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.

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[1]. 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[2].

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[3]. Normal stomach mucosa is characterized by the production of MUC5AC by the surface epithelial mucous cells and MUC6 by the gastric glands[4]. MUC2 is the secreted mucin present predominantly in small and large intestine and confined to goblet cells[5]. 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[6-8]. A decrease of MUC2 expression has been reported in poorly differentiated colorectal adenocarcinoma[9]. The gastric mucin MUC5AC has been reported to be expressed in colorectal adenocarcinoma[10]. 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[11]. The expression of CD10 has not been examined in colorectal carcinoma. 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[12]. However, expression of these molecules has not been investigated in colorectal adenocarcinoma.

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 ± 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 ± 7.53 vs 58.6 ± 3.53, P = 0.229 at t-test). MUC1/DF3 and MUC1/CORE were immunolocalized on the membrane and/or cytoplasm of tumor cells.
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.

### 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            | 3/6 (50)          | 10/10 (100)        |
| MUC1/CORE           | 1/6 (16.7)        | 7/10 (70)          |
| MUC2                | 6/6 (100)         | 10/10 (100)        |
| MUC5AC/CHL2         | 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. *A P-value below 0.05 was considered significant. MAC: Mucinous adenocarcinoma.

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[13-15]. 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[7]. 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 wide to moderate differentiation in the colon and rectum[16-20]. In addition, MUC5AC has also been reported to be expressed in 56%-63% cases of colorectal MAC[17,18]. On the other hand, Kocer et al. 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 results by Kocer et al.[19], 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[16,18]. 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...
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.
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MUC5AC are useful markers to discriminate high-grade MAC from low-grade
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