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Expression of Mucin Carbohydrates and Core Proteins in Carcinomas of the Ampulla of Vater: Their Relationship to Prognosis

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We examined the expression of carbohydrate antigens which are associated with the earliest steps in mucin glycosylation (Tn and sialosyl-Tn) and the expression of the mucin core protein antigens associated with MUC1 gene product (DF3 antigen) as well as MUC2 gene product (intestinal-MRP antigen) in tissues from 38 patients with carcinoma of the ampulla of Vater, in order to determine whether these mucin antigens are available as tumor markers or not, and to evaluate whether their expression is correlated with the biological behavior of the carcinomas or not. DF3 antigen showed a relatively high expression rate (61%) in the carcinoma tissues, but was rarely expressed in the non-neoplastic epithelium around the carcinomas in the region of the ampulla of Vater. Tn and sialosyl-Tn antigens showed high expression rates in the carcinoma tissues (86% and 84% each), whereas they showed rare or no expression in the non-neoplastic epithelium around the carcinomas, except for highly restricted expression in the duodenal villous epithelium. The patients with positive DF3 expression in the carcinoma showed significantly poorer survival than those with negative DF3 expression ($P < 0.05$), whereas the patients with positive intestinal-MRP expression in the carcinoma showed significantly more favorable survival than those with negative intestinal-MRP expression ($P < 0.05$). The expression rate of DF3 antigen was significantly higher in the cases with deep invasion into the pancreas (89%) than in those with no or minimal invasion (52%) ($P < 0.05$). In contrast, the expression rate of intestinal-MRP antigen was significantly higher in the cases with no or minimal invasion into the pancreas (38%) than in those with deep invasion (0%) ($P < 0.05$). In conclusion, the expression of DF3, Tn and sialosyl-Tn antigens is an effective histopathological indicator for carcinomas in the area of the ampulla of Vater, and the expression of DF3 and intestinal-MRP antigens is a useful indicator of the prognosis of the patients.

Key words: Carcinomas of the ampulla of Vater — Mucin antigen — Immunohistochemistry — Prognosis

Mucins are high-molecular-weight glycoproteins with oligosaccharides connected to serine or threonine residue(s) of the protein backbone (apomucin) by *O*-glycosidic linkages.[1] Alterations in the glycosylation of mucins have been described in various types of cancer.[1–13] These alterations include incomplete glycosylation resulting in accumulation of core oligosaccharides such as Tn antigen, and aberrant glycosylation resulting in disaccharides such as sialosyl-Tn (STn) antigen.[1–4] Many investigators have reported that Tn and STn antigens are useful tumor markers in various organs.[1,4–13] We also previously reported that Tn and STn antigens are useful indicators of malignancy in the pancreas, intrahepatic bile duct and ovary.[14–16] However, the expression of Tn and STn antigens in carcinomas of the ampulla of Vater has not been investigated systematically.

Moreover, little is known about the expression of different types of apomucin in the area of the ampulla of Vater. During recent years, several biochemical studies on the structures and organ specificities of mucin core proteins have been reported.[17–20] Our previous studies of the pancreatic and intrahepatic bile duct tumors disclosed that dominant expression of MUC1 apomucin was seen in invasive ductal carcinomas of the pancreas and cholangiocarcinomas of the liver with marked invasive growth and a poor prognosis, whereas dominant expression of MUC2 apomucin was seen in intraductal papillary tumors of the pancreas and bile-duct cystadenocarcinomas of the liver with expansive growth and a favorable prognosis.[14,15] These findings suggest that the pattern of apomucins expression is associated with the biological properties of neoplasms, and is useful to predict the prognosis of the patients. We are interested in investigating the relationship between the expression of MUC1 and MUC2 apomucins in carcinomas of the am-

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pulla of Vater and the prognosis of the patients. In the present study the expression of Tn, STn, DF3 and intestinal-MRP antigens in carcinomas of the ampulla of Vater was investigated systematically to determine whether the expression of these mucin antigens is useful as a histopathological indicator for the carcinomas, and to evaluate whether the expression of these apomucins is correlated with the biological behavior of the carcinomas.

MATERIALS AND METHODS

Tissue samples Tissue samples of carcinomas of the ampulla of Vater were obtained from 38 patients (17 males and 21 females) undergoing pancreatoduodenectomy in the Kagoshima-shi Medical Association Hospital from January 1985 to March 1994. The mean age of the patients was 64.9 years (range: 35–84 years). All the resected specimens were fixed in formalin, sectioned thinly into serial slices, and embedded in paraffin. We examined all the hematoxylin-eosin-stained sections from each slice to determine the origin of the carcinomas. The criteria in "General Rules for Surgical and Pathological Studies on Cancer of Biliary Tract" were used for diagnosis of the carcinoma of the ampulla of Vater in the present study.[21] According to the criteria, the carcinomas of the ampulla of Vater were distinguished from pancreas head carcinomas, lower bile duct carcinomas and periampullary duodenal carcinomas. The site of origin of each carcinoma is listed in Table I. The paraffin blocks containing the main lesions were cut into 4 μm serial sections for immunohistochemical staining of mucin antigens.

