ORIGINAL ARTICLE

Prognostic significance of mucins in colorectal cancer with different DNA mismatch-repair status

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Background: Expression of mucin antigen MUC1 and down regulation of MUC2 are associated with adverse prognosis in colorectal cancer (CRC), but their prognostic significance with respect to differing DNA mismatch repair (MMR) status is poorly understood.

Objective: To determine the prognostic significance of MUC1 and MUC2 in CRC with different MMR statuses.

Methods: Using the tissue microarray (TMA) technique, a series of 1420 unselected, non-consecutive CRC resections was subdivided into three groups: (1) MMR-proficient; (2) MLH1-negative; and (3) presumed hereditary non-polyposis colon cancer (HNPCC). Immunohistochemical analysis of MUC1 and MUC2 expression (>0%) and loss (0%) was performed, and the results were correlated with clinicopathological parameters.

Results: In MMR-proficient CRC, MUC1 expression was more frequently found in tumours with higher tumour stage ($p=0.004$) and higher tumour grade ($p=0.041$) and loss of MUC2 was associated with higher tumour stage ($p=0.028$), node stage ($p=0.001$), presence of vascular invasion ($p=0.028$) and worse survival ($p=0.034$). In MLH1-negative CRC, MUC2 loss was associated with the presence of lymph node metastasis ($p=0.028$) and worse survival ($p=0.015$), but there was no association between MUC1 expression and clinicopathological features. In presumed HNPCC, MUC1 expression and MUC2 loss were not associated with clinicopathological parameters.

Conclusions: Mucins have a prognostic significance in sporadic CRC, but not in hereditary CRC. Loss of MUC2 is an adverse prognostic factor in MMR-proficient and MLH1-negative CRC, whereas MUC1 expression is associated with tumour progression in MMR-proficient CRC only.

Mucins are membrane-associated or secretory high-molecular-weight glycoproteins expressed by epithelial tissues and characterised by variable nucleotide tandem repeat subdomains that provide sites for O-glycosylation.¹ Among the several mucin antigens, such as MUC1, MUC2, MUC3, MUC4, MUC5AC and MUC5B, which have analysed in relation to the adenoma–carcinoma sequence of the colorectum,^{2–4} MUC1 and MUC2 are the best characterised.

The MUC1 gene on chromosome 1q21–24 encodes a transmembrane glycoprotein^{5–6} that is widely distributed in glandular epithelia and most strongly expressed in the crypt base of the colonic mucosa.⁷ MUC1 is involved in cell adhesion, signal transduction, maintenance of cellular polarity and immune modulation.^{1–8–11} MUC1 expression has been found in colorectal adenomas and carcinomas, and a correlation with increasing dysplasia was detected in most of these studies.^{12–16} Increased MUC1 expression is correlated with higher incidence of lymph node and liver metastasis,^{17–19} tumour progression and worse prognosis.^{20–23}

The MUC2 gene is located on chromosome 11p15.5 and encodes a gel-forming mucin that is the main secretory mucin in the colorectum and specific to goblet cells.^{24–25} Down regulation of MUC2 was detected in non-mucinous carcinomas arising within adenomas, whereas increased MUC2 expression was mainly observed in villous adenomas and mucinous carcinomas.^{2–14–26} Although several studies have focused on the prognostic significance of mucins in colorectal cancer (CRC), only a few investigations have concentrated on their prognostic value in CRC stratified by microsatellite status. Serrated polyps show an upregulation of secretory mucins MUC2 and MUC5A,²⁷ and an increased MUC2 expression was detected in sporadic microsatellite instability-high (MSI-H) cancers of the colorectum.²⁸ These results suggest that serrated

polyps may represent precursors of MSI-H cancers and explain the overexpression of mucinous cancers among MSI-H colorectal cancers.^{27–28}

The aim of this study was to determine the prognostic significance of MUC1 and MUC2 expression in a large series of CRC ($n=1420$) stratified into mismatch-repair(MMR)-proficient, MLH1-negative and presumed hereditary non-polyposis colon cancer (HNPCC).

