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.

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 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-μm-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 (Novocasta, 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 antirabbit 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 immuno-peroxidase 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 χ² 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 cytoplasmas 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...
(≤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 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).
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.

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

<table>
<thead>
<tr>
<th>Category</th>
<th>Patients, n</th>
<th>MUC16 negative &lt;5%</th>
<th>MUC16 positive ≥5%</th>
<th>p value</th>
<th>Mesothelin negative &lt;5%</th>
<th>Mesothelin positive ≥5%</th>
<th>p value</th>
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<td>24 (61.5)</td>
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<td>16 (48.5)</td>
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<td>25 (75.8)</td>
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<td>7 (35.0)</td>
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<td>16 (44.4)</td>
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<td>29 (80.6)</td>
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<td>14 (51.8)</td>
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<td>21 (77.8)</td>
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<td>18 (75)</td>
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<td>19 (48.7)</td>
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<td>11 (50)</td>
<td>0.782</td>
<td>17 (77.3)</td>
<td>5 (22.7)</td>
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<td>33 (80.5)</td>
<td>8 (19.5)</td>
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<td>Perineural invasion</td>
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<td>24 (60)</td>
<td>16 (40)</td>
<td>0.110</td>
<td>30 (75)</td>
<td>10 (25)</td>
<td>0.259</td>
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<td>9 (39.1)</td>
<td>14 (60.9)</td>
<td></td>
<td>20 (87)</td>
<td>3 (13)</td>
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</table>

Figures in parentheses are percentages.
Invasion 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 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.

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Disclosure Statement

The authors have no conflicts of interest to disclose.

References


