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Prediction of Prognosis in Gallbladder Carcinoma by Mucin and p53 Immunohistochemistry

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Mucin core proteins are known to be present in various organs and are specifically expressed with carcinogenesis and closely associated with the prognoses of various malignant tumors in the digestive tract such as colorectal cancer. The present study evaluated correlations between mucin and p53 expression and prognosis of gallbladder cancer using surgically resected tissue specimens from 26 patients with gallbladder carcinoma surgically treated at our hospital. Immunohistochemical staining was performed using MUC1, MUC2, and p53 monoclonal antibody. The level of antigen expression in the lesion was classified into four stages: none($-$), slight($+$), moderate ($++$), and severe ($+++$). According to the UICC classification, histopathological grading, levels of T, N, and M factors, and tumor stages were compared with regard to the correlations with mucin and p53 expression. All cases were classified into two groups according to the results of mucin immunohistochemistry: group A (MUC1, $\geq++$; and MUC2, $\leq+$) and group B (MUC1, $<++$; or MUC2, $>+$). Postoperative survival periods were compared between the two groups and p53-positive and -negative groups. Neither histological grading nor T factor correlated with mucin or p53 expression, respectively. Moreover, neither N factor nor M factor correlated with mucin or p53 expression. Furthermore, stage grouping did not correlate with mucin or p53 expression. However, when the correlation between the postoperative survival period and mucin expression was evaluated, the mean postoperative surgical period was significantly shorter in Group A than in Group B (1.02 years in Group A vs 2.92 years in Group B; $P = 0.016$). There was no relationship between postoperative survival period and p53 positivity. Mucin expression was independent of various tumor growth factors and clearly reflected the prognosis of gallbladder cancer. Because the relative malignancy of gallbladder cancer could be evaluated by examining the level of glycoprotein expression in tumor tissue, mucin could be a more important marker than p53 for predicting prognosis in gallbladder carcinoma using surgically resected tissue specimens.

KEY WORDS: gallbladder carcinoma; mucin; p53; prognosis.

Despite the recent improvement in treatment results and prognosis in gallbladder cancer, the prognosis of advanced gallbladder cancer frequently remains poor even with extended surgery (1). However, some advanced gallbladder cancers have been increasingly found to have a good prognosis. It has been shown that mutations in the oncosuppressor gene p53 are closely related to the histologic type and stage of cancer and to patient survival (2–6). On the other hand, mucin core proteins are found in various organs of the body and have been reported to be closely related to the prognosis of many malignant tumors of the

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gastrointestinal tract such as colorectal cancer (7–11). In this study, we performed immunohistochemical staining of paraffin sections of surgical specimens; examined the relationships between the expression of mucin/p53 and the tumor invasion factor, progression factor, stage, and prognosis; and determined what findings in surgical specimens predicted the prognosis.

MATERIALS AND METHODS

Materials. We examined paraffin sections of tumor invasive fronts that had been diagnosed on HE-stained sections of surgical specimens from 26 patients (11 men and 15 women, with a mean age of 64.3 years) who had undergone surgery under a diagnosis of gallbladder cancer at Tokushima University Hospital between 1992 and 2000 and had been histopathologically diagnosed with gallbladder cancer.

Immunohistochemistry. Serial sections were immunostained with monoclonal antibodies against MUC1, MUC2, and p53 protein. For immunostaining of mucin, antibodies MY.1E12 (kindly provided by Prof. Tatsuro Irimura, The University of Tokyo) and CCP58 (PHARMINGEN Co. Ltd., CA) were diluted 100 and 50 times, respectively, before application. The antibody used for p53 was DO-7 (DACO) and was diluted 50 times. For enhancement of the immunoreactivity of CCP58 and p53, sections were pretreated in a microwave oven; and deparaffinized sections were transferred to 0.01 *M* citrate buffer at pH 6 and heated in a 500-W microwave oven for 20 min. To inactivate endogeneous peroxidase, all sections were treated in methanol containing 0.3% hydrogen peroxidase for 10 min. After washing with PBS, the sections were incubated overnight with each monoclonal antibody at 4°C. They were then washed with PBS and incubated with biotinylated goat anti-rabbit IgG antibody (Vector Laboratories, Inc.) and avidin–biotin complex for 40 min at room temperature. The reactions were visualized by brownish 3,3′-diaminobenzidine, and the sections were counterstained with methyl green.