MATERIAL AND METHODS

Tissue microarray construction

A tissue microarray (TMA) of 1420 unselected, non-consecutive CRC was constructed as described previously.²⁹ Formalin-fixed, paraffin wax-embedded tissue blocks of CRC resections were retrieved from the archives of the Institute of Pathology (University Hospital of Basel, Basel, Switzerland) the Institute of Clinical Pathology (Basel, Switzerland) and the Institute of Pathology (Stadtspital Triemli, Zürich, Switzerland). Tissue cylinders, each with a diameter of 0.6 mm were punched from morphologically representative tissue areas of each donor tissue block and combined into one recipient paraffin wax block (3×2.5 cm) using a home-made semiautomated tissue arrayer. Failure of analysis was related to TMA technology, including a fraction of missing samples or those containing only a few tumour cells.

Clinicopathological data and tumours

All clinicopathological data on CRCs were systematically re-evaluated by one pathologist (LTe). CRCs were stratified as

Abbreviations: CRC, colorectal cancer; HNPCC, hereditary non-polyposis colon cancer; MMR, mismatch repair; MUC, mucin; MSI-H, microsatellite instability-high; TMA, tissue microarray

likely sporadic and microsatellite stable (expressing MLH1, MSH2 and MSH6), likely sporadic microsatellite instability-high (MLH1-deficient and >55 years of age) and probable HNPCC (loss of MSH2 and/or MSH6 at any age or loss of MLH1 at ≤55 years of age). These immunohistochemical groups showed a good fit with the known clinicopathological features associated with these subsets of CRC. In particular, the MLH1-negative group was associated with advanced age, predilection for female gender and proximal colon, large tumour size and poor differentiation. The presumed HNPCC group was young and showed no gender difference, and there was a predilection for the proximal colon compared with the MMR-proficient group. Although it is possible that a small proportion of presumed sporadic MSI-H and HNPCC cases were incorrectly assigned, the overall findings are likely to be valid in view of the large numbers of samples and the good fit with clinico-pathological features. Table 1 summarises the clinicopathological data of the different subsets of CRC. Any disagreement between the clinicopathological features and the numbers of available tissue punches shown in table 1 is due to missing clinicopathological data.

Normal colon tissue

To compare the MUC1 and MUC2 expression in CRC with that in normal colon mucosa, a control group of 57 tissue samples from a normal colon was included in the study.

Immunohistochemistry of TMA

Sections of TMA blocks 4 µm thick were transferred to an adhesive-coated slide system (Instrumedics, Hackensack, New Jersey, USA) to facilitate the transfer of TMA sections to slides and to minimise tissue loss. Standard indirect immunoperoxidase procedures were used for immunohistochemistry (ABC-Elite, Vector Laboratories, Burlingame, California, USA). A total of 1420 CRC and 57 normal colonic mucosa samples were immunostained for MUC1 (clone 139H2, dilution 1:100,

Monosan), MUC2 (clone Ccp58, dilution 1:100, Monosan, Cedarlane Laboratories, Hornby, Ontario, Canada), MLH1 (clone MLH-1; dilution 1:100; BD Biosciences Pharmingen), MSH2 (clone MSH-2; dilution 1:200; BD Biosciences Pharmingen) and MSH6 (clone 44; dilution 1:400; BD Biosciences Pharmingen). After dewaxing and rehydration in deionised water, sections for immunostaining were subjected to antigen retrieval by heating in a microwave oven (1200 W, 15 min) in 0.001 mol/l EDTA, pH 8.0, for MLH1 and MSH2 and in 0.01 mol/l citrate buffer, pH 6.0, for MSH6. Endogenous peroxidase activity was blocked using 0.5% H₂O₂. After transfer to a humidified chamber, the sections were incubated with 10% normal goat serum (Dako Cytomation, Mississauga, Canada) for 20 min and incubated with primary antibody at room temperature for 1 h (MUC1 and MUC2). Subsequently, the sections were incubated with peroxidase-labelled polymer (K4005, EnVision+System-HRP (AEC); DakoCytomation) for 30 min at room temperature. For visualisation of the antigen, the sections were immersed in 3-amino-9-ethylcarbazole+ substrate-chromogen (K4005, EnVision+System-HRP (AEC); DakoCytomation) for 30 min and counterstained lightly with Gill's haematoxylin.

