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Prognostic value of *c-erb*B-2 expression in uterine cervical carcinoma

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Abstract

***Aims*: To study the pattern of expression and prognostic importance of *c-erb*B-2 protein in cervical carcinoma.**

***Methods*: Sixty two cases of stage IB/IIA cervical carcinoma, representing the three main tumour types, were investigated immunohistochemically for the presence of *c-erb*B-2 protein expression, using a monoclonal antibody (CB11) to its internal domain. Follow up of at least five years' duration was available in all cases.**

***Results*: Definite membrane staining was seen in 38·7% of cases. There was a strong correlation with poor survival ($p < 0·0001$) particularly. For those with adenocarcinomas, this was the case when nodal metastases were present. In contrast, for squamous carcinomas and adenosquamous carcinomas, the association with a poor prognosis was most apparent in those patients without lymph node metastases.**

***Conclusions*: These findings raise the possibility that immunostaining for *c-erb*B-2 protein could be used as a prognostic marker and may help identify those patients for whom early adjuvant treatment might be beneficial.**

*c-erb*B-2 (also known as *HER2* or *neu*) is a proto-oncogene which encodes a 185–190 kilodalton glycoprotein molecule that is closely related in structure to epidermal growth factor receptor (EGF),[1–4] which is encoded by the oncogene *c-erb*B-1. Recently, Lupu *et al* identified a protein, gp30, which specifically inhibits the growth of cells that overexpress *c-erb*B-2 and which binds to the putative receptor.[5] It maps to human chromosome 17.[6]

The gene product, which has tyrosine kinase activity, is localised to the cell membrane with extracellular, transmembrane, and intracellular domains.[1] Various specific antibodies have been raised to the extra- and intracellular domains. Repeated studies have shown a good correlation between amplification of the *c-erb*B-2 gene and positive immunostaining for its protein product in the cells using these specific antibodies,[7–12] although protein product overexpression can sometimes occur in the absence of gene amplification.[9 12]

In breast carcinomas between 9% and 33% of invasive tumours overexpress the gene product[7 9 10 12–20] and there is strong evidence that overexpression is associated with increased tumour aggression.[9 10 14 19–24] It has, however, recently become apparent that the prognostic importance of *c-erb*B-2 staining is of value only in patients without lymph node metastases and that no additional prognostic information is provided in patients with nodal metastases.[25]

In a study of normal and neoplastic cervical, vulval, and vaginal tissue increased staining was found in intraepithelial neoplasia and weak staining was present in a high proportion of the invasive cervical carcinomas.[26] Brumm *et al* found that all 17 cases of CIN III showed at least focal positivity and six of eight squamous cell carcinomas of the cervix uteri were positive for *c-erb*B-2 protein, with two cases being strongly positive.[27]

Methods

A total of 62 cases of stage IB/IIA cervical carcinoma were retrieved from the files of the Department of Reproductive Pathology, St Mary's Hospital, Manchester.

Different tumour types behave differently.[28] The three main tumour types (squamous carcinoma, adenosquamous carcinoma, and adenocarcinoma) were therefore equally represented. Tissue was formalin fixed and paraffin 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.[29]

All of the patients had undergone Wertheim's hysterectomy, without prior radiotherapy. Note was made of the presence or absence of lymph node metastases. Follow up information of between five and 11 years' duration was available for all patients.

For immunohistochemical staining, sections were dewaxed, washed in TBS (TRIS–buffered saline) and the endogenous peroxidase blocked by 3% hydrogen peroxide in methanol for five minutes at room temperature. After washing in TBS, sections were incubated overnight at 4°C with the monoclonal antibody NCL-CBll (Novocastra laboratories) at a concentration of 1 in 50. This is a mouse monoclonal antibody raised against the internal domain of the *c-erb*B-2 protein. After further washing in TBS, sections were exposed to biotinylated rabbit anti–mouse immunoglobulin (Dakopatts UK) at a concentration of 1 in 400 for two hours at room temperature. After rinsing with TBS, avidin-biotin complex/horseradish peroxidase (Dakopatts UK) was applied for 30 minutes. The sections were again rinsed in TBS and the colour was developed using a standard diaminobenzidine technique. The slides were then

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rinsed for 10 minutes in running tap water, counterstained with Mayer's haematoxylin, dehydrated and mounted.

A breast carcinoma known to express *c-erb*B-2 oncoprotein strongly was used as a positive control and negative controls involved the omission of the primary antibody. When staining was assessed, only those cases showing definite membranous positivity were accepted. Tumours were classed as being either positive or negative.

The results were tabulated, put into numerical form, and analysed using Kendall's Tau 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 as measured by death rate.

