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Abnormal subcellular distribution of mature MUC2 and *de novo* MUC5AC mucins in adenomas of the rectum: Immunohistochemical detection using non-VNTR antibodies to MUC2 and MUC5AC peptide

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Anti-mucin variable number tandem repeat (VNTR) antibodies have been used previously to demonstrate the *de novo* presence of MUC5AC and MUC6 mucin in colorectal adenomas and increased synthesis of MUC2, the major secreted mucin in normal colorectal mucosa. Here we examined secreted mucins in tubular, tubulovillous and villous adenomas of the rectum using non-VNTR antibodies designed to assess mature mucin. Mucin gene messenger RNAs were detected by *in situ* hybridization. The anti-MUC2 non-VNTR antibody in the goblet cells of adenomas revealed a staining pattern of increased cytoplasmic, Golgi and membrane staining with no change in goblet vesicle reactivity compared with normal controls. In addition, blank goblet cell vesicle immunostaining for MUC2 was found in the transitional mucosa adjacent to all types of adenoma. Although a trend to overexpression of MUC2 was observed with *in situ* hybridization this was not detected with immunohistology. *De novo* synthesis of MUC5AC, but not MUC5B or MUC6 mucin was seen in all adenomas and transitional mucosa using immunohistochemistry. There was no correlation of MUC2 or MUC5AC mucin with polyp size or the grade of dysplasia using the non-VNTR antibodies.

This study demonstrates that anti-mucin non-VNTR antibodies reveal a different subcellular-localization in rectal adenomas compared with normal colorectal mucosa. Further, this pattern is in contrast to that reported for anti-mucin VNTR antibodies. Combined use of these reagents may benefit future assessment of these cancers.

Keywords: **mucin, MUC2, MUC5AC, immunohistochemistry, *in situ* hybridization, tubular, tubulovillous and villous adenomas, variable number tandem repeat**

Introduction

Neoplastic transformation leads to the alteration of mucin gene expression, which is apparent in colorectal cancer tissue and colorectal cancer lines [1–8]. Two recent publications underline the relevance of this class of molecules in cancer. The deletion of the major intestinal secreted mucin gene, Muc2 in mice has been shown to give rise to the development of intestinal tumours [9] and in addition, MUC11 and 12 are down regulated in colorectal cancer [8].

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The mucin family may be divided into secretory and membrane-associated types. At least four mucin genes are clustered on chromosome 11p15.5, these are MUC2, MUC5AC, MUC5B and MUC6 which all code for the secretory, gel forming mucins found overlying mucosal surfaces [10]. The normal human colorectum is characterised by goblet cell specific MUC2 and membrane-associated MUC4 with lesser amounts of MUC1 and MUC3 mucins [1,11–13]. Additional mucin genes have been detected recently in the human gastrointestinal tract, including MUC11 and 12 [8], MUC13 [14], and MUC17 [15]. The downregulation of MUC11 and 12 in colorectal cancer further emphasizes a significant role for mucins in neoplastic transformation [8].

The biosynthesis of mucins involves extensive post-translational glycosylation of the mucin polypeptide to yield very high molecular weight mature mucins. The mature secretory mucins are the components that lend mucous secretions their viscoelastic properties. This gel forming property is essential to their function as cytoprotective agents at mucosal surfaces throughout the gastrointestinal tract.

The molecular characterization of the family of mucin (MUC) genes [10] has facilitated the design of probes for use in *in situ* hybridization and Northern blotting and supplied peptide sequence information for the design of gene specific antibodies. The histological and biochemical use of these reagents has improved the understanding of the degree and localization of mucin gene product synthesis in normal and cancer tissue and cancer cell lines [1,11,16,17].

