

## Comparison between colorectal low- and high-grade mucinous adenocarcinoma with MUC1 and MUC5AC

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### Abstract

**AIM:** To explore useful prognostic factors for mucinous adenocarcinoma (MAC) in the colon and rectum.

**METHODS:** MAC was divided into low- and high-grade types based on the degree of structural differentiation; low-grade MAC arisen from well to moderately differentiated adenocarcinoma and papillary carcinoma, and high-grade MAC from poorly differentiated adenocarcinoma and signet ring cell carcinoma. Immunohistochemically, the expression of 2 types of MUC1 (MUC1/DF and MUC1/CORE), MUC2, 2 types of MUC5AC (MUC5AC/CHL2 and HGM), MUC6, CDX2, and CD10 was examined in 16 cases of MAC consisting of 6 low- and 10 high-grade types.

**RESULTS:** MUC1/DF3 was expressed in 3 of 6 low-

grade MAC (50%) and 10 of 10 high-grade MAC (100%). MUC1/CORE was expressed in 1 of 6 low-grade MAC (16.7%) and 7 of 10 high-grade MAC (70%). MUC2 was expressed in all MAC regardless of the grade. MUC5AC was expressed in 6 of 6 low-grade MAC (100%) and 4 of 10 high-grade MAC (40%). HGM was expressed in 5 of 6 low-grade MAC (83.3%) and 6 of 10 high-grade MAC (60%). Expression of MUC6 and CD10 was undetected in all MAC regardless of the grade. CDX2 was expressed in 5 of 6 low-grade MAC (83.3%) and 7 of 10 high-grade MAC (70%). Taken together, MUC1/DF3 was expressed significantly more frequently in high-grade MAC than in low-grade, and MUC5AC/CHL2 was expressed significantly more frequently in low-grade MAC than in high-grade.

**CONCLUSION:** It is proposed that MUC1/DF3 and MUC5AC/CHL2 immunostaining is useful to discriminate high-grade MAC from low-grade MAC.

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**Key words:** Mucinous adenocarcinoma; Colon; Rectum; MUC1; MUC5AC

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### INTRODUCTION

Mucinous adenocarcinoma (MAC) is defined as a carcinoma with mucin composing more than 50% of the

lesion and characterized by pools of extracellular mucin that contain malignant epithelium as acinar structures, strips of cells or single cells. MAC is not classified into subtypes in the World Health Organization Classification of 'Tumors of the Digestive System'<sup>[1]</sup>. However, it has been reported that MAC can be divided into two types based on the degree of structural differentiation. One type of MAC is low-grade MAC arisen from well to moderately differentiated adenocarcinoma and papillary carcinoma, and the other high-grade MAC arisen from poorly differentiated adenocarcinoma and signet ring cell carcinoma<sup>[2]</sup>.

Mucins are the major component in the mucus gel on epithelial surfaces with a characteristic organ- and cell type-specific distribution. MUC1 is a large cell surface mucin glycoprotein expressed by most glandular and ductal epithelial cells<sup>[3]</sup>. Normal stomach mucosa is characterized by the production of MUC5AC by the surface epithelial mucus cells and MUC6 by the gastric glands<sup>[4]</sup>. MUC2 is the secreted mucin present predominantly in small and large intestine and confined to goblet cells<sup>[4]</sup>. It has also been reported that altered mucin expression is a feature of precancerous and cancer cells. For example, the expression of MUC1 is up-regulated in a variety of carcinomas including colorectal carcinoma<sup>[5-8]</sup>. A decrease of MUC2 expression has been reported in poorly differentiated colorectal adenocarcinoma<sup>[8]</sup>. The gastric mucin MUC5AC has been reported to be expressed in colorectal adenocarcinoma<sup>[8]</sup>. In addition, CD10, a membrane-bound zinc metallopeptidase, is a small intestinal type-brush border marker and is expressed in the intestinal phenotype of gastric carcinoma<sup>[9]</sup>. The expression of CD10 has not been examined in colorectal MAC. CDX2, an intestine-specific transcription factor, is expressed in the nuclei of normal colorectal tissue, colorectal adenocarcinoma, and mucinous types of adenocarcinoma of ovarian and lung origin<sup>[10,11]</sup>. However, expression of these molecules has not been investigated to compare low-grade MAC with high-grade MAC. To find useful prognostic factors of colorectal MAC, we here immunohistochemically examine the expression of MUC1, MUC2, MUC5AC, CD10 and CDX2 in relation to the grade of colorectal MAC.