The ampulla of Vater is anatomically complex and adenocarcinoma may arise from one of three types of epithelium: duodenal mucosa (DM), terminal pancreatic duct (TPD) and terminal common bile duct (TCBD). We used non-neoplastic epithelium of DM, TPD and TCBD, which could be observed in 38, 16 and 12 cases examined, respectively, as the non-neoplastic counterpart.

Antibodies Monoclonal antibody (mAb) 91S8 (mouse IgM, ascites, developed in the laboratory of Dr. Y. S. Kim) was used to detect Tn antigen.[22] STn antigen was detected with mAb TKH2 (mouse IgG, purified ascites, Ohtsuka Pharmaceutical Co., Ltd., Diagnostic Division, Tokushima).[4] mAb DF3 (mouse IgG, Centocor CA15-3, Toray-Fuji Bionics, Lot No. 12445, Tokyo) was used to identify the mammary-type apomucin-related antigen,[23–27] although mAb DF3 binding to protein may be enhanced by the presence of carbohydrates.[23] Polyclonal antibody, anti-MRP (rabbit IgG, developed in the laboratory of Dr. Y. S. Kim), reacts with the 23-amino-acid, threonine-rich tandem repeat peptide of intestinal-type apomucin (intestinal-MRP antigen).[28]

Biotinylated affinity-purified horse antimouse IgG, goat antimouse IgM, goat antirabbit IgG and avidin-biotinylated horseradish peroxidase complex (ABC complex) were purchased from Vector Laboratories (Burlingame, CA) as the Vectastain ABC Kit.

Staining procedure Immunohistochemical stainings were done on formalin-fixed, paraffin-embedded tissue sections by an immunoperoxidase method using the ABC complex[29] as described previously,[5, 14–16] with some modification. 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 rehydration in decreasing concentrations of ethanol in water, the sections were washed in 0.01 mol/liter phosphate-buffered saline (PBS, pH 7.4). Then, 2% goat or horse serum in PBS was applied for 30 min at room temperature to prevent non-specific staining. In the staining using each antibody, the sections were incubated with primary antibodies [91S8, 1:2000; TKH2, 1:200; DF3, 1:10; anti-MRP, 1:600 dilutions, respectively, in PBS with 1% bovine serum albumin (PBS-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 the sections were then exposed to ABC complex for 30 min. The sections were washed with PBS three times, reacted with diaminobenzidine substrate for 10 to 30 min for visualization, rinsed with tap water, stained with hematoxylin and mounted. Negative controls had PBS, nonimmune mouse or rabbit serum in place of the primary antibodies.

Evaluation of the results by scoring We observed the whole area of carcinomas by sequentially examining low-power ($\times$ 10) optical fields in the sections stained with the antibodies and calculated the approximate percentage of positively stained neoplastic cells. The results were graded as follows: $-$; less than 5% of neoplastic cells stained; $+$; 5–50% of neoplastic cells stained; and $++$; over 50% of the neoplastic cells stained. Moreover, we evaluated the staining of cytoplasm, cell surface and associated secretory products (luminal contents). Cells were considered to be positive when at least one of these components was positive.

Comparison of the results with survival of the patients Survival of the patients was compared between the group with positive mucin antigens expression and the group with negative mucin antigens expression according to the Kaplan-Meier method. The significance of the difference of survival between the two groups was tested by use of the log rank test and Breslow-Gehan-Wilcoxon test. The data on mucin antigens expression were analyzed in connection with various pathological prognostic factors, such as tumor size, grade of pancreas invasion and lymph node status.

RESULTS

Expression patterns and staining intensity of each mucin antigen in the carcinomas are summarized in Table I, which also shows clinicopathological data (site of origin of carcinoma, tumor size, grade of pancreas invasion, lymph node status and stage) and the results of follow-up of each patient studied. The differences in the expression patterns of mucin antigens did not reflect the origin of the carcinoma, as shown in Table I.

