

• LIVER CANCER •

## Expression of MUC1 and its significance in hepatocellular and cholangiocarcinoma tissue

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### Abstract

**AIM:** To investigate the relation between MUC1 expression, distribution, and prognosis in hepatocellular and cholangiocarcinoma (HCC and CC) and cirrhotic liver tissues, and their significance in HCC and CC diagnosis.

**METHODS:** Expression and distribution of MUC1 were examined by immunohistochemical assay with anti-MUC1 mAb in 59 samples of HCC and 37 samples of CC, 20 samples of cirrhotic liver tissues, and 10 samples of normal liver tissues, seeking possible associations between MUC1 positive expression, distribution in HCC and CC (primary liver cancer, PLC) cases and the studied clinical data.

**RESULTS:** Immunohistochemical analysis of MUC1 expression showed that in the 96 PLC samples, 68 (70.8%) were strong positive, and 6 (6.2%) were weak positive. Only 4 in the 20 cirrhotic liver tissues were found to be weak positive, while no expression of MUC1 was detected in normal liver tissues. Apparently, the high expression rate of MUC1 in PLC tissues was statistically significant in comparison to that in cirrhotic and normal liver tissues. The expressed MUC1 protein, stained in dark brownish or brownish-yellow particles, chiefly localized on the cancer cell membranes or in cytoplasm. In the 68 strong positive samples, 40 were detected on cell membrane and the other 28 were in cytoplasm. In addition, follow-up studies of those PLC cases demonstrated that MUC1 expression on cell membrane or in cytoplasm was closely associated with PLC prognosis. The expression of MUC1 in PLC had little statistical significance in respect of the pathological types and sizes of the tumors, but a strong

relationship regarding histological differentiation, metastasis of lymph nodes, portal canal emboli, and post-operational recurrence of the carcinomas. After 3 years of tumor excision, the metastasis rate in MUC1 positive expression group (67.6%) was much higher than that in MUC1 weak expression group (33.3%) and negative expression group (31.8%), and thus the survival rate in MUC1-positive expression group was significantly different from that in weak and negative expression groups.

**CONCLUSION:** Expression and localization of MUC1 proteins in primary liver carcinomas (PLCs) may act as prognostic markers, and MUC1 molecules might be helpful in differential diagnosis.

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**Key words:** MUC1; Primary liver carcinoma; Prognosis; Immunohistochemistry

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### INTRODUCTION

Primary liver carcinoma (PLC) is one of the most frequent malignant tumors in clinics, and its tendency to invade and metastasize is the paramount reason for high recurrence after excision, greatly affecting the survival rate of PLC suffers. Even with the developments in PLC therapies, the overall effect is far from radical cure on demand<sup>[1]</sup>. Thus, it is quite necessary and important to explore new approaches for early diagnosis and immunological treatment of PLC.

MUC1, also known as polymorphic epithelial mucin, is a group of glycoproteins with high molecular mass. One important characteristic of MUC1 gene is the polymorphism. The second expressed exon within the MUC1 coding genes contains a variable number of tandem repeats (VNTRs), and every VNTR is composed of a 20-amino acid peptide motif as VTSAPDTRPAPGSTAPPAGH, constituting main antigenic determinants in this region. Usually MUC1 is expressed at a very low level on normal adenocytes, chiefly localized on gland cell surfaces or in gland cavities by excretion, and thus not recognized by the host immune system<sup>[2-4]</sup>. It has been found that MUC1 is aberrantly expressed in the forms of misglycolization or incomplete

glycolization, in many tumor tissues like breast, stomach, and colon cancers. These abnormal MUC1 molecules reveal new protein epitopes or carbohydrate antigens, distributed all around the cancer cell surface, and may be recognized by the immune system as notable tumor-associated antigens<sup>[5-9]</sup>. As a tumor marker, MUC1 has been applied in breast cancer and other tumors for their diagnosis and biological treatment<sup>[10-13]</sup>.

During the process of malignant transformation and invasion of tumor cells, the changes of MUC1 glycolization influence the biological behavior of tumor cells<sup>[14]</sup>. It was also reported that MUC1 positive expression is an important prognostic indicator in breast cancer and other tumor patients<sup>[15-17]</sup>. However, the expression levels of MUC1 in PLC and cirrhotic liver tissues and their correlation with carcinogenesis still remain to be elucidated. In this study, we used immunohistochemical assay to detect MUC1 expression in PLC and cirrhotic liver tissues, and further investigated the potential significance of MUC1 in PLC diagnosis and immunological treatment.