## MATERIALS AND METHODS

### Ethics

This work has been carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. All patients provided written informed consent.

### Cases

Sixteen patients with colorectal MAC underwent surgical resection at the Hospital of Hyogo College of Medicine between 2001 and 2005. These 16 MAC were divided into 6 low- and 10 high-grade tumors based on the degree of structural differentiation. Each specimen was examined by 2 authors (T.N. and T.T.) with a multiheaded microscope to reach a consensus on pathological diagnosis. Six

patients were male and 10 female with a mean age of  $62.1 \pm 14.5$  years (44-90 years) (Table 1).

### Immunohistochemistry

The immunohistochemical analysis was carried out according to the method described previously<sup>[12]</sup>. Briefly, tissues embedded in paraffin were cut into 5- $\mu$ m-thick sections. The sections were heated in 10 mmol/L sodium citrate buffer (pH 6.0) at 95°C for 40 min to facilitate antigen retrieval. For immunohistochemical analysis of MUC1, 2 monoclonal antibodies, MUC1/DF3 (15-3, Toray-fuji Bionics, Tokyo, Japan) that identified the core peptide of MUC1 with sialyl oligosaccharides and MUC1/CORE (Ma695, Novocastra Lab, Newcastle Upon Tyne, UK) that recognized the core peptide of MUC1, were used. For immunohistochemical analysis of MUC5AC, MUC5AC/CLH2 (CLH2, Novocastra Lab) that identified only MUC5AC without glycosidic modification and HGM (45M1, Novocastra Lab) that recognized MUC5AC regardless of glycosidic modification were used. The sections were incubated with the primary antibodies of MUC1/DF3, MUC1/CORE, MUC2 (Ccp58, Novocastra Lab), MUC5AC/CLH2, HGM, MUC6 (CLH5, Novocastra Lab), CD10 (56C6, Novocastra Lab), or CDX2 (CDX-2-88, Biogenex, San-Roman, CA, USA). Then, the sections were subjected to the Envision kit (Dako, Glostrup, Denmark) including the secondary antibodies. Immunoreacted cells were visualized with diaminobenzidine tetrahydrochloride, and nuclei were lightly counterstained with hematoxylin. Positive controls comprised normal pancreatic duct cells for MUC1/DF3 and MUC1/CORE, mature goblet cells of normal colonic mucosa for MUC2, normal gastric mucosa for MUC5AC/CLH2 and HGM, normal pyloric gland for MUC6, normal intestinal mucosa for CDX2, and normal small intestinal mucosa for CD10.

### Scorings of immunohistochemical results

Immunohistochemical stains were graded by the presence of positively stained tumor cells as follow: -, less than 5% of tumor cells; +, 5% to 50% of tumor cells; and ++, over 50% of tumor cells. The cases showing + and ++ were evaluated as "positive".

### Statistical analysis

The value was shown as mean  $\pm$  SE. Statistical analysis was carried out by the Student's *t*-test or  $\chi^2$  test (Excel: Microsoft, Redmond, WA, USA). A *P*-value below 0.05 was considered significant.

## RESULTS

Clinical and immunohistochemical characteristics of each case are shown in Table 1. There was no significant difference between patients with low-grade MAC and those with high-grade MAC in both sex ratio (M:F = 2:4 *vs* 4:6) and age distribution ( $67.8 \pm 7.53$  *vs*  $58.6 \pm 3.53$ , *P* = 0.229 at *t*-test). MUC1/DF3 and MUC1/CORE were immunolocalized on the membrane and/or

