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Original Article

Carcinoma of ectopic liver tissue shares characteristics with combined hepatocellular-cholangiocarcinoma

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Abstract: Carcinoma of ectopic liver tissue (CELT) is rare and only 49 cases have been reported. Most of patients (39, 79.6%) were Asian, especially from eastern Asia. We reported here the first two cases of CELT with stem-cell features. Both of the patients complained abdominal pain. The levels of serum AFP were significantly elevated. Endoscopy and CT scans revealed nodular lesions in the upper abdomen. Histologically, the tumor cells arranged in thin trabeculae and pseudoglands. Immunohistochemical staining demonstrated that they simultaneously expressed typical markers of hepatocyte (Hep Par 1 and/or AFP), cholangiocyte (CK19) and hepatic progenitor cells (HPCs) (EpCAM). The majority of malignant cells showed nuclear positivity for p53. Radiology examinations revealed that there was no significant abnormality in the mother liver and testis. Laboratory tests showed no virus infection (HBV, HCV and HIV). Furthermore, the medical and family histories of the patients were silent. The pathological diagnosis of CELT with stem-cell features was established. A review of the published literature revealed that 17 of 28 available cases (60.7%) complained irregular pain of the diseased region. The serum AFP levels of most patients (29/36, 80.6%) were elevated over 200 ng/ml and decreased dramatically after the surgical resection. The serum AFP level has been proposed as an indicator of metastasis or recurrence. Mother liver was the most common secondary site (7/14, 50%). The risk factors between CELT and hepatocellular carcinoma differ essentially. HBV and HCV infection had been demonstrated in three and two patients respectively. There was no definite cirrhosis observed in CELT. The prognosis of patients with CELT is more favorable as compared to other AFP-producing tumors originating in mother liver and testis. We hypothesized that HPCs are involved in the pathogenesis of CELT. The incomplete vascular and bile duct systems result in the chronic injury of ectopic liver tissue, which impairs the regenerative capacity of hepatocytes. Consequently, HPCs arise in or near the canal of Hering (COH) and proliferate to compensate the loss of parenchymal cells. However, the genomic changes may increase susceptibility of HPCs to the detrimental microenvironment and finally result in the malignant transformation of HPCs.

Keywords: Carcinoma of ectopic liver tissue, hepatic progenitor cell, pathogenesis, combined hepatocellular-cholangiocarcinoma, AFP

Introduction

Ectopic liver tissue (ELT) accounts for approximately 0.02% up to 0.47% of the general population with two major types: 1. Accessory liver lobe, which is attached to the mother liver by a stalk. 2. Ectopic liver, which is situated outside the mother liver completely [1-3]. Accumulating evidence indicate that ELT are more prone to carcinogenesis because of the incomplete vascular and ductal system. Although several hypotheses have been proposed regarding the histogenesis of carcinomas in ELT, the underlying mechanisms are still debated.

Combined hepatocellular-cholangiocarcinoma (CHCC) is a rare form of primary liver cancers and has been subdivided into two major types,

