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ORIGINAL SCIENTIFIC REPORT

Post-resection Prognosis of Combined Hepatocellular Carcinoma-Cholangiocarcinoma According to the 2010 WHO Classification

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Abstract

Background Combined hepatocellular carcinoma and cholangiocarcinoma (cHC) has wide histological diversity. We intended to investigate the prognostic influence of tumor types of cHC.

Methods We analyzed the clinical and pathological features of cHC along 2010 WHO classification. Study group was 100 cHC patients who underwent primary resection. Control group comprised 200 propensity score-matched patients with intrahepatic cholangiocarcinoma (ICC).

Results In cHC group, tumor diameter was 4.4 ± 2.8 cm and 95 patients had single tumor. They were classified as classical type in 46 and subtypes with stem cell (SC) features in 54. Subtypes with SC features included typical in 16, intermediate cell in 22, and cholangiolocellular in 16. Their 1- and 3-year tumor recurrence rates were 31.7 and 59.8%; and 1- and 3-year patient survival rates were 92.5 and 77.3%, respectively. Tumor recurrence ($p = 0.008$) and patient survival ($p = 0.005$) rates were different according to tumor types. Further stratification by subtypes with SC features resulted in prognostic stratification in tumor recurrence ($p = 0.045$) and patient survival ($p = 0.042$). However, tumor stage was the only independent risk factor for tumor recurrence and patient survival. Comparing with ICC control group, cHC group showed no significant difference in rates of tumor recurrence ($p = 0.523$), but better survival outcomes ($p = 0.008$). Median post-recurrence patient survival period was 20 months in cHC patients and 6 months in ICC patients ($p = 0.001$).

Conclusions Our results indicated that there would be close relationship between the post-resection prognosis and histological types according to the 2010 WHO classification, but these histological types did not become an independent prognostic factor.

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Introduction

Combined hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC) is an uncommon form of primary liver cancer containing components of both HCC and ICC. The disease entity of combined HCC–CC (cHC) was reported in 1903. In 1949, this tumor was first classified as the double tumors, combined type, and mixed type [1]. In 1985, a revised classification was proposed to

include collision type, transitional type, and fibrolamellar tumors [2].

With advances in the field of molecular biology, the cancer stem cell theory of solid neoplasms has been accepted more widely. Primary liver cancers, including HCC, ICC, and cHC, are thought to originate from hepatic progenitor cells (HPCs). HPCs are liver-specific adult stem cells that are activated when mature hepatocytes and/or cholangiocytes are damaged. HPCs can differentiate into either hepatocytes or cholangiocytes. The theory that cHC originates from HPCs was adopted in the 2010 World Health Organization (WHO) classification, which includes two main histological forms as the classical type and subtypes with stem cell (SC) features [3].

We previously presented the post-resection outcomes of cHC, in which we did not find significant statistical difference in post-resection prognosis according to the old classifications [4]. These old classifications of cHC are regarded as kinds of histopathological classifications that are not associated with prognostic discrimination. In our previous study [4], we had attempted to reclassify surgical specimens according to the subtype definitions of the 2010 WHO classification through specific immunohistochemical staining of paraffin-embedded specimen sections, but it was very difficult to reclassify them through such a retrospective method. Thus, we established a study cohort of new patients diagnosed with cHC whose pathologic diagnoses were made prospectively according to the 2010 WHO classification.

Because of the low incidence of cHC and recent adoption of the new classification, only a few studies have presented the clinicopathological characteristics and prognosis of cHC patients along the 2010 WHO classification [5–7]. To our knowledge, this study is the first to include patients who were prospectively diagnosed with cHC according to the 2010 WHO classification.

The purposes of this study were to investigate the clinical and pathological features of patients with cHC classified according to the 2010 WHO classification and to know whether the tumor types have any prognostic influence or not.

Patients and methods

Patients

We searched the institutional liver cancer surgery database extensively to find cHC patients whose diagnosis was made along the 2010 WHO classification. We identified 135 cHC patients during a 3-year study period from July 2012 to June 2015. During this study period, 1932 patients with HCC, 270 patients with ICC, and a small number of patients with rare liver tumors also underwent hepatic resection [8]; thus, cHC comprised approximately 5.8% of all primary liver malignancies.

Of the 135 patients with cHC, 33 were diagnosed using the old classifications and were thus excluded. Of the 102 patients who were diagnosed using the 2010 WHO classification, two patients had concurrent ICC, and thus they were excluded due to its unfavorable prognosis. On the other hand, another two patients also had concurrent small HCC, but they were included due to its more favorable prognosis than that of cHC [4]. Finally, 100 patients with cHC were selected as the study group in this study. The medical records of these patients were retrospectively reviewed after approval of the Institutional Review Board of our institution (AMC IRB 2015-0641).

We followed up the patients until January 2016 through review of medical records. The follow-up period for each patient was $\geq$7 months or up to patient death. Routine preoperative work-up and post-resection follow-up protocols for primary liver malignancies have been described elsewhere [4, 9–13].

Pathologic diagnosis according to the 2010 WHO classification

This study used the 2010 WHO classification [3], in which cHC tumors are divided as the classical type and subtypes with SC features. The classical type meets the traditional definition of cHC. Subtypes with SC features are further classified as typical, intermediate cell, and cholangiolocellular subtypes. The subtypes were classified based on the major components after immunohistochemical staining for HepPar1, CD10, CD34, cytokeratin 7, cytokeratin 19, carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), nuclear cell adhesion molecule (NCAM1/CD56), epithelial cell adhesion molecule (EpCAM), reticulin, KIT (CD117), and others [3]. More than 5 tumor blocks were used to determine the major subtypes.

