Expression of MUC1 and MUC2 Mucins in Gastric Carcinomas: Its Relationship with the Prognosis of the Patients

Tamiharu Utsunomiya, Suguru Yonezawa, Hideki Sakamoto, Hiroshi Kitamura, Shuichi Hokita, Takashi Aiko, Sadao Tanaka, Tatsuro Irimura, Young S. Kim, and Eiichi Sato

Departments of Pathology [T. U., S. Y., H. S., H. K., E. S.] and Surgery [S. H., T. A.], Kagoshima University School of Medicine, Kagoshima 890-8520, Japan; Department of Pathology, Kagoshima Medical Association Hospital, Kagoshima 890-0064, Japan [S. T.]; Department of Cancer Biology and Molecular Immunology, Faculty of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113-0033, Japan [T. I.]; and Gastrointestinal Research Laboratory, Veterans Affairs Medical Center and University of California, San Francisco, California 94121 [Y. S. K.]

ABSTRACT

Our previous immunohistochemical studies for the expression of MUC1 mucin antigen (which was detected by monoclonal antibody DF3) and MUC2 mucin antigen (which was detected by polyclonal antibody anti-MRP) in pancreatic and intrahepatic bile duct tumors demonstrated that invasive carcinoma with poor outcome showed a pattern of MUC1+ and MUC2− expression, whereas many of the noninvasive tumors with favorable outcome showed a pattern of MUC1− and MUC2+ expression. To clarify the relationship between the expression of these mucin antigens and the biological properties of gastric cancers, the expression of MUC1 and MUC2 mucin antigens was examined immunohistochemically in 136 patients with gastric cancer invading the submucosa or the deeper layer, and the survival of the antigen-positive and antigen-negative patient groups was compared using the Kaplan-Meier method. For MUC1 mucin expression, different glycoforms of MUC1 were examined using four monoclonal antibodies (NCL-MUC-1-CORE, DF3, MY.1E12, and HMFG-1). The patients with MUC1+ mucin antigen staining in the carcinoma showed significantly worse survival than those with MUC1− mucin antigen staining. In contrast, the patients with MUC2+ mucin antigen staining in the carcinoma showed significantly better survival than those with MUC2− mucin antigen staining. In conclusion, MUC1 antigen expression was associated with a poor outcome in patients with gastric cancer, irrespective of its glycosylation status, and MUC1 is thus considered to be a useful prognostic factor for poor outcome in patients. In contrast, MUC2 antigen expression is a prognostic factor associated with a favorable outcome in patients. In addition, combined evaluation of the MUC1 and MUC2 mucin staining is clinically useful to predict outcome in patients with gastric cancer.

INTRODUCTION

The synthesis and secretion of mucin are common features of glandular epithelial tissues, and it has been reported that many cancer-associated antigens are mucin antigens. Mucins are high molecular weight glycoproteins with oligosaccharides attached to the apomucin backbone by O-glycosidic linkages.

Our previous immunohistochemical studies for MUC1 mucin expression detected by MAb DF3 and MUC2 mucin expression detected by polyclonal antibody anti-MRP in pancreatic and intrahepatic bile duct tumors demonstrated that invasive carcinoma with a poor outcome showed a pattern of MUC1+ and MUC2− expression, whereas many of the noninvasive tumors with a favorable outcome showed a pattern of MUC1− and MUC2+ expression. Therefore, we have been interested in studying the relationship between the expression of these mucins and the biological properties of various human cancers.

Our recent study indicated that gastric carcinoma patients with DF3+ expression in their carcinoma cells showed significantly decreased survival compared to patients who were negative for DF3 expression (1). MAb DF3, which was used in the study described above, identifies MUC1 apomucin; however, MAb DF3 binding to the MUC1 core protein may be enhanced by the presence of carbohydrates (2). There are differences in the glycosylation of MUC1 in the different human tumors and normal cell types. MUC1 expressed by breast carcinomas is poorly glycosylated in the MUC1 mucin, whereas normal breast tissue shows little or no expression of the MUC1 mucin core peptide (3–5). This phenomenon is explained in part by the finding that MUC1 core peptide epitopes are masked by carbohydrate side chains produced by normal breast epithelial cells (4, 6), whereas the carbohydrate side chains of MUC1 produced by breast adenocarcinomas are shorter or less densely distributed than those produced by normal cells (7). On the other hand,