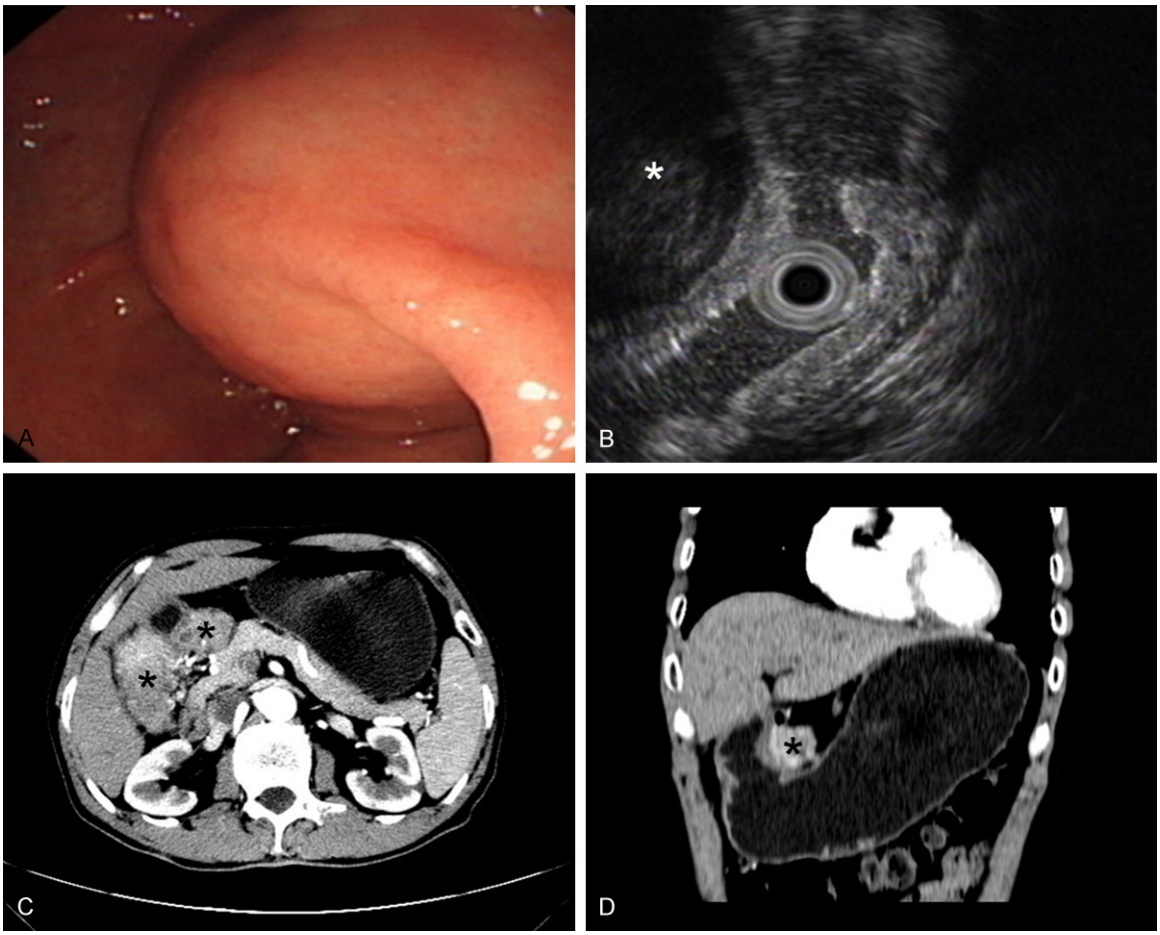

Figure 1. Case 1. A. Endoscopic examination found that the covering membrane of the lesion was protruding without ulcer. B. The result of EUS indicated a submucosa lesion (*). C, D. Spiral CT revealed an irregular mass locating under the mucus membrane of gastric antrum (*). There was no close relationship between the mass and the mother liver.

including classical type and subtypes with stem cell features. In published literatures the incidence of CHCC varies from 3.3% to 10.1% [4, 5]. It should be distinguished from other malignant tumors of primary liver because of the different prognosis and tailored therapeutic strategies. A distinctive feature of CHCC is its simultaneous expression of typical markers of hepatocyte (HepPar 1 or AFP) and cholangiocyte (CK7 or CK19). Furthermore, the malignant tumor cells can be pinpointed by hepatic progenitor cell (HPC, also known as oval cell in rodents, OC) markers such as EpCAM and NCAM.

We describe here two cases of carcinoma of ELT (CELT) sharing characteristics with CHCC with stem-cell features. Both cases were asyptomatic despite abdominal pain. An irregular mass had been confirmed in the following CT scans. The preoperative diagnosis was an AFP-producing carcinoma, because laboratory examinations showed the elevated AFP levels. Histologically, the tumors consisted of irregular trabecular and pseudoglandular pattern. Malignant cells co-expressed Hep Par 1, CK19, EpCAM and p53. It thus seems that, at least in our cases, HPC may contribute to the malignant transformation of ELT.

