Expression of *c-erbB-2* oncogene product in Barrett's adenocarcinoma: Pathological and prognostic correlations

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Abstract

Aims—To establish the prevalence of *c-erbB-2* protein expression in a surgical series of Barrett's adenocarcinomas; and to correlate this expression with clinico-pathological data and prognosis.

Methods—Sixty six surgical specimens of Barrett's adenocarcinomas were included in this retrospective study. Blocks of the tumour and of non-dysplastic Barrett's mucosa were stained with a polyclonal antibody specific for the intracytoplasmic domain of the *c-erbB-2* protein.

Results—Seven of 66 tumours showed membrane staining for the *c-erbB-2* protein. The non-dysplastic Barrett's mucosa was negative in all cases. There was no difference between *c-erbB-2* positive and negative tumours with regard to mean age, sex ratio, percentage of alcohol misusers, percentage of smokers, tumour differentiation, depth of invasion, lymph node response, and proliferative activity, assessed by the percentage of tumour cells positive with the MIB-1 antibody directed against the Ki-67 antigen. All *c-erbB-2* positive tumours were of Lauren's intestinal type compared with negative *c-erbB-2* tumours. Patients with *c-erbB-2* positive tumours had a significantly poorer prognosis than patients with negative tumours.

Conclusions—The prevalence of Barrett's adenocarcinomas expressing *c-erbB-2* found in this study (11%) was similar to that observed in published series of gastric adenocarcinomas. *c-erbB-2* protein expression could be an important prognostic indicator in Barrett's adenocarcinoma.

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c-erbB-2 is a proto-oncogene that codes for a 185 kilodalton glycoprotein, with high homology with the epidermal growth factor receptor.¹ The *c-erbB-2* gene product is thought to be activated by specific ligand(s), resulting in the stimulation of cytoplasmic tyrosine kinase activity and causing signal transduction in the cell.^{2,3} Amplification of the *c-erbB-2* gene occurs in various tumours, including adenocarcinomas of the stomach.^{4,5}

The immunohistochemical staining of the membranes of tumour cells using specific

antibodies directed against *c-erbB-2* protein, is a reliable marker of *c-erbB-2* gene amplification.⁶ This staining has been reported as a prognostic indicator in some tumours—notably in adenocarcinoma of the breast.⁷ Its prognostic importance, however, in gastric adenocarcinoma is debatable, and no data are available regarding Barrett's adenocarcinoma.⁸⁻¹¹ The incidence of Barrett's adenocarcinoma is increasing,¹² and its prognosis is currently based on pathological features, mainly the degree of infiltration and lymph node status.¹³

Methods

Oesophagogastrectomy specimens from 66 consecutive patients with an adenocarcinoma which had developed in Barrett's oesophagus were retrieved from the files of the Department of Pathology, Hôpital Beaujon, Clichy, France (n = 56), and the Department of Pathology, McGill University, Montreal, Canada (n = 10). All patients had a potentially curative resection performed during the period 1976-91. The follow up period ranged from 0.4 to 123 months, with a mean of 26 months. Minimum follow up was at least 12 months for patients operated on in 1991 and who were still alive at the time of this study (n = 3), and 24 months for patients operated on in 1990 (n = 4). The tumour was regarded as having developed in Barrett's oesophagus when Barrett's mucosal changes were observed between the tumour and either the squamous oesophageal mucosa, or the gastric mucosa.¹⁴

Tissues had been fixed in 100% formol saline (n = 46) or Bouin's fixative (n = 20) and embedded in paraffin wax. Blocks of the main tumour and of non-dysplastic mucosa from around the tumour were stained in each case with a polyclonal antibody directed against the intracytoplasmic domain of the *c-erbB-2* oncoprotein (A485, Dakopatts, Glostrup, Denmark). In 30 cases areas of epithelial dysplasia, mostly high grade, were present adjacent to the carcinoma. Dewaxed sections were incubated with A485 at a dilution of 1 in 200 in TRIS-buffered saline for 40 minutes. Endogenous peroxidase was not inhibited. A standard avidin-biotin-peroxidase visualisation method was used. Haematoxylin was used to counterstain nuclei lightly. Negative control slides were prepared in each case by omitting the primary antiserum. The inclusion in each run of a known positive case of intraductal breast

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carcinoma was used as a positive control. Results of *c-erbB-2* immunohistochemical staining were reported as positive only when membrane staining was present.

The proliferative activity of tumour cells was assessed immunohistochemically on paraffin wax sections of formalin fixed speci-

Figure 1 *c-erbB-2* positive Barrett's adenocarcinoma with immunoreactivity localised on basolateral membranes of tumour cells.

