcinoma to define groups with either a good prognosis (requiring no further treatment) or where the outcome is likely to be poor and where more intensive treatment might be beneficial.

Already identified as important prognostic factors in early stage cervical carcinoma (stage IB/IIA) are tumour volume, lymph node status, lymphatic permeation and pregnancy. Tumour type is not of confirmed value in predicting clinical outcome. The pattern of behaviour of the different tumour types is not,

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Tumour cells can produce and are able to respond to their own growth factors. One such factor is epidermal growth factor (EGF), first purified from the submandibular salivary gland of male mice.¹ EGF is found in almost all body fluids under normal physiological conditions.² It is known to promote cell proliferation while inhibiting terminal differentiation and conversely can cause dose dependent inhibition of cell proliferation. Different aspects of growth and differentiation were described in certain cell culture models and in vivo, following EGF treatment.³⁻⁵

The specific receptor for EGF (EGFR) was first purified from the A431 cell line which was derived from an epidermoid carcinoma of the vulva. It is a 170 000 kilodalton trans-

Department of Reproductive Pathology, St Mary's Hospital, Manchester

R J Hale
C H Buckley
J Williams
H Fox

ICRF Oncology Group, Hammersmith Hospital, London
W J Gullick

Department of Obstetrics and Gynaecology, Royal Victoria Hospital, Blackpool
F L Wilcox

Correspondence to: Dr R J Hale, Department of Reproductive Pathology, St Mary's Hospital, Hathersage Road Manchester M13 0JH

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however, the same and the prognostic and biological importance of this requires further investigation.²⁵

Methods

A total of 62 cases of early stage cervical carcinoma (all but one being stage IB) treated by Wertheim's hysterectomy at St Mary's Hospital, Manchester, form the basis of this study. None of the patients had received prior radiotherapy.

The different tumour types have different behavioural patterns²⁵ and, therefore, the three main tumour types (squamous carcinoma, adenosquamous carcinoma, and adenocarcinoma) were equally represented.

Note was made of the presence or absence of lymph node metastases in each case. Follow up information of between 5 and 11 years' duration was available for all patients.

Tissue was formalin fixed, paraffin processed, and wax embedded. Using haematoxylin and eosin stained sections and periodic acid Schiff/alcian blue staining, with and without predigestion with diastase, the tumours were typed according to the criteria of Buckley and Fox.²⁶

For immunohistochemical staining, sections were dewaxed and a standard indirect avidin-biotin immunoperoxidase technique was then applied. A rabbit polyclonal antibody 12E²⁷ at a concentration of 4 µg/ml was used, the sections being incubated for 30 minutes at room temperature.

Human placental tissue was used as a positive control and negative controls involved the omission of the primary antibody.

Membrane staining was semiquantitatively graded on a four point scale according to the intensity of staining (0 negative; + weak; ++ moderate, and +++ strong).

The results were tabulated, put into numerical form, and analysed using Kendall's τ_B and C (SPSS package for IBM compatible computers). A significant correlation between two parameters was taken at the 95% confidence limit, where $p < 0.05$. Particular note was made of any correlation with clinical outcome, measured by death rate.

Results

The distribution of tumour types is shown in the table.

In most cases where ectocervical squamous epithelium was included positive membrane staining (+) was present in the basal and parabasal layers. Therefore, those tumours with grades 0 or + were regarded as negative

and those with grades ++ and +++ were deemed positive for overexpression of EGFR.

Positive staining was detected in 21 (34%) cases. The numbers in each tumour type are shown in the table, positivity being seen most frequently in the squamous cell carcinomas ($p = 0.019$). Staining was variable between and within the tumours. In only three cases did more than 75% of the cells stain (fig 1). In the remainder positivity was restricted to scattered small groups of cells randomly distributed throughout the tumour. In squamous cell carcinomas, however, there was a tendency for staining to be localised to the peripheral cells of the infiltrating tongues of tumour (fig 2). The pattern of staining was not directly related to outcome, the important factor being the presence or absence of EGFR overexpression.

