MUC1, MUC2 and MUC5AC expression in hepatocellular carcinoma with cardiac metastasis

YU-SHENG LIN[1], SHIH-MING JUNG[2], CHUN-NAN YEH[3], YUNG-CHANG CHEN[4], FENG-CHUN TSAI[5], TZU-FANG SHIU[6], HSUEH-HUA WU[6], PYNG-JING LIN[5] and PAO-HSIEN CHU[6]

[1]Department of Internal Medicine, Chang Gung Memorial Hospital, Taoyuan; Departments of [2]Pathology, [3]Surgery, [4]Nephrology, Divisions of [5]Thoracic and Cardiovascular Surgery, [6]Cardiology, Department of Internal Medicine, Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Taipei, Taiwan

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Abstract. Advanced hepatocellular carcinoma is characterized by a poor prognosis, and the choice of therapy is complicated in cases with cardiac metastasis due to the questionable benefits of surgery. Since many studies have indicated that mucin (MUC) expression plays an important role in cancer metastasis and recurrence, we investigated mucin expression in hepatocellular carcinoma patients with cardiac metastasis compared with primary hepatocellular carcinoma to confirm the nature of the malignancy. Over a 6-year period, the expression patterns of MUC1, MUC2 and MUC5AC in tumor samples from hepatocellular carcinoma patients with cardiac metastasis were assessed using immunochemistry. The results were compared with findings from a group characterized by a more favorable prognosis; those with primary hepatocellular carcinoma without recurrence. Pathologic examinations indicated that patients with hepatocellular carcinoma and cardiac metastasis had more vascular invasion (P=0.004) and less section-free zone involvement (P<0.001) than those with primary hepatocellular carcinoma. MUC1 expression was significantly higher in hepatocellular carcinoma with cardiac metastasis (P<0.005). In conclusion, the expression of mucins, especially MUC1, confirms the malignant nature of hepatocellular carcinoma with cardiac metastasis. It is recommended that such patients receive aggressive therapy.

Introduction

Hepatocellular carcinoma (HCC) is the most common primary malignant tumor of the liver (1). Extrahepatic metastasis is frequently observed in advanced HCC, and results in a poorer prognosis. Metastasis to the heart is rare, but more common in advanced HCC patients, and the choice of therapy is complicated due to questionable benefits of surgery versus drug treatment.

Our previous study suggested that aggressive cardiac surgery may improve the survival rate for patients with HCC and cardiac metastasis (2). However, the poor prognosis for HCC patients with cardiac metastasis may be due to the malignant nature of the disease itself. Therefore, our goal was to find biomarkers to confirm malignancy in patients with HCC and cardiac metastasis. In many tissues, mucins (MUC), which are polysaccharide-containing proteins, play a role in the cell membranes and protect the epithelium from injury (3,4). At least 20 mucins have been identified, and recent studies suggest that some are important in cancer metastasis and prognosis (3-5). A previous report demonstrated that MUC1 and MUC5AC are not strongly expressed in normal liver tissue or HCC (4).

We hypothesized that mucins play a major role in metastasis, and that mucin expression is a predictor of a poor prognosis in patients with advanced HCC and cardiac metastasis. To test this hypothesis, we compared mucin expression patterns between cases with primary HCC and those with HCC and cardiac metastasis.

Materials and methods

This retrospective study was conducted between January 1993 and December 2006, and comprised 6 consecutive HCC patients with cardiac metastasis who had received surgical management. The study was approved by our institutional review board (CGMH96048B). As a control group with a more favorable prognosis, 22 patients were enrolled who had been diagnosed with primary HCC, had received resection, and had been recurrence-free for at least 6 years. Clinical data on age, gender, presenting symptoms, site of metastasis, surgical procedures, α fetal protein (AFP), liver cirrhosis and vascular invasion status were acquired. Follow-up data were compiled from clinical records and standardized telephone interviews.

