

Clinicopathologic Study on Combined Hepatocellular Carcinoma and Cholangiocarcinoma: with Emphasis on the Intermediate Cell Morphology

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Combined hepatocellular carcinoma and cholangiocarcinoma (combined HCC-CC) is a rare subtype of primary liver cancer. We investigated the histopathologic features of transitional or intermediate areas in 21 combined HCC-CCs and immunophenotypes using different hepatic progenitor cell markers (CK7, CK19, c-kit, NCAM, and EpCAM). Major histologic findings of transitional or intermediate areas of 21 combined HCC-CCs included strands/trabeculae of small, uniform, oval-shaped cells with scant cytoplasm and hyperchromatic nuclei embedded within an abundant stroma, small cells with an antler-like anastomosing pattern, and solid nests of intermediate hepatocyte-like cells surrounded by small cells in periphery, in order of frequency. The intermediate area of one tumor was composed predominantly of spindle cells arranged in short fascicles. Immunophenotype of tumor cells with intermediate morphology suggested a progenitor cell origin for this tumor. Clinical findings of combined HCC-CC showed a closer resemblance with those of HCC than those of CC. In univariate analysis, tumor size, TNM stage, and serum alpha-fetoprotein levels showed a significant association with poor patient survival. Serum alpha-fetoprotein level was an independent prognostic indicator in multivariate analysis. In conclusion, an awareness of the clinicopathologic features, specifically the various morphologic features of intermediate areas in this tumor, is essential for prevention of potential misdiagnosis as another tumor.

Key Words: Carcinoma; Hepatocellular; Cholangiocarcinoma

INTRODUCTION

Combined hepatocellular carcinoma and cholangiocarcinoma (combined HCC-CC) is a rare subtype of liver cancer displaying components of both hepatocellular and cholangiocellular carcinoma. The World Health Organization (WHO) classification defines combined HCC-CC, classical type as a tumor containing unequivocal elements of both hepatocellular carcinoma (HCC) and cholangiocarcinoma (CC), which are intimately admixed; this tumor should be distinguished from separate HCC and CC arising in the same liver (1). Recent classification of combined HCC-CC is usually based on three categories (2): a) separate type, in which the tumor consists of varying degrees of a combination of moderately differentiated HCC with a trabecular pattern and CC with a tubular pattern and abundant fibrous stroma; both elements are clearly separated and coincide with the collision type of gross classification; b) transitional type, in which the CC element with its tubular pattern and fibrous stroma is contiguous with HCC elements having a tra-

becular or solid pattern; this shares transition features between HCC and CC elements; c) intermediate type, in which HCC elements and CC elements are almost indistinguishable, and the tumor cells are hyperchromatic, ovoid to round, and show a solid growth and/or a cord-like pattern; they are considered to be intermediate in form between HCC and CC. According to WHO classification, tumor cells of combined HCC-CCs with predominantly immunophenotypical features of stem/progenitor cells are regarded as combined HCC-CCs with stem cell features. WHO classification divided combined HCC-CC with stem cell features into three subtypes according to their histopathology and immunophenotype: a) combined HCC-CC with stem-cell features, typical subtype; b) combined HCC-CC with stem-cell features, intermediate-cell subtype; c) combined HCC-CC with stem-cell features, cholangiolocellular subtype (1).

Both the transitional and intermediate type of combined HCC-CC contained a variable number of tumor cells with intermediate morphology between HCC and CC. Tumor cells with intermediate morphology consisted of strands or trabeculae of small,

uniform cells with scant cytoplasm and hyperchromatic nuclei (1, 3), or proliferating tumor cells with an antler-like anastomosing pattern resembling the canal of Hering within a desmoplastic stroma (1, 3, 4), or solid nests comprising intermediate hepatocyte-like cells with scant cytoplasm in the center with peripheral small cells (1, 5). According to its unique histologic features and frequent expression of hepatic progenitor cell markers, such as CK7, CK19, c-kit, NCAM, and EpCAM in intermediate cells, combined HCC-CC is thought to originate from the ductules and/or canal of Hering, where hepatic progenitor cells are located (1, 3-6). However, the clinicopathologic features of combined HCC-CC, specifically the histopathologic and immunohistochemical features of transitional or intermediate areas in combined HCC-CC have not been fully elucidated.

In the present study, clinicopathologic characteristics were studied in 21 resected cases of combined HCC-CC. In addition, an immunohistochemical analysis was performed using primary antibodies to CK7, CK19, c-kit, NCAM, and EpCAM in transitional or intermediate areas of the combined HCC-CC.