Table 1 Clinical and immunohistochemical characteristics

Case	Location	Sex	Age	Grade	MUC1/DF3	MUC1/CORE	MUC2	MUC5AC/CHL2	HGM	MUC6	CD10	CDX2
1	T	F	71	Low	-	-	++	+	+	-	-	-
2	R	M	71	Low	-	-	++	++	+	-	-	++
3	A	F	83	Low	+	-	++	++	++	-	-	++
4	A	F	90	Low	+	+	++	++	+	-	-	++
5	R	F	48	Low	+	-	++	+	++	-	-	++
6	R	M	44	Low	-	-	++	+	-	-	-	++
7	R	F	75	High	++	++	++	+	+	-	-	-
8	T	F	57	High	++	+	++	-	+	-	-	++
9	R	M	47	High	++	+	+	-	-	-	-	+
10	R	M	63	High	+	-	++	+	+	-	-	++
11	A	M	57	High	+	-	++	-	-	-	-	-
12	A	F	68	High	+	+	++	-	-	-	-	++
13	T	F	48	High	++	+	+	+	++	-	-	+
14	R	F	75	High	++	+	++	-	+	-	-	-
15	R	M	48	High	++	+	+	-	-	-	-	+
16	A	F	48	High	+	-	++	+	+	-	-	++

T: Transverse colon; R: Rectum; A: Ascending colon; F: Female; M: Male. Immunohistochemical stains were graded by the presence of positively stained tumor cells as follow: -, less than 5% of tumor cells; +, 5% to 50% of tumor cells; and ++, over 50% of tumor cells.

Table 2 Summary of immunostaining

Antibodies	Low-grade MAC (%)	High-grade MAC (%)
MUC1/DF3 <sup>1</sup>	3/6 (50)	10/10 (100)
MUC1/CORE	1/6 (16.7)	7/10 (70)
MUC2	6/6 (100)	10/10 (100)
MUC5AC/CHL2 <sup>1</sup>	6/6 (100)	4/10 (40)
HGM	5/6 (83.3)	6/10 (60)
MUC6	0/6 (0)	0/10 (0)
CD10	0/6 (0)	0/10 (0)
CDX2	5/6 (83.3)	7/10 (70)

Proportions (%) of cases evaluated as "positive" are shown. Statistical analysis between low- and high-grade MAC was carried out by  $\chi^2$  test. <sup>1</sup>A *P*-value below 0.05 was considered significant. MAC: Mucinous adenocarcinoma.

intracytoplasmic lumen of tumor cells. MUC1/DF3 was positive in 3 of 6 low-grade MAC (50%) and 10 of 10 high-grade MAC (100%). MUC1/CORE was positive in 1 of 6 low-grade MAC (16.7%) and 7 of 10 high-grade MAC (70%). MUC2 was immunolocalized in the cytoplasm of tumor cells. MUC2 was expressed in all MAC regardless of the grade. MUC5AC/CHL2 and HGM were immunolocalized in the cytoplasm of tumor cells with goblet or columnar cell features. MUC5AC/CHL2 was positive in 6 of 6 low-grade MAC (100%) and 4 of 10 high-grade MAC (40%). HGM was positive in 5 of 6 low-grade MAC (83.3%) and 6 of 10 high-grade MAC (60%). MUC6 and CD10 were not detected in any MAC regardless of the grade. CDX2 was immunolocalized in the nucleus of tumor cells. CDX2 was positive in 5 of 6 low-grade MAC (83.3%) and 7 of 10 high-grade MAC (70%). Taken together, MUC1/DF3 was expressed significantly more frequently in high-grade MAC than in low-grade ( $P = 0.131$ ,  $\chi^2$  test), and MUC5AC/CHL2 was expressed significantly more frequently in low-grade MAC than in high-grade ( $P = 0.164$ ,  $\chi^2$  test) (Table 2). Representative immunostaining patterns of MUC1/DF3, MUC2, and MUC5AC/CHL2 in low- and high-grade MAC are shown in Figure 1.