Case presentation

Case 1

A 61-year-old Chinese man, in good health, had an abdominal pain and was submitted to The Fourth Affiliated Hospital of Nanchang University for further investigation. Endoscopy found that the covering mucus membrane of the lesion was protruding without ulcer (**Figure**

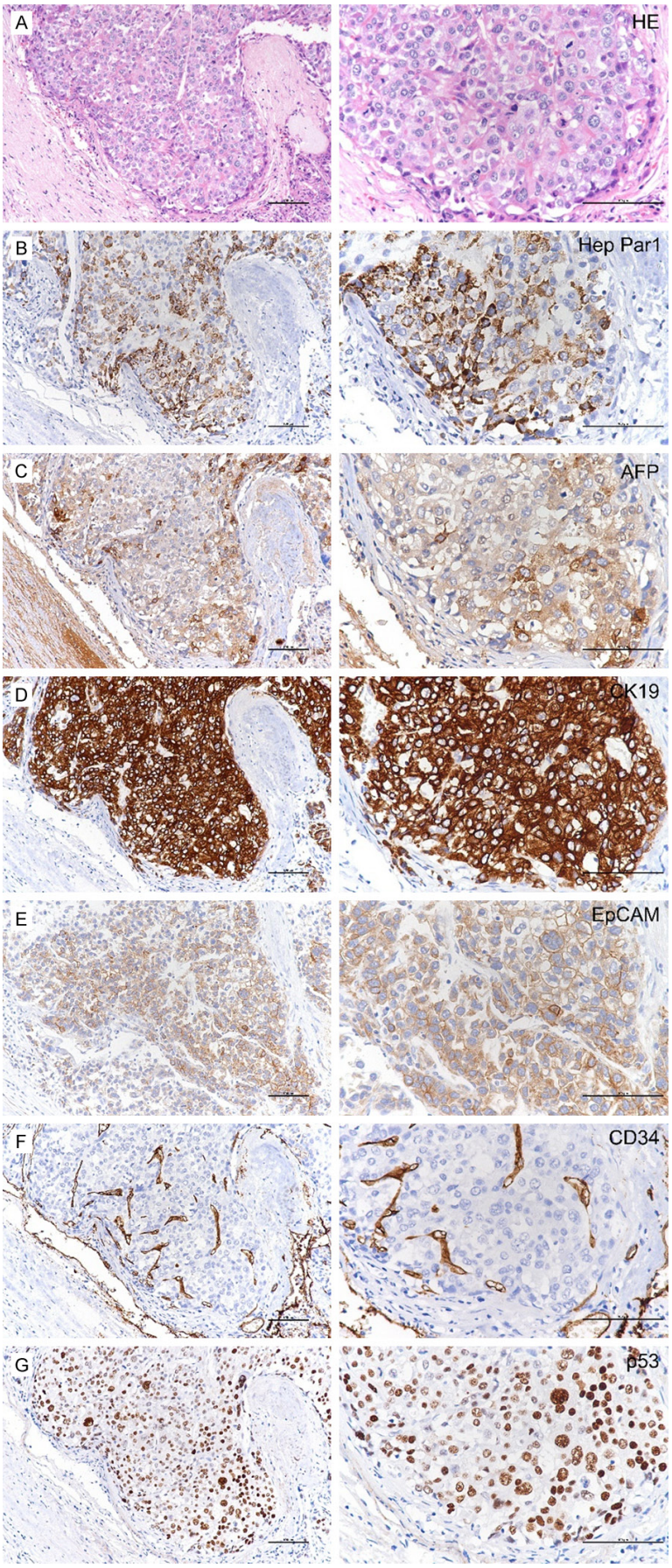

Figure 2. Case 1. A. H&E staining exhibited the characteristically trabecular and pseudoglandular patter of CELT with stem-cell features (original magnification: left × 200; right × 400). B, C. The malignant cells expressed typical hepatocyte markers such as Hep Par1 and AFP (original magnification: left × 200; right × 400). D. They also displayed positivity for cholangiocyte marker CK19 (original magnification: left × 200; right × 400). E. The membrane positivity for EpCAM indicated that these cells may derive from HPCs (original magnification: left × 200; right × 400). F. The lining endothelium of sinusoidal structures expressed CD34 (original magnification: left × 200; right × 400). G. The majority of tumor cells displayed strong-diffuse positivity for p53 (original magnification: left × 200; right × 400).