Only in adenocarcinomas was it possible to demonstrate any correlation between positive staining and the presence of lymph node metastases ($p = 0.028$).

Considering all three tumour types as a whole, there was a significant correlation between positive staining and death rate ($p = 0.003$). This was also true for patients without lymph node metastases ($p < 0.001$) but not for those with lymph node metastases (fig 3). However, this correlation seems to be due entirely to the influence of EGFR in adenosquamous carcinomas.

In patients with squamous cell carcinoma (fig 4) there was no correlation between positive staining and outcome, either overall or in those patients with lymph node metastases. However, a trend did exist between positive staining and outcome where nodal metastases were absent, although this failed to reach significance.

In the group of patients with adenosquamous carcinoma, there were significantly more deaths in patients with positive staining, both overall ($p = 0.005$) and in those lacking lymph node metastases ($p < 0.001$). When lymph node metastases were present no such correlation could be shown (fig 5).

In patients with adenocarcinoma no correlation could be demonstrated between staining pattern and clinical outcome, either overall or where nodal metastases were or were not present (fig 6). However, in this group of patients there was a very strong correlation between outcome and nodal status such that no deaths have occurred in those patients lacking nodal metastases (at the time of writing). Obviously this makes it very difficult to demonstrate any impact that EGFR expression may have on prognosis.

Discussion

Unlike breast carcinomas, where the expression of EGFR has been widely studied,¹⁵⁻²⁴ EGFR expression in cervical carcinoma has been the subject of only a limited number of papers. It has been shown that squamous cell carcinomas of the cervix frequently express EGFR,^{12 28-30} often at a high concentration.³¹ In one study, 41% of the cases showed over-

Distribution of tumour types and the number staining for EGFR in each group

Tumour type	Staining for EGFR		Total
	Positive	Negative	
Squamous carcinoma	10 (50%)	10 (50%)	20
Adenosquamous Carcinoma	7 (33%)	14 (66%)	21
Adenocarcinoma	4 (19%)	17 (81%)	21
Overall	21	41	62

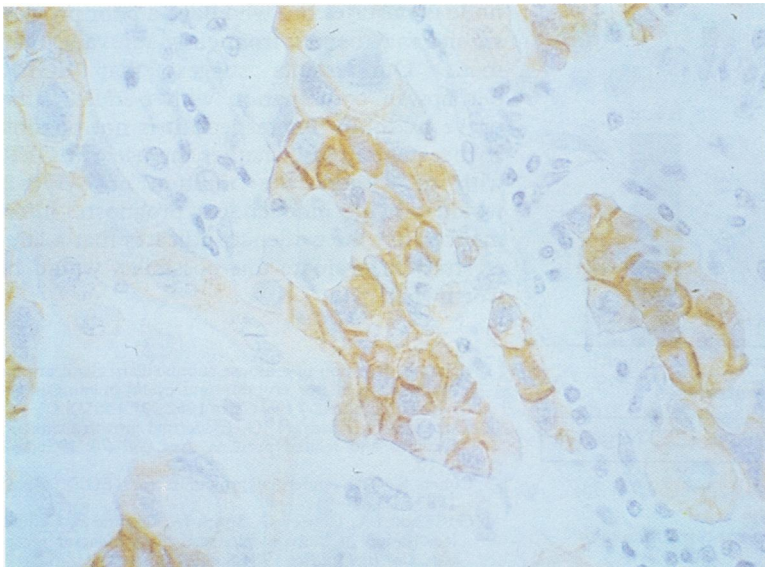


Figure 1 Prominent membrane staining in poorly differentiated adenocarcinoma indicating overexpression of EGFR.

