hepatocellular-cholangiocarcinoma, subtypes with stem cell features, was significantly high compared with the ChC component of combined hepatocellular-cholangiocarcinoma, classical type (Table 4). Combined hepatocellular-cholangiocarcinoma, stem cell features, cholangiolocellular subtype, had a significantly high expression of CK19 compared with the ChC component of combined hepatocellular-cholangiocarcinoma, classical type (Table 4). Moreover, the IHC score of CD133, EpCAM, and vimentin in combined hepatocellular-cholangiocarcinoma,

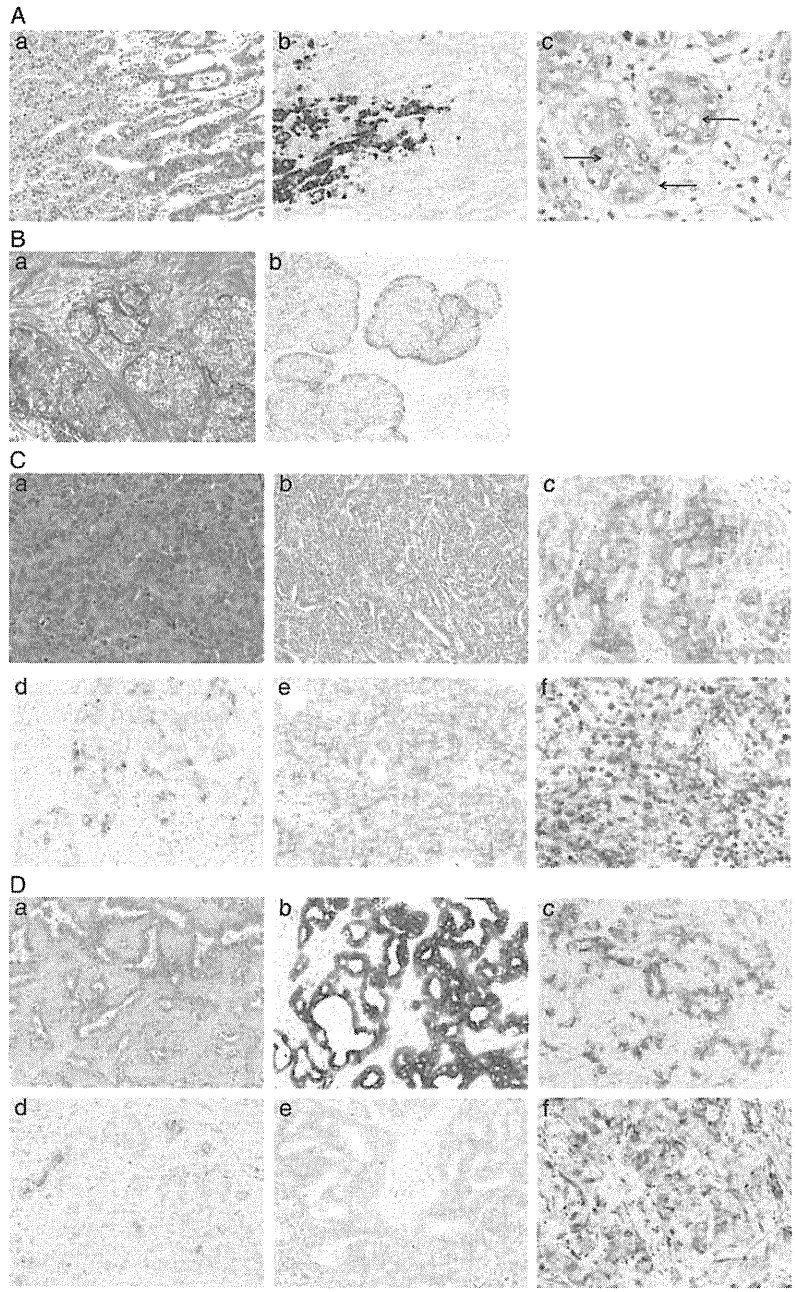

FIGURE 1. *(continued)*

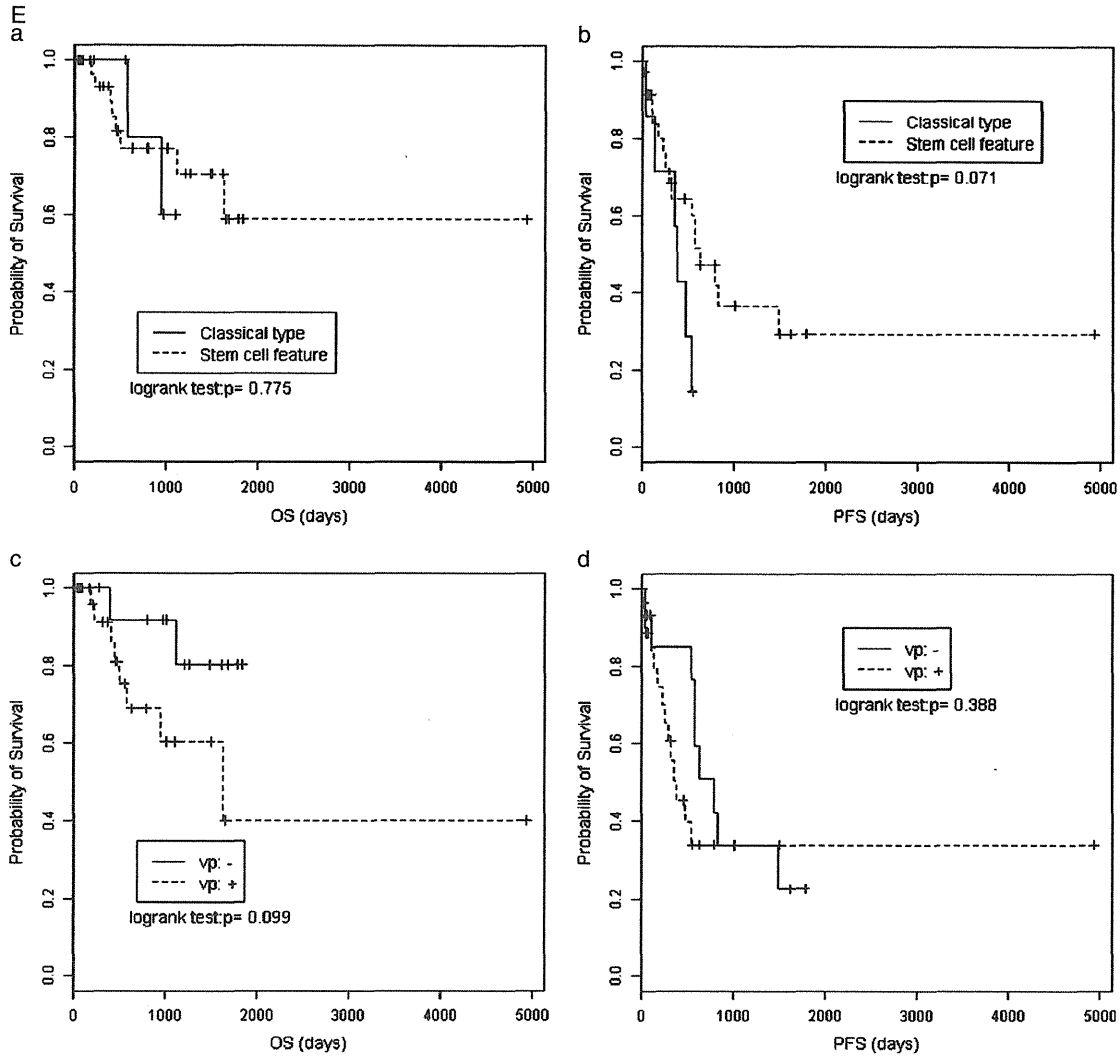

FIGURE 1. A, Representative microscopic findings of combined hepatocellular-cholangiocarcinoma, classical type. The HCC (left) and ChC (right) components were contiguous with transitional features at the boundary (a). Immunochemical stain for HerPar-1 was positive only in the HCC component (b). Mucin production was observed in the lumina surface of small glands of the ChC component (c: arrows, mucicarmine stain). B, Microscopic findings of combined hepatocellular-cholangiocarcinoma, stem cell features, typical subtype. The tumor showed a nested growth pattern with peripheral clusters of small cells that had a high nucleus:cytoplasm ratio in the sclerotic stroma (a). EpCAM showed a predominantly circumferential staining pattern (b). C, Representative microscopic findings of combined hepatocellular-cholangiocarcinoma, stem cell