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Immunohistochemical Examination of a Resected Advanced Hilar Cholangiocarcinoma Arising in a 29-Year-Old Male without Primary Sclerosing Cholangitis

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Key Words
Cholangiocarcinoma · Youth · Immunohistochemistry · S-1 · Gemcitabine

Abstract
A 29-year-old man with advanced hilar cholangiocarcinoma was successfully treated with an extended right lobectomy. The carbohydrate antigen 19-9 (CA19-9) level was elevated to 939 IU/l, and the pathological findings revealed moderately differentiated tubular adenocarcinoma which involved almost the entire thickness of the hepatic duct and the adjacent liver tissue (T3) and which was associated with lymph node metastasis (N1). It was a stage IIB (T3N1M0) tubular adenocarcinoma according to UICC pathological staging. Immunohistochemical examination revealed that Ki-67, cyclin D1, and MMP-7 were positive, and 14-3-3σ and p27 were negative. The pathological and immunohistochemical findings indicated high malignant potential indicating poor prognosis. We administrated the postoperative adjunct gemcitabine combined with S-1 chemotherapy. The patient is alive without recurrence and doing well two years after surgery. We also review other reports of cholangiocarcinoma patients aged less than 30 years.

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Introduction

Hilar cholangiocarcinoma (HC) is a rare tumor characterized by slow growth; the average patient age at clinical diagnosis of HC is 60–79 years, and HC in patients aged less than 30 years has not been reported in Japan [1]. Despite the reputed slow tumor growth rate and uncommon hematogenous metastasis, the propensity for extensive local invasion of the hepatic hilum and the limited resolution of hepatic hilar imaging initially accounted for low overall resectability [2]. Complete resection remains the only therapy that offers the possibility of long-term survival, and hepatic resection is a critical component of the surgical approach.

Several reports have described the prognostic factors for cholangiocarcinoma [2–9], which include operative resectability [3–6], portal vein invasion [7], lymph node metastasis [8], and age [9]. In this paper, we report a case of a resected advanced HC arising in a 29-year-old male without primary sclerosing cholangitis (PSC) with an immunohistochemical examination of prognostic factors and review other reports of cholangiocarcinoma patients aged less than 30 years.

Case Report

A 29-year-old man presented with cholecystolithiasis was admitted to our hospital; his condition was detected by ultrasonography. Physical examination revealed no palpable mass in the abdomen. His laboratory findings upon admission are shown in **table 1**. Although the serum carcinoembryonic antigen (CEA) level was within the normal range, the carbohydrate antigen 19-9 (CA19-9) level was elevated to 939 IU/l.

Abdominal ultrasonography and abdominal computed tomography revealed a hypovascular mass measuring 2.5 cm at the hilum and dilation of the intrahepatic bile duct of the right lobe (**fig. 1**a). An endoscopic retrograde cholangiopancreatography (ERCP) showed complete obstruction of the right hepatic duct and narrowing of the common hepatic duct (fig. 1b). A fluorodeoxyglucose (FDG) positron emission tomographic scan revealed a high FDG uptake with a maximal standardized uptake value of 5.8 (fig. 1c). A HC was suspected based on these findings. We recommended surgery and performed an extended right lobectomy with bile duct resection. The tumor had invaded to the right branch of the portal vein and the right lobe was atrophic at operation (fig. 1d). The operation time was 7 h and 25 min. Blood loss was 425 ml and no blood transfusion was administered. The resected specimen was a tumor 2.5 cm in diameter and involving the right hepatic duct and right portal vein (fig. 1e).

The pathological findings revealed a moderately differentiated tubular adenocarcinoma, which involved almost the entire thickness of the hepatic duct and the adjacent liver tissue (T3) and which was associated with lymph node (N1) metastasis. It was a stage IIB (T3N1M0) tubular adenocarcinoma according to UICC pathological staging.

The sections were examined immunohistochemically with primary antibodies using the streptavidin peroxidase complex method. The antibodies and the immunohistochemical results are summarized in **table 2** and figure 2. Ki-67 [10] and cyclin D1 [11] were stained cancer cell nuclei, and the labeling indices were 24 and 30.4%, respectively (**fig. 2**b, c). MMP-7 [12] densely stained the cytoplasm of almost all cancer cells (fig. 2d), whereas 14-3-3σ [13] and p27 [14] staining were negative.

The pathological and immunohistochemical findings revealed that this tumor exhibited a high malignant potential, indicating poor prognosis. We administrated the postoperative adjunct gemcitabine (GEM) combined with S-1 chemotherapy on postoperative day 37. We orally administrated 40 mg S-1 twice daily for seven days followed by a 1-week blank. We administrated 1,000 mg GEM in a 1-hour intravenous infusion on day 1, and this cycle was repeated every 14 days. The patient is alive without recurrence and doing well two years after surgery.