Survival comparison with ICC

To objectively compare the post-resection outcomes, a control group of ICC patients was narrowly selected through propensity score matching. The inclusion conditions were as follows: single tumor with 2–6 cm in size; R0 resection; mass-forming growth type; and 7th American Joint Committee on Cancer (AJCC) tumor stage I or II.

We calculated the sample numbers of the ICC control groups by using MedCalc (version 15.11.4; MedCalc Software, Ostend, Belgium). The sample numbers of the control group were estimated with a type I error (α) of 0.05 and type II error (β) of 0.20, in addition to a difference in long-term survival rates of 18% and a ratio of sample size

Table 1 Clinicopathological features of patients with combined hepatocellular carcinoma-cholangiocarcinoma according to the 2010 WHO classification

	Combined hepatocellular carcinoma-cholangiocarcinoma					$A + B + C + D$	Intrahepatic cholangiocarcinoma (E)	p value	p value
	Classical type (A)	Subtypes with stem cell features						A versus $B + C + D$	$A + B + C + D$ versus E
		Typical (B)	Intermediate cell (C)	Cholangiolocellular (D)	$B + C + D$				
Number	46	16	22	16	54	100	200		
Age (years)	53 ± 12.0	52.6 ± 7.8	54.3 ± 10.4	59.2 ± 9.3	55.2 ± 9.6	54.2 ± 10.7	56.1 ± 9.1	0.316	0.109
Sex (M/F)	41/5	10/6	13/9	12/4	35/19	76/24	138/62	0.005	0.206
Background liver disease								0.137[b]	0.000[b]
Hepatitis B virus infection	29	10	12	7	29	58	29		
Hepatitis C virus infection	4	1	0	1	2	6	1		
Others[a]	13	5	10	8	23	36	170		
Serum AFP								0.205	NA
Mean (ng/mL)	131.9 ± 362.5	48,549.5 ± 108,820.9	415.8 ± 759.2	22.1 ± 9.3	12,271.3 ± 62,248.2	671.8.2 ± 46,046.5	NA		
Median (ng/mL)	7.9	57.0	28.5	2.7	7.8	9.2	NA		
Serum PIVKA-II								0.463	NA
Mean (mAU/mL)	839.5 ± 3570.3	1924.7 ± 5482.9	2080.2 ± 6230.3	22.3 ± 8.3	1542.1 ± 5155.9	1182.6 ± 4404.1	NA		
Median (mAU/mL)	40	26	32	21	28	32	NA		
Serum CA 19-9								0.861	0.001
Mean (ng/mL)	27.0 ± 55.1	12.5 ± 8.3	45.2 ± 103.4	7.9 ± 6.4	24.3 ± 67.5	25.5 ± 62.0	66.9 ± 97.3		
Median (ng/mL)	10.5	8.5	13.1	5.5	8.5	9.8	29.3		
ICG-R15 (%)	13.0 ± 7.4	10.9 ± 1.9	10.9 ± 5.4	8.9 ± 4.0	10.4 ± 4.3	11.6 ± 6.0	10.3 ± 5.6	0.000	0.065
MELD score	7.7 ± 1.6	7.5 ± 1.8	7.3 ± 1.7	7.8 ± 1.8	7.5 ± 1.7	7.6 ± 1.7	7.3 ± 1.3	0.548	0.091
Preoperative locoregional treatment (n)	12	3	3	2	8	20	16	0.160	0.262
Anatomical resection (n)	39	14	19	16	49	88	172	0.361	0.631
R0 resection (n)	41	15	20	16	51	92	200	0.468	0.001
Tumor size (cm)									
Mean (cm)	4.5 ± 3.3	4.4 ± 3.1	4.4 ± 2.2	4.5 ± 1.9	4.4 ± 2.3	4.4 ± 2.8	4.4 ± 1.7	0.015	1.000
Median (cm)	3.0	3.0	3.7	4.1	3.8	3.5	4.3		
≤5 cm (n)	31	11	16	10	37	68	156		
>5 cm (n)	15	5	6	6	17	32	44		
Tumor number								0.659	0.004
Single (n)	43	16	20	16	52	95	200		
Multiple (n)	3	0	2	0	2	5	0		
Lymphovascular invasion (n)	17	8	8	2	18	35	55	0.701	0.182
Perineural invasion (n)	4	2	4	0	6	10	59	0.749	0.000
Lymph node metastasis (n)	4	0	0	1	1	5	0	0.177	0.004

Table 1 continued

	Combined hepatocellular carcinoma-cholangiocarcinoma					$A + B + C + D$	Intrahepatic cholangiocarcinoma (*E*)	*p* value *A* versus *B* + *C* + *D*	*p* value *A* + *B* + *C* + *D* versus *E*
	Classical type (*A*)	Subtypes with stem cell features		Cholangiolocellular (*D*)	$B + C + D$				
		Typical (*B*)	Intermediate cell (*C*)						
7th AJCC tumor stage								0.512[c]	0.028[c]
I	26	8	12	14	34	60	145		
II	15	8	10	1	19	34	55		
III	1	0	0	0	0	1	0		
IV	4	0	0	1	1	5	0		
Tumor recurrence rate								0.014	0.523
At 1 year	48.1%	18.7%	33.0%	12.5%	18.7%	31.7%	31.2%		
At 3 years	69.4%	100%	34.8%	31.8%	52.1%	59.8%	50.4%		
Patient survival rate								0.003	0.008
At 1 year	90.1%	100%	90.2%	93.3%	94.1%	92.5%	80.5%		
At 3 years	47.8%	100%	84.6%	93.3%	92.0%	77.3%	53.6%		

AFP alpha-fetoprotein, *PIVKA-II* proteins induced by vitamin K antagonist or absence-II, *CA 19-9* carbohydrate antigen 19-9, *ICG-R15* indocyanine green retention test at 15 min, *MELD* model for end-stage liver disease, *AJCC* American Joint Committee on Cancer, *NA* not available

[a] Including normal liver, idiopathic chronic hepatitis, alcoholic liver disease and others

[b] Viral hepatitis versus others

[c] Stage I versus other advanced stages

of 2. These values gave an estimated sample size of 200 for the control group.