MATERIALS AND METHODS

Patients and immunohistochemistry

This study was based on 21 consecutive cases of combined HCC-CCs, which were resected at the Department of Pathology of Chonbuk National University Hospital between January 1999 and December 2010. In each case, clinical features, including patient age at diagnosis, sex, etiology, serological data, background liver disease, microvessel invasion, presence of metastasis, and follow-up data were obtained from hospital records. Tumors were staged according to the 2010 American Joint Committee on Cancer (AJCC) tumour-node-metastasis (TNM) classification (7). The follow-up period was determined from the date of initial surgery until the date of the last follow-up or death.

Tumor pathology was reexamined and representative paraffin blocks containing cancer cells with intermediate morphology between HCC and CC were selected. Morphologic criteria proposed by the WHO and Kojiro were used in classification of the tumor (1, 2). For each sample, CC areas were confirmed by immunoreactivity for CK7 and/or CK19 (Dako, Carpinteria, CA, USA) and HCC differentiation was confirmed by immunoreactivity for HepPar 1 (Dako). The following pathologic features were evaluated in each case: 1) major histologic features of transitional or intermediate areas; 2) degree and type of inflammatory cell infiltrate; 3) degree of desmoplastic stroma; 4) microvessel invasion. For each case, the major histologic feature was defined as the morphology of the largest tumor element composing the transitional or intermediate area. The degree of inflammation and desmoplastic stroma were semiquantitatively classified into 4 groups, as follows: -, when inflammatory cells were minimum or extracellular stroma was scanty; +, when less

than 10% of tumor cells were infiltrated by inflammatory cells or surrounded by fibrous stroma; ++, when 11%-50% of tumor cells were infiltrated by inflammatory cells or surrounded by fibrous stroma; +++, when more than 50% of tumor cells were infiltrated by inflammatory cells or surrounded by fibrous stroma.

Antibodies used in immunohistochemistry included CK7, CK19, c-kit (DAKO), NCAM/CD56 (Novocastra, Newcastle, UK), and EpCAM (Calbiochem, La Jolla, CA, USA). Samples showing staining of at least 10% of tumor cells with intermediate morphology were defined as positive, and the intensity of immunoreactivity was graded as weak (+), moderate (++), and strong (+++).

Statistical analysis

Survival analyses were performed using the Kaplan-Meier method, and differences in survival between different clinical groups were determined by the log-rank test. Cox proportional hazards regression analysis was performed for estimation of the impact of clinicopathologic factors on survival of patients. *P* values less than 0.05 were considered to indicate statistical significance. The SPSS version 15.0 statistical software program (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

Ethics statement

This study protocol was approved by the institutional review board of Chonbuk National University Hospital (No: 201102026). Because this was a retrospective study, informed consent was exempted by the board.

RESULTS

Clinical features

The 21 patients consisted of 15 men and 6 women with a mean age of 59 yr (range 44-75 yr). Seventeen (81%) of the 21 patients were positive for hepatitis B virus surface antigen; none of the patients was positive for anti-hepatitis C virus antibody. Surrounding liver tissue showed liver cirrhosis in 13 (62%) patients and chronic hepatitis with varying degrees of fibrosis in 8 (38%) patients. One patient had chronic alcoholic hepatitis. Two patients with unknown etiology also showed findings of chronic hepatitis. Serum α -fetoprotein (AFP) levels, which were available for 20 patients, ranged from 2.46 to 75,680 ng/mL (mean 5,723 ng/mL). AFP level was greater than 15 ng/mL in 16 (80%) out of 20 patients examined (Table 1). These clinical characteristics of combined HCC-CC showed a closer resemblance to those of HCC than to those of CC. Eight (38%) patients had lymph node or distant metastasis.