Evaluation of immunoreactivity for each antibody was based on the extent of staining of cancer cells: 0% (−), none; 1–10% (+), mild; 11–50% (++), moderate; and >50% (+++), strong. In mucin staining, lesions showing MUC1 expression of ++ or more and MUC2 expression of + or below were classified as belonging to group A, and the remaining lesions as belonging to group B. In p53 staining, lesions showing an expression of + or more were regarded as positive. These groups of lesions were compared for histopathological grading, TNM classification, stage grouping, and postoperative survival time. Gallbladder cancers were evaluated according to the TNM Classification of Malignant Tumors (ICD-O C23.9) of the UICC.

Statistical Analysis. Categorical data were analyzed using chi-square test and survival rates were analyzed using the Kaplan–Meier method. P values <0.05 were considered significant.

TABLE 1. PATIENT CHARACTERISTICS IN EACH MUCIN EXPRESSION GROUP

	Group A (n = *10*)	*Group B* (n = *16*)	P *value*
Gender (M/F)	2/8	9/7	N.S.*
Age	59.90 ± 12.17	67.06 ± 9.93	N.S.†
p53 expression (+/−)	5/5	5/11	N.S.*
Stage grouping			
I	0	3	
II	2	6	
III	3	5	N.S.†
IV	5	2	

*Chi-square test with Yate's correlation.
†*t*-text.

TABLE 2. RELATIONSHIP BETWEEN HISTOLOGICAL TYPE AND EXPRESSION OF EACH ANTIBODY

	G1	*G2*	*G3*	*G4*
Group A ($n = 10$)	4	4	1	1
Group B ($n = 16$)	11	3	2	0
p53 positive ($n = 10$)	5	3	1	1
p53 negative ($n = 16$)	10	4	2	0
Total ($n = 26$)	15	7	3	1

RESULTS

Table 1 shows the characteristics of all patients. No correlation was noted between histopathological grading and mucin or p53 expression (Table 2). No correlation was found between mucin expression and the T factor (T1a = 3, T2 = 15, T3 = 7, T4 = 1) ($P = 0.421$; Table 3), the N factor (N0 = 12, N1 = 7, N2 = 7) ($P = 0.058$; Table 4), or the M factor (M0 = 25, M1 = 1) ($P = 0.420$; Table 5). Similarly, there was no correlation between p53 expression and the T, N, or M factors. In addition, no correlation was found between stage grouping (I = 3, II = 8, III = 8, IV = 7) and mucin or p53 expression ($P = 0.140$; Table 5).

The mean postoperative survival time (1.02 years) for group A mucin expression was significantly shorter than that (2.92 years) for group B mucin expression ($P = 0.016$; Figure 1). No significant correlation was noted between p53 expression and postoperative survival (Figure 2).

Patient 10 was a 48-year-old woman who underwent surgery with a preoperative diagnosis of stage IVB disease. The resected specimen was histopathologically diagnosed as G3, T3, N2, M0, and stage IVB. Mucin staining showed that the lesion was strongly positive for MUC1

TABLE 3. RELATIONSHIP BETWEEN T FACTOR AND EXPRESSION OF EACH ANTIBODY

	T1a	*T2*	*T3*	*T4*
Group A ($n = 10$)	0	6	4	0
Group B ($n = 16$)	3	9	3	1
p53 positive ($n = 10$)	1	5	3	1
p53 negative ($n = 16$)	2	10	4	0
Total ($n = 26$)	3	15	7	1

TABLE 4. RELATIONSHIP BETWEEN T FACTOR AND EXPRESSION OF EACH ANTIBODY

	N0	*N1*	*N2*
Group A ($n = 10$)	2	3	5
Group B ($n = 16$)	10	4	2
p53 positive ($n = 10$)	5	2	3
p53 negative ($n = 16$)	7	5	4
Total ($n = 26$)	12	7	7

and negative for MUC2 (group A) (Figures 3A and B). She died of peritoneal recurrence of gallbladder cancer after a postoperative survival period of 482 days.