1 The abbreviations used are: MAb, monoclonal antibody; ABC, avidin-biotinylated horseradish peroxidase complex.

Received 4/16/98; revised 8/10/98; accepted 8/17/98.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported in part by Grants-in-Aid 08457064, 09470053, and 09877038 and the Monbusho International Scientific Research Program, Special Cancer Research from the Ministry of Education, Science, Sports and Culture, Japan [to S. Y.]; by the Kodama Memorial Fund for Medical Research, Japan [to S. Y.]; by an Osuka Pharmaceutical Co., Ltd. grant, Gastric Mucin Research Forum, Japan [to S. Y.]; and by USPHS Grant CA 24321 from the National Cancer Institute, the Department of Veterans Affairs Medical Research Service, and the Theodore Betz Foundation [to Y. S. K.]

2 To whom requests for reprints should be addressed, at Second Department of Pathology, Faculty of Medicine, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890-8520, Japan. Phone: 81-99-275-5270; Fax: 81-99-265-7235; E-mail: syoneza@med4.kufm.kagoshima-u.ac.jp.

2605-2614, November 1998
colorectal carcinomas show a high level of expression of fully glycosylated MUC1 mucin in the advanced stages or in the metastatic lesions (8). Thus, it is of interest to investigate the relationship between the glycosylation of MUC1 mucin expressed in gastric cancer tissues and the outcome in patients with gastric carcinoma.

In the present study, we examine the expression of different glycoforms of MUC1 mucins by using four MAbs (NCL-MUC-1-CORE, DF3, MY.1E12, and HMFG-1) in gastric cancer tissues and show that MUC1 expression is associated with a poor outcome in patients with gastric cancer, irrespective of their glycosylation status. We also examine the expression of MUC2 mucin in the same gastric cancer tissues and show that MUC2 mucin expression is a favorable prognostic factor in patients with gastric carcinoma.

MATERIALS AND METHODS

Tissue Samples. Surgical specimens of 136 gastric cancers (from 86 males and 50 females) invading the submucosal layer or the deeper layer were obtained from the files of the Department of Pathology, Kagoshima University School of Medicine and the Department of Pathology, Kagoshima-shi Medical Association Hospital. The mean age of the patients was 62.5 years (range, 32–91 years). The pathological staging was achieved using the unified Japanese Classification of Gastric Carcinoma (9). Histologically, there were 68 cases of intestinal-type cancer (T1, 17 cases; T2, 46 cases; and T3, 5 cases) and 68 cases of diffuse-type cancer (T1, 5 cases; T2, 48 cases; and T3, 15 cases). Lymph node metastasis was found in 87 patients (46 patients with intestinal-type cancer and 41 patients with diffuse-type cancer). Clinical outcome data were available for 136 gastric cancer patients. The patients who died of other diseases were excluded from the analysis in the current study. All specimens were fixed in formalin, embedded in paraffin, and cut into 4-μm-thick sections for immunohistochemistry in addition to the usual H&E staining.

Antibodies. The antibodies used to detect the mucin antigens are listed in Table 1. For MUC1 expression, different glycoforms of MUC1 were examined by four MAbs (NCL-MUC-1-CORE, DF3, MY.1E12, and HMFG-1). MAb NCL-MUC-1-CORE recognizes the MUC1 mucin core peptide [(TR-PAPG) Data sheet; Novocastra Laboratories, Ltd., New Castle, United Kingdom]. MAb DF3 identifies the MUC1 core peptide; however, the MAb DF3 binding to protein may be enhanced by the presence of sialyl oligosaccharides (2). A new MAb, MY.1E12, is specific for sialylated-MUC1 mucin (10). The binding of MAb HMFG-1 to core protein epitopes is influenced by the carbohydrate chains (4), and HMFG-1 detects fully glycosylated MUC1 mucin, and its binding is particularly affected by sialic acid (4, 11, 12).

For simple representation, MUC1-CORE, DF3, MY.1E12, and HMFG-1 antigens were used for the MUC1 mucin antigens detected by MAbs NCL-MUC-1-CORE, DF3, MY.1E12, and HMFG-1 in this study, respectively. MRP antigen was used for the MUC2 mucin antigen detected by polyclonal antibody anti-MRP.

