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ORIGINAL ARTICLE

MUC5AC-NEGATIVE PHENOTYPE IS CORRELATED WITH POOR PATIENT PROGNOSIS OF PANCREAS HEAD DUCTAL CARCINOMA.

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Abstract Both pancreas head ductal carcinoma (PHDC) and distal bile duct carcinoma (DBDC) are located within the pancreas head/intra-pancreatic bile duct region, and are the most aggressive malignancies with poor patient prognosis. In the present study, we demonstrated clinicopathological features and patients prognosis of PHDC/DBDC. We examined total 87 surgically resected cases of PHDC (40 cases) and DBDC (47 cases). PHDC showed frequent neural invasion (85.0%) and lymph node metastasis (77.5%), compared with DBDC (57.4% and 40.4% respectively), resulting in the poorer prognosis (P=0.0219) than DBDC. In addition, PHDC expressed MUC2 (10.0%) and MUC6 (25.0%) less frequently, compared with DBDC (36.2% and 55.3%, respectively). MUC5AC-negative PHDC exhibited significantly poorer patient's prognosis, compared with MUC5AC-positive PHDC (P=0.0111), MUC5AC-positive DBDC (P=0.000162), and MUC5AC-negative DBDC (P=0.00416). In conclusion, MUC5AC-negative PHDC showed significantly poor patient's prognosis.

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Key words: mucin; MUC5AC; pancreas cancer; bile duct cancer; patient prognosis.

Introduction

Pancreas head ductal carcinoma (PHDC) is one of the most lethal diseases, while both PHDC and distal bile duct carcinoma (DBDC) are aggressive malignancies with poor patient prognosis [1-4]. Despite of the advances in surgical techniques and adjuvant therapy, the 5-year survival rates of PHDC and DBDC are approximately 7% and 20%, respectively. PHDC and DBDC frequently exhibit obstructive jaundice due to the bile duct stenosis/obstruction; *i.e.*, PHDC involves intra-pancreatic bile duct, while DBDC invade pancreas head tissue. Both PHDC and DBDC are located within the pancreas head/intra-pancreatic bile duct region (Fig. 1), and the clinical differential diagnosis between PHDC and DBDC is difficult. However, the differential diagnosis between PHDC and DBDC is very important because of the different patient's prognosis of these tumors.

Mucin is a family of high molecular weight glycoproteins, produced by epithelial tissues. The mucin shares the common features of having an extensive tandem repeat region, and a peptide domain containing a high percentage of serine and threonine [5-7]. Recently, many human mucin genes have been distinguished by cDNA cloning as follows: MUC1, MUC2, MUC3A, MUC3B, MUC4, MUC5AC, MUC5B, MUC6, MUC7, MUC8, MUC12, MUC13, MUC15, MUC16, MUC17, MUC19, and MUC20 [8]. Although some mucins are membrane-bound due to the presence of a hydrophobic membrane-spanning domain that favors retention in the plasma membrane, most mucins are secreted onto mucosal surfaces.

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It is necessary to make the pathological differential diagnosis between PHDC and DBDC in order to perform effective therapeutic strategies for these malignant tumors. In this study, we analyzed the mucin phenotype and clinical outcome of 40 surgically-resected cases of PHDC, compared with 47 cases of DBDC.

Materials and Methods

Patients

We investigated 87 surgically resected cases of PHDC and DBDC treated between January 2007 and December 2012, after obtaining each patient's informed consent with to use their clinical records and pathology specimens at Hirosaki University Hospital. Survival data were obtained from hospital medical charts, and median observation period was 26.4 months (87 cases). The series consisted of 47 men and 40 women with a median age of 66.7 years (range 31-83 years). Curative resection (pancreaticoduodenectomy or pylorus-preserving pancreaticoduodenectomy or subtotal stomach-preserving pancreaticoduodenectomy) and regional lymph node dissection were performed. The present study followed the principles of the World Medical Association Declaration of Helsinki 1964.

