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Expression of Biliary Antigen and Its Clinical Significance in Hepatocellular Carcinoma

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Abstract

In order to classify the hepatocellular carcinomas (HCCs) which had diverse clinicopathologic characteristics, we divided HCCs into two groups according to the expression of biliary antigen on the basis of the hypothesis that the hepatocyte and biliary epithelial cell originate from the same precursor cell, and then we investigated the clinical and pathologic characteristics in the two groups. Forty HCC cases with no preoperative treatment and at least two-year follow-up data were selected among 202 cases of HCC files from 1991 to 1995. Expression of biliary antigen (AE1, cytokeratin 19), p53, AFP, and Ki-67 in the tumor tissue were assessed by immunohistochemistry. Positive cytokeratin 19 was noted in one case (2.5%); AE1 was detected in 40% of patients; p53 was overexpressed in 20% of patients; and AFP was detected in 45% of patients. No statistical difference between the biliary antigen positive group (16 cases) and the negative group (24 cases) were noted in terms of mean age, sex, presurgical serum AFP level, Child class, and tumor size. HBsAg positive rate was 66.7% for the biliary antigen (−) group and 93.8% for the biliary antigen (+) group with a statistically significant difference ($p=0.048$). The number of cases for Edmonson-Steiner grade I/II and III/IV were 15 and 9 in the biliary antigen (−) group, and 4 and 12 in the biliary antigen (+) group, respectively, with a statistically significant difference ($p=0.024$). The 1, 3 and 5-year disease-free survival rates were 69.7, 40.9 and 40.9% for the biliary antigen (−) group and 73.7, 39.1, 39.1% for the biliary antigen (+) group with no statistically significant difference. The 1, 3 and 5-year overall survival rates were 91.7, 73.8, 66.4% for the biliary antigen (−) group and 68.8, 34.4, 34.4% for the biliary antigen (+) group, with a significantly greater overall survival rate for the biliary antigen negative group ($p=0.045$). Poor histopathological differentiation, a high HBsAg positive rate and poor overall survival rate were noted in the biliary antigen positive group and the differences were statistically significant. In conclusion, HCCs with positive biliary antigen, which originates from more primitive cells, is suggested to be more aggressive than HCCs with negative biliary antigen.

Key Words: Hepatocellular carcinoma, biliary antigen, clinical significance

INTRODUCTION

In general, hepatocellular carcinoma (HCC) has been considered a single disease based on the assumption that it originates from a single cell type. However, its diverse clinical and pathobiological behavior suggests that HCC is a heterogeneous disease. It is possible that different stages of differentiation may have a different susceptibility to carcinogens. HCCs, transformed from cells at different stages of differentiation, may also have different clinical and pathobiological patterns. Recently, Wu et al suggested that cells at different phases of differentiation, when transformed by carcinogens or genetic events into malignant cells, might retain their phenotypic markers.[1] Malignant transformation of the progenitor cells may give rise to HCC with both hepatocyte and biliary differentiation markers.

HEP-PAR-reactive antigen is a hepatocytic differentiation marker and it is detected in 99.7% of HCCs while AE1, cytokeratin-7 and cytokeratin-19 are biliary differentiation markers that are detected in 29.3% of HCCs.[1] AE1 (cocktail of cytokeratin 10, 14, 15, 16, 19) and cytokeratin-19 are well known tumor markers that can discriminate hepatocellular carci-

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noma and cholangiocellular carcinoma.[2-7]

Several data in recent years suggest that HCCs expressing the biliary antigen appear to be more aggressive, clinicially and pathobiologically.[1] This is supposed to be used as the predictive marker of the possibility of recurrence and treatment results after surgical resection of HCC. However, there are no reports about the relationship between the expression of the biliary antigen and prognostic factors, the tumor markers in HCC and about the overall survival rate and the disease-free survival rate of the HCC after curative resection according to the expression of biliary antigen.

We investigated the factors influencing the expression of biliary antigen in HCC patients who received curative resection and examined the differences in clinical courses and survival rates according to the expression of biliary antigen.

MATERIALS AND METHODS

Patient population

From January 1991 to December 1995, 202 patients underwent hepatectomy due to HCC in the Department of Surgery, Yonsei University College of Medicine. Among them, 40 patients without any presurgical procedure and with at least two-year follow-up data and paraffin-embedded tissue blocks were examined. The factors influencing biliary antigen expression and the differences in clinical courses and survival rates according to biliary antigen expression were investigated. Clinicopathologic variables of sex, age, HBsAg, serum alpha-fetoprotein (AFP), Child class, tumor size, histologic grades were checked through medical records, retrospectively. Each patient's survival state was obtained by medical records, telephone, and mail, while follow-up data was recorded until death of the patient or until January 1st, 1998.

