Significance of MUC1 and MUC2 mucin expression in colorectal cancer

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Abstract

Aims—To compare the lineage specific distribution of MUC1 and MUC2 mucins in normal colorectal mucosa and adenocarcinoma and to identify pathological correlations.

Methods—Paraffin wax sections from 51 colorectal cancers were examined for the expression of MUC1 and MUC2, non-O-acetyl sialic acid and the carbohydrate epitopes Le^x, Le^y, sialosyl-Le^x, sialosyl-Tn, and Tn using standard histochemical methods.

Results—MUC1, Le^x and Le^y co-localised with columnar cell secretions, whereas MUC2, mild periodic acid Schiff and sialosyl-Tn co-localised with goblet cell mucin in both normal and malignant tissues. Sialosyl-Le^x and Tn were associated with both lineages. In normal tissues MUC1, Le^x and Le^y showed only trace expression by crypt base columnar cells. Cancers could be classified into four phenotypes (MUC2+/MUC1–, MUC2+/MUC1+, MUC2–/MUC1+, MUC2–/MUC1–). Particular phenotypes showed significant correlations with cancer type, lymph node spread and peritumoral lymphocytic infiltration and trends falling short of significance in relation to grade of differentiation and contiguous adenoma.

Conclusions—Classification of colorectal cancer by means of lineage specific function may be relevant to both pathogenesis and prognosis.

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Keywords: colorectal cancer, MUC1, MUC2, pathogenesis, prognosis.

Seven genes for the protein component of epithelial mucins have been cloned.^{1,2} Of these, MUC2,³ MUC3,⁴ and MUC4⁵ are expressed by intestinal goblet cells, although MUC2 predominates within colorectal goblet cell mucin.⁶ Interesting relations between MUC2 expression and the pathogenesis of colorectal neoplasia have been suggested.⁷ MUC2 is expressed by adenomas and mucinous carcinomas.⁷ Down regulation of MUC2 is seen in non-mucinous adenocarcinomas arising within adenomas, whereas cancers considered to develop de novo do not express MUC2.⁷

MUC1 codes for non-secretory, cell membrane associated glycoproteins expressed along the columnar cell apexes in a variety of glandular tissues.¹ MUC1 is the apomucin for epithelial membrane antigen and related structures

identified with monoclonal antibodies raised against the membrane component of human milk fat globules.^{8,9} MUC1 expression is up regulated in a variety of cancers, including colorectal cancer.¹⁰

Monoclonal antibodies have been raised against the repetitive amino acid sequences specific to the various apomucins.¹¹ Antibodies directed against MUC1 bind to the glycosylated protein, but unmasking of the apomucin through periodate oxidation enhances immunoreactivity.¹² Antibodies to MUC2 react only with the non-glycosylated apomucin, giving a characteristic perinuclear pattern of immunolocalisation within goblet cells.⁴

It has been suggested previously that MUC1 and MUC2 could be related to specific cell lineages within colorectal cancer, namely columnar cells and goblet cells, respectively.¹³ Mucin-like material within colorectal cancer would therefore comprise MUC1, MUC2 or mixtures of both depending on lineage differentiation. It was further predicted that particular carbohydrate structures might be co-expressed with MUC1 and MUC2 and that these would show characteristic patterns of localisation. Specifically, MUC1 and non-sialylated type 2 blood group substances (Le^x and Le^y) would be expressed within the glycocalyx of columnar cells, whereas MUC2 and sialylated blood group structures—for example, sialosyl-Tn, would be related to extracellular material with the typical morphological characteristics of secretory mucin.¹³

The aim of this study was to confirm the putative lineage specific co-expression of the carbohydrate and protein components of epithelial glycoproteins and to investigate relations with pathological parameters relevant to type, grade and stage of colorectal cancer.

Methods

Representative samples of paraffin wax embedded tissue were obtained from the files of 48 patients treated consecutively for colorectal cancer in 1992 at Auckland Hospital. The material included three synchronous cancers giving a total of 51 blocks, 41 of which included normal mucosa. Contiguous adenoma was present in eight samples. Sections, 4 µm thick, were cut and mounted on poly-L-lysine coated slides. These were stained with haematoxylin and eosin, mild periodic acid Schiff (PAS) (specific for non-O-acetyl sialic acid)¹⁴ and a series of monoclonal antibodies visualised by the stepwise addition of biotinylated anti-mouse antibody followed by Streptavidin-biotin peroxidase labelled com-

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plex (Dako, Glostrup, Denmark; Serotec, Oxford, UK) and finally development with diaminobenzidine.