Pathological analysis

All surgically resected specimens were routinely fixed with 10% formalin, then embedded in paraffin, and stained with hematoxylin and eosin (H&E) for pathological evaluation. The following histological features were assessed: depth of invasion (T-grade), histological type, lymphatic invasion, venous invasion, neural invasion, and lymph nodal metastasis. Degrees of lymphatic, venous and neural invasions were classified as follows: 0, no invasion; 1, mild invasion; 2, moderate invasion; and 3, severe invasion. These data were evaluated according to our previous study [2, 9] with reference to the World Health Organization classification [1], and staged according to the TMN classification of the International Union Against Cancer (UICC) [10]. We also investigated mucin phenotypes of PHDC and DBDC using immunohistochemical procedure described as follows.

Immunohistochemistry

For histological examination, PHDC/DBDC specimens were routinely fixed with formalin, embedded in paraffin, thin-sectioned. Four-μm-thick sections were mounted on saline-coated glass slides. Immunohistochemical examination was performed on deparaffinized sections using the standard avidin-biotin-peroxidase complex method with automated immunostainer (Benchmark XT; Ventana Medical System, Tucson, AZ, USA) according to our previous study [9, 11]. We used MUC1, MUC2, MUC5AC and MUC6 to clarify mucin expression of PHDC and DBDC. The antibodies used are listed in Table 1.

Evaluation of immunohistochemistry

Two investigators (SS, HK) simultaneously assessed the immunohistochemical results without any patient's clinicopathological data. Luminal membranous immunoreactivities of the tumor were judged as positive for MUC1, and

Table 1 Antibodies for immunohistochemistry

Antigen	Monoclonal/polyclonal	Clone	Dilution	Source
MUC1	Monoclonal, mouse	Ma695	1 : 50	Novocastra
MUC2	Monoclonal, mouse	Ccp58	1 : 50	Novocastra
MUC5AC	Monoclonal, mouse	CLH2	1 : 100	Novocastra
MUC6	Monoclonal, mouse	CLH5	1 : 100	Novocastra

Table 2 Clinicopathological factors and mucin expression of PHDC/DBDC

Variables	PDAC	DBDA	*P*-Value
Age			
＜65	19	17	0.285
≧65	21	30	
Gender			
Male	16	31	0.0155*
Female	24	16	
Histological differentiation			
well, mod, pap	33	40	0.97
por, others	7	7	
Depth of invasion			
T1	1	6	0.118
T2, 3, 4	39	41	
Lymphatic invation			
ly0, 1	6	29	1
ly2, 3	34	18	
Venous invasion			
v0, 1	13	25	0.0525
v2, 3	27	22	
Neural invation			
ne0, 1	6	20	0.0103*
ne2, 3	34	27	
Lymph node metastasis			
pN(−)	9	28	0.00108*
pN(+)	31	19	
MUC expression			
MUC1			
+	35	43	0.798
−	5	4	
MUC2			
+	4	17	0.00955*
−	36	30	
MUC5AC			
+	19	30	0.126
−	21	17	
MUC6			
+	10	26	0.00421*
−	30	2	

PHDC, pancreas head ductal carcinoma; DBDC, distal bile duct carcinoma well, well-differentiated adenocarcinoma; mod, moderately differentiated adenocarcinoma; pap, papillary adenocarcinoma; por, poorly differentiated adenocarcinoma. *Statistically significant: $P<0.05$.

cytoplasmic immunoreactivities as positive for MUC2, MUC5AC and MUC6. According to the above immunohistochemical expression of each mucin, the cases were divided into two groups; a negative group in which < 10% of tumor cells were stained, and a positive group in which ≧ 10% were stained.

Statistical analysis

Statistical comparisons between two groups were analyzed using both Chi square test and Fisher's exact test. Survival curves were constructed using the Kaplan-Meier method and differences in survival were evaluated using log-rank test. The relative prognostic factors were analyzed with the Cox's proportional hazards regression model. Differences were considered