1A). Endoscopic ultrasound (EUS) found a submucosa lesion, indicating gastrointestinal stromal tumor (GIST) (**Figure 1B**). However, this preoperative diagnosis did not supported by laboratory studies that the serum AFP was elevated up to 11,802 ng/ml. Spiral CT revealed an irregular mass locating under the mucus membrane of gastric antrum (**Figure 1C**, **1D**). Enhanced-contrast CT was performed to further investigate the nature of the mass. The lesion showed characteristic enhancement patterns of hepatocellular carcinoma (HCC): washin and washout (**Figure 1D**). It is interesting that the mother liver and the testis showed no remarkable abnormality during CT scans. Further investigations confirmed none of the following attributable risk factors for HCC: hepatitis B virus (serum DNA and surface antigen), hepatitis C virus (serum RNA), alcohol and oral drugs. His past medical history and family history were silent. At laparotomy, a

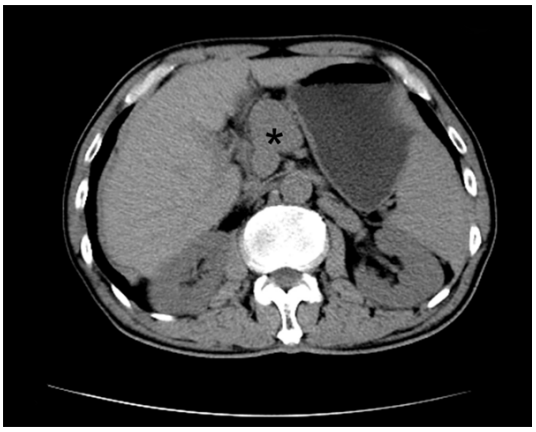

Figure 3. Case 2. The CT scan confirmed a heterogeneous mass between the liver, stomach and pancreas (*).

Table 1. Summary of Primary Antibodies Used for Immunohistochemistry

Antibody	Isotope	Supplier	Dilution
EpCAM	IgG1 (mouse)	DAKO	1:150
CK7	IgG1 (mouse)	DAKO	1:150
CK19	IgG2a (mouse)	DAKO	1:150
CK20	IgG2a (mouse)	DAKO	1:150
Hep Par1	IgG1 (mouse)	DAKO	1:150
AFP	IgG (rabbit)	DAKO	1:250
CD34	IgG1 (mouse)	DAKO	1:150
p53	IgG2b (mouse)	DAKO	1:100

mass, without any connection with the mother liver, was resected. The surface of the mother liver was normal.

The lesion appeared as a lobulated nodule with focal necrosis, approximately 21 cm in greatest diameter. The cut surface displayed a gray color after fixation with formalin. The tumor invaded into the serous membrane and muscular layer of the gastric antrum but not into mucous membrane. Histologically, the tumor was composed of polygonal epithelioid cell, arranged in the irregular trabecular and pesudograndular pattern (**Figure 2A**). Serial sections were cut in 4-mm slices and incubated with primary antibodies overnight at 4°C (**Table 1**). The malignant cells were simultaneously positive for hepatocytic (Hep Par1 and AFP) and cholangiocytic (CK19) markers (**Figure 2B-D**). Furthermore, EpCAM, a HPC/OC marker, drew the outlines of some tumor cells (**Figure 2E**). CD34 positivity demonstrated a sinusoidal pattern surrounding neoplastic cells (**Figure 2F**). More than 60% of malignant cells showed strong nuclear staining for p53 (**Figure 2G**). CK7 and CK20 stains were negative (data not shown). The final pathological diagnosis in this patient was CELT with stem-cell features.

The serum AFP dropped to 1,656 ng/ml on post-operative day 5 and was within normal limit on day 32. Upon further follow-up, he is now alive and without any signs of metastasis or recurrence almost five months after the initial operation.

