Clinicopathological features of cholangiolocarcinoma and impact of tumor heterogeneity on prognosis: A single institution retrospective study

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Abstract. Cholangiolocarcinoma (CLC) is an extremely rare tumor classified as a subtype of small duct-type intrahepatic cholangiocarcinoma (iCCA). There are few detailed reports on CLC and the prognostic impact of tumor heterogeneity is not clear. Between April 2006 and June 2022, of the 774 primary liver cancer resection cases who presented at Kanazawa University Hospital, 14 patients were pathologically diagnosed with CLC through immunohistochemical analysis of their molecular and biological features. Clinicopathological features and prognoses were evaluated retrospectively. Additionally, tumor heterogeneity was assessed and tumors were classified into pure and partial types according to the CLC component proportion in a single tumor. Chronic liver disease was observed in nine patients (64.3%). All tumors were mass-forming, and pathological R0 resection was achieved in 11 patients (78.6%). Tumor heterogeneity was classified as pure in 11 (78.6%) and partial in three (21.4%) patients. The median follow-up was 59.5 months (12-114 months). There was no difference in the 5-year disease-specific survival rates between the pure and partial (90.0% vs. 100.0%; P=0.200) types, but rates were significantly higher in the R0 resection group compared with those in the R1 resection group (100.0% vs. 50.0%; P=0.025). In conclusion, these results suggest that it is important for CLC patients to achieve curative resection, and CLC may have a good prognosis regardless of the proportion of CLC components in a single tumor.

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Introduction

Intrahepatic cholangiocarcinoma (iCCA) is the second most prevalent type of primary liver cancer, comprising ~15% of cases of primary liver cancers (1). Recently, iCCA have been classified into small- and large-duct types. Cholangiolocellular carcinoma (CoCC) is an extremely rare tumor that accounts for <1% of all primary liver cancers (2). CoCC has been categorized as a small-duct type iCCA, renamed cholangiolocarcinoma (CLC), based on the 2019 World Health Organization classification (3). In clinical practice, large-duct type iCCA frequently recur despite curative surgical resection and are resistant to chemotherapy or other drugs owing to their high invasiveness (4,5). Therefore, large-duct type iCCA have a worse prognosis than small-duct type iCCA (6). In contrast, CLC has a better prognosis after curative resection than iCCA, because it is less invasive (7).

iCCAs arise from every part of the intrahepatic biliary system, including the peribiliary glands (8). In contrast, CLCs are hypothesized to originate from hepatic stem cells or hepatic progenitor cells (HPCs) occupying the canals of Hering or cholangioles and cells of the peripheral branches of the intrahepatic bile ducts (9,10). Liver damage and chronic stimulation have been implicated in the activation of HPCs and carcinogenesis (9).

Histologically, large-duct type iCCA are characterized by cuboidal or columnar cells with mucus production constituting an irregular ductal or tubular arrangement (11). In contrast, CLC is characterized by small cuboidal cells without mucus, forming angular small ductular, antler-like or branched arrangement patterns with abundant hyalinized fibrous stroma (11). However, in clinical practice, tumors with mixed CLC and iCCA, with varying degrees of differentiation are often encountered. Hence, it is essential to elucidate the significance of tumor heterogeneity related to CLC components.

Several histological variants of iCCAs have been previously reported. Recently, iCCA tumor heterogeneity has been the focus of attention because it is among the factors contributing to iCCA resistance to therapy (12-14). However, it is unclear

how tumors with mixed CLC and iCCA differ clinically from iCCAs that do not contain any CLC components. A previous study defined pure CLC as a tumor comprising >80% of CLC components within a single tumor (15,16). We hypothesized that tumors containing various proportions of iCCA and CLC in a single tumor would have different recurrence or survival rates after resection compared with pure iCCA (not containing any CLC components).

In previous years, the pathological diagnosis of CLC has been made not only by hematoxylin and eosin (H&E) staining but also by evaluating molecular biology findings by immunostaining (15). However, most previous reports on the results of clinicopathological features and surgical outcomes for CLC included patients with CLC diagnosed with only H&E staining and few reports included patients with CLC evaluated based on molecular biology findings (2,17,18).

The present study was performed to identify the CLC component within an iCCA tumor diagnosed by assessing not only morphological features but also molecular biological features by immunostaining and reviewing the clinicopathological features, surgical outcomes and prognosis. Moreover, the impact on prognosis of tumor heterogeneity due to CLC components was evaluated.

Materials and methods

Study design. The present study was a retrospective observational study conducted at a single institution. This study was approved by the Kanazawa University Ethics Committee (approval no. 3221-2) and was performed in accordance with the ethical standards stated in the Declaration of Helsinki. Informed consent for this study was obtained from all participants, from Kanazawa University Hospital's website, as an opt-out option. All data, including immunostaining, were obtained from medical records.