Table 3 Clinicopathogical factors and MUC5AC expression of PHDC/DBDC

Variables	MUC5AC(−)	MUC5AC(+)	*P*-Value
Age			
＜65	18	18	0.317
≧65	20	31	
Gender			
Male	22	25	0.523
Female	16	24	
Histological differentiation			
well, mod, pap	30	43	0.415
por, Other	8	6	
Depth of invasion			
T1	2	5	0.461
T2, 3, 4	36	44	
Lymphatic invation			
ly0, 1	10	25	0.0197*
ly2, 3	28	24	
Venous invasion			
v0, 1	13	25	0.116
v2, 3	25	24	
Neural invation			
ne0, 1	10	16	0.521
ne2, 3	28	33	
Lymph node metastasis			
pN(−)	12	25	0.0688
pN(+)	26	24	
MUC expression			
MUC1			
+	34	44	1
−	4	5	
MUC2			
+	1	20	1
−	37	29	
MUC6			
+	5	31	1
−	33	18	

PHDC, pancreas head ductal carcinoma; DBDC, distal bile duct carcinoma
well, well-differentiated adenocarcinoma; mod, moderately differentiated adenocarcinoma; pap, papillary adenocarcinoma; por, poorly differentiated adenocarcinoma. *Statistically significant: $P<0.05$.

to be significant if the p-value was less than 0.05. All statistical evaluations were performed using R (http://www.r-project.org), and PASW statistics software (version 18.0; SPSS, Inc., Chicago, IL, USA).

Results

Clinicopathological factors and mucin expression of PHDC/DBDC

Clinicopathological factors and mucin expression of PHDC/DBDC are summarized in Tables 2 and 3. Both of PHDC and DBDC were mainly composed differentiated adenocarcinoma, i.e., well-differentiated, moderately differentiated or papillary adenocarcinoma (Fig. 1). The majorities of PHDC/DBDC were pT2 - pT4 advanced cancers, *i.e.*, PHDC more than 2 cm in size, and DBDC beyond the bile duct wall. On the other hand, PHDC significantly showed frequent neural invasion (ne2,3: 34/40, 85.0%) and lymph node metastasis (pN(+): 31/40, 77.5%),

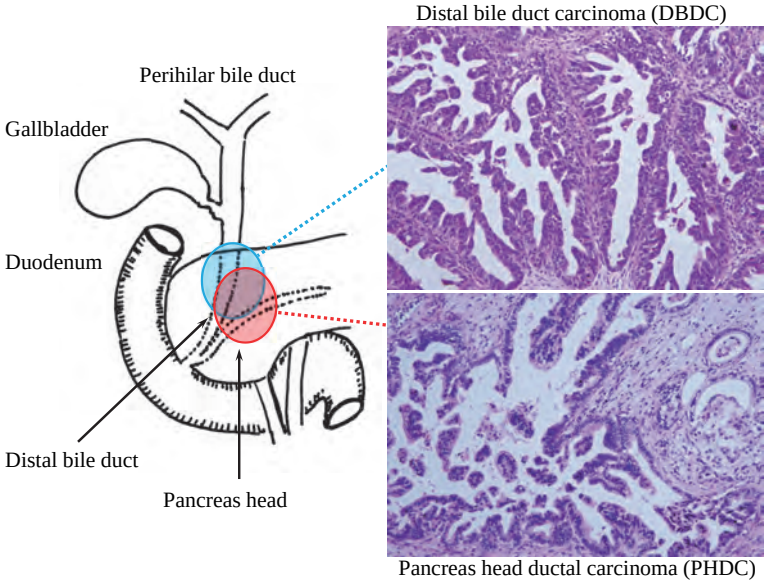

Fig. 1 Location of pancreas head ductal carcinoma (PHDC) and distal bile duct carcinoma (DBDC). Both PHDC and DBDC are located within the pancreas head/intra-pancreatic bile duct region, and frequently exhibit obstructive jaundice due to the bile duct stenosis/obstruction. Histologically, PHDC and DBDC are mainly composed differentiated adenocarcinoma.