Expression of each mucin antigen in non-neoplastic tissues and carcinomas Table II shows the expression of four mucin-associated antigens in the non-neoplastic epithelium of DM (38 cases), TPD (16 cases) and

Table I. Expression Patterns and Staining Intensity of Tn, STn, DF3 and Intestinal-MRP (I-MRP) Antigens in Carcinomas of the Ampulla of Vater, Prognostic Pathological Factors in Each tumor, and Follow-up of Each Patient Studied

Case	Age	Sex	Mucin antigen				Site[a]	TS[b]	LN[c]	PI[d]	Stage[e]	Outcome	
			Tn	STn	DF3	I-MRP							
1	60	M	++	++	++	−	A	A	+	+	III	dead	8 m
2	67	M	++	++	++	−	Ac	B	+	+	IV	dead	13 m
3	74	F	++	++	++	−	A	A	−	−	II	dead	2 m
4	74	F	++	++	++	−	Acb	B	+	+	III	dead	21 m
5	62	M	++	++	++	−	Ac	A	−	−	I	alive	75 m
6	73	M	++	++	++	−	Ac	A	+	−	II	alive	33 m
7	80	F	++	++	++	−	Abp	A	+	+	III	alive	20 m
8	69	F	++	+	++	−	Acd	A	−	−	II	alive	13 m
9	63	F	+	++	++	−	Acb	A	−	−	II	alive	21 m
10	35	M	+	++	++	−	Acb	B	+	−	III	dead	21 m
11	59	F	+	+	++	−	A	A	+	−	III	dead	13 m
12	73	M	+	+	++	−	A	B	+	+	II	dead	1 m
13	63	M	−	++	++	−	A	B	+	+	III	dead	18 m
14	79	F	++	+	++	−	Acd	B	−	−	I	dead	59 m
15	62	F	+	−	++	+	Ac	A	+	−	II	dead	48 m
16	72	F	+	+	++	+	Abc	B	+	−	II	dead	12 m
17	70	F	+	+	++	+	Acd	A	−	−	II	alive	25 m
18	84	M	+	+	+	+	A	A	−	−	II	alive	27 m
19	60	M	++	−	+	−	Acd	B	−	−	III	alive	74 m
20	69	F	+	−	+	−	Abc	B	+	−	II	dead	2 m
21	70	M	+	+	+	−	A	B	+	−	II	dead	26 m
22	60	M	+	+	+	−	A	B	+	+	IV	dead	1 m
23	73	F	+	+	+	−	A	B	+	+	III	dead	6 m
24	70	M	++	++	−	+	Abc	A	−	−	III	alive	22 m
25	62	F	++	+	−	+	Acd	A	−	−	II	alive	17 m
26	60	F	++	+	−	+	Adc	B	+	−	II	alive	79 m
27	73	F	+	++	−	+	Abc	A	−	−	III	alive	11 m
28	46	F	+	+	−	+	Abd	B	+	−	III	alive	22 m
29	61	M	−	+	−	+	A	A	−	−	I	alive	65 m
30	56	F	++	+	−	−	Acd	B	+	+	III	dead	10 m
31	69	F	++	+	−	−	A	A	−	−	I	alive	7 m
32	47	F	+	−	−	−	Acd	A	−	−	I	dead	58 m
33	73	M	+	++	−	−	Abc	B	+	−	II	alive	17 m
34	45	M	+	++	−	−	Ac	A	−	−	II	alive	63 m
35	58	M	+	+	−	−	Ab	B	+	−	II	dead	15 m
36	69	F	−	+	−	−	Acd	A	−	−	II	alive	11 m
37	57	M	−	−	−	−	Ac	A	+	−	III	alive	51 m
38	70	F	−	−	−	−	Ab	A	−	−	I	alive	57 m

a) Site: Origin of carcinoma according to the criteria in "General Rules for Surgical and Pathological Studies on Cancer of Biliary Tract."
b) TS (tumor size): A, >2 cm; B, 2 cm<.
c) LN (lymph node status): −, negative lymph node metastasis; +, positive lymph node metastasis.
d) PI (degree of pancreas invasion): −, no or minimal invasion (<0.5 cm); deep invasion (>0.5 cm).
e) The criteria in "General Rules for Surgical and Pathological Studies on Cancer of Biliary Tract."

Table II. Positive Expression of Tn Antigen, STn Antigen, DF3 Antigen and Intestinal-MRP (I-MRP) Antigen (%)

		Tn	STn	DF3	I-MRP
Duodenal mucosa (DM)	(n=38)	34 (89)	37 (97)	0 (0)	37 (97)
Terminal pancreatic duct (TPD)	(n=16)	3 (19)	0 (0)	2 (13)	0 (0)
Terminal common bile duct (TCBD)	(n=12)	0 (0)	0 (0)	0 (0)	1 (8)
Carcinomas of the ampulla of Vater (Ca)	(n=38)	33 (86)	32 (84)	23 (61)	11 (29)

TCBD (12 cases) and in the carcinomas of the ampulla of Vater (38 cases).

