CLINICAL RESEARCH

Sialomucins at resection margin and likelihood of recurrence in colorectal carcinoma

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Abstract

Oncogenic transformation of colonic epithelium is accompanied by changes in surface carbohydrate, notably an increased secretion of sialomucins at the expense of the normally predominant sulphomucins. In a multicentre prospective trial the correlation between the presence of sialomucins at the resection margin and the subsequent development of local recurrence was studied in 250 patients who had undergone "curative" resection for colorectal carcinoma with a mean follow up period of 14 months. Nineteen of 70 patients (27.1%) with a sialomucin predominant pattern at either resection margin developed local recurrence compared with 15 of 180 patients (8.3%) with a mixed or sulphomucin predominant pattern ($p < 0.01$). Increased sialomucin staining at the resection margins was associated with reduced survival in these patients ($p < 0.01$). At a mean of 14 months of follow up 153 patients (85%) were alive in the sulphomucin group and 53 patients (76%) were alive in the sialomucin group. Regression analysis predicted five year survivals of 32.8% and 18.9% for the sulphomucin and sialomucin groups respectively.

Abnormal mucus production at the resection margin in patients treated for colorectal carcinoma appears to identify those with a higher risk of local recurrence and reduced survival.

Introduction

Colorectal carcinoma ranks second only to carcinoma of the lung as the main cause of death from cancer in the Western World. Recent

five year survival figures are around 30-32%.^{1,2} Treatment failures are often due to local tumour recurrence, but currently available tumour markers are of limited value in detecting this at an early stage. The incidence of tumour recurrence varies from 12% to 34%,^{1,3-6} and three quarters of recurrences occur within two years of operation.^{3,4,7,8} Several mechanisms have been proposed to explain the development of local recurrence: inadequate excision of the primary tumour³; implantation of viable malignant cells at the suture line; or the continuing process of carcinogenesis in the remaining bowel.^{9,10}

Management of this disease should be improved by clearly defining the various risk factors associated with a poor prognosis. Broder classified tumours histologically on the degree of cell dedifferentiation and showed that this grading affected the outcome.¹¹ Dukes staged rectal cancer according to the degree of tumour spread, and again the prognosis correlated with the staging.¹² We have used the degree of tumour fixity and local tumour penetration for staging of colorectal carcinomas and to determine prognosis.^{13,14} Nevertheless, other indices are urgently required to identify early those patients who are at high risk of tumour recurrence after radical surgery.

Filipe¹⁵ used histochemical methods¹⁶ to detect abnormal mucus production around colonic tumours, notably an increase in sialomucins at the expense of the normally predominant sulphomucins. This abnormal mucus production was associated with a pre-malignant change of the bowel.¹⁵ The abnormal pattern may extend for a considerable distance from the tumour edge¹⁶ into morphologically normal mucosa, reaching the proximal or distal resection margin in 15% of resected specimens.^{17,18} Recent retrospective studies^{14,19} have suggested a relation between local tumour recurrence and the presence of excessive sialomucin at the resection margins. This paper reports the initial results of a prospective multicentre study designed to confirm or refute this relation.

Patients and methods

A prospective study of patients in eight centres undergoing "curative" resection of colorectal cancer was begun in January 1983. The centres were the Hammersmith Hospital, London; Bristol Royal Infirmary, Bristol; King Edward VII Hospital, Windsor; Heatherwood Hospital, Ascot; Royal Berkshire Hospital, Reading; St Peter's Hospital, Chertsey; Southmead Hospital, Bristol; and Wexham Park Hospital, Slough. Recruitment ended in December 1983 when 250 patients had been entered.

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After removal of the tumour bearing bowel small specimens were taken from each resection margin and fixed in 10% formaldehyde. Sections of 5 μm^3 were then stained with haematoxylin and eosin and high iron diamine-alcian blue at pH 2.5 for specific acid mucins.¹⁵ By this technique the normal mucosa consisting predominantly of sulphomucins is stained brown and the abnormal mucosa consisting predominantly of sialomucins is stained blue. Three patterns of staining were recognised at the resection margin: sialomucin predominant (overwhelmingly blue), mixed sialomucin-sulphomucin (both blue and brown), and sulphomucin predominant (brown). Only the sialomucin predominant pattern was considered definitely abnormal. All slides were studied independently by two surgeons and one pathologist and the staining patterns documented prospectively. Subsequently all patients were followed up in the outpatient clinic and investigated as appropriate on clinical suspicion of recurrent cancer. No patient was lost from follow up and none had adjuvant chemotherapy or radiotherapy before the onset of tumour recurrence.