Pathological diagnosis of CLC. CLC was pathologically defined as the proliferation of tumor cells with insufficient mucus composed of small tubular glands, antler-like or anastomotic patterns with abundant fibrous stroma by H&E staining, as previously described (11,19). H&E staining was performed according to our institutional protocol. In brief, after deparaffinization, 4 μm sections were stained with hematoxylin solution for 5 min, and rinsed under running water for 15 min. Then the sections were stained with eosin solution for 2 min and followed by dehydration with graded alcohol and clearing in xylene. In addition to the morphological findings identified following H&E staining, CLC cells were further identified using immunohistochemistry and mucicarmine staining. Positive epithelial membrane antigen (EMA) staining in the glandular lumen, positive neural cell adhesion molecule 1 (NCAM1) staining and the absence of mucin production were observed in CLC (15,20). In contrast, positive EMA staining in the cytoplasm, negative NCAM1 expression, and mucin production are usually observed in iCCA (15,20). For the immunohistochemical analysis of EMA and NCAM1, resected samples were immediately fixed in 10% neutral buffered formalin at room temperature for 24 h, embedded in paraffin and sliced into 4 μm sections. For antigen retrieval, sections were treated with 0.05 M citric acid buffer (pH 6) at 95˚C for 20 min in a microwave oven. After blocking endogenous peroxidase activity by 3% hydrogen peroxide solution at room temperature for 10 min, the sections were incubated with primary antibodies against EMA clone E29 (cat. no. IR629; Dako; Agilent Technologies, Inc.) and NCAM1 (cat. no. 418191; Nichirei Biosciences, Inc.) at 4˚C overnight. Envision + solution (Dako; Agilent Technologies, Inc.) was then applied for 30 min at room temperature and the reaction products were visualized using 3, 3'-diaminobenizidine tetrahydrochloride (MilliporeSigma) and H_2O_2. After that the sections were counterstained with hematoxylin for 1 min at room temperature. Sections were observed under a BX51 optical microscope (Olympus Corporation).

The CLC component was distinguished from the iCCA component based on the morphological characteristics identified using H&E staining and immunostaining.

Selection of patients with CLC. Between April 2006 and June 2022, 774 patients underwent liver resection for primary liver cancer in Kanazawa University Hospital. This period was determined by the availability of the clinical data to be analyzed in the present study. Of the 774 patients, only 65 (8.4%) were diagnosed with iCCA after surgery. Of these 65 iCCA patients, only 14 (21.5%) patients containing CLC component in a single tumor met the selection criteria and were defined as patients with CLC. Inclusion criteria were: i) That curative resection was intended and ii) that the CLC component should be present in >5% of a single tumor. Exclusion criteria were: i) No synchronous cancer in other organs, ii) no preoperative chemotherapy, and iii) no hepatocellular carcinoma component in a tumor. Pathological diagnosis and evaluation were independently performed by four board-certified pathologists.

Classification of pure type and partial type CLC. Based on the pathological proportion of the CLC component within a single iCCA tumor, CLC were classified into pure and partial types. A tumor in which the CLC component occupied >95% of the whole tumor was defined as a pure type and the rest (CLC component ≤95%) were defined as a partial type. For the classification, a board-certified expert pathologist blindly performed morphological evaluation of all H&E slides and immunostaining to determine the percentage of CLC component. Representative cases with the pathological findings of the CLC component in pure-type CLC (Fig. 1A) and the iCCA component in partial-type CLC (Fig. 1B) are presented.

Clinicopathological evaluation of CLC patients. Each patient was pathologically staged according to the 8th edition of the Union for International Cancer Control (UICC) Staging System (21) after surgery. The gross morphology of the tumor was classified according to the general rules for the clinical and pathological study of primary liver cancer (22). The location of the tumor in the liver was classified as hilar or peripheral, depending on the presence or absence of contact with the hepatic hilum (between the right side of the umbilical portion of the left portal vein and the left side of the origin of the right posterior portal vein) based on preoperative computed tomography (CT) and pathological findings of the resected specimen, as previously described (23). The extent and number of lymph nodes dissected were in accordance with

Table I. Patient characteristics.