Through an extensive search of the ICC database at our institution, we identified 200 ICC patients who met the abovementioned conditions during the period from January 2005 to December 2013 [4, 8, 10]. We followed up these ICC control patients until December 2015 through review of medical records.

Statistical analysis

Continuous variables were analyzed by using the Student *t* test or analysis of variance (ANOVA) test depending on the types of distribution. Incidence variables were compared by the χ^2 test or Fisher exact test. Survival curves were estimated by the Kaplan–Meier method and compared by using the log-rank test. Cox proportional hazard regression analysis was used to obtain hazard ratio [HR] and 95% confidence interval (CI). A *p* value less than 0.05 was considered to be statistically significant. We used SPSS (version 22; IBM, New York, NY) for statistical analyses.

Results

Clinicopathological features

The clinical features of 100 patients pathologically diagnosed of cHC are summarized in Table 1. Their mean age was 54.2 ± 10.8 years (range: 30–81), and 72 were male. Most patients had been preoperatively diagnosed with HCC, and thus, some of them initially underwent transcatheter arterial chemoembolization (TACE) ($n = 19$) or radiofrequency ablation ($n = 1$). The extents of liver resection are summarized in Table 2. Regarding curative resection, R0 resection was performed in 92 patients and R1 resection in 8. The pathological findings of cHC patients are summarized in Table 1. The diameter of tumors was 4.4 ± 2.8 cm. Ninety-five patients had a solitary tumor. Two patients had a concurrent solitary HCC <2 cm in size. The cHCs were classified as the classical type in 46 patients and as subtypes with SC features in 54. Subtypes with SC features were divided as typical subtype ($n = 16$), intermediate-cell subtype ($n = 22$), and cholangiolocellular subtype ($n = 16$; Fig. 1).

Post-resection prognosis

No patients died of perioperative complications. During the follow-up period of 18.6 ± 8.9 months (range: 5–43), tumor recurrence occurred in 42 patients. Their 1-, 2-, and 3-year tumor recurrence rates were 31.7, 48.8, and 59.8%,

Table 2 Extents of liver resection in 100 patients with combined hepatocellular carcinoma-cholangiocarcinoma

Types of resection		Patient no.
Anatomical resection (*n*)		88 (88%)
	Right hepatectomy	15
	Left hepatectomy	17
	Right anterior sectionectomy	15
	Right posterior sectionectomy	18
	Central bisectionectomy	6
	Left lateral sectionectomy	10
	Left medial sectionectomy	3
	Caudate lobectomy	2
	Right trisectionectomy	2
Non-anatomical resection (*n*)	Partial hepatectomy[a]	12 (12%)
Concurrent bile duct resection (*n*)		3 (3%)
Laparoscopic resection (*n*)		8 (8%)

[a] Including subsegmentectomy and non-anatomical partial hepatectomy

respectively (Fig. 2a). During the follow-up period, 15 patients died and their cause of death was tumor recurrence. Their 1-, 2-, and 3-year overall patient survival rates were 92.5, 80.5, and 77.3%, respectively (Fig. 2b).

Tumor staging according to the 7th AJCC system identified 60 patients in stage I, 34 in stage II, 1 in stage III, and 5 in stage IV. The curves of tumor recurrence and patient survival showed definite prognostic stratification according to the 7th AJCC tumor staging system ($p = 0.002$ for tumor recurrence and $p < 0.001$ for patient survival; Fig. 3).

The most common site of the first post-resection recurrence was intrahepatic recurrence. The common sites and corresponding treatment for first recurrence are summarized in Table 3. In principle, we have tried to provide every available treatment for recurrence. However, no specific treatment was provided to 5 of 42 patients with tumor recurrence (11.9%) because of the poor general condition and/or unwillingness of the patients.

Risk factor analysis for post-resection prognosis

Univariate analysis revealed that significant risk factors for tumor recurrence were the 7th AJCC tumor stage ($p < 0.001$), perineural invasion ($p < 0.001$), lymph node metastasis ($p = 0.001$), and types of the 2010 WHO classification ($p = 0.008$), but not tumor size $\geq$5 cm ($p = 0.054$), lymphovascular invasion ($p = 0.101$), tumor number ($p = 0.461$), anatomical resection ($p = 0.522$), and macroscopic curative resection ($p = 0.673$). Multivariate analysis revealed that the AJCC tumor stage was the only independent risk factor for tumor recurrence ($p = 0.031$; HR = 1.62; 95% CI = 1.05–2.51).

Univariate analysis also revealed that significant risk factors for patient survival were the AJCC tumor stage ($p < 0.001$), lymph node metastasis ($p < 0.001$), perineural invasion ($p = 0.005$), types of the 2010 WHO classification ($p = 0.005$), tumor number ($p = 0.053$), anatomical resection ($p = 0.006$), and lymphovascular invasion ($p = 0.007$), but not tumor size $\geq$5 cm ($p = 0.324$) and macroscopic curative resection ($p = 0.844$). Multivariate analysis revealed that the AJCC tumor stage was the only independent risk factor for patient survival ($p = 0.019$; HR = 2.24; 95% CI = 1.14–4.38).