Patient 25 was a 74-year-old man who underwent surgery with a preoperative diagnosis of stage III. The resected specimen was histopathologically diagnosed as G2, T3, N0, M0, and stage III. Mucin staining showed that the lesion was strongly positive for MUC1 and moderately positive for MUC2 (group B) (Figures 4A and B). He has survived for 1440 days after surgery.

DISCUSSION

Studies investigating the relationship between p53 mutations and gallbladder cancer have reported that although the rate of p53 overexpression varies from 30.6 to 72.7% (2–6, 12), p53 overexpression is closely related to histologic type (5–7), tumor grade (5), and survival time (5). On the other hand, its overexpression has been reported not to be correlated with the prognosis (2, 12). In this study, the degree of p53 expression was not significantly associated with the histologic type, T factor, N factor, stage, or postoperative survival time. It is speculated that p53 mutations play important roles in the transition from a premalignant to a malignant lesion but are not directly involved in the progression and metastasis of the tumor itself.

On the other hand, the mucin core protein is known to be present in various organs of the body and to be expressed specifically in association with malignant transformation (13, 14), and has been reported to be closely related to the prognosis of many gastrointestinal malignant tumors

TABLE 5. RELATIONSHIP BETWEEN STAGE GROUPING AND EXPRESSION OF EACH ANTIBODY

	Stage			
	I	*II*	*III*	*IV*
Group A ($n = 10$)	0	2	3	5
Group B ($n = 16$)	3	6	5	2
p53 positive ($n = 10$)	1	3	3	3
p53 negative ($n = 16$)	2	5	5	4
Total ($n = 26$)	3	8	8	7

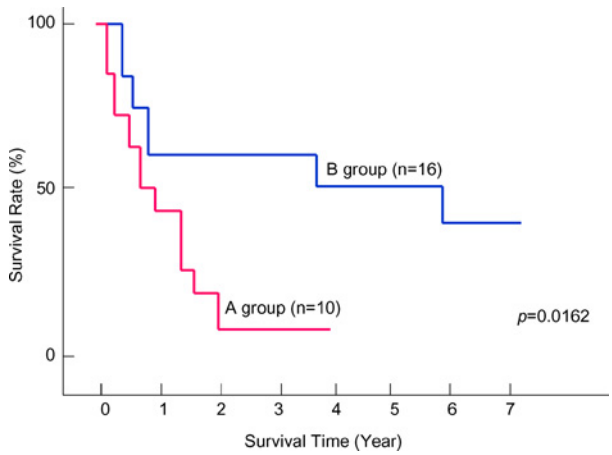

Fig 1. Relationship between mucin expression and postoperative survival time. Group A had a significantly poorer prognosis (mean survival, 1.02 years) compared with group B (mean survival, 2.92 years).

such as colorectal cancer (7–11). It was concluded that in gallbladder cancer, lymphatic vessel invasion and lymph node metastasis were significantly correlated with stainability for DF3, making it a good indicator (15). Sasaki *et al.* (16) performed mucin staining on 55 sections, including gallbladder cancer, and demonstrated a correlation between the histologic type of gallbladder cancer and the expression of MUC2. Yonezawa *et al.* also reported that mucin was a useful tumor marker in showing a high rate of MUC1 (DF3, MY1E12) expression in gallbladder cancer but could not demonstrate its correlation with the stage or prognosis at that time. However, they noted that MUC2 (anti-MRP)-positive pancreatic and intrahepatic bile duct tumors had a relatively better prognosis than their negative counterparts (10) and later demonstrated correlations of the intensities of MUC1 and MUC2 staining with