features, intermediate cell subtype. The tumor was composed of small, oval-shaped cells with a trabecular, solid nested (a) or ill-defined glandular proliferative (b) pattern. CK19 was diffusely positive for tumor cells (c). Tumor cells showed variable staining for CD133 (d), EpCAM (e), and vimentin (f). D, Representative microscopic findings of combined hepatocellular-cholangiocarcinoma, stem cell features, cholangiolocellular subtype. The tumor cells showed a tubular structure with marked fibrous stroma (a). Immunostains with CK19 (b) and vimentin (f) showed positive reaction in the cytoplasm at various degrees. CD133 (d) was positive for the lumina surface of glandular structures. The expression of CD56 (c) and EpCAM (e) was observed in the cell surface of tumor cells. E, Kaplan-Meier curve demonstrating that OS and PFS of patients with combined hepatocellular-cholangiocarcinoma, classical type, were not statistically different from those of patients with combined hepatocellular-cholangiocarcinoma, subtypes with stem cell features (a and b). However, combined hepatocellular-cholangiocarcinoma, classical type, tended to show shorter PFS compared with combined hepatocellular-cholangiocarcinoma, subtypes with stem cell features (b). There was no significant difference in OS and PFS between the patients with and without portal vein permeation (c and d). However, cases with portal vein permeation had a tendency to shorter OS compared with cases without portal vein permeation (d).

