Pathobiological Implications of MUC16/CA125 Expression in Intrahepatic Cholangiocarcinoma-Mass Forming Type

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Key Words

Cholangiocarcinoma • MUC16 • CA125 • Prognosis

Abstract

Objectives: MUC16 carries the peptide epitope CA125, which is well known as a marker of ovarian cancer. High serum levels of MUC16 (CA125) have been reported not only in patients with ovarian cancer but also in patients with liver diseases. We evaluated the expression of MUC16 in intrahepatic cholangiocarcinoma-mass forming type (ICC-MF) tissues. **Methods:** We examined the expression of MUC16 by immunohistochemical analyses using the monoclonal antibody M11 in ICC-MF tissues from 63 patients. To compare the prevalence of each mucin expression by clinicopathological features, appropriate statistical analysis was performed. **Results:** MUC16 was detected in 48% of samples (30/63). After adjusting for the effects of other prognostic factors, multivariate survival analysis revealed that MUC16 expression is a significant independent factor of poor prognosis ($p = 0.005$). **Conclusion:** The current results indicate that MUC16 expression is a prognostic factor of poor survival in ICC-MF.

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Introduction

Intrahepatic cholangiocarcinoma (ICC) is an intrahepatic malignancy with biliary epithelial differentiation. ICC is the second most common primary hepatic malignancy after hepatocellular carcinoma. ICC is grossly classifiable into three types: mass-forming type (ICC-MF), periductal infiltrating type and intraductal growth type. ICC-MF is the most common type, and presents as a gray to gray-white nodular or mass lesion [1]. ICC-MF has a worse prognosis than periductal infiltrating and intraductal growth types [2].

ICC-MF has always been a difficult-to-treat tumor and most patients with this tumor have an unfavorable prognosis. Complete surgical resection of the tumor is the only way to improve the cure rate; however, many patients who underwent curative resection have a poor outcome [3, 4]. Thus, investigation of the prognostic factor is very valuable in the treatment of patients with ICC-MF.

Mucins are high molecular-weight glycoproteins having oligosaccharides attached to the serine or threonine residues of the mucin core protein backbone by O-glycosidic linkages [5]. Our immunohistochemical studies of mucin expression in various human tumors have demon-

strated that MUC1 expression is related to invasive tumor proliferation and a poor patient outcome [2, 6, 7]. On the other hand, MUC2 expression is related to noninvasive tumor proliferation and a favorable patient outcome [2, 6, 7]. MUC4 expression in pancreatobiliary carcinomas is also an independent prognostic factor of poor survival [8–10].

MUC16 carries the peptide epitope CA125, a well-known marker of ovarian cancer. As highly O-glycosylated repeats are the landmark of the mucin family of glycoproteins, CA125 was also named 'MUC16' [11–13]. High serum levels of MUC16 were reported not only in patients with ovarian cancer but also in patients with liver cirrhosis, liver cell tumors or extrahepatic bile duct tumors [14].

In the present study, we demonstrate that MUC16 expression is an independent factor of poor prognosis in patients with ICC-MF.

Patients and Methods

Patients

Surgically resected ICC-MF tissues from 63 patients (33 males, 30 females) were studied. All tissue specimens were retrieved from the files of the Department of Pathology, Faculty of Medicine, Kagoshima University, from 1986 to 2006. Twenty-seven of the 63 patients had also been included in our previous study [8]. The mean age of the patients was 67.4 years (range: 41–85 years). The study was approved by the Human Investigation Committees of Kagoshima University Faculty of Medicine and Kagoshima University Hospital.

All specimens were fixed in formalin, embedded in paraffin and cut into 4- μ m-thick sections for the usual hematoxylin and eosin staining and were also studied by immunohistochemistry.

Antibodies for Immunohistochemistry

MUC16 was detected by mouse monoclonal antibody (mAb), M11 (mouse IgG, Dako Cytomation, Glostrup, Denmark). For the comparative study, mesothelin, MUC1, MUC2 and MUC4 were detected by mAb 5B2 (Novocastra, Newcastle, UK), mAb DF3 (mouse IgG, Toray-Fuji Bionics, Tokyo, Japan), mAb Ccp58 (Novocastra) and mAb 8G7 (generated by S.K.B., one of the authors), respectively.

Biotinylated affinity-purified horse anti-mouse IgG, goat anti-rabbit IgG and avidin-biotinylated horseradish peroxidase complex (ABC) were purchased from Vector Laboratories (Burlingame, Calif., USA) as the Vectastain Elite ABC kit.

