Mucin in primary liver carcinomas: Combined hepatocellular-cholangiocarcinoma or variant hepatocellular carcinoma

SHIGERU HIROTA MD, MINORU NOMOTO MD, SADAHIRO HOSOBÉ MD,
YUTAKA AOYAGI MD, HITOSHI ASAKURA MD

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OBJECTIVE: To investigate whether the presence of mucin defines a combined hepatocellular-cholangiocarcinoma or merely a variant of usual hepatocellular carcinoma (HCC).

METHODS: From 1979-92, 124 cases of usual HCC were studied at Niigata University Hospital, Niigata City, Japan, and several affiliated hospitals. Histological diagnoses were determined according to World Health Organization (WHO) criteria. Hematoxylin and eosin stain, periodic acid-Schiff (PAS) stain, PAS stain after diastase digestion (D-PAS) and silver stain tests were performed. Cases containing D-PAS-positive substances were also stained by Alcian blue (AB) stain, high iron diamine (HID) stain and concanavaline A paradox-3 type (ConA3) stain. The classification of mucin was determined by AB, HID and ConA3 stains.

RESULTS: Mucin was recognized in the area of HCC by mucin stains in 25 of 124 cases. Two forms of mucin existence were classified: extracellular and intracellular. Mucins were classified by histochemical stains into three

types: sulfomucin, sialomucin and neutral mucin.

CONCLUSIONS: According to the WHO histological classification of primary carcinoma of the liver, mucin existence is characteristic of intrahepatic cholangiocarcinoma. But if mucin exists in morphologically usual HCC, it is better to diagnose it as a variant of HCC (with mucin) rather than as a combined hepatocellular-cholangiocarcinoma.

Key Words: *Hepatocellular carcinoma, Mucin*

Mucine dans les cancers primitifs du foie : cholangiocarcinome hépatocellulaire combiné ou variante du cancer hépatocellulaire

OBJECTIF : Déterminer si la présence de mucine suffit pour distinguer un cholangiocarcinome hépatocellulaire d'une simple variante du cancer hépatocellulaire habituel.

MÉTHODES : Entre 1979 et 1992, 124 cas de cancer hépatocellulaire ont été étudiés à l'hôpital universitaire de Niigata au Japon et dans plusieurs hôpitaux affiliés. Les diagnostics histologiques ont été posés conformément aux critères établis par l'Organisation mondiale de la santé (OMS). Les techniques de coloration par hématoxyline et éosine, de coloration périodique acide de Schiff (PAS), la coloration PAS après digestion

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Departments of Gastroenterology and Pathology, Akita Red Cross Hospital, Nakadouri Akita City; and The Third Department of Internal Medicine, Niigata University, Niigata City, Japan

Correspondence and reprints: Dr Shigeru Hirota, Department of Gastroenterology, Akita Red Cross Hospital, 1-4-36 Nakadouri Akita City, Japan. Telephone 81-188-343361, fax 81-188-358100

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de la diastase (D-PAS) et les tests de coloration à l'argentaffine ont été effectués. Certains cas renfermant des substances D-PAS-positives ont également été colorés à l'aide de bleu alcian (AB), de diamine ferrique élevée (HID) et de concanavaleine A de type paradoxe-3 (ConA3). La classification de la mucine a été déterminée par les colorations AB, HID et ConA3.

RÉSULTATS : La mucine a été reconnue dans les zones atteintes par le carcinome hépatocellulaire par les colorations de la mucine dans 25 cas sur 124. Deux formes de mucine ont été identifiées : la mucine extracellulaire et la mucine intracellu-

laire. Les mucines ont été classifiées en trois types par coloration histochimique : sulfomucine, sialomucine et mucine neutre.

CONCLUSION : Selon la classification histologique de l'OMS du cancer primitif du foie, la présence de mucine est caractéristique d'un cholangiocarcinome intrahépatique. Or, si la mucine est présente dans le carcinome hépatocellulaire de morphologie typique, il est préférable de poser un diagnostic de variante du carcinome hépatocellulaire (avec mucine) plutôt qu'un diagnostic de cholangiocarcinome hépatocellulaire combiné.

Primary carcinoma of the liver can be classified histologically as hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (CC) and combined hepatocellular-cholangiocarcinoma (HCC-CC). HCC is characterized by trabecular patterns of hepatocyte-like cells with stroma of sinusoid-like capillaries and sometimes bile production. CC is characterized by glandular structures with abundant fibrous stroma. Mucin production is thought to characterize CC. Combined HCC-CC is a tumour containing unequivocal elements of both HCC and CC (1). In this study, we classified HCC into two groups by mucin stains and compared the two groups from clinical and histopathological points of view.