Clone 139H2 reacted with MUC1, which was purified and deglycosylated before immunisation of Balb/c mice, but the dominant epitope of 139H2 has not yet been determined.

All cases were scored by one experienced pathologist (AL). Cases in which the interpretation of the immunohistochemical analysis was difficult were discussed with a second experienced pathologist (JRJ). Cytoplasmic and/or membranous MUC1 and MUC2 were scored as the number of positive tumour cells divided by the total number of tumour cells (0–100%). Staining intensity was scored as + = weak, ++ = moderate and +++ = strong. MUC1 and MUC2 expression was defined as >0% positive tumour cells and MUC1 and MUC2 loss as 0% positive tumour cells.

Table 1 Clinicopathological characteristics of 1420 patients with colorectal cancer

	Total (%)	MMR proficient (%)	MLH1 negative (%)	Presumed HNPCC (%)	p Value
Number	1420	1197 (84.3)	141 (9.9)	82 (5.8)	<0.001
Median (range) age (years)	71 (30, 96)	71 (30, 96)	76 (56, 93)	60 (37, 82)	<0.001
Gender	1414 (100)	1191 (100)	141 (100)	82 (100)	
Male	673 (47.6)	595 (50.0)	41 (29.1)	37 (45.1)	<0.001
Female	741 (52.4)	596 (50)	100 (70.9)	45 (54.9)	
Anatomical site of the tumour	1401 (100)	1178 (100)	141 (100)	82 (100)	
Right colon	489 (34.9)	344 (29.2)	112 (79.4)	33 (40.2)	<0.001
Left colon	430 (30.7)	383 (32.5)	24 (17)	23 (28.1)	
Rectum	482 (34.4)	451 (38.3)	5 (3.6)	26 (31.7)	
Median (range) tumour size	4.5 (0.4–17)	4.5 (0.4–15.0)	5.5 (1.5–17.0)	5.0 (2.5–13.0)	<0.001
T stage	1387 (100)	1165 (100)	140 (100)	82 (100)	
T1	62 (4.5)	60 (5.2)	0	2 (2.4)	<0.001
T2	203 (14.6)	189 (16.2)	6 (4.3)	8 (9.8)	
T3	899 (64.8)	740 (63.5)	97 (69.3)	62 (75.6)	
T4	223 (16.1)	176 (15.1)	37 (26.4)	10 (12.2)	
Node stage	1263 (100)	1142 (100)	139 (100)	82 (100)	
N0	711 (52.2)	587 (51.4)	79 (56.8)	45 (54.9)	0.593
N1	358 (26.3)	308 (27.0)	29 (20.9)	21 (25.6)	
N2	294 (21.6)	247 (21.6)	31 (22.3)	16 (19.5)	
Tumour grade	1385 (100)	1165 (100)	139 (100)	81 (100)	
G1	31 (2.2)	27 (2.3)	2 (1.4)	2 (2.5)	<0.001
G2	1177 (85)	1010 (86.7)	103 (74.1)	64 (79.0)	
G3	177 (12.8)	128 (11.0)	34 (24.5)	15 (18.5)	
Histological type	1420 (100)	1197 (100)	141 (100)	82 (100)	
Mucinous	119 (8.4)	89 (7.4)	19 (13.5)	11 (13.4)	0.012
Non-mucinous	1301 (91.6)	1108 (92.6)	122 (86.5)	71 (86.6)	
Vascular invasion	1385 (100)	1166 (100)	138 (100)	81 (100)	
No	1002 (72.4)	834 (71.5)	104 (75.4)	64 (79)	0.244
Yes	383 (27.6)	332 (28.5)	34 (24.6)	17 (21)	
Mean (SE) (months) 5-year survival,	31.90 (0.77)	31.87 (0.81)	28.40 (2.56)	20.39 (1.62)	<0.001

HNPCC, hereditary non-polyposis colon cancer; MMR, mismatch-repair.

Statistical analysis

Clinicopathological characteristics across CRC groups were analysed using the Kruskal–Wallis test for age and tumour size and by the χ^2 test for gender, anatomical site, T (tumour) stage, N (node) stage, grade and vascular invasion. MUC1 and MUC2 expression and staining intensity and their association with clinicopathological features were evaluated using the χ^2 test or Fisher's exact test where appropriate. Kaplan–Meier survival analysis and the log rank test were performed to identify differences in 5-year survival times between CRC groups and across groups by MUC1 and MUC2 expression. Values of $p < 0.05$ were considered significant. All analyses were carried out using SAS V.9.1.

