

Oestrogen receptors in colorectal carcinoma

P M Dawson, S Shousha, S D Blair, G D Carter, J Jones, J Alagband-Zadeh, N A Theodorou

Abstract

The oestrogen receptor content of colorectal adenocarcinoma was investigated using an established ligand binding biochemical assay and two more recently introduced techniques using specific monoclonal antibodies (Abbott ER-EIA and ER-ICA assay kits). Twenty nine tumours were investigated by the ligand binding assay. Only one (3.4%) tumour gave a weakly positive result (11 fmol/mg cytosol protein); the rest were all negative. Where sufficient tissue was available, the receptors were also determined by a quantitative immunoassay in 18 patients and an immunohistochemical method in 13 patients. The results were similarly all negative.

It is concluded that most colorectal carcinomas, irrespective of sex, are oestrogen receptor negative, and it is thus unlikely that hormonal manipulation would have an influence on the course of the disease.

The association between breast cancer and other extra-mammary neoplasms, particularly colorectal carcinoma, is well established.¹ This, coupled with the presence of some similar epidemiological features in the two tumours, suggest that there may be a common aetiological factor.² In this context some investigators have recently reported the presence of oestrogen receptors in up to 30% of colorectal carcinomas,^{3,4} and the possibility that these tumours may be influenced by appropriate endocrine manipulation has been raised.⁵ The scientific literature, however, also contains reports of studies which failed to show the presence of oestrogen receptors in colorectal cancer.^{6,7}

This discrepancy, together with the recent introduction of monoclonal antibodies that can be used for assessing the oestrogen receptor content of tumours by quantitative enzyme immunoassay, as well as immunohistological staining of tissue sections, has prompted us to investigate the presence of oestrogen receptors in 29 colorectal carcinomas using a standard ligand binding assay and to validate the results using these other techniques.

Methods

Tumour tissue from 29 patients with adenocarcinoma of the colon and rectum was studied (12 men, 17 women, mean age 66.2 (SD 12.8)

years). Two patients (one male, one female) only had recurrent tumour tissue available for study. A representative piece of tumour was selected by PMD immediately after removal of the specimen at operation. The tissue was immediately immersed in liquid nitrogen and the receptor content of each tumour measured by a ligand binding assay. Where sufficient tissue was available, receptors were also determined by a quantitative enzyme immunoassay and an immunohistochemical technique (see below).

LIGAND BINDING ASSAY

Oestrogen and progesterone receptors were measured by a method conforming to the guidelines recommended by the EORTC Breast Cancer Cooperative Group.⁸ Briefly, biopsy specimens were pulverised frozen by a microdismembrator (Braun Instruments; FT Scientific Instruments Ltd, Brendan, Gloucestershire). A crude cytosol was prepared by mixing the tissue powder with phosphate buffer (0.01 M NaH₂PO₄, 1.5 mM EDTA, 3.0 mM NaN₃, 10 mM monothioglycerol, 30% v/v glycerol, pH 7.4; tissue weight:buffer volume = 1:8) and centrifuging at 2000 × *g* for 20 minutes. Two hundred microlitre aliquots of crude cytosol were incubated with seven different concentrations of ³H-E₂ (oestrogen receptors) or ³H-Org 2058 (progesterone receptors) ranging between 0.5 and 5 × 10⁻⁹ M. Non-specific binding was determined by incubating with a 200-fold excess of diethylstilboestrol (oestrogen receptors) or Org-2058 (progesterone receptors).

After incubation overnight at 4°C unbound steroid was removed with dextran coated charcoal (final concentrations, 0.25% Norit A and 0.025% dextran T70). Scatchard plot analysis¹⁰ was performed to calculate the concentration of receptor binding sites (fmol/mg protein) and the equilibrium dissociation constant (K_d). The lower limit of sensitivity of this method is about 10 fmol/mg.

QUANTITATIVE ENZYME IMMUNOASSAY

Eighteen specimens were examined by this method using the Abbot ER-EIA monoclonal antibody kit for the quantitative measurement of oestrogen receptor content. In this method beads coated with rat monoclonal anti-oestrogen receptor are incubated with specimens (tissue cytosols prepared as above), or the appropriate standards, or control. During incubation, oestrogen receptors bind to the solid phase. Unbound materials are removed by aspiration and washing. Bound

Charing Cross
Hospital, London W6
Department of
Gastrointestinal
Surgery

P M Dawson
S D Blair
N A Theodorou

Department of
Histopathology
S Shousha

Department of
Chemical Pathology
G D Carter
J Jones
J Alagband-Zadeh

Correspondence to:
Mr P M Dawson,
Department of
Gastrointestinal Surgery,
Charing Cross Hospital,
Fulham Palace Road,
London W6 8RF, England.