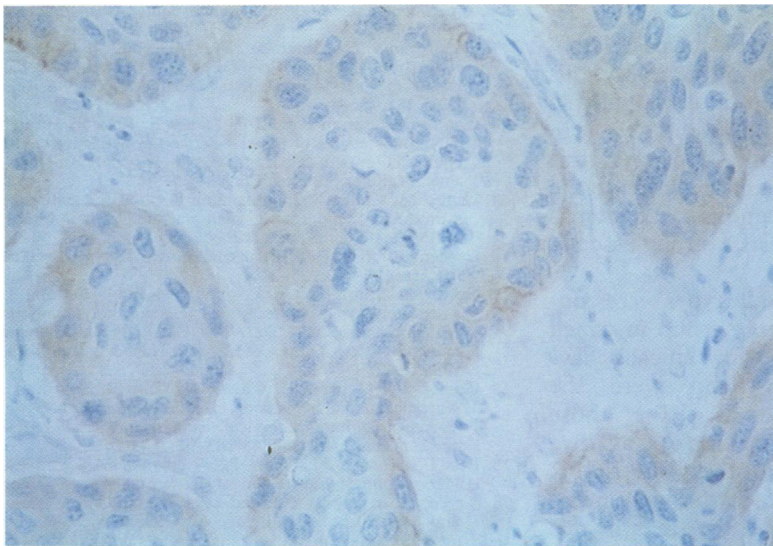


Figure 2 Squamous cell carcinoma showing patchy membrane staining with anti EGFR predominantly at the periphery of the tumour lobules.

expression of EGFR,³¹ a proportion similar to that found in the present study.

Overexpression of EGFR correlate with a worse prognosis according to Pfeiffer *et al*,³² who suggested that EGFR expression may be indicative of the biological aggressiveness of cervical squamous cell carcinomas. More recently, in a study in which clinical behaviour was measured by the presence or absence of lymph node metastases, no correlation could be identified between EGFR expression in squamous cell carcinomas of the cervix and nodal status.³³ It was concluded that EGFR was, therefore, unlikely to be a useful prognostic marker.

To our knowledge, the present study is the first to investigate EGFR expression and its possible prognostic value in all three main types of cervical carcinoma.

We have shown that EGFR overexpression is most frequently seen in squamous carcinomas and least frequently in adenocarcinomas.

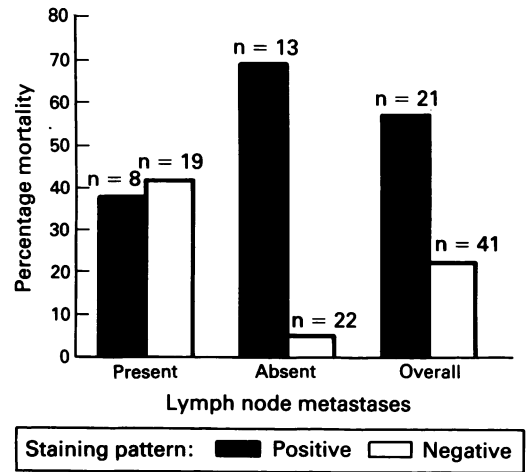


Figure 3 EGFR in cervical carcinoma: correlation with prognosis.

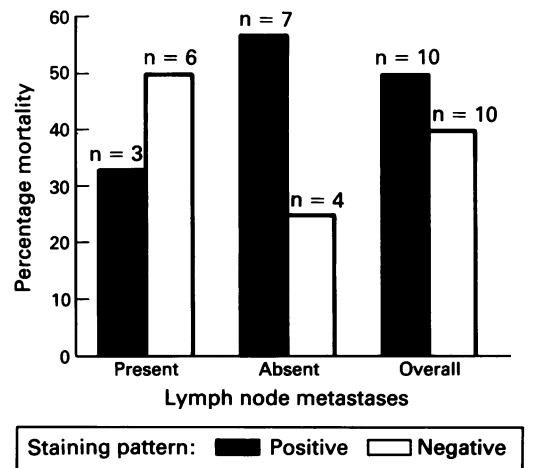


Figure 4 EGFR in squamous cell carcinoma: correlation with prognosis.