Immunohistochemical staining for MUC1, MUC2 and MUC5AC. Assessment of MUC1 (1:100), MUC2 (1:100) and MUC5AC (1:100) was carried out using kits from Dako

Correspondence to: Dr Pao-Hsien Chu, The First Cardiovascular Division, Department of Internal Medicine, Chang Gung Memorial Hospital, 199 Tun-Hwa North Road, Taipei 105, Taiwan
E-mail: pchu@adm.cgmh.org.tw

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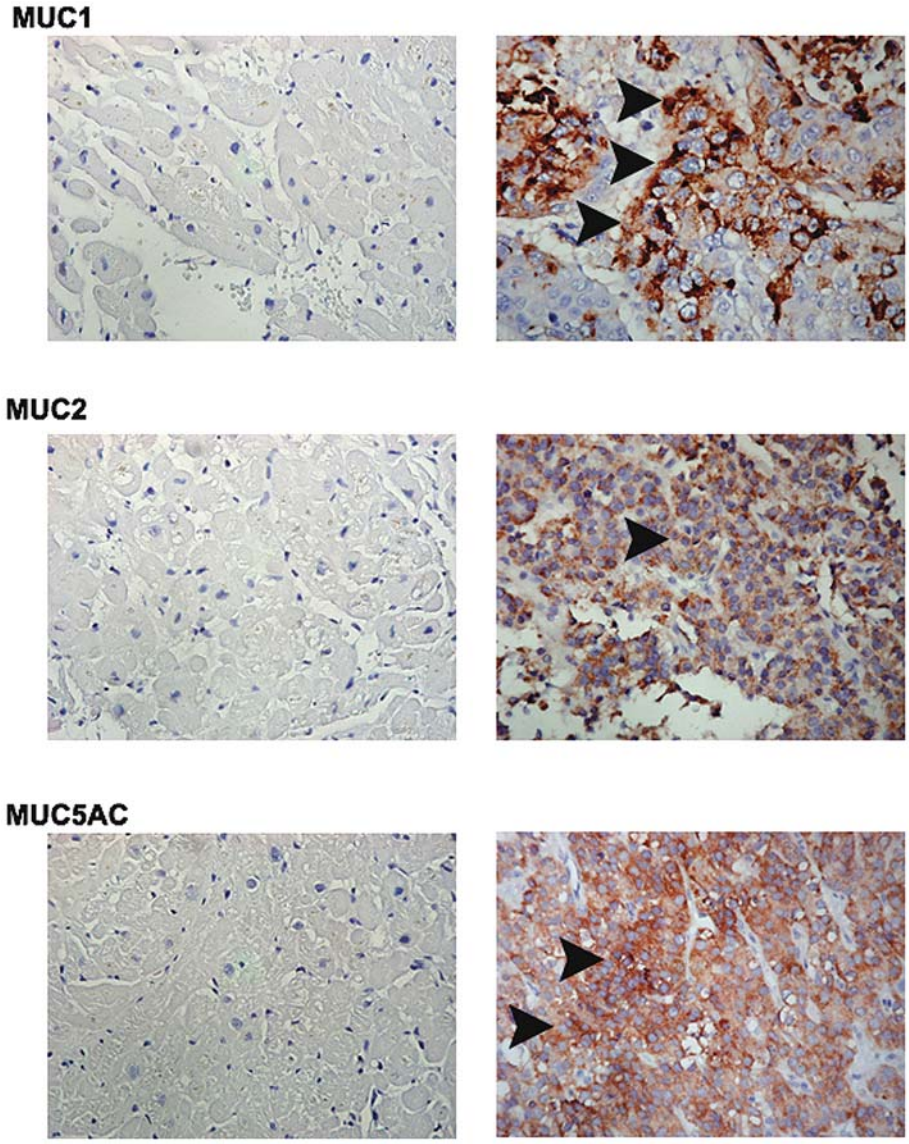

Figure 1. Representative immunohistological studies showing typical positive mucin staining (MUC1, MUC3 and MUC5AC) in hepatocellular carcinoma with cardiac metastasis (arrowheads, right column). Myocardium was used as a negative control group (left column). Original magnification, x400.

A/S (Denmark) according to the manufacturer's instructions. Negative controls included sections stained with phosphate-buffered saline without the primary antibody, and previously-obtained sections of myocardium (5) (Fig. 1).

The proportion of cells that stained positive for mucin expression was determined and reported as the number of positively-stained cells per 100 HCC cells. Sections with a score of 0 (<5% positive staining cells) were defined as immunohistochemically negative; all others were defined as immunohistochemically positive. The NPar test was used to statistically evaluate the results.

Results

Clinical findings. Clinical data, including age, gender, virus carrier status, liver cirrhosis status, AFP and cancer size, are listed in Table I. Patients in the primary HCC group were asymptomatic and screened using an abdominal echogram. Patients in the group with HCC and cardiac metastasis included those who were asymptomatic (84%) and those who presented with heart failure (16%). In HCC with cardiac metastasis, the involved cardiac areas included the right atrium (100%), right ventricle (33%), inferior vena cava (33%) and left atrium (16%).

