Internal Medicine
The Japanese Society of Internal Medicine
doi: 10.2169/internalmedicine.9021-17
Intern Med 56: 3033-3040, 2017
http://internmed.jp

【 CASE REPORT 】

Cholangiolocellular Carcinoma in a Young Patient Who Showed Sustained Virological Response after Treatment for Hepatitis C Virus Infection

Mitsutaka Osawa[1], Satoshi Saitoh[1], Shunichiro Fujiyama[1], Yusuke Kawamura[1], Hitomi Sezaki[1], Tetsuya Hosaka[1], Norio Akuta[1], Masahiro Kobayashi[1], Yoshiyuki Suzuki[1], Fumitaka Suzuki[1], Kenji Ikeda[1], Keiichi Kinowaki[2], Takeshi Fujii[2], Toshio Fukusato[3], Fukuo Kondo[3] and Hiromitsu Kumada[1]

Abstract:
A 35-year-old male patient who showed sustained virological response (SVR) following treatment for hepatitis C virus infection developed liver cancer. The lesion was identified by imaging studies, with atypical findings suggestive of hepatocellular carcinoma. Partial hepatectomy was performed and the histopathological diagnosis was cholangiolocellular carcinoma (CLC). Only a few cases of CLC have been described in young patients who achieved SVR. Hepatologists should recognize the potential development of CLC even in young patients who achieve SVR, and the need for a close follow-up by imaging studies. In addition, true characteristics and cell origin of CLC were discussed in this report.

Key words: cholangiolocellular carcinoma, hepatitis C virus, young patient, sustained virological response

(Intern Med 56: 3033-3040, 2017)
(DOI: 10.2169/internalmedicine.9021-17)

Introduction

Sustained virological response (SVR) reduces the chance of liver cancer in patients with hepatitis C virus (HCV) infection. However, a proportion of patients who achieve SVR still develop hepatocellular carcinoma (HCC), including young patients. On the other hand, cholangiolocellular carcinoma (CLC) is comparatively uncommon liver malignancy. We report a rare case of a young patient who had previously achieved SVR but was later diagnosed with CLC.

Case Report

The male patient developed acute lymphocytic leukemia (ALL) in 1986, and was found to have HCV infection at the time of autologous bone marrow transplantation at another hospital in 1994. He had been followed-up for chronic hepatitis C (genotype 1b, IL28B polymorphisms TG) at our hospital since February 2002. Previous interferon monotherapy, followed by interferon and ribavirin combination therapy, failed to eradicate HCV. On October 2014, he underwent 24 weeks of daclatasvir plus asunaprevir combination therapy after being confirmed to harbor the NS5A-Y93 wild type virus, and achieved SVR. He was followed up regularly at the outpatient clinic and underwent imaging study regularly.

He was admitted to our hospital in June 2016, aged 35, for management of a liver tumor that had been found on a follow-up magnetic resonance imaging (MRI). Physical examination on admission showed no specific findings. The medical history was negative for alcohol abuse and smoking. He received blood transfusion at the age of five. The family history was negative apart from a paternal grandfather with hepatoma. Hepatitis B virus surface antigen (HBsAg) and HCV-RNA were negative, and other laboratory test including tumor marker were almost normal (Table).

[1]Department of Hepatology, Toranomon Hospital, Japan, [2]Department of Pathology, Toranomon Hospital, Japan and [3]Department of Pathology, Teikyo University Hospital, Japan
Received: February 15, 2017; Accepted: March 27, 2017; Advance Publication by J-STAGE: September 25, 2017
Correspondence to Dr. Satoshi Saitoh, sa3110@f2.dion.ne.jp

Table. Laboratory Data on Admission.

Peripheral blood		Fasting blood glucose	99 mg/dL
Leukocyte count	4,200/μL	Triglyceride	173 mg/dL
Erythrocyte count	457×10⁴/μL	Total cholesterol	200 mg/dL
Hemoglobin	14.1 g/dL	HDL cholesterol	42 mg/dL
Hematocrit	42.0%	LDL cholesterol	128 mg/dL
Platelet count	19.3×10⁴/μL	Na	142 mmol/L
Blood chemical examination		K	4.1 mmol/L
Total Protein	7.5 g/dL	Cl	106 mmol/L
Albumin	4.8 g/dL	Ca	9.7 mg/dL
Creatine kinase	104 IU/L	P	3.3 mg/dL
Total bilirubin	0.7 mg/dL	Fe	192 μg/dL
Direct bilirubin	0.0 mg/dL	UIBC	164 μg/dL
Aspartate aminotransferase	24 IU/L	Ferritin	120 μg/L
Alanine aminotransferase	26 IU/L	Tumor marker	
Lactate dehydrogenase	222 U/L	CEA	1.1 μg/L
Alkaline phosphatase	274 U/L	AFP	4 μg/L
γ-Glutamyltranspeptidase	52 U/L	PIVKA-II	34 AU/L
Cholinesterase	402 U/L	Coagulation tests	
Amylase	62 IU/L	Prothrombin time	109.8%
Blood urea nitrogen	11 mg/dL	PT INR	0.96
Creatinine	0.85 mg/dL	Hepaplastin time	72.3%
Uric acid	6.9 mg/dL	Virus marker	
Ammonia	35 μg/dL	HCV RNA	-
C-reactive protein	0.0 mg/dL	HBsAg	-
Hyaluronic acid	27 μg/L	ICG15	17%

