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CLINICOPATHOLOGIC FEATURES AND DIAGNOSIS OF COMBINED HEPATOCELLULAR AND CHOLANGIOCARCINOMA

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Hepatoma cases (N=130) were analyzed through histochemical and immunohistochemical staining. There were 99 cases of hepatocellular carcinoma (HCC), 15 cholangiocarcinoma (CC), and 16 combined HCC and CC (HCC+CC). The clinical features and the cases accompanied with hepatitis and/or liver cirrhosis in the non-tumor liver tissue of HCC+CC Group were between HCC Group and CC Group. Histologically, there were 4 cases with trabecular, 4 with pseudoglandula, 3 with solid type in HCC+CC Group. In these 11 cases, the CC area was less than 10% of the neoplasm. The cases were classified as HCC+CC type I. There was no obvious stroma fibrosis. The rest 5 cases of HCC+CC showed tubular carcinoma. The CC area took up over 10% of the tumor. These cases were designed as HCC+CC type II. There was significant fibrosis in the stroma so that its CC area is indistinguishable from that of CC cases. The CC area of all HCC+CC cases was positive to mucin and EMA staining, the same to that of CC cases. Near 70% of the HCC+CC cases had intracytoplasmic glycogen in the HCC area. The HCC area was mucin and EMA negative, similar to that of HCC cases. We also observed transition areas between HCC and CC in both of the type I and type II HCC+CC cases. The areas were mucin negative but EMA positive. We concluded that HCC+CC had HCC and CC area, with the characteristics of HCC of CC respectively. Histochemical mucin staining and immunohistochemical EMA staining were valuable in detection and diagnosis of the HCC+CC.

Key words: Combined hepatocellular and Cholangiocarcinoma, Diagnosis, Histochemistry, Immunohistochemistry.

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The most common malignancy of the liver is hepatocellular carcinoma (HCC), followed by cholangiocarcinoma (CC).[1, 2] Histologically, HCC shows trabecular, pseudoglandular, solid or sinusoid structure. HCC can also produces bile.[2, 3] These features and identification of alpha-fetoprotein (AFP) formation by the cancer cells are evidence of HCC.[4, 5] On the other hand, CC is characterized by glandular structure formation and mucin production. Histochemical mucin staining is an effective method of showing the CC component.[5] Carcinoma with both features of the HCC and CC is called combined HCC and CC (HCC+CC), but there is no generally accepted criteria for its diagnosis and classification.

Histological typing of tumors of the liver by the WHO only mentioned that HCC+CC has the characteristics of HCC and CC,[5] while AFIP classified the HCC+CC as a special subtype of HCC.[6] Epithelial membrane antigen (EMA) was reported helpful in the diagnosis and classification of the hepatoma.[7] There is no research on the significance of the EMA on the classification of HCC+CC. We used histochemistry and immunohistochemistry to classify the HCC+CC cases, discuss their clinicopathological features and

pathogenesis.

MATERIALS AND METHODS

The surgical materials were collected from hepatoma cases (N=130) of the Shanghai Medical University, China and Okayama University Medical School, Japan. The materials were fixed in 10% formalin, embedded in paraffin. Serial sections 4 μm thick were made and stained with hematoxylin and eosin, PAS, colloid iron and Masson trichrome. Immunohistochemistry included EMA and AFP staining. The antibodies to EMA and AFP were obtained from Dako Japan (Kyoto, Japan). Biotin labeled second antibodies and horseradish labeled avidin were purchased from Nichilei, Co. (Tokyo, Japan). After routine immunohistochemical reaction, the sections were incubated in 3, 3'diaminobenzidine (DAB, Sigma, St. Luis, MO, USA) in 0.003% hydrogen peroxidase solution. The nuclear were counterstained with hematoxylin, sealed for observation. Negative controls included substitution of the primary antibodies by non-immune serum from the same animal species.

The amount of the glycogen in the cancer cells or the mucin in the tumor were graded into: no (-), small amount (+), medium amount (++), and large amount (+++).

The immuohistochemical staining intensity of the cancer cells were classified as: negative (-), weakly (+), moderately (++), strongly (+++) stained.

The appearance of stroma fibrosis was based on the comparison with those of the normal liver. Stroma fibrosis indicated the cancerous stroma obviously exceeded that of the normal liver.

Upon histological observation, HCC cases were classified into four subtypes of trabecular, glandular, solid and sclerotic. Well differentiated HCC has more cytoplasm with fine acidophilic granules, sometimes with bile production. There may be intracytoplasmic glycogen, or positive staining to the AFP shown by histochemical or immunohistochemical staining. There is no mucin, the tumor is negative or only a few cancer cell positive to EMA staining.

The CC was divided into tubular, papillary or cystic adenocarcinoma. The tumor is characterized by glandular structure, mucin production. The cancer cell has no obvious glycogen. Fibrous tissue proliferation or fibrosis is common in the stroma.