Pathological findings. Microscopic pathologic findings for primary HCC and for HCC with cardiac metastasis included capsule invasion, vascular invasion, section-free area, daughter nodule and tumor thrombi (Table II). Only differences in the section-free area ($P<0.001$) and the vascular invasion ratio ($P=0.04$) were significant.

Expression of MUC1, MUC2 and MUC5AC. Expression of MUC in the two study groups is listed in Table III. MUC1, MUC2 and MUC5AC showed similar expression trends, with higher values in the cardiac metastasis group. MUC5AC showed little difference between groups, but followed the same trend. Values for MUC1 and MUC2 were higher in the experimental than in the control groups, but the difference was only significant for MUC1 ($P<0.005$).

Table I. Clinical data for primary hepatocellular carcinoma and hepatocellular carcinoma with cardiac metastasis.

Variables	Primary HCC without recurrence (n=22)	HCC with cardiac metastasis (n=6)	NPar test
Age (years)	52±13	54±9	P>0.05
Gender (%)			P>0.05
Males	18 (82)	4 (67)	
Females	4 (18)	2 (33)	
Virus carrier status (%)			P>0.05
HBV	12 (55)	1 (17)	
HCV	4 (18)	1 (17)	
Non-B Non-C	3 (14)		
Liver cirrhosis (%)			P>0.05
Non-cirrhosis	12 (56)	1 (17)	
Child A	8 (36)	2 (34)	
AFP (ng/dl)			P>0.05
<100	7 (32%)	3 (50%)	
>100	7 (32%)		
Cancer size (%)			P>0.05
<3 cm^3	5 (23)		
>3 cm^3	15 (68)	2 (34)	

HCC, hepatocellular carcinoma; AFP, α fetal protein.

Table II. Pathologic findings in primary hepatocellular carcinoma and hepatocellular carcinoma with cardiac metastasis.

Variables	Primary HCC without recurrence (n=22)	HCC with cardiac metastasis (n=6)	NPar test
Daughter nodule (%)			P>0.050
Not found	18 (81)	2 (34)	
Tumor thrombi (%)			P>0.050
Yes	3 (14)	1 (17)	
Capsule invasion (%)			P>0.050
Yes	11 (50)	1 (17)	
Pseudoglandular pattern (%)			P>0.050
No	6 (27)	2 (30)	
Vascular invasion (%)			P=0.004
Yes	2 (9)	1 (17)	
Section zone (%)			P<0.001
Free	22 (100)		
Not free		1 (17)	

HCC, hepatocellular carcinoma; section zone, the cancer-free area >1 cm from the cutting border.

Table III. Immunohistochemical expression of MUC1, MUC2 and MUC5AC in primary hepatocellular carcinoma and hepatocellular carcinoma with cardiac metastasis.

Variables	Primary HCC without recurrence (n=22)	HCC with cardiac metastasis (n=6)	NPar test
MUC1 (%)	0.68±3.19	10.83±16.25	P<0.005
MUC2 (%)	1.59±4.72	15.00±36.7	P>0.050
MUC5AC (%)	15.09±22.08	18.33±22.06	P>0.050

HCC, hepatocellular carcinoma.

Discussion

Clinical data, including virus carrier status, liver cirrhosis status, AFP level and tumor size, were not correlated with outcome in primary HCC and HCC with cardiac metastasis.

Pathologic analysis of tissues revealed that neither HCC capsule invasion status nor daughter nodule status were correlated with performance between primary HCC and HCC with cardiac metastasis. However, differences in vascular invasion status and section-free status were markedly significant in primary HCC and HCC with cardiac metastasis. These differences also confirmed the malignant nature of HCC with cardiac metastasis.

In immunochemical assays, MUC1, a transmembrane mucin transcribed from 1q21, is found on normal epithelial cells and is increased in cancer cells (3,6). The traditional intracellular role of MUC1 is the modulation of adhesion, which protects against pathogens. However, in cancer cells MUC1 contributes to metastasis (7,8). MUC1 is not expressed in normal liver tissue but is expressed in progenitor hepatocytes and in cancer cells (9), possibly signalling metastasis and poor prognosis. In the current study, the expression of MUC1 was significantly higher in HCC patients with cardiac metastasis than in those with primary HCC alone. Patients with HCC and cardiac metastasis had a poorer prognosis, possibly corresponding to a more terminal stage.