CEA: serum carcinoembryonic antigen, AFP: alpha-fetoprotein, PIVKA-II: Protein induced by vitamin K absence or antagonist II

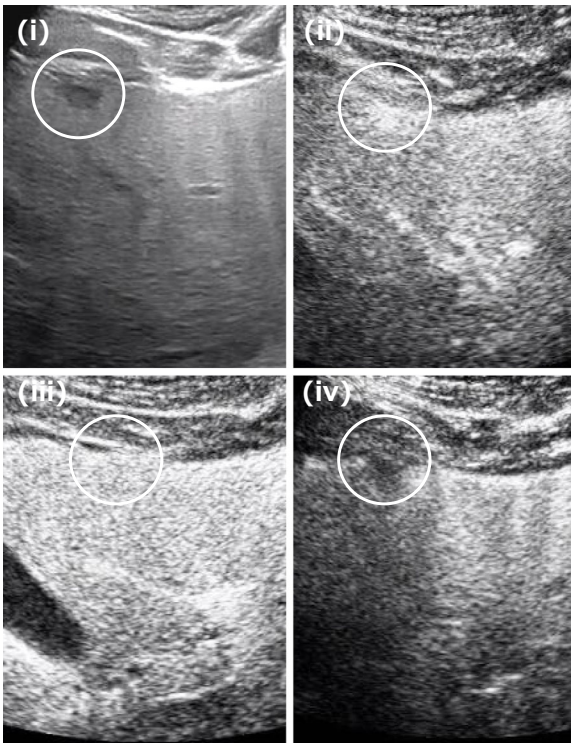

Figure 1. B-mode ultrasonography (US) showed an 8-mm hypoechoic space-occupying lesion in S4 (i). On contrast-enhanced US with sonazoid, the space-occupying lesion showed hyper-enhancement in the early vascular phase (ii), prolonged enhancement in the late vascular phase (iii), and defect in the Kupffer phase (iv).

B-mode ultrasonography (US) showed hepato-renal echo contrast and an 8-mm hypoechoic space-occupying lesion (SOL) in segment 4 (S4). Contrast-enhanced US with Sonazoid also showed hypervascular enhancement in the vascular phase and defect in the post vascular phase (Fig. 1). Plain computerized tomography (CT) showed ill-defined low density area in S4, and dynamic CT showed hyper-enhancement in the early phase and hyper-iso-enhancement in the late phase (Fig. 2). On MRI, the nodule in S4 showed low intensity on T1-weighted images, high intensity on T2-weighted images, and high intensity in diffusion-weighted images. Gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA)-enhanced MRI showed hyper-enhancement in the early phase, hypo-enhancement in the late phase, and defect in the hepatobiliary phase (Fig. 3). Based on these clinical and imaging findings, the tumor was diagnosed as atypical HCC. Laparoscopic partial hepatectomy was performed at our hospital.

Macroscopically, the tumor was a single whitish nodular lesion, measuring 6×6 mm, with regular margin and capsule (Fig. 4i). Microscopic examination showed tumor cells, with the formation of small and irregularly tortuous glands in the tumor tissues (Fig. 4ii). A small area of this tumor showed large cancer duct (Fig. 4iii). However, this large cancer ducts were less than 10% of the whole tumor. Background liver showed mild chronic hepatitis (F1/A1, by the New Inuyama Classification). In addition, approximately 50% fatty change, mild lobular inflammation, and histological