RESULTS

Normal colonic mucosa

In normal colonic mucosa, MUC1 expression was found in 10% of cases, whereas MUC2 expression was detected in 100% of tissues.

Comparison of MUC1 and MUC2 expression with the DNA MMR status

Different frequencies of MUC1 expression occurred across the MMR-proficient, MLH1-negative and presumed HNPCC subsets (58.2%, 75.6% and 82.9%, respectively ($p < 0.001$)). There was no significant difference of MUC2 expression between the three CRC subsets (49.3%, 53.0% and 41.7%, respectively; $p = 0.3$).

MMR-proficient CRC

Table 2 shows the association of MUC1 and MUC2 and the clinicopathological parameters in MMR-proficient CRC. MUC1 expression was more frequently detected in tumours with higher T stage, particularly with T4 tumours ($p = 0.004$), and with higher tumour grade ($p = 0.041$). There was a small but significant increase in frequency of MUC1 expression among mucinous tumours ($p = 0.008$). There was no association between MUC1 expression and tumour site, N stage, vascular invasion and survival.

Loss of MUC2 was more frequently found in T2 and T3 tumours ($p = 0.028$) and was associated with higher N stage ($p = 0.001$). Loss of MUC2 was significantly associated with the presence of vascular invasion and with worse survival ($p = 0.028$ and $p = 0.034$, respectively). A significantly larger number of mucinous carcinomas was found to express MUC2, whereas non-mucinous tumours were more likely to show loss of MUC2 ($p < 0.001$). Right-sided CRCs were associated with MUC2 expression, whereas MUC2 loss was more frequently detected in left-sided colonic and rectal adenocarcinomas ($p = 0.005$).

MLH1-negative CRC

Table 3 shows the association of MUC1 and MUC2 and the clinicopathological parameters in MLH1-negative CRC. Loss of MUC2 was more frequently observed in stage N2 (19 cases of loss vs 9 cases of expression), whereas MUC2 expression was more likely to be found in stage N0 (42 vs 33 cases; $p = 0.028$). Additionally, MUC2 loss was associated with worse survival ($p = 0.015$ fig 1). As in MMR-proficient CRC, mucinous carcinomas were associated with MUC2 expression, whereas non-mucinous tumours were predominantly found to have loss of MUC2 expression ($p < 0.001$). In mucinous and in non-mucinous cancers, there were more cases with presence than with absence of MUC1 expression ($p = 0.044$). There was no further significant association between MUC1 expression and the clinicopathological parameters, although MUC1 expression showed a trend towards being a favourable feature in this subset. This may be explained by the association between MUC1 and mucinous histology and therefore coexpression of MUC1 and MUC2.

Presumed HNPCC

Table 4 shows the association of MUC1 and MUC2 and the clinicopathological parameters in presumed HNPCC. MUC2 expression was more frequently found in mucinous tumours (7 vs 2 cases), whereas the frequency of MUC2 loss was higher in non-mucinous CRC (40 vs 23 cases; $p = 0.019$). There was no

Table 2 Association of MUC1 and MUC2 and the clinicopathological parameters in mismatch-repair R-proficient colorectal cancer