Case 2

A 65-year-old Chinese man was admitted to his neighborhood hospital with abdominal pain. A CT scan revealed a heterogeneous mass between the liver, stomach and pancreas (**Figure 3**). He was subsequently transferred to The Third Affiliated Hospital of Nanchang University for further evaluation. The level of preoperative serum AFP was elevated (70.8 ng/ml). However, the patient was examined with a contrast-enhanced CT scan and an ultrasound without findings of significant diseases in the mother liver and testis. Subsequently, the mass was resected *en bloc* through laparoscopy. The surgery confirmed that the tumor was absolutely separated from the mother liver and other abdominal organs. Laboratory tests showed negative results for virus infection (HBV, HCV and HIV). The patient dined any history of alcohol/oral drugs abusing, parasite infection and neoplasms.

The nodular mass was encapsulated in fibrous tissue. It had been fixed in 4% buffered formalin and embedded in paraffin by conventional techniques. Then, immunohistochemistry were performed on serial sections with antibodies against hepatocyte, cholangiocyte and HPC markers (**Table 1**). Neoplastic cells exhibited strong-diffuse reactivity for Hep Par 1, CK19 and p53 (**Figure 4A**, **4B**, **4D**, **4G**). However, they did not express AFP (**Figure 4C**). EpCAM was detected in some, but not all tumor cells, indicating that these targeted cells may be bipotential (**Figure 4E**). CD34-positivity was detected in lining endothelium of sinusoidal structures (**Figure 4F**). Metastatic gastrointestinal tumors were ruled out, because the tumor cells did not express CK7 and CK20 (data not shown). The

