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Increased expression of MUC1 in advanced pancreatic cancer

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Background. MUC1 is associated with tumor invasion and metastasis, and is expressed in pancreatic cancer with a high frequency. This study explored whether MUC1 expression affected the survival of patients with pancreatic cancer. ***Methods.*** Tissue specimens obtained from 70 patients with invasive ductal carcinoma of the pancreas, in pTNM stage III or IV, were immunostained with the anti-MUC1 monoclonal antibody DF3. The results of immunostaining were determined to be positive when more than 50% of the total cancer cells were positively stained. Association of the expression of the DF3 epitope with clinicopathological parameters or patients' survival was statistically evaluated. ***Results.*** The incidence of positivity of MUC1 expression was 55.7% (39/70) and this incidence was significantly higher in pTNM stage IV than in stage III (odds ratio [OR], 4.015; 95% confidence interval [CI], 1.459–11.0541; $P = 0.0076$). As there was a clear difference in overall survival between pTNM stages III and IV ($P = 0.0016$), the effect of MUC1 expression on survival was separately evaluated in each stage. It was shown that the expression of MUC1 was associated with unfavorable overall survival in stage IV ($P = 0.0197$). ***Conclusions.*** Our data suggest that the expression of MUC1 may be related to the progression of pancreatic cancer.

Key words: MUC1, pancreatic cancer, invasive ductal carcinoma, pTNM stage, overall survival

Introduction

MUC1 is a heavily glycosylated type I transmembrane protein mainly expressed in epithelial cells.[1] The extracellular domain consists of 30 to 90 tandemly repeated sequences, with one repeat containing 20 amino acids with at least three *O*-glycosylation sites.[1,2] Part of the repeat sequence (APDTRPA), which is highly immunogenic in mice, was shown to be a tumor-associated epitope. It was then revealed that a number of monoclonal antibodies (mAbs) established against tumor cells recognized this epitope. The expression of MUC1 has been extensively studied using these mAbs, and has been demonstrated to be related to the development of a variety of tumors, including esophageal,[3] gastric,[4] colorectal,[5] and breast cancers.[6] We have shown that the overexpression of MUC1 by cDNA transfer enhances the in vitro motility and invasiveness of gastric cancer MKN45[7] and pancreatic cancer S2-013 cells.[8] Furthermore, MUC1 transfectants of MKN45 and S2-013 cells transplanted into nude mice showed increased in vivo invasiveness[7] and spontaneous lung metastasis,[8] respectively. There is accumulating evidence that MUC1 expression in gastric cancer is associated with a poor outcome.[3,9–12] In contrast, the relation of MUC1 expression to prognosis in invasive ductal carcinoma (IDC) of the pancreas, which is the most common type of pancreatic cancer, remains unknown. The expression of MUC1 in normal pancreas and IDC tissues has thus far been revealed by in situ hybridization and immunohistochemistry.[13–17] Although the staining intensity of anti-MUC1 mAb has been evaluated in three previous reports,[15–17] there has been no study of the association of MUC1 expression with clinical outcome. We therefore performed immunohistochemical detection of MUC1 in IDC of the pancreas to clarify whether MUC1 expression is relevant to clinicopathological parameters and clinical outcomes.

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Subjects, materials, and methods

Human tissue samples

Tissue specimens were obtained from 70 patients with IDC surgically operated on at Kagoshima University Hospital, Hamamatsu Western Medical Center, and Yamaguchi University Hospital between 1990 and 1998. Only patients whose tumors had been in pTNM stage[18] III or IVa-b were included in the study, to reduce the effect of tumor stage on patients' survival. The mean age of the patients was 63 years (range, 30–79 years), and there were 45 men and 25 women. The median length of the follow-up of the patients alive at the end of follow-up was 77.2 months (range, 36–127 months). The survival period after surgical operation was used as survival time for statistical analysis. The frequency of each histological grade[18] of IDC was as follows: pG1 (well-differentiated type), 44/70 (62.9%); pG2 (moderately differentiated type), 18/70 (25.7%); pG3 (poorly differentiated type), 8/70 (11.4%); and pG4 (undifferentiated type), 0/70 (0%). Formalin-fixed and paraffin-embedded tissue sections (5-μm-thick) were used for immunohistochemical study, with an anti-MUC1 mAb (DF3; Dako, Carpinteria, CA, USA).