Figure 2 Heterogeneity of *c-erbB-2* protein expression with contiguous positive and negative areas.

mens ($n = 46$) using the MIB-1 antibody (Immunotech, Marseille, France), specific for the Ki-67 antigen.¹⁵ An enhancement method based on microwave overheating of tissue sections was used, according to Shi *et al.*¹⁶ Sections were counted at high power ($\times 400$) using an eyepiece graticule, with at least 1000 tumour cell nuclei counted in each case.

The medical history of each patient was obtained, including alcohol and tobacco consumption. The following tumour variables were recorded: tumour differentiation, degree of infiltration, evidence of vascular invasion, presence of lymph node metastases, TNM stage according to the International Union Against Cancer (UICC),¹⁷ and tumour type in Lauren's classification of gastric adenocarcinomas.¹⁸ Lauren's classification divides carcinoma of the stomach into intestinal and diffuse types. The intestinal type resembles cancers of the large intestine and is usually associated with extensive intestinal metaplasia of the mucosa. The diffuse type is composed of single tumour cells or small abortive glands that diffusely infiltrate the gastric wall.

Statistical analysis of the results was performed using χ^2 test with Yates's correction when necessary, Student's *t* test, the Kaplan Meier method, and the logrank test. The level of significance was 0.05.

Results

Membrane staining for *c-erbB-2* protein was present in seven (11%) tumours. No cytoplasmic staining was observed. The rate of *c-erbB-2* positive tumours was not influenced by the type of fixative, with three positive cases among 20 tumours fixed in Bouin's solution and four positive cases among 46 formalin fixed tumours (not significant). The staining was localised to the basolateral membranes of the tumour cells (fig 1). In all positive cases the *c-erbB-2* staining was limited to one or several tumour parts, with all malignant cells positive in those areas; the rest of the tumour was unstained (fig 2). In *c-erbB-2* positive cases with a distinct differentiation pattern the staining was limited to the better differentiated areas. In 30 cases with dysplastic areas around the edge of the tumour, there was no staining of dysplastic glands, even in three cases in which the adjacent adenocarcinoma was *c-erbB-2* positive. The non-dysplastic Barrett's mucosa was negative in all cases.

Clinicopathological characteristics of *c-erbB-2* positive and negative cases are summarised in the table. There was no clinical difference between the *c-erbB-2* positive and negative cases, regarding mean age, sex ratio, percentage of alcohol misusers and percentage of smokers. Tumour differentiation and invasion (in the different layers, in lymph nodes, in vessels, and using the TNM staging system) were similar in the two groups. All the *c-erbB-2* positive cases were of Lauren's intestinal type, compared with 61% of the negative cases. This difference did not reach significance ($p = 0.12$). Among the formalin

fixed tumours, the MIB-1 labelling index was similar in the four *c-erbB-2* positive cases and in the 42 *c-erbB-2* negative cases (table). Moreover, when considering only *c-erbB-2* positive cases, the MIB-1 labelling index was similar in *c-erbB-2* positive and negative areas (data not shown).

Tumours with positive *c-erbB2* staining had a poorer outcome at one (29%), two (14%), and five years (0%), compared, respectively, with 63%, 40%, and 33% in the *c-erbB-2* negative group ($p < 0.05$) (fig 3).

Percentage of clinicopathological features and *c-erbB-2* staining in 66 cases of Barrett's adenocarcinoma

	<i>c-erbB-2</i> - (n = 59)	<i>c-erbB-2</i> + (n = 7)	p Value
Clinical features			
Age years (mean (SD))	64.2(12.3)	58.5(13.7)	p = 0.26
Sex (male %)	93	100	p = 0.89
Alcohol consumption	45	67	p = 0.53
Tobacco consumption	60	83	p = 0.48
Pathological features			
Differentiation			p = 0.67
Well	39	43	
Moderate	27	43	
Poor	34	14	
Extension			p = 0.76
Mucosa	15	0	
Submucosa	13	14	
Muscularis propria	17	29	
Adventitia	55	57	
Superficial*	27	14	p = 0.78
Invasive	73	86	
Lymph node metastases			p = 0.89
Absent	48	43	
Present	52	57	
Vascular invasion			p = 0.96
Absent	36	43	
Present	63	57	
pTNM stage			p = 0.73
I	27	14	
IIA	20	29	
IIB	8	14	
III	39	14	
IV	6	29	
Lauren's type			p = 0.12
Intestinal	61	100	
Diffuse	22	0	
Mixed or unclassified	17	0	
MIB-1 (Ki-67) labelling index† (mean (SD))	72.3 (13)%	78.7 (3)%	p = 0.34

*Infiltration limited to the mucosa and submucosa.
†Only performed in 46 formalin fixed cases.