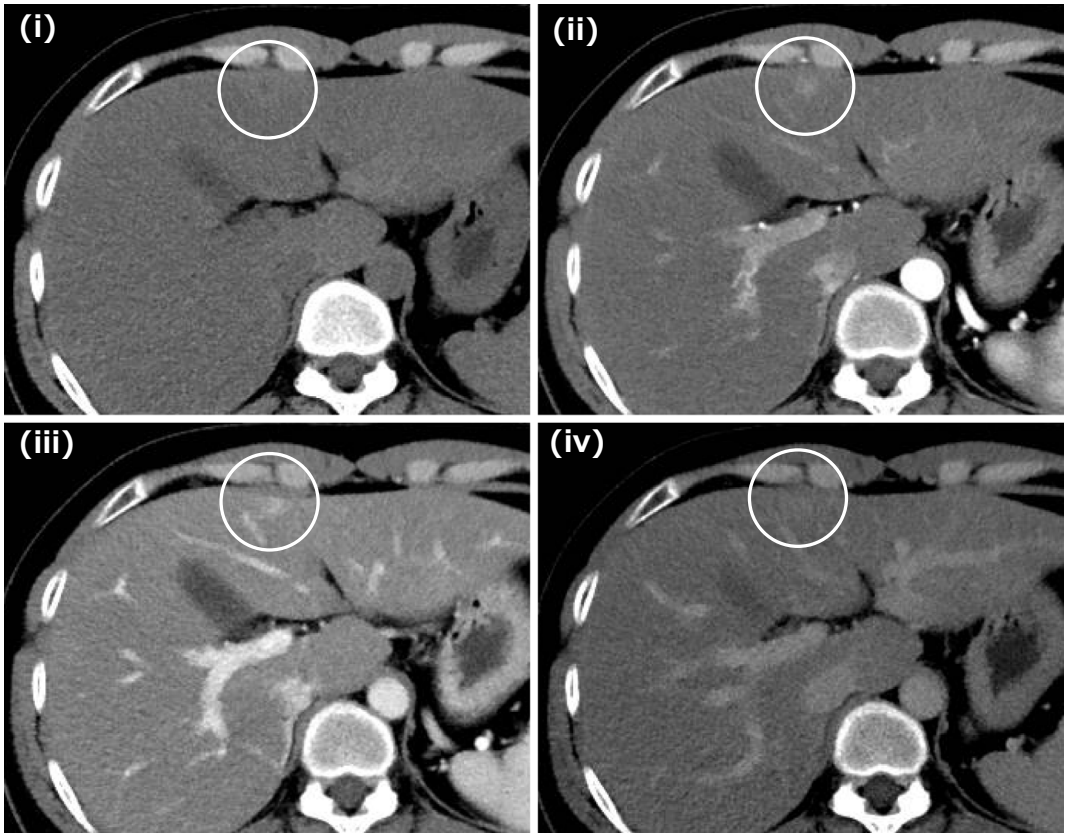

Figure 2. **Plain CT showed unclear boundary, low-density area in S4 (i). Dynamic CT showed hyper-enhancement in the arterial phase (ii), hyper-enhancement in the portal phase (iii), and iso-enhancement in the delayed phase (iv).**

findings resembling hepatocellular ballooning were observed (corresponding to Steatosis 2, Hepatocyte ballooning 0, lobular inflammation 1, fibrosis stage 2, in Brunt's classification of steatohepatitis) (Fig. 4iv).

Immunohistochemically, all of the tumor cells were positive for cytokeratin (CK) 7, CK19, and epithelial membrane antigens (EMA) with an intraluminal staining pattern in the gland, and negative for HepPar1, alpha-fetoprotein (AFP) and Arginase1 (Fig. 5). Based on these histopathological and immunohistochemical findings, this tumor was diagnosed as CLC.

Because CLC has been speculated to be originated from cholangiolocells which have characteristics of hepatic stem/progenitor cells, additional morphometric and immunohistochemical studies were performed. Cancer ducts were classified into 3 patterns (i) very small ducts, (ii) small ducts and (iii) large ducts. Non-neoplastic biliary ducts were classified as (iv) cholangioles, (v) interlobular ducts of small size (ILD-S) and (vi) interlobular ducts of medium size (ILD-M) (Fig. 6) (1). Morphometrically, even a small cancer duct was apparently larger than a non-neoplastic very small cholangiole (21 μm vs. 11.8 μm, Fig. 6i and iv). Many of the very small and small cancer ducts (Fig. 6i and ii) showed the similar sizes to those of ILD-S and ILD-M (Fig. 6i, ii, v and vi). These duct sizes were within the range of ILD-S (15-40 μm) and ILD-M (40-100 μm) (1-3). A large cancer duct showed diameters almost 20 times of a cholangiole (228 μm vs. 11.8 μm, Fig. 6iii and iv).

Following the description of WHO Classification (4), immunohistochemical markers i.e. c-Kit, CD56 and EpCAM were used to examine the stem cell characteristics of this tumor (Fig. 7). The cancer ducts showed positivity of these markers. However, non-neoplastic ILD-S and ILD-M also showed positivity of these markers.

Discussion

Previous studies indicated that HCC after SVR among HCV patients aged 40 or younger is extremely rare and few reports of such cases have been published previously (5, 6). Furthermore, CLC is also an uncommon form of liver cancer with a reported incidence among resected primary liver cancer of 0.56% (7). The clinical and histogenetic features of CLC had not been fully characterized, probably due to the low prevalence, although they have been recently clarified following advancements in imaging technology.

CLC was first described by Steiner and Higginson in 1959. It was thought to originate from canals of Hering, which locate between interlobular duct and bile canaliculus (8). Interlobular duct was also speculated to be a cell origin of CLC in this paper. However, only cholangiole origin theory has been emphasized due to several studies of stem cell features of cholangiole and CLC (7, 9, 10). Finally, CLC was categorized as one of the subtypes with