Case	Age, years	Sex	Underlying liver disease	CEA, ng/ml (≤5.0)	CA19-9, ng/ml (≤37.0)	AFP, ng/ml (≤10.0)	PIVKA-II, mAU/ml (<40.0)	Tumor location	Type of liver resection	Number of lymph nodes removed
1	80	F	HCV	2.5	19	5	17	peripheral	Partial hepatectomy (S5/6)	-
2	62	M	NL	3.4	76	5	22	hilar	Left hepatectomy	+ (8,12)
3	73	M	HCV	4.5	45	202	24	peripheral	Left lateral sectionectomy	-
4	75	M	NL	4.2	41	5	18	peripheral	Left hepatectomy	+ (12)
5	62	M	NL	4.2	24	5	31	peripheral	Partial hepatectomy (S7/8)	-
6	79	F	HCV	2	59	3	753	hilar	Left extended hepatectomy with bile duct resection	+ (12)
7	70	F	LC(C)	0.9	44	4	11	peripheral	Partial hepatectomy (S5/6)	-
8	69	M	NL	4.8	4	2	17	peripheral	Right posterior sectionectomy	-
9	63	M	HBV	1.9	23	89	27	peripheral	S7 segmentectomy	-
10	78	F	NASH	2	7	4	14	Peripheral	Laparoscopic partial hepatectomy (S8)	-
11	78	M	NL	1.8	24	4	16	hilar	Left extended hepatectomy with bile duct resection	+ (3,7,8,12,13a)
12	71	M	HBV	2.1	25	7	16	peripheral	Laparoscopic S3 segmentectomy	-
13	66	M	HCV	1.5	17	4	16	peripheral	S7 segmentectomy	-
14	70	M	Alcoholic	5.3	208	6	21	peripheral	Laparoscopic partial hepatectomy (S8)	-

HCV, hepatitis C virus; NL, normal liver; LC, liver cirrhosis; NASH, non-alcoholic steatohepatitis; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; AFP, alpha-fetoprotein; PIVKA-II, protein levels induced by the absence of vitamin K or antagonist-II.

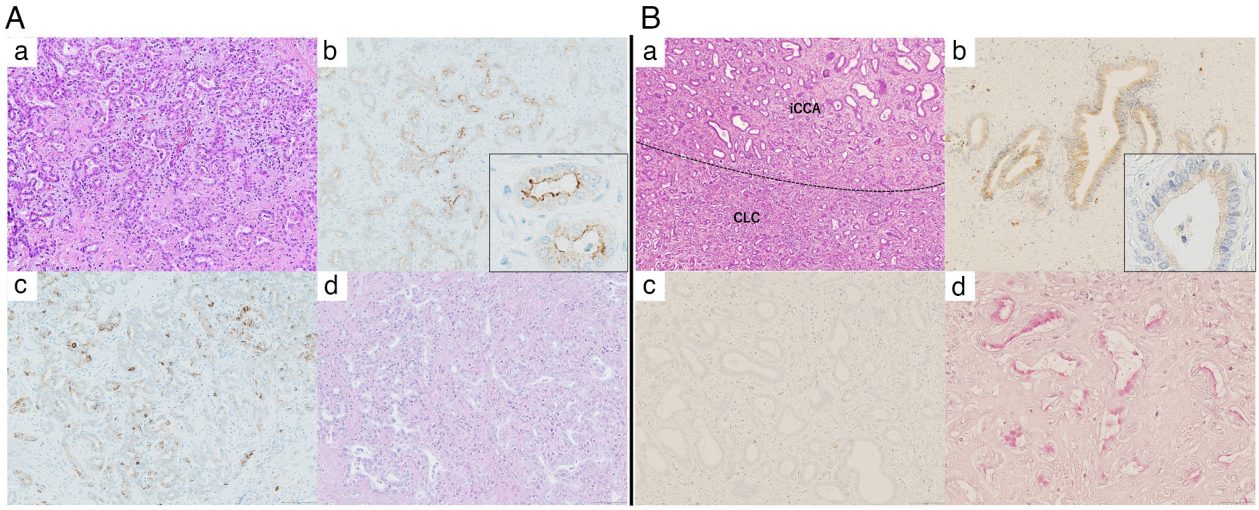

Figure 1. Histopathological findings of CLC. Histopathological findings indicating the CLC component (pure-type CLC). (A-a) Small tubular gland-forming cells proliferate in antler-like and anastomosing patterns with abundant fibrous stroma (hematoxylin and eosin staining; magnification, x100). (A-b) Immunohistochemical result for EMA is positive in the apical membrane of the tumor glands (magnification, x100). The inset shows image of grandular duct (magnification, x400). (A-c) Immunohistochemical result for NCAM1 is positive in the tumor cells (magnification, x100). (A-d) Mucin production is not observed in tumor cells stained with mucicarmine (magnification, x100). Histopathological findings indicating the iCCA component (partial-type CLC). (B-a) The iCCA and CLC components within a single tumor. Proliferation of atypical large tubular glands with fibrous stroma is identified in the iCCA component (hematoxylin and eosin staining; magnification, x100). (B-b) Immunohistochemical result for EMA is positive in the cytoplasm of tumor cells (magnification, x100). The inset shows image of grandular duct (magnification, x400). (B-c) Immunohistochemical result for NCAM1 is negative in tumor cells (magnification, x100). (B-d) Mucin production is observed in tumor cells on mucicarmine staining (magnification, x100). CLC, cholangiolocarcinoma; NCAM1, neural cell adhesion molecule 1; EMA, epithelial membrane antigen; iCCA, intrahepatic cholangiocarcinoma.