TABLE 3. HCC Component of Combined Hepatocellular-Cholangiocarcinoma Classical Type, Versus Combined Hepatocellular-Cholangiocarcinoma, Subtypes With Stem Cell Features

Antibody	WHO	IHC Score 0	1	2	3	4	*P* (Overall)	*P* (Classical vs. SCs)
CK7	HCC-comp-classical	3	1	4	0	2	0.001	—
	SC-int	1	2	3	7	19		0.0033
	SC-CLC	0	0	0	0	11		< 0.001
CK19	HCC-comp-classical	9	0	1	0	0	< 0.001	—
	SC-int	3	2	5	5	17		< 0.001
	SC-CLC	2	0	0	0	9		< 0.001
EMA	HCC-comp-classical	10	0	0	0	0	< 0.001	—
	SC-int	1	0	7	3	21		< 0.001
	SC-CLC	0	0	1	1	9		< 0.001
HepPar-1	HCC-comp-classical	0	3	1	1	5	< 0.001	—
	SC-int	23	6	3	0	0		< 0.001
	SC-CLC	11	0	0	0	0		< 0.001
CD56	HCC-comp-classical	10	0	0	0	0	0.0191	—
	SC-int	21	6	3	1	1		0.4228
	SC-CLC	3	2	1	2	3		0.0018
c-kit	HCC-comp-classical	10	0	0	0	0	0.2065	—
	SC-int	3	2	5	5	17		1
	SC-CLC	9	2	0	0	0		0.4762
CD133	HCC-comp-classical	10	0	0	0	0	0.0052	—
	SC-int	27	3	2	0	0		0.7464
	SC-CLC	4	3	1	0	3		0.0067
EpCAM	HCC-comp-classical	10	0	0	0	0	0.0042	—
	SC-int	17	1	4	4	6		0.1772
	SC-CLC	2	0	2	0	7		< 0.001
Vimentin	HCC-comp-classical	10	0	0	0	0	0.0071	—
	SC-int	23	2	2	2	3		0.9214
	SC-CLC	2	2	4	2	1		< 0.001

P-value (overall) was calculated by the Fisher exact test for comparison among HCC component of CHC-classical, SC-int, and SC-CLC.

P-value was for pairwise comparison between HCC component of CHC-classical and SC-int or SC-CLC.

HCC-comp-classical indicates HCC component of combined hepatocellular-cholangiocarcinoma, classical type; SC-int, combined hepatocellular-cholangiocarcinoma, stem cell features, intermediate cell subtype; SC-CLC, combined hepatocellular-cholangiocarcinoma, stem cell features, cholangiolocellular subtype.

stem cell features, cholangiolocellular subtype, was significantly high compared with the ChC component of combined hepatocellular-cholangiocarcinoma, classical type, and combined hepatocellular-cholangiocarcinoma, stem cell features, intermediate cell subtype (Tables 4, 5).

The HCC component and the ChC component of combined hepatocellular-cholangiocarcinoma, classical type, were evaluated separately. The former component consisted of tumor cells with trabecular and/or pseudoglandular structures, suggesting well-differentiated or moderately differentiated HCC (Fig. 1Aa, left). This component showed significantly high expression of HerPar-1 (Fig. 1Ab), followed by CK7 and CK19. There were no expressions of the other markers. The latter component had an irregular tubular pattern with small-sized to medium-sized lumina (Fig. 1Aa, right). Mucin production was confirmed with mucicarmine stain at the lumina surface (Fig. 1Ac). This component showed high expression of biliary markers and low expression of HerPar-1 (Fig. 1Ab), CD56, c-kit, and EpCAM. No expressions of CD133 and vimentin were observed. In 9 of 10 cases, portal vein permeation was found around the tumor. Tumor casts of the intraportal vein were composed solely of the HCC component in 2 cases and both HCC and ChC components in 7 cases. Two of 10 cases had minor components, each of which was a combined hepatocellular-cholangiocarcinoma, stem cell features, intermediate cell subtype–like, and combined hepatocellular-cholangiocarcinoma, stem cell features, cholangiolocellular subtype–like component.

Only 1 case was classified into combined hepatocellular-cholangiocarcinoma, stem cell features, typical subtype. This case was accompanied by the HCC component and the ChC component adjacent to combined hepatocellular-cholangiocarcinoma, stem cell features, typical subtype. This tumor showed a nested pattern with peripheral clusters of small cells that had a high N/C ratio and hyperchromatic nuclei. Tumor cells located inside nests mimicked mature hepatocytes and had a

TABLE 4. ChC Component of Combined Hepatocellular-Cholangiocarcinoma Classical Type, Versus Combined Hepatocellular-Cholangiocarcinoma, Subtypes With Stem Cell Features