Biotinylated affinity-purified horse antimouse IgG, goat antirabbit IgG, and ABC were purchased from Vector Laboratories (Burlingame, CA), as was the Vectastain ABC Kit.

Staining Procedure. Immunohistochemical stainings were done on formalin-fixed, paraffin-embedded tissue sections by an immunoperoxidase method using the ABC (13) as described previously (1, 14, 15). Briefly, each section was deparaffinized with xylene. Endogenous peroxidase was blocked by incubating the sections in 0.3% hydrogen peroxide in absolute methanol at room temperature for 30 min. After hydration in decreasing concentrations of ethanol in water, the sections were washed in 0.01 mol/liter PBS (pH 7.4), and then 2% horse or goat serum in PBS was applied for 30 min at room temperature to prevent nonspecific staining. In the staining using each antibody, the sections were incubated with dilutions of the primary antibodies (NCL-MUC-1-CORE, 1:100; DF3, 1:200; MY.1E12, 1:200; HMFG-1, 1:100; and anti-MRP, 1:600) in PBS with 1% BSA for 16 h at 4°C. The sections were washed three times with PBS, incubated with the biotinylated secondary antibodies, and washed three times with PBS. All of the sections then received ABC for 30 min. After washing with PBS three times, the sections were finally reacted with diaminobenzidine substrate for 10–30 min for visualization, rinsed with tap water, counterstained with hematoxylin, and mounted. Reaction products were not present when nonimmune serum or PBS was used instead of the primary antibodies.

Evaluation of the Results by Scoring. The results of the antibody stainings were evaluated by the percentage of positively stained neoplastic cells. The immunostaining result was considered positive if at least 5% of the cells were stained. When less than 5% of neoplastic cells were stained, the immunostaining result was considered negative. To compare the expression of antigens in a more comprehensive manner, the degree of positive expression was graded as follows: (a) +, 5–50% of the neoplastic cells were stained; and (b) ++, >50% of the neoplastic cells were stained.

Statistical Analysis. For an evaluation of the expression rates of mucin antigens, statistical analysis using Fisher’s exact test was performed. A probability of $P < 0.01$ or $P < 0.05$ was considered statistically significant. The survival of the patients
was compared between the group with positive mucin antigen expression and the group with negative mucin antigen expression according the Kaplan-Meier method. The significance of the difference in survival between the two groups was tested using the log-rank test.

The correlation of mucin expression with nodal status or histology (intestinal-type cancer versus diffuse-type cancer) was analyzed statistically. The correlation of the degree of staining with the depth of invasion or with the prognosis of patients was also analyzed statistically.

**Combined Evaluation of MUC1 and MUC2 Expression.** Combined evaluation of MUC1 and MUC2 expression was examined in every MUC1 mucin (MUC1-CORE, DF3, MY.1E12, and HMFG-1) and MUC2 mucin (MRP). The survival of the patients was compared between the group with the MUC1+ and MUC2− expression pattern and the group with the MUC1− and MUC2+ expression pattern.

**RESULTS**

**Expression of the Mucin Antigens in Nonneoplastic Gastric Mucosa**

Table 2 shows the expression rate of each mucin antigen in the nonneoplastic epithelium of foveolar epithelium (64 cases), the fundic gland (42 cases), the pyloric gland (32 cases), and intestinal metaplasia (35 cases). MUC1-CORE antigen was expressed in the fundic glands in 16 of 42 (38%) specimens examined, but it was not expressed in the other areas in all of the cases. DF3 antigen was expressed in the fundic glands in all of the specimens examined, but it was not expressed in the other areas in many cases. However, DF3 antigen expression was observed in the foveolar epithelium in 14 of 64 (22%) cases. MY.1E12 antigen was expressed in the fundic glands in 41 (98%) specimens, in foveolar epithelium in 7 (11%) specimens, and the pyloric glands in 8 (25%) specimens. HMFG-1 antigen was expressed in the fundic glands in 34 (81%) specimens, in foveolar epithelium in 25 (39%) specimens, and in the pyloric glands in 12 (38%) specimens. MRP antigen was not expressed in the normal gastric mucosa, except for a faint positive staining in the fundic glands in all of the specimens examined.