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Discussion

Because of the lack of early symptoms, most patients are diagnosed with HC at an advanced stage, by which it is found that the cancer has often metastasized or invaded the adjacent liver, portal vein, or hepatic artery. Despite a reputed slow tumor growth rate and uncommon hematogenous metastasis, the propensity for extensive local invasion of the hepatic hilum and the limited resolution of hepatic hilar imaging initially accounted for the low overall resectability. As a consequence of limited resectability of HC, the prognosis is poor, and surgical management is primarily palliative. Rea et al. reported on patients with HC undergoing hepatic and biliary tract resection and regional lymphadenectomy with Roux-en-Y hepaticojejunostomy. The actual 5-year survival rate of patients was 26%, and the few clinicopathological factors were associated with survival [2].

A right hepatectomy most likely enables the en bloc resection of the hepatic ductal confluence and its surrounding structures because the confluence lies on the right side of the hepatic hilum. Kondo et al. analyzed tumor recurrence after R0 resections and indicated a low frequency of local recurrence and a high frequency of peritoneal seeding, contrary to previous reports describing locoregional recurrence as the most common mode [5]. Hasegawa et al. also described that surgery is the only potentially curative treatment for hilar bile duct cancer [6]. Aggressive surgical approaches including radical hepatectomy based on the precise knowledge of the surgical anatomy of the hepatic hilum and meticulous surgical technique can achieve a 77% R0 rate and a relatively low rate of local recurrence. They identified lymph node metastases as another independent prognostic factor. Kondo et al. reported a high R0 rate (95%) in 40 major hepatectomy cases with a low local recurrence rate [5]. In contrast, Hasegawa et al. reported that more than half (60%) of the patients with R0 operations developed distant metastases [6]. These results indicated the limitation of the surgeries, which are able to achieve only local control of the tumor.

Portal vein invasion was previously a main cause for HC unresectability. Ebata et al. performed combined hepatobiliary and portal vein resection in patients with advanced HC and concluded that adjuvant therapy is important because of the modest improvement in survival following portal vein resection [7]. Furthermore, it was also reported that R0 resection remains the best treatment for ensuring long-term survival, and lymph node status is the most important prognostic factor following R0 resection [8].

Several authors have reported cholangiocarcinomas in patients aged less than 39 years (**table 3**) [9, 15–17]. Yeh et al. indicated that age is a risk factor for cholangiocarcinoma and that only one patient in the younger cholangiocarcinoma group survived for 24.2 months, whereas the remaining patients died within 12 months [9]. Bjornsson and Angulo reported eight cases of cholangiocarcinoma with PSC and five without PSC [17]. Although three of eight cholangiocarcinoma patients with PSC were alive for 7 months to 7 years, all cholangiocarcinoma patients without PSC died within 18 months. Cholangiocarcinoma in younger patients had reached a highly advanced stage and these patients showed a lower incidence of operation than older cholangiocarcinoma patients.

Clinicopathologically, our case may have had a poor prognosis because of age, portal vein invasion, and lymph node metastasis. In this report, we immunohistochemically examined the cell-regulating factors (cyclin D1, Ki-67, 14-3-3σ, and p27), growth factors (c-erbB2 and EGFR), and MMPs (MMP-2 and MMP-7). We found that this patient not only showed Ki-67 [10], cyclin D1 [11] and MMP-7 [12] overexpression, but also

decreased 14-3-3σ [13] and p27 [14] expression. These findings demonstrated that the cancer exhibited a high proliferative and invasive activity.

From the pathological findings, we concluded an R0 operation in our case; however, portal vein invasion and lymph node metastasis were also present. Additional treatment modalities such as systemic adjuvant chemotherapy must be developed to control systemic cancer cell micrometastasis for improving the survival of patients with HC. Results of a recent phase II study revealed that GEM [18, 19] and S-1 [20] therapies are effective for cholangiocarcinoma. Furthermore, a combination therapy with GEM and S-1 was well tolerated and yielded a significantly high response rate in metastatic [21] and resected [22] pancreatic cancers. Our patient received adjuvant GEM combined with S-1 therapy, showing no side effects and good drug tolerance. There was no evidence of tumor recurrence two years after surgery. He is doing well and is employed.

In conclusion, we reported a 29-year-old male patient who underwent a major hepatectomy for advanced HC. He is alive without any recurrence and doing well two years after surgery. We conclude that an R0 operation and adjuvant chemotherapy could be an effective treatment strategy for advanced HC.