	MUC1+, n (%)	MUC1–, n (%)	p Value	MUC2+, n (%)	MUC2–, n (%)	p Value
Site						
Right colon	180 (31.2)	108 (25.8)	0.181	167 (33.6)	124 (24.3)	0.005
Left colon	182 (31.5)	140 (33.5)		150 (30.2)	173 (33.9)	
Rectum	215 (37.3)	170 (40.7)		180 (36.2)	213 (41.8)	
Tumour stage			0.004			0.028
T1	30 (5.2)	24 (5.8)		35 (7.1)	15 (3.0)	
T2	81 (14.1)	81 (19.6)		78 (15.9)	86 (17)	
T3	359 (62.7)	264 (63.9)		306 (62.3)	330 (65.4)	
T4	103 (18)	44 (10.7)		72 (14.7)	74 (14.7)	
Node stage			0.75			0.001
N0	292 (52)	215 (53.4)		270 (56.6)	238 (47.5)	0.466
N1	150 (26.7)	110 (27.3)		123 (25.8)	143 (28.5)	
N2	120 (21.3)	78 (19.4)		84 (17.6)	120 (24)	
Grade			0.041			
G1	12 (2.1)	9 (2.2)		12 (2.4)	7 (1.4)	<0.001
G2	488 (85.2)	374 (90.1)		432 (87.8)	451 (89.1)	
G3	73 (12.8)	32 (7.7)		48 (9.8)	48 (9.5)	
Type			0.008			
Mucinous	52 (8.8)	19 (4.5)		53 (10.6)	11 (2.1)	0.028
Non-mucinous	536 (91.2)	402 (95.5)		449 (89.4)	506 (97.9)	
Vascular invasion			0.771			
Absence	417 (72.7)	298 (71.8)		368 (74.7)	346 (68.4)	0.034
Presence	157 (27.3)	117 (28.2)		125 (25.3)	160 (31.6)	
Mean (SE) 5-year survival	32.2 (1.3)	34.2 (1.3)	0.088	33.2 (1.3)	30.5 (1.2)	

Table 3 Association of MUC1 and MUC2 and the clinico-pathological parameters in MLH1-negative colorectal cancer

	MUC1+, n (%)	MUC1-, n (%)	p Value	MUC2+, n (%)	MUC2-, n (%)	p Value
Site			0.575			0.29
Right colon	78 (81.3)	23 (74.2)		58 (82.9)	47 (75.8)	
Left colon	14 (14.6)	7 (22.6)		11 (15.7)	11 (17.7)	
Rectum	4 (4.2)	1 (3.2)		1 (1.4)	4 (6.5)	
T stage			0.142			0.148
T1	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	
T2	4 (4.2)	2 (6.5)		5 (7.1)	1 (1.6)	
T3	70 (73.7)	17 (54.8)		51 (72.9)	42 (67.7)	
T4	21 (22.1)	12 (38.7)		14 (20.0)	19 (30.7)	
N stage			0.768			0.028
N0	54 (56.3)	17 (56.7)		42 (60.9)	33 (54.1)	
N1	21 (21.9)	5 (16.7)		18 (26.1)	9 (14.8)	
N2	21 (21.9)	8 (26.7)		9 (13.0)	19 (31.1)	
Grade			0.702			0.405
G1	1 (1.1)	1 (3.2)		2 (2.9)	0 (0.0)	
G2	69 (72.6)	22 (71.0)		50 (72.5)	45 (73.8)	
G3	25 (26.3)	8 (25.8)		17 (24.6)	16 (26.2)	
Type			0.044			<0.001
Mucinous	17 (17.7)	1 (3.2)		17 (24.3)	2 (3.2)	
Non-mucinous	79 (82.3)	30 (96.8)		53 (75.7)	60 (96.8)	
Vascular invasion			0.186			0.076
Absence	72 (76.6)	20 (64.5)		55 (80.9)	41 (67.2)	
Presence	22 (23.4)	11 (35.5)		13 (19.1)	20 (32.8)	
Mean (SE) 5-year survival	31.4 (3.2)	16.1 (2.1)	0.116	37.1 (3.9)	20.4 (2.6)	0.015

association between MUC1 and MUC2 expression/loss and the clinicopathological parameters.

Staining intensity

MUC1 and MUC2 staining intensity were not independently associated with the clinicopathological data.

DISCUSSION

The subdivision of a large series of CRC into MMR-proficient, MLH1-negative and presumed HNPCC groups allowed us to analyse the possible differential associations of MUC1 and MUC2 expression with respect to tumour progression and prognosis in CRC. Although the subdivision was based on the clinicopathological data and immunohistochemical analysis, there is no evidence that unequivocally abnormal immunohistochemistry for DNA mismatch repair proteins in CRC should be confirmed by microsatellite instability testing if optimised laboratory procedures and appropriate interpretation are guaranteed.³⁰