## MATERIALS AND METHODS

### Clinical data

Ninety-six paraffin-embedded PLC samples were collected from patients undergoing surgery in our hospital from 1990 to 2001. Hepatocellular carcinoma (HCC) was found in 59 patients, and cholangiocarcinoma (CC) in 37 patients. Sixty-six samples were from male patients and 30 from female patients, aged 35-68 years (averaged 41.5 years). Lymph node metastasis was confirmed by pathological examination. No chemotherapy or radiotherapy was given before tumor excision. In the 20 samples of cirrhotic tissues, 12 were from male patients and 8 female patients, aged 19-70 years (averaged 40.7 years). Ten samples of normal liver tissues from 10 cases of hepatic angiomas served as normal controls. All tissue sections were stained with hematoxylin and eosin (H&E).

The diagnosis of HCC was primarily based on history of chronic hepatitis, results of AFP detection, and space occupying lesions in the liver on ultrasonography and CT. The pathological diagnosis of HCC and CC was confirmed by H&E staining of the tissue sections. Patients were regularly examined by ultrasonography and CT after PLC surgery. Tumor recurrence was defined as new focus was detected, and metastasis was defined when lymph nodes of hepatic portal vein became swollen or new foci were found in distal organs.

### Preparation of tissue samples

For routine sections, tissue samples were immersion-fixed in 40 g/L buffered formaldehyde for hours, and dehydrated through graded alcohols. After paraffin wax embedding, sections of 5  $\mu$ m thickness were cut and mounted on coated glass slides. Then H&E as well as immunohistochemical staining were performed.

### Immunohistochemical assay

Endogenous peroxidase blocker and normal house serum were added to all sections for 30-min incubation at room

temperature to minimize non-specific staining, and antigen restoration was performed by microwave method. Mouse anti-human MUC1 mAb working at 1:100 dilution was purchased from Antibody Diagnostica Inc., USA, and ABC diagnostic kit was purchased from Santa Cruz, USA. Detection procedures were performed according to the kit instructions. The cover slide was treated with goat anti-mouse bridging antibody (1:200) for 30 min at 37 °C. Finally, diaminobenzidine tetrachloride was used for color development and the slides were counterstained with hematoxylin. Dehydration, clearing, covering, and light microscopy were performed routinely. Blank test and replacement test were set for negative controls.

### Result determination

Brownish-yellow particles under light microscope were considered positive. Three positive levels according to positive cell percentage in five high power, randomized, observation fields were classified: Level 0: cells without stained particles (negative MUC1 expression, -), Level 1: positive cell percentage less than 25% (weak MUC1 expression, +), and Level 2: positive cell percentage more than 25% (strong MUC1 expression, ++).

### Statistical analysis

All data were analyzed by SPSS 11.0 (SPSS Inc., USA). Statistical methods included  $\chi^2$ -test, Fisher's exact test, and the Kruskal-Wallis test.  $P < 0.05$  was considered statistically significant.

## RESULTS

### MUC1 expression in primary liver carcinoma, cirrhotic liver, and normal liver tissues

In the 96 tested PLC samples, 68 were strong positive (Figures 1A and B) and 6 were weak positive. Only 4 in the 20 cirrhotic liver tissues were found to be weak positive (Figure 1C), while no expression of MUC1 was detected in normal liver tissues (Figure 1D). Apparently, the high expression rate of MUC1 in PLC tissues was statistically significant in comparison to that in cirrhotic and normal liver tissues ( $P < 0.05$ , Table 1).