Pathologic features

Gross appearance of combined HCC-CC is classified into three major types (2): collision type, HCC-predominant type, and

CC-predominant type. Among the 21 cases of combined HCC-CC, 12 (57.1%) were the HCC-predominant type, and 9 (42.9%) were the CC-predominant type. Maximum tumor diameter ranged from 1.4 to 8.6 cm (mean \pm SD, 4.6 \pm 2.3 cm). Pathologic findings are summarized in Table 2. Sixteen resected specimens

showed only a single tumor nodule, whereas 5 contained two or three nodules. Histologically, 19 of the tumors were defined as transitional type and the remaining two cases were intermediate type, according to the classification scheme of Kojiro (2). None was classified as separate type. According to the WHO

Table 1. Clinical characteristics of 21 patients with combined hepatocellular and cholangiocarcinoma

No.	Age (yr)/sex	Etiology	Liver cirrhosis	Tumor size (cm)	Serum AFP (ng/mL)	Metastasis	Death (months)
1	47/F	HBV	+	5	7,733		+ (4.6)
2	65/M	HBV	-	3	350		+ (2.1)
3	63/M	HBV	+	7	12,598		+ (11.17)
4	53/M	Alcohol	-	4.5	115		- (112)
5	62/M	HBV	+	6	5.3		+ (19.5)
6	61/M	HBV	+	2.5	36		+ (35.2)
7	45/F	HBV	-	5.1	348	Peritoneum	+ (1.37)
8	75/M	UK	-	7	1,426	Retroperitoneum	+ (0.47)
9	53/M	HBV	-	8.5	419	Lymph node, omentum	+ (1.83)
10	47/M	HBV	+	5.6	4,523	Lymph node	+ (10)
11	45/M	HBV	-	8.6	72	Lymph node, peritoneum	+ (5.3)
12	44/F	HBV	-	6	10,848		+ (2.43)
13	63/M	HBV	+	1.4	85		- (54.43)
14	65/M	HBV	+	2.3	53	Abdominal wall	+ (6.03)
15	63/M	HBV	+	3	10		+ (14.6)
16	65/F	HBV	+	1.8	118		- (46)
17	73/F	HBV	+	8.5	75,680	Lung	+ (1.27)
18	67/M	UK	-	2.9	Not checked		- (18.87)
19	64/F	HBV	+	3.1	7.1		+ (3.47)
20	53/M	HBV	+	2.4	34	Lymph node	+ (14.03)
21	56/M	UK	+	2.3	2.46		- (10.23)

HBV, hepatitis B virus; UK, unknown.

Table 2. Pathologic characteristics of 21 patients with combined hepatocellular and cholangiocarcinoma

No.	Classification by Kojiro	WHO type	Gross classification	Major intermediate feature [†]	Fibrosis	Inflammation	MI	Other histology
1	Transitional	Classical	HCC-type	1)	+++	+	+	
2	Transitional	Classical	CC-type	2)	+++	+++ (N)	-	1)
3	Transitional	Classical	HCC-type	3)	+++	+	+	
4	Transitional	Classical	CC-type	1)	+++	+++	+	2)
5	Transitional	Classical	CC-type	1)	+++	+	+	2)
6	Transitional	Classical	CC-type	1)	+++	+	-	
7	Transitional	Classical	HCC-type	1)	+++	+	+	
8	Transitional	Classical	CC-type	2)	+++	+	+	1)
9	Transitional	Classical	CC-type	1)	+++	+++	+	2) + SA
10	Transitional	Classical	HCC-type	4)	+++	+	+	1)
11	Transitional	Classical	HCC-type	1)	++	+++	+	
12	Transitional	Classical	HCC-type	1)	++	+	+	
13	Intermediate	Stem cell*	CC-type	1)	+++	+++	-	S
14	Transitional	Classical	HCC-type	2)	+++	+++ (N)	+	1)
15	Transitional	Classical	CC-type	2)	++	+++ (N)	+	1)
16	Transitional	Classical	HCC-type	1)	+++	+	+	
17	Transitional	Classical	HCC-type	1)	+++	+++ (N)	+	2) + SA
18	Transitional	Classical	CC-type	1)	++	+++ (N)	-	
19	Transitional	Classical	HCC-type	1)	+++	+	+	2) + S
20	Transitional	Classical	HCC-type	1)	-	-	+	2) + S
21	Intermediate	Stem cell [†]	HCC-type	3)	++	+++	+	

*Combined HCC-CC with stem cell features, intermediate-cell subtype; [†]Combined HCC-CC with stem cell features, typical subtype; 1), strands or trabeculae of small, uniform cells with scant cytoplasm and hyperchromatic nuclei; 2), tubular pattern like cholangiolocellular component; 3), solid nests of hepatocyte-like cells surrounded by small dark cells in periphery; 4), spindle-shaped cells arranged in short fascicles, mimicking mesenchymal tumor cells; (N), neutrophils; MI, microvessel invasion; SA, sarcomatous change; S, focal spindle tumor cells area.

classification (1), 19 (90%) were combined HCC-CC, classical type, and 2 (10%) were combined HCC-CC with stem cell features.