Our study shows that in MMR-proficient CRC, MUC1 expression and MUC2 loss are markers of tumour progression

and worse survival, whereas in MLH1-negative CRC only loss of MUC2 is an adverse prognostic factor. In MMR-proficient and MLH1-negative CRC, down regulation of MUC2 was associated with worse survival in a univariate analysis. In undertaking survival analysis, we acknowledge an important limitation of this study, specifically, the lack of information regarding metastasis (M) stage and use of adjuvant treatment. These variables, particularly M stage, would be expected to have a major influence on survival and should be included in multivariate analysis. In this study, therefore, we undertook only univariate analysis with the limited aim of establishing possible associations between survival and expression of MUC1 and MUC2, and possible differences across three subtypes of CRC.

MUC1 expression could be demonstrated in only 10% of normal colonic samples, whereas MUC2 expression was detected in all normal epithelial tissues. Additionally, both MUC1 and MUC2 expression occurred more frequently in mucinous tumours. MUC2 expression was more frequent in MLH1-negative CRC than in the other CRC subsets.

Our findings are in partial agreement with previous studies investigating MUC1 and MUC2 expression in colorectal mucosa and CRC. In normal colorectum MUC1 expression, has been detected by northern blotting and in situ hybridisation.⁴⁻³¹ Manne *et al*²² found MUC1 expression in 8.4% (14 of 166 cases)³² and Matsuda *et al* found MUC1 expression in 0% (0 of 86) of the colorectal mucosa by immunohistochemical analysis; in other studies the exact percentage was not stated.¹⁴⁻²⁸ MUC2 expression has also been detected in the majority of cases including normal colorectal mucosa.²²⁻³²

Several studies have shown that MUC1 expression is implicated in progression and metastasis of CRC.¹⁸⁻¹⁹⁻²¹⁻²³⁻³³ In a recent study on 243 CRCs, only MUC1 was an independent prognostic factor.²⁰ In another study, only MUC1 expression at the invasive tumour front was an independent adverse prognostic factor, whereas MUC1 overexpression was associated with worse survival in a univariate analysis.²¹

MUC2 down regulation has been associated with tumour progression through the adenoma-carcinoma sequence,²⁶ and MUC2 expression is more frequently associated with sporadic

Figure 1 Kaplan-Meier 5-year survival curve for MUC2 expression in MLH1-negative colorectal cancer.

Table 4 Association of MUC1 and MUC2 and the clinicopathological parameters in presumed hereditary nonpolyposis colon cancer

	MUC1+, n (%)	MUC1-, n (%)	p Value	MUC2+, n (%)	MUC2-, n (%)	p Value
Site			0.449			1.0
Right colon	6 (50.0)	21 (36.2)		12 (40.0)	16 (38.1)	
Left colon	4 (33.3)	17 (29.3)		8 (26.7)	12 (28.6)	
Rectum	2 (16.7)	20 (34.5)		10 (33.3)	14 (33.3)	
T stage			0.732			0.862
T1	2 (3.5)	0 (0.0)		0 (0.0)	1 (2.4)	
T2	5 (8.6)	2 (16.7)		3 (10.0)	4 (9.5)	
T3	43 (74.1)	9 (75.0)		23 (76.7)	32 (76.2)	
T4	8 (13.8)	1 (8.3)		4 (13.3)	5 (11.9)	
N stage			0.869			0.085
N0	32 (55.2)	6 (50.0)		21 (70.0)	19 (45.2)	
N1	15 (25.9)	4 (33.3)		4 (13.3)	14 (33.3)	
N2	11 (19.0)	2 (16.7)		5 (16.7)	9 (21.4)	
Grade			0.743			0.081
G1	2 (3.5)	0 (0.0)		0 (0.0)	2 (4.8)	
G2	43 (75.4)	10 (83.3)		20 (68.4)	35 (83.3)	
G3	12 (21.1)	2 (16.7)		9 (31.0)	5 (11.9)	
Type			0.531			0.019
Mucinous	6 (10.3)	2 (16.7)		7 (23.3)	2 (4.76)	
Non-mucinous	52 (90.0)	10 (83.3)		23 (76.7)	40 (95.2)	
Vascular invasion			0.18			0.375
Presence	42 (73.9)	11 (91.7)		24 (82.8)	31 (73.8)	
Absence	15 (26.3)	1 (8.3)		5 (17.2)	11 (26.2)	
Mean (SE) 5-year survival	19.83 (1.9)	(only 1 death)	–	19.88 (2.8)	21.1 (2.4)	0.357