Antibody	WHO	IHC Score 0	1	2	3	4	*P* (Overall)	*P* (Classical vs. SCs)
CK7	ChC-comp-classical	1	0	1	1	7	0.4029	—
	SC-int	1	2	3	7	19		0.7811
	SC-CLC	0	0	0	0	11		0.0902
CK19	ChC-comp-classical	3	0	1	3	3	0.182	—
	SC-int	3	2	5	5	17		0.3419
	SC-CLC	2	0	0	0	9		0.0418
EMA	ChC-comp-classical	2	1	3	0	4	0.1327	—
	SC-int	1	0	7	3	21		0.0868
	SC-CLC	0	0	1	1	9		0.1081
HepPar-1	ChC-comp-classical	9	1	0	0	0	0.3226	—
	SC-int	23	6	3	0	0		0.5557
	SC-CLC	11	0	0	0	0		0.4762
CD56	ChC-comp-classical	7	2	1	0	0	0.1154	—
	SC-int	21	6	3	1	1		1
	SC-CLC	3	2	1	2	3		0.1702
c-kit	ChC-comp-classical	9	0	1	0	0	0.1327	—
	SC-int	3	2	5	5	17		0.4572
	SC-CLC	9	2	0	0	0		0.4762
CD133	ChC-comp-classical	10	0	0	0	0	0.0052	—
	SC-int	27	3	2	0	0		0.7464
	SC-CLC	4	3	1	0	3		0.0067
EpCAM	ChC-comp-classical	9	0	0	1	0	0.0092	—
	SC-int	17	1	4	4	6		0.3558
	SC-CLC	2	0	2	0	7		< 0.001
Vimentin	ChC-comp-classical	10	0	0	0	0	0.0071	—
	SC-int	23	2	2	2	3		0.9214
	SC-CLC	2	2	4	2	1		< 0.001

P-value (overall) was calculated by the Fisher exact test for comparison among ChC component of CHC-classical, SC-int, and SC-CLC.

P-value was for pairwise comparison between ChC component of CHC-classical and SC-int or SC-CLC.

ChC-comp-classical indicates ChC component of combined hepatocellular-cholangiocarcinoma, classical type; SC-int, combined hepatocellular-cholangiocarcinoma, stem cell features, intermediate cell subtype; SC-CLC, combined hepatocellular-cholangiocarcinoma, stem cell features, cholangiolocellular subtype.

TABLE 5. Combined Hepatocellular-Cholangiocarcinoma Stem Cell Features, Intermediate Cell Subtype Versus Cholangiolocellular Subtype

		IHC Score					
Antibody	WHO	0	1	2	3	4	*P*
CK7	SC-int	1	2	3	7	19	0.2014
	SC-CLC	0	0	0	0	11	
CK19	SC-int	3	2	5	5	17	0.2802
	SC-CLC	2	0	0	0	9	
EMA	SC-int	1	0	7	3	21	0.868
	SC-CLC	0	0	1	1	9	
HepPar-1	SC-int	23	6	3	0	0	0.1758
	SC-CLC	11	0	0	0	0	
CD56	SC-int	21	6	3	1	1	0.0284
	SC-CLC	3	2	1	2	3	
c-kit	SC-int	3	2	5	5	17	0.1191
	SC-CLC	9	2	0	0	0	
CD133	SC-int	27	3	2	0	0	0.0023
	SC-CLC	4	3	1	0	3	
EpCAM	SC-int	17	1	4	4	6	0.0362
	SC-CLC	2	0	2	0	7	
vimentin	SC-int	23	2	2	2	3	0.0069
	SC-CLC	2	2	4	2	1	

P-value was calculated by the Fisher exact test for comparison between SC-int and SC-CLC.

SC-int indicates combined hepatocellular-cholangiocarcinoma, stem cell features, intermediate cell subtype; SC-CLC, combined hepatocellular-cholangiocarcinoma, stem cell features, cholangiolocellular subtype.

partial clear cell change (Fig. 1Ba). Immunohistochemically, biliary markers (CK7, CK19, and EMA), CD56, c-kit, and EpCAM (Fig. 1Bb) were positive for both cells mimicking hepatocytes and peripheral small cells. However, EpCAM showed a predominantly circumferential staining pattern. HepPar-1 was positive only inside the nests.

Thirty-two cases were classified into combined hepatocellular-cholangiocarcinoma, stem cell features, intermediate cell subtype. This type of tumor showed strands/trabeculae of small, uniform, round to oval cells with scanty cytoplasm and hyperchromatic nuclei (Figs. 1Ca, b). Mucin production was not observed in tumor cells. Most cases had expressions of biliary markers, such as CK7, CK19 (Fig. 1Cc), and EMA. The cases with HepPar-1 and CD56 expression were relatively limited in number. CD133 (Fig. 1Cd) and EpCAM (Fig. 1Ce) expressions were observed in some cases. Vimentin, which is a representative marker of mesenchymal cells, was also positive in some cases (Figs. 1Cf). The tumor cells with vimentin expression showed an epithelial growth pattern and lacked a mesenchymal growth pattern. Fifteen, 6, and 1 case was accompanied by HCC-like, ChC-like, and combined hepatocellular-cholangiocarcinoma, stem cell features, cholangiolocellular subtype–like components, respectively, as minor components.