Immunohistochemical staining

The histologic grade of HCC was subclassified by Edmonson-Steiner classification while polyclonal antibodies AE1 (Dako, Carpinteria, CA, USA) and monoclonal antibodies against cytokeratin-19 (Dako, Carpinteria, CA, USA) were used as phenotypic markers of biliary differentiation. MIB-1 (Ki-67, Immunotech, Westbrook, ME, USA) was used as a cell proliferation marker. In addition, AFP (Dako, Carpinteria, CA, USA) and p53 (Dako, Carpinteria, CA, USA) were also determined.

All tissue sections were fixed in 10% buffered formaldehyde solution, processed by routine methods and stained with hematoxylin-eosin. Immunohistochemical staining was carried out as described previously.[6] The primary antibodies used were AE1 at a dilution of 1 : 50, cytokeratin-19 at a dilution of 1 : 75, AFP at a dilution of 1 : 100, p53 at a dilution of 1 : 40, and Ki-67 at a dilution of 1 : 75. Diaminobenzidine was the colorizing agent used and the sections were counter-stained with Harris' hematoxylin. Negative control experiments were carried out by substituting the primary antibody with phosphate-buffered saline.

The expression of AE1, cytokeratin-19, AFP, and p53 were semiquantitatively evaluated as follows: those having positive staining in less than 5% were regarded as negative; between 5% and 25% as 1+; between 25% and 50% as 2+; and greater than 50% as 3+.

The proliferation activity was evaluated as Ki-67 labeling indices (LIs). At least 1,000 nuclei were selected randomly using a monoclonal eye-piece grid and a mechanical stage to avoid recounting. Nuclei with well-defined brown granular staining were considered to be positive. Ki-67 LIs were expressed as a percentage of positive nuclei per all nuclei counted.

Statistical analysis

All collected data were analyzed using a statistical program (SPSS for Windows). Positive staining for AE1 or cytokeratin-19 was categorized as HCC-biliary antigen (BAg) (+), and negative staining was categorized as HCC-BAg (−). For statistical analysis, t-test was used to compare the HCC-BAg (+) and HCC-BAg (−) groups in terms of age and tumor size, and chi-square test was used for sex, HBsAg, serum AFP, Child class, and histologic grade. The Kaplan-Meier method was used to calculate the disease-free and overall survival rates, while the differences between the curves were assessed using the Log rank test. A p-value <0.05 was considered statistically significant.

RESULTS

Of 40 patients, 26 patients were male and 14 were female, and the mean age was 52 (range, 22−68) years. The mean tumor size was 5 cm (range, 1−13 cm), and 22 cases were less than 5 cm in size and 18 cases were more than 5 cm. The HBsAg positive rate was 77.5%. The median value of serum AFP was 145.9 IU/ml (range, 0.9−16584.2 IU/ml) and 20 cases were less than 100 IU/ml and 20 cases were higher. According to the Child classification, 33 patients belonged to the class A and 7 to class B. According to the Edmonson-Steiner's histologic grade, 4 cases belonged to grade I, 15 to grade II, 17 to grade III, and 4 to grade IV (Table 1).

Positive reaction to cytokeratin-19 was noted in one case (2.5%). For AE1, 1+ was noted in 12 cases (30.0%) and 2+ in 4 cases (10.0%) (Fig. 1). For p53, 1+ was noted in 3 cases (7.5%), 2+ in 2 cases (5.0%), and 3+ in 3 cases (7.5%). For AFP, 1+ was noted in 10 cases (25.0%), 2+ in 6 cases (15.0%), and 3+ in 2 cases (5.0%).

Comparison between HCC-biliary antigen +) and HCC-biliary antigen (−)

There was no statistical difference in the expression of p53 and AFP between the HCC-BAg (+) group and the HCC-BAg (−) group. As well, there was no statistical difference in the Ki-67 index indicating the cellular proliferation between the two groups (Table 2).

In the clinicopathologic parameters, such as mean age, sex, preoperative serum AFP, Child class and tumor size, there were no statistical differences. But in the biliary antigen positive group, the HBsAg positive rate was significantly higher and poorer cellular differentiation was noted than in the biliary antigen negative group (p=0.048, p=0.024, respectively) (Table 3).

The 1, 3 and 5-year disease-free survival rates were 69.7, 40.9, and 40.9% for the HCC-BAg (−) group and 73.7, 39.1, 39.1% for the HCC-BAg (+) group, with no statistically significant difference (Fig. 2). The 1, 3 and 5 year overall survival rates were 91.7, 73.8,

Table 1. Clinical and Histologic Characteristics of Patients

Characteristics		Number (%) (n=40)
Sex	Male	26 (65)
	Female	14 (35)
Tumor size	≤5 cm	22 (55)
	>5 cm	18 (45)
HBsAg	positive	31 (77.5)
	negative	9 (22.5)
Serum-AFP	≤100 IU/ml	20 (50)
	>100 IU/ml	20 (50)
Child class	A	33 (82.5)
	B	7 (17.5)
	C	0 (0.0)
Histologic grade*		4 (10)
	II	15 (37.5)
	III	17 (42.5)
	IV	4 (10)

* Edmonson-Steiner grade.