Antibody reagents designed to specifically identify mucin gene products may be directed to either the characteristic variable number tandem repeat (VNTR) or non-VNTR peptide domains. The VNTR and non-VNTR antibodies have selective reactivity with the mucin gene products produced during biosynthesis. Precursor mucin forms can be detected with VNTR antibodies, but these do not react with the mature, fully glycosylated molecules [13,18,19]. This is in contrast to the recently developed non-VNTR antibodies which are not subject to interference by glycosylation and are intended to react with mucin molecules at all stages of biosynthesis [20–23]. As the organelles responsible for mucin synthesis are discretely arranged within the cell it follows that both the localization of precursor and mature mucin forms, and hence the reactivity of anti-VNTR and non-VNTR mucin antibodies is also expected to reflect the physical separation of these subcellular compartments.

Colorectal cancer is a major healthcare problem in the UK with over 24,000 deaths per year. In 5% of cases there is a clear genetic cause such as familial adenomatous polyposis (FAP) and hereditary non-polyposis colon cancer (HNPCC), while the remaining sporadic cancers are thought to develop from adenomas [24]. Adenomatous polyps are benign pre-malignant lesions with a progressively increasing malignant potential from tubular through tubulovillous to villous adenomas. The associated sequential expression of dysplasia may finally lead to invasive adenocarcinoma [25]. A feature of colorectal carcinomas and all but the smallest adenomas is the presence of a surrounding transitional zone mucosa, which is believed to arise as a result of factors derived from the neighbouring tumours.

Changes in mucin biology have been reported in adenomas of the large bowel at histological, biochemical and molecular biological levels [14,26–28]. These changes have identified the *de novo* synthesis of both gastric mucins MUC5AC and MUC6 [26,27] and over expression of the major mucin, MUC2, messenger RNA and gene product [26,27,29,30]. The changes in MUC2, MUC5AC and other mucin products have been correlated with villous histology, adenoma size and dysplasia [27,28]. All of these studies have relied on the use of anti-mucin VNTR antibodies and have thus selected for a limited population of mucin forms.

The purpose of this study was to evaluate the pattern of the secreted MUC genes in rectal adenomas and transitional mucosa using rationally designed, gene product specific rabbit polyclonal antisera raised to hapten linked synthetic peptide from non-tandem repeat, low glycosylation domains. As the binding of these novel anti-MUC2, -MUC5AC and -MUC5B reagents is independent of the glycosylation status of the mucin they can be used directly to document the levels of these mucins in tissue sections.

Patient groups

Recent cases of formalin fixed and paraffin embedded locally excised adenomas of the rectum were obtained in a search of the archives of the Department of Histopathology, Bristol Royal Infirmary. These were classified according to World Health Organization guidelines as tubular adenomas ($n = 16$), tubulovillous adenomas ($n = 9$) and villous adenomas ($n = 14$) [31]. The rectal adenomas were classified by size (<0.5 cm, 0.5–1.0 cm, 1.0–3.0 cm and >3.0 cm), type, architecture and degree of dysplasia (mild, moderate and severe). Also a control group of histologically normal colorectal mucosal tissue ($n = 21$) was obtained. Tissues were fixed by routine methods in formol saline for up to 2 days and embedded using standard histological techniques. Selected normal human mucosal tissues, submandibular gland, bronchus, gastric mucosa, jejunum, ileum and colon were used as positive and negative controls for both *in situ* hybridization and immunohistology. Ethical approval for all experiments was obtained from the United Bristol Hospital Trust Ethics Committee.

Materials

Immunohistochemistry reagents

The anti-mucin antibodies were used as reported before [32–34]. Anti-MUC2 (LUM2-3) and anti-MUC5AC (LUM5-1) were described previously [20, 22]. Anti-MUC6 (LUM6-1) rabbit polyclonal antiserum was raised against the VNTR derived peptide sequence for MUC6 An anti-MUC5B rabbit polyclonal antiserum [35] was kindly donated by Dr D. Thornton and Dr J. Sheehan, University of Manchester, U.K.

Secondary antibody, a goat anti-rabbit horse radish peroxidase conjugate was purchased from Dako, UK.

