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MUC1, MUC2, and MUC5AC Mucin Core Protein Expression in Ulcerative Colitis-associated Colorectal Carcinoma

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Summary. **Mucin core protein expression in ulcerative colitis-associated colorectal carcinoma (UC-CRC) has not been investigated to date. We immunohistochemically examined expression of MUC2, MUC5AC, and MUC1 mucin core proteins in 8 UC-CRCs (4 mucinous carcinomas and 4 well and/or moderately differentiated adenocarcinomas) in order to characterize their mucin phenotypes. We then compared these with sporadic colorectal carcinomas (sporadic CRCs) (30 mucinous carcinomas and 60 well and/or moderately differentiated adenocarcinomas) in order to confirm whether any specific cellular lineage differentiation occurs in UC-associated carcinogenesis. MUC2, MUC5AC, MUC1 are normally synthesized in goblet cells of gastrointestinal tract, in gastric foveolar epithelium, and in columnar and goblet cells of the colorectum, respectively. UC-CRC exhibited extensive expression of MUC2 (50-100%), MUC5AC (50-75%), and MUC1 (75%), irrespective of histological type, and MUC2+/MUC5AC+/MUC1+ was the most common mucin phenotype (4/8). Six of 8 UC-CRCs had the MUC2+ phenotype, and co-expression of MUC5AC or MUC1 was demonstrated in 5 of these. These findings were similar to those of sporadic mucinous CRCs. The present results indicate that both UC-CRCs and sporadic mucinous CRCs are predominated by cancer cells of goblet cell lineage (MUC2+) with gastric differentiation (MUC5AC+), and up-regulation of gel-forming mucin MUC2 and MUC5AC would account for the frequently observed mucinous histology. This suggests that UC-associated carcinogenesis may originate from MUC5AC+ gastric metaplasia and is similar to UACL (ulcer-associated cell lineage) generated in colorectal mucosa exhibiting long-term chronic inflammation.**

Key words—ulcerative colitis, colorectal carcinoma, MUC1, MUC2, MUC5AC, mucin phenotype.

INTRODUCTION

Mucins are high molecular weight glycoproteins consisting of oligosaccharide chains attached to a protein backbone (core protein) by covalent linkage[1]. Twelve types of mucin (MUC) genes, MUC1 to MUC12, encoding mucin core proteins have been identified[2] and immunohistochemical examination of these proteins has led to a mucin cellular phenotype-based approach to gastrointestinal diseases[2)3)]. In colorectal neoplasms, MUC1, MUC2, and MUC5AC core protein expression has been demonstrated to be relevant to cell lineage differentiation, histogenesis, progression, and genetic alterations[3]. The MUC1 gene is located on chromosome 1q21-24[4] and encodes transmembrane glycoproteins expressed along the luminal membranes of columnar cells in various glandular tissues[5]. In the colorectum, MUC1 is synthesized in both columnar and goblet cells[6]. The MUC2 and MUC5AC genes encode a gel-forming mucin, and are located in a cluster of chromosome

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Abbreviations—CRC, colorectal carcinoma; UC, ulcerative colitis; UC-CRC, UC-associated colorectal carcinoma.

11p15.5[7]. MUC2 is synthesized in goblet cells of the gastrointestinal tract[8] and MUC5AC is normally expressed in gastric foveolar epithelium[9].

Decreased MUC2 expression and increased MUC1 expression are common in colorectal carcinoma[10], while markedly increased MUC2 expression is seen in mucinous carcinoma[10,11]. In colorectal tubular adenomas, MUC1 expression is higher with increasing grade of atypia whereas that of MUC2 is reduced[12]. On the other hand, constantly increased MUC2 expression may be seen in villous adenoma, indicating a mucinous histogenetic continuum[11]. Aberrant expression of gastric mucin MUC5AC is observed in serrated adenoma[13,14], which implies gastric differentiation. MUC5AC is also expressed in mucinous carcinoma[15]. Furthermore, increased expression of MUC2 and MUC5AC is closely correlated with genetic MSI (microsatellite instability)-high colorectal cancers[15].

It is well known that colorectal carcinoma is a major complication of long-term ulcerative colitis (UC)[16,17]. UC-associated carcinoma (UC-CRC) has unique histological characteristics and is more likely to be mucinous, poorly differentiated and multicentric when compared to sporadic CRC[18]. UC-CRC is thought to originate from unequivocal intramucosal neoplastic changes termed as dysplasia[19], which are generated in mucosa after repeated episodes of injury and repair in long-term chronic inflammation[20]. To date, study of mucin core protein expression in UC has been limited to the MUC2 expression of inflammatory mucosa[21,22,23], and no investigation has studied this in UC-CRC.