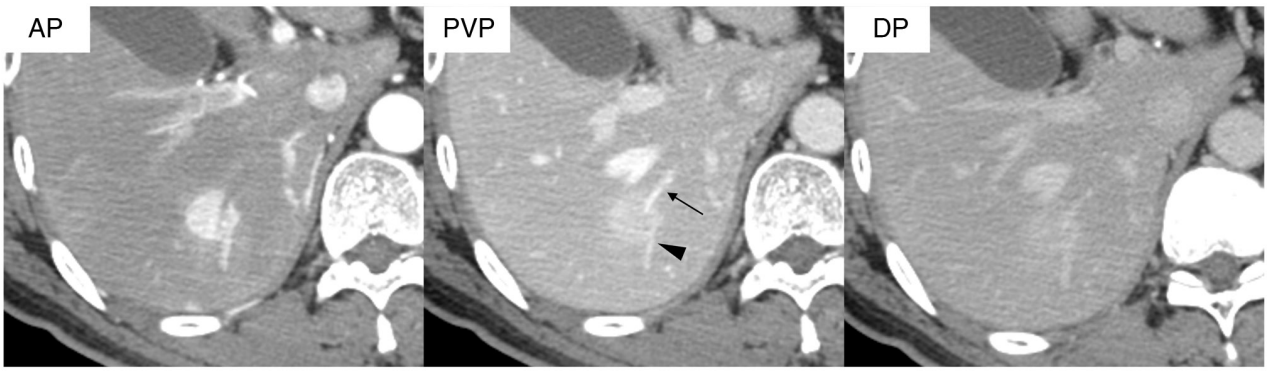

Figure 2. Abdominal dynamic computed tomography findings of cholangiolocarcinoma. The tumor shows hypervascularity and enhancement during the AP and prolonged enhancement during the PVP and DP. The presence of intratumoral pre-existing vessels, such as the portal vein (arrowhead) and hepatic vein (arrow), is also observed. AP, arterial phase; PVP, portal venous phase; DP, delayed phase.

the Japanese Society of Biliary Surgery classification (24). Postoperative complications were classified according to the Clavien-Dindo classification (25).

Follow-up of patients with CLC. All the patients were followed up after surgery as an outpatient in our institution for >5 years (unless they died or dropped out). In principle, contrast-enhanced CT and blood examination including tumor markers were performed every 3-6 months for evaluation of recurrence. The survival period was defined as the date of surgery to death or last contact. If recurrence was detected, systemic chemotherapy, local treatment if suitable or best supportive care was considered depending on the condition of the patient.

Statistical analysis. Overall survival (OS), recurrence-free survival (RFS), and disease-specific survival (DSS) rates were calculated using the Kaplan-Meier method, and the log-rank test was used for comparison. $P<0.05$ was considered to indicate a statistically significant difference. Statistical analyses were performed using Graph Pad Prism (9.4.1; Dotmatics).

Results

Patient characteristics. The characteristics of all patients are presented in Table I. The median age of these patients was 70.5 (range, 62-80) years. There were 10 males (71.4%) and four females (28.6%). Nine patients (64.3%) had a chronic liver injury (alcohol, n=1; chronic hepatitis due to hepatitis B virus, n=2; chronic hepatitis due to hepatitis C virus, n=4; liver cirrhosis due to hepatitis C virus, n=1; and nonalcoholic steatohepatitis, n=1). The liver function of all patients was well preserved based on the Child-Pugh classification

Table II. Preoperative patient characteristics.

Variable	Value
CLC, no.	14
Sex male, no. (%)	10 (71.4)
Age, median (range), year	70.5 (62-80)
Underlying liver disease, no. (%)	
Present	9 (64.3)
Alcohol	1 (11.1)
HBV	2 (22.2)
HCV	5 (55.6)
NASH	1 (11.1)
CEA	
Median (range), ng/ml	2.3 (0.9-5.3)
>5.0 ng/ml, no. (%)	1 (7.1)
CA19-9	
median (range), ng/ml	24.5 (4.0-208.0)
>37.0 ng/ml, no. (%)	6 (42.9)
AFP	
median (range), ng/ml	5.0 (2.0-202.0)
>10.0 ng/ml, no. (%)	2 (14.3)
PIVKA-II	
median (range), ng/ml	17.5 (11.0-753.0)
≥40.0 mAU/ml, no. (%)	1 (7.1%)
Preoperative diagnosis of CLC, no. (%)	8 (57.1)
Number of tumor, no. (%)	
single	13 (92.8)
multiple	1 (7.1)

CLC, cholangiolocarcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; NASH, non-alcoholic steatohepatitis; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; AFP, alpha-fetoprotein; PIVKA-II, protein levels induced by the absence of vitamin K or antagonist-II.