Expression of Tn antigen: In the non-neoplastic counterparts, Tn antigen was expressed exclusively in the supranuclear areas of both columnar epithelium and goblet cells of DM in 34 (89%) of 38 cases, and exclusively in the supranuclear areas of TPD epithelium in 3 (19%) of 16 cases, but was not expressed in TCBD (0%). Most of the carcinomas (33 of 38 cases, 86%) showed positive Tn expression mainly in the cytoplasm of the papillary structures (Fig. 1A), tubular structures (Fig. 2A) and poorly differentiated areas.

Expression of STn antigen: In the non-neoplastic counterparts, STn antigen was expressed exclusively in the mucigen droplets of the goblet cells of DM in 37 (97%) of 38 cases, but was not expressed in TPD or TCBD (0%). Most of the carcinomas (32 of 38 cases, 84%) showed positive STn expression in the cytoplasm, cell apices and luminal contents. The positive STn expression was seen in the papillary structures (Fig. 1B), tubular structures (Fig. 2B) and poorly differentiated areas. Simultaneous expression of Tn and STn antigens was seen in 29 (76%) of 38 cases examined (Table I).

Expression of DF3 antigen: In the non-neoplastic counterparts, DF3 antigen was expressed exclusively at the cell apex of TPD only in 2 (13%) of 16 cases, but was not expressed in DM or TCBD (0%). Twenty-three of the 38 carcinomas (61%) showed positive DF3 expression in the cytoplasm, cell apices and luminal contents. The expression of DF3 antigen was occasionally negative in the papillary structures (Fig. 1C), but was frequently positive in the tubular structures or in the poorly differentiated areas showing invasive growth (Fig. 2C).

Expression of intestinal-MRP antigen: In the non-neoplastic counterparts, intestinal-MRP antigen was expressed exclusively in the supranuclear areas of the goblet cells of DM in 37 (97%) of 38 cases, and also exclusively in the supranuclear areas of TCBD only in 1 (8%) of 12 cases, but was not expressed in TPD (0%). Eleven of the 38 carcinomas (29%) showed positive intestinal-MRP antigen expression mainly in the cytoplasm. The expression of intestinal-MRP antigen was seen frequently in the papillary structures which were usually observed in the surface areas of the carcinomas (Fig. 1D), but was occasionally negative in the tubular structures or in the poorly differentiated areas showing invasive growth (Fig. 2D).

Comparison of the mucin antigens expression with survival of the patients Fig. 3 showed a comparison of the survival of the patients between the group with positive mucin antigens expression and the group with negative mucin antigens expression according to the Kaplan-Meier method.

The group with positive Tn expression in the carcinoma tended to show poor survival compared with that with negative Tn expression, but there was no significant difference between the groups (Fig. 3A). The group with positive STn expression in the carcinoma also tended to show poor survival compared with that with negative STn expression, but there was no significant difference (Fig. 3B).

The patients with positive DF3 expression in the carcinoma showed significantly poor survival compared with those showing negative DF3 expression (Fig. 3C) ($P<0.05$). In contrast, the patients with positive intestinal-MRP expression in the carcinoma showed significantly more favorable survival than those having negative intestinal-MRP expression (Fig. 3D) ($P<0.05$).

Comparison of the mucin antigens expression with pathological prognostic factors

Relationship between tumor size and mucin antigens expression: There was no apparent relationship between the tumor size and the pattern of expression of mucin antigens (Table I).

Relationship between grade of pancreas invasion and mucin antigens expression (Table III): The expression rate of DF3 antigen was significantly higher in the cases with deep invasion into the pancreas (89%) than in the cases with no or minimal invasion (52%) ($P<0.05$). In contrast, the expression rate of intestinal-MRP antigen was significantly higher in the cases with no or minimal invasion into the pancreas (38%) than in the cases with deep invasion (0%) ($P<0.05$). The expression of Tn and STn antigens did not correlate with the grade of pancreas invasion.

Relationship between lymph node status and the expression of mucin antigens (Table IV): The expression rate of DF3 antigen was higher in the cases with positive lymph