Analysis of prognosis according to the 2010 WHO classification

Tumor classification into the classical type and subtypes with SC features revealed a definite prognostic stratification of tumor recurrence rates ($p = 0.008$) and patient survival rates ($p = 0.005$; Fig. 4a, b). Further stratification by subtypes with SC features also resulted in noticeable prognostic stratification of tumor recurrence rates ($p = 0.045$) and patient survival rates ($p = 0.042$; Fig. 4c, d).

To avoid the confounding effects of different tumor stages, 67 patients were selected with following inclusion conditions of curative resection, solitary tumor $\leq$6 cm, no lymph node metastasis, and no adjacent organ invasion. After application of this narrow selection, the prognostic stratification along the 2010 WHO classification was no longer statistically significant in tumor recurrence rates ($p = 0.167$) and patient survival rates ($p = 0.898$) probably due to small sample number and reduced prognostic differences from the lowered rates of tumor recurrence and patient death (Fig. 4e, f).

Comparison of prognosis with ICC control group

Comparison of the prognoses of 100 patients with cHC and 200 patients with ICC revealed that there was no significant difference in tumor recurrence rates ($p = 0.523$), but the cHC group showed better survival outcomes ($p = 0.008$; Fig. 5a, b).

As mentioned above, 67 cHC patients were selected to avoid the confounding effects of different tumors and then compared with the ICC control group. There was no significant difference in tumor recurrence rates ($p = 0.585$), but the cHC group showed a much better survival outcome ($p = 0.002$; Fig. 5c, d). Thereafter, the cHC group was