Figure 3 Survival of patients with *c-erbB-2* positive Barrett's adenocarcinoma (n = 7) v patients with *c-erbB-2* negative Barrett's adenocarcinoma (n = 59)

Discussion

In this study we have shown, by immunohistochemistry, an increased *c-erbB-2* protein expression in 11% (7/66) of Barrett's adenocarcinomas. In two published studies of *c-erbB-2* protein expression in adenocarcinomas of the oesophagus 11 of 15 (73%) and six of 10 (60%) adenocarcinomas overexpressed the *c-erbB-2* protein, a figure much higher than ours.^{19 20} In the present series dysplastic (n = 30) and non-dysplastic (n = 66) Barrett's mucosa around the tumour were *c-erbB-2* negative. This result compares well with the features observed in gastric mucosa around gastric cancers^{4 9 11 21 22} and in normal adult tissues,²³ but again differs from those observed by Jankowski *et al* in patients with Barrett's oesophagus.¹⁹ In this latter study Barrett's mucosa and gastric mucosa were *c-erbB-2* positive, in nine (60%) and two (13%) respectively, of 15 patients with non-malignant Barrett's oesophagus. This positivity, reported as mild or weak, could be explained by the different material used in the studies—that is, frozen specimens of Jankowski *et al*¹⁹ and Al-Kasspooles *et al*,²⁰ paraffin wax rather than embedded material used in the present series. Such weak positivity could not be detected by immunohistochemistry applied to paraffin wax embedded tissues, which may only permit the detection of abnormal overexpression of *c-erbB-2* protein.²⁴ Moreover, the external domain of *c-erbB-2* protein may be more accessible to staining in paraffin wax embedded tissues than intracellular components, which may be another explanation for the low rate of *c-erbB-2* positive cases that we observed in our study.

The prevalence of *c-erbB-2* positivity that we observed in cases of Barrett's adenocarcinoma (11%) was similar to that found in gastric adenocarcinoma with 11% (10/93) and 9% (8/87) *c-erbB-2* positive gastric cancers in two recent series that used immunohistochemistry on paraffin wax embedded material.^{8 10} In these two studies *c-erbB-2* positive adenocarcinomas were all of the intestinal type. In our study we applied Lauren's classification¹⁸ of gastric adenocarcinoma to Barrett's adenocarcinoma, and again we found that only intestinal type cancers were *c-erbB-2* positive. *c-erbB-2* positive tumours seem to form a subset of intestinal type tumours, both in the stomach and in Barrett's oesophagus. However, only a few intestinal type tumours overexpress *c-erbB-2*. In gastric carcinoma this percentage is greater in high risk populations (40% in Japan)²⁵ than in low risk populations (19% in the United Kingdom).⁸ This latter result compares with the level that we observed in the present series of Barrett's adenocarcinoma (16%). These interpopulation variations of *c-erbB-2* overexpression favour the hypothesis that *c-erbB-2* positive gastric carcinomas are a subgroup of intestinal type tumours whose aetiology is environmental.⁸ It has been suggested that Barrett's carcinoma has aetiological and pathogenic factors in common with gastric

cancer—notably with cancer of the cardia.²⁶ It could therefore be of interest to know whether *c-erbB-2* positive gastric and oesophageal cancers share common environmental pathogenetic factors, but larger prospective studies are needed to answer this question.

The prognostic value of *c-erbB-2* protein overexpression has been diversely appreciated in gastric adenocarcinoma. Jain *et al*⁸ have suggested that *c-erbB-2* overexpression was associated with better prognosis, while in a larger study, Yonemura *et al* found that overexpression was associated with a worse prognosis,⁹ as in breast adenocarcinoma.⁷ Recently, two groups did not find any prognostic value for *c-erbB-2* protein overexpression^{11,27}; on the other hand, in a study of 220 early gastric cancers Yonemura *et al* found that positive *c-erbB-2* staining was associated with lymph node metastasis.²⁸ A complete confirmation of prognostic value will require a greater number of gastric cancer cases to be studied. In this study the overexpression of *c-erbB-2* protein by Barrett's adenocarcinoma seemed to correlate with lower survival. *c-erbB-2* positivity, moreover, was related neither to the depth of tumour invasion, nor to the presence of lymph node metastases, two prognostic markers of Barrett's adenocarcinoma,¹² suggesting that it may have an independent prognostic value. This independent prognostic value can only be demonstrated by multivariate analysis involving a large number of patients and avoiding confounding variables, such as later presentation of patients with *c-erbB-2* positive tumours. Tumours which overexpress *c-erbB-2* may be associated with a higher degree of proliferation than *c-erbB-2* negative tumours.²⁹ This hypothesis has been verified in breast adenocarcinoma using flow cytometry.³⁰ But the results that we have obtained regarding the Ki-67 labelling index do not support this hypothesis in Barrett's adenocarcinoma.

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