Staining Procedure

Immunohistochemical stainings were done by an immunoperoxidase method using the ABC complex as described previously [2, 6, 9, 15]. For the retrieval of MUC4, the sections were treated in a water bath at 80°C for 20 min in 0.01 M citrate buffer (pH 6.0). For the retrieval of the other epitopes, the sections were treated at 120°C for 5 min in 0.01 M citrate buffer (pH 6.0). In the staining using each antibody, the sections were incubated with

dilutions of the primary antibodies (M11, 1:100; OC125, 1:50; 5B2, 1:100; DF3, 1:10; Ccp58, 1:100; 8G7, 1:3,000) in PBS with 1% bovine serum albumin for 16 h at 4°C.

Evaluation of the Results by Scoring

The results of the immunohistochemical stainings were evaluated by calculating the percentage of positively stained carcinoma cells. The immunostaining was considered positive if at least 5% of the carcinoma cells were stained. When less than 5% of the carcinoma cells were stained, the case was considered as negative.

Statistical Analysis

To compare the prevalence of each mucin expression by clinicopathological features, statistical analysis was performed using the χ^2 test and Fisher's exact test, where appropriate. Survival curves for subgroups by MUC16 expression status were drawn by the Kaplan-Meier method using recurrence and decease as endpoints. The difference in the probability of clinical prognosis between subgroups was examined by the log-rank test. Univariate and multivariate survival analyses were also performed using the Cox proportional-hazards model. A probability of <0.05 was considered statistically significant.

Results

MUC16, Mesothelin, MUC1, MUC2 and MUC4 Expression in Normal Epithelium of the Intrahepatic Bile Duct

MUC16, mesothelin, MUC1, MUC2 and MUC4 were not expressed in normal epithelium of the intrahepatic bile duct.

MUC16, Mesothelin, MUC1, MUC2 and MUC4 Expression Rate in ICC-MF Tissues

Figure 1 shows a representative expression pattern of MUC16 in ICC-MF. MUC16 was expressed in the cell apices and/or cytoplasm of the carcinoma cells in 30 (48%) of the 63 ICCs-MF cases (fig. 1b). Mesothelin was expressed in the cytoplasm in 13 cases (21%); MUC1 was expressed in the cytoplasm and/or membrane of the carcinoma cells in 58 cases (92%); MUC2 was expressed in the cytoplasm in 13 cases (21%), and MUC4 was expressed in the cytoplasm in 19 cases (30%).

Relationship between MUC16, Mesothelin, MUC1, MUC2 and MUC4 Expression and Clinicopathological Features

The relationship between MUC16, mesothelin, MUC1, MUC2 and MUC4 expression and clinicopathologic features is summarized in table 1 and online supplementary table 1 (for all online supplementary material, see www.karger.com/doi/10.1159/000335164). Younger patients

Fig. 1. Representative microscopic features of ICC-MF. **a.** Hematoxylin and eosin staining shows atypical epithelial cells composed of irregular tubular structure. **b** MUC16 is expressed in the cell apices and/or cytoplasm of the carcinoma cells.

(<65 years old) and perineural invasion showed more frequent MUC16 expression, but these associations were not statistically significant ($p = 0.064$ and 0.110 , respectively). Mesothelin expression was more frequently observed in ICC-MF with smaller tumors (<4 cm), and this association was marginally significant ($p = 0.055$). MUC4 expression was frequently observed in larger tumors. On the other hand, MUC1 and MUC2 expressions were not related to any of the clinicopathological features (online suppl. table 1).

Relationship between MUC16, Mesothelin, MUC1, MUC2 and MUC4 Expression and Clinical Prognosis

Among the 63 patients examined, 43 patients died during the follow-up period, including 1 case who died from an unrelated cause. Median and mean lengths of survival of patients with ICC-MF who were operated on were 12 and 20 months, respectively. The prognosis of patients with MUC16 expression ($n = 30$) was significantly poorer than that of MUC16-negative patients ($n = 33$) ($p = 0.03$ by the log-rank test; fig. 2). The prognosis of patients with MUC4 expression ($n = 19$) was significantly poorer than that of MUC4-negative patients ($n = 44$) ($p = 0.031$; online suppl. fig. 1). Mesothelin, MUC1 and MUC2 expression was not related to the patients' prognosis.

Univariate Analysis of Prognostic Factors

The results of univariate analysis of prognostic factors of ICC-MF are summarized in table 2. Large tumors, the

Fig. 2. Correlation between MUC16 expression and cumulative survival rate in 63 patients with ICC-MF as determined by the Kaplan-Meier method. The survival of patients with MUC16 expression was worse than that of MUC16-negative patients ($p = 0.03$).

presence of lymph node metastasis, lymphatic permeation, venous invasion and perineural invasion were significantly unfavorable prognostic factors in patients with ICC-MF. Furthermore, patients with MUC16 and MUC4 expression had a poorer prognosis than MUC16- and MUC4-negative patients ($p = 0.03$ and 0.04 , respectively).