The antibodies were directed against the following epitopes: MUC1 (MUSE11, undiluted supernatant¹²), MUC2 (CCP58, ascites, diluted 1 in 1000⁷), Le^x or CD15 (CMRF7, ascites, diluted 1 in 400¹⁵), sialosyl-Le^x (Km-93, diluted 1 in 100, Serotec), sialosyl-Tn (TKH2, diluted 1 in 100, Dako), and Tn (Hb-Tn1, diluted 1 in 50, Dako). The epitope Le^y was demonstrated by means of the biotinylated lectin *Ulex europaeus* agglutinin-1 (UEA-1) (Sigma, Poole, Dorset, UK) at a dilution of 20 µg/ml and visualised with the Streptavidin-biotin peroxidase labelled complex. Immunostaining for MUC1 was preceded by periodate oxidation. Known positive and negative tissue controls were included with each staining run.

The extent of each reaction was graded semiquantitatively as follows: 0,1 = <5% of cells; 2 = 5–30% of cells; 3 = 30–60% of cells; 4 = >60% of cells. These scores were used to demonstrate correlations between protein (MUC1 and MUC2) and carbohydrate markers by means of the Kendall rank coefficient. For the purposes of relating MUC1 and MUC2 reactivity with pathological variables, cancers were regarded as positive when the score was at least 3. On this basis, cancers were classified as MUC2+/MUC1–, MUC2+/MUC1+, MUC2–/MUC1+, and MUC2–/MUC1–. These phenotypes were related to a comprehensive set of clinical and pathological variables¹⁶ and statistical analysis was performed by the χ^2 test or Fisher's test, as appropriate, comparing the index phenotype with the rest.

Results

In normal mucosa MUC1 was observed along the apical membrane of crypt base cells (fig 1). This was a consistent finding, but was not observed without the unmasking effect of periodate oxidation. Of 51 cancers, 38 (74.5%) showed obvious MUC1 up regulation (score 3–4), evident with or without periodate oxidation. MUC1 was expressed intraluminally, within the glycocalyx (fig 2) and in intracytoplasmic lumina. The intraluminal material was dense, eosinophilic and admixed with necrotic debris. It was variably mild PAS positive. Le^x, Le^y (UEA-1) and sialosyl-Le^x showed similar, but not identical patterns of distribution. In normal mucosa, Le^x was expressed within the apical cytoplasm of crypt base columnar cells, whereas Le^y was observed in the glycocalyx (like MUC1). Crypt base expression of sialosyl-Le^x occurred rarely, focally and limited to the mucosa immediately adjacent to cancer. Superficially located goblet cells were occasionally noted to express sialosyl-Le^x. Expression of all three markers was up regulated in colorectal cancer. Le^x and Le^y were localised similarly to MUC1, though Le^x also showed cytoplasmic staining. Not all MUC1 positive cancers expressed Le^x. Sialosyl-Le^x expression was more extensive, including material with the morphological characteristics of extracellu-

Figure 1 MUC1 expression within the glycocalyx of crypt base columnar cells (arrow). Immunoperoxidase with MUSE11.

lar mucin, as well as the glycocalyceal pattern described for MUC1, Le^x and Le^y.

MUC2 was demonstrated in the perinuclear cytoplasm of normal and malignant goblet cells (figs 3 and 4). Of 51 cancers, 24 (47.0%) had a MUC2 score of 3 or more. MUC2 was not evident in either the goblet cell theca or extracellular mucin. In cancers, MUC2 expression was associated with the presence of lumi-

Figure 2 MUC1 expression within the glycocalyx of moderately differentiated adenocarcinoma. Immunoperoxidase with MUSE11.