In this study, we immunohistochemically examined expression of MUC1,MUC2, and MUC5AC mucin core proteins in UC-CRCs to characterize the mucin phenotypes, and compared these phenotypes to those of sporadic CRC in order to confirm whether any specific cellular lineage differentiation occurs in UC-associated carcinogenesis.

MATERIALS AND METHODS

Histologic diagnosis

UC-CRC was defined as epithelial neoplasm surrounded by UC mucosa and invading the submucosa or below. Intramucosal epithelial neoplasm in UC was diagnosed as dysplasia according to the standardized classification of Riddell et al[19]. Diagnosis of CRC (UC-CRC and sporadic CRC) followed the Japanese General Rules for Clinical and Pathological Studies on Cancer of the Colon, Rectum and Anus[24]. Histological type of CRC was first classified as mucinous carcinoma or adenocarcinoma, and the latter was subclassified as well-, moderately-, or poorly-differentiated.

Samples

Colectomy specimens of 8 CRCs were obtained from 6 patients with UC. Clinicopathological features of the samples are shown in Table 1. The specimens were fixed in formalin, and each specimen was cut into 5-mm step sections. All UC-CRCs were advanced carcinomas. Four of the 8 UC-CRCs were mucinous carcinomas and 4 were well and/or moderately differentiated adenocarcinomas. Six UC-CRCs associated dysplasia. As a control, 30 mucinous carcinomas and 60 well and/or moderately differentiated adenocarcinomas were selected from 449 sporadic advanced CRCs resected surgically at the First Department of Surgery of Niigata University. Of the 449 CRCs, all 30 mucinous carcinomas (comprising 6.7%) were selected and 60 adenocarcinomas were selected randomly. Representative sections were then selected and 3-μm-thick serial slices were prepared for hematoxylin and eosin (HE) staining and immunohistochemistry.

Immunohistochemistry

Immunostaining was performed using the streptavidin-biotin immunoperoxidase method (Histofine SAB-PO kit Nichirei, Japan). Monoclonal antibodies used were Ma552 (Novocastra, UK) against MUC1, Ccp58 (Novocastra, UK) against MUC2, and HGM 45M1 (Novocastra, UK) against MUC5AC. Immunostaining for MUC1 was preceded by periodate oxidation[10]. Staining patterns of the mucin core proteins were scored semi-quantitatively as 0 (negative), 1+ (<5% cells), 2+ (5-30% of cells), 3+ (30-60% of cells) and 4+ (>60% cells). A staining score of 3+ or more was taken as positive[10].

Statistical analysis

Statistical analyses were performed using Fisher's exact test. Differences were considered significant at p values of less than 0.05.

RESULTS

The immunohistochemical results for the 8 UC-CRCs from 6 UC patients are shown in Table 1 and are summarized in Tables 2 and 3.

MUC2, MUC5AC, and MUC1 expression (staining

Table 1. Summary of clinicopathological features and immunohistochemical results for UC-CRCs

Case	Age	Sex	Type of UC	Duration of UC (yrs)	No. of UC-CRC	Site	Gross type	Size	Histology	Depth	Dysplasia	Mucin expression of UC-CRC		
												MUC2	MUC5AC	MUC1
1	63	M	unknown	?	①	Rb	type 4	100×65	muc	se	+	+	+	-
2	23	F	total colitis	8	①	C	type 3	45×30	muc	ss	-	+	+	+
					②	S	type 2	120×75	wel>mod	mp	+	+	+	+
3	52	M	total colitis	10	①	Rs	type 2	60×48	mod	ss	+	-	-	+
4	35	F	total colitis	14	①	Ra	type 4	90×40	muc	ss	+	+	-	+
5	47	F	total colitis	16	①	Ra	type 1	120×80	well	mp	+	+	+	+
6	77	F	total colitis	19	①	Rb	type 4	120×100	muc	a	+	+	+	+
					②		type 2	65×35	mod	a	-	-	-	+

Site: C, cecum; S, sigmoid colon; Ra, upper rectum; Rb, lower rectum.
Histology: wel, well differentiated; mod, moderately differentiated; muc, mucinous.
Depth: mp, proper muscle layer; ss, subserosa; se, exposed to the serosa; a, adventitia.
MUC2, MUC5AC, MUC1 staining score of 3 or more was taken as positive.