In situ hybridization

Terminal deoxytransferase kit, yeast tRNA and proteinase K were from Roche, Lewes, UK. [^{35}S]-Deoxy-(α-thio) adenine triphosphate (35 MBq/mmol), [^{14}C] labeled radioactive standards and Hyperfilm MP were obtained from Amersham International plc, Amersham, UK. Complementary 48 mer oligonucleotide probes were designed from the tandem repeat domains of MUC2, MUC5AC, MUC5B, MUC6 and a 45

mer negative control against TRK as reported earlier [32–34, 36]. A 48 mer, TGGTGGAGCTGGTGTAGTTGCAGAAGGTGTGGGTGGGGCAGCTGTGGT was designed from the MUC7 tandem repeat sequence. Oligonucleotides were synthesized and purified by Prof. L. Hall, Dept Biochemistry, University of Bristol, UK.

Methods

Immunochemistry

All immunohistochemical techniques were as reported before [32–34]. Briefly, the tissues were deparaffinised using standard techniques. Endogenous peroxidase activity was blocked by incubation of 3% (v/v) hydrogen peroxidase in distilled water for 20 min and washing in tap water for 1 min. Antigen retrieval was performed by pressure cooking at 121°C for 85 seconds in 10 mM citrate buffer pH 6.0 and the tissue sections left to cool at room temperature for 20 min. The MUC2 antisera also required reduction in 10 mM dithiothreitol in 10 mM Tris/HCl pH 8.0 at 37°C for 30 min. This reduction step was omitted for the MUC5AC (LUM5-1), MUC5B and MUC6 (LUM6-1) antisera. The primary rabbit polyclonal antisera were incubated at dilutions of 1 in 3,000 for the MUC2, 1 in 800 for the MUC 5AC, 1 in 2,500 for the MUC5B and 1 in 1,000 for the MUC6 in phosphate buffered saline (PBS) for 1 hour at room temperature and then washed three times 5 min in PBS and incubated with secondary reagent, 1 in 100 goat anti-rabbit horse radish peroxidase conjugate (Dako) in PBS for 25 min at room temperature. After washing for three times 5 min in PBS, sections were developed in 0.6 mg/ml 3-3 diaminobenzidine/0. 03% (v/v) hydrogen peroxide in PBS, rinsed in water, counterstained with haematoxylin, dehydrated, cleared and mounted.

Control staining with test tissue samples gave the expected patterns with each of the anti-mucin antibodies. As a further control, competition studies using purified human colonic and respiratory mucins were performed as described [32]. These showed the ability to abolish the MUC2 (LUM2-3) colonic epithelial staining with 1 mg/ml purified colonic mucin (i.e. MUC2), but not with purified 1 mg/ml respiratory mucin. The specific reactivity of anti-MUC5AC (LUM5-1) in bronchial epithelial tissue was abolished with 1 mg/ml respiratory mucin (i.e. MUC5AC), but not with purified colonic mucin. Bovine serum albumin at 10% had no effect on either LUM2-3 or LUM5-1 binding. The immunochemical staining of mucin gene product was assessed by a single pathologist (BFW) and scored from 0 to 3 according to the degree in the control epithelium, transitional mucosa and adenomatous sites. This assessment was also made for the goblet and absorptive cell phenotypes.

In situ hybridization

In situ hybridization was carried out as before [32–34,36] Oligonucleotides were end labeled with a terminal deoxytransferase kit according to the manufacturers instructions. In brief, 4 μm sections were cut onto gelatin-coated slides, deparaffinised and rehydrated before proteinase K digestion and paraformaldehyde fixation. After prehybridisation in triethanolamine and acetic anhydride, dehydration and delipidation the sections were rehydrated before air-drying. Hybridization was performed by incubating sections with 90 μl of hybridization buffer containing 4×10^5 cpm of [^{35}S]d-ATP labeled probe, covered with squares of labfilm and incubated for 16–20 hours at 42°C.