Table 1. Laboratory findings in the present case

Peripheral blood counts		Biochemistry	
RBC, $\times 10^4$	458	TP, g/dl	7.1
Hb, g/dl	14.4	Alb, g/dl	3.8
Ht, %	41.6	T-Bil, g/dl	0.6
Plt, $\times 10^4$	19.4	AST, IU/l	33
WBC	4,740	ALT, IU/l	54
Coagulation		ALP, IU/l	166
PT, %	104	g-GTP, IU/l	209
APTT, s	35.2	FBS, mg/dl	90
Immunology		BUN, mg/dl	6.5
CRP, mg/dl	0.3	Cr, mg/dl	0.8
Tumor markers		Na, mEq/l	139
CEA, ng/ml	2.1	K, mEq/l	4.1
CA19-9, U/ml	939	Cl, mEq/l	101

Table 2. Immunohistochemical examination of the resected specimen

Antigen	Positivity	Prognosis	Reference
Ki-67	30.4%	poor	10
p53	0%		
14-3-3σ	0%	poor	13
Cyclin D1	24%	poor	11
c-erbB2	negative		
EGFR	negative		
p27	<10%	poor	14
MMP-2	negative		
MMP-7	>90%	poor	12

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Table 3. Cases with cholangiocarcinoma under 30 years old

Authors	Country	n	Age	PSC	Reference
Kitagawa et al.	Japan	1	24	?	15
Yeh et al.	Taiwan	1	28	?	9
Klein et al.	USA	1	25	–	16
Bjornsson and Angulo	USA	5	21–25	–	17
Present report	Japan	1	29	–	–

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Fig. 1. Imaging and operative findings. **a** A computed tomography scan demonstrated hypovascular masses (arrows) at the hilum and slight dilation of the intrahepatic bile duct of the right lobe. **b** An ERCP showed complete obstruction of the right hepatic duct and narrowing of the common hepatic duct (arrow). **c** A FDG positron emission tomographic scan; the mass showed high FDG uptake with a maximal standardized uptake value of 5.8. **d** Operative findings. The tumor invasion into the right branch of the portal vein (arrows). **e** A resected specimen. The tumor was 2.5 cm in diameter and involved the right hepatic duct (HD) and right portal vein (PV).