A. Six patients (42.9%) had elevated carbohydrate antigen 19-9 (CA19-9) levels (>37.0 ng/ml), and two (14.3%) had elevated alpha-fetoprotein (AFP) levels (>10.0 ng/ml). Only one patient (7.1%) had elevated carcinoembryonic antigen (CEA) protein levels (>5.0 ng/ml) and one patient (7.1%) had elevated levels of protein induced by the absence of vitamin K or antagonist-II (PIVKA-II; ≥40.0 mAU/ml), respectively. CLC was diagnosed preoperatively in eight of 14 patients (57.1%) based on characteristic imaging features, including hypervascularity and enhancement during the arterial phase (rim arterial phase hyperenhancement; AP), prolonged enhancement in the delayed phase, and the presence of intratumoral pre-existing vessels, such as the portal and hepatic veins, on dynamic contrast-enhanced CT. A representative of these finding is presented for one patient with CLC (Fig. 2). According to the classification by tumor location from the preoperative CT findings, there were 11 peripheral-type (78.6%) and three hilar-type CLCs (21.4%). Preoperative patient characteristics were presented in Table II.

Laparoscopic hepatectomy was performed in three patients (21.4%), all of whom had peripheral-type CLC based on the preoperative image findings. Anatomical liver resection was performed in nine patients (left extended hepatectomy, n=2; left hepatectomy, n=2; right posterior sectionectomy, n=1; left lateral sectionectomy, n=1; and segmentectomy, n=3), and partial hepatectomy was performed in five patients. Lymph node dissection was performed in four patients (28.6%), which consisted of all three patients with hilar-type CLC and one with peripheral-type CLC. Two patients (14.3%) with hilar-type CLC underwent extrahepatic bile duct resection. The median operative time and blood loss were 367 min and 310 ml, respectively. The intraoperative transfusion rate was 21.4%. Early postoperative complications with Clavien-Dindo classification IIIa occurred in three patients (21.4%; bile leakage, n=2; pleural fluid, n=1). The median length of hospital stay was 21 days. No postoperative deaths occurred within 90 days.

Pathological findings and prognosis. The pathological findings and prognosis are presented in Table III. The gross type of all the resected CLCs based on macroscopic findings was the mass-forming (MF) type. One patient (7.1%) had two lesions in the resected specimen (Fig. 3A). According to the classification of tumor heterogeneity by the CLC component proportion, there were 11 patients with pure (78.6%) and three with partial (21.4%) type CLCs (Fig. 3B). According to the pathological classification of tumor location, there were 11 peripheral-type (78.6%) and three hilar-type (21.4%) CLCs (Fig. 3C).

The median maximum diameter of the primary tumors was 25.5 mm (Fig. 3D). Lymph node metastasis was detected in one patient (7.1%) and vascular invasion was detected in 12 patients (85.7%). Based on the eighth edition of the UICC, two, 11 and one patient had stage IA, II and IIIB CLC, respectively.

R0 resection was achieved in 11 patients (78.6%) and R1 resection in 3 patients (21.4%, Fig. 3E). Two of the three patients (66.6%) who underwent R1 resection had hilar-type CLC. These patients underwent left extended hepatectomy and extrahepatic bile duct resection; one was surgical margin-positive at the bile duct transection and dissected surface (case 6) and the other was surgical margin-positive at the transection surface (case 11). Another case of R1 resection was a peripheral-type, and the patient underwent laparoscopic partial hepatectomy with tumor exposure on part of the transection surface (case 14).

In the median follow-up period of 59.5 (range, 12-114) months, recurrence of CLC occurred in two patients with positive surgical margins (cases 6 and 11). In one patient (case 6), distant lymph node metastases (para-aortic lymph nodes) and peritoneal dissemination were observed 14 months postoperatively. Although chemotherapies with tegafur-gimeracil-oteracil potassium, gemcitabine and paclitaxel were administered sequentially, the disease progressed, and the patient died 31 months postoperatively. In one patient (case 11), a local recurrence was detected on the transection surface of the liver 63 months postoperatively. The patient did not wish treatment for recurrence and died 64 months postoperatively. Recurrence of lymph node metastasis was observed in only one patient with hilar-type CLC. There were no CLC recurrences in patients who underwent R0 resection. The cumulative 5-year

Table III. Pathological findings and prognosis.