Eleven cases were classified into combined hepatocellular-cholangiocarcinoma, stem cell features, cholangiolocellular subtype. These cases demonstrated admixtures of small monotonous glands and antler-like anastomosing patterns embedded within a thick desmoplastic stroma (Fig. 1Da). Mucin production was not observed in tumor cells. All cases had plural biliary marker expressions [CK7, CK19 (Fig. 1Db), and EMA]. HepPar-1 was not positive for tumor cells with combined hepatocellular-cholangiocarcinoma, stem cell features, cholangiolocellular subtype. c-kit expression was observed in a relatively limited area. CD56 (Fig. 1Dc), CD133 (Fig. 1Dd), and EpCAM (Fig. 1De) expressions were observed in several cases. Vimentin expression was also observed in some cases (Fig. 1Df). Six cases had minor components. Five of them were accompanied by an HCC-like component, and 1 of them was accompanied by a ChC-like component.

Clinical and Follow-up Data

There was no significant difference in clinical outcome between combined hepatocellular-cholangiocarcinoma of classical type and subtypes with stem cell features (Figs. 1Ea, b) and between combined hepatocellular-cholangiocarcinoma, stem cell features of intermediate cell subtype and cholangiolocellular subtype. However, combined hepatocellular-cholangiocarcinoma, classical type, tended to show shorter PFS compared with combined hepatocellular-cholangiocarcinoma, subtypes with stem cell features (Fig. 1Eb). There was no statistical difference in OS and PFS between the patients with and without portal vein permeation (Fig. 1Ec, d). However, cases with portal vein permeation had a tendency to shorter OS compared with cases without portal vein permeation (Fig. 1Ec).

DISCUSSION

Combined hepatocellular-cholangiocarcinoma was reported to be a primary malignant liver tumor by Wells in 1903. Allen and Lisa[10] subclassified combined hepatocellular-cholangiocarcinoma into mixed and combined type. In 1985, Goodman et al[25] classified combined hepatocellular-cholangiocarcinoma into collision type (type I), transitional tumor (type II), and fibrolamellar tumor (type III). Moreover, Taguchi et al[28] classified combined hepatocellular-cholangiocarcinoma into 3 histologic types according to the combination pattern of HCC and ChC elements and also the presence of the transitional features of both elements. Although these classifications have been proposed, the histogenesis of combined hepatocellular-cholangiocarcinoma had remained unclear for many years. However, recent advances of HPC investigations have provided new insight. It has been shown that murine mature polypoid hepatocytes have a stem cell–like regenerative capacity and that even human hepatocytes are highly regenerative.[29] The adult liver harbors facultative biopotential progenitors that give rise to an intermediary cell type, described as oval cells, which are thought to differentiate into both biliary epithelium and hepatocytes.[30] Oval cells have been linked to the subsequent development of hepatic malignancies in animal models.[5,8] The existence of human cell populations that have similar characteristics to animal oval cells has been confirmed, and they have been termed HPCs.[7] HPCs have been identified in several pathologic liver conditions, such as

hepatitis, cirrhosis, focal nodular hyperplasia, and hepatocellular adenoma.[31–34] As HPCs have bipotential—that is, being capable of differentiation into either hepatocytes or cholangiocytes[31,35,36]—the hypothesis that combined hepatocellular-cholangiocarcinoma is derived from HPCs is easily acceptable.[37]

Combined hepatocellular-cholangiocarcinoma, classical type, corresponds with type I of Goodman's classification.[25] Ten cases were classified into this type of tumor in our serial cases. Combined hepatocellular-cholangiocarcinoma, classical type, is composed of a typical HCC area and a typical ChC area. The possible histogenesis is as follows: (i) HCC and ChC arise independently and separately; (ii) HCC or ChC arises first and transforms to ChC or HCC, respectively, in various degrees; and (iii) malignant transformation of HPCs occurs, and they differentiate completely and incompletely to HCC and ChC.

Combined hepatocellular-cholangiocarcinoma, stem cell features, typical subtype, is newly adopted in the latest WHO classification. Originally, this type of tumor was reported by Theise et al.[11] Subsequently, Fujii et al[38] reported 21 cases of scirrhous HCCs, stem cell features. They described small tumor cells located at peripheral tumor nests that had a similar characteristic with side population cells isolated from HCC cell lines Huh7 and PLC5. Moreover, Ki-67 labeling index was higher in small tumor cells located at the periphery of tumor cell nests than in the central part of tumor cells mimicking HCC cells.[34] Although tumor cells of HCC or combined hepatocellular-cholangiocarcinoma adjacent to sclerotic stroma sometimes show small ductular proliferation, the Ki-67 labeling index of the reactive ductular epithelium under a pathogenic condition is generally much less than that of small tumor cells located at the periphery of tumor cell nests.[38,39] However, only 1 case was classified into combined hepatocellular-cholangiocarcinoma, stem cell features, typical subtype, in our present study. A case with typical features showing combined hepatocellular-cholangiocarcinoma, stem cell features, typical subtype, might be rare. Further studies should be conducted to clarify the clinicopathologic significance of this tumor.