The study attempted to correlate sialomucin staining at the resection margins with local tumour recurrence and cancer related death in 250 patients followed up for six to 30 months (mean 14 months). Statistical analyses were by χ^2 test and Fisher's exact probability test. Details of follow up, recurrence of tumour, and cause of death were recorded for all patients from each of the participating centres. Perioperative deaths and unrelated deaths were excluded. Life table survival was correlated with the absence or presence of sialomucin in the resection margin against time. Life table curves were subjected to regression analysis (Minitab) and predicted five year survival figures calculated.

Results

Of the 250 patients, 70 (28%) had a sialomucin predominant staining pattern at one or other colorectal resection margin (table I). This pattern was more common in association with Dukes's stage B tumours and less common with right sided tumours, but otherwise no correlation was found with stage, grade, or site of the carcinoma.

Within the short period of surveillance (mean 14 months) 54 patients (22%) developed recurrent carcinoma. Recurrence occurred at the anastomosis in six, in the vicinity of the original site of tumour (locoregional) in 12, and at distant sites in 20; a further 16 showed evidence of both local and distant disease (table II). Among the 34 patients with local recurrence there were nine whose original tumours had arisen in the right colon (that is, proximal to the splenic flexure), 13 with tumours of the left colon, and 12 with rectal tumours. The diagnosis of recurrent carcinoma rested on biopsy

in 26, on ultrasound scan of the liver in 11, on computed tomography of the pelvis in eight, and on clinical examination alone in the remaining nine patients. Nineteen patients were subjected to further laparotomy; five had recurrent tumours excised, two had metachronous colorectal cancers excised, and in the remainder no further resection was possible. Ten patients received radiotherapy and 25 no treatment. At the time of reporting, 44 of the 54 patients with recurrent carcinoma had died.

There was a highly significant correlation between the presence of the sialomucin predominant pattern at the resection margin and the development of early local recurrence (table II). Local recurrence refers to tumour recurrence at or in the vicinity of the operative site but excluding peritoneal or hepatic metastases. Nineteen of 70 patients (27.1%) with this histological pattern developed local recurrence as opposed to 15 of 180 patients (8.3%) with mixed or sulphomucin predominant ("normal") mucosa ($p<0.01$). Likewise, there was a clear correlation between the presence of the sialomucin predominant pattern at the resection margin and cancer related death (figure). There were 49 deaths (10.9%) related to either local or

Survival of patients with (○) and without (●) sialomucin at resection margin.

regional recurrence of disease. Twenty one (27.2%) occurred in the sialomucin group and 28 (13.7%) in the sulphomucin group ($p<0.02$; $\chi^2=6.246$). The figure shows the life table analysis for both groups. At a mean follow up of 14 months 153 patients (85%) in the sulphomucin group had survived while 53 (76%) in the sialomucin group had survived. Regression analysis predicted five year survivals of 32.8% in the sulphomucin group and 18.9% in the sialomucin group.

Discussion

This study showed a strong correlation between predominant sialomucin staining at one or other resection margin and the subsequent risk of tumour recurrence ($p<0.001$) and cancer related death ($p<0.01$). Thus staining the resection margins with high iron diamine-alcian blue is a reliable, predictive method that identifies patients with a poor prognosis.

It has been suggested that increased sialomucins reflect a transformation to fetal epithelium,²⁰ since in embryonic and early fetal life large bowel mucosa mainly secretes sialomucins. In addition, the altered mucus production has been shown to occur at an early stage in carcinogenesis in experimental tumour models.^{21, 22} Filipe showed that these mucus changes reflect an increase in sialic acids,¹⁵ though the role of sialic acid is not clear.

It is doubtful whether these mucin changes are tumour specific, as they have been reported in inflammatory bowel conditions in man²³ and after enteric bypass in rats.²⁴ Nor do we know whether these changes are primary or secondary phenomena in the multi-stage process of carcinogenesis. Nevertheless, the presence of abnormal mucus at the resection margin is associated with a higher incidence of local tumour recurrence and reduced survival. It is therefore a potentially important prognostic factor and one that is independent of the site, stage, or differentiation of the tumour. Abnormal mucin production in bowel remote from the tumour might reflect either a more "malignant" type of carcinoma or a continuing process of carcinogenesis in the remaining bowel after potential curative resection. Patients with abnormal staining at the resection margins may possibly be candidates for more aggressive treatment, such as subtotal colectomy or prophylactic adjuvant treatment. Lastly, staining with high iron diamine-alcian blue is a simple, practical tool of low cost and high sensitivity.