All tumors had histopathologic features that were intermediate between those of HCC and CC throughout or revealed a gradual transition from tubular CC areas toward HCC elements showing trabecular patterns. In these intermediate areas, a variable number of histologic features was observed; these included strands/trabeculae of small, uniform, oval-shaped cells with scant cytoplasm and hyperchromatic nuclei embedded within an abundant stroma (Fig. 1A), tubular arrangement of ovoid to round small cells with eosinophilic cytoplasm and mild atypia, mimicking the canal of Hering (Fig. 1B), nests of hepatocyte-like cells surrounded by small dark cells in the periphery (Fig. 1C), focal or scattered areas of abrupt glandular formation com-

posed of cuboidal or columnar tumor cells with scant basophilic cytoplasm (Fig. 1D), and spindle-shaped cells arranged in short fascicles, mimicking mesenchymal tumor cells (Fig. 1E, F). The most frequent major histologic feature of intermediate or transitional areas (14 out of 21, 67%) was strands/trabeculae of small, oval-shaped cells with scant cytoplasm, and hyperchromatic nuclei in a background of desmoplastic stroma (Table 2). Four of 21 (19%) tumors showed major histologic features consisting of small cells with vague gland-like structures resulting in an 'antler-like' appearance in an abundant fibrous stroma. The transitional area of one tumor was composed predominantly of spindle cells with scant cytoplasm arranged in short fascicles (Fig. 1E, F). This unusual finding of spindle tumor cells with a streaming pattern was also focally observed in the other three tumors (Fig. 1G). Abundant infiltrates of inflam-