Immunohistochemistry

Immunostaining was performed by an immunoperoxidase method, as described previously,[19] with some modifications. Briefly, after being deparaffinized, each section was treated with Target Retrieval Solution (Dako, Tokyo, Japan) for 10 min at 105°C. The section was then rehydrated and incubated with fresh 0.3% hydrogen peroxide in methanol for 20 min at room temperature and then washed with phosphate-buffered saline (PBS). Normal rabbit serum (5%) was applied for 20 min and removed by blotting. The sections were incubated with primary antibody for 60 min at room temperature, washed three times in buffer, and incubated with secondary antibody (peroxidase-conjugated rabbit anti-mouse immunoglobulin diluted 1/40 in PBS) for 30 min at room temperature. After three washes, the sections were incubated with diaminobenzidine tetrahydrochloride in 0.03% hydrogen peroxide for 5–10 min, washed, counterstained with hematoxylin or methylgreen, rinsed in tap water, and mounted. The results of immunostaining were judged to be positive, irrelevant of staining pattern, when more than 50% of the total cancer cells were positively immunostained. Diluted mouse serum (1:200) was used as a negative control antibody for the primary antibody.

Statistical analysis

Fisher's exact test was used to compare categorical data between the MUC1-positive and MUC1-negative groups. Odds ratios (ORs) and 95% confidence intervals (CIs) were also calculated. The cumulative overall survival of patients was assessed by the Kaplan-Meier method, and comparison of the MUC1-positive and MUC1-negative survival curves was performed using the log-rank test. A P value of less than 0.05 was considered significant.

Results

Tissue specimens with positively immunostained areas of more than 50% of the cancer region were arbitrarily determined as positive. Most cancer cells were positively immunostained in the tissue specimens judged as positive, as shown in Fig. 1A. Apical and cytoplasmic staining patterns were predominantly observed in histological grades pG1 and pG3, respectively. Most tumors of pG2 grade had both patterns. Positive immunoreactivity for the DF3 epitope of MUC1 was identified in 39 of the 70 tumor specimens (55.7%). There was no association of MUC1 expression with age, sex, or histological grade, but the incidence of MUC1 positivity in pTNM stage IV (which includes both stages IVa and IVb) was significantly higher than that in stage III (OR, 4.015; 95% CI, 1.459–11.054; $P = 0.0070$; Table 1).

We then statistically assessed the effect of MUC1 expression on overall survival. Patients with MUC1-positive tumors had a worse outcome compared with patients whose tumors were MUC1-negative ($P = 0.0170$; Fig. 2A). However, because a clear difference in overall survival was observed between pTNM stages III and IV ($P = 0.0016$; Fig. 2B), the effect of MUC1 expression on survival was separately evaluated in each stage. It was revealed that MUC1 expression was associated with an unfavorable overall survival in stage IV ($P = 0.0197$; Fig. 2D), but not in stage III (Fig. 2C).

Discussion

IDC of the pancreas has a very poor prognosis, because early detection is difficult and effective treatments are not established.[20] Because most of the patients with IDC are thus in advanced stages at diagnosis, we tested only IDC tissue specimens from patients with stage III or IV in this study. It was shown that the expression of MUC1 (DF3 epitope) in more than 50% of the tumor region of the tissue specimens was associated with the pTNM stage of IDC. The incidence of MUC1 positivity was significantly higher in stage IV than in stage III. The

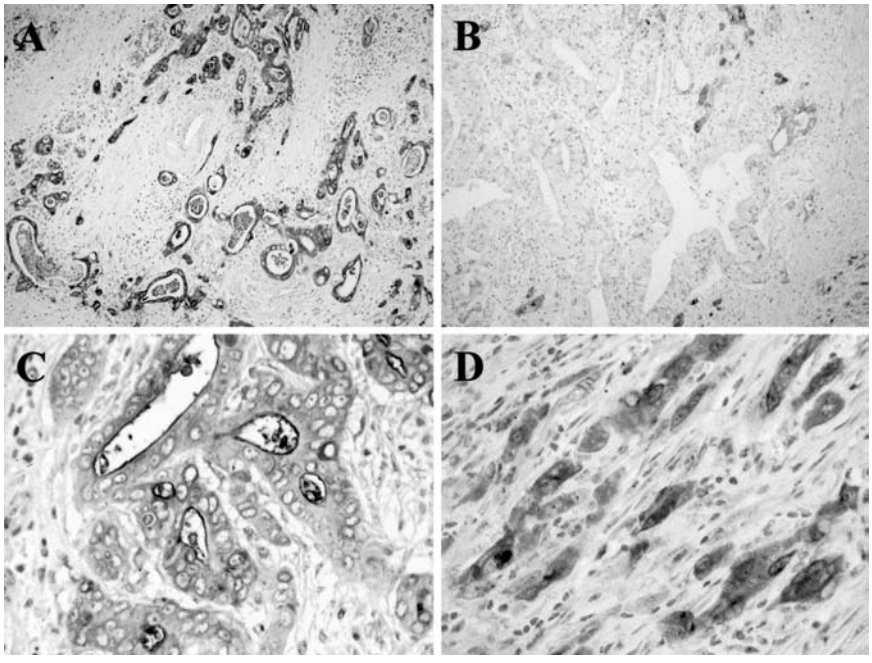

Fig. 1A–D. Immunohistochemical detection of DF3 epitope in pancreatic cancer tissues. Representative cases judged to be positive (**A**) and negative (**B**) are shown; also, apical (cytoplasm was also positively immunostained) (**C**) and cytoplasmic (**D**) staining patterns are shown. **A**, **B** ×200; **C**, **D** ×400