Table 2. Mucin core protein expression in UC-associated and sporadic colorectal carcinomas

	n	MUC2+	MUC5AC+	MUC1+
UC-CRC				
mucinous	4	4(100%)	3(75.0%)	3(75.0%)
wel, mod	4	2(50.0%)	2(50.0%)	3(75.0%)
Sporadic CRC				
mucinous	30	30(100%) *	21(70.0%) *	24(80.0%)
wel, mod	60	4(6.7%) *	1(1.7%) *	45(75.0%)

MUC2, MUC5AC, MUC1 staining score of 3 or more was taken as positive.
*, $p<0.05$.

Table 3. MUC phenotypes of UC-associated and sporadic colorectal carcinomas

Mucin expression			UC-CRC		Sporadic CRC	
MUC2	MUC5AC	MUC1	mucinous	wel, mod	mucinous	wel, mod
+	+	+	2(50.0%)	2(50.0%)	17(56.7%)	0
+	+	-	1(25.0%)	0	4(13.3%)	0
+	-	+	1(25.0%)	0	7(23.3%)	4(6.7%)
+	-	-	0	0	2(6.7%)	0
-	+	+	0	0	0	1(1.7%)
-	+	-	0	0	0	0
-	-	+	0	2(50.0%)	0	42(70.0%)
-	-	-	0	0	0	13(21.6%)
			4(100%)	4(100%)	30(100%)	60(100%)

MUC2, MUC5AC, MUC1 staining score of 3 or more was taken as positive.

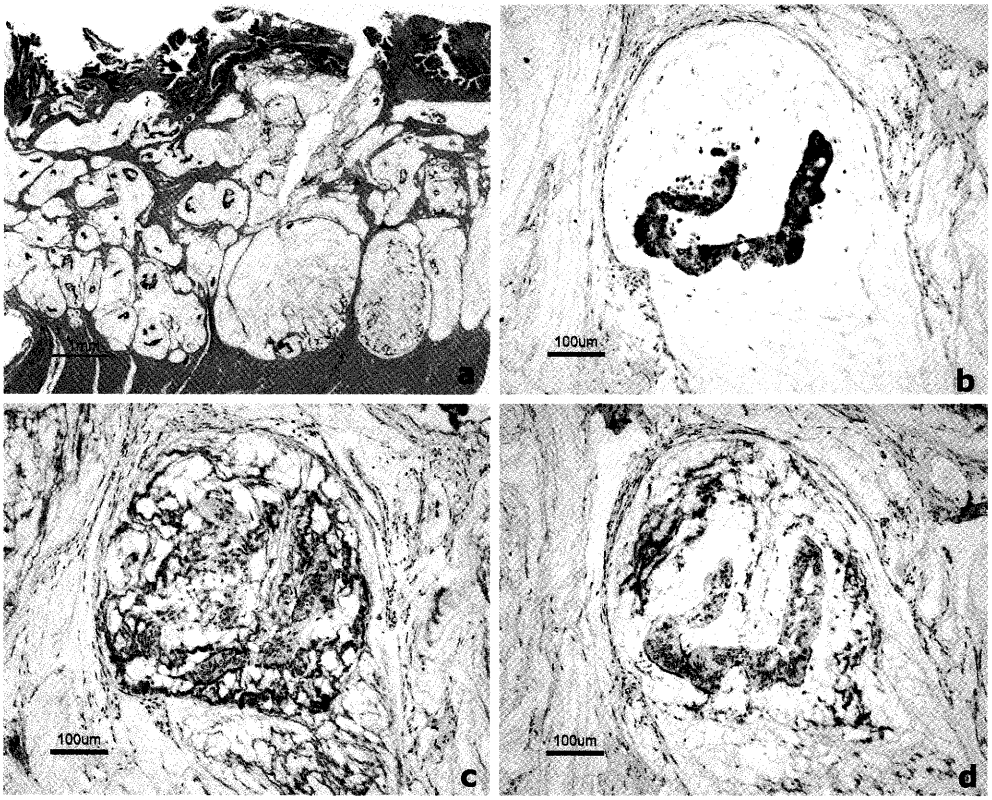

Fig. 1. Mucinous UC-CRC (**a**) (Case 6-①). Immunostaining for MUC2 (**b**), MUC5AC (**c**), and MUC1 (**d**) in a representative area of (**a**). Cancer epithelium floats in the substantial interstitial mucous component. Three mucin species are strongly expressed within the cancer epithelium (MUC2+/MUC5AC+/MUC1+ phenotype).

score of 3 or more) was seen in 50-100%, 50-75%, and 75%, respectively, of UC-CRCs and there was no significant difference in the incidence of mucin core protein expression by histological type (Table 2) (Fig 1 and Fig. 2). In sporadic CRCs, mucinous carcinoma showed a similar pattern of mucin core protein expression as the UC-CRCs, but well and/or moderately differentiated types exhibited a reduction of MUC2 (6.7%) and MUC5AC (1.7%) positivity. The differences between UC-CRC and sporadic CRC with regard to MUC2 and MUC5AC expression of well and/or moderately differentiated types were statistically significant. MUC1 expression was consistently high in UC-CRC and sporadic CRC (75-80%).