The expression of MUC2 has been associated with elevated metastasis rates in various types of cancer (10). We observed a similar pattern, although the difference was not significant, despite our having found a much higher expression level in cardiac metastasis.

MUC5AC, which is expressed mainly in the trachea or gastric mucosa, has been associated with a fair prognosis, but is a predictor of post-operative disease recurrence (11-13). In our study, MUC5AC expression differed little between groups, although it exhibited higher levels in HCC with cardiac metastasis.

Taken together, the results suggest that the expression of MUC1 in HCC with cardiac metastasis is correlated with

prognosis and metastasis, supporting our hypothesis that mucins play a major role in the prognosis of HCC patients with cardiac metastasis. The expression of MUC1 suggests more extensive metastasis and a poorer prognosis in such patients.

Our study had certain limitations. First, the study sample was small, as cases of HCC with cardiac metastasis are rarely observed. However, these patients require more aggressive therapy due to an extensive pattern of metastasis. Second, although mucins such as MUC1 suggest a possible mechanism of metastasis and recurrence in HCC, the details regarding this mechanism remain unknown. Further clarification is therefore required.

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References

1. Parkin DM, Pisani P and Ferlay J: Estimates of the worldwide incidence of 25 major cancers in 1985. Int J Cancer 80: 827-841, 1999.
2. Lin YS, Jung SM, Tsai FC, Yeh CN, Shiu TF, Wu HH, Lin PJ and Chu PH: Hepatoma with cardiac metastasis: An advanced cancer requiring advanced treatment. World J Gastroenterol 13: 3513-3516, 2007.
3. Hattrup CL and Gendler SJ: Structure and function of the cell surface (tethered) mucins. Annu Rev Physiol 70: 431-457, 2007.
4. Sasaki M, Ikeda H and Nakanuma Y: Expression profiles of MUC mucins and trefoil factor family (TFF) peptides in the intrahepatic biliary system: physiological distribution and pathological significance. Prog Histochem Cytochem 42: 61-110, 2007.
5. Chu PH, Jung SM and Yeh TS: MUC1, MUC2 and MUC5AC expressions in cardiac myxoma. Virchows Arch 446: 52-55, 2005.
6. Carraway KL III, Funes M, Workman HC and Sweeney C: Contribution of membrane mucins to tumor progression through modulation of cellular growth signaling pathways. Curr Top Dev Biol 78: 1-22, 2007.
7. Ahmad R, Raina D, Trivedi V, Ren J, Rajabi H, Kharbanda S and Kufe D: MUC1 oncoprotein activates the IκB kinase ß complex and constitutive NF-κB signaling. Nat Cell Biol 9: 1419-1427, 2007.
8. You JF, Hsieh LL, Changchien CR, Chen JS, Chen JR, Chiang JM, Yeh CY, Hsieh PS, Fan CW, Liu CT and Tang R: Inverse effects of mucin on survival of matched hereditary nonpolyposis colorectal cancer and sporadic colorectal cancer patients. Clin Cancer Res 12: 4244-4250, 2006.
9. Yuan SF, Li KZ, Wang L, Dou KF, Yan Z, Han W and Zhang YQ: Expression of MUC1 and its significance in hepatocellular and cholangiocarcinoma tissue. World J Gastroenterol 11: 4661-4666, 2005.
10. Sonoda H, Yamamoto K, Kushima R, Yamamoto H, Naitoh H and Okabe H: Detection of lymph node micrometastasis in pN0 early gastric cancer: efficacy of duplex RT-PCR with MUC2 and TFF1 in mucosal cancer. Oncol Rep 16: 411-416, 2006.
11. Boonla C, Sripa B, Thuwajit P, Cha-On U, Puapairoj A and Miwa M: MUC1 and MUC5AC mucin expression in liver fluke-associated intrahepatic cholangiocarcinoma. World J Gastroenterol 11: 4939-4946, 2005.
12. Yu CJ, Yang PC, Shun CT, Lee YC, Kuo SH and Luh KT: Overexpression of MUC5 genes is associated with early post-operative metastasis in non-small-cell lung cancer. Int J Cancer 69: 457-465, 1996.
13. Yu CJ, Shih JY, Lee YC, Shun CT, Yuan A and Yang PC: Sialyl lewis antigens: association with MUC5AC protein and correlation with post-operative recurrence of non-small cell lung cancer. Lung Cancer 47: 59-67, 2005.