The subtype of combined hepatocellular-cholangiocarcinoma, stem cell features, intermediate cell subtype, corresponds to cases previously reported as liver carcinoma of the intermediate (hepatocyte-cholangiocyte) phenotype.[26] Tumor cells are morphologically and immunophenotypically composed of intermediate cells between HCC and ChC. The histogenesis is thought to be transformed in HPCs. In our present study, 32 cases were classified into combined hepatocellular-cholangiocarcinoma, stem cell features, intermediate cell subtype. Tumor cells immunochemically show the positive reaction of not only biliary markers but also HPC markers. Moreover, vimentin, which is a representative mesenchymal marker, was also positive in some cases. Nakanuma et al[40] reported that peripheral ChC is divided into ductular type and duct type. The former had histologic resemblance to reactive bile ductules and showed expression of CD56 and vimentin.[40,41] Furthermore, they mentioned that the ChC component of combined hepatocellular-cholangiocarcinoma shared the features of the ductular type of ChC. These findings strongly support the hypothesis that combined hepatocellular-cholangiocarcinoma originates from HPCs. However, the precise mechanism and significance of the expression of the mesenchymal marker vimentin are still elusive.

Cholangiolocellular carcinoma was categorized as a subtype of ChC on the basis of the previous WHO classification. In the latest WHO classification, cholangiolocellular carcinoma is classified into combined hepatocellular-cholangiocarcinoma, stem cell features, cholangiolocellular subtype. Cholangiolocellular carcinoma was originally reported by Steiner and Higginson in 1959.[27] As cholangiolocellular carcinoma is a rare malignant liver tumor accounting for 0.56% to 1% of all primary liver cancer cases, serial reports are limited in number.[6,9,27] Although cholangiolocellular carcinoma is thought to originate from canals of Hering, Komuta et al[6] provided reliable evidence that cholangiolocellular carcinoma originates from HPCs. IHC findings in our study were similar to those in previous reports. The expression of vimentin was also confirmed in combined hepatocellular-cholangiocarcinoma, stem cell features, cholangiolocellular subtype, and in combined hepatocellular-cholangiocarcinoma, stem cell features, intermediate cell subtype. Collectively, these results strongly suggest that combined hepatocellular-cholangiocarcinoma, stem cell features, cholangiolocellular subtype, originates from HPCs.

Thirty-four (63%) of 54 cases were associated with HBV and/or HCV infection. Previous reports on combined hepatocellular-cholangiocarcinoma also showed that 58% to 93% cases were affected by HBV and/or HCV infection.[26,28,37,42] Komuta and colleagues reported that 30% of cases of cholangiolocellular carcinoma were infected with HBV and/or HCV.[17] The findings of previous reports and our present study support the hypothesis that chronic hepatitis virus infection might be associated with various types of combined hepatocellular-cholangiocarcinoma. However, currently the precise mechanisms of the carcinogenesis of combined hepatocellular-cholangiocarcinoma and chronic hepatic damage due to HBV and/or HCV remain unclear.

Several reports document combined hepatocellular-cholangiocarcinoma as an aggressive tumor with poor outcome, compared with HCC.[28,43–47] In this study, no significant differences were found in patient outcome between combined hepatocellular-cholangiocarcinoma, classical type, and combined hepatocellular-cholangiocarcinoma, subtypes with stem cell features. Although vascular invasion, satellite lesion, number of tumors, and tumor size were listed as worse prognostic factors in combined hepatocellular-cholangiocarcinoma,[43,44,47] there was no significant difference in patient outcome between the cases with and without portal vein permeation. The insufficient number of cases and the diversity of stages at diagnosis or postoperative treatment made it difficult to make any

generalizations about the clinical outcome. Moreover, as we did not compare the outcome among combined hepatocellular-cholangiocarcinoma and other primary liver tumors, it might be difficult to address the aggressiveness of combined hepatocellular-cholangiocarcinoma in this study.

Our results validate the latest WHO classification of combined hepatocellular-cholangiocarcinoma with regard to morphologic features and immunophenotypes. However, clinical aspects, including the prognosis, of each subtype remain unknown as described in the WHO classification.[24] To enforce this classification, combined hepatocellular-cholangiocarcinomas should be strictly categorized. In the present study, we defined the predominant histologic pattern ($\geq 50\%$) as its pathologic diagnosis for the sake of expedience when a tumor contained plural histologic patterns. However, there is no definitive description in the WHO classification that the tumor amount should be reflected in pathologic diagnosis and handled as a minor histologic component. The description that HCC-like and/or ChC-like areas are frequently present at the periphery of combined hepatocellular-cholangiocarcinoma, stem cell features, cholangiolocellular subtype, is seen in the WHO classification. In fact, minor histologic components were observed in 31 (57.4%) of 54 cases of combined hepatocellular-cholangiocarcinoma. The ratio of having minor histologic components was significantly higher in combined hepatocellular-cholangiocarcinoma, subtypes with stem cell features, compared with combined hepatocellular-cholangiocarcinoma, classical type, in this study (Table 2). At present, the clinicopathologic significance of the minor component is unclear. However, recently Komuta et al[48] reported that intrahepatic cholangiocarcinoma (ICC) with histologic diversity (hepatic differentiation area and/or ductular areas) tended to show better prognosis compared with ICC without histologic diversity. In addition, they mentioned that ICC with histologic diversity had a similar molecular profile to cholangiolocellular carcinoma. We showed that combined hepatocellular-cholangiocarcinoma was usually composed of a complex heterogenous mixture of histologic subtypes in this study. Therefore, describing all components that may be present might be a tentative method at present while the significance of the minor component remains elusive.