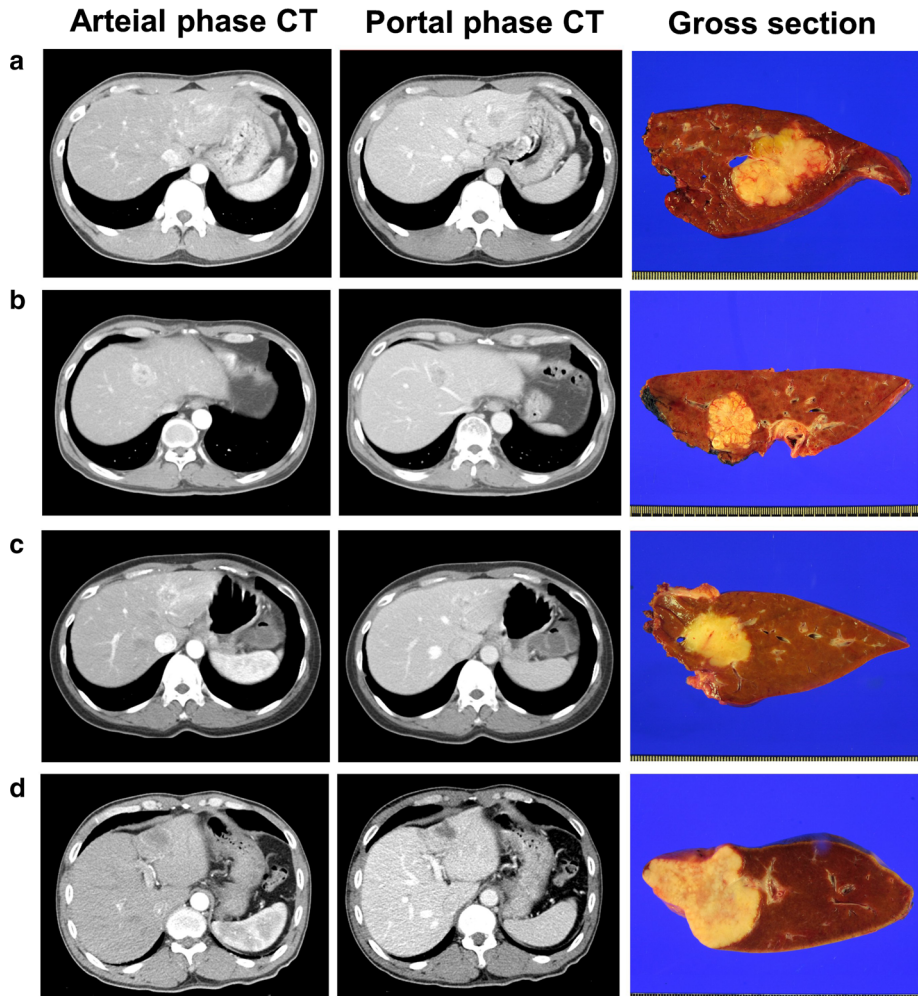

Fig. 1 Preoperative computed tomography (CT) findings and gross photographs of the surgical specimens. **a** A case of combined hepatocellular carcinoma-cholangiocarcinoma (cHC), classical type: a 35-year-old male patient showed a 4-cm-sized ill-defined lesion in the left liver, which was diagnosed as atypical hepatocellular carcinoma (HCC) or intrahepatic cholangiocarcinoma (ICC). The mass was composed of 20% HCC of Edmondson-Steiner grade III/II and 80% moderately differentiated adenocarcinoma. Immunohistochemical (IHC) staining results were polyclonal CEA positive at the canalicular and membranous staining; HepPar1 positive in the HCC component; cytokeratin 7 (CK 7) and cytokeratin 19 (CK 19) positive in the adenocarcinoma component; CD56 positive in a few cells; and CD117 negative. **b** A case of cHC, subtype of stem cell (SC) features, typical: a 48-year-old female patient showed a 3.5-cm-sized suspected HCC lesion in the left liver. IHC staining results were CK 7 and CK 19 focally positive; HepPar1 focally positive; polyclonal CEA positive in canalicular and cytoplasmic patterns; CD56 focally positive; and CD117 negative. **c** A case of cHC, subtype of SC features, intermediate cell: a 42-year-old female patient showed a 2-cm-sized ICC in the left liver and a 1-cm-sized hemangioma in the right liver. IHC staining results were CK 19 heterogeneously positive; HepPar1 negative; and polyclonal CEA positive in mixed membranous and cytoplasmic patterns. **d** A case of cHC, subtype of SC features, cholangiolocellular: a 51-year-old male patient showed a 4-cm-sized mass suspicious of cHC or ICC in the left liver. IHC staining results were CK 7 and CK 19 strongly positive; c-Kit, CD56 and HepPar1 negative; and polyclonal CEA positive in canalicular and cytoplasmic patterns

divided into the classical type and subtypes with SC features, with no significant difference in tumor recurrence rates ($p = 0.343$) among two study subgroups and one control group. In contrast, the cHC group of subtypes with SC features showed a definitely better survival outcome ($p = 0.001$), but nearly no or only marginal survival difference was observed between the cHC group of the classical type and the ICC control group ($p = 0.058$; Fig. 5e, f).

Post-recurrence patient survival rates were compared between the cHC patients ($n = 42$) and ICC patients ($n = 106$) showing tumor recurrence after resection, in which the median post-recurrence patient survival period was 6 months in CCC group and 20 months in all cHC group ($p = 0.001$; Fig. 6a); and 20 months in patients with cHC of classical type and >24 months in patients with cHC of subtypes with SC features ($p = 0.209$; Fig. 6b).

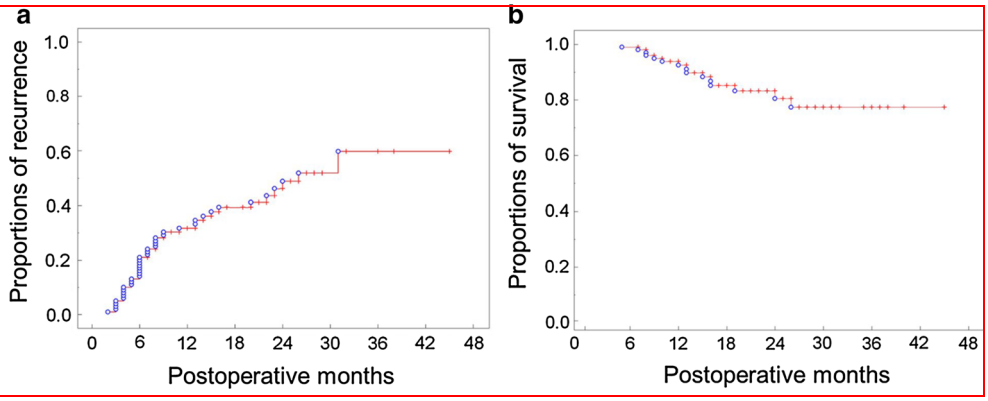

Fig. 2 Tumor recurrence (**a**) and overall patient survival (**b**) curves for 100 patients with combined hepatocellular carcinoma-cholangiocarcinoma

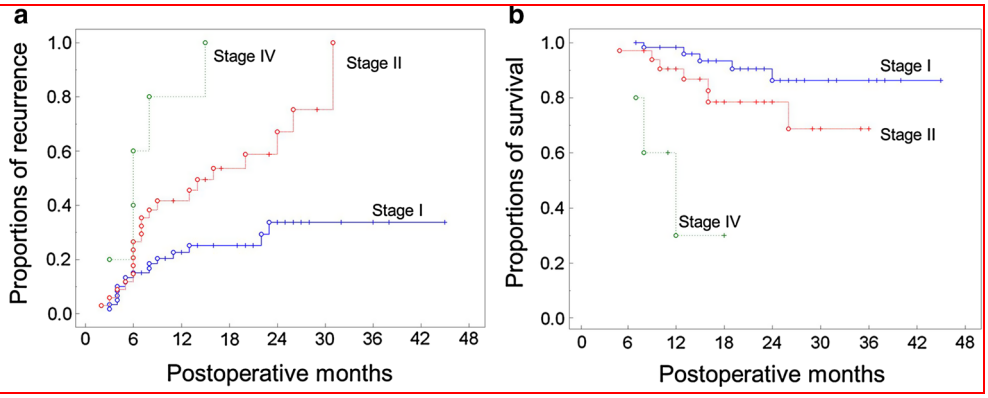

Fig. 3 Tumor recurrence (**a**) and overall patient survival (**b**) curves according to the 7th AJCC tumor staging system for combined hepatocellular carcinoma-cholangiocarcinoma

Table 3 Treatment for the first recurrence after resection in 42 patients with combined hepatocellular carcinoma-cholangiocarcinoma

Sites of first recurrence	Patient no.
Intrahepatic recurrence	30 (71.4%)
TACE	19
Repeat resection	4
Chemotherapy	4
No specific treatment	3
Intra- and extrahepatic recurrence[a]	5 (11.9%)
TACE	1
Chemotherapy	3
No specific treatment	1
Pulmonary metastasis	3 (7.1%)
Chemotherapy	3
Intraperitoneal extrahepatic metastasis	4 (9.5%)
Chemotherapy	3
No specific treatment	1