Case no.	Tumor no.	CLC type (CLC component)	Tumor location	Size (mm)	Lymph node metastasis	Vascular invasion	T (UICC)	Stage (UICC)	Surgical margin	Recurrence site	Treatment for recurrence	Follow-up period (months)	Death (cause of death)
1	1	Pure	peripheral	35	n.a.	+	2	II	R0	-	-	114	no
2	1	Pure	hilar	52	-	+	2	II	R0	-	-	103	no
3	1	Partial (50%)	peripheral	20	n.a.	+	2	II	R0	-	-	113	no
4	1	Pure	peripheral	25	-	+	2	II	R0	-	-	57	no
5	1	Partial (15%)	peripheral	35	n.a.	+	2	II	R0	-	-	57	yes (other disease)
6	1	Pure	hilar	26	+	+	3	IIIB	R1	lymph node, peritoneum	chemotherapy	31	yes (CLC)
7	1	Pure	peripheral	32	n.a.	+	2	II	R0	-	-	95	no
8	1	Pure	peripheral	23	n.a.	-	1a	IA	R0	-	-	93	no
9	1	Pure	peripheral	19	n.a.	+	2	II	R0	-	-	59	yes (pneumonia)
10	1	Pure	peripheral	15	n.a.	+	2	II	R0	-	-	60	no
11	2	Pure	hilar	50	-	+	2	II	R1	local	BSC	64	yes (CLC)
12	1	Pure	peripheral	17	n.a.	+	2	II	R0	-	-	38	no
13	1	Pure	peripheral	16	n.a.	-	1a	IA	R0	-	-	19	no
14	1	Partial (25%)	peripheral	28	n.a.	+	2	II	R1	-	-	12	no

BSC, best supportive care; CLC, cholangiolocarcinoma; n.a., not available; UICC, 8th edition of the Union for International Cancer Control (UICC) Staging System.

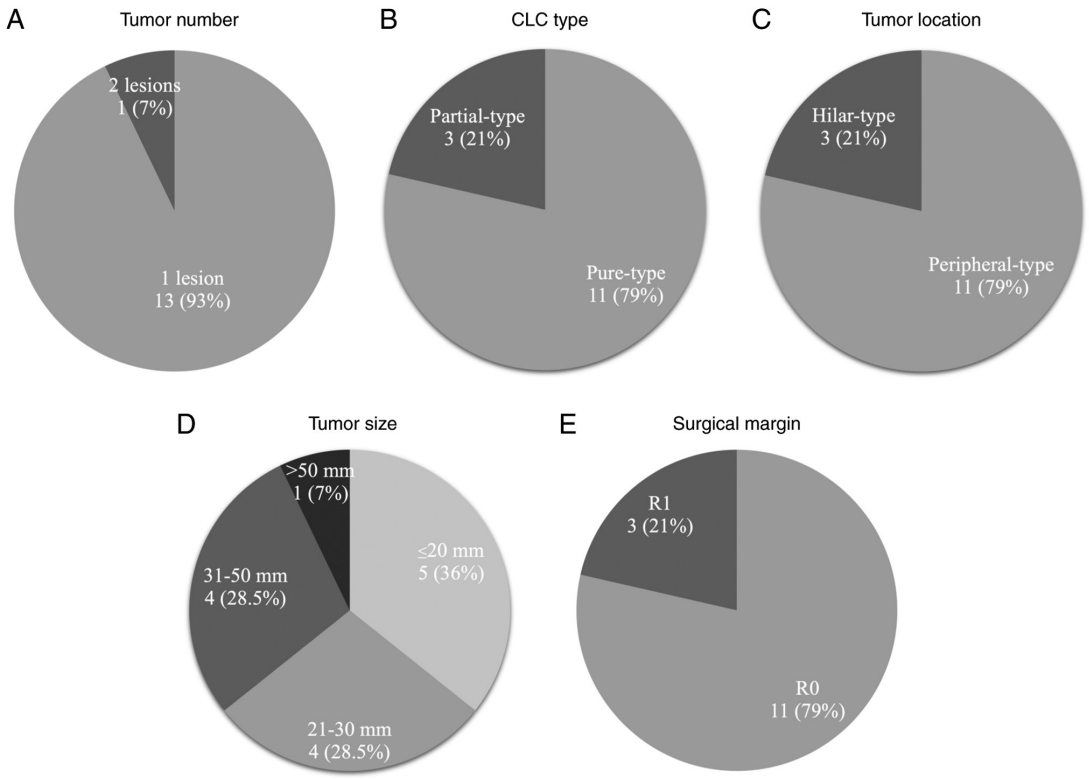

Figure 3. Pathological characteristics of patients with CLC. (A) Tumor number of each patient. (B) CLC types classified according to the proportion of CLC components. (C) Tumor location based on the pathological findings. (D) The maximum diameter of tumor. (E) Pathological surgical margin of the resected specimen. CLC, cholangiolocarcinoma.

