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HER2 (*c-erb*B-2) oncoprotein expression in colorectal adenocarcinoma: an immunohistological study using three different antibodies

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Abstract

Paraffin wax sections of 70 surgically resected colorectal adenocarcinomas were examined for the overexpression of HER2/*c-erb*B-2 oncoprotein using three different specific antibodies and the avidin-biotin immunoperoxidase technique. The patients included 38 men and 32 women aged between 47 and 80 years. The tumours were derived from various parts of the large intestinal tract, and represented all three stages of Dukes' classification and the three histological grades of differentiation. Many tumour sections also included adjacent normal or transitional mucosa. Eight tubular adenomas found in the colectomy specimens in association with some carcinomas were also examined. No positive membrane staining was seen in any of the 70 carcinomas, four adenomas, two hyperplastic polyps, nor in the adjacent normal or transitional mucosa.

It is suggested that the overexpression of *c-erb*B-2 gene product is unlikely to be as common and as pronounced in colorectal adenocarcinoma as it is in ductal carcinoma of the breast.

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Studies of the overexpression of HER2 (*c-erb*B-2) oncogene and its protein product ($p185^{HER2}$) in colorectal carcinoma conflict with a reported prevalence varying from 0-30%.[1-5] This immunohistological study aimed at investigating the prevalence of $p185^{HER2}$ overexpression in a series of colorectal tumours of various stages and degrees of differentiation, and from various parts of the large intestine, using three different specific antibodies to HER2 (*c-erb*B-2) oncoprotein.

Methods

Paraffin wax sections of 70 surgically resected colorectal adenocarcinomas were examined using the avidin-biotin immunoperoxidase technique and three different specific antibodies. The first was the monoclonal antibody 4D5 (Genentech, San Francisco, California, USA), raised against the extra-cellular domain of the HER2 gene product[6] the second was the polyclonal antibody 21N, which was raised to a synthetic peptide from the predicted sequence of the gene product. The third antibody was the polyclonal antibody HER 14 (Genentech) which was also raised to a synthetic peptide derived from the 14 c-terminal residues of $p185^{HER2}$. Many of the sections examined contained normal or transitional mucosa as well as the tumour. Four tubular adenomas and two hyperplastic polyps discovered in the colectomy specimens were also included in the study. A case of *c-erb*B-2 positive breast carcinoma was used as a positive control.

Sections were dewaxed in xylene and rehydrated in graded alcohol. They were then incubated for 30 minutes with either 10% normal swine serum in TRIS-buffered saline (TBS), for sections intended to be stained with the polyclonal antibodies, or with 10% normal rabbit serum in TBS for sections intended to be stained with the monoclonal antibody 4D5. This was followed by overnight incubation with the specific antibodies at 4°C. The concentrations used were 1:50 for 21N, 1:100 for 4D5 and 1:500 for HER 14. On the following day sections were rinsed with TBS and then incubated for 30 minutes with a 1 in 250 dilution of biotinylated swine anti-rabbit immunoglobulin (Dako UK) for sections treated with 21N, or biotinylated rabbit anti-mouse immunoglobulin (Dako UK) for sections treated with 4D5. After rinsing with TBS, sections were incubated for 60 minutes with avidin-biotin complex HRP (Dako UK), rinsed, then incubated for six minutes with diaminobenzidine (Sigma UK), counterstained with Harris's haematoxylin, dehydrated in graded alcohol, and mounted with ralmount (BDH UK).

A case was considered positive only when dark brown cell membrane staining was obtained. As generally accepted, cytoplasmic staining alone was not enough to consider the case positive.[4 7]

Results

The patients included 38 men and 32 women, ranging in age from 47 to 80 years (mean 62 years). The sites of the tumours are shown in the table. Five tumours were well differentiated, 49 were moderately, and 16 were poorly differentiated. Four tumours were Dukes' stage A, 34 were B, and 32 were C.

No positive cell membrane staining was made by any of the antibodies in any of the 70

Sites of tumours

Site	*Number of cases*
Caecum	11
Ascending colon	10
Transverse colon	3
Descending colon	6
Sigmoid colon	14
Rectum	26
Total	70

tumours or their surrounding transitional or normal mucosa, or in the four tubular adenomas or two hyperplastic polyps examined. Cytoplasmic staining of tumour cells, which most authors do not consider as enough evidence for the amplification of the oncogene,[4 7] was seen in cases stained with the polyclonal antibodies. With 21N, this cytoplasmic staining was faint and diffuse, and was seen in only five cases. With HER 14, the staining was focal, granular, and of a variable intensity, and was seen in 24 cases. Similar granular cytoplasmic staining was seen with the latter antibody in stromal and normal epithelial cells of many of the cases. There was no cytoplasmic staining with 4D5.

Discussion

The absence of immunohistologically demonstrable HER2 (*c-erb*B-2) oncoprotein in all the tumours examined in this study is more in line with the findings of McCann *et al*,[4] who were able to show the presence of the protein, using 21N antibody, in paraffin wax sections of only one out of 23 (4%) colorectal carcinomas. However, the findings are different from those of D'Emilia *et al*,[2] who found the oncoprotein also using the 21N antibody, in paraffin wax sections of eight out of 40 (20%) colonic carcinomas, and in 29 out of 37 (78%) colonic adenomas. Our findings are also different from those of Natali *et al*,[5] who found the protein, using the monoclonal antibody 4D5, in frozen sections of 10 out of 34 (29%) colorectal adenocarcinoma.

It is unlikely that the discrepancies in the results are due to differences in the sensitivity of the antibodies or the use of paraffin wax rather than frozen sections. We used three different antibodies and all gave, essentially, the same result; one of the antibodies used was the 21N which D'Emilia *et al*[2] used on paraffin wax sections and reported a 20% positive rate in their cases. This antibody, in particular, has been repeatedly and successfully used for the demonstration of the oncoprotein in paraffin wax sections of breast carcinoma.[7] It is also unlikely that the discrepancies are due to differences in the way the tumours were handled postoperatively, as $p185^{HER2}$ has been found to be fairly stable in the tumours up to 24 hours after their resection.[8] Although there may be inherent differences in the tumours selected for study by different investigators, it seems more likely that the discrepancies are due to differences in the interpretation of the results. Cytoplasmic staining, diffuse or localised to the Golgi region of tumour cells, was seen in some of our cases; but in accordance with other investigators[4 7] this, in the absence of cell membrane staining, was not considered sufficient to qualify a case as positive. This interpretation of the staining results is thought to reflect more accurately HER2 oncogene amplification within the tumour cells.[9] In this respect it may be worth mentioning that in three published studies only a total of four out of 149 (2·7%) colorectal adenocarcinoma examined in all three studies showed *c-erb*B-2 oncogene amplification (0/31,[1] 2/69,[3] and 2/49[10]); an overall prevalence which is much closer to ours and McCann's[4] than the much higher prevalence reported by D'Emilia[2] and Natali.[5]

Immunohostologically demonstrable cell membrane staining for HER2 (*c-erb*B-2) oncoprotein seems to be extremely uncommon in colorectal adenocarcinoma, thus ruling out the possibility of its general use as a prognostic marker for colorectal carcinoma as has been suggested for breast and ovarian carcinoma.

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