TABLE I—Correlation between sialomucin staining and tumour stage, grade, and site. Except where stated otherwise figures are numbers (percentages) of patients

	Sialomucin predominant (n=70)	Mixed or sulphomucin predominant (n=180)	p Value
Mean age in years (SD)	66.9 (11.8)	65.8 (10.4)	NS
Sex (M:F)	32:38	82:98	NS
Stage:			
A (n=13)	2 (3)	11 (6)	NS
B (n=153)	51 (73)	102 (57)	
C (n=84)	17 (24)	67 (37)	
Grade:			
Well (n=35)	11 (16)	24 (13)	NS
Moderate (n=173)	47 (67)	126 (70)	
Poor (n=25)	7 (10)	18 (10)	
Unspecified (n=17)	5 (7)	12 (7)	
Site:			
Right* (n=80)	14 (20)	66 (37)	NS
Left* (n=88)	30 (43)	58 (32)	
Rectum (n=82)	26 (37)	56 (31)	

* Right refers to caecum, ascending colon, hepatic flexure, and transverse colon; left refers to splenic flexure, descending colon, and sigmoid colon.

TABLE II—Mucus staining characteristics and local tumour recurrence in 34 patients. Figures are numbers (percentages) of patients

Tumour recurrence	Sialomucin (n=70)	Sulphomucin (n=180)
Local or regional (n=12)	4 (5.7)	8 (4.4)
Anastomotic (n=6)	5 (7.1)	1 (0.6)
Local plus metastases (n=16)	10 (14.3)	6 (3.3)
Total	19 (27.1)	15 (8.3)

($\chi^2=13.62$; $p<0.01$)

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Subacute sclerosing panencephalitis: detection of measles virus RNA in appendix lymphoid tissue before clinical signs

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Abstract

An appendix removed 15 days before onset of symptoms of subacute sclerosing panencephalitis was examined retrospectively for measles virus ribonucleic acid (RNA). Tissue sections hybridised in situ to a cloned measles virus probe of deoxyribonucleic acid specific for nucleocapsid protein showed that many cells of the lymphoid tissue contained measles virus RNA. In contrast, only a few infected lymphoid cells were detected in three out of six seropositive controls and none in three seronegative infants.

A widespread chronic viral infection of the immune system, established after measles, may promote or even initiate nerve cell infection in subacute sclerosing panencephalitis.

Introduction

The lymphotrophy of measles virus has recently been described in subacute sclerosing panencephalitis, a delayed neurological disease,

viral ribonucleic acid (RNA) being detected in nerve cells and in both circulating and brain perivascular lymphocytes.¹ We obtained an appendix sample from a patient in whom the first signs of subacute sclerosing panencephalitis appeared after the appendix operation. This afforded an opportunity to examine the interactions between measles virus and lymphoid tissue before the onset of the neurological disease. We report our results using in situ hybridisation and a measles virus specific probe¹ in an attempt to detect viral RNA sequences in the appendix tissue.

Subjects and methods

The patient, a 10 year old girl with a history of measles at the age of 2, underwent appendicectomy for abdominal pain. The appendix was fixed in Bouin's solution and conserved in paraffin. Fifteen days after operation she began to have myoclonic jerks and subacute sclerosing panencephalitis was diagnosed. She died three years after the first manifestation of the disease. Appendix tissue from six subjects seropositive for measles virus and three seronegative newborn infants was used as controls.

Tissue sections (10 µm) were deparaffinised in xylene and dehydrated. After incubation in lithium carbonate they were treated with 0.2M hydrochloric acid and proteinase K² 25 mg/l. The techniques of hybridisation, washings, and autoradiography after six to eight weeks of exposure have been described.¹ The reaction medium contained 0.2 mg probe per l, 200 mg salmon sperm deoxyribonucleic acid per l, and 1 g/l each of CV1 cell RNA, yeast RNA, and *Escherichia coli* RNA.

Results

In tissue taken from the patient before the onset of subacute sclerosing panencephalitis a large number of cells hybridised with the specific viral probe. In lymph follicles (fig (a)) measles virus RNA was found in numerous cells both in the mantle zone and in the germinal centre. Other cells showing hybridisation were present throughout the mucosa, and measles virus RNA was detected in the nuclei of Lieberkühn gland epithelial cells (fig (b)).

Occasional hybridising mononuclear cells were found in blood capillaries where the endothelial cell nuclei also reacted with the probe (fig (c)). Only a

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