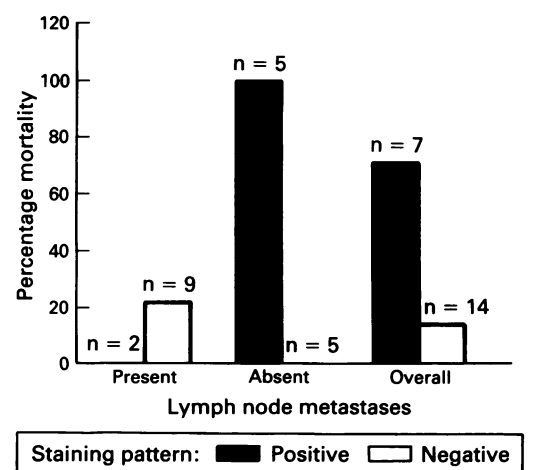


Figure 5 EGFR in adenosquamous carcinoma: correlation with prognosis.

Considering all three tumour types as a whole, like Hayashi and co-authors,³³ no association could be shown between EGFR expression and nodal metastases. Only in adenocarcinomas was there a correlation

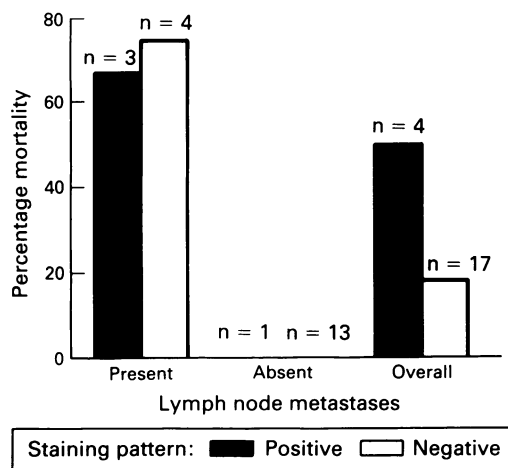


Figure 6 EGFR in adenocarcinoma: correlation with prognosis.

adenocarcinomas was there a correlation between EGFR expression and the presence of lymph node metastases.

Overall, there was a significant correlation between EGFR expression and mortality, this being particularly prominent in those patients lacking lymph node metastases and absent in those with nodal metastases. However, this seems to have been almost entirely due to the influence of adenosquamous carcinomas where EGFR expression has a strong association with prognosis.

When the different tumour types were considered individually the only group in which any significant correlation with clinical behaviour could be demonstrated were the adenosquamous carcinomas. In those patients lacking nodal metastases all five whose tumours stained positively for EGFR died of disease while all five who were negative were alive and disease free. It is in this subgroup, particularly, that the evaluation EGFR expression may prove to be of clinical value. A similar pattern of behaviour was seen in the squamous carcinomas, but this failed to reach significance.

These results were compared with those obtained when *c-erbB-2* expression was studied on the same group of patients.³⁴ There was a close correlation between the results obtained with both antibodies ($p < 0.001$). This was particularly so in certain subgroups. Four of the six patients with squamous carcinoma and all five patients with adenosquamous carcinomas which stained positively with both antibodies and who lacked lymph node metastases have died of disease.

The identification of patients at high risk of recurrence and death from disease remains an important factor in patient management. In early stage cervical carcinoma one of the most important prognostic indicators is lymph node status.²⁵ However, in absolute terms equal numbers of deaths from disease occur in patients with and without lymph node metastases.³⁵ Therefore, it is important to try and identify those patients who lack lymph

node metastases but who have a poor prognosis and may benefit from early adjuvant treatment. Our results suggest that EGFR, perhaps in combination with *c-erbB-2*, may prove useful in this respect. It is not possible at this stage to say whether the use of EGFR, with or without the addition of *c-erbB-2*, would provide independent prognostic information, but the evidence indicates that a larger study to evaluate this possibility would be worthwhile.

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