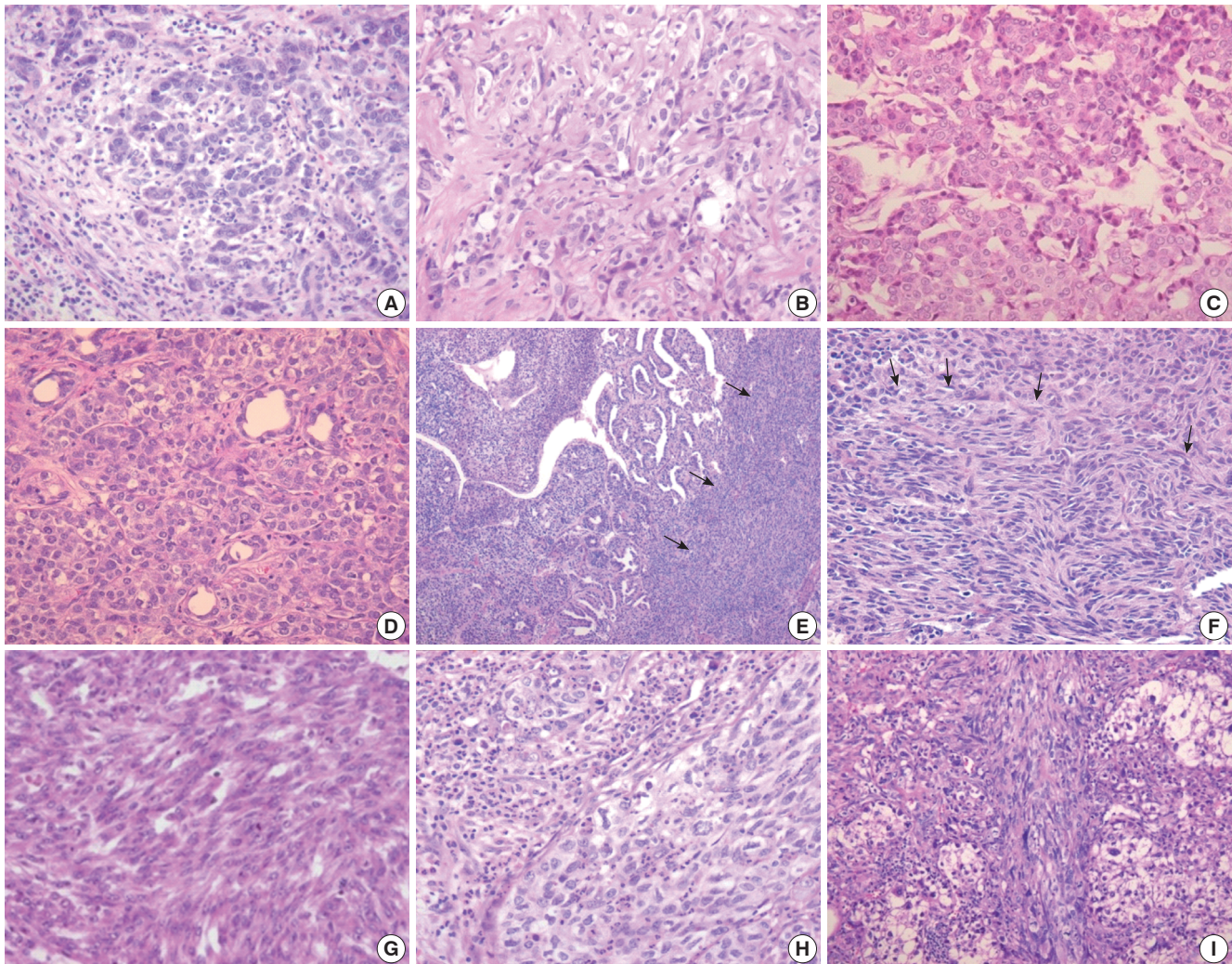


Fig. 1. Histologic features of transitional or intermediate areas in combined HCC-CC. (A) Strands of small, uniform cells with scant cytoplasm and hyperchromatic nuclei within desmoplastic stroma (H&E, $\times 400$). (B) Proliferating tumor cells with an antler-like anastomosing pattern (H&E, $\times 400$). (C) Solid nests comprised of intermediate hepatocyte-like cells in the center with peripheral small cells (H&E, $\times 400$). (D) Scattered foci of abrupt glandular formation composed of cuboidal tumor cells (H&E, $\times 400$). (E) The transitional area of the tumor is composed predominantly of spindle cells arranged in short fascicles (arrows) (H&E, $\times 100$). (F) Transition of small, oval cells to spindle cells, suggesting the same cellular origin of mesenchymal like spindle cells (arrows) (H&E, $\times 400$). (G) Spindle cells with a streaming pattern in case 13 (H&E, $\times 400$). (H) A massive neutrophilic infiltration around the nest of tumor cells (H&E, $\times 400$). (I) Sarcomatous change of tumor cells (H&E, $\times 200$).

matory cells around tumor cells, composed primarily of lymphocytes, plasma cells, and neutrophils, were observed in 10 (48%). In five of these ten tumors, inflammatory cells were composed predominantly of neutrophils (Fig. 1H). Plenty of desmoplastic stroma, which encased the strands or trabeculae of tumor cells, was observed in 15 (71%) tumors. Seventeen (81%) tumors showed features of microvessel invasion. Two tumors showed sarcomatous change (Fig. 1H).

Results of immunohistochemical staining are tabulated in Table 3. In non-tumor tissues, all five of the markers showed strong expression in proliferating bile ductules that were thought to be derived from hepatic progenitor cells. NCAM expression was also observed in peripheral nerve tissues. Tumor cells with intermediate morphology showed immunoreactivity to at least one of the five examined antibodies. Sixty-two percent (13 of

21), 81% (17 of 21), 43% (9 of 21), 19% (4 of 21) and 86% (18 of 21) of the tumors showed positive staining for CK7, CK19, c-kit, NCAM, and EpCAM, respectively. Tumor cells showed cytoplasmic immunoreactivity for CK7, CK19, and c-kit, while NCAM and EpCAM were mainly expressed on the tumor cell membrane (Fig. 2).

Outcome

Follow-up intervals ranged from 0.47 to 112 months. Sixteen patients died, and 8 of 21 patients showed local recurrence or distant metastasis. Median survival (95% confidence interval) of patients with combined HCC-CC was 10.0 months (1.5-18.5). The 5-yr survival rate in patients with combined HCC-CC was 19.4% \pm 9.2%. In univariate analysis, tumor size (< 3 cm vs \geq 3 cm), TNM stage (I and II vs III and IV), and serum AFP levels (< 400 ng/mL vs \geq 400 ng/mL) showed a significant association with poor patient survival ($P = 0.018$, $P = 0.041$, $P = 0.010$, respectively). Serum AFP level was an independent prognostic indicator ($P = 0.017$) in multivariate analysis (Table 4). Other factors, including age, sex, pathologic N classification, major intermediate features, cirrhosis, and microvessel invasion did not show any significant effect on patient prognosis.

