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Original

MUC1 Expression in Colorectal Cancer is Associated with Malignant Clinicopathological Factors

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Abstract: This study aimed to evaluate the frequency, distribution, and corresponding histology of MUC1 expression in colorectal cancer and examine its association with clinicopathological factors. MUC1 expression was confirmed in 86 of 169 surgically resected colorectal cancers (51%), although the ratio of MUC1-positive cells was less than 5% in 33 cases (20%), 5–50% in 46 cases (27%), and greater than 50% in only 7 cases (4%). None or less than 5% of MUC1 expression cases were classified as L-group cancers (116 cases, 69%), while cancers showing higher than 5% expression were classified into the H-group (53 cases, 31%). Analysis of the intratumoral distribution of positive cells in the H-group cases showed MUC1 expression distributed predominantly in the upper layers in 3 cases (6%), in the lower layers in 18 cases (34%), and in all layers in 32 cases (60%). MUC1 expression was observed in various histomorphological cancer forms, but the most frequent expression was noted in the monolayer cuboidal (pancreatobiliary-type) neoplastic glands. Considering the relationship between MUC1 expression and clinicopathological factors, H-group cases demonstrated significantly larger lesions showing a greater number of ulcerated-type cancers, deeper invasion, poorer differentiation, higher frequency of budding, and higher rate of lymph node metastasis than L-group cancers. Furthermore, there was a difference of 10% between the H-group and L-group with regard to the frequency of relapse/tumor mortality three years after surgery. In colorectal cancer, MUC1 expression increases with progression of the tumor indicating that it is one of the useful indicators of malignancy and may facilitate appropriate treatment regimens; however, as its expression is heterogeneous and localized, it will be necessary to confirm the state of MUC1 expression by case.

Key words: colorectal cancer, MUC1, immunohistochemistry, clinicopathological study

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Introduction

Up to the early 1990s, there was an increase in the rate of colorectal cancer in Japan that coincided with the westernization of eating habits and increased prevalence of overweight and obese individuals Thereafter, the rate began to level off and presently, approximately 105,000 people are diagnosed with colorectal cancer every year. In recent years, advancements in medical care and diagnostic technologies have resultsed in colorectal cancers being detected at an early stage, and improved surgical or endoscopic resection has increased the number of cases with complete cure. However, the number of deaths still exceeds 40,000 people each year[1].

In accordance with the progression of colorectal cancers, chemotherapy is frequently used as an adjuvant treatment or as part of multidisciplinary therapy[2]. Although the effectiveness of chemotherapy has improved over the years, results remain unsatisfactory[3]. Further improving the cure rates of cancer chemotherapy will require that the treatment regimen and drugs used to be carefully selected according to the specific traits of tumor cells, such as their sensitivity to specific anticancer drugs and the presence of tumor-associated antigens. This importance of personalized treatment will also be considered in the treatment of colorectal cancer, because some colorectal cancers show a heterogeneous cell lineage and partially or predominantly lack the characteristics of an intestinal-type cancer.

MUC1 is a mucin antigen highly expressed in pancreatobiliary-type cancer, such as ductal adenocarcinoma of the pancreas or bile duct, and is a marker of heterogeneity in colorectal cancer. We recently decided to investigate MUC1 expression in colorectal cancer because it is a marker for poor prognosis[4], its expression may affect the selection of chemotherapeutic agents and use of MUC1-cytotoxic T-lymphocyte (CTL) immunotherapy[5], and little is reported about MUC1 expression in colorectal cancer tissues to date[6, 7].

This study investigated the frequency and distribution of MUC1 expression in colorectal cancer with respect to the corresponding histology, and examined the association of these findings with the patient's pathological prognostic factors.

Materials and Methods

Objectives

The study comprised 169 of 203 cases of surgical resection conducted from 2006 to 2007 at the authors' respective facilities and histologically diagnosed as colorectal adenocarcinoma (excluding cases of serous membrane-invasive cancers and directly invasive cancers to other organs). The patients' average age was 71.2 years (36–92 years), with a male : female ratio of 1 : 0.6. The average tumor diameter was 43.7 mm, with tumor localized in the cecum in 19 cases, in the colon in 100, and in the rectum in 50. Based on the invasion depth (T classification[8]), 21 cases were classified as T1, 35 as T2, and 113 as T3. Lymph node metastases were noted in 51 cases. Seventy-four cases were being treated with adjuvant

chemotherapy (5 cases were unconfirmed). Furthermore, outpatient clinical records of 129 patients for three years after surgery were examined to assess post-operative prognosis.