Table 1. Association of MUC1 expression with clinicopathological parameters

Clinicopathological parameters	MUC1 staining (positive/total)	MUC1-positive (%)	P^a	OR (95% CI)
Age at diagnosis (years)				
<60	15/22	68.2	NS	
≥60	24/48	50.0		
Sex				
Male	25/45	55.6	NS	
Female	14/25	56.0		
Histological grade				
pG1	27/44	61.4	NS	
pG2–3	12/26	46.2		
pTNM stage				
III	10/28	35.7	0.0076	4.015 (1.459–11.054)
IV	29/42	69.0		

OR, odds ratio; CI, confidence interval; NS, not significant
[a]Statistical analysis was performed by Fisher's exact test

relationship between MUC1 expression and clinical outcome was then evaluated, revealing that MUC1-positive patients had a worse survival rate compared to MUC1-negative ones in pTNM stage IV. The reason why no significant difference in survival was observed in stage III may be due to the smaller sample size and lower frequency of MUC1 expression compared to stage IV. Further studies will be required to confirm this lack of difference. Our data suggested that the expression of MUC1 (DF3 epitope) may be related to the progression of IDC.

Although mAb DF3 recognizes the peptide core sequence of MUC1,[21] it was shown that oligosaccharides could be involved in the structure of the DF3 epitope.[22,23] Hull et al.[22] demonstrated that DF3 antigen purified from breast cancer BT-20 cells contained peanut agglutinin-binding disaccharide, a component of T antigen, and its mono- and disialylated derivatives. Immature glycosylation of tumor mucins results in the simplification of oligosaccharide structures and the accumulation of precursors, such as the core oligosaccharides of T or Tn antigen and precursor type I.[24,25] Thus, the prognostic significance of the DF3 epitope shown here could reflect tumor-associated alterations in the *O*-linked oligosaccharides of MUC1 protein. Balague et al.,[16] by using the anti-MUC1 synthetic peptide mAb BC-1, reported that more than 50% of pancreatic cancer cells were positively stained in nine out of ten (90%)