DISCUSSION

Combined hepatocellular carcinoma and cholangiocarcinoma (combined HCC-CC) is a rare form of primary liver carcinoma that includes coexistence of the composition and characteristics of HCC and CC in the same tumor (1). According to the WHO classification, it is defined as a tumor containing unequivocal, intimately mixed elements of both hepatocellular carcinoma (HCC) and cholangiocarcinoma (CC); this tumor should be distinguished from separate HCC and CC arising in the same liver (1). Most combined HCC-CCs have transitional or intermediate areas, in which HCC and CC elements are practically indistinguishable, making diagnosis a challenge (1, 2). In order to prevent diagnosis as another tumor, awareness of the various

Table 3. Immunohistochemical results of intermediated cells of combined hepatocellular and cholangiocarcinoma

No.	Immunohistochemistry of intermediate cells				
	CK7	CK19	EpCAM	c-kit	NCAM
1	-	+++	-	-	-
2	-	-	+++	-	-
3	++	+++	+++	+	-
4	++	+++	+++	-	-
5	++	+++	+++	-	-
6	++	++	+++	++	-
7	-	+++	-	-	-
8	+	+++	+++	+	+
9	-	-	++	-	-
10	+	+++	+++	-	-
11	-	+++	-	-	-
12	-	+++	+++	-	-
13	+++	+++	++	+	-
14	+++	+++	+++	++	-
15	++	+++	+++	+	-
16	+++	+++	+++	++	-
17	+++	+++	+++	++	++
18	+	+++	+++	-	-
19	++	+++	+++	++	++
20	-	-	+++	-	++
21	-	-	+++	-	-

Table 4. Cox proportional hazard analyses of factors associated with 21 patients with combined hepatocellular and cholangiocarcinoma

Factors	No. (%)	Univariate model			Multivariate model*		
		HR	95% CI	P value	HR	95% CI	P value
Tumor size	< 3.0	7 (33.3)	1				
	\geq 3.0	14 (66.7)	4.68	1.30-16.79	0.018		
T	1, 2	8 (38.1)	1				
	3, 4	13 (61.9)	3.34	1.05-10.62	0.041		
M	No	16 (76.2)	1				
	Yes	6 (33.8)	8.10	2.23-29.45	0.001		
Stage	I, II	8 (38.1)	1				
	III, IV	13 (61.9)	3.34	1.05-10.62	0.041		
AFP (ng/mL)	< 400	13 (65.0)	1		1		
	\geq 400	7 (35.0)	4.65	1.45-14.91	0.010	4.44	1.31-15.05

*Multivariate model was adjusted for age (< 61, \geq 61 yr) and sex (male, female). HR, hazard ratio; CI, confidence interval; AFP, α -fetoprotein.