Accepted for publication
13 September 1989

Table 1 Sites of tumour, operations performed, and Dukes' staging and differentiation in 29 patients with colorectal cancer

Site	No (%)	Operation	No (%)
Caecum	5 (17.2)	Right hemicolectomy	10 (34.5)
Ascending	3 (10.3)	Left hemicolectomy	3 (10.3)
Transverse	3 (10.3)	Sigmoid colectomy	8 (27.6)
Descending	2 (6.9)	Anterior resection	6 (20.7)
Sigmoid	9 (31.0)	Anterioresection	2 (6.9)
Rectum	7 (24.1)		
Total	29		29

Dukes	No (%)	Differentiation	No (%)
A	3 (10.3)	Well	1 (3.4)
B	12 (41.4)	Moderate	20 (68.9)
C	10 (34.5)	Poor	8 (27.6)
D	4 (13.8)		
Total	29		29

oestrogen receptor is labelled by incubating with a second antibody conjugated with horse-radish peroxidase. After washing, the beads are incubated with enzyme substrate solution (H_2O_2 and ortho-Phenylenediamine-HCl) to develop a colour which is a measure of bound oestrogen receptor conjugate. The intensity of the colour is read using a spectrophotometer set at 492 nm. A standard curve is obtained plotting the oestrogen receptor concentration of the standard *v* the absorbance. The oestrogen receptor concentrations of the specimens and the controls can be determined by interpolation.

IMMUNOHISTOLOGY

Thirteen cases were examined by this method using the Abbott ER-ICA monoclonal antibody kit. Frozen sections, 5 μ m thick, from tumour tissue which had been stored in liquid nitrogen were thaw mounted on to slides. Sections were immediately fixed in formaldehyde-phosphate buffered saline for 10 minutes, rinsed in phosphate buffered saline, followed by further fixation in cold methanol for five minutes and cold acetone for three minutes.

Table 2 Results of three methods of receptor analysis in 29 patients with colorectal cancer

Case No	Ligand binding		Quantitative enzyme immunoassay oestrogen receptor fmol/mg	Immunohistology Oestrogen receptor
	Oestrogen receptors (fmol/mg)	Progesterone receptors (fmol/mg)		
1	<10	<10	4	Negative
2	<10	<10	4	Negative
3	<10	<10	2	Negative
4	<10	<10	2	
5	<10	<10	7	Negative
6	<10	<10	3	
7	<10	<10	2	Negative
8	<10	<10	2	
9	<10	<10	2	
10	<10	<10	2	Negative
11	<10	<10	2	Negative
12	<10	<10	2	Negative
13	<10	<10	6	Negative
14	<10	<10	3	Negative
15	<10	<10	4	Negative
16	<10	<10	6	
17	<10	<10	4	Negative
18	<10	<10	4	Negative
19	<10	<10		
20	<10	<10		
21	<10	<10		
22	11	116 (low affinity)		
23	<10	<10		
24	<10	<10		
25	<10	<10		
26	<10	<10		
27	<10	<10		
28	<10	<10		
29	<10	<10		

The sections were then washed thoroughly in phosphate buffered saline. Sections were then incubated in a moist chamber for 15 minutes with the kit's blocking reagent. This was drained and the primary antibody applied for 30 minutes, followed by 30 minutes' incubation in bridging antibody, then 30 minutes' incubation in peroxidase-antiperoxidase (HRP) complex. The sections were washed thoroughly in phosphate buffered saline after each antibody incubation. The reaction was developed by incubating the sections in the chromogen substrate solution for six minutes. They were then washed in distilled water and counterstained briefly with alum haematoxylin. Positive controls included slides of known positive cells supplied with the kit and treated identically. Negative controls were tumour sections, treated as described, except for substituting the primary antibody for control antibody, also supplied with the kit. The nuclei of oestrogen receptor positive cells stained dark brown, while those of negative cells stained blue with the haematoxylin.

Results

The sites of carcinoma, operations performed, Dukes' staging and differentiation are shown in table 1. The results of the three receptor assays are shown in table 2. Of the 29 tumours examined by the ligand binding assay, only one patient (3.4%) had detectable oestrogen receptor content (11 fmol/mg cytosol protein). This tumour was also rich in low affinity progesterone receptors (116 fmol/mg cytosol protein). The lesion was a recurrent caecal carcinoma in a 43 year old woman which had invaded the urogenital tracts. None of the remaining primary tumour specimens showed specific high affinity oestrogen or progesterone receptors by the ligand binding technique. All 18 cases examined with the solid phase enzyme immunoassay and all 13 cases examined with the immunohistological technique were oestrogen receptor negative (table 2). These cases did not include the weakly positive case detected by the ligand binding assay.

Discussion

Our findings strongly suggest that most colorectal adenocarcinomas are oestrogen receptor negative, irrespective of the patient's sex, site of tumour or its degree of differentiation. The very low oestrogen receptor content detected in the only positive case in this study is probably of no practical importance. Of the 29 cases examined, 18 were studied with two different techniques, and 13 by three different techniques. All methods gave identical results. They are all established methods in which well characterised reagents and appropriate controls are used, and which are successfully used for the determination of oestrogen receptor content in breast tumours.