Immunohistochemistry

MUC1 expression was examined immunohistochemically. The staining was performed using the BENCHMARK (Ventana Medical Systems, Inc. Tucson, AZ, USA) automated avidin-biotin complex detection immunostaining system. In each case, 3-μm, ultrathin sections were cut from 1-2 formalin-fixed paraffin-embedded tissue blocks for immunostaining. These slices were stained with anti-MUC1 monoclonal antibody (Ma695, Novocastra, Newcastle upon Tyne, UK; diluted 1 : 100) and subsequently observed under an optical microscope. For each section, the ratio of MUC1-positive cells to tumor cells was calculated. Two types of positive reactions were observed in the tumor cells. The first was a thick, outer-layer staining of the tumor cell membranes, while the second was a light, occasionally dense, diffuse, granular staining of the cytoplasm. As the latter positive reaction was observed in the normal epithelium, only the former positive reaction was considered significant for MUC1 detection.

Clinicopathological examination

We first examined the frequency and distribution of MUC1 expression in the cancer tissue, and then compared these with MUC1 expression in lymph node metastatic lesions from the study patients, 59 pancreatic cancers, and 10 small intestinal cancers. Next, histomorphological features showing MUC1 expression were evaluated. Finally, the relationship between MUC1 expression and clinicopathological factors, especially those related to unfavorable prognosis, was investigated. For statistical analysis, chi-square tests and Student's t-tests were used, and a P-value < 0.05 was considered significant.

Results

Frequency and distribution

MUC1 expression was confirmed in 86 cases (51%); however, the proportion of MUC1-positive cells was 0% in 83 of the tumor samples (49%), 1-5% in 33 cases (20%), 5-50% in 46 cases (27%), and greater than 50% in only 7 cases (4%). Hence, the negative and extremely low ($< 5\%$) MUC1-expressing cases (hereafter known as the MUC1 L-group) comprised approximately 70% of the total number of cases (Table 1). In the other 53 cases with higher MUC1 expression ($> 5\%$ of tumor cells, hereafter known as the MUC1 H-group), we analyzed the intratumoral distribution of MUC1 expression and found that 3 cases (6%) showed expression predominantly in the upper layers, 18 cases (34%) in the lower layers, and 32 cases (60%) in all layers (Table 2). Based on the invasion depth (T classification), only 1 of 21 T1 cancer cases (5%), 11 of 35 T2 cancer cases (31%), and 41 of 113 T3 cancer cases (36%) were from the MUC1 H-group (Table 3). Of the 51

Table 1. Frequency of MUC1 expression in colorectal cancer (n=169)

MUC1 expression	n (%)	Ratio of MUC1 positive cells	n (%)	Grouping	n (%)
Negative	83 (49%)	0%	83 (49%)	MUC1 L-group	116 (69%)
Positive	86 (51%)	< 5%	33 (20%)		
		5-50%	46 (27%)	MUC1 H-group	53 (31%)
		50% ≤	7 (4%)		

Table 2. Distribution of MUC1 positive cells in colorectal cancer (MUC1 H-group) (n=53)

Distribution	n (%)
Upper layer	3 (6%)
Upper and lower layers	32 (60%)
Lower layer	18 (34%)

Table 3. Relation between invasion depth (T stage) and MUC1 expression

	MUC1 H-group (n=53)	MUC1 L-group (n=116)
T1 (n=21)	1/21 (5%)	20/21 (95%)
T2 (n=35)	11/35 (31%)	24/35 (69%)
T3 (n=113)	41/113 (36%)	72/113 (64%)

Table 4. Comparison of MUC1 expression among colorectal, small intestinal and pancreatic cancer

	Colorectal cancer (n=169)	Small intestinal cancer (n=10)	Pancreatic cancer (n=59)
Frequency (MUC1 positive cell ratio of > 50%)	7 (4%)	3 (30%)	41 (70%)

cases of lymph node metastases, 18 of 22 cases (82% ; 3 cases untested) in the H-group continuously exhibited high MUC1 expression (> 5%), while in the L-group, 10 of 26 cases (38%) revealed high MUC1 expression. In the comparison of colorectal cancers with pancreatic or small intestinal cancers, we found that 7 of 169 colorectal cancers (4%) showed a MUC1-positive cell ratio of > 50%, compared to 41 of 59 pancreatic cancers (70%) and 3 of 10 small intestinal cancer (30%) (Table 4).