Mucin messenger RNA was detected on microscope slides by apposition to Hyperfilm MP for 7 days. [^{14}C]-labeled radioactive standards were also put down and films were processed automatically in an Agfa Gevaert Curix 402 processor. Slides were dipped in Ilford K 5 emulsion and exposed for 2 to 3 weeks in a desiccation chamber before development. Sections were counterstained with methyl green pyronin and mounted. Light and dark field photomicrography was performed using a Leitz Dialux 22 EB microscope.

A semi-quantitative assessment of mucin gene expression was made visually from autoradiographic films and scored 0 to ++++ according to the degree of hybridization in the epithelium. The response of the autoradiographic film was calibrated using [^{14}C] microscale standard (Tocris Cookson, Langford, UK). The localization of the mucin gene expression was confirmed by emulsion autoradiography.

The specificity of the oligonucleotide probes for the detection of mucin messenger RNA by *in situ* hybridization was supported by a predominantly epithelial disposition of mucin hybridization as reported before [32]. Example slides were digested with ribonuclease A prior to hybridization as described earlier [34], which abolished the signal obtained. In addition, competition studies using a fifty fold excess of the same unlabelled probe or an irrelevant probe gave the appropriate response. Negative results were obtained for *in situ* hybridization in normal rectal mucosa and rectal adenomas with MUC7 as an internal control. A low background for *in situ* hybridization was achieved by using an optimized hybridization temperature and with stringent post hybridization washing at a high temperature near to the calculated probe melting temperature.

Mucin gene expression was largely reproducible between replicates indicating that variations in tissue fixation, duration of storage and prehybridisation processing of paraffin embedded archival mucosal tissue had no significant effect on the detection of mucin messenger RNA. However, high or low expression was seen with individual specimens.

Statistical analysis

Analysis was carried out using Unistat software using the Mann Whitney U test for non-parametric data as before [32].

Results

Mucin immunohistochemistry

Immunohistochemistry with anti-MUC2 sera revealed a different staining pattern between control and adenomas. Control

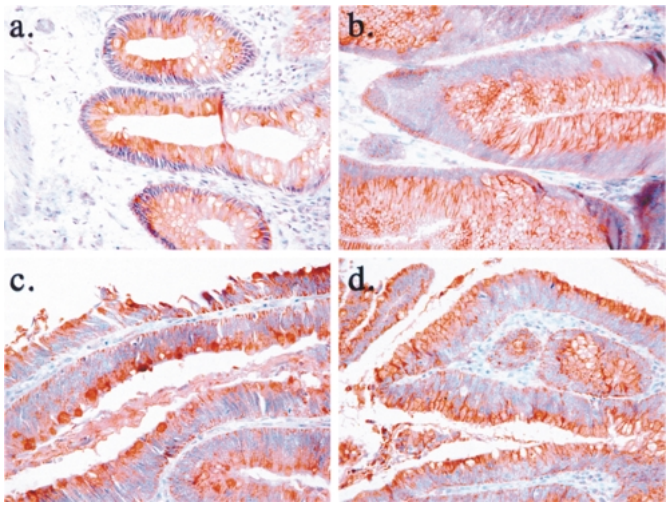

Figure 1. Immunohistological detection of MUC2 in normal and rectal adenomas using the LUM 2-3 antibody. The staining patterns for normal colorectum (a), tubular adenoma (b), tubulovillous adenoma (c) and villous adenoma (d) are shown. Original magnification was ×400 in all cases.

colorectum staining of goblet cells was intense and showed a granular pattern for most vesicles with minor staining in the apical and vesicle membranes, cytoplasm and Golgi (perinuclear region) (Figure 1a, Tables 1 and 2). Occasional absorptive cells showed low-level MUC2 stain (Table 1). Control colorectum did not show any significant binding of anti-MUC5AC (Figure 2a, Table 1) anti-MUC5B or anti-MUC6 antibodies.