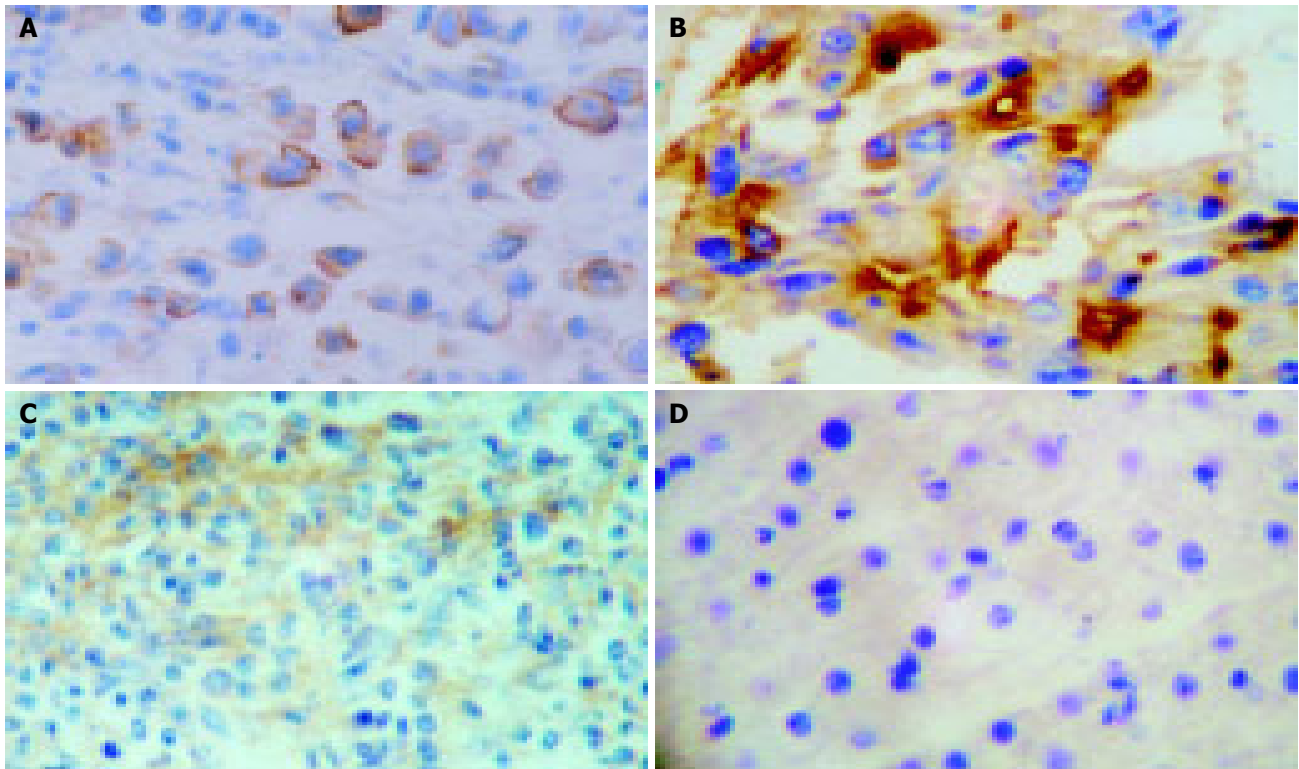
**Table 1** Expression of MUC1 in PLC, cirrhotic, and normal liver tissues

Group	n	Expression of MUC1		
		-	+	++
Normal liver	10	10	0	0
Cirrhotic liver	20	16	4	0
PLC	96	22	6	68 <sup>a</sup>

<sup>a</sup> $P < 0.05$  vs cirrhotic and normal liver tissues.

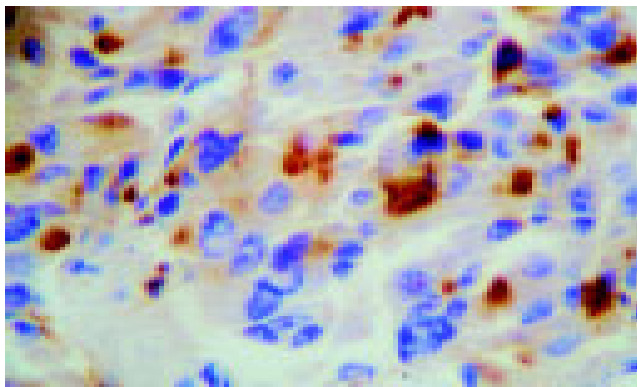
### MUC1 expression was associated with PLC pathology

MUC1 was both expressed in HCC (Figure 2) and CC tissues with no statistical difference between them ( $P > 0.05$ ), demonstrating that MUC1 gene expression was not associated with histological classification of the hepatic tumors. The



**Figure 1** Characterization of MUC1 expression in PLC and cirrhotic liver tissues as well as normal liver tissues by immunohistochemical staining. **A:** Overexpression of MUC1 on cell membranes (×400); **B:** positive staining of

MUC1 in cytoplasm (×400); **C:** MUC1 weak expression in cirrhotic liver tissues (×400); **D:** MUC1 negative expression in normal liver tissues (×400).