Table 1 Relations between MUC phenotypes and pathological variables

	MUC2+/MUC1−	MUC2+/MUC1+	MUC2−/MUC1+	MUC2−/MUC1−
Number (%)	9 (17.6%)	15 (29.4%)	23 (45.1%)	4 (7.8%)
Sex				
Male	4 (44.4%)	7 (46.7%)	10 (43.5%)	2 (50.0%)
Female	5 (55.6%)	8 (53.3%)	13 (56.5%)	2 (50.0%)
Age (years)	66.8 ± 16.5	68.9 ± 12.6	68.4 ± 9.8	65.0 ± 9.8
Site				
Right colon	2 (22.2%)	5 (33.3%)	9 (39.1%)	1 (25.0%)
Left colon	5 (55.6%)	4 (26.7%)	10 (43.5%)	3 (75.0%)
Rectum	2 (22.2%)	6 (40.0%)	4 (17.4%)	0 (0%)
Spread				
Intramural	2 (22.2%)	1 (6.7%)	3 (13%)	0 (0%)
Extramural	7 (77.8%)	14 (93.3%)	20 (87%)	4 (100%)
Lymph node spread				
Yes	1 (11.1%)*	7 (46.7%)	12 (52.2%)	4 (100%)
No	8 (88.9%)	8 (53.3%)	11 (47.8%)	0 (0%)
Grade				
Well	0 (0%)	1 (6.7%)	4 (17.4%)	0 (0%)
Moderate	9 (100%)	11 (73.3%)	12 (52.2%)	3 (75.0%)
Poor	0 (0%)	3 (20.0%)	7 (30.4%)	1 (25.0%)
Mucinous cancer				
Yes	1 (11.1%)	4 (26.7%)*	0 (0%)	0 (0%)
No	8 (88.9%)	11 (73.3%)	23 (100%)	4 (100%)
Expanding				
Yes	6 (66.7%)	12 (80.0%)	17 (73.9%)	2 (50.0%)
No	3 (33.3%)	3 (20.0%)	6 (26.1%)	2 (50.0%)
Lymphocytes				
Yes	1 (11.1%)	2 (13.3%)	8 (34.8%)*	0 (0%)
No	8 (88.9%)	13 (86.7%)	15 (65.2%)	4 (100%)
Residual adenoma				
Positive	1 (11.1%)	4 (26.7%)	3 (13.0%)	0 (0%)
Negative	8 (88.9%)	11 (73.3%)	20 (87.0%)	4 (100%)
Jass group				
1	2 (22.2%)	2 (13.3%)	8 (34.8%)	0 (0%)
2	3 (33.3%)	7 (46.7%)	2 (8.7%)	0 (0%)
3	4 (44.4%)	3 (20.0%)	7 (30.4%)	2 (50.0%)
4	0 (0%)	3 (20.0%)	6 (26.1%)	2 (50.0%)

*p < 0.05 (Fisher’s exact test).

nal and interstitial material having the features of secretory mucin: lightly basophilic on staining with haematoxylin and eosin and foamy or

wispy. The same material was mild PAS positive and expressed both sialosyl-Tn and sialosyl-Le^x.

Figure 3 MUC2 expression within normal colon showing restriction of expression to the perinuclear cytoplasm of goblet cells. Immunoperoxidase with CCP58.

Figure 4 MUC2 expression within moderately differentiated colorectal cancer limited to the perinuclear cytoplasm of cells of goblet lineage. The goblet cell theca (arrow) is unstained. Immunoperoxidase with CCP58.

Tn expression was either cytoplasmic when it was associated with MUC1 or within the Golgi zone in areas of MUC2 positivity. Glycocalyceal and intraluminal Tn was infrequent. Scores for MUC1 showed significant positive correlations with scores for Le^x and cytoplasmic Tn and a significant negative correlation with mild PAS. Scores for MUC2 showed a significant positive correlation with mild PAS, sialosyl-Tn and Golgi zone Tn, and a significant negative correlation with Le^x (Kendall rank coefficients not shown). Sialosyl-Le^x scores did not correlate with either MUC1 or MUC2, being strongly coexpressed with both. Despite their similar patterns of immunolocalisation in normal and malignant tissues, scores for MUC1 and Le^x did not show a significant correlation. MUC1 and MUC2 scores showed a significant negative correlation. Relations between MUC1/MUC2 phenotypes and pathological data are shown in table 1.