High level goblet cell staining was found in all three types of adenoma with anti-MUC2 sera and was increased relative to controls for tubulovillous adenomas, $p < 0.02$ (Table 1, Figure 1b–d). In tubular adenomas the subcellular localization in goblet cells of MUC2 immunostaining was lower in vesicles compared to controls (Table 2), ($p < 0.5$) and shifted more to the cytoplasm ($p < 0.01$) and Golgi ($p < 0.005$) while membrane staining was decreased $p < 0.003$ (Table 2). This wider distribution of staining in membranes ($p < 0.01$) cytoplasm ($p < 0.01$) and Golgi ($p < 0.01$) compartments was maintained in tubulovillous adenomas (Table 2). Villous adenomas also showed a different pattern of staining to the control colorectum with shifts from a predominantly goblet vesicle location to the membrane ($p < 0.02$), cytoplasmic ($p < 0.005$) and Golgi ($p < 0.003$) compartments (Table 2, Figure 1d).

MUC2 staining in the transitional mucosal (TM) crypts adjacent to all adenomas showed moderate goblet cell MUC2 staining and in normal crypts immediately adjacent to transitional mucosa this returned to the moderate to high levels observed for the control colon. The pattern of immunostaining in TM showed blank goblet cell vesicles not apparent in control tissue and is shown in Figure 3 for a tubular adenoma. The absorptive cells of tubular adenomas showed low level MUC2 staining for all adenomas (Table 1). Also there was no change in anti-MUC2 binding to absorptive cells in those specimens with adjacent transitional crypts.

Neo-synthesis of goblet cell MUC5AC mucin was observed in all adenomas. This was detected at a moderate level (Table 1, Figure 2b), $p < 0.002$ in all of the tubular adenomas. A weak cytoplasmic stain was seen in most cells with occasional strong punctate vesicular or periplasmic reactivity and a small proportion of cells showed an apical stain. Where present the TM crypts adjacent to tubular adenomas showed a sporadic, low level of goblet cell MUC5AC mucin. No significant synthesis of MUC5AC could be detected in the goblet cells of normal crypts adjacent to TM crypts.

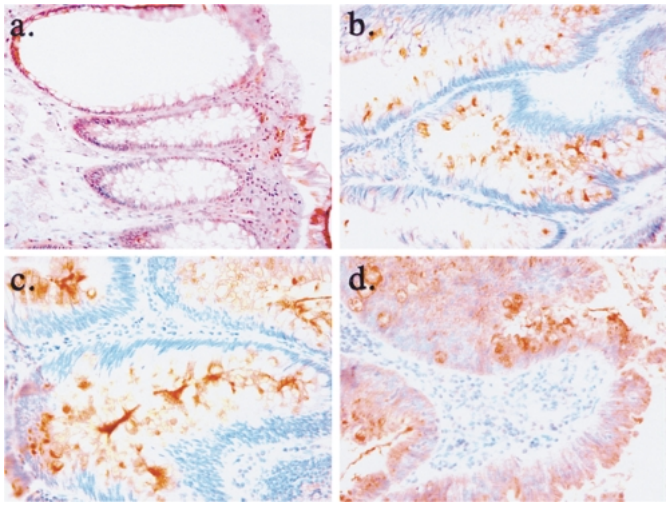

Figure 2. Immunohistological detection of MUC5AC in normal and rectal adenomas using the LUM 5-1 antibody. The staining patterns for normal colorectum (a), tubular adenoma (b), tubulovillous adenoma (c) and villous adenoma (d) are shown. Original magnification was ×400 in all cases.