HCC+CC was diagnosed when the cancer tissue has evident HCC and CC area, the cancer cells have the markers of HCC and CC separately.[5, 9] We named those tumors with less than 10% of CC area, more than 90% of HCC area as HCC+CC type I; while that with more than 10% of CC area as HCC+CC type II.

RESULTS

Present series includes 99 cases of HCC, CC 15 cases, HCC+CC 16 cases. Table 1 shows the clinical data of these cases.

Histologically, most of the HCC showed trabecular and solid type. Fourteen of 15 CC were tubular adenocarcinoma. HCC+CC had trabecular (N=4), pseudoglandular (N=4), solid (N=3) and tubular (N=5) types. The CC area of the former three types of HCC+CC took less than 10% of the tumor, belonging to the HCC+CC type I. The main morphological finding was that a part of the HCC nests had CC differentiation. The CC area mainly located in the peripheral part of the HCC, without obvious stromal fibrosis (Figure 1a–c). Pseudoglandular HCC+CC had CC area of more than 10% of the tumor, namely HCC+CC type II. The stroma of HCC+CC type II had obvious fibrosis (Figure 2a–c). The morphology of the CC area in the HCC+CC type II is indistinguishable from that of CC. The transitional area between the HCC and CC area was seen in both HCC+CC type I (Figure 1a) and type II. Hepatitis and/or liver cirrhosis were observed in the non-cancer tissue of 87/99 of the HCC cases, 13/16 of HCC+CC cases. The liver cirrhosis were only seen in 3/15 of the CC cases ($P<0.001$). The HCC area of HCC+CC was EMA negative, but positive for glycogen in 68.7% (11/16) cases. In all HCC+CC cases, the CC area was similar to the CC cases, showing positive mucin and EMA staining in the cancerous glandular structure (Figure 1b, 2b). In the transitional, area between HCC and CC the cancerous glandular structure showed positive EMA staining without obvious mucin. EMA positive cells were see in a few HCC cases, but the positive cells were scattered in the cancer, positive cells in clusters or nests were rarely seen. No positive EMA staining was observed in the pseudoglandular structure of the HCC cases (Table 2).

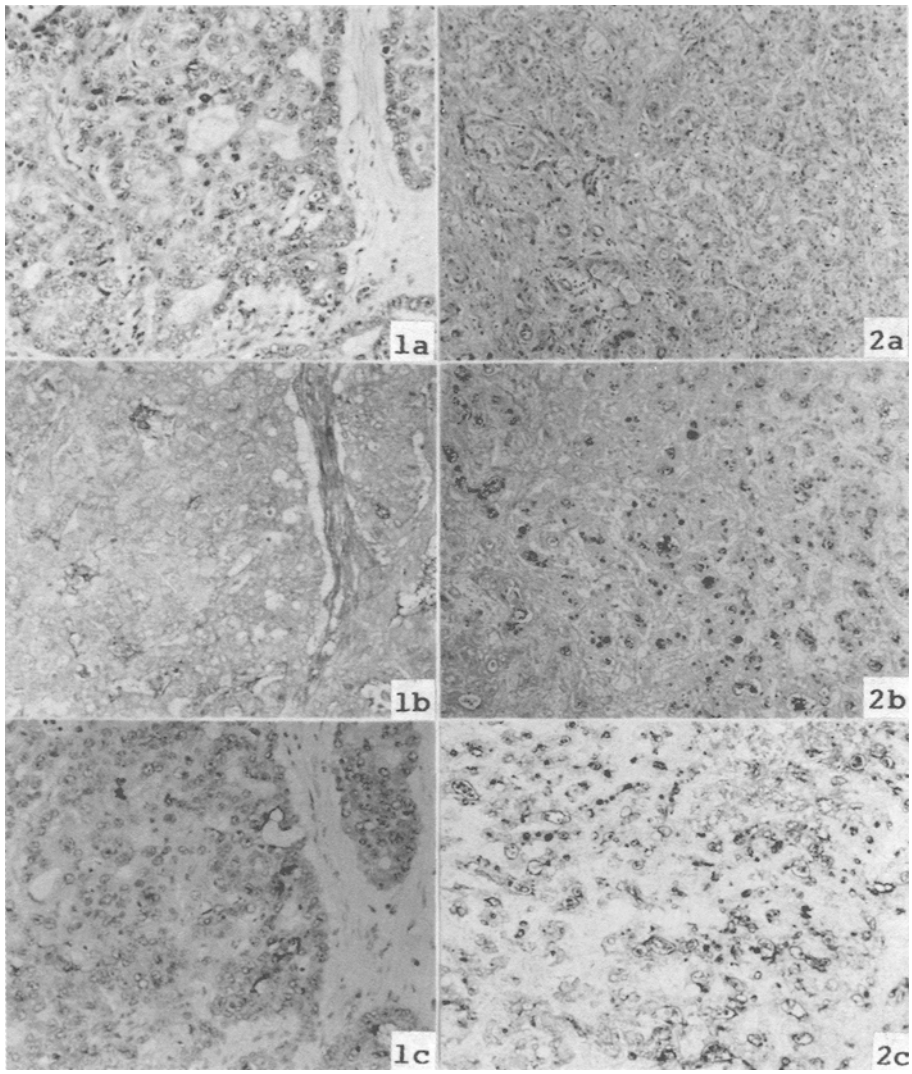

Fig. 1a. Combined hepatocellular carcinoma (HCC) and cholangiocarcinoma (CC) mostly composed of HCC area (HCC+CC type I). There is glandular structure at the peripheral part of the carcinoma (arrow). H.E. × 100.