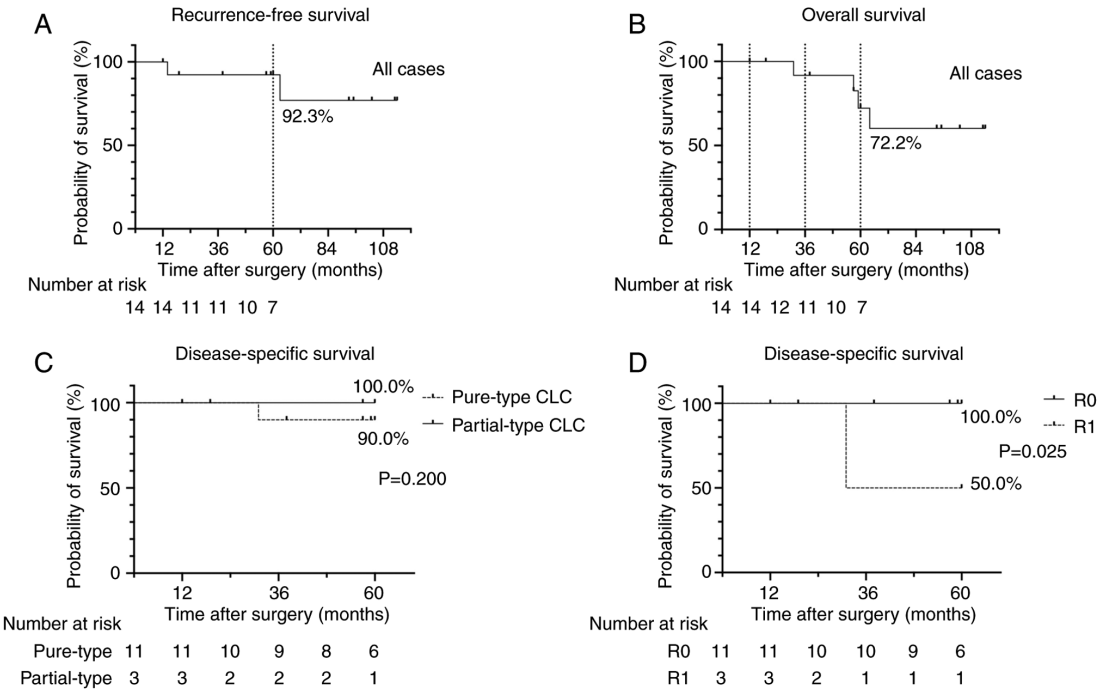

Figure 4. Survival analysis. Kaplan-Meier survival analysis of (A) recurrence-free and (B) overall survival of all cases. (C) Comparison of 5-year disease-specific survival between pure-type and partial-type cholangiolocarcinoma. (D) Comparison of 5-year disease-specific survival between the R0 and R1 resection groups.

RFS rate in the 14 patients after surgery was 92.3% (Fig. 4A). The cumulative 1, 3 and 5-year OS rates in the 14 patients were 100, 91.7 and 72.2%, respectively (Fig. 4B). Comparison of the cumulative 5-year DSS rate between patients with pure-type and partial-type CLC showed no significant differences (Fig. 4C). The cumulative 5-year DSS rate was significantly higher in the

R0 resection group (100.0%) than that in the R1 resection group (Fig. 4D).

Discussion

CLC is an extremely rare primary liver tumor that accounts for ~0.6% of all primary liver tumors (2), and to the best of our knowledge, there are few reports on the clinicopathological features of patients with CLC. In the present study, 14 patients with CLC who underwent resection at a single institution were included. Additionally, all CLC tumors were diagnosed using molecular biology markers with immunostaining in addition to morphological evaluation of H&E staining.

Regarding the characteristics of underlying liver disease, a previous study reported that >50% of patients with CLC had chronic liver injury (26). The frequency of chronic liver injury in patients with CLC has also been reported to be higher than that in patients with iCCA (27). In the present study, nine patients (64.3%) had an underlying chronic liver injury, most of whom had chronic viral hepatitis. These results support the hypothesis that chronic inflammation is associated with the development of CLC. With regards the characteristics of CLC in blood tests, it has been reported that the number of patients with abnormal CA19-9 levels is lower among those with CLC than among those with iCCA (7). In the present study, only six of the patients (42.9%) had elevated CA19-9; however, this was the most frequently elevated of the tumor markers.