Discussion

It would seem reasonable to assume that the normal counterpart of secretory material within colorectal cancers is goblet cell mucin, or principally MUC2 mucin. Yet, this is true only for a subset of cancers, of which mucinous adenocarcinoma would be an obvious example. This study indicates that non-mucin-like intraluminal secretions, glycocalyceal material and the mucins of intracytoplasmic lumina are up regulated, membrane associated glycoproteins coded for by MUC1. The normal counterpart is the trace secretion associated with the apical cytoplasm and glycocalyx of crypt base columnar cells. The existence of such a population of secretory columnar cells has been known for many years through ultrastructural studies, the cells being termed differentiating cells,¹⁷ intermediate cells¹⁸ and, in species other than humans, vacuolated columnar cells.¹⁹ This is the first description of MUC1 immunolocalisation along the apical membrane of these cells (fig 1), although biochemical studies have confirmed the presence of MUC1 in normal colorectal mucosa.²⁰ Demonstration of MUC1 within colorectal cancers (fig 2) did not require pretreatment with periodic acid, perhaps because the apomucin is less heavily glycosylated as well as up regulated. Recapitulation of coexpressed MUC1, Le^x and Le^y within crypt base columnar cells by malignant columnar cells indicates a histogenetic link between these populations. Sialosyl-Le^x is known to be coexpressed with MUC1 in tumours, but not in normal mucosa²⁰; this could arise through abnormal sialylation of Le^x. As the precursor of core structure 3,¹³ Tn would be expected to co-localise with all classes of glycoprotein.

MUC2 expressing cells (fig 4) were conspicuous in 47% of cancers, sometimes in association with MUC1 cells (table 1). MUC2 scores were higher when there was MUC1 up regulation. Coexpression of MUC2 with sialosyl-Tn and sialosyl-Le^x is consistent with previous reports.²¹⁻²³

Grouping the cancers into the four possible phenotypes, a number of interesting trends, some of statistical significance, emerged. The

lack of a significant association with grade of differentiation is of interest given the fact that lineage differentiation must account for the different MUC phenotypes. The pattern closest to normal (MUC2+/MUC1-) occurred in nine cancers, of which only one was associated with lymph node metastases (Fisher's exact test, $p = 0.019$). Others have demonstrated the converse relation between MUC1 up regulation and cancer spread.²⁴ The phenotype MUC2+/MUC1+ included four of the five mucinous cancers (the other being MUC2+/MUC1-) (Fisher's exact test, $p = 0.02$). It is possible that hypersecretion of all mucin species (perhaps including MUC3 and MUC4) is a characteristic of mucinous carcinoma. There was a trend for residual adenoma (tubulovillous and villous) to be associated with MUC2+/MUC1+ cancers (Fisher's exact test, $p = 0.16$). This fits not only with the high frequency of MUC2 expression in mucinous carcinomas, but with their histogenetic relation to villous adenomas.⁷ It remains to be shown whether a proportion of MUC2- cancers are of de novo origin.⁷ In this regard, the histogenetic link between normal crypt base columnar cells and MUC1+ cancers (see earlier) is of potential interest. Could de novo cancers arise directly from this population of immature columnar cells?

The association between peritumoral lymphocytic infiltration and MUC2-/MUC1+ cancers (Fisher's exact test, $p = 0.04$) could be due to removal of the immunosuppressant effect of MUC2 mucus. MUC2 mucins bear the sialosyl-Tn antigen which is known to mediate the inhibition of natural killer cell cytotoxicity.²⁵ Furthermore, the fact that the MUC1 antigen (when expressed by tumours) is recognised by cytotoxic T cells could be relevant to this finding also.²⁶ The MUC2-/MUC1+ cancers were unusual in being mainly distributed in Jass group 1 (those with a pronounced lymphocytic infiltrate) or Jass groups 3 and 4 (poor prognostic groups).¹⁶

Whilst peripheral to the molecular changes fundamental to the process of oncogenesis, the characterisation of colorectal cancer mucin provides new opportunities for marking different cell lineages and developing classifications that may be relevant to the pathogenesis and behaviour of colorectal cancer.

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