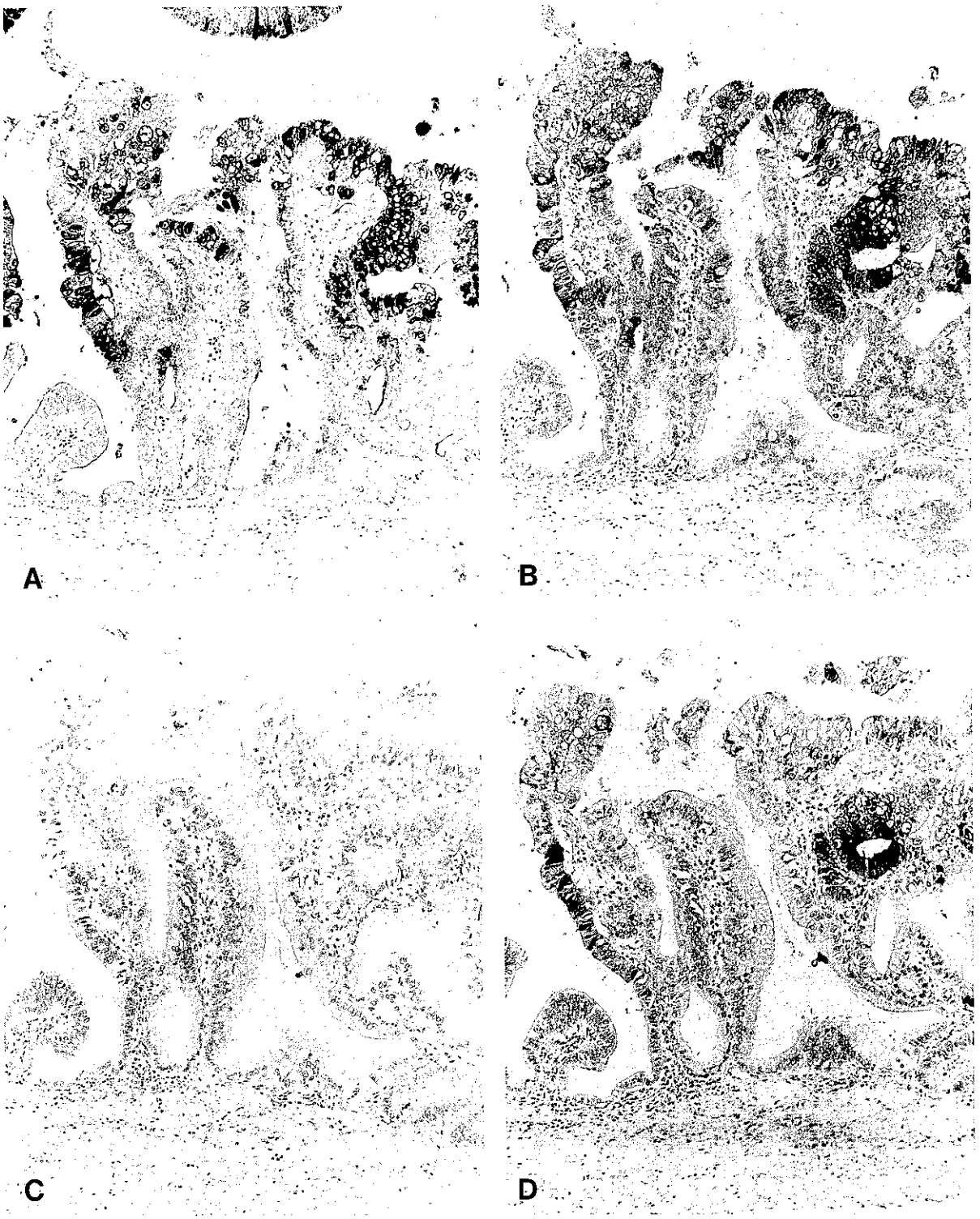

Fig. 1. Expression of each mucin antigen in the surface area of the carcinoma showing papillary configuration, serial sections of case No.25: Tn antigen (A), STn antigen (B), DF3 antigen (C) and intestinal-MRP antigen (D). Tn, STn and intestinal-MRP antigens were expressed in the supranuclear areas and/or cytoplasm (A, B, D), whereas DF3 antigen was not expressed in this area (C). ×90.