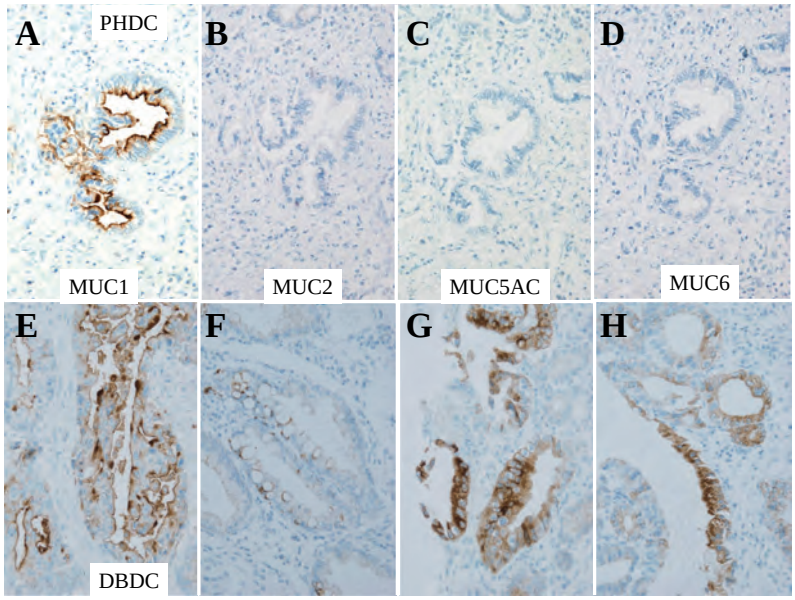

Fig. 2 Representative mucin expression of pancreas head ductal carcinoma (PHDC) and distal bile duct carcinoma (DBDC). A case of PHDC (A)-(D), and a case of DBDC (E)-(H). Immunochemical staining of MUC1 (A, E), MUC2 (B, F), MUC5AC (C, G), and MUC6 (D, H). PHDC (A)-(D) shows MUC1 positive (A), MUC2 negative (B), MUC5AC negative (C), and MUC6 negative (D). DBDC (E)-(H) shows MUC1 positive (E), MUC2 positive (F), MUC5AC positive (G), and MUC6 positive (H).

compared with DBDC (ne2,3: 27/47, 57.4%; and pN(+): 19/47, 40.4%). PHDC expressed MUC2 (4/40, 10.0%) and MUC6 (10/40, 25.0%) less frequently, compared with DBDC (MUC2: 17/47, 36.2%; and MUC6: 26/47, 55.3%) (Fig. 2). MUC5AC expression was inversely correlated with lymphatic invasion (P=0.0197) (Table 3). Patient's prognosis of PHDC was significantly

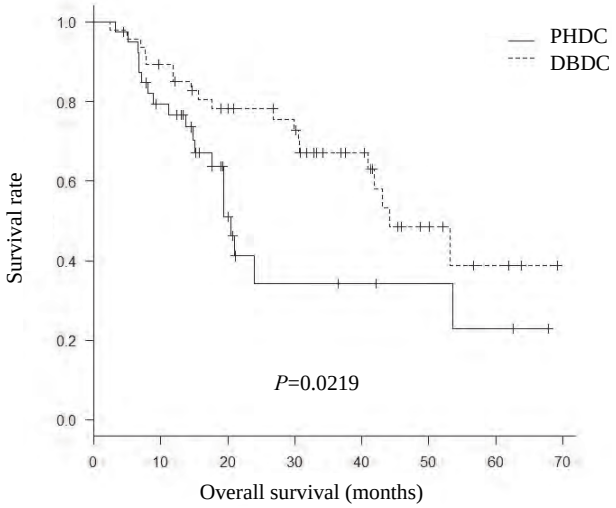

Fig. 3 Cumulative survival of patients with pancreas head ductal carcinoma (PHDC) or distal bile duct carcinoma (DBDC). Patient's prognosis of PHDC is significantly poorer than that of DBDC.

poorer than that of DBDC (P=0.0219) (Fig. 3).

Prognostic factors of PHDC and DBDC

Prognostic factors of PHDC/DBDC are summarized in Tables 4 and 5. Patient's prognosis of PHDC was associated with venous invasion (v2,3) and MUC5AC (Table 4). Especially, MUC5AC-negative phenotype was significantly correlated with patient's prognosis of PHDC, and became an important prognostic factor based on the multivariate analysis. On the other hand, patient's prognosis of DBDC was associated with lymphatic/venous invasion (ly2,3; and v2,3) and lymph nodal metastasis (pN(+)), while there was no significant association between patient's prognosis and mucin expression (Table 5). Interestingly, MUC5AC-negative PHDC exhibited significantly poorer patient's prognosis, compared with MUC5AC-positive PHDC, MUC5AC-positivve DBDC, and MUC5AC-negative DBDC (Fig. 4).