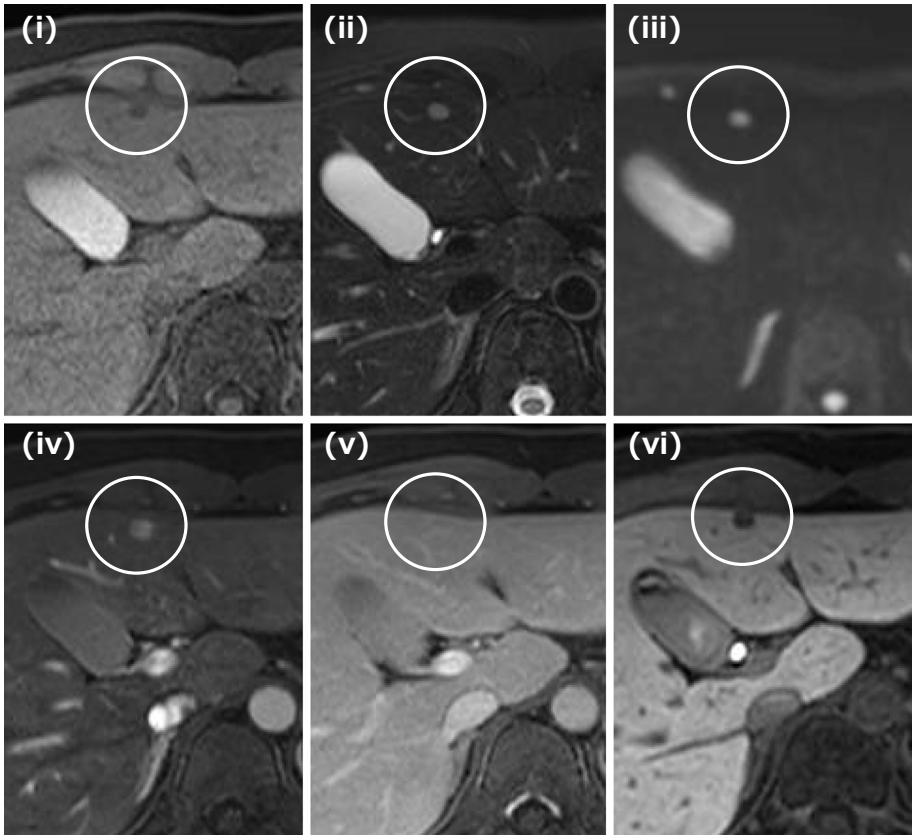

Figure 3. **MRI showed low, high, and high intensity nodule in T1 (i), T2 (ii), and diffusion (iii) weighted images. Gd-EOB-DTPA-enhanced MRI showed hyper-enhancement in the arterial predominant phase (iv), iso-hypo-enhancement in the portal predominant phase (v), and defect in the hepatobiliary phase (vi).**

stem cell features of combined heparocellular-cholangiocarcinoma (hepato-biliary tumor) in the 2010 WHO classification of tumors (4). However, CLC is classified as a cancer of non-combined type primary liver cancer (pure biliary tumor) in the General Rules for the Clinical and Pathological Study of Primary Liver Cancer, 6th edition (11). The WHO classification and the Japanese general rule show serious difference in the interpretation of CLC, namely hepato-biliary tumor or pure biliary tumor. In addition, Maeno and Kondo recently described the papers which suggest interlobular duct origin theory (non-stem cell origin theory) (2, 3).

As can be seen from the above, the exact definition of CLC has not been established yet. To avoid confusion in clinical practice, there is no doubt a need for a broadly acceptable classification of liver malignancy.

Histopathological examination of the present case, which consisted mostly of thin cancer ducts, showed various findings of CLC, i.e. so called antler-like anastomozing patterns, and replacement growth pattern at the boundary division between the cancerous and non-cancerous tissues. Immunohistochemistry also showed that the cancer cells were positive for markers of cholangiocytes (CK7 and CK19), negative for markers of hepatocytes (Hep-Parl and Arginase1), and positive for membranous pattern of EMA, which has been considered a specific feature of CLC (2, 12). These findings are well compatible with the diagnosis of CLC in the Japanese general rule. However, they were not necessarily applicable for the diagnosis of "combined hepatocellular cholangiocarcinoma", with stem-cell features, cholangiolocellular type of the 2010 WHO classification of tumors. This case was classified as a pure biliary tumor and was not classified as "combined hepatocellular cholangiocarcinoma", because no hepatocellular components were found. Recently, Kondo et al. classified CLC into two subtypes, i.e. (i) pure biliary type and (ii) hepato-biliary type (combined carcinoma type) (13). The present case is classified into pure biliary type.

Furthermore, stem cell features were not sufficiently proved in this case. The cancer ducts were apparently larger than non-neoplastic cholangioles. Their sizes corresponded to those of interlobular ducts (non-stem cell ducts). Immunohistochemical studies using stem cell markers also failed to prove the stem cell features of this tumor. Although c-Kit, CD56 and EpCAM were positively stained in this tumor to some degree, they were also positive in the non-neoplastic and non-stem cell bile ducts (interlobular ducts). These markers may not be sufficiently specific markers for the proof of stem cell features (3).