1b. There is mucin in the glandular structure of the same area as Figure 1a (arrow). Colloid iron staining, × 100.

1c. Immunohistochemical staining shows the positive EMA reaction in the glandular structure (The same area as Figure 2a). The positive staining mainly located along the luminal side of the cancer cells (arrow). × 100.

Fig. 2a. Combined HCC and CC with substantial CC area (HCC+CC type II). The CC area composed of glandular structure. There is obvious fibrosis in the stroma. H.E. × 100.

2b. There is large amount of mucin in the glandular structure. (The same area as Figure 2a). Colloid iron staining, × 100.

2c. Immunohistochemical staining shows strong positive EMA reaction in the glandular structure. The luminal side of the cancer cells has strongest positive staining, × 100.

Table 1. The clinical data of the 130 hepatoma cases

	Age range yr. ($\bar{x}\pm s$)	Male : Female*	HBV infection**	High serum AFP***
HCC (N=99)	21–78 (48.9±12.4)	91: 8	66	45
CC (N=15)	35–77 (51.7±11.1)	7: 8	1	0
HCC+CC (N=16)	34–59 (48.0± 8.5)	14: 2	3	5

*x^2=7.527, P<0.05; **x^2=27.949, P<0.001; ***x^2=11.828, P<0.001.

Table 2. Histochemical and immunohistochemical staining results of the hepaoma cases

	Glycogen*		Mucin **		EMA***	
	Positive	Negative	Positive	Negative	Positive	Negative
HCC (N=99)	7	92	99	0	92	7[b]
CC (N=15)	14	1[a]	0	15	0	15
HCC+CC (N=16)	5	11	0	16	0	16

*x^2=62.062, P<0.001; **x^2=129.855, P<0.001; ***x^2=98.042, P<0.001.

[a]a few cells with small amount of glycogen; [b]a few cells weekly positive.

DISCUSSION

Hepatocellular carcinoma and cholangiocarcinoma seen in the same liver was divided into three categories: 1. Solitary tumors appears in different locations (double primary cancer); 2. Neighboring tumors meet and fuse with each other accompanying with their growth (collision tumors); and 3. histological features of the HCC and CC find in the same tumor (Mixed or combined type of HCC and CC).[9,10] The combined HCC and CC discussed in present work was diagnosed according to the criteria of Liver Cancer Study Group of Japan.[9] There are the histological features of both HCC and CC. The HCC and CC areas are "connected" and the transitions between the two areas can be seen. So that our series of HCC +CC belongs to category 3.

The mechanism of HCC+CC formation is obscure. The transformed hepatocyte can "differentiate into adenocarcinoma (CC)" *in vivo*.[11] In liver cancer occurred in the experimental animal, the differentiation of the same atypical hyperplasia or cancer "differentiates" to both of the HCC and CC.[12] So that we suggest that the HCC+CC type I was formed by "differentiation" of small amount of cancer cells to CC during the progress of HCC. The cancer cells proliferate in the direction of both HCC and CC, or large amount of cancer cells in HCC or CC progress and "differentiate" in the directions of other types resulted in HCC+CC type II.

We also noticed that the hepatoma with obvious fibrous stroma may be CC, HCC+CC, or fibrolamella or sclerosing HCC. There are 15, 5, and 3 cases respectively in present series. The definite diagnosis is only possible after histochemical or immunohistochemical staining.

The hepatocytes and cancer cells of HCC was reported to be EMA negative.[13] Present study shows that small amount of the cancer cells in some HCC cases may be weekly EMA positive, but the glandular structure is negative. Conversely, all of the glandular structures in CC had strong EMA staining. In the cases of HCC+CC, the transitional area was EMA positive, without mucin production. We considered the area to be the real transition or intermediate state of the HCC and CC. Thus, the EMA staining may be used as a marker of CC "differentiation". We used this marker, combined with glycogen and mucin staining, found that small amount of cases originally classified as HCC had CC "differentiation" area, which was EMA and mucin positive, glycogen negative. These areas were generally less than 10% of the tumor. The cases were classified as HCC+CC type I. Those carcinomas with obvious CC "differentiation" (taken up more than 10% of the tumor) were sorted as HCC+CC type II.

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