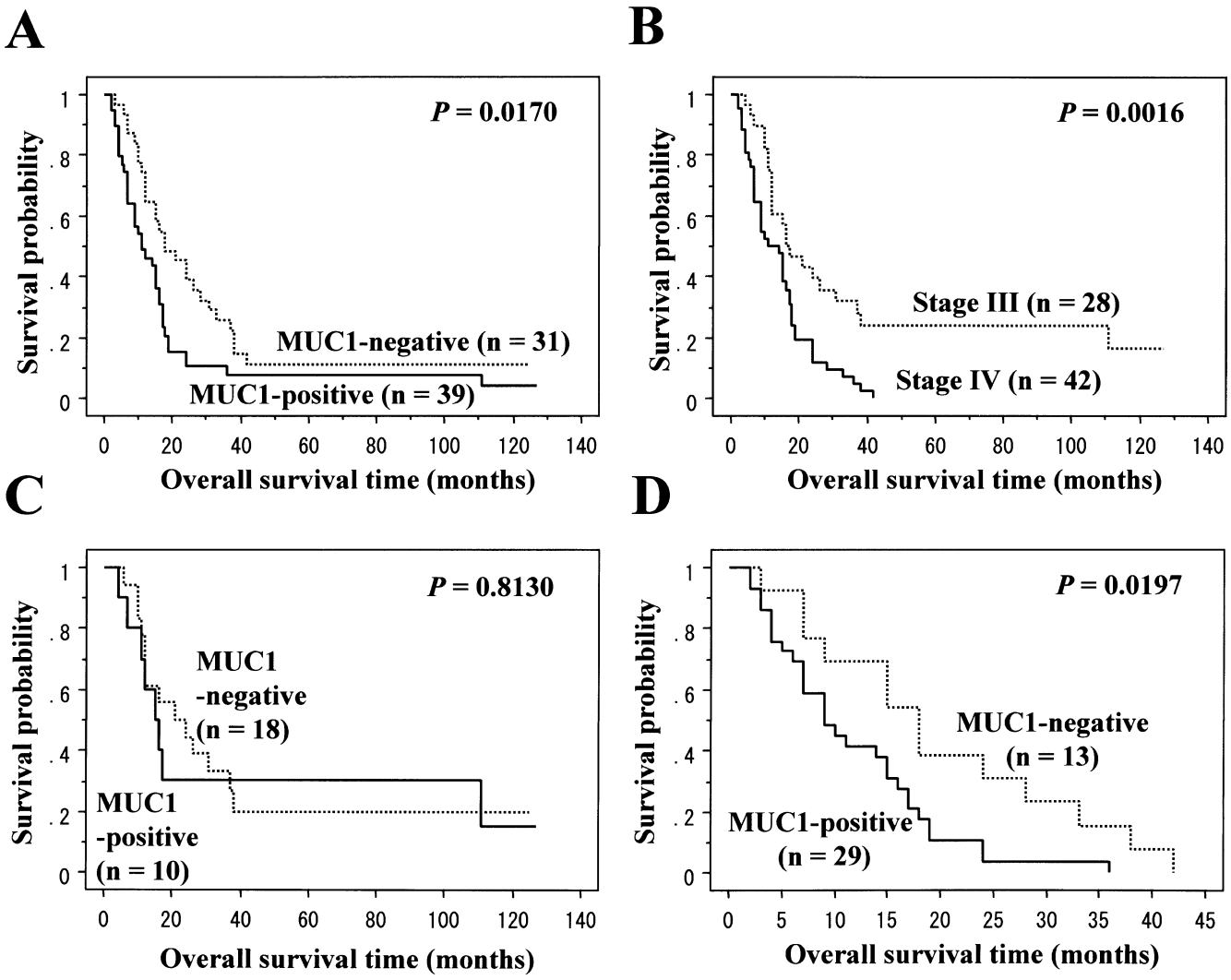

Fig. 2A–D. Kaplan-Meier survival curves for pancreatic cancer patients stratified by MUC1 expression or stage. **A** Overall survival of patients with MUC1-positive tumors (n = 39) and those with MUC1-negative tumors (n = 31). **B** Overall survival of patients with pTNM stage III (n = 28) and those with stage IV (n = 42). **C** Overall survival of patients with MUC1-positive tumors (n = 10) and those with MUC1-negative tumors (n = 18) in stage III. **D** Overall survival of patients with MUC1-positive tumors (n = 29) and those with MUC1-negative tumors (n = 13) in stage IV

tissue specimens. In contrast, in studies with mAb DF3,[14,15,17] the expression levels of the epitope tended to be various in each case of IDC. We therefore attempted to evaluate the expression of the DF3 epitope in IDC of the pancreas.

In this study, we adopted a cutoff level of 50%, meaning that immunostaining with mAb DF3 was judged to be positive when the rate of positive cancer cells in a tissue specimen was more than 50%. In a recent review of immunohistochemical studies of MUC1 in breast cancer,[6] it was seen that this cutoff level was the one most frequently used in previous studies. It is reasonable to expect that molecules affecting the development of cancer and the prognosis of patients would be widely expressed in surgically resected cancer tissues.

The mechanism by which DF3 epitope expression affects the progression of IDC is unclear. However, the finding seems consistent with our previous one that the overexpression of MUC1 in pancreatic cancer S2-013 cells resulted in enhanced in vivo spontaneous lung metastasis.[8] It is hypothesized that, by its anti-adhesive activity, MUC1 expressed on the surfaces of cancer cells enhances their detachment from the primary site, and accelerates distant metastasis, probably through binding to E-selectin[26] and/or intercellular adhesion molecule-1 (ICAM-1)[27,28] of endothelial cells. The anti-adhesive activity of MUC1 is based on its linear and rigid structure, for which the presence of oligosaccharides is essential, and is due to the overall negative charge as a result of extensive sialylation of the oli-

gosaccharides.[29] We have shown that the treatment of MUC1 transfectants with benzyl-α-GalNAc, an *O*-glycosylation inhibitor, abolished the enhanced effects of MUC1 on the in vitro motility and invasiveness of gastric and pancreatic cancer cells.[7,8] Burdick et al.,[30] by immunoprecipitation and immunoblotting, demonstrated that tumor-associated oligosaccharides such as sialyl-Lewis^a and sialyl-Lewis^x were newly synthesized on MUC1 protein after MUC1 cDNA transfer to cultured colon cancer cells. This suggests the importance of oligosaccharides synthesized on MUC1 protein in cancer metastasis. Expression of the DF3 epitope in pancreatic cancer tissues reflects dysregulated oligosaccharide biosynthesis, as described above. Thus, the presence of abnormal oligosaccharides on MUC1 may play an important role in the development of pancreatic cancer.

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