In conclusion, combined hepatocellular-cholangiocarcinoma is a neoplasm with wide histologic diversity and shows immunophenotypic expression of not only biliary markers but also HPC markers to various degrees, indicating that the histogenesis of combined hepatocellular-cholangiocarcinoma could be strongly associated with HPCs. Our results practically validate the latest WHO classification of combined hepatocellular-cholangiocarcinoma with regard to pathologic features. However, the complex mixture of histologic subtypes has presented a challenge to the classification of combined hepatocellular-cholangiocarcinoma. Further expanded studies using a large cohort should be conducted to confirm the utility of this classification.

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REFERENCES

1. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med.* 1997;3:730–737.
2. Lapidot T, Sirard C, Vormoor J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature.* 1994;367:645–648.
3. Reya T, Morrison SJ, Clarke MF, et al. Stem cells, cancer, and cancer stem cells. *Nature.* 2001;414:105–111.
4. Marquardt JU, Factor VM, Thorgeirsson SS. Epigenetic regulation of cancer stem cells in liver cancer: current concepts and clinical implications. *J Hepatol.* 2010;53:568–577.
5. Fausto N. Mouse liver tumorigenesis: models, mechanisms, and relevance to human disease. *Semin Liver Dis.* 1999;19:243–252.
6. Komuta M, Spee B, Vander Borght S, et al. Clinicopathological study on cholangiolocellular carcinoma suggesting hepatic progenitor cell origin. *Hepatology.* 2008;47:1544–1556.
7. Roskams TA, Theise ND, Balabaud C, et al. Nomenclature of the finer branches of the biliary tree: canals, ductules, and ductular reactions in human livers. *Hepatology.* 2004;39:1739–1745.
8. Sell S. The role of determined stem-cells in the cellular lineage of hepatocellular carcinoma. *Int J Dev Biol.* 1993;37:189–201.
9. Shiota K, Taguchi J, Nakashima O, et al. Clinicopathologic study on cholangiolocellular carcinoma. *Oncol Rep.* 2001;8:263–268.
10. Allen RA, Lisa JR. Combined liver cell and bile duct carcinoma. *Am J Pathol.* 1949;25:647–655.
11. Theise ND, Yao JL, Harada K, et al. Hepatic 'stem cell' malignancies in adults: four cases. *Histopathology.* 2003;43:263–271.
12. Becker G, Schmitt-Graeff A, Ertelt V, et al. CD117 (c-kit) expression in human hepatocellular carcinoma. *Clin Oncol (R Coll Radiol).* 2007;19:204–208.
13. Haraguchi N, Ishii H, Mimori K, et al. CD13 is a therapeutic target in human liver cancer stem cells. *J Clin Invest.* 2010;120:3326–3339.
14. Kim H, Choi GH, Na DC, et al. Human hepatocellular carcinomas with "Stemness"-related marker expression: keratin 19 expression and a poor prognosis. *Hepatology.* 2011;54:1707–1717.
15. Lee ES, Han EM, Kim YS, et al. Occurrence of c-kit+ tumor cells in hepatitis B virus-associated hepatocellular carcinoma. *Am J Clin Pathol.* 2005;124:31–36.
16. Ma S, Chan KW, Hu L, et al. Identification and characterization of tumorigenic liver cancer stem/progenitor cells. *Gastroenterology.* 2007;132:2542–2556.
17. Sasaki M, Tsuneyama K, Ishikawa A, et al. Intrahepatic cholangiocarcinoma in cirrhosis presents granulocyte and granulocyte-macrophage colony-stimulating factor. *Hum Pathol.* 2003;34:1337–1344.
18. Yamashita T, Budhu A, Forgues M, et al. Activation of hepatic stem cell marker EpCAM by Wnt-beta-catenin signaling in hepatocellular carcinoma. *Cancer Res.* 2007;67:10831–10839.
19. Yamashita T, Forgues M, Wang W, et al. EpCAM and alpha-fetoprotein expression defines novel prognostic subtypes of hepatocellular carcinoma. *Cancer Res.* 2008;68:1451–1461.
20. Yamashita T, Ji J, Budhu A, et al. EpCAM-positive hepatocellular carcinoma cells are tumor-initiating cells with stem/progenitor cell features. *Gastroenterology.* 2009;136:1012–1024.
21. Yang W, Yan HX, Chen L, et al. Wnt/beta-catenin signaling contributes to activation of normal and tumorigenic liver progenitor cells. *Cancer Res.* 2008;68:4287–4295.
22. Yang ZF, Ho DW, Ng MN, et al. Significance of CD90+ cancer stem cells in human liver cancer. *Cancer Cell.* 2008;13:153–166.
23. Zhu Z, Hao X, Yan M, et al. Cancer stem/progenitor cells are highly enriched in CD133+CD44+ population in hepatocellular carcinoma. *Int J Cancer.* 2010;126:2067–2078.
24. Theise ND, Nakashima O, Park YN, et al. *WHO Classification of Tumours of the Digestive System.* Lyon: IARC Press; 2010:225–227.