**Figure 2** Positive expression of MUC1 protein in HCC samples (×400).

sizes of solid tumors were not different in the MUC1 expression either ( $P>0.05$ ). However, MUC1 expression in well differentiated tumor tissues were significantly different from that in moderately and poorly differentiated tissues ( $P<0.05$ ), and so did in lymph node metastases, portal vein embolis, and 3-year post-operation recurrence ( $P<0.05$ ). The association of MUC1 expression and PLC clinical pathology behavior is illustrated in Table 2.

#### Localization of MUC1 in PLC tissues and prognosis

By immunohistochemical assay, the expressed MUC1 proteins in liver carcinoma tissues were stained as dark brownish or brownish-yellow particles, and mainly localized on the cancer cell membranes (Figure 1A) or in cytoplasm (Figure 1B). Table 3 shows the association of strong MUC1

expression and the prognosis of 68 cases of hepatic tumor.

#### MUC1 expression and PLC metastasis

Three years after surgery, metastasis occurred in 55 out of

**Table 2** MUC1 expression and PLC clinical pathology behavior

Clinical pathology	n	MUC1 expression			P
		-	+	++	
Histological classification					
HCC	59	17	4	38	>0.05
CC	37	5	2	30	
Histological differentiation					
Well <sup>a,c</sup>	30	14	2	14	<0.01
Moderate	40	6	2	32	
Poor	26	2	2	22	
Lymph node metastasis					
Yes	52	5	2	45	<0.01
No	44	17	4	23	
Portal vein embolism					
Yes	32	1	2	29	<0.01
No	64	21	4	39	
Size of solid tumor (cm)					
<5	49	15	2	32	>0.05
≥5	47	7	4	36	
Recurrence (3 yr)					
Yes	65	9	4	52	<0.01
No	31	13	2	16	

<sup>a</sup> $P<0.05$  vs moderately differentiated HCC. <sup>c</sup> $P<0.05$  vs poorly differentiated HCC.

the 96 PLC patients: intra-hepatic metastasis in 30 cases, pulmonary metastasis in 12 cases, osseous metastasis in 4 cases, and lymph node metastasis in 9 cases. The metastasis rate in MUC1 strong positive expression group (67.6%) was much higher than that in MUC1 negative expression group (31.8%,  $P < 0.01$ , Table 4).

**Table 3** MUC1 localization in PLC tissues and prognosis of PLC

Clinical pathology	<i>n</i>	MUC1 expression		<i>P</i>
		Membrane	Cytoplasm	
Lymph node metastasis				
Yes	45	23	22	<0.05
No	23	17	6	
Portal vein embolism				
Yes	29	11	18	<0.01
No	39	29	10	
Survival rate (yr)				
<3	50	17	33	<0.01
≥3	18	13	5	

**Table 4** MUC1 expression and post-operation metastasis

MUC1 expression	n	Rate of metastasis (%)
Negative	22	31.8
Weak positive	6	33.3
Strong positive <sup>b</sup>	68	67.6

<sup>b</sup> $P < 0.01$  vs negative.

### Positive MUC1 expression in PLC tissues and survival rate

Follow-up data were collected from 86 of the 96 PLC patients (89.6%). The survival rate in MUC1 strong positive expression group was significantly different from that in MUC1 weak positive or negative expression group ( $P < 0.05$ , Table 5).

**Table 5** Positive MUC1 expression in PLC tissues and survival of PLC patients

MUC1 expression	n	Survival rate (%)			
		6 mo	12 mo	24 mo	36 mo
Negative	22	86.4	72.7	59.1	50.0
Weak positive <sup>a</sup>	6	50.0	33.3	33.3	33.3
Strong positive <sup>a</sup>	68	51.5	44.1	29.4	26.4

<sup>a</sup> $P < 0.05$  vs negative.

## DISCUSSION

To investigate the possible correlation among MUC1 expression and localization and prognosis in PLC and cirrhotic liver tissues, we carried out immunohistochemical assay to detect the MUC1 expression in 96 samples of PLC hepatic tissues. The results showed that MUC1 was strongly expressed on PLC cell membrane or in cytoplasm, while weak and negative expressions were found in human

cirrhotic liver tissues, and no expression was found in normal liver tissues. The difference was of statistical significance. In addition, MUC1 expression increased during the process of transformation from benign cells to malignant cells, which is in accordance with the common understanding of hepatic carcinoma resulting from liver cirrhosis.