In the intestinalized gastric mucosa, which was seen in 35 of 64 (55%) specimens, MUC1-CORE, DF3, MY.1E12, and HMFG-1 antigens were not expressed, even in the cases showing positive MUC1 mucin antigen expression in the foveolar epithelium. On the other hand, MRP antigen was expressed in the supranuclear region of the goblet cells in all of the specimens.

**Expression of the Mucin Antigens in Carcinoma Tissue**

Fig. 1 shows representative immunohistochemical stainings in MUC1+ (A) and MUC1− (B) carcinomas and in MUC2+ (C) and MUC2− (D) carcinomas.

Table 3 shows the expression rates of each mucin antigen in carcinoma tissues by the depth of tumor invasion (T1, T2, and T3) and the degree of staining. The total expression rate of the DF3 antigen was not correlated with the depth of tumor invasion, but the ++ expression rate of the DF3 antigen was significantly higher in T2 (35%) and T3 (15%) than it was in T1 (0%). The total expression rate of the MY.1E12 antigen was significantly higher in T2 (78%) than it was in T1 (55%). In particular, the ++ expression rate of the MY.1E12 antigen was significantly higher in T2 (52%) than it was in T1 (23%). The expression rate of the MRP antigen was significantly higher in T1 (64%) than it was in T2 (30%) or T3 (30%). Both the + and ++ expression rates of the MRP antigen were significantly higher in T1 (41 and 23%) than they were in T2 (19 and 11%). The expression of the other MUC1-CORE and HMFG-1 antigens was not correlated with the depth of tumor invasion.

Intratumoral heterogeneity of antigen expression was observed from nest to nest in several cases of intestinal-type cancer, whereas it was not seen in the diffuse-type cancer. However, there was no apparent difference between the degree of expression in the whole area and that in the invasive edge.

**Expression of the MUC1-CORE Antigen.** In the 136 patients with gastric carcinomas, the MUC1-CORE antigen was expressed in 18 (13%) patients, but it was not expressed in 118 (87%) patients. The patients with positive MUC1-CORE expression in the carcinoma showed significantly poorer survival than those with no MUC1-CORE expression (Fig. 2A; P < 0.01), although the expression rates of MUC1-CORE showed no significant relationship to the depth of tumor invasion (Table 3).

**Expression of the DF3 Antigen.** DF3 antigen was expressed in 75 (55%) patients, but it was not expressed in 61 (45%) patients. The patients with positive DF3 expression in the carcinoma showed significantly poorer survival than those with no DF3 expression (Fig. 2B; P < 0.01). As mentioned above, the ++ expression rate of the DF3 antigen was significantly higher in the T2 and T3 cases than it was in the T1 cases (Table 3).

**Expression of the MY.1E12 Antigen.** MY.1E12 antigen was expressed in 100 (74%) patients, but it was not expressed in 36 (26%) patients. The patients with positive MY.1E12 expression in the carcinoma showed significantly poorer survival than those with no MY.1E12 expression (Fig. 2C; P < 0.05). As mentioned above, the expression rate of the MY.1E12 antigen in the T2 cases was significantly higher than that in the T1 cases (Table 3).

| Table 2 | Expression of the mucin antigens in nonneoplastic gastric mucosa |
|----------------|------------------|------------------|------------------|------------------|------------------|
|            | MUC1-CORE (%)    | DF3 (%)          | MY.1E12 (%)      | HMFG-1 (%)       | MRP (%)          |
| Foveolar epithelium (n = 64) | 0 | 22 | 11 | 39 | 0 |
| Fundic gland (n = 42) | 38 | 100 | 98 | 81 | 0 |
| Pyloric gland (n = 32) | 0 | 0 | 25 | 38 | 0 |
| Intestinal metaplasia (n = 35) | 0 | 0 | 0 | 0 | 100 |
Expression of the HMFG-1 Antigen. HMFG-1 antigen was expressed in 105 patients (77%), but it was not expressed in 31 patients (23%). The patients with positive HMFG-1 expression in the carcinoma showed significantly poorer survival than those with no HMFG-1 expression (Fig. 2D; $P < 0.05$), although the expression rates of the HMFG-1 antigen showed no significant relationship to the depth of tumor invasion (Table 3).