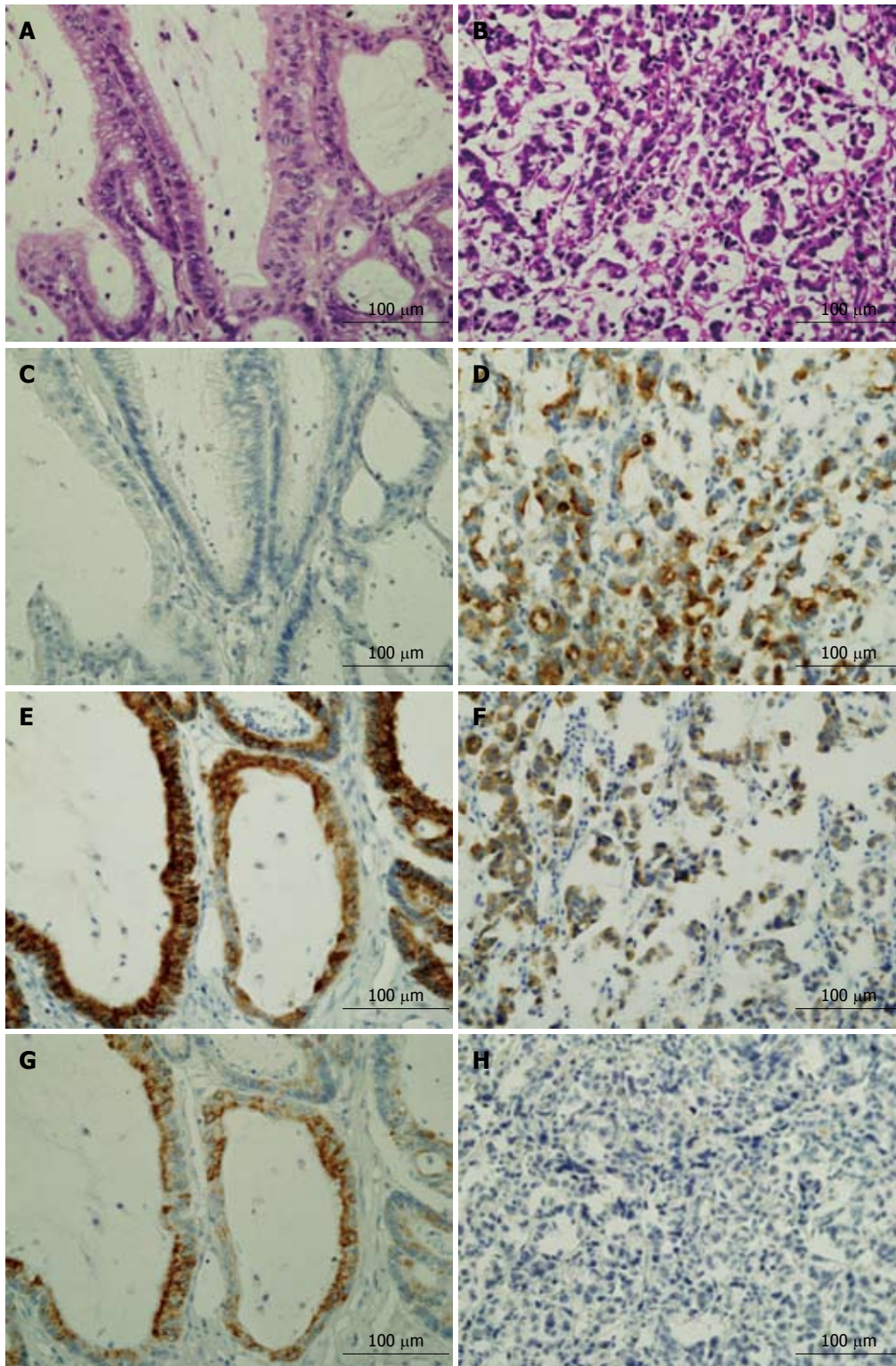
## DISCUSSION

It has been reported that MUC1 is frequently expressed in invasive carcinomas, but not non-invasive carcinomas in various tissues, suggesting that the expression of MUC1 is related to increasing tendency for malignancy and invasion<sup>[13-15]</sup>. In the non-specific conventional adenocarcinoma of colon and rectum, MUC1 is considered to be a prognostic marker and served as a biological feature associated with the aggressiveness of advanced carcinomas<sup>[7]</sup>. We report that MUC1/DF3 was expressed significantly more frequently in high-grade MAC than in low-grade MAC in the colon and rectum. These results support that MUC1 is one of the indices of malignancy of tumors, and suggest that MUC1/DF3 immunostaining is useful to distinguish between low- and high-grade MAC.