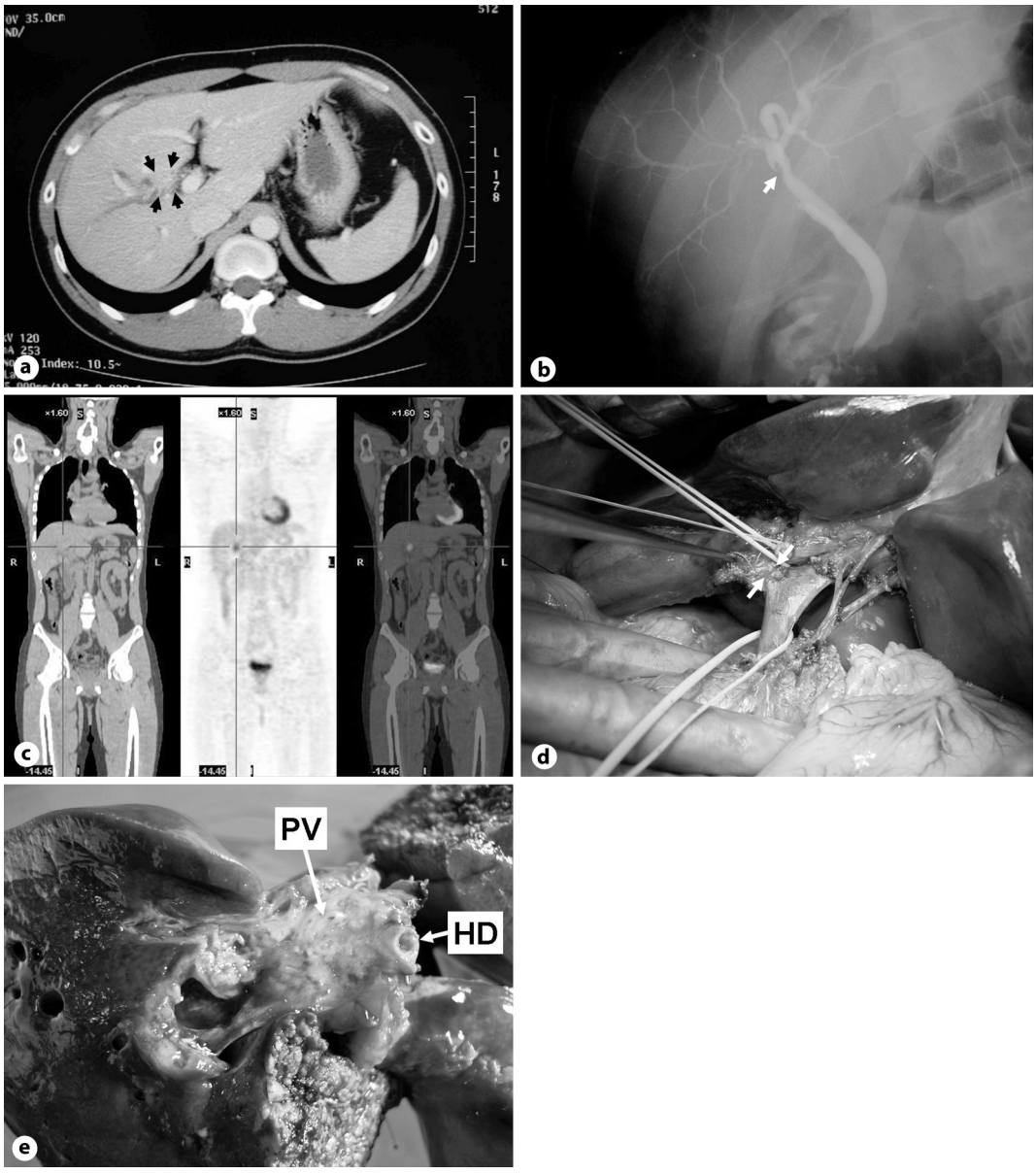

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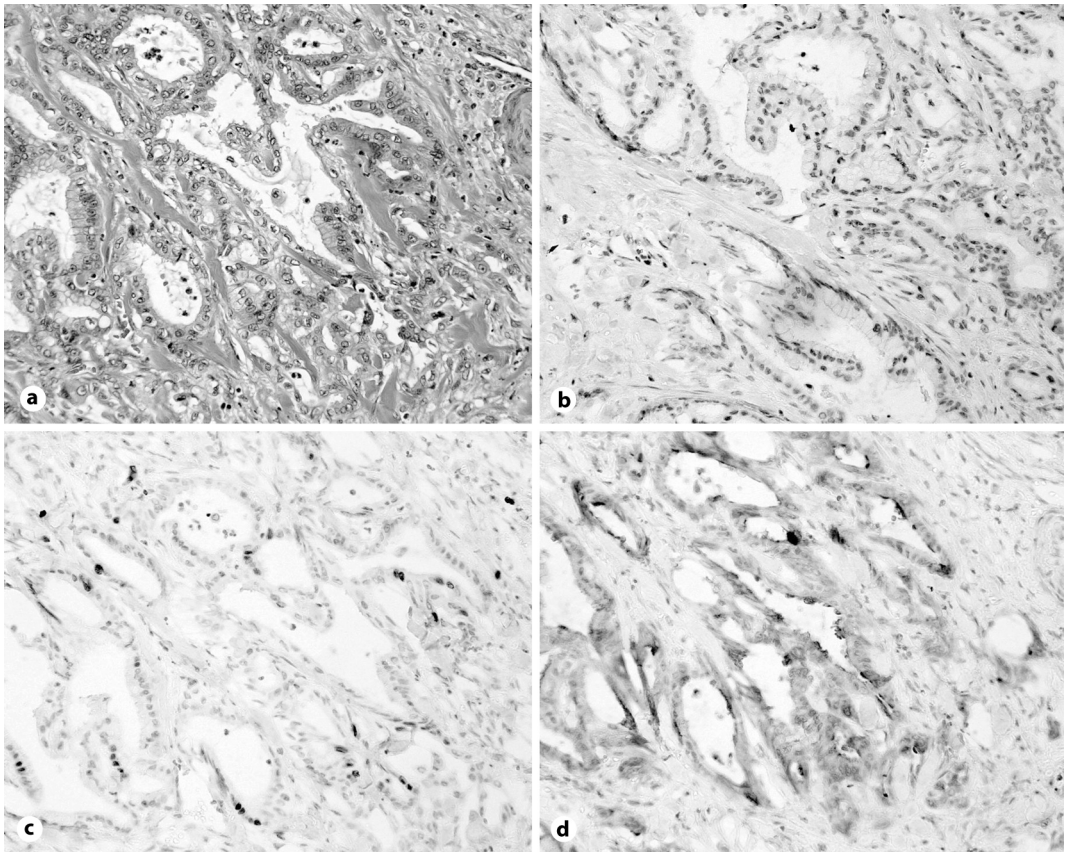
Fig. 2. Pathological and immunohistochemical findings of the resected specimen. **a** Hematoxylin and eosin staining revealing a moderately differentiated tubular adenocarcinoma. **b** Ki-67 staining of cancer cell nuclei; the labeling index was 24%. **c** Cyclin D1 staining of cancer cell nuclei; the labeling index was 30.4%. **d** Dense MMP-7 staining of the cytoplasm of almost all cancer cells.



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