Table 1. Summary of the data on MUC16 and mesothelin expression and clinicopathological features of 63 cases of ICC-MS

Category	Patients, n	MUC16			Mesothelin		
		negative <5%	positive ≥5%	p value	negative <5%	positive ≥5%	p value
Age							
<65 years, n	24 (38.1)	9 (37.5)	15 (62.5)	0.064	17 (70.8)	7 (29.2)	0.189
≥65 years, n	39 (61.9)	24 (61.5)	15 (38.5)		33 (84.6)	6 (20.6)	
Gender							
Men, n	33 (52.4)	17 (51.5)	16 (48.5)	0.885	25 (75.8)	8 (24.4)	0.458
Women, n	30 (47.6)	16 (53.3)	14 (46.7)		25 (83.3)	5 (16.7)	
Tumor size							
<4 cm, n	20 (31.7)	11 (55.0)	9 (45.0)	0.777	13 (65.0)	7 (35.0)	0.055
≥4 cm, n	43 (68.3)	22 (51.2)	21 (48.8)		37 (86.0)	6 (14.0)	
Surgical margin							
Negative, n	36 (57.1)	20 (55.6)	16 (44.4)	0.560	29 (80.6)	7 (19.4)	0.787
Positive, n	27 (42.9)	13 (48.2)	14 (51.8)		21 (77.8)	6 (22.2)	
Intrahepatic metastasis							
Negative, n	47 (74.6)	26 (55.3)	21 (44.7)	0.424	37 (78.7)	10 (21.3)	0.829
Positive, n	16 (25.4)	7 (43.8)	9 (56.3)		13 (81.3)	3 (18.7)	
Lymph node metastasis							
Negative, n	39 (61.9)	21 (53.9)	18 (46.1)	0.767	31 (79.5)	8 (20.5)	0.976
Positive, n	24 (38.1)	12 (50)	12 (50)		19 (79.2)	5 (20.8)	
Lymphatic permeation							
Negative, n	24 (38.1)	13 (54.2)	11 (45.8)	0.824	18 (75)	6 (25)	0.502
Positive, n	39 (61.9)	20 (51.3)	19 (48.7)		32 (82.1)	7 (17.9)	
Venous invasion							
Negative, n	22 (34.9)	11 (50)	11 (50)	0.782	17 (77.3)	5 (22.7)	0.764
Positive, n	41 (65.1)	22 (53.7)	19 (46.3)		33 (80.5)	8 (19.5)	
Perineural invasion							
Negative, n	40 (63.5)	24 (60)	16 (40)	0.110	30 (75)	10 (25)	0.259
Positive, n	23 (36.5)	9 (39.1)	14 (60.9)		20 (87)	3 (13)	

Figures in parentheses are percentages.

Multivariate Analysis of Prognostic Factors

The results of multivariate analysis of prognostic factors of ICC-MF are summarized in table 3. After adjusting for the effects of other clinicopathological factors, only MUC16 expression was a significant independent prognostic factor of poor survival ($p = 0.005$).

Discussion

The serum levels of CA125 are high in patients with ovarian cancer as well as in patients with liver cirrhosis, liver cell tumor and extrahepatic bile duct tumors [11–13]. Immunohistochemical studies show high expression of CA125 in tumoral tissues in colon adenocarcinoma, breast carcinomas, malignant mesothelioma, uterine adenoma-

toid tumors, lung bronchoalveolar carcinoma and ovarian endometrioid and serous carcinomas [14, 16–19]. However, there is no study on CA125 expression in ICC-MF.

Around 2000, CA125 was identified as MUC16. The present study demonstrated that MUC16/M11 is expressed in ICC-MF although it is not expressed in normal liver tissue including normal intrahepatic bile duct epithelium. The antibody M11 recognizes a mucin-like glycoprotein larger than 200 kDa, expressing the CA125 epitope [13, 20–22]. We found for the first time that MUC16/M11 expression is significantly related to poor survival in patients with ICC-MF, and that MUC16/M11 expression is a statistically significant independent prognostic factor of poor survival in patients with ICC-MF.