Discussion

Both PHDC and DBDC are located within the pancreas head/intra-pancreatic bile duct region. In the present study, we demonstrated frequent neural invasion and lymph node metastasis of PHDC, resulting in the poorer prognosis of PHDC, compared with DBDC. This is the first report to clarify the MUC5AC-negative phenotype as a significant prognostic factor of PHDC.

Clinically, surgeons divide pancreas cancers into the two groups, *i.e.*, pancreas head cancer (PHDC) and pancreas body/tail cancer, and perform different treatments, *e.g.*, pancreaticoduodenectomy for PHDC, and distal pancreatectomy for the pancreas body/tail cancer. They also classify extrahepatic bile duct cancers into the two groups, *i.e.*, perihilar bile duct cancer and distal bile duct cancer (DBDC), and perform different treatments, e.g., hepatectomy for perihilar bile duct cancer, and pancreaticoduodenectomy for DBDC. The location and the treatment of PHDC/DBDC are similar. However, PHDC significantly showed frequent neural invasion and lymph node metastasis, resulting into the poorer prognosis, compared with DBDC.

Mucin is a heterogeneous group of high molecular weight glycoproteins with many

Table 4 Univariate and multivariate analysis of prognostic factors of PHDC

Univariate analysis		
Variables	Values (%)	*P*-Value
Age		
＜65	19 (47.5)	0.266
≧65	21 (52.5)	
Gender		
Male	16 (40)	0.333
Female	24 (60)	
Histological differentiation		
well, mod, pap	33 (82.5)	0.143
por, others	7 (17.5)	
Depth of invasion		
T1	1 (2.5)	0.338
T2, 3, 4	39 (97.5)	
Lymphatic invation		
ly0, 1	6 (15)	0.0706
ly2, 3	34 (85)	
Venous invasion		
v0, 1	13 (32.5)	0.0155*
v2, 3	27 (67.5)	
Neural invation		
ne0, 1	6 (15)	0.291
ne2, 3	34 (85)	
Lymph node metastasis		
pN(−)	9 (22.5)	0.16
pN(+)	31 (77.5)	
MUC expression		
MUC1		
+	35 (87.5)	0.411
−	5 (12.5)	
MUC2		
+	4 (10)	0.513
−	36 (90)	
MUC5AC		
+	19 (47.5)	0.0111*
−	21 (52.5)	
MUC6		
+	10 (25)	0.134
−	30 (75)	

Multivariate analysis			
Variables	HR	(95%CI)	*P*-Value
MUC5AC			
+	0.294	0.108-0.795	0.0159*
-	3.4	1.256-9.202	

PHDC, pancreas head ductal carcinoma; well, well-differentiated adenocarcinoma; mod, moderately differentiated adenocarcinoma; pap, papillary adenocarcinoma; por, poorly differentiated adenocarcinoma; HR, hazard ratio; CI, confidence interval. *Statistically significant: $P<0.05$.

carbohydrate side chains. The mucin shares the common features of having an extensive tandem repeat region, and a peptide domain containing a high percentage of serine and threonine [5-7]. Recently, 19 human mucin genes have been assigned to the MUC gene family. MUC1, located in 1q21-23, is a transmembrane mucin and is expressed in pancreatobiliary cancer, and may function as an anti-adhesion molecule that inhibits homotypical cell aggregation and adhesion to the extracellular matrix, promoting cell invasion [12]. MUC2, MUC5AC and MUC6, which are clustered within the 11p15 locus, are expressed in intestinal goblet cells, gastric foveolar cells and gastric pyloric gland cells [13, 14], respectively. Histological phenotypes are