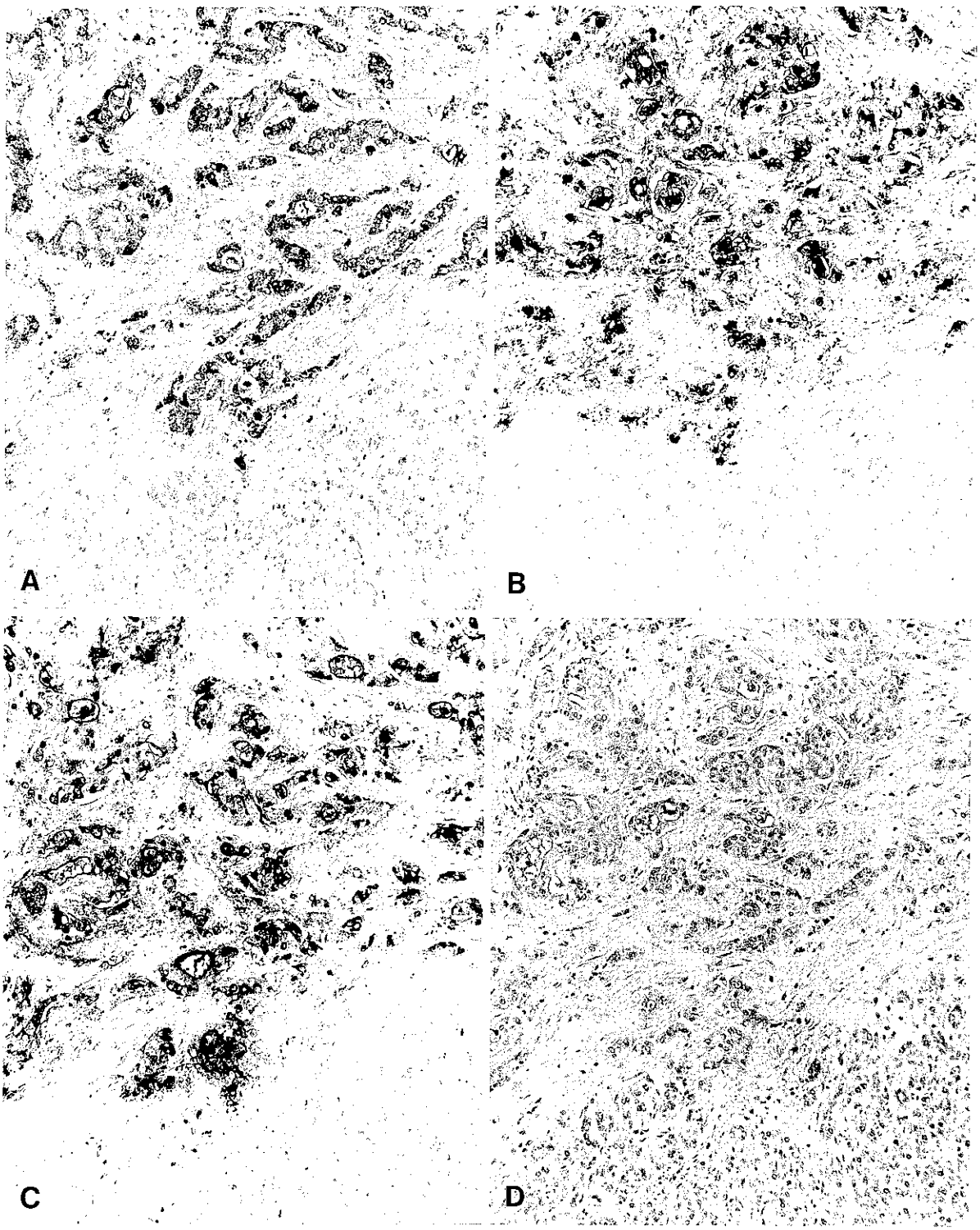

Fig. 2. Expression of each mucin antigen in the deep area of the carcinoma showing tubular configuration and invading the pancreas (lower side), serial sections of case No.2: Tn antigen (A), STn antigen (B), DF3 antigen (C) and intestinal-MRP antigen (D): Tn antigen was expressed mainly in the cytoplasm (A). STn and DF3 antigens were expressed mainly at the cell apex and in the cytoplasm (B, C). Intestinal-MRP antigen was not expressed in this area (D). ×90.