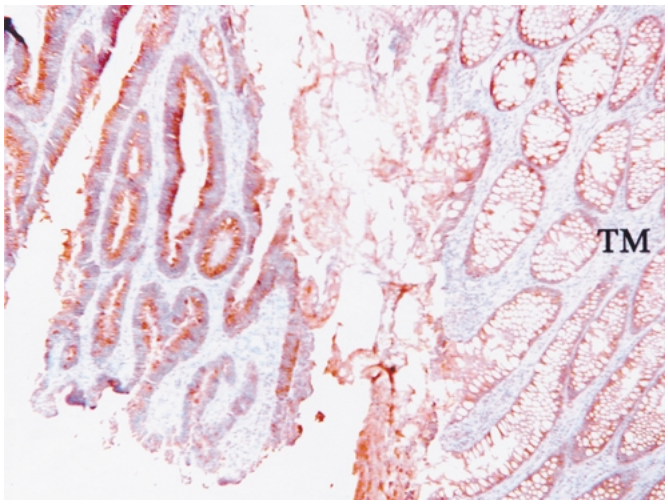

Figure 3. Immunohistological detection of MUC2 in tubulovillous adenoma with adjacent transitional mucosa (TM) using the LUM 2-3 antibody. Original magnification is ×400.

Table 1. Immunohistochemical detection of MUC2 and MUC5AC mucin gene product in control colorectum and rectal adenomas with non-VNTR antibodies

	MUC2		*MUC 5AC*	
	Goblet	*Absorptive*	*Goblet*	*Absorptive*
Control colorectum				
Mucosa	2.6 (0.15)	0.7 (0.11)	0.2 (0.11)	0.1 (0.07)
n	18/18	12/18	3/21	1/21
Tubular adenomas				
Adenoma	2.5 (0.13)	0.9 (0.09)	1.5 (0.16)	0.2 (0.14)
n	15/15	10/15	16/16	3/16
Tubulovillous adenomas				
Adenoma	3.0 (0.00)	1.0 (0.00)	1.9 (0.12)	0.3 (0.18)
n	8/8	8/8	8/8	2/8
Villous adenomas				
Adenoma	2.6 (0.16)	0.9 (0.10)	1.7 (0.15)	0.5 (0.17)
n	10/10	8/10	10/10	4/10

A relative assessment of mucin gene product in goblet and absorptive cells was made visually by conventional light microscopy from immunohistochemical stains of tissue sections using the non-VNTR antibodies. The MUC2 and MUC5AC were scored in control specimens and in adenomatous crypts. The degree of staining is presented on a scale of 0 to 3. A score of 1 corresponds to the minimum, 3 to the maximum positive and 0 to the absence of staining. Values are the mean (SEM) of the total number of cases examined. Number $n =$ the number of specimens (as a proportion of the total number) with positive staining.

All tubulovillous adenomas had moderate to high levels $p < 0.001$ of MUC5AC (Table 1). Staining was slightly stronger than seen in the tubular adenomas (Table 1) and was located in cytoplasmic, vesicular and perinuclear regions (Figure 2c). Minor amounts of MUC5AC gene product were detected in transitional mucosal crypts and the absorptive cells of tubulovillous adenomas (Table 1) and its TM.

Table 2. Subcellular localization of MUC2 mucin within goblet cells of the control colorectum and rectal adenomas with non-VNTR antibodies

	Mean score	*Membrane cytoplasm*	*Golgi*	*Goblet vesicle*
Control	0.25	0.33	0.33	1.75
n	3/12	4/12	4/12	12/12
Tubular	0	1.44	1.33	1.22
n	0/9	9/9	9/9	9/9
Tubulovillous	1.38	1.25	1.12	1.63
n	8/8	8/8	8/8	8/8
Villous	0.86	2.00	1.57	1.14
n	6/7	7/7	7/7	7/7

The subcellular localization of MUC2 mucin in the crypts from control and adenomatous goblet cells was determined by immunohistochemistry using the non-VNTR MUC2 antibody. Relative tissue staining levels were assessed by conventional microscopy and scored on a scale of 0 to 3. A score of 1 corresponds to the minimum, 3 to the maximum positive and 0 to the absence of staining. Scores are the mean of the total number of cases examined. Number $n =$ the number of specimens (as a proportion of the total number) with positive staining.

The villous adenomas all showed moderate to high levels of goblet cell MUC5AC mucin compared with controls (Table 1, Figure 2d) $p < 0.002$. Most cells showed cytoplasmic and punctate staining. Much of the stain was perinuclear. Apical labeling was lower compared with tubulovillous adenomas but similar to tubular adenomas (Figure 2d). Specimens with adjacent TM showed a reduction in goblet cell MUC5AC mucin to a sporadic, low level in the TM. Absorptive cells had minor staining of MUC5AC mucin in villous adenomas (Table 1) with no significant reaction in adjacent TM.