MATERIALS AND METHODS

From 1979-92, 124 cases of usual HCC (91 autopsy cases and 33 surgical resection cases; 97 males and 27 females, ages from 17 to 78 years old) were studied at Niigata University Hospital, Niigata City, Japan and several other affiliated hospitals. Histological diagnoses were determined according to World Health Organization (WHO) criteria (1). Specimens were fixed in 10% formaldehyde solutions and embedded in paraffin wax. Sections measuring 4 µm were stained with hematoxylin and eosin stain, periodic acid-Schiff (PAS) stain, PAS stain after diastase digestion (D-PAS) and silver stain. Samples containing D-PAS-positive substances were also stained by Alcian blue (AB) stain, high

TABLE 1
Clinical and histopathochemical features

#	Age (years)	Sex	HBsAg	Anti-HBs	Anti-HCV	S-AFP (ng/mL)	S-CEA (ng/mL)	D-PAS stain	AB stain	HID stain	ConA3 stain	Background	Histology	Type	Mucin
1	55	M	+	ND	+	25	ND	+	—	—	+	LC	Mod	E	NCon
2	54	M	—	—	—	542	3.4	+	+	+	—	CPH	Mod	E	Sulfo
3	52	M	+	—	—	311	3.7	+	—	—	+	CAH	Mod	E	NCon
4	62	M	+	—	—	40	ND	+	—	—	+	LC	Mod	E	NCon
5	62	M	—	—	—	11,550	4.9	+	+	+	+	LC	Poor	E	NCon, Sulfo
6	51	M	—	+	+	58	5.2	+	+	—	+	CAH	Un	E	NCon, Sialo
7	65	M	+	—	—	28,400	4.0	+	+	—	—	LC	Mod	E	Sialo
8	60	M	—	—	ND	21,000	3.0	+	+	—	—	CAH	Mod	E	Sialo
9	46	M	+	—	ND	92	ND	+	+	+	—	LC	Mod	E	Sulfo
10	49	F	+	—	ND	659	4.9	+	+	—	—	LC	Mod	E	Sialo
11	62	F	—	—	ND	355,000	ND	+	+	—	+	LC	Mod	I	NCon, Sialo
12	35	M	ND	+	+	490	35.2	+	+	+	—	LC	Poor	I	Sulfo
13	55	M	+	—	+	46,900	3.3	+	+	—	—	LC	Poor	E	Sialo
14	57	M	+	—	+	160	5.1	+	—	—	+	CAH	Mod	E	NCon
15	62	M	—	+	+	7470	3.6	+	+	—	—	LC	Mod	I	Sialo
16	58	M	—	+	+	300,000	ND	+	—	—	+	LC	Poor	E	NCon
17	56	M	+	ND	—	2.7	8.4	+	—	—	+	LC	Well	E	NCon
18	40	F	—	—	—	22,000	6.1	+	+	—	—	CAH	Mod	E	Sialo
19	64	M	—	—	ND	13	2.7	+	—	—	+	LC	Mod	E	NCon
20	36	F	—	+	+	94,000	3.8	+	—	—	+	LC	Mod	E	NCon
21	62	M	—	—	ND	2880	ND	+	—	—	+	LC	Poor	E	NCon
22	57	M	—	—	ND	250,000	3.3	+	+	+	—	CAH	Mod	E	Sulfo
23	70	M	+	—	ND	24	2.2	+	—	—	+	LC	Poor	E	NCon
24	51	M	—	+	+	16,700	1.6	+	—	—	+	LC	Mod	E	NCon
25	58	M	ND	ND	ND	296	ND	+	+	—	—	LC	Mod	E	Sialo

AB Alcian blue; CAH Chronic active hepatitis; ConA3 Concanavaleine A paradox-3 type; CPH Chronic persistent hepatitis; D-PAS Diastase digestion periodic acid-Schiff; E Extracellular type; F Female; HBsAg Hepatitis B surface antigen; HCV Hepatitis C virus; HID High iron diamine; I Intracellular type; LC Liver cirrhosis; M Male; Mod Moderately differentiated; NCon Neutral mucin concanavaleine A paradox-3 type stain; ND Not described; Poor Poorly differentiated; S-AFP Serum alpha-fetoprotein; S-CEA Serum carcinoembryonic antigen; Sialo Sialomucin; Sulfo Sulfomucin; Well Well differentiated; Un Undifferentiated