Chemotherapy regimens included gemcitabine, 5-fluorouracil, sorafenib or others

TACE transcatheter arterial chemoembolization

[a] Including lung and intraperitoneal metastasis

Discussion

The cHC is a rare tumor that comprised approximately 5.8% of primary liver malignancies in the present study, which is much higher than the 1.1% incidence in our previous study [4]. The reasons for such large difference in incidence between our previous and present studies may include a possible incompleteness of data collection in our old database, a recent increased preference for surgical resection, even for small or huge solitary liver tumors, and a probable actual increase in its incidence or patient concentration to a high-volume liver cancer center [8]. In the literature, cHC accounts for 0.8–14.3% of primary liver malignancies, with incidences widely varying among studies [14–16]. According to a population-level analysis in the USA, 52,825 patients had HCC, 7181 patients had ICC, and 465 patients had cHC between 1988 and 2009; thus, the proportion of cHC was 0.8% [17].

The two old classifications of cHC are regarded as histological classifications [1, 2], and their types were proven to not be associated with prognostic discrimination. Advances in HPC research provide some new insights into the development of cHC. As HPCs have bipotential

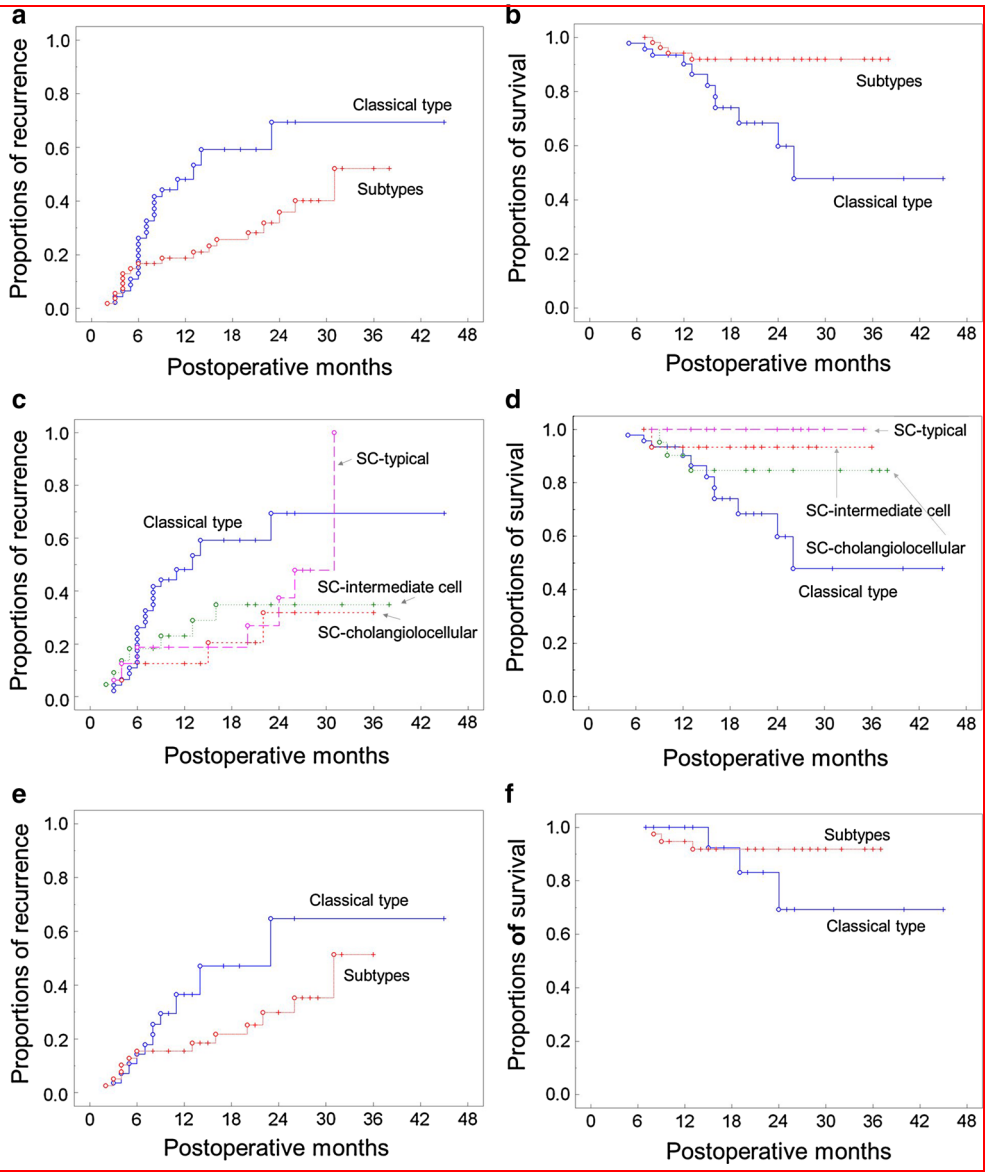

Fig. 4 Tumor recurrence and overall patient survival curves according to the 2010 WHO classification of combined hepatocellular carcinoma-cholangiocarcinoma: classical type versus subtypes with stem cell (SC) features (**a**, **b**); further stratification of subtypes with SC features (**c**, **d**); and classical type versus subtypes with SC features after avoiding confounding tumor stage-associated effects in 67 patients (**e**, **f**)

characteristics, the hypothesis that cHC is derived from HPCs is understandable. It was suggested that cHC originates from HPCs which can develop into HCC or ICC [18–20].