The anti-MUC5B, and anti-MUC6 antisera were completely negative in tubular, tubulovillous and villous adenomas (data not shown).

Mucin messenger RNA expression in the control colorectum and adenomas

The expression of the mucin genes MUC2, MUC5AC, MUC5B, MUC6 and MUC7 was determined in control rectal tissues by *in situ* hybridization. In the control colorectum expression of MUC2 transcripts was at an intermediate level with no expression of MUC5AC, MUC5B, MUC6 and MUC7 (Table 3). There was no detectable expression of MUC5AC transcripts in the majority of control rectum specimens (Table 3). In contrast neo-expression of MUC5AC was seen in most of the tubular and villous and all the tubulovillous adenomas. Minor MUC5B expression was observed only in the tubulovillous adenomas, control, tubular and villous tissues were negative (Table 3). No expression of the mucin genes MUC6 and MUC7 was detected in rectal adenomas.

Table 3. Mucin gene expression in rectal adenomas

	MUC 2	*MUC 5AC*	*MUC 5B*	*MUC 6*	*MUC 7*	*TRK*
Control rectum						
	++	0	0	0	0	0
n	7	7	7	7	7	7
Tubular adenomas						
	++	+	0	0	0	0
n	12	12	10	14	14	14
Tubulovillous adenomas						
	++	++	+	0	0	0
n	7	6	6	7	7	7
Villous adenomas						
	+++	++	0	0	0	0
n	13	13	8	13	13	13

A semi-quantitative assessment of mucin gene expression determined by *in situ* hybridization was made visually from autoradiographic films and scored on a scale of 0 to ++++. Where 0 is negative and ++++ is the maximum positive. The scores shown are the average intensity for the samples examined.

MUC2, MUC5AC mucin and transcripts in relation to adenoma size and dysplasia grade

Comparison of adenoma polyp size and MUC2 and MUC5AC mucin and transcript levels showed no relationship (data not shown). As expected a clear skew in the numbers of adenomas represented in the four size groups chosen (<0.5 cm, 0.5–1.0 cm, 1.0–3.0 cm and >3.0 cm) was seen, with small polyps <0.5 cm present only in the tubular adenoma group and large polyps, >3.0 cm appearing only in the villous adenoma group. However, MUC5AC transcript was absent in the three smallest polyps in the tubular adenoma group.

The severity of dysplasia was scored as mild moderate and severe but no correlation was detected with the dysplasia grade in adenomas and MUC2 or MUC5AC mucin detected by immunohistology or *in situ* hybridization (data not shown). A bias in the distribution of severity was detected as expected with most adenomas studied being either mild or moderate grade, while all the examples of severe dysplasia were in the villous adenoma group.

Discussion

This study presents an analysis of mature MUC2 and MUC5AC mucins in rectal adenomas using novel non-VNTR antibodies. It expands previous work using VNTR antibodies that are not expected to react with the mature fully glycosylated mucins and which give an incomplete view of the mucins present in these tissues. The non-VNTR antibodies show differences in the levels of mucin and their subcellular distribution compared with normal colorectal tissue and implicates defective cellular processing in the process of malignant transformation at these stages in the adenoma-carcinoma sequence. A lack of correlation with polyp size and dysplastic grade is in contrast to results reported with VNTR reagents [27–29] and further underlines the differences dependent on the nature of the mucin products detected.

The design of anti-mucin antibodies to MUC2 and MUC5AC based on peptide sequences outside the VNTR domains is intended to provide reagents that react with mucin molecules at all stages of their biosynthesis. The staining pattern with the non-VNTR, anti-MUC2 antibody, in normal control colorectal tissue showed strong reaction with most goblet cell vesicles. In addition, the cytoplasmic, Golgi, goblet vesicle and membrane compartments all stained for MUC2 (Table 2) indicating that precursor, underglycosylated mucin and mature, stored mucin, can be detected by this antibody as has been suggested from biochemical studies [19,20,22]. In contrast VNTR reagents may only detect precursor mucins [13,19,27,37,38].