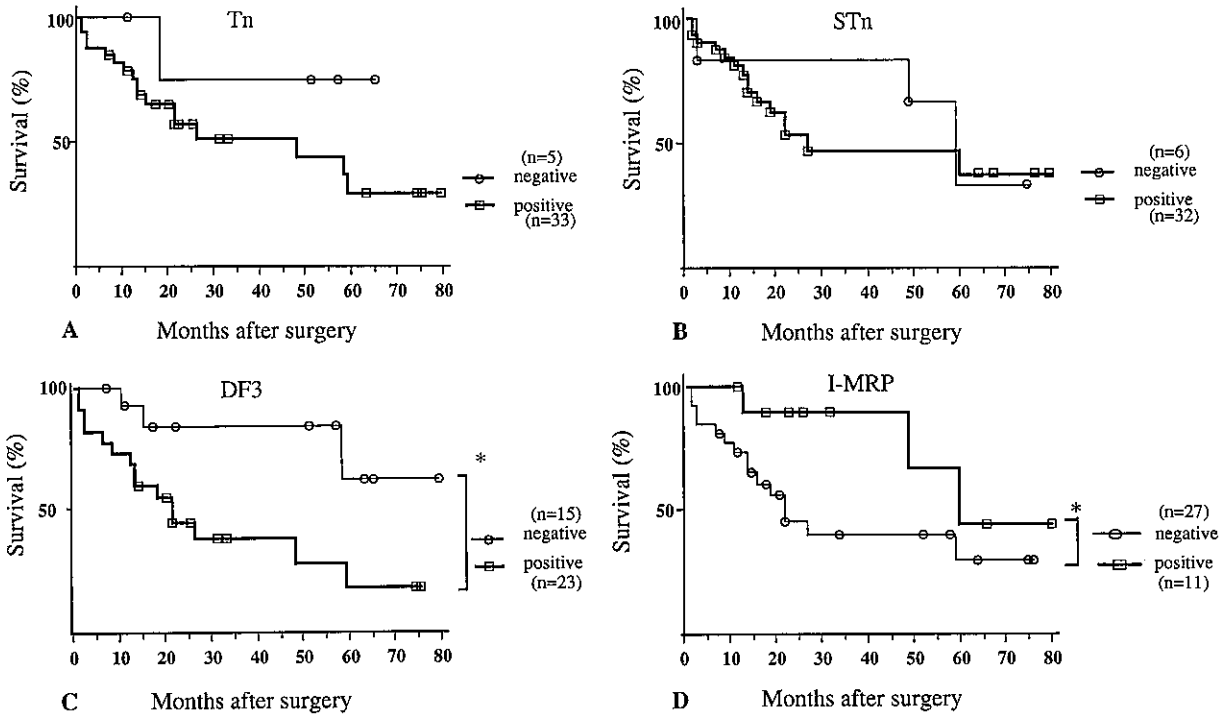

Fig. 3. Comparison of survival between the group with positive mucin antigens expression and the group with negative mucin antigens expression according to the Kaplan-Meier method: Tn antigen (A), STn antigen (B), DF3 antigen (C) and intestinal-MRP antigen (D). The patients with positive Tn and STn expression in the carcinoma tended to show poor survival compared with those having negative Tn and STn expression, although there was no statistically significant difference between these groups (A, B). The patients with positive DF3 expression in the carcinoma showed significantly poorer survival than those with negative DF3 expression (C). In contrast, the patients with positive intestinal-MRP expression in the carcinoma showed significantly more favorable survival than those with negative intestinal-MRP expression (D). $*P<0.05$.

Table III. Relationship between Grade of Pancreas Invasion and Mucin Antigens Expression (%)

		Tn	STn	DF3	I-MRP
With no or minimal invasion	(n=29)	25 (86)	23 (79)	15 (52)[a]	11 (38)[b]
With deep invasion	(n=9)	8 (89)	9 (100)	8 (89)[a]	0 (0)[b]

a) Significant difference between 'with no or minimal invasion (<0.5 cm)' and 'with deep invasion (>0.5 cm)' in the expression of DF3 antigen ($P<0.05$).
b) Significant difference between 'with no or minimal invasion (<0.5 cm)' and 'with deep invasion (>0.5 cm)' in the expression of intestinal-MRP (I-MRP) antigen ($P<0.05$).

Table IV. Relationship between Lymph Node Status and Mucin Antigens Expression (%)

		Tn	STn	DF3	I-MRP[a]
With negative metastasis	(n=17)	14 (82)	14 (82)	8 (47)	7 (41)
With positive metastasis	(n=21)	19 (90)	18 (86)	15 (71)	4 (19)

a) Intestinal-MRP.

node metastasis (71%) than in the cases with negative lymph node metastasis (47%), whereas the expression rate of intestinal-MRP antigen was higher in the cases with negative lymph node metastasis (41%) than in the cases with positive lymph node metastasis (19%), although there was no statistically significant difference between the expression rates in these groups. The expression of Tn and STn antigens showed no correlation with the lymph node status.

DISCUSSION

In the present study, the expression of mucin core region carbohydrate antigens associated with the early steps of the mucin glycosylation pathway (Tn and STn antigens) and that of MUC1 and MUC2 apomucins were examined systemically in carcinomas of the ampulla of Vater in comparison with the expression of the mucin antigens in the non-neoplastic counterparts, i.e., DM, TPD and TCBD.

Among the four mucin associated antigens examined, DF3 antigen was an effective histopathological tumor marker, because DF3 antigen showed a relatively high expression rate (61%), but was not expressed in the non-neoplastic epithelium except for low expression (13%) in TPD in the region of ampulla of Vater. We have previously reported that expression of Tn and STn antigens serves as a highly effective histopathological tumor marker in the pancreas,[14] intrahepatic bile duct,[15] and ovary.[16] It has been reported that three-fourths of colon cancer cases expressed both Tn and STn antigens simultaneously.[1] In the present study, Tn and STn antigens showed high expression rates in the carcinoma tissues in the region of the ampulla of Vater, and simultaneous expression of Tn and STn antigens was seen in 29 (76%) of 38 cases examined (Table I). On the other hand, non-neoplastic TPD and TCBD showed rare or no expression of Tn and STn antigens. Although the non-neoplastic DM showed positive Tn and STn expression in most of the cases examined, the distribution of both antigens was highly restricted to the supranuclear area of the epithelium or mucigen droplets of the goblet cells, respectively. Thus, a very good distinction between the carcinoma tissues and the non-neoplastic tissues could be obtained by the immunohistochemical staining method using these antibodies.