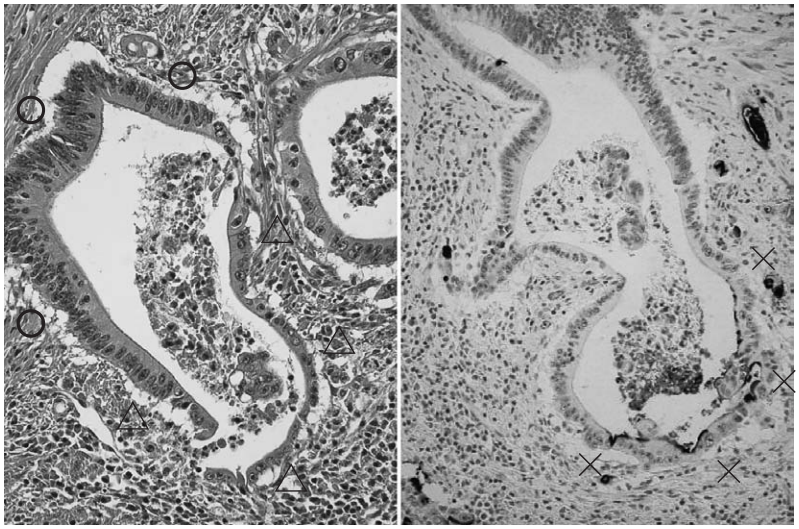

Fig. 1. Histological and MUC1 immunostaining of colorectal cancer.
Note the monolayer cuboidal neoplastic glands (pancreatobiliary-type adenocarcinoma) (△) transformed from columnar neoplastic glands with pseudostratified spindle-shaped nuclei (intestinal-type adenocarcinoma) (○). MUC1 expression is seen only in the pancreatobiliary type glands (×).

MUC1 expression and histological morphology

MUC1 expression was observed in various histological types of colorectal cancer, such as in cancer cells showing cribriform structure, small or budding cancer cell nests, invasive micropapillary carcinoma components, mucinous carcinoma components, or scirrhous tubular adenocarcinoma components (similar to pancreatic cancer). In particular, we noticed frequent expression of MUC1 in the monolayer, cuboidal neoplastic glands (pancreatobiliary-type adenocarcinoma) transformed from columnar neoplastic glands with pseudostratified spindle-shaped nuclei and densely colored cytoplasm (intestinal-type adenocarcinoma) (Fig. 1). MUC1 expression in common intestinal-type tubular adenocarcinomas was also noticed, although it was infrequent.

MUC1 expression and clinicopathological factors

We compared the clinicopathological factors in 53 cases (31%) of the MUC1 H-group with those in 116 cases (69%) of the MUC1 L-group (Table 5). No significant difference was observed in age, gender, or tumor localization between the groups; however, the MUC1 H-group exhibited significantly greater tumor size than the MUC1 L-group (5.2 cm vs. 4.0 cm diameter, respectively), more ulcerated-type tumors (91% vs. 76%, respectively), deeper infiltration depth for T2 and T3 cancer cases (98% vs. 83%, respectively), lower differentiation state (moderately to poorly differentiated: 74% vs. 57%, respectively), greater frequency of budding (58% vs. 41%, respectively), and higher rate of lymph node metastasis

Table 5. MUC1 expression and clinicopathological factors

	MUC1 H-group	MUC1 L-group	P-value
n	53	116	–
Age (years)	73 (36-92)	70 (39-92)	NS
Gender (male : female)	37 : 16	68 : 48	NS
Tumor size (cm)	5.2 (1.7-14)	4.0 (0.9-9)	0.001
Protuberant type vs. ulcerated type (%)	9% vs. 91%	24% vs. 76%	0.03
Localization, left side colon vs. right side colon (%)	68% vs. 32%	66% vs. 34%	NS
Invasion depth, T1 vs. T2 + T3 (%)	2% vs. 98%	17% vs. 83%	0.005
Differentiation, tub1 vs. tub2-por (%)	26% vs. 74%	43% vs. 57%	0.04
High budding (%)	58%	41%	0.03
Lymph node metastasis (%)	43%	21%	0.005
Recurrence/tumor mortality at 3 years post-operation (%)	35%	25%	NS

NS ; not significant

Table 6. MUC1 expression and clinicopathological factors in T3 cancers

	MUC1 H-group	MUC1 L-group	P-value
n	41	72	–
Tumor size (cm)	5.8 (2.5-14)	4.8 (10-90)	0.01
Protuberant type vs. ulcerated type (%)	5% vs. 95%	10% vs. 90%	NS
Differentiation, tub1 vs. tub2-por (n)	20% vs. 80%	33% vs. 67%	NS
High budding (%)	59%	50%	NS
Lymph node metastasis (%)	47%	27%	0.03
Recurrence/tumor mortality at 3 years post-operation (%)	42%	35%	NS