There are discrepant results in reports on MUC1 expression and PLC histological differentiation types. Sasaki and Nakanuma<sup>[18]</sup> reported that MUC1 core protein is expressed in intrahepatic bile duct carcinoma, but not in HCC. Cao *et al.*<sup>[19]</sup> demonstrated that MUC1 is remarkably expressed in HCC cells and can be considered as an indicator of HCC prognosis. In our test, MUC1 expression was not associated with either histological classification or size of the tumors. Based on our testing data, we take it that MUC1 can be used as one of the helping indicators early diagnosis of PLC, by hepatic puncture biopsy before PLC surgery.

In previous studies, it was suggested that high-level MUC1 expression is reversely correlated with prognosis of tumor patients. For example, MUC1 expression is closely related with prognosis of breast cancer sufferers<sup>[3,20]</sup>. However, possible association(s) of MUC1 expression levels in PLC liver tissues with the prognosis still remains uncertain. From our test results, it is clear that MUC1 expression levels are quite different in well-differentiated tumor tissues and moderately- or poorly-differentiated tumor tissues, and so are the differences among MUC1 expression and lymph node metastasis, portal vein embolism, and post-operation recurrence. Expression and localization of MUC1 proteins in PLC may act as different prognostic markers of PLC. We also found that the prognosis was worse in cytoplasm expression group than in cell surface expression group. It is reasonable to deduce that MUC1 molecule on cell surface may stimulate protective immunities (for instance, specific cytotoxic T lymphocytes) from the body to fight against the tumor cells.

Intra-hepatic tumor invasion in portal vein system with subsequent metastasis is the major cause of morbidity and mortality in patients with PLC. In our work, it was apparent that MUC1 expression was associated with PLC cell infiltration and metastasis as well. The possible mechanisms might be as follows. (1) E-cadherin is a transmembrane glycoprotein that mediates calcium-dependent, inter-cellular adhesion and is specifically involved in epithelial cell-to-cell adhesion. In cancers, decreased E-cadherin expression is one of the alterations that characterize the invasive phenotype, and the data support its role as a tumor suppressor. Studies have shown that aberrant E-cadherin expression is associated with the acquisition of invasiveness and more advanced tumor stage for many cancers including lung cancer, prostate cancer, gastric cancer, and breast cancer, indicating that MUC1 promotes tumor metastasis by downregulating E-cadherin expression and its binding to beta-catenin<sup>[21-23]</sup>. (2) MUC1 acts as anti-cell adhesion molecules. High density of filamentous MUC1 molecules expressed on tumor cell surface might prevent binding between membrane-anchored ligands and corresponding receptors, minimize intercellular interactions induced by integrin in extracellular matrix, thus facilitating cancer cell invasion<sup>[24,25]</sup>. (3) Sialyl Lewis epitopes on MUC1 molecules function as

ligands to E-selectin in injured or inflammatory vascular endothelial cells, and facilitate tumor cell adhesion, infiltration, and metastasis<sup>[26]</sup>.

Currently, carcinectomy is still one of the major treatments for PLC, but has limitations for PLC patients of life expansion and quality promotion. MUC1 molecules play a double role in tumor genesis and development<sup>[4]</sup>. On one hand, aberrant MUC1 expression influences inter-cellular adhesions via surface molecule interactions, and makes easier for tumor cell growth and metastasis. On the other hand, as a hapten with newly formed glycan or peptide epitopes because of incomplete glycolization, MUC1 induces anti-tumor immune responses, and may be a target for tumor immunotherapy. It was reported that clinical MUC1 vaccination trials for breast cancer are in progress<sup>[27]</sup>. We also discovered that MUC1 gene vaccination induces specific cytotoxic T lymphocyte responses in mice<sup>[28]</sup>. MUC1-targeted tumor therapeutical vaccination may be effective for PLC treatment.

To conclude, we used immunohistochemical assay to detect MUC1 expression in PLC, cirrhotic and normal liver tissues, and the results show that MUC1 may be an indicator for PLC diagnosis and prognosis. As HCC is common in China<sup>[29-31]</sup> and most Asian countries<sup>[32-35]</sup>, further investigations on MUC1 roles in PLC genesis and development are of particular significance in providing new ways of PLC treatment.

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