TABLE 2
Comparison of clinical features

	# of cases	Mean age (range in years)	Male:female ratio	HBsAg	Anti-HCV	Serum AFP: >1000 ng/mL	Serum AFP: <20 ng/mL	Serum CEA: >10 ng/mL
Group A (HCC-mucin-positive)	25	55.2 (35-70)	21:4	10/23 (43.5%)	9/16 (56.3%)	12/25 (48.0%)	2/25 (8.0%)	1/18 (5.6%)
Group B (HCC-mucin-negative)	99	58.9 (17-78)	76:23	24/90 (26.7%)	37/65 (56.9%)	28/97 (28.9%)	12/97 (12.3%)	2/40 (5.0%)

AFP Alpha-fetoprotein; CEA Carcinoembryonic antigen; HBsAg Hepatitis B surface antigen; HCC Hepatocellular carcinoma; HCV Hepatitis C virus

TABLE 3
Comparison of histopathological background

	Liver cirrhosis					Chronic active hepatitis					Chronic persistent hepatitis				
	n	Well	Mod	Poor	Un	n	Well	Mod	Poor	Un	n	Well	Mod	Poor	Un
Group A	18/25 (72.0%)	1/18 (5.6%)	11/18 (61.1%)	6/18 (33.3%)	0/18 (0%)	6/25 (24.0%)	0/6 (0%)	5/6 (83.3%)	0/6 (0%)	1/6 (16.7%)	1/25 (4.0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)
Group B	60/99 (60.6%)	0/60 (0%)	45/60 (75.0%)	11/60 (18.3%)	4/60 (6.7%)	33/99 (33.3%)	0/33 (0%)	22/33 (66.7%)	9/33 (27.3%)	2/33 (6.0%)	6/99 (6.1%)	1/6 (16.7%)	2/6 (33.3%)	2/6 (33.3%)	1/6 (16.7%)

Group A comprised 25 hepatocellular carcinoma-mucin-positive cases and group B comprised 99 hepatocellular carcinoma-mucin-negative cases. Mod Moderately differentiated; Poor Poorly differentiated; Well Well differentiated; Un Undifferentiated

iron diamine (HID) stain (2) and concanavaline A paradox-3 type (ConA3) stain (3). Twenty-five cases were positive as determined by mucin stains. Mucin classification was determined by AB, HID and ConA3 stains. Mucins were classified into three types: sulfomucin (AB-positive, HID-positive, ConA3-negative), sialomucin (AB-positive, HID-negative, ConA3-negative) and neutral (AB-negative, HID-negative, ConA3-positive) (2,3). HCC was classified into two groups based on mucin existence in the area of HCC: group A – HCC-mucin-positive and group B – HCC-mucin-negative.

RESULTS

Twenty-five of the 124 cases were HCC-mucin-positive (Table 1). The other 99 cases were HCC-mucin-negative. Clinical data and background liver disease are shown in Tables 2 and 3.

In a review of the 25 HCC-mucin-positive cases, two types of mucin were classified: extracellular (n=22, 88%) – mucin was recognized inside the extracellular space surrounded by carcinoma cells of HCC (Figures 1,2); and intracellular (n=3, 12%) – mucin was recognized inside the carcinoma cells of HCC (Figure 3).

The characteristics of mucin were classified by AB, HID and ConA3 stains. The results showed sulfomucin in four cases (16.0%); sialomucin in seven cases (28.0%); and neutral mucin ConA3-positive in 11 cases (44.0%). Two cases of the extracellular type contained sialomucin and neutral mucin ConA3-positive in different areas (8.0%). Similarly, one of the intracellular types contained sulfomucin and neutral mucin ConA3-positive (4.0%) (Table 4).

There was no correlation between mucin characteristics and histopathological differentiation; similarly, there was no correlation between histopathological differentiation and mucin form (χ^2 , $P<0.05$).

No difference was found between group A (25 cases of

HCC-mucin-positive) and group B (99 cases of HCC-mucin-negative) in terms of age, sex, positive ratio of hepatitis B surface antigen, positive ratio of antihepatitis C virus, serum alpha-fetoprotein level, serum carcinoembryonic antigen level and background liver diseases (Student's *t* test, $P<0.05$).