In 2010 WHO classification with the conceptual adoption of HPC origin, cHCs are divided into two types as classical type and subtypes with SC features [3]. First, cHC of the classical type found in 46 patients (46%) in this study. The classical type contains typical areas of both HCC and ICC. Its possible histogeneses are as follows: HCC and ICC develop independently and separately; HCC develops first and transforms into ICC or vice versa; and malignant change of HPCs occurs and then they differentiate to HCC and ICC in variable degrees.

Regarding cHC with SC features, the typical subtype is newly adopted. This subtype was reported to be rare [6], but it is not so uncommon because 16 patients (16%) in this study had this subtype tumor. The intermediate-cell subtype corresponds to the patients who had been known as liver carcinoma of the intermediate (hepatocyte–cholangiocyte) phenotype [21]. Our study included 22 patients (22%) with this subtype. Cholangiolocellular carcinoma was classified as a subtype of ICC at the previous versions of WHO classification, but, in the 2010 WHO classification, it is classified as the cholangiolocellular subtype with SC features belonging to the cHC category. Cholangiolocellular carcinoma is considered a rare malignant liver tumor [22], but 16 patients (16%) had this cancer in this study. So far, classification of various subtypes with SC features still appears to be challenging and requires further validation through large cohort studies [6].

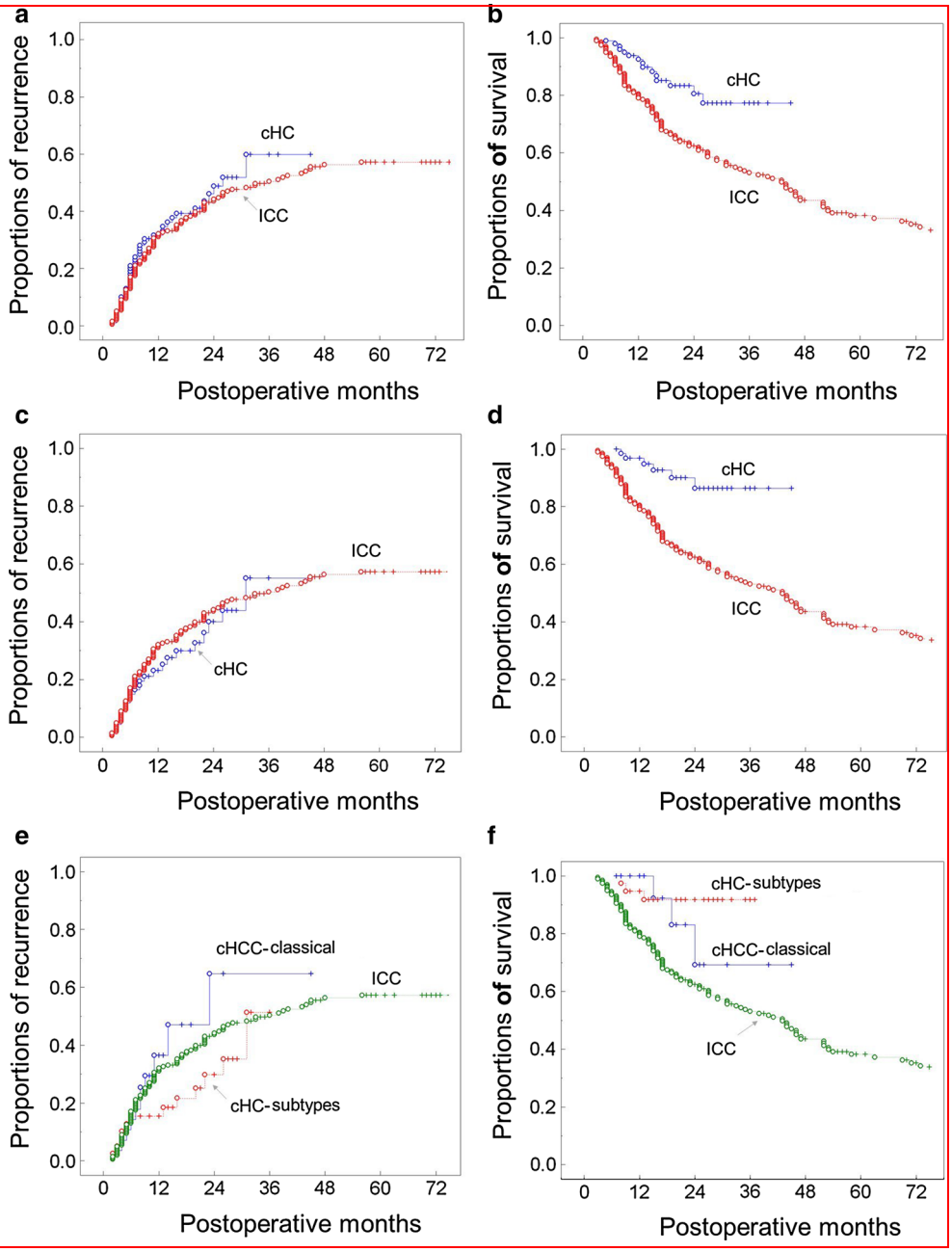

Fig. 5 Tumor recurrence and overall patient survival curves in the combined hepatocellular carcinoma-cholangiocarcinoma (cHC) study group ($n = 100$) and the intrahepatic cholangiocarcinoma (ICC) control group ($n = 200$) (**a**, **b**); comparison after avoiding confounding tumor stage-associated effects in cHC patients ($n = 67$) (**c**, **d**); and comparison after further subdivision of cHC patients into the classical type ($n = 28$) and subtypes with stem cell (SC) features ($n = 39$) (**e**, **f**)