The pattern of expression of MUC1 apomucin (DF3 antigen) and that of MUC2 apomucin (intestinal-MRP antigen) showed an inverse relationship with respect to the survival of the patients with carcinoma of the ampulla of Vater, as shown in Fig. 3C and 3D. The patients with positive DF3 expression in the carcinoma showed significantly poorer survival than patients having negative DF3 expression (Fig. 3C). In contrast, the patients with positive intestinal-MRP expression in the carcinoma showed significantly more favorable survival than those with negative intestinal-MRP expression (Fig. 3D). The high expression rate of DF3 antigen in the carcinomas with deep invasion into the pancreas (Table III) and with positive lymph node metastasis (Table IV) may be related to the poor prognosis of the patients with positive DF3 expression. On the other hand, the high expression rate of intestinal-MRP antigen in the carcinomas with no or minimal pancreas invasion (Table III) and with negative lymph node metastasis (Table IV) may be related to the favorable prognosis of the patients with positive intestinal-MRP expression. These findings are similar to our previous findings on pancreatic and intrahepatic bile-duct tumors, demonstrating that MUC1 apomucin expression is a significant feature in the invasive carcinomas with poor prognosis, whereas MUC2 apomucin expression is dominant in the non-invasive tumors with favorable prognosis.[14, 15] Since the region of the ampulla of Vater develops from the hepatic diverticulum from the caudal part of the foregut in the embryo, from where pancreas head and hepatobiliary system arise,[30, 31] the pattern of apomucins expression in the carcinomas of the ampulla of Vater may be expected to show similarity to that in tumors of these organs.

In a previous immunohistochemical study, we also noted the expression of MUC2 apomucin in mucinous carcinoma of the breast, which has less frequent lymph node metastasis and a more favorable outcome compared with invasive ductal carcinoma.[32] In mucinous carcinomas, MUC2 apomucin expression is thought to be strongly related to the formation of the special histologic configuration of this type and to the production of abundant extracellular mucin, since MUC2 glycoprotein is a secretory mucin having no transmembrane domains, according to the current working model of the MUC2 glycoprotein.[33, 34] Komaki *et al.* suggested that floating of cancer cells in abundant mucus without having contact with the stroma is important for a favorable prognosis in mucinous carcinoma patients.[35] They also commented that abundant mucus within the tumor acts as a barrier to cancerous extension in mucinous carcinomas. Hence, the MUC2 gene expression may be related with the favorable prognosis of the patients with mucinous carcinoma through the production of abundant mucin. In intraductal papillary tumors of the pancreas and bile-duct cystadenocarcinoma of the liver too, the expression of MUC2 apomucin seems to play an important role through the production of abundant extracellular mucin, forming the characteristic configuration of non-invasive and expansive growth which may be related with the favorable prognosis of the patients. However, in carcinomas of the ampulla of Vater examined in the present study, there was no production of abundant mucin. Fur-

ther investigation is necessary to explain the mechanism of the better outcome of the patients with positive MUC2 apomucin expression than those with negative expression in the carcinoma of the ampulla of Vater.

MUC1 mucin contains transmembrane domains and is synthesized as a membrane protein rather than as a secreted protein.[20] Recently, Nakamori *et al.* reported that highly glycosylated "mature" MUC1 mucin may be a marker of progression and metastasis of human colorectal carcinoma.[36] Since the binding of mAb DF3 to protein seems to be enhanced by the presence of carbohydrates,[23] DF3 antigen may be a glycosylated "mature" MUC1 mucin. As seen in colorectal carcinoma,[36] the positive DF3 expression may also be related with invasive biological character of the carcinoma of the ampulla of Vater.

Itzkowitz *et al.* reported that colon cancer patients showing positive STn expression showed a poor prognosis compared with the patients showing negative STn expression.[9] We found that positive Tn expression is related with poor prognostic factors such as positive lymph node metastasis or large tumor size in the scirrhous subtype of mammary invasive ductal carcionoma.[37] In the present study the patients with positive Tn and STn expression in the carcinoma tended to show poor survival compared with those having negative Tn and STn expression, although there was no significant difference between the two groups (Figs. 3A and 3B). Further study of Tn and STn expression in large numbers of carcinomas of the ampulla of Vater may determine whether Tn and STn expression can serve as a prognostic indicator.

In summary, although the number of patients studied was small, our current results suggest that the expression of DF3 and intestinal-MRP antigens is a useful indicator of the biological properties of carcinomas of the ampulla of Vater and may be available to predict the prognosis of patients with the carcinoma. Further, the expression of DF3, Tn and STn antigens is an effective histopathological indicator for carcinomas in the area of the ampulla of Vater. The results presented in this study provide a basis for further clinically oriented or fundamental studies to elucidate the underlying mechanism of the mucin antigens expression in carcinomas of the ampulla of Vater.

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