Expression of the MRP Antigen. MRP antigen was expressed in 48 (35%) patients, but it was not expressed in 88 (65%) patients. The patients with positive MRP expression in the carcinoma showed significantly better survival than those with no MRP expression (Fig. 2E; $P < 0.05$). As mentioned above, the expression rate of the MRP antigen was significantly higher in the T1 cases than it was in the T2 or T3 cases (Table 3).

Fig. 1 Representative immunohistochemical stainings in MUC1 (MY.1E12)-positive (A) and -negative (B) carcinomas and in MUC2 (MRP)-positive (C) and -negative (D) carcinomas.
Survival Related to the Combined Status of MUC1 and MRP.

Antigen Expression and Nodal Status

The expression rates of MUC1-CORE (18%) and DF3 (62%) in the primary tumors of the patients with positive lymph node metastasis (n = 87) were significantly higher than those (MUC1-CORE, 4%; DF3, 43%) in the patients with negative lymph node metastasis (n = 49; P < 0.05, respectively). In contrast, the expression rate of MRP (28%) in the primary tumors of the patients with positive lymph node metastasis was significantly lower than that (49%) in the patients with negative lymph node metastasis (P < 0.05). The expressions of MY.1El2 and HMFG-1 showed no significant correlation with nodal status.

Antigen Expression and Histological Type

There was no significant difference in patient survival between intestinal-type cancer (n = 68) and diffuse-type cancer (n = 68) as analyzed by the Kaplan-Meier analysis and log-rank test (data not shown), although the early-stage cases (T1) were more numerous in intestinal-type cancer (25%) than in the diffuse-type cancer (7%; P < 0.01). The expression rates of DF3 (71%), MY.1El2 (84%); and HMFG-1 (88%) in intestinal-type cancer were significantly higher than those (DF3, 40%; MY.1El2, 63%; HMFG-1, 66%) in diffuse-type cancer (P < 0.01, respectively). The expressions of MUC1-CORE and MRP showed no significant correlation with histological type.

The Degree of Positive Expression and Prognosis

Patients with ++ expression of MY.1El2 in the carcinoma showed significantly poorer survival than those with + expression of MY.1El2 (P < 0.01; Fig. 2C, inset). The other antigens examined showed no significant difference in patient survival between patients with ++ expression and those with + expression (data not shown).

Survival Related to the Combined Status of MUC1 and MUC2 Expression

The results of the combined evaluation of MUC1 and MUC2 expression were shown in Fig. 3, A (MUC1-CORE and MRP), B (DF3 and MRP), C (MY.1El2 and MRP), and D (HMFG-1 and MRP). The patients with the MUC1+ and MUC2− expression pattern always showed the best outcome.

In contrast, the patients with the MUC1− and MUC2+ expression pattern always showed the best outcome. This phenomenon is seen in every MUC1 mucin examined (MUC1-CORE, DF3, MY.1El2, and HMFG-1; Fig. 3, A–D).

**DISCUSSION**

In the current study, we examined the expression of different glycoforms of MUC1 mucin antigens detected by four MAbs (NCL-MUC1-CORE, DF3, MY.1El2, and HMFG-1) in 136 patients with gastric carcinomas invading the submucosal layer or the deeper layer of the stomach. A comparison of the survival of the antigen-positive patient group and the antigen-negative patient group by using the Kaplan-Meier method indicated that the patients whose tumors were positive for NCL-MUC1-CORE, DF3, MY.1El2, and HMFG-1 antibodies showed significantly worse survival compared to patients whose tumors showed negative staining with these antibodies. Because these four MAbs recognize either only the core peptide or different glycoforms of MUC1 mucin, these results indicate that MUC1 mucin antigen expression was associated with a poor outcome in patients irrespective of its glycosylation status, and MUC1 is thus considered to be a useful prognostic marker for poor outcome in patients with gastric cancers.