In UC-CRCs, as well as in sporadic CRCs, MUC2 staining was localized in the perinuclear cytoplasm of cancer cells (Fig. 1b and 2b) irrespective of whether the cells were goblet or non-goblet types, and the thecae of goblet cells were negative. MUC5AC expression was seen in the apical cytoplasm (Fig. 1c and 2c), intracytoplasmic mucous vacuoles, or thecae of goblet cells. MUC1 was expressed within the apical membrane (Fig. 1d and 2d) and in the intracytoplasmic lumina. Interstitial mucous content was positive for MUC5AC and MUC1 (Fig. 1c and d), but negative for MUC2 (Fig. 1b).

Eight mucin phenotype patterns were observed by combining MUC2, MUC5AC, and MUC1 expression (Table 3). In UC-CRCs, the most common mucin phenotype was MUC2+/MUC5AC+/MUC1+ (50.0% for mucinous and well and/or moderately differentiated types) (Fig. 1 and Fig. 2), and in the MUC2+ phenotype, MUC5AC or MUC1 were co-expressed in 5 of 6 cases (83.3%). Sporadic mucinous CRCs exhibited a similar pattern that the most common phenotype was MUC2+/MUC5AC+/MUC1+ (17/30, 56.7%), and the MUC2+ phenotype was co-expressed with MUC5AC or MUC1 in 21 of 30 (70.0%) and 24 of 30 (80.0%), respectively. The majority (70%) of sporadic well and/or moderately differentiated CRCs possessed the MUC2-/MUC5AC-/MUC1+ phenotype.

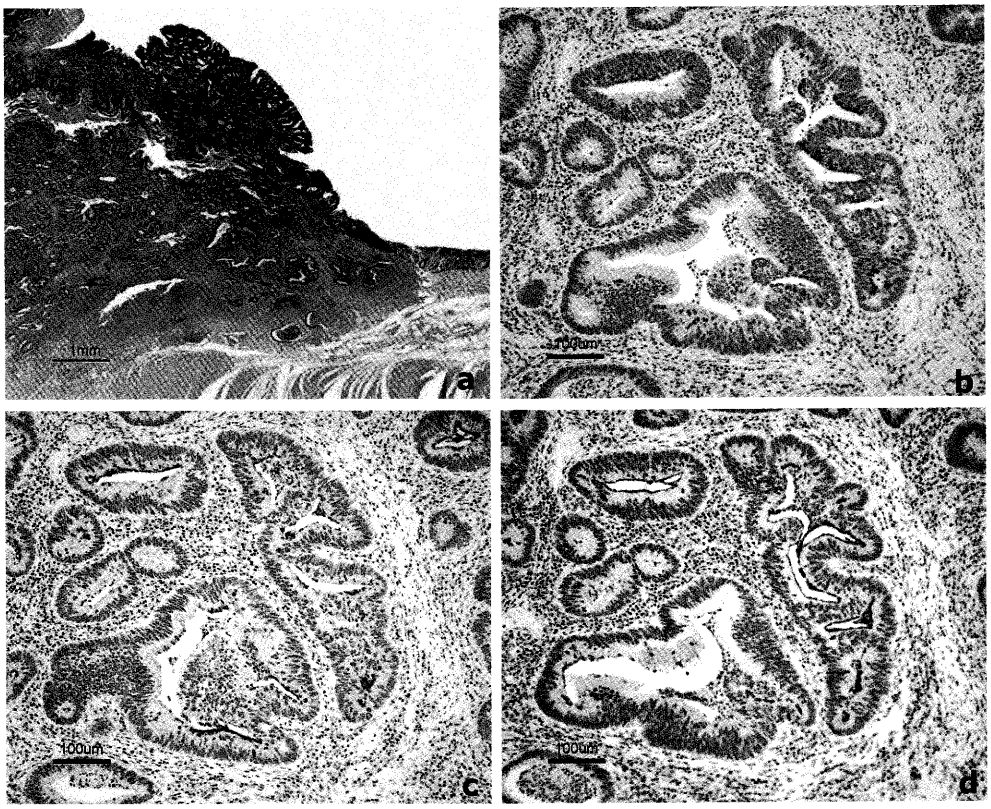

Fig. 2. Well differentiated UC-CRC (**a**) (Case 5-①). Immunostaining for MUC2 (**b**), MUC5AC (**c**), and MUC1 (**d**) in a representative area of (**a**). Three mucin species are strongly expressed within the cancer epithelium (MUC2+/MUC5AC+/MUC1+ phenotype).