These results are consistent with those of some published reports^{6,7} but not with others.^{3,4} The cause of this discrepancy is not obvious. It has been suggested that the surgical technique and the time interval during which the tumour

is subjected to ischaemia before it is immersed in liquid nitrogen may influence the results of receptor assays.¹⁰ In this study the tumours were immersed in liquid nitrogen as soon as they were removed, in exactly the same way as breast tumours are dealt with in this hospital, without any deleterious effect on their oestrogen receptor content. It is therefore unlikely that the brief period of ischaemia to which the tumours are inevitably subjected would have any clinically important effect on oestrogen receptor concentrations unless these were extremely low to start with.

Another possible source for the discrepancies noted in published oestrogen receptor assay results could be the histological types of tumours examined. In the breast associations have been found between certain histological types of carcinoma and their oestrogen receptor content.^{11 12} All our colorectal tumours were of the usual adenocarcinoma type. None was of the much less common pure mucinous or of the rare signet ring adenocarcinoma type, and the oestrogen receptor content of these uncommon varieties may therefore be different.

Another possibility is that the specific epitopes recognised by the monoclonal antibodies used in two of the assays are not present in the oestrogen receptor of colorectal carcinomas as these monoclonal antibodies are produced specifically for the detection of oestrogen receptor in breast and female genital tract tissue. This seems unlikely as the same two monoclonal antibodies have been reported to be used for the demonstration of oestrogen receptor content in gastric adenocarcinoma.¹³ The ligand binding assay has also been used successfully for the demonstration of oestrogen receptor in other tissues, such as stomach¹³ and prostate,¹⁴ as well as in one of our colonic tumours (case 22).

It is concluded that most colorectal adenocarcinomas of the usual type, in both sexes, are

oestrogen receptor negative, although an occasional case may show unimportant concentrations of the receptor protein. It therefore seems unlikely that endocrine manipulations would have an influence on the course of these tumours.

We thank Mrs Gillian C Miller for her valuable technical assistance. This study was partly funded by the Peel Medical Research Trust.

- 1 Herrmann JB. Breast cancer and associated extramammary malignant neoplasms. *Am J Surg* 1972;124:620-8.
- 2 Berg JW. Can nutrition explain the pattern of international epidemiology of hormone dependant cancers. *Cancer Res* 1975;35:3345-50.
- 3 Sica V, Nola E, Contieri E, et al. Estradiol and Progesterone receptors in malignant gastrointestinal tumours. *Cancer Res* 1984;44:4670-4.
- 4 Francavilla A, DiLeo LA, Conte PD. Nuclear and cytosolic estrogen receptors in human colonic carcinoma and in surrounding non-cancerous colonic tissue. *Gastroenterology* 1987;93:1301-6.
- 5 McClendon JE, Appleby D, Clandon DB, et al. Colonic neoplasms: tissue estrogen receptor and carcinoembryonic antigen. *Arch Surg* 1977;112:240-1.
- 6 Wobbes T, Beeks LVAM, Koenders AMJ. Estrogen and progesterone receptors in colonic cancer? *Dis Colon Rectum* 1984;27:591-2.
- 7 d'Istria M, Fasano G, Catnagno F, et al. Androgen and progesterone receptors in colonic and rectal cancers. *Dis Colon Rectum* 1986;29:263-5.
- 8 EORTC Breast Cancer Cooperative Group. Revision of the standards for the assessment of hormone receptors in human breast cancer. *Eur J Cancer* 1980;16:1513-5.
- 9 Scatchard G. The attraction of proteins for small molecules and ions. *Ann NY Acad Sci* 1949;51:660-72.
- 10 Rosenthal LJ. Discrepant oestrogen receptor protein levels according to surgical technique. *Am J Surg* 1979;138:680-1.
- 11 Silversward C, Gustafsson SA, Humla S, Nordenskjold B, Wallgren A, Wrang O. Oestrogen receptor concentrations in 269 cases of histologically classified human breast cancer. *Cancer* 1980;45:2001-5.
- 12 Shousa S, Backhouse CM, Alaghband-Zadeh J, Burn JI. Alveolar variant of invasive lobular carcinoma of the breast. A tumour rich in oestrogen receptors. *Am J Clin Pathol* 1986;85:1-5.
- 13 Yokozaki H, Takekura N, Takanashi A, et al. Estrogen receptors in gastric adenocarcinoma: a retrospective immunohistochemical analysis. *Virchows Arch (Pathol Anat)* 1988;413:297-302.
- 14 Emtage LA, Dunn PJS, Rowse AD. Androgen and oestrogen receptor status in benign and neoplastic prostate disease. Study of prevalence and influence on time to progression and survival in prostate cancer treated by hormonal manipulation. *Br J Urol* 1989;63:627-33.