CRC showing DNA MSI-H,^{25 28 34} suggesting that regulation of MUC2 is implicated in tumorigenesis in MMR-proficient and MLH1-negative CRC, but in different ways. MLH1-negative or sporadic MSI-H CRC has been associated with serrated polyps that show upregulation of both MUC2 and MUC5AC.²⁷ Additionally, the association of MUC2 expression with poor differentiation fits with the known tendency for MSI-H cancers to be poorly differentiated.^{28 35–38}

In a previous study, the relationship between MUC phenotypes (MUC2+/MUC1–, MUC2+/MUC1+, MUC2–/MUC1+ and MUC2–/MUC1–) and the pathological variables in 51 unselected CRCs were analysed.¹⁴ The pattern closest to normal MUC2+/MUC1– was more frequently associated with early tumour stages and the phenotype MUC2+/MUC1+ with mucinous cancers. These findings could be partially confirmed in this study. MUC2+/MUC1+ was more frequently found in mucinous carcinomas, but no association was detected between the different mucin phenotypes and tumour stages (data not shown).

The different results between our study and previous studies may relate to small case numbers, differences in antigen retrieval and staining procedures, and lack of standard evaluation systems for declaring a case as positive or negative for MUC1 and MUC2 expression. In previous studies that analysed MUC1 and MUC2 expression in CRC by immunohistochemistry, three different scoring systems were used.^{14 20–22 26 28} This study attempted to resolve the contradictions by analysing a large number of unselected CRC cases (n = 1420) and stratifying these according to MMR repair status. Additionally, we applied a simple descriptive scoring system already used in a previous study,³⁹ and avoided a complex and often arbitrary composite scoring system (ie, multiplying the percentage of positive cells by the staining intensity). Indeed, an association between staining intensity and the clinicopathological data was not observed in our study.

Use of TMA technology raises the questions of whether a minute tissue sample (0.6 mm) per tumour is representative of the tumour as a whole and how many punches per tumour should be taken. Several arguments favour the use of TMA

technology. First, although the results obtained using TMAs approach the findings in large sections as more samples are analysed,^{40–43} it is conceivable that reproducing the findings of large sections is not an optimal endpoint for the validation of the method, because even large sections contain only a small fraction of the entire tumour mass (1/10 000).²⁹ Second, several studies have shown previously well-established associations between molecular features and clinicopathological end points in TMAs using only one spot per tumour.^{40 43 44} Third, the greater objectivity of the staining interpretation on one small tissue fragment and the high level of standardisation are probable factors that may compensate for the disadvantage of the small sample size.²⁹ Fourth, it is often impossible to include several punches per tumour using small tissue specimens as donor tissue and therefore it is better to collect data from large series of tumours to determine the prognostic significance of protein expression.⁴⁵ Fifth, in the specific case of MUC1 and MUC2, Matsuda *et al*²² have demonstrated that immunostaining of preoperative biopsy samples is useful for evaluating the immunoreactivity of the whole tumour.

In summary, our study has shown that both MUC1 expression and MUC2 loss are adverse prognostic factors in MMR-proficient CRC. In MLH1-negative CRCs, loss of MUC2 was associated with advanced N stage and reduced survival, whereas MUC1 expression showed trends towards associations with a favourable outcome, perhaps because of its association with mucinous differentiation and MUC2 expression.

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Take-home messages

- Mucins have a prognostic significance in sporadic mismatch-repair (MMR)-proficient and deficient colorectal cancer, but not in hereditary non-polyposis colorectal cancer.
- In mismatch-repair (MMR)-proficient colorectal cancer, MUC1 expression and MUC2 loss are adverse prognostic factors.
- In MLH1-negative colorectal cancer, loss of MUC2 is associated with advanced N (node) stage and reduced survival.
- In MLH1-negative colorectal cancer, MUC1 expression shows trends towards associations with a favourable outcome. This may be explained by the association between MUC1 and MUC2 expression in mucinous carcinomas, which are over-represented in this subset.

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