Table 5 Univariate and multivariate analysis of prognostic factors of DBDC

Univariate analysis		
Variables	Values (%)	*P*-Value
Age		
＜65	17 (36.2)	0.0651
≧65	30 (63.8)	
Gender		
Male	31 (66)	0.911
Female	16 (34)	
Histological differentiation		
well, mod, pap	40 (85.1)	0.0016*
por, others	7 (14.9)	
Depth of invasion		
T1	6 (12.8)	0.0795
T2, 3, 4	41 (87.2)	
Lymphatic invation		
ly0, 1	29 (61.7)	0.000404*
ly2, 3	18 (38.3)	
Venous invasion		
v0, 1	25 (53.2)	0.000209*
v2, 3	22 (46.8)	
Neural invation		
ne0, 1	20 (42.6)	0.307
ne2, 3	27 (57.4)	
Lymph node metastasis		
pN(−)	28 (59.6)	0.000237*
pN(+)	19 (40.4)	
MUC expression		
MUC1		
+	43 (91.5)	0.589
−	4 (8.5)	
MUC2		
+	17 (36.2)	0.591
−	30 (63.8)	
MUC5AC		
+	30 (63.8)	0.842
−	17 (36.2)	
MUC6		
+	26 (55.3)	0.475
−	21 (44.7)	

Multivariate analysis			
Variables	HR	(95%CI)	*P*-Value
Lymphatic invation			
ly0, 1	0.208	0.0734-0.594	0.00334*
ly2, 3	4.786	1.682-13.62	
Venous invasion			
v0, 1	0.252	0.0855-0.744	0.0125*
v2, 3	3.964	1.344-11.69	

DBDC, distal bile duct carcinoma; well, well-differentiated adenocarcinoma; mod, moderately differentiated adenocarcinoma; pap, papillary adenocarcinoma; por, poorly differentiated adenocarcinoma; HR, hazard ratio; CI, confidence interval. *Statistically significant: $P<0.05$.

thought to modulate the biological behavior of carcinoma cells such as tumor progression. Previous studies have reported relationships between mucin phenotypes and progression/invasion of pancreas cancer [15-17]. A few reports mentioned that MUC5AC are associated with tumor progression [18, 19], and *in vivo* tumorigenicity of the pancreas cancer cells [20]. MUC5AC gene is thought to up-regulate the cell proliferation. The other study reported controversial results that MUC5AC mRNA was expressed in the majority of intraductal papillary mucinous neoplasms

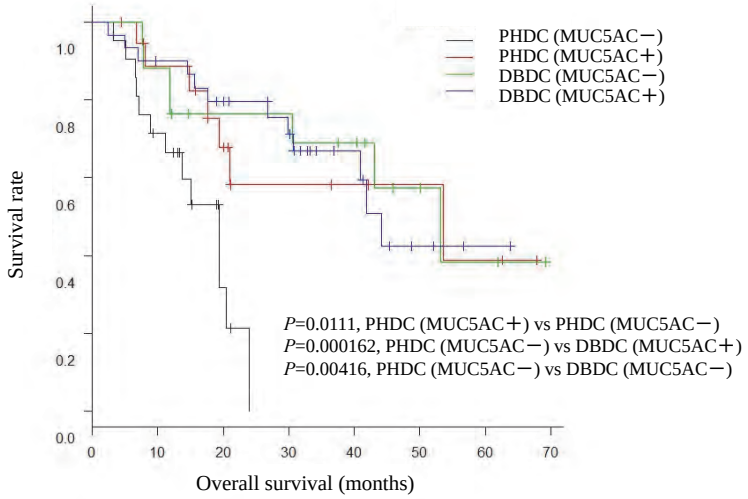

Fig. 4 MUC5AC expression and cumulative survival of patients with pancreas head ductal carcinoma (PHDC) or distal bile duct carcinoma (DBDC). MUC5AC-negative PHDC exhibits significantly poorer patient's prognosis, compared with MUC5AC-positive PHDC, MUC5AC-positive DBDC, and MUC5AC-negative DBDC.

of pancreas (low-grade malignant tumor), but less frequently in the invasive ductal carcinoma (high-grade malignancy) [18]. Our present study clarified that MUC5AC was inversely correlated with patient's prognosis of PHDC, and have supported the latter study [18, 19]. We speculated that MUC5AC is a secretory mucin, and may play a role a member of extracellular matrix suppressing the lymphatic invasion of PHDC. However, definite molecular functions of MUC5AC have not extensively elucidated in the pancreas cancer, and will be needed in the near future. In conclusion, MUC5AC-negative PHDC showed significantly poor patient's prognosis.

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