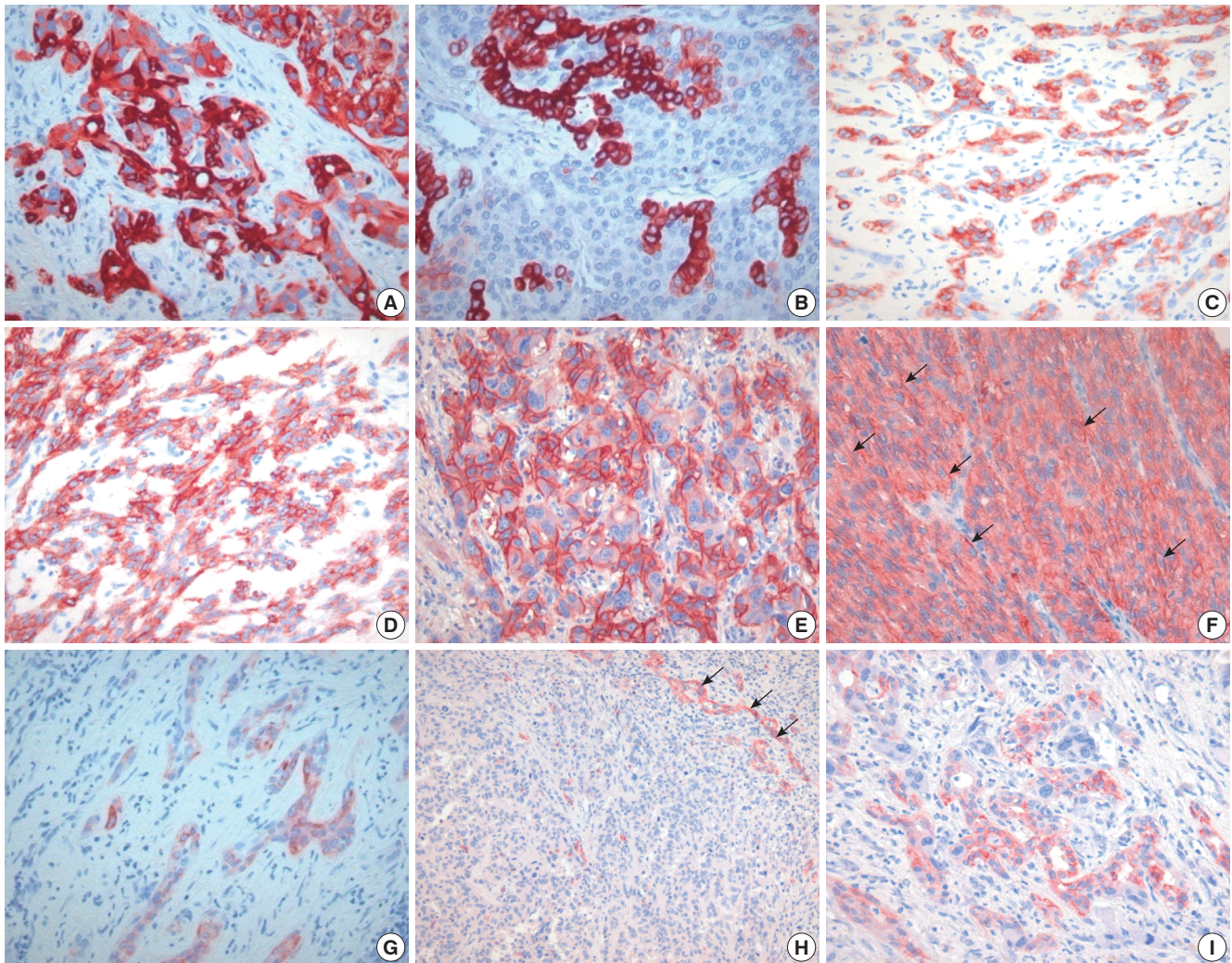


Fig. 2. Immunohistochemical features of transitional or intermediate areas in combined HCC-CC. (A) Strong expression of CK19 in tumor cells consisting of strands or trabeculae ($\times 400$). (B) Peripheral small cells were positive for CK19, whereas central intermediate hepatocyte-like cells were negative for CK19 ($\times 400$). (C) Cholangiolocarcinoma-like areas with CK7 immunoreactivity ($\times 200$). (D) Intermediate type carcinoma cells showed strong immunoreactivity for CK7 ($\times 400$). (E) Strong membranous staining of EpCAM ($\times 400$). (F) Spindle carcinoma cells showed accentuated membrane staining for EpCAM (arrows) ($\times 400$). (G) C-kit positive tumor cells with an antler-like anastomosing pattern ($\times 400$). (H) Positive immunoreactivity for NCAM in reactive ductular cells served as internal positive controls. Most tumor cells reveal negative immunoreactivity for NCAM ($\times 200$). (I) NCAM positive tumor cells in a cholangiolocellular pattern ($\times 400$).

morphologic features of intermediate areas in this tumor is essential. In order to provide a fuller and more precise definition of the morphological characteristics of this tumor, we investigated histologic and immunohistochemical findings of transitional or intermediate areas in combined HCC-CC.

In this study, the most frequent major histologic feature of tumor cells in intermediate areas was small and oval-shaped cells with hyperchromatic nuclei and scant cytoplasm. They were arranged in trabeculae, solid nests, or strands, and set within a desmoplastic stroma. The second most common pattern was that of small cells with oval nuclei, growing in a tubular cord-like anastomosing pattern, the so-called 'antler-like' pattern. The third major feature was nests of central intermediate-hepatocyte like cells with peripheral clusters of small cells. An interesting finding was the arrangement of spindle tumor cells in short

fascicles, simulating mesenchymal tumor cells. One tumor was composed predominantly of spindle cells, with a streaming pattern, and three other tumors showed focal spindle cell areas. To the best of our knowledge, such a finding has not been specifically described in combined HCC-CCs before. In addition, in the area of intermediate cancer cells, we found massive neutrophilic infiltrate surrounding nests or cords of tumor cells. Sasaki et al. (8) described frequent massive neutrophilic infiltration in intrahepatic cholangiocarcinoma elements of combined HCC-CCs associated with frequent expression of granulocyte colony-stimulating factor (G-CSF) and granulocyte macrophage colony-stimulating factor (GM-CSF), and stem cell markers in cancer cells. They proposed that up-regulation of GM-CSF and G-CSF of cancer cells may reflect the hepatic stem cell phenotype and be responsible for prominent neutrophilic infiltration. How-

ever, intratumoral or peritumoral neutrophilic infiltrations in various malignant tumors, including HCC, have been reported (9, 10). A conclusion regarding the role of cancer cells in tumoral neutrophilic infiltration of combined HCC-CCs should be interpreted with caution.