The MUC1 mucin is a transmembrane glycoprotein with an extracellular domain consisting of a variable number of highly conserved tandem repeats of 20 amino acids, a transmembrane domain, and a cytoplasmic tail of 69 amino acids (16–21). Overexpression of MUC1 by cultured cells inhibits their aggregation, possibly because of its large, extended, and rigid structure (22). MUC1 expressed in tumors may function as an adhesion molecule that inhibits cell-cell adhesion, inducing the release of cells from the tumor (22, 23). In these manners, MUC1 mucin expression may be associated with the invasive or metastatic properties of carcinoma cells, resulting in a poor prognosis for patients with gastric cancers with MUC1 expression. A recent experimental study by Suwa et al. (24) showed that cultured gastric cancer cells acquired increased motility and invasive property when they were transfected with the MUC1 gene. These findings are consistent with our observations demonstrating high expression of MUC1 mucin (MUC1-CORE and DF3) in the primary gastric cancers of patients with lymph node metastasis.
Fig. 2. Comparison of survival between the groups with positive and negative expression of the MUC1 mucins [MUC1-CORE (A), DF3 (B), MY.1E12 (C), and HMFG-1 (D)] and the MUC2 mucin [MRP (E)] as determined by the Kaplan-Meier method. The patients with positive MUC1 expression in the carcinoma showed significantly poorer survival than did those with no MUC1 expression (A–D). In contrast, the patients with positive MUC2 expression in the carcinoma showed significantly better survival than did those with negative MUC2 expression (E). Statistical analysis for the correlation of the degree of staining with the prognosis of the patients demonstrated that the patients with ++ expression of MY.1E12 in the carcinoma showed significantly poorer survival than did those with + + expression of MY.1E12 (C, inset). The other antigens examined showed no significant difference between the survival of the patients with + + expression and those with + expression. □ and ○, patients who were alive at the last follow-up.
metastasis and poor prognosis for patients with positive MUC1 expression (all MUC1 mucins examined) in the gastric cancers. Generally, intestinal-type cancers behave more passively than diffuse-type cancers (25–27). However, in the present study, there was no significant difference in patient survival between intestinal-type and diffuse-type cancers, despite the prevalence of the early-stage cases in the intestinal-type cancer. Therefore, the expression of MUC1 mucin (DF3, MY.1E12, and HMFG-1) in patients with intestinal-type cancer is considered to affect the survival of patients with this type cancer to the same level as patients with diffuse-type cancer.

The exposure of cryptic polypeptide epitopes recognized by T and B cells (28–31) may result in carcinoma-specific cytotoxic lymphocytes that recognize MUC1 mucin core peptides (16). These epitopes serve as antigenic determinants for many anti-MUC1 mucin MAbs (3, 4, 6, 32–37). On the other hand, tumor cells expressing sialomucin were shown to be less sensitive to cytolysis by human lymphokine-activated killer lymphocytes (38–41). Thus, high levels of cell surface sialomucin may be related to an escape from immune killing, resulting in an increased metastatic colonization of carcinoma cells (41, 42). In the present study, the expression rate of sialylated MUC1 mucin detected by MAb MY.1E12 was significantly higher in T2 than in T1. The + + expression rate of the DF3 antigen, whose epitope seems to have sialyl oligosaccharides on the core peptide (2), was also significantly higher in T2 and T3 than in T1 (Table 3). Therefore, sialylation of the MUC1 mucin might be related to the invasiveness of gastric cancer cells and, consequently, to the poor survival of the patients whose tumors express the sialylated MUC1 epitope. In particular, the apparent poor survival of patients with + + expression of MY.1E12 in the carcinoma (Fig. 2C, inset) may indicate that a dominant expression of sialylated MUC1 mucin detected by this MAb is related to cancer cell invasion, inducing poor outcome in the patients.

On the other hand, the expression of the other MUC1 mucin antigens (MUC1-CORE and HMFG-1) was not correlated with the depth of tumor invasion (Table 3). Because the glycosylation levels of MUC1-CORE and HMFG-1 antigens are different, the production of MUC1 mucin peptide itself may also