The characteristic radiological findings of CLC include hypervascularity in the AP, peritumoral enhancement (either ring-like or wedge-shaped in the AP), small intratumoral portal tracts, rare peripheral bile duct dilatation and prolonged staining in the equilibrium phase (28). However, the imaging findings of CLC are similar to those of iCCA or HCC, owing to CLC tumor heterogeneity (29). Therefore, many patients with CLC tend to be misdiagnosed with iCCA or HCC preoperatively. In a study performed by Ariizumi *et al* (7), 34% of patients with CLC were preoperatively diagnosed with iCCA and 55% with HCC. In the present study, eight patients (57.1%) were suspected to have CLC preoperatively based on these characteristic imaging findings. Although preoperative diagnosis based on imaging findings alone is challenging, it is crucial to consider CLC in the differential diagnosis from a comprehensive perspective.

Although an appropriate therapeutic strategy for CLC has not yet been established, curative resection is reported to be the more effective treatment compared with chemotherapy or hepatic arterial infusion (7). The prognosis of CLC is reported to be better after curative resection than that of iCCA (7). The present study also demonstrated that the prognosis of all CLC patients was favorable, with a 5-year OS rate of 72.2%. However, recurrences of CLC were observed in two patients. Both individuals exhibited hilar-type, positive vascular invasion, and positive surgical margins (R1 resection) pathologically. In contrast, no recurrence was observed in the patients who underwent pathological R0 resection. Moreover, the 5-year DSS rate was 100.0% in these patients. These results indicate that R0 resection is an important therapeutic strategy for CLC, due to the complete removal of cancerous tissue. In the present study, all patients with hilar-type CLC underwent major hepatectomy with lymph node dissection. In contrast, most patients with peripheral-type CLC do not undergo lymph node dissection in the present study. Only one of the patients with CLC developed lymph node metastatic recurrence, and this was the only patient positive for lymph node metastasis at the time of surgery. These results suggest that the rate of lymph node positivity in CLC is low. Moreover, even in cases without lymph node dissection, lymph node metastasis can be considered negative at the time of surgery since no lymph node recurrence was found during the observation period.

Pathological examination indicates that CLC is often composed of a CLC component and an iCCA component in variable proportions (30), which complicates CLC diagnosis. Komuta *et al*. (16) defined CLC as a condition when the CLC component accounted for >90% of the whole tumor, but the definition of 'a significant proportion of CLC components' has not been determined previously. In the present study, pure-type CLC (CLC component >95%) and partial-type CLC (CLC component ≤95%) were defined as indicated. As a result, in partial-type CLC patients, each tumor containing 15, 25 or 50% CLC components, was categorized into groups. No recurrence was observed in any patient. Furthermore, there was no statistically significant difference in the DSS between patients with pure- and partial-type CLC. Few previous reports have compared prognoses focusing on CLC heterogeneity. The present study showed that partial-type CLC, comprising a small portion of the CLC component (15-50%), was similar to pure-type CLC in prognosis. This was because even in partial-type CLC a better prognosis was observed when R0 resection was achieved.

The present study has certain limitations. First, the number of patients with CLC included in the present study was small because of the rarity of CLC and the single institution nature of the study. Multivariate analyses could not be performed for prognostic comparisons because of the small sample size. Secondly, as this was a retrospective observational study, lymph node dissections were not performed in all patients; therefore, the assessment of lymph node metastasis could not be performed reliably. In addition, selection bias or information bias may exist due to this study design. Third, due to the long study time period, it is possible that medical developments during that period may have affected perioperative outcomes and prognosis.

In conclusion, the clinicopathological features and prognoses of 14 patients with CLC who underwent resection and were diagnosed based on molecular biological and immunohistochemical findings, are reported. Patients with CLC were categorized into pure and partial type CLC based on tumor heterogeneity, and the results suggest that regardless of the proportion of CLC components, patients with resected CLC may have a favorable prognosis. Curative resection is crucial to achieve a better prognosis in patients with CLC. Further case accumulation and assessment in a larger study are necessary to validate the findings of the present study.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

HS, SN and SY contributed to study design, drafting the manuscript, acquisition and analysis of all clinical data and confirm the authenticity of all the raw data. RG, TT, RT, MO, KKa, ST and IM contributed to collecting data, conception and design of the study, and drafting and revising the manuscript. KKo contributed to evaluation of imaging data. KH contributed to evaluation of pathological findings. All authors read and approved the final manuscript.

Ethics approval and consent to participation

This study was conducted in accordance with the ethical standards stated in the Declaration of Helsinki and with approval from the Kanazawa University Ethics Committee (approval no. 3221-2). Informed consent for this study was obtained from all participants from Kanazawa University Hospital's website as an opt-out option.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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