Hepatic progenitor cells (HPCs) are small epithelial cells with an oval nucleus, scant cytoplasm, and are located in the bile ductules and canal of Hering (3-6, 11, 12). In chronic liver disease, HPCs are activated, meaning that they proliferate and differentiate toward hepatocytic and/or biliary lineages. Not only do HPCs and their progeny have a distinct morphology, they also express several stem cell markers (3-6, 11, 12). The most common markers facilitating detection of HPCs are CK7, CK19, c-kit, and NCAM (1, 11). EpCAM is also an epithelial cell adhesion molecule previously identified as a marker for HPCs of adult liver and oval cells (6). In this study, all of the proliferating ductular cells in matched surrounding non-tumor tissue showed strong CK7, CK19, c-kit, NCAM, and EpCAM immunoreactivity. All tumor cells with intermediate morphology, regardless of their different growth patterns in 21 tumors, showed positivity to one of the five examined antibodies, CK7, CK19, c-kit, NCAM, and EpCAM. Similar to our results, previous studies have reported that all combined HCC-CCs show a variable amount of immature-appearing intermediate cells that have morphological and immunohistochemical features of both hepatocytes and cholangiocytes. Intermediate cells sometimes resemble HPCs or reactive bile ductules in the non-tumoral liver and have shown frequent expression of HPC markers (1-6, 11-14). Taken together, these findings strongly suggested that intermediate elements in combined HCC-CC might be derived from HPCs that have features that are intermediate between those of hepatocytes and cholangiocytes.

Findings from the current study demonstrated that combined HCC-CCs share many clinical similarities with HCC. The most similar findings included sex ratio, frequent serologic positive result for HBV, association with chronic liver disease, including cirrhosis, and an elevated serum AFP level. Clinical features of combined HCC-CCs in patients of Eastern countries are more similar to those of HCC than to CC (2-4, 14, 15), whereas in the West, combined HCC-CCs have been reported to show more CC-like features (13, 16). In many reports, mostly from Asian countries, cirrhosis is associated with 50%-70% of cases and the prevalence of HBV or HCV related cases is also similar to that of ordinary HCC (2-4, 14, 15). However, Tickoo et al. (13) have reported that combined HCC-CCs showed many differences from HCC, including absence of cirrhosis, rarity of serum hepatitis B or C marker positivity, and normal to only mildly elevated serum AFP levels. Jarnagin et al. (16) have also demonstrated that the demographic and clinical features of patients with combined HCC-CCs were similar to those of patients with CC. In contrast, in Italy, the rate of HCV antibody positivity, presence of cirrhosis or

chronic hepatitis, and elevated serum AFP are comparable to values generally reported in Asian countries (17). This discrepancy is not easily explained, but can be linked to the different etiologic roles according to the geographic situation or the presence of other carcinogenesis mechanisms.

The biologic behavior of combined HCC-CC remains unclear. According to some reports, combined HCC-CCs have a prognosis that lies between that of HCC and CC (12, 13, 15). In contrast, several studies have demonstrated that survival of patients with combined HCC-CC was poorer than that of patients with HCC or CC (16, 18-20). This discrepancy in prognosis of combined HCC-CCs patients might be explained by differences in tumor stage, presence of cirrhosis, hepatitis B or C infection, and an insufficient number of patients with combined HCC-CC in each reported series.

In conclusion, our study highlights the need for awareness of the various morphologic features of intermediate areas in this tumor for prevention of potential misdiagnosis as another tumor.

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AUTHOR SUMMARY

Clinicopathologic Study on Combined Hepatocellular Carcinoma and Cholangiocarcinoma: with Emphasis on the Intermediate Cell Morphology

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We specifically investigated the histopathologic features of transitional or intermediate areas in 21 combined HCC-CCs and immunophenotypes with five different hepatic progenitor cell markers. In addition to major types, one tumor was composed of spindle cells arranged in short fascicles, a feature that has not been previously described in combined HCC-CC. In order to prevent diagnosis as another tumor, an awareness of the various morphologic features of intermediate areas in this tumor is essential.