No clear increase in MUC2 immunochemical staining was seen in the adenoma groups relative to the normal control colon (Table 1), in agreement with the work of Biemer Huttmann [37], but in contrast to that found by others [28,29], using tandem repeat anti-MUC2 antibodies. However, *in situ* hybridization analysis suggests an increased expression of MUC2 messenger RNA in the tubulovillous and villous adenomas (Table 3), supporting earlier findings [26,29], but contrary to Ho et al. [28]. These discrepancies in transcript and protein remain to be explained, but may be related to mucin processing including post translational modifications such as glycosylation [1] and proteolytic cleavage [23,39,40].

The use of the non-VNTR antibodies shows a change in the subcellular staining of MUC2 within goblet cells from a predominantly goblet vesicle localization in the control colon to a wider distribution, notably in the cytoplasm in all three groups of adenomas (Figure 1, Table 2). The result strongly suggests that alterations in the cellular processing of mucins occur in the adenoma-carcinoma sequence and that the new antibodies are essential to visualize these processes. These observations may be due to several events. Firstly, an alteration in the rate of mucin turnover leading to an accumulation of MUC2, secondly, abnormal subcellular trafficking of MUC2 between compartments, involving precursor, intermediate and mature MUC2

mucin and thirdly switching on of cytoplasmic, endocytic mediated secretion pathways in addition to the established goblet vesicle routes.

The upregulation of MUC5AC mucin and transcript occurs in all types of adenoma (Tables 1 and 3, Figure 2) and confirms earlier data [26,27,41]. The mucin is localized to goblet cells as reported before [37] and no marked increase in MUC5AC occurs with increasing villosity (Tables 1 and 3 and [27]). These results suggest that the induction of MUC5AC is a common and early event in adenoma formation. We have shown that no general field change of MUC5AC is associated with colorectal adenomas [33]. Furthermore, analysis of MUC5AC in colorectal carcinomas has shown that synthesis of this mucin gene is a feature of adeno- and mucinous carcinomas with greater production in the well-differentiated adenocarcinomas [32].

Mature MUC2 and MUC5AC detected with in the non-VNTR antibodies gave no significant correlation with polyp size and the grade of dysplasia in contrast to results with VNTR antibodies [27,28]. Thus VNTR antibodies are superior in detecting these relationships in adenomas. This result suggests that the precursors or low-glycosylated mucin forms detected by these antibodies, are a better marker for polyp size and dysplastic grade. The poor correlation with the non-VNTR antibodies may be explained by the wider range of precursor, intermediate and mature mucin forms detected by this antibody. Thus the larger pools of intermediate and mature mucins may mask any underlying increase in the levels of MUC2 precursors in adenomas.

The anti-VNTR antibodies are more sensitive to detect alterations in the half life and/or abundance of MUC2 precursors in the cells and are of greater value in detecting mucin precursor relationships.

The *de novo* synthesis of MUC5AC in adenomas indicates that those regulatory factors responsible for this action can regulate this gene independently of the other secretory mucins at the chromosome 11p15.5 locus. This mirrors the bacterial regulation of MUC5AC hypersecretion reported recently in respiratory mucosa [42].

In conclusion, this work shows that detection of the secreted mucins MUC2 and MUC5AC using non-VNTR antibodies reveals abnormalities in their cellular processing during the adenoma-carcinoma sequence. It shows that changes in the biology of these mucins are associated with the early events of malignant transformation in rectal adenomas. Future analysis of the biosynthesis of MUC2, MUC5AC and other mucins will be assisted by the combined use of both VNTR and non-VNTR antibodies to assess histologically and biochemically the relative levels of precursors, intermediate and mature mucins.

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