NS ; not significant

(43% vs. 21%, respectively). These findings indicated a strong correlation between MUC1 expression and malignancy factors. Furthermore, a difference of 10% in the frequency of relapse/tumor mortality between the H-group (35%) and the L-group (25%) was observed three years after surgery. In an examination of only T3 cancer cases, several factors failed to show statistically significant differences, but there remained a significant difference in lymph node metastasis between the H-group (47%) and the L-group (27%) (Table 6). Adjuvant chemotherapy was administered almost equally to patients in both groups, namely 26 cases in the MUC1 H-group (51%, 2 cases unknown) and 48 cases in the MUC1 L-group (42%, 3 cases unknown).

Discussion

The histological structure of colorectal cancers is fairly uniform, with the majority showing intestinal-type tubular adenocarcinomas with high expression of the intestinal markers CDX2[9] and MUC2[10]. On the other hand, the MUC1 examined in this study is con-

sidered a pancreatobiliary marker and is commonly expressed in normal pancreatobiliary epithelium cells and pancreatobiliary-type adenocarcinoma. Therefore, when an unknown hepatic tumor is encountered, coimmunostaining of CDX2, MUC2, and MUC1 would be useful to distinguish between colorectal and pancreatobiliary cancers[11]. However, MUC1 expression has been reported at various frequencies (32-50%[4, 12]) in colorectal cancer[13]. The reasons for the difference in positivity rates may include differences in the MUC1 antibody used, the significant positive reactions graded, and in standards for classifying positive cases. In considering all levels of MUC1 expression, our study found MUC1 expression in approximately half of the cases. However, only 4% of these (7 of 169 cases) showed a MUC1-positive cell ratio of greater than 50%. This result was in contrast with 70% (41 of 59 cases) of pancreatic cancers which exhibited MUC1 positivity at a ratio of greater than 50% in additional studies. Thus, unlike for pancreatobiliary cancers, MUC1 expression in colorectal cancer can only be considered a secondary trait.

However, even if MUC1 expression in colorectal cancer is a minor phenomenon, its expression should not be overlooked. In this study, MUC1 expression was clearly observed in 53 cases classified into the MUC1 H-group, of which all except one were either T2 or T3 advanced cancer cases. Therefore, the frequency of MUC1 expression tended to be higher with the stage of the tumor, consistent with a previous study[14]. Furthermore, the MUC1-positive cells were mostly throughout all layers or predominantly in the lower layers, both of which included the invasive fronts. This distribution pattern has been already noted by Baldus *et al*[15] and Hiraga *et al*[16], who reported MUC1 expression as an independent factor indicative of unfavorable prognosis. Moreover, we confirmed that the majority of tumors in the MUC1-H group exhibited high levels of continuous MUC1 expression in lymph node metastatic lesions, and that even in the MUC1-L group, 38% showed high levels of MUC1 expression.

This study also aimed to clarify the relationship between MUC1 expression and clinicopathological factors. To date, MUC1 is known to be a significant marker of unfavorable prognosis in colorectal cancer[4]. In this study, we found that the MUC1 H-group exhibited a significantly greater tumor size, greater number of ulcerated-type tumors, deeper infiltration depth, lower states of differentiation, greater frequency of budding, and higher rate of lymph node metastasis than the MUC1 L-group. These findings indicate a strong correlation between MUC1 expression and malignant factors (Table 5). In an examination of only T3 cancer cases, although the number of cases was small and several factors lacked statistical significance, the MUC1 H-group samples tended to show a stronger relationship with malignant factors than samples from the MUC1 L-group, as well as a significant difference in lymph node metastases[17] (Table 5). Thus, these results indicate that MUC1 expression in colorectal cancer is related to malignancy[18], affects localized progression of the cancer and its metastasis, and is useful as a tumor marker and treatment target[19, 20].

Finally, MUC1 expression was found in various histological features of colorectal cancer,

making it impossible to identify specific morphological features of the tumor tissues that favor MUC1 expression. However, the findings of frequent MUC1 expression in the monolayer cuboidal (pancreatobiliary-type) neoplastic glands, which were often transformed from intestinal type glands, was considered important and a subject for future study.

Conclusion

In colorectal cancer, MUC1 expression increases with development of the tumor, and exhibits a strong correlation with malignant factors. Thus, MUC1 is a useful malignant marker and an indicator for treatment regimen. However, its expression is heterogeneous and localized; therefore, it will be necessary to confirm the state of MUC1 expression with tissues including the invasive front or metastasis on a case-by-case basis.

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