According to Kondo et al., many of CLC cases may not

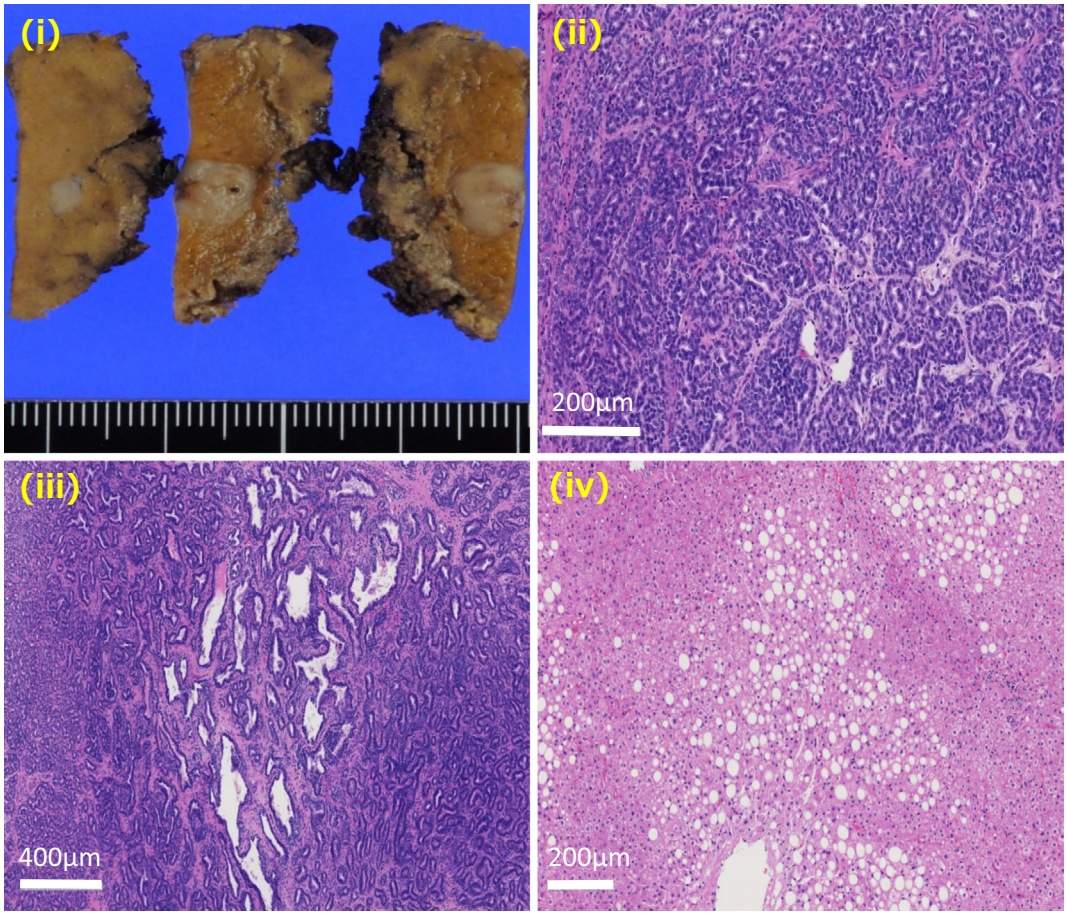

Figure 4. **Macroscopically, the resected specimen was a 6 mm diameter tumor, single nodular type, whitish in color with regular margins and covered with a capsule (i). Microscopic examination showed small monotonous and irregularly tortuous glands in the cancerous component (ii and iii), and severe fatty changes in the non-cancerous component (iv).**

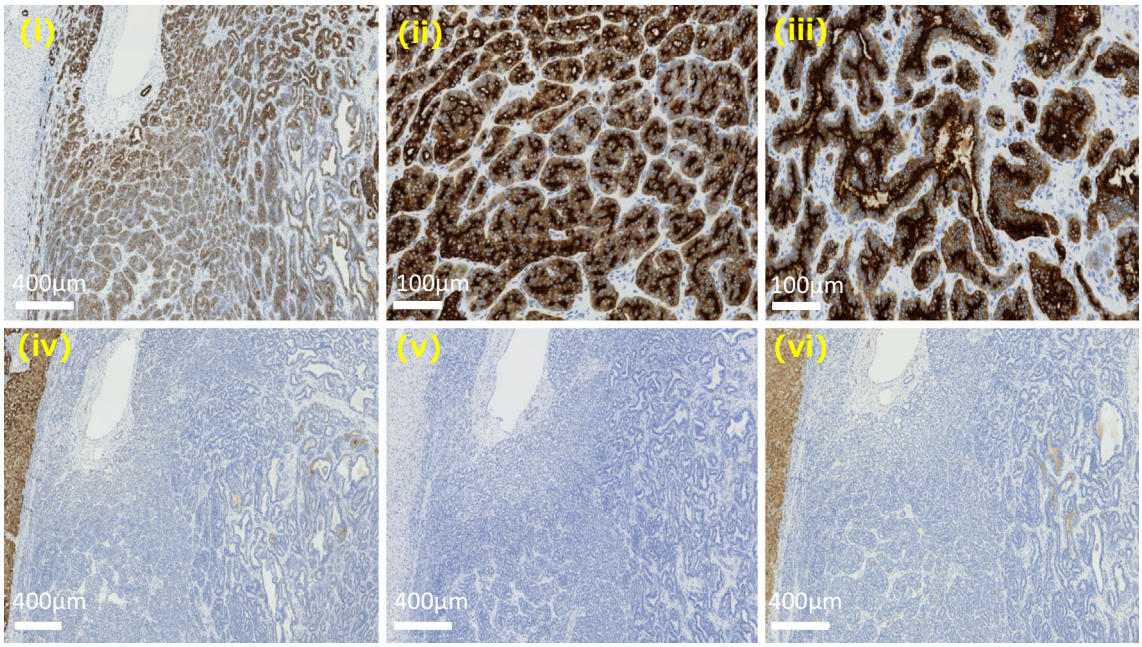

Figure 5. **Immunohistochemical staining showed positivity of cancer cells for CK19 (i) and luminal membranous pattern for EMA (ii and iii), and negativity for HepPar1 (iv), AFP (v) and Arginase1 (vi).**