Table 2 Percentage death rate with respect to c-erbB-2 staining in patients with and without lymph node metastases in each tumour type and overall

	c-erbB-2 staining	
Tumour type	*Positive*	*Negative*
Lymph node metastases present:		
Squamous carcinoma	50% (2/4)	40% (2/5)
Adenosquamous carcinoma	33% (1/3)	13% (1/8)
Adenocarcinoma	83% (5/6)	0% (0/1)
Overall	62% (8/13)	21% (3/14)
Lymph node metastases absent:		
Squamous carcinoma	80% (4/5)	17% (1/6)
Adenosquamous carcinoma	83% (5/6)	0% (0/4)
Adenocarcinoma	— (0/0)	0% (0/14)
Overall	82% (9/11)	4% (1/24)
Total:		
Squamous carcinoma	67% (6/9)	27% (3/11)
Adenosquamous carcinoma	67% (6/9)	8% (1/12)
Adenocarcinoma	83% (5/6)	0% (0/15)
Overall	71% (17/24)	11% (4/38)

Results

The distribution of tumour types, the numbers with and without lymph node metastases, and clinical outcome are shown in table 1. Membranous staining was detected in 24 cases (38·7%), the proportions being similar in each tumour type. The proportion of positively staining cells varied from only occasional groups to, in some tumours, as many as 80% of the neoplastic cells.

Overall, a significant correlation existed between positive staining and a poor outcome, as measured by death rate ($p < 0.0001$), this being true for patients both with ($p = 0.0188$) and without ($p < 0.0001$) lymph node metastases. A significant correlation between positive staining and a poor prognosis was also seen in all three individual tumour types (squamous carcinoma, $p = 0.0430$; adenosquamous carcinoma, $p = 0.0031$; adenocarcinoma, $p < 0.0001$ (table 2).

No significant correlation was detected between lymph node status and *c-erb*B-2 staining except in adenocarcinomas where there was significantly more staining in the tumours of patients in whom lymph node metastases had been identified ($p < 0.0001$), with only one of the seven cases in this group failing to stain.

In patients with nodal metastases there was no correlation between *c-erb*B-2 overexpression and outcome in either the squamous ($p = 0.6248$) or adenosquamous ($p = 0.4909$) groups. Five of the six patients with adenocarcinomas and positive staining died ($p < 0.0001$).

Where lymph node metastases were absent, positive staining for *c-erb*B-2 was significantly related to poor outcome in squamous carcinomas ($p < 0.0371$) and in adenosquamous carcinomas ($p < 0.0238$). In adenocarcinomas all 14 tumours lacking nodal metastases were negative for *c-erb*B-2 protein.

Table 1 Distribution of cases by tumour type, presence, or absence of lymph node metastases and clinical outcome

	Lymph node metastases				
	Present		*Absent*		
Tumour type	*Alive*	*Dead*	*Alive*	*Dead*	*Total*
Squamous carcinoma	5	4	6	5	20
Adenosquamous carcinoma	9	2	5	5	21
Adenocarcinoma	2	5	14	0	21
Overall	16	11	25	10	62

Discussion

In this study 38% of all cervical carcinomas showed definite membrane staining for *c-erb*B-2 oncoprotein. This compares with an incidence of between 0% and 33% in breast and ovarian carcinomas in other series.[7 9 10 12–20 30 31] In contrast, in a study of endometrial adenocarcinomas, in which frozen tissue rather than formalin fixed tissue was used, all 95 cases were found to have at least light to moderate staining.[32] The same authors showed light staining in 25 of 26 cervical squamous carcinomas, the remaining case showing heavy staining.[26] This latter patient was the only one with distant metastases.

In most studies overexpression of *c-erb*B-2 oncoprotein in breast carcinomas correlated with a poor prognosis,[9 10 14 22 24 25 33] although this has not been everyone's experience.[13 15 19 34 35] *c-erb*B-2 protein positivity has also been associated with a poor short term prognosis in gastric carcinoma[36] and with advanced stage disease in endometrial cancer.[32] Studies of *c-erb*B-2 protein expression in ovarian neoplasms have yielded contradictory results[9 30 31 37 38] and it is probably of little prognostic value in such neoplasms. This study is, to our knowledge, the first in which the prognostic implications of *c-erb*B-2 protein expression in cervical carcinomas have been examined.

When the three tumour types were considered as a single group, a significant association between *c-erb*B-2 protein expression and a poor prognosis, both overall and in patients with and without lymph node metastases, was shown. This effect was particularly striking for patients with squamous and adenosquamous carcinomas and in whom lymph node metastases were absent.

In contrast, all patients with adenocarcino-

mas in which *c-erb*B-2 protein expression was identified had nodal metastases, and only one of the seven cases in which lymph node metastases were present failed to show *c-erb*B-2 expression.

In a previous study we showed that, overall, lymph node metastases were an independent prognostic factor in cervical carcinoma but that this effect is not appreciable in adenosquamous carcinomas, is significant in squamous carcinomas, and is profound in adenocarcinomas.[28] Therefore, in adenocarcinomas the striking association between *c-erb*B-2 expression, lymph node metastases, and prognosis seems to be almost inseparable. This may have important implications when management of patients with adenocarcinoma is being considered after the initial biopsy, but prior to definitive treatment, as *c-erb*B-2 staining could provide useful prognostic information without the need for extensive pelvic surgery.

The identification of patients at high risk of recurrence and death from disease is an important step in planning management strategies. Numerous factors have been identified as having prognostic importance but there is still room for improvement. One of the major factors predicting outcome is lymph node status. However, in real terms an equal number of deaths occur in node negative as in node positive patients who have stage IB disease.[39] Our results in squamous and adenosquamous carcinomas are interesting because they suggest the possibility that we may be able to identify patients without lymph node metastases but who may have a poor prognosis and who may benefit from early adjuvant treatment.

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