25. Goodman ZD, Ishak KG, Langloss JM, et al. Combined hepatocellular-cholangiocarcinoma. A histologic and immunohistochemical study. *Cancer*. 1985;55:124–135.
26. Kim H, Park C, Han KH, et al. Primary liver carcinoma of intermediate (hepatocyte-cholangiocyte) phenotype. *J Hepatol*. 2004;40:298–304.
27. Steiner PE, Higginson J. Cholangiolocellular carcinoma of the liver. *Cancer*. 1959;12:753–759.
28. Taguchi J, Nakashima O, Tanaka M, et al. A clinicopathological study on combined hepatocellular and cholangiocarcinoma. *J Gastroenterol Hepatol*. 1996;11:758–764.
29. Zaret KS, Grompe M. Generation and regeneration of cells of the liver and pancreas. *Science*. 2008;322:1490–1494.
30. Fausto N, Campbell JS. The role of hepatocytes and oval cells in liver regeneration and repopulation. *Mech Dev*. 2003;120:117–130.
31. Roskams TA, Libbrecht L, Desmet VJ. Progenitor cells in diseased human liver. *Semin Liver Dis*. 2003;23:385–396.
32. Clouston AD, Powell EE, Walsh MJ, et al. Fibrosis correlates with a ductular reaction in hepatitis C: roles of impaired replication, progenitor cells and steatosis. *Hepatology*. 2005;41:809–818.
33. Eleazar JA, Memeo L, Jhang JS, et al. Progenitor cell expansion: an important source of hepatocyte regeneration in chronic hepatitis. *J Hepatol*. 2004;41:983–991.
34. Roskams T, De Vos R, Desmet V. 'Undifferentiated progenitor cells' in focal nodular hyperplasia of the liver. *Histopathology*. 1996;28:291–299.
35. Roskams T, De Vos R, Van Eyken P, et al. Hepatic OV-6 expression in human liver disease and rat experiments: evidence for hepatic progenitor cells in man. *J Hepatol*. 1998;29:455–463.
36. Theise ND, Saxena R, Portmann BC, et al. The canals of Hering and hepatic stem cells in humans. *Hepatology*. 1999;30:1425–1433.
37. Zhang F, Chen XP, Zhang W, et al. Combined hepatocellular cholangiocarcinoma originating from hepatic progenitor cells: immunohistochemical and double-fluorescence immunostaining evidence. *Histopathology*. 2008;52:224–232.
38. Fujii T, Zen Y, Harada K, et al. Participation of liver cancer stem/progenitor cells in tumorigenesis of scirrhous hepatocellular carcinoma–human and cell culture study. *Hum Pathol*. 2008;39:1185–1196.
39. Fabris L, Cadamuro M, Guido M, et al. Analysis of liver repair mechanisms in Alagille syndrome and biliary atresia reveals a role for notch signaling. *Am J Pathol*. 2007;171:641–653.
40. Nakanuma Y, Sasaki M, Ikeda H, et al. Pathology of peripheral intrahepatic cholangiocarcinoma with reference to tumorigenesis. *Hepatol Res*. 2008;38:325–334.
41. Kozaka K, Sasaki M, Fujii T, et al. A subgroup of intrahepatic cholangiocarcinoma with an infiltrating replacement growth pattern and a resemblance to reactive proliferating bile ductules: 'bile ductular carcinoma'. *Histopathology*. 2007;51:390–400.
42. Zuo HQ, Yan LN, Zeng Y, et al. Clinicopathological characteristics of 15 patients with combined hepatocellular carcinoma and cholangiocarcinoma. *Hepatobiliary Pancreat Dis Int*. 2007;6:161–165.
43. Jarnagin WR, Weber S, Tickoo SK, et al. Combined hepatocellular and cholangiocarcinoma: demographic, clinical, and prognostic factors. *Cancer*. 2002;94:2040–2046.
44. Koh KC, Lee H, Choi MS, et al. Clinicopathologic features and prognosis of combined hepatocellular cholangiocarcinoma. *Am J Surg*. 2005;189:120–125.
45. Ng IO, Shek TW, Nicholls J, et al. Combined hepatocellular-cholangiocarcinoma: a clinicopathological study. *J Gastroenterol Hepatol*. 1998;13:34–40.
46. Lee CC, Wu CY, Chen JT, et al. Comparing combined hepatocellular-cholangiocarcinoma and cholangiocarcinoma: a clinicopathological study. *Hepatogastroenterology*. 2002;49:1487–1490.
47. Yano Y, Yamamoto J, Kosuge T, et al. Combined hepatocellular and cholangiocarcinoma: a clinicopathologic study of 26 resected cases. *Jpn J Clin Oncol*. 2003;33:283–287.
48. Komuta M, Govaere O, Vandecaveye V, et al. Histological diversity in cholangiocellular carcinoma reflects the different cholangiocyte phenotypes. *Hepatology*. 2012;55:1876–1888.