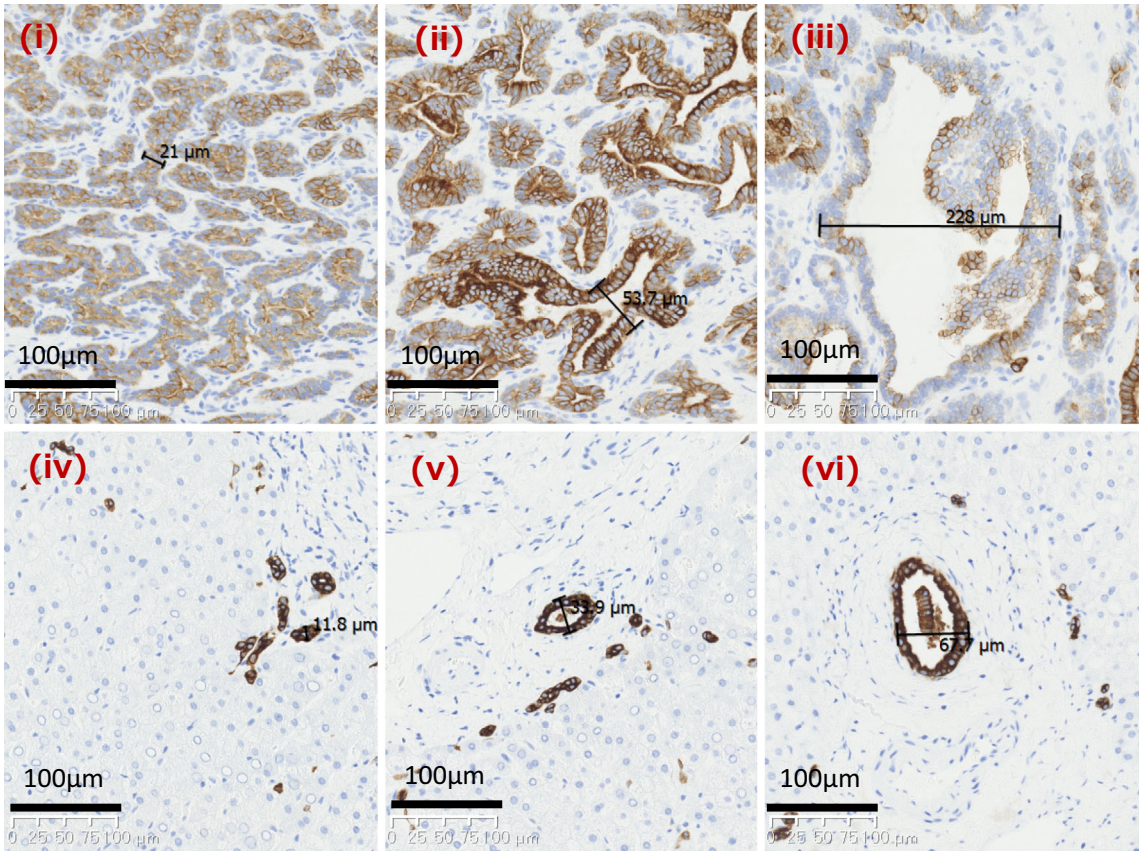

Figure 6. **Comparison of sizes of cancer ducts and various non-neoplastic biliary ducts. (i), (ii), (iii): Cancer ducts. (i) very small duct, (ii) small duct, (iii) large duct. (iv), (v), (vi): Non-neoplastic biliary ducts. (iv) cholangiole, (v) interlobular duct of small size (ILD-S) and (vi) interlobular duct of medium size (ILD-M). A very small cancer duct [(i), 21µm] and a small cancer ducts [(ii), 53.7µm] were clearly larger than a non-neoplastic cholangiole [(iv), 11.8µm]. The sizes of these very small and small cancer ducts were similar to those of non-neoplastic interlobular ducts [(v), 33.9µm, (vi), 67.7µm]. Cancer ducts of large size [(iii), 228µm>100µm] existed in this tumor. However, these were less than 10% of the whole cancer ducts.**

be true CLC (cholangiole origin) and are pseudo-CLC (interlobular duct origin). Assessing the size of the cancer duct, positivity for c-Kit and coexistence of an ordinary intrahepatic cholangiocarcinoma (ICC) component is useful for the differentiation between true and pseudo-CLC (3). More than 90 % of cancer ducts of the present case showed the diameter between 15 µm and 100 µm (the size of interlobular duct). The rest of the cancer ducts were thicker than 100 µm (the duct size of ordinary ICC). Almost all the tumor cells were negative for c-Kit. Only a few tumor cells were positive. It may be very reasonable to speculate that this tumor originated from interlobular duct.