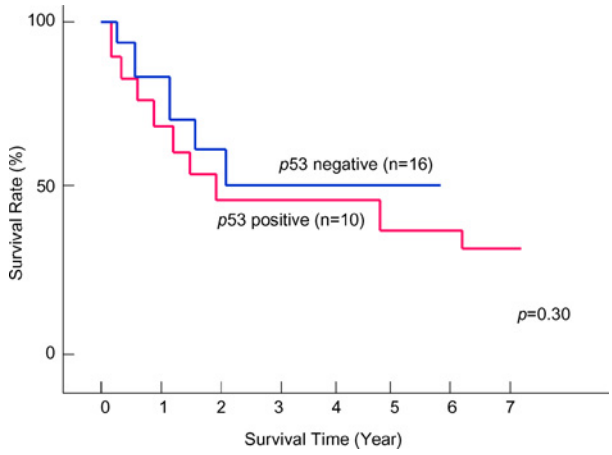

Fig 2. Relationship between p53 expression and postoperative survival time. No significant correlation was noted.

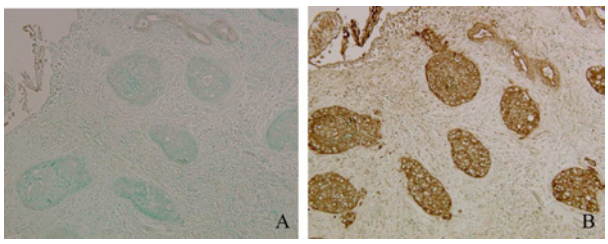

Fig 3. Mucin staining in case 10. The lesion was strongly positive for MUC1 (A) and negative for MUC2 (B).

the prognosis of cholangiocarcinoma and gastric carcinoma (9, 14). Using anti-MUC1 and anti-MUC2 antibodies, Yamoto *et al.* (17) performed in situ hybridization as well as immunostaining and showed that mucin expression differed between dysplasia and cancer and between histologic types of cancer, suggesting that MUC1 reflects histological differentiation, increased proliferative activity, and invasiveness, whereas MUC2 reflects a decrease in proliferative activity.

Our data showed no significant correlation of mucin or p53 expression with the histologic type, depth of invasion, TNM factors, or stage of gallbladder cancer. However, the group with strong MUC1 and weak MUC2 expression had a significantly shorter survival time. Most deaths were due to peritoneal or hepatic recurrence, strongly suggesting the high proliferative and metastatic activity of cancer cells.

Change in the expression status of cell surface sugar chains in association with malignant transformation has been shown in various epithelial tumors and is thought to be an important factor in determining prognosis. Analysis of their expression status is expected to determine the relative degree of malignancy of gallbladder cancer, making it an important marker for predicting the prognosis from surgical specimens. In addition, further studies in many more surgical specimens are expected to show that evaluation of mucin expression can determine the decision whether to perform postoperative adjuvant chemotherapy.

In conclusion, the results of this study suggest that mucin expression is an important prognostic factor for gallbladder cancer, independent of tumor invasion factor, progression factor, and stage, and is a more sensitive marker than p53 expression.

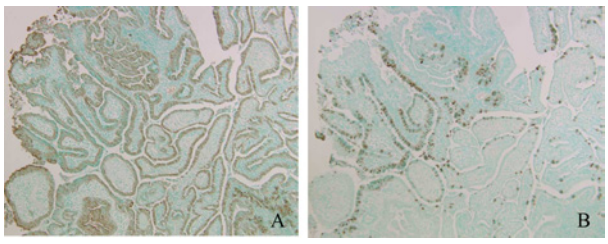

Fig 4. Mucin staining in case 25. The lesion was strongly positive for MUC1 (A) and moderately positive for MUC2 (B).

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