MUC5AC is expressed in adenoma and conventional adenocarcinoma with well to moderate differentiation in the colon and rectum<sup>[16-20]</sup>. In addition, MUC5AC has also been reported to be expressed in 56%-63% cases of colorectal MAC<sup>[17,18]</sup>. On the other hand, Kocer *et al*<sup>[19]</sup> have reported that the absence of MUC5AC expression in tumors can be a prognostic factor for more aggressive adenocarcinoma in the colon and rectum. In this study, we found that the frequency of MUC5AC/CHL2 expression was significantly lower in high-grade MAC compared with low-grade MAC. These findings are consistent with the reports by Kocer *et al*<sup>[19]</sup>, and indicate that decreases in MUC5AC expression are a prognostic marker for aggressive and advanced MAC.

MUC2 has been reported to be expressed in poorly differentiated adenocarcinoma in the colon and rectum<sup>[16-18]</sup>. In this study, MUC2 was expressed in all colorectal MAC regardless of the grade. These results indicate that MUC2 is a positive marker for colorectal MAC but is not suitable to distinguish between low-grade MAC and high-grade MAC. MUC6 and CD10 were not detected in any MAC regardless of the grade. These molecules may be negative





**Figure 1 Histological characteristics of MAC.** A, C, E, and G show histological characteristics of case #1 (low-grade MAC) and B, D, F and H those of case #8 (high-grade MAC). C and D: Immunostained with MUC1/DF3. E and F: Immunostained with MUC2; G and H: Immunostained with MUC5AC/CHL2.

markers for colorectal MAC.

In summary, we compared immunohistochemical expression of MUC1, MUC2, MUC5AC, MUC6, CD10 and CDX2 between low- and high-grade MAC, and found that increased MUC1 and decreased MUC5AC expressions are related to malignant potential of

colorectal MAC. Since the expression of MUC1/DF3 and MUC5AC/CHL2 differed significantly between low- and high-grade MAC in the colon and rectum, it is proposed that MUC1/DF3 and MUC5AC/CHL2 immunostaining is useful to distinguish between these two types of MAC.

## COMMENTS

### Background

Mucinous adenocarcinoma (MAC) is characterized by pools of extracellular mucin that contain malignant epithelium. MAC can be divided into two types based on the degree of structural differentiation; low-grade MAC arisen from well to moderately differentiated adenocarcinoma and papillary carcinoma, and high-grade MAC arisen from poorly differentiated adenocarcinoma and signet ring cell carcinoma. However, useful markers for the differential diagnosis of low- and high-grade MAC in the colon and rectum have not been identified.

### Innovations and breakthroughs

The immunohistochemical expression of 2 types of MUC1 (MUC1/DF and MUC1/CORE), MUC2, 2 types of MUC5AC (MUC5AC/CHL2 and HGM), MUC6, CDX2, and CD10 was compared between low- and high-grade MAC. MUC1/DF3 was expressed significantly more frequently in high-grade MAC than in low-grade, and MUC5AC/CHL2 was expressed significantly more frequently in low-grade MAC than in high-grade. These results indicate that increased MUC1 and decrease MUC5AC expressions are related to malignant potential of colorectal MAC.

### Applications

It is proposed that MUC1/DF3 and MUC5AC/CHL2 immunostaining is useful to discriminate high-grade MAC from low-grade MAC in the colon and rectum.

### Terminology

MAC is defined as a carcinoma with mucin composing more than 50% of the lesion. Mucin, a high molecular weight glycoprotein, is the major component in the mucus gel on epithelial surfaces with a characteristic organ- and cell type-specific distribution. Mucin binds to pathogens as part of the immune system.

### Peer review

This is a good descriptive study in which authors compared the expression of mucins (MUC1, MUC2, MUC5AC, and MUC6), CD10, and CDX2 between low- and high-grade MAC. The results are interesting and indicate that MUC1 and MUC5AC are useful markers to discriminate high-grade MAC from low-grade MAC in the colon and rectum. The combination of markers in this study is quite unique.

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