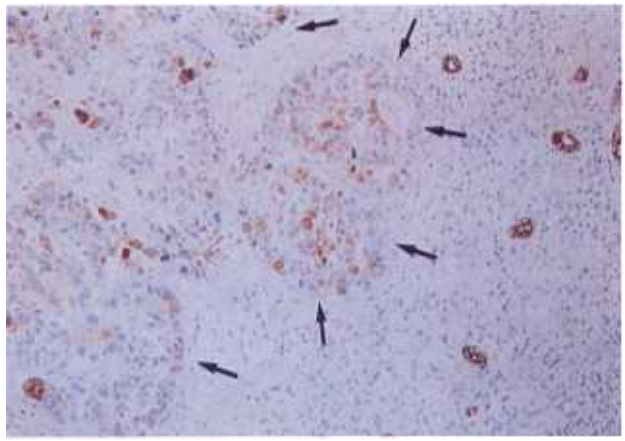

Fig. 1. AE1 expression in hepatocellular carcinoma (arrows). The bile ducts in the non-tumor tissue also showing AE1 expression (LASB, ×200).

Table 2. p53 and AFP Expression According to the Biliary Antigen Expression

		HCC-BAg (	HCC-BAg (+)	p-value
p53	positive	5		0.395
	negative	19		
AFP	positive	9	9	0.077
	negative	15	7	
Ki-67	index*	48.3	57.3	0.568

HCC, hepatocellular carcinoma; BAg, biliary antigen.
* No. of positive cells/1000 cells.

Table 3. Relation between the Expression of Biliary Antigen and Clinical Characteristics

	HCC-BAg (−)	HCC-BAg (+)	p-value
Age (yr, mean)	54.3	48.8	0.107
Sex (M/F)	18/6	8/8	0.105
HBsAg (+/−)	16/8	15/1	*0.048*
s-AFP (≤100/>100 ng/ml)	14/10	6/10	0.197
Child class (A/B)	21/3	12/4	0.407
Tumor size (≤5/>5 cm)	14/10	8/8	0.604
HCC grade* (I, II/III, IV)	15/9	4/12	*0.024*

HCC, hepatocelluar carcinoma; BAg, biliary antigen.
* Edmonson-Steiner grade.

66.4% for the HCC-BAg (−) group and 68.8, 34.4, 34.4% for the HCC-BAg (+) group, with a significantly greater overall survival rate for the HCC-BAg (−) group (p=0.045) (Fig. 3).

DISCUSSION

HCC is one of the most common malignant tumors in Korea. It is difficult to cure, and its prognosis is very poor.[8,9] Hepatic resection has been the most effective treatment modality for HCC, however the resection rate is low (3−30%), and the five-year survival rate after the curative resection is reported as 10−40%.[10] Also, the recurrence rate after curative resection is 30−76%, which is very high.[11-13] In Korea, the resection rate is 20% and the five-year survival rate is reported as 23−42%.[14]

There is increasing evidence that the stem cell of the liver and the two major cell types of hepatocytes and biliary epithelial cells are believed to originate from the same progenitor cells.[15] As well, combined hepatocellular-cholangiocarcinomas have been explained as bidirectional differentiation of a neoplastic hepatic stem cell population. If this hypothesis is true, HCC can be divided into the HCC derived from progenitor cells and the HCC derived from hepatocytes, though they have same histologic features.[16] Since the progenitor cell can differentiate into both hepatocytes and biliary epthelial cells, it can express both hepatocyte differentiation markers and biliary differentiation markers. However the hepatocyte does not express biliary differentiation markers. The HCC cases of malignant transformation from the progenitor cell express both hepatocyte differentiation markers and biliary differentiation markers, therefore HCCs can be classified by the presence or absence of biliary antigens.

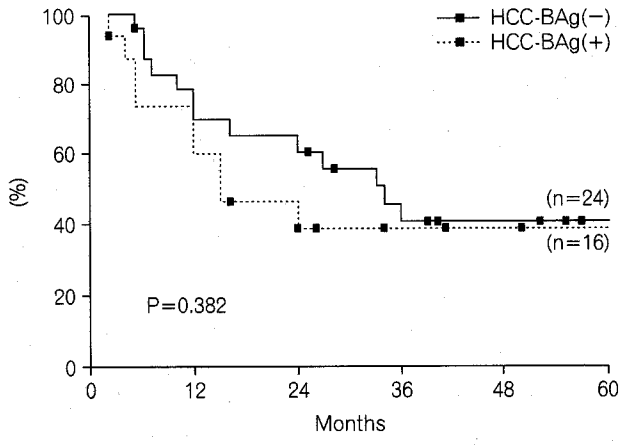

Fig. 2. Disease-free survival of patients with HCC with and without biliary antigen (BAg).