DISCUSSION

According to the usual histopathological classification of primary carcinoma of the liver, mucin production can be recognized in CC, CC areas of combined HCC-CC7 and bile duct cystadenocarcinoma, but not in HCC. Mucin production is one characteristic of CC (1,4). There is a lot of evidence that these two types of liver carcinoma (HCC and CC) differ in their etiological, epidemiological, clinical and histopathological features (5-7). Combined HCC-CC7 presents both characteristics histopathologically.

Allen and Lisa (8) reported five cases of combined HCC-CC and reviewed 11 others from the literature. They defined combined HCC-CC based on the three types of tumours: separate masses of HCC and CC; contiguous but independent masses of HCC and CC; and an intimate intermingling of hepatocellular and glandular elements. Also Goodman and Ishak (9) classified combined HCC-CC into three types: collision tumours, apparently a coincidental occurrence of both HCC and CC in the same patient; transitional tumours, in which there are areas of intermediate differentiation and an identifiable transition between HCC and CC; and fibrolamellar tumours, which resemble the fibrolamellar variant of HCC but also contain mucin-producing pseudoglands. In their classification, one common criterion of CC was the production of mucin.

Wada et al (10) reported that intrahepatic metastatic lesions of HCC showing a trabecular growth pattern produced both bile and mucin. They diagnosed the metastatic lesion as combined HCC-CC because of the presence of mucin.

Figure 1) Extracellular type of mucin existence. **A** Large spaces can be seen in hepatocellular carcinoma, which is surrounded by sinusoid-like capillaries. Hematoxylin and eosin stain. **B** Mucin is stained with red inside the large spaces. Diastase digestion periodic acid-Schiff stain. **C** Mucin, composed of sialomucin, is stained blue. Alcian blue stain ($\times 330$)

Figure 3) Intracellular type of mucin existence. **A** Hepatocellular carcinoma surrounded by sinusoid-like capillaries can be seen. Hematoxylin and eosin stain. **B** Part of carcinoma cells of hepatocellular carcinoma is stained with blue. Mucin is composed of sialomucin. Alcian blue stain ($\times 409$)

Figure 2) Left Extracellular type of mucin existence. **A** Mucin is stained with red in the small spaces between carcinoma cells. Diastase digestion periodic acid-Schiff stain. **B** Mucin is stained with brown inside the small spaces between carcinoma cells of hepatocellular carcinoma. Mucin is composed of neutral mucin. Concanavaline A paradox-3 type stain ($\times 419$)

TABLE 4
Relationship between characteristics of mucin and forms of mucin existence

	Sulfomucin	Sialomucin	NConA3-positive	Sulfomucin/ConA3-positive*	Sialomucin/ConA3-positive*
Extracellular type (n=22)	3 (13.6%)	6 (27.2%)	11 (50.0%)	1 (4.6%)	1 (4.6%)
Intracellular type (n=3)	1 (33.3%)	1 (33.3%)			1 (33.3%)

*Two kinds of mucin exist in different areas of hepatocellular carcinoma. ConA3 Concanavaline A paradox-3 type stain; NConA3 Neutral mucin concanavaline A paradox-3 type stain

Robert and colleagues (11) concluded that mucin can be found within the pseudogland-like structures of HCC metastatic to the ovary. In combined HCC-CC, Uekusa et al (12) recognized mucin inside the pseudogland-like structures and inside the carcinoma cells in the area of HCC, but these workers did not find the stains helpful for discrimination. These three reports show a similar contradiction: histopathologically, the diagnosis was HCC, but mucin was recognized. Mucin presence changed the diagnoses in these reports.

Twenty-five of the 124 HCC cases in this study were HCC-mucin-positive (group A) and the other 99 cases were HCC-mucin-negative (group B). There was no difference between the groups concerning clinical features and histopathological backgrounds. If mucin stains had not been per-

formed, the two groups would have been regarded as usual HCC. According to WHO criteria (1), the 25 HCC-mucin-positive cases must be diagnosed as combined HCC-CC rather than HCC because they contain mucin. Mucin existence is important enough to change diagnoses. Goodman and Ishak (9), and Popper and Shaffner (13) have questioned this criterion about mucin production.

Apparent in this study were some problems concerning mucin; for example, mucin characteristics were not fixed. Also, it was unclear whether the mucin was derived from carcinoma cells (14), stroma or mucinous degeneration (15). In addition, bile duct cystadenocarcinoma and CC contain sialomucin predominantly (16).

If the origin of mucin is not clear, it is inappropriate to change the morphological diagnoses.

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