The unique clinical features of CLC can be explained by the interlobular duct origin theory (3). Because interlobular duct is the smallest bile duct, except for the cholangiole and bile canaliculus, carcinoma of interlobular duct origin will not cause dilatation of the peripheral bile duct. For the same reason, the carcinoma may present as the mass forming type. If chronic inflammation of the peripheral portal tracts accelerates the carcinogenesis of cholangiocytes of interlobular ducts, the coexistence of CLC and chronic liver disease is reasonable. The present case was infected with HCV and showed steatohepatitis in the major part of non-cancerous tissues.

Accumulation of new case reports of CLC in recent years has allowed the characterization of imaging findings and enhanced the diagnosis. On B-mode US, CLC generally appears as a heterogeneous hypoechoic lesion with unclear border and without dilatation of bile ducts. The space-occupying lesion often show hypervascular enhancement from interior or periphery to the entire tumor with portal venules penetrating the lesion and without fast wash-out in the arterial-portal predominant phase, and a distinct defect in the post-vascular phase on contrast-enhanced US with sonazoid. On MRI, CLC mainly shows low intensity on T1-weighted images, high intensity on T2-weighted images, and high intensity on diffusion-weighted images. In many cases, the tumor shows hyper-enhancement of the periphery or the entire lesion in the early phase, hyper-iso-enhancement in the delayed phase on dynamic CT or MRI. In addition, CLC is mostly described as a clear defect in the hepatobiliary phase on Gd-EOB-DTPA-enhanced MRI (14-16). In our case, al-

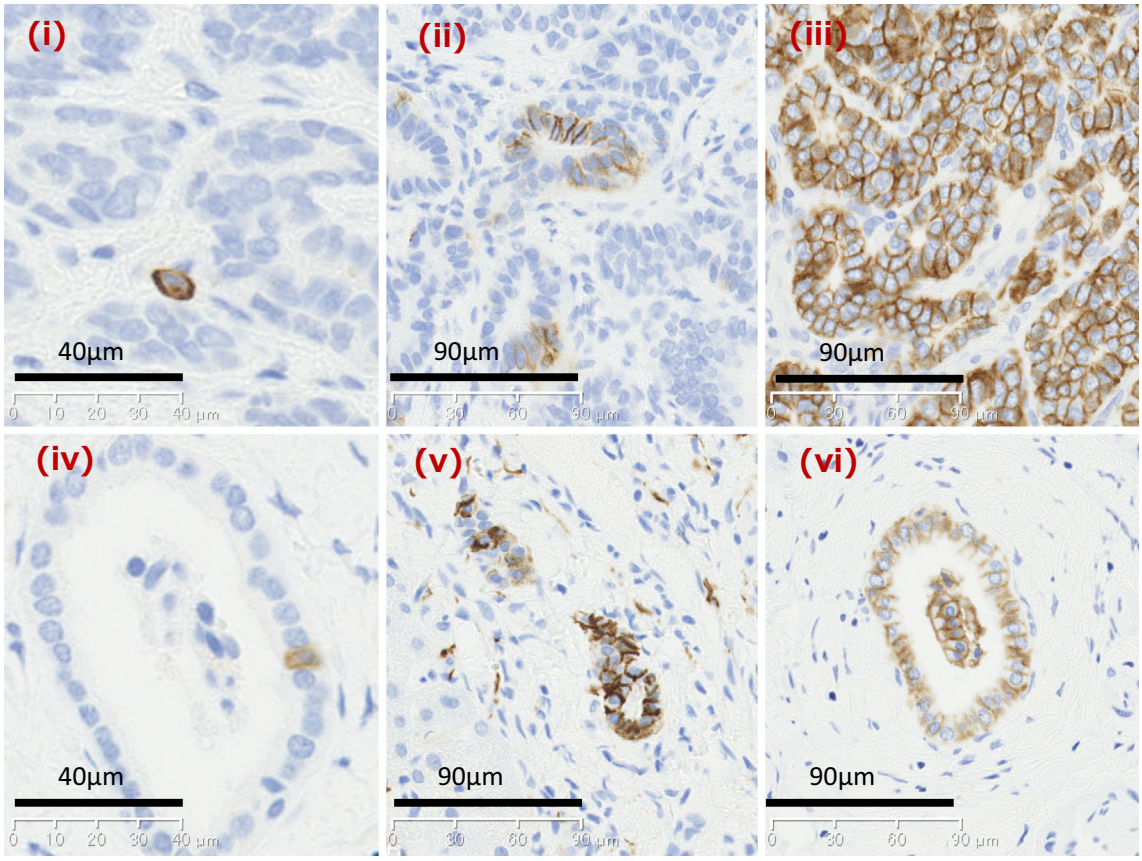

Figure 7. **Immunohistochemical staining of stem cell markers in cancer ducts (i), (ii), (iii) and non-neoplastic ducts (iv), (v), (vi). (i) c-Kit was positive in only a few cancer cells. However, it was also positive in non-neoplastic interlobular duct cells (iv). CD56 and EpCAM were positive in cancer cells [(ii), (iii)], however, they were also positive in non-neoplastic interlobular duct cells [(v), (vi)].**

most all of imaging findings applied to published features of CLC except for portal venules penetrating the tumor.