The concept that cHC originates from HPCs was adopted in the 2010 WHO classification. At that time, the prognosis for cHC without SC features was thought to be worse than for pure HCC, but the prognosis for cHC with SC features was unknown with conflicting evidence based on small series and number of patients [3]. There are two medium-volume retrospective studies regarding prognosis of cHC along the 2010 WHO classification so far. Ikeda et al. [5] suggested that patients with subtypes with SC features showed poorer survival outcome than those with classical type. In contrast, Akiba et al. [6] suggested that there was no significant difference in survival outcomes between patients with classical type and subtypes with SC features. After simulating the data in these two studies, we presumed that such confusing results on the clinical impact of subtypes with SC features might be associated with small sample number and difficulty in the retrospective subtype classification because some patients might show ambiguous immunohistochemical findings. Sasaki et al. [7] reported that all 63 cHC specimens concurrently showed all three subtypes of SC features in various amounts and combinations, but each tumor was retrospectively classified by the major type of histology along the 2010 WHO classification. They were thus

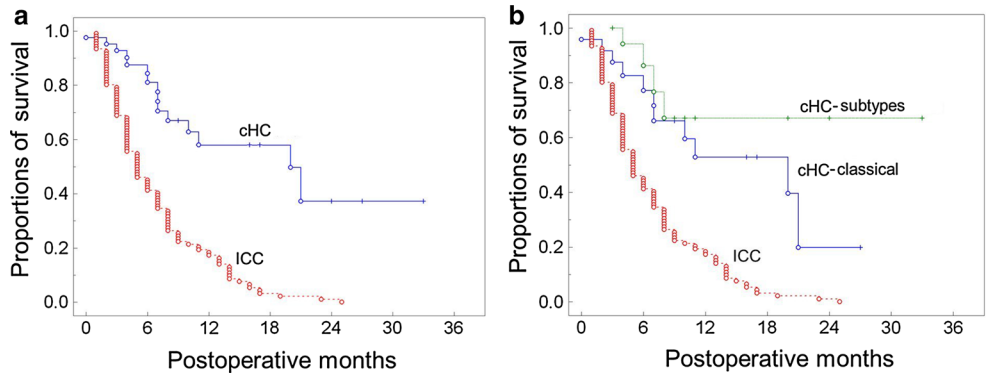

Fig. 6 Post-recurrence patient survival curves in the combined hepatocellular carcinoma-cholangiocarcinoma (cHC) study group ($n = 42$) and the intrahepatic cholangiocarcinoma (ICC) control group ($n = 106$) (**a**); and comparison after subdivision of cHC patients into the classical type ($n = 24$) and subtypes with stem cell features ($n = 18$) (**b**)

classified as the classical type ($n = 4$; 6.3%) and typical ($n = 3$; 4.8%), intermediate ($n = 28$; 44.4%), and cholangiolocellular ($n = 27$; 42.9%) subtypes. The proportions of each type varied widely, even in the above-mentioned three Japanese studies.

To avoid a bias from the small sample number and retrospective re-classification, we established a new cohort of 100 patients with cHC in the present study. To our knowledge, this study is the first and the largest study with patients who were prospectively diagnosed with cHC according to the 2010 WHO classification. We recognized that the prognostic influence of subtypes with SC features became more evident than in other studies, indicating that the presence of SC features may be closely associated with favorable tumor biology. However, multivariate analyses presented that the histological type of cHC according to the 2010 WHO classification did not become an independent prognostic factor after resection.

To validate the prognostic impact of cHC types classified according to the 2010 WHO classification, we compared their prognostic outcomes with a propensity score-matched control group of ICC. The post-resection outcome of tumor recurrence and patient survival in the subgroup with cHC of the classical type is quite similar to that of patients with ICC, but subtypes with SC features showed definitely improved survival outcomes [5, 23]. These results support the belief that cHC of the classical type and ICC may share similarly aggressive tumor biology, but that subtypes with SC features may have a less aggressive tumor nature.

Such differences in overall post-resection survival rates were associated with the post-recurrence survival period. In cHC patients with subtypes of SC features, the survival period after tumor recurrence appeared longer than in cHC patients with the classical type or the ICC control group, indicating the higher efficacy in recurrence treatment and the less aggressive tumor biology [4]. According to a study regarding the treatment modalities for primary cHC [24], hepatic-directed therapy, such as TACE, transarterial radioembolization, percutaneous ablation, and hepatic arterial infusion pump, showed superior objective responses over systemic chemotherapy alone.

This study has some limitations. This is a single-center retrospective study in a hepatitis B virus-endemic area. It is necessary to validate the prognostic influence of subtypes with SC features in other geographic regions to extend our results to patients with cHC of various causes. The follow-up period was relatively short due to the recent adoption of the 2010 WHO classification. The strong point of this study is that all patients were completely traced without any loss during follow-up. Multi-regional multicenter collective studies with sufficiently long-term follow-up duration are necessary to know the prognostic impact of subtypes with SC features.

In conclusion, cHC is a neoplasm with wide histological diversity, indicating a strong association with HPCs. Our results indicated that there would be a close relationship between the post-resection prognosis and the histological types according to the 2010 WHO classification, but the histological type was not an independent prognostic factor after resection.

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Compliance with ethical standards

Conflict of interest There were no authors who have conflict of interest to disclose.

Author contributions HS and JDH designed the study; HSM, SGW, LYJ, KKH, ACS, MDB, YES, HTY, PGC, LHC, LYS, and LSG

contributed to data acquisition and statistical analysis; and HS and JDH drafted and revised the article. All authors approved the submitted version of manuscript.

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