Tumor size, surgical margin, intrahepatic metastasis, lymph node metastasis, vascular invasion, lymphatic in-

Table 2. Results of survival analysis by the univariate Cox proportional-hazards model

Covariate	Recurrence or death/total	Hazard ratio	95% confidence interval
Age			
<65 years	17/24	1.00	reference
≥65 years	28/39	1.10	0.60–2.02
Sex			
Male	20/33	1.00	reference
Female	25/30	1.48	0.82–2.68
Tumor size			
<4 cm	9/20	1.00	reference
≥4 cm	36/43	2.88	1.38–6.01
Lymph node metastasis			
Negative	24/39	1.00	reference
Positive	21/24	2.82	1.50–5.32
Lymphatic permeation			
Negative	12/24	1.00	reference
Positive	33/39	3.35	1.70–6.60
Venous invasion			
Negative	13/22	1.00	reference
Positive	32/41	2.16	1.13–4.16
Perineural invasion			
Negative	23/40	1.00	reference
Positive	22/23	2.98	1.59–5.58
Intrahepatic metastasis			
Negative	31/47	1.00	reference
Positive	14/16	1.72	0.91–3.25
Surgical margin			
Negative	24/36	1.00	reference
Positive	21/27	1.47	0.81–2.67
MUC16 expression			
Negative	20/33	1.00	reference
Positive	25/30	2.23	1.23–4.06
Mesothelin expression			
Negative	37/50	1.00	reference
Positive	8/13	0.85	0.39–1.84
MUC1 expression			
Negative	1/5	1.00	reference
Positive	44/58	4.17	0.57–30.3
MUC2 expression			
Negative	35/50	1.00	reference
Positive	10/13	1.02	0.50–2.05
MUC4 expression			
Negative	24/44	1.00	reference
Positive	16/19	1.91	1.03–3.54

vasion and perineural invasion have been reported as prognostic factors in patients with ICC-MF [9, 23–25]. The prognosis of patients with MUC16 or MUC4 expression was significantly poorer than that of MUC16- or MUC4-negative patients. The univariate analysis of prognostic factors in the present study showed that large

Table 3. Results of the survival analysis by the multivariate Cox proportional-hazards model

Covariate	Hazard ratio ¹	95% confidence interval ¹	p value ²
MUC16-positive	2.92	1.35–6.31	0.005
Mesothelin-positive	1.35	0.54–3.41	0.529
MUC1-positive	3.34	0.43–25.8	0.168
MUC2-positive	1.23	0.58–2.62	0.600
MUC4-positive	1.73	0.83–3.60	0.147

¹ Hazard ratios and 95% confidence intervals were obtained by the Cox proportional-hazards model using age (continuous), tumor size, surgical margin, intrahepatic metastasis, lymph node metastasis, lymphatic permeation, venous invasion, and perineural invasion as covariates.

² p values were obtained by likelihood ratio test.

tumors, the presence of lymph node metastasis, lymphatic permeation, venous invasion and perineural invasion were significantly unfavorable prognostic factors. Furthermore, patients with MUC16 and MUC4 expression showed a poorer prognosis than MUC16- or MUC4-negative patients. In the multivariate analysis, only MUC16 expression ($p = 0.005$) was a significant independent prognostic factor of poor survival after adjusting for the effects of other clinicopathological factors.

Mesothelin is a 40-kDa glycoprotein, and the interaction between mesothelin and MUC16 could facilitate peritoneal metastasis of ovarian tumors in vitro [22]. However, in our study, there was no significant difference in survival between mesothelin-positive and negative patients. Evaluation of the combined expression of MUC16 and mesothelin showed no significant result. These findings suggest that an interaction between MUC16 and mesothelin is not a major factor in the malignant potential of ICC-MF.

In the clinicopathological study, we concluded that expression of MUC16 in ICC-MF is an independent prognostic factor of poor survival and a useful marker to predict the outcome of patients with ICC-MF who had surgical resection of the tumor. MUC16-positive patients with ICC-MF should be followed up carefully.

Acknowledgements

The authors are grateful to Mr. Yoshiharu Atsui, Ms. Yukari Nishimura, Ms. Sayuri Yoshimura and Ms. Izumi Houjou for their excellent technical assistance. They wish to thank the Joint

Research Laboratory of Kagoshima University Graduate School of Medical and Dental Sciences for the use of their facilities. This work was supported by Scientific Research (C) grant 21590399 to M. Higashi and Scientific Research on Priority Areas grant 20014022 and Scientific Research (C) grant 20590345 to S. Yonezawa from the Ministry of Education, Science, Sports, Culture and Technology, Japan, and a grant from the Kodama Memorial Foundation to M. Higashi and also by a JSPS Fellowship Grant-in-Aid to N. Yamada (219447), and to S. Kitamoto (239349). The

study was also supported by the International Educational Research Support Project for Islands, Environment and Medicine from Kagoshima University to S. Yokoyama.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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