Fortunately, CLC is largely associated with relatively favorable prognosis (17). A few case reports have also described slowly growing (over several years) CLC (18). In our case, we cannot exclude the possibility that the CLC had already existed at the time of achievement of SVR in August 2015. However, US did not identify any neoplastic lesion at least 3 months before the tumor was identified for the first time on MRI. The present case highlights the fact that liver cancer should be ruled out through regular clinical examinations and imaging studies, even in young patients who achieve SVR.

Author's disclosure of potential Conflicts of Interest (COI).
Fumitaka Suzuki: Honoraria, Bristol-Myers Squibb. Yoshiyuki Suzuki: Honoraria, Bristol-Myers Squibb. Kenji Ikeda: Honoraria, Dainippon Sumitomo Pharma and Eisai. Hiromitsu Kumada: Honoraria, MSD, Bristol-Myers Squibb, Gilead Sciences, AbbVie, GlaxoSmithKline and Dainippon Sumitomo Pharma.

References

1. Roskams T, Desmet VJ, Verslype C. Development, structure and function of the liver. In: MacSween's Pathology of the Liver. 5th ed. Burt AD, Portman BC, Ferrel LD, Eds. Churchill Livingstone Elsevier, Philadelphia, 2007: 1-73.
2. Maeno S, Kondo F, Sano K, Takada T, Asano T. Morphometric and immunohistochemical study of cholangiolocellular carcinoma: Comparison with non-neoplastic cholangiole, interlobular duct and septal duct. J Hepatobiliary Pancreat Sci **19**: 289-296, 2012.
3. Kondo F, Fukusato T. Pathogenesis of cholangiolocellular carcinoma: possibility of an interlobular duct origin. Intern Med **54**: 1685-1694, 2015.
4. Theise ND, Nakashima O, Park YN, Nakanuma Y. Combined hepatocellular-cholangiocarcinoma. In: World Health Organization Classification of Tumors. WHO Classification of Tumors of the Digestive System. Bosman FT, Carneiro F, Hruban RH, Theise ND, Eds. International Agency for Research on Cancer, Lyon, 2010: 225-227.
5. Asahina Y, Tsuchiya K, Nishimura T, et al. α-fetoprotein levels after interferon therapy and risk of hepatocarcinogenesis in chronic hepatitis C. Hepatology **58**: 1253-1262, 2013.
6. Ikeda M, Fujiyama S, Tanaka M, et al. Risk factors for development of hepatocellular carcinoma in patients with chronic hepatitis C after sustained response to interferon. J Gastroenterol **40**: 148-156, 2005.
7. Shiota K, Taguchi J, Nakashima O, Nakashima M, Kojiro M. Clinicopathologic study on cholangiolocellular carcinoma. Oncol Rep **8**: 263-268, 2001.
8. Steiner PE, Higginson J. Cholangiolocellular carcinoma of the liver. Cancer **12**: 753-759, 1959.
9. Theise ND, Saxena R, Portmann BC. The canals of Hering and hepatic stem cells in humans. Hepatology **30**: 1425-1433, 1999.

10. Komuta M, Spee B, Borght S, et al. Clinicopathological study on cholangiolocellular carcinoma suggesting hepatic progenitor cell origin. Hepatology **47**: 1544-1556, 2008.
11. Liver Cancer Study Group of Japan. The general rules for the clinical and pathological study of primary liver cancer. 6th ed. Kanehara, Tokyo, 2015 (in Japanese).
12. Kanamoto M, Yoshizumi T, Ikegami T, et al. Cholangiolocellular carcinoma containing hepatocellular carcinoma and cholangiocellular carcinoma, extremely rare tumor of the liver: a case report. J Med Invest **55**: 161-165, 2008.
13. Kondo F, Fukusato T, Tokairin T, Saito K, Soejima Y. Chapter 11: Cholangiolocellular carcinoma: Is it a subtype of cholangiocarcinoma or combined hepatocellular cholangiocarcinoma?. In: Pathology of the Bile Duct. Nakanuma Y, Ed. Springer Natur, Singapore, 2017: 139-147.
14. Mori N, Ichikawa T, Hashimoto J, et al. Cholangiolocellular carcinoma of the liver exhibiting high F-18 FDG uptake. Tokai J Exp Clin Med **41**: 60-64, 2016.
15. Joshita S, Ichijo T, Suzuki F, et al. A case of well-differentiated cholangiolocellular carcinoma visualized with contrast-enhanced ultrasonography using Sonazoid. Hepatol Res **39**: 207-212, 2009.
16. Asayama Y, Tajima T, Okamoto D, et al. Imaging of cholangiolocellular carcinoma of the liver. Eur J Radiol **75**: e120-e125, 2010.
17. Ariizumi S, Kotera Y, Katagiri S, et al. Long-term survival of patients with cholangiolocellular carcinoma after curative hepatectomy. Ann Surg Oncol **21** (Suppl 3): S451-S458, 2014.
18. Koga Y, Nagahama H, Tateyama M, et al. A case of cholangiolocellular carcinoma combined with intrahepatic cholangiocarcinoma diagnosed after 4 years follow-up for hepatic hemangioma. Nihon Shokakibyo Gakkai Zasshi (Jpn Soc Gastroenterol) **109**: 231-239, 2012 (in Japanese, Abstract in English).