DISCUSSION

In this study, we demonstrated for the first time the cellular mucin phenotypes of UC-CRCs by immunohistochemically examining MUC2, MUC5AC, and MUC1 expression. Extensive expression of these three mucin species is characteristic of mucin phenotypes of UC-CRC, regardless of histological type. The mucin phenotype MUC2+/MUC5AC+/MUC1+ was confirmed in 50% of mucinous and non-mucinous UC-CRCs. The cellular mucin phenotypes of UC-CRCs were similar to those of sporadic mucinous CRCs. In sporadic CRCs, all (30/30) mucinous carcinomas possessed the MUC2+ phenotype and 17 of 30 (56.7%) possessed the MUC2+/MUC5AC+/MUC1+ phenotype, whereas 42 of 60 (70.0%) non-mucinous (well and/or moderately differentiated) CRCs showed expression of MUC1 alone, which is consistent with previous studies[10,11,19].

MUC2 and MUC5AC are the core proteins for gel-forming mucin[7], and their up-regulation in cancer cells would cause over-production of cellular and extracellular mucous. The present findings of extensive MUC2 and MUC5AC expression in sporadic mucinous CRCs are consistent with the substantial accumulation of a mucous component within the tumor, and the finding that UC-CRCs also demonstrate similar mucin phenotypes would account for its frequent mucinous histology, as reported in the literature[18]. A possible reason that the 2 UC-CRCs with the MUC2+/MUC5AC+ phenotype in the present study (case 2-② and case 5) remained non-mucinous is that the depth of cancer invasion was relatively shallow, being limited to the proper muscle coat (mp). Accumulation of a mucous component within the tumor may be determined not only by the capacity for mucin production and secretion of cancer cells but also by inhibition of the release of secreted mucous to the lumen of the bowel wall. The latter phenomenon usually occurs when the cancer epithelium is trapped below the proper muscle coat. We speculate that the entrapment of cancer epithelium was not sufficient to result in mucinous his-

tology in these 2 UC-CRCs, despite their MUC2+/MUC5AC+ phenotype.

Carcinoma phenotypes defined by mucin core protein expression would correspond to their cell lineage and differentiation[3]. The present results indicate that UC-CRCs largely contain in cancer cells of goblet cell lineage with gastric differentiation. Most (6/8) of the UC-CRCs expressed MUC2 (MUC2+ phenotype), which is synthesized in goblet cells[8], and co-expression of MUC1 or MUC5AC was demonstrated in 5 of the 6 UC-CRCs with the MUC2+ phenotype (Table 2). MUC1, as well MUC2, is synthesized by goblet cells in the colorectum[6], and MUC5AC expression is normally limited to the gastric foveolar epithelium[9].

The mucin phenotype of carcinomas may also be relevant to their histogenesis. Sporadic mucinous CRCs are considered to be derived from either villous adenoma or serrated adenoma that share a common MUC2+[11] or MUC2+/MUC5AC+[13,14,15] phenotype, respectively. However, the histogenesis of UC-CRCs, which are known to be derived from dysplasia[19], cannot be identical to that of sporadic mucinous CRCs, despite the similarity of their mucin phenotypes. Although not in the colorectum, aberrant MUC5AC expression is observed in the regenerative ileal epithelium following mucosal injury[25,26] known as UACL (ulcer-associated cell lineage)[27], or so-called (pseudo) pyloric gland metalasia[28]. As a possible link between MUC5AC expression in UC-CRCs and what is known of colorectal neoplastic processes, we speculate that MUC5AC+ gastric metaplasia similar to UACL may develop in colorectal mucosa experiencing long-term chronic inflammation, and may play a role in the initial stages of UC-associated carcinogenesis. In order to confirm this notion, investigation of the mucin phenotypes of the dysplasia and inflammatory mucosa adjoining and surrounding UC-CRCs is necessary.

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