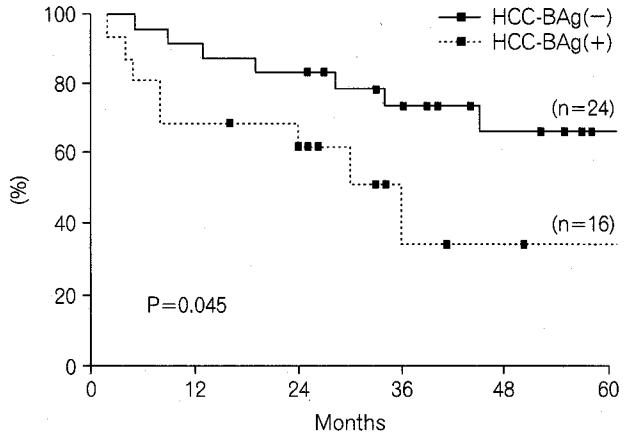

Fig. 3. Overall survival of patients with HCC with and without biliary antigen (BAg).

Cytokeratins are catalogued into 19 different cytokeratin polypeptides.[17] Human hepatocytes contain only cytokeratins Nos. 8 and 18 in the catalogue of Moll, whereas cytokeratins Nos. 10, 14, 15, 16, and 19 are contained in many epithelia, including bile duct epithelium.[17] So, the HCCs which are immunoreactive with monoclonal antibody against cytokeratin 19 and with polyclonal antibody AE1 may be derived

from progenitor cells. In the previous reports, the positive rates of the biliary antigen in the HCC were 29-50%.[1,2] In this study, 16 of 40 cases expressed the biliary antigen and the positive rate was 40%.

Mutation of the p53 gene is frequently found in diverse human cancers. It has been reported that the mutation rate of the p53 gene is about 50-60% in lung cancer, 50% in colorectal cancer, 50% in extrahepatic bile duct cancer, and 40% in esophageal cancer.[18-20] In HCC, overexpression of p53 has been shown in 5-24% of Caucasian patients,[21,22] 31% of Hong Kong Chinese,[23] and 61% of Chinese patient.[24] p53 mutation is usually more frequently detected in tumors with poorer differentiation, greater tumor size and presence of giant cells in HCCs.[23] In some reports, overexpression of p53 protein is reported to be a poorer prognostic indicator,[25,26] however different results have also been reported.[22,27] In this study, overexpression of p53 protein was noted in 8 of 40 cases (20%), and there was no relation between the overexpression of p53 protein and the expression of biliary antigen in HCCs.

Serum AFP is also known to be related to the prognosis.[28] Our results revealed that the expression of AFP was noted in 18 cases (45%), and preoperative serum AFP was above 100 IU/ml in 20 cases (50%). There was no statistically significant relationship between the expression of biliary antigen and the expression of AFP, although the incidence of AFP expression was relatively lower in the HCC-BAg (-) group. In previous reports, HCC-BAg (+) showed a significant increase in the expression of AFP,[1] so further studies might be needed with more cases.

Ki-67 is expressed during G1, increases during the cell cycle and rapidly declines after mitosis. Therefore, its expression represents cellular proliferation. Prior studies revealed that the cases with a low Ki-67 index had significantly longer disease-free survival,[29] and that the expression of Ki-67 was an independent prognostic marker in patients with HCC.[30] In this study, the Ki-67 index was 57.3 in HCC-BAg (+) and 48.3 in HCC-BAg (-) and there was no statistical significance between the two groups, although there was a slightly increasing tendency in the HCC-BAg (+) group.

The HBsAg positive rate was significantly higher in the HCC-BAg (+) group than in the HCC-BAg (-) group (66.7% vs 93.8%, p=0.048) while HCCs with positive biliary antigen had poorer cellular differentiation (p=0.024). The proliferation of progenitor cells was reported in hepatitis B virus associated human hepatocarcinogenesis.[31]

In comparison of the biliary antigen expression to survival, patients with HCC-BAg (+) didn't have a different disease-free survival rate compared to HCC-BAg (-) patients, but HCC-BAg (+) patients had a poorer overall survival rate (p=0.045). In association with poorer histologic grade and the increasing tendency of Ki-67, this observation suggests that HCC-BAg (+) may be more aggressive compared to HCC-BAg (-).

In conclusion, HCCs with positive biliary antigen expression originating from more primitive cells are suggested to be more aggressive than HCCs with negative biliary antigen expression. To determine its clinical usefulness as a prognostic factor will require further study of more HCC cases with long-term follow up.

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