Fig. 3 Combined evaluation of MUC1 and MUC2 expression. Patient survival was compared between the group with the MUC1+ and MUC2− expression pattern and the group with the MUC1− and MUC2+ expression pattern in each MUC1 mucin and MUC2 mucin [MUC1-CORE and MRP (A), DF3 and MRP (B), MY.1E12 and MRP (C), and HMFG-1 and MRP (D)]. The patients with the MUC1+ and MUC2− expression pattern always showed the worst outcome. In contrast, the patients with the MUC1− and MUC2+ expression pattern always showed the best outcome. □, ◊, △, and ◉, patients who were alive at the last follow-up.
be related to poor prognosis of patients with gastric cancer, in the different mechanism from the sialylated-MUC1 mentioned above. Overexpression of MUC1 on the membrane of cultured cells may also inhibit interaction between cytotoxic lymphocytes and tumor cells (43). Cells with overexpression of MUC1 showed an inhibition of integrin-mediated cell adhesion to the extracellular matrix (44). A recent report by Agrawal et al. (45) showed that cancer-associated MUC1 mucin and synthetic tandem repeats of MUC1 mucin core peptide can suppress human T-cell-proliferative responses, and high levels of MUC1 mucin are correlated with immunosuppression in adenocarcinoma patients. The immune suppression by MUC1 mucin may also result in the poor prognosis of patients with gastric cancer. The survival of patients with positive expression of nonglycosylated MUC1-CORE in the carcinoma was particularly poor (Fig. 2A) compared with those with positive expression of the other glycosylated MUC1 mucin antigens (DF3, MY.1E12, and HMFG-1; Fig. 2, B–D). The suppression of human T-cell-proliferative responses by the MUC1 mucin core peptide (45) might be related to the particularly poor prognosis of MUC1-CORE-positive patients.

MUC2 is a secretory mucin that forms large polymers as a result of disulfate bonding. MUC2 has a high viscosity in solution (46). Our results indicate that in contrast to MUC1 mucin antigen expression, MUC2 antigen expression seems to be a prognostic factor associated with a favorable outcome in patients with gastric cancers (Fig. 2E). Our previous studies on tumors of the pancreas and intrahepatic bile duct showed that MUC2 was expressed mainly in the noninvasive-type tumors (14, 15). In the present study on gastric carcinomas, MUC2 expression was seen in many cases of early-stage gastric carcinoma (T1). The significantly high expression of MUC2 (MRP antigen) in early-stage cancers (T1; Table 3) and the significantly low expression of MUC2 in primary gastric cancers with lymph node metastasis are consistent with the favorable survival of patients with MUC2 expression observed in this study. The cysteine-rich domains of MUC2 (47, 48) may play a role in regulating cell proliferation (48), which may in turn contribute to the low malignant potential of gastric cancers with positive MUC2 expression.

Mucin glycoproteins are heavily glycosylated, with approximately 50–85% of the total molecular weight composed of carbohydrates (49). MUC2 expression in gastric cancers was usually intracytoplasmic and was not seen in the secreted mucin fractions (Fig. 1C). The observed pattern of the intracytoplasmic expression suggests the following possibilities: (a) precursor forms of the MUC2 polypeptide backbone are not heavily glycosylated in early compartments of the Golgi; therefore, these forms are readily detected by anti-MRP; and (b) heavily glycosylated MUC2 mucin is present in secreted fractions and is difficult to detect with antibodies due to the blocking of epitope binding on the polypeptide backbone by carbohydrate side chains. Further investigation of carbohydrate structures linked to the MUC2 polypeptide backbone in the secreted mucin may be important to our understanding of the biological role of secreted and extracellular mucin in gastric cancers. The anti-MRP antibody recognizes the MUC2 core protein (50, 51). No antibody is available for specific glycoforms of MUC2. The relationship between the degree of MUC2 glycosylation and prognosis would also be an important area for future study.

The combined evaluation of MUC1 and MUC2 expression, as shown in Fig. 3, A–D, could show very clear differences in outcome between the group of patients with MUC1+ and MUC2− expression and the group of patients with MUC1− and MUC2+ expression. The combined evaluation of MUC1 and MUC2 mucin staining may be useful clinically to predict the outcome in patients with gastric cancer. Recently, Ho et al. (52) reported that gastric cancers contain apomucin antigens other than MUC1 and MUC2 mucins, and that more advanced stage gastric cancers express greater numbers of apomucin antigens. The relationship between the other apomucin antigens and the outcome of patients would also be an interesting future area of study.

ACKNOWLEDGMENTS

We are grateful to Dr. Joyce Taylor-Papadimitriou (Imperial Cancer Research Fund Laboratories, London, United Kingdom) for kindly providing the MAAb HMFG-1 used in this study and also to Yoshiharu Atsuji and Yoshiko Arimura for excellent technical assistance.

REFERENCES


Expression of MUC1 and MUC2 mucins in gastric carcinomas: its relationship with the prognosis of the patients.

T Utsunomiya, S Yonezawa, H Sakamoto, et al.

*Clin Cancer Res* 1998;4:2605-2614.