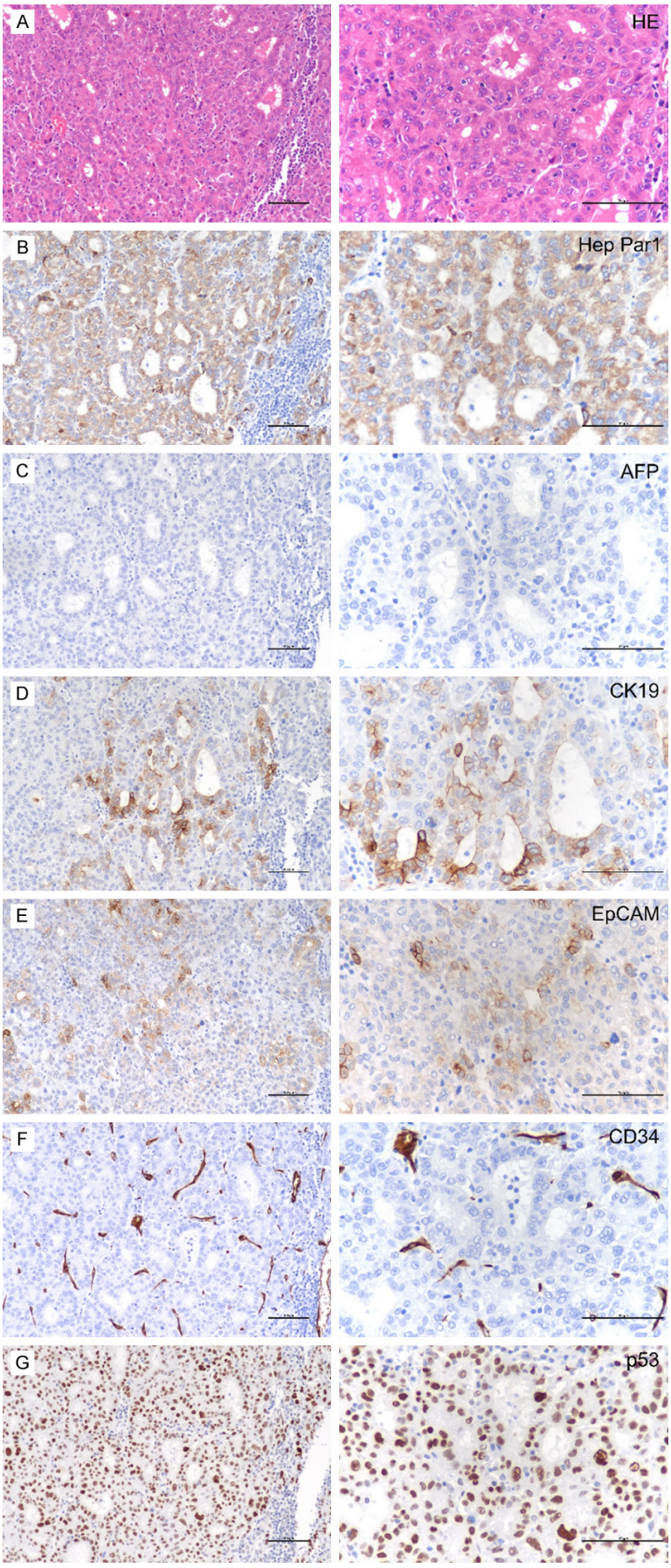

Figure 4. Case 2. A. Histologically, the irregular thin trabecular pattern with pseudolands was typical feature of CELT with stem-cell features (original magnification: left × 200; right × 400). B, C. The malignant cells showed immunopositivity for Hep Par1, but not AFP (original magnification: left × 200; right × 400). D. CK19 reactivity was detected mainly in the pseudoglandular pattern (original magnification: left × 200; right × 400). E. EpCAM drew outlines of the progenitor cell-like tumor cells (original magnification: left × 200; right × 400). F. CD34 positivity demonstrated a sinusoidal pattern surrounding neoplastic cells (original magnification: left × 200; right × 400). G. Tumor cells were strongly positive for p53 (original magnification: left × 200; right × 400).

diagnosis of CELT with stem-cell features was established.

The patient was discharged from our hospital on post-operative day 19. Serum AFP levels decreased rapidly and were within normal limits on day 30. He had been doing well for the past four months and showed no signs of recurrence or metastasis.

Discussion

CELT is rare. To date, there are only 49 cases reported in English literatures (**Table 2**). The male-to-female ratio was approximately 2.8 to 1, with a strong male preponderance. Thirty-nine patients (79.6%) came from Asian countries/area, especially from East Asia such as Japan, Korea, Taiwan area and China mainland. The other ten patients (20.4%) came from Europe. The patient's age at diagnosis ranged from 34 to 81, most of them are over age 50 (44, 89.8%). The symptoms of CELT are always non-specificity. Among 28 available cases,

Table 2. Clinical and pathological data of patients with CELT

Case No.	Year	Sex/ Age (year)	Country (Area)	Complain	Tumor Location	Virus marker		AFP (ng/ml)	Mother Liver	Normal EL	IHC					Metastasis/Recurrence	Reference
						HBV	HCV				Hep Par1	AFP	CK7	CK19	Other		
1	1969	M/57	Japan	N/A	left upper abdomen retroperito-neum	N/A	N/A	N/A	cirrhosis	N/A	N/A	N/A	N/A	N/A	N/A	liver (4 months)	[6]
2	1973	M/63		N/A	below right lobe	N/A	N/A	18	cirrhosis	N/A	N/A	N/A	N/A	N/A	N/A	lung (18 months)	
3	1977	M/67		N/A	between stomach, pancreas and liver	-	N/A	4000	cirrhosis	N/A	N/A	N/A	N/A	N/A	N/A	- (0 month)	
4	1980	F/53		N/A	between stomach and left lobe	+	N/A	510	cirrhosis	N/A	N/A	N/A	N/A	N/A	N/A	- (0 month)	
5	1982	M/77		N/A	between stomach and left lobe	-	N/A	-	chronic hepatitis	N/A	N/A	N/A	N/A	N/A	N/A	multiple metastasis (0 month)	
6	1984	M/51		N/A	between spleen, colon and liver	-	N/A	-	cirrhosis	N/A	N/A	N/A	N/A	N/A	N/A	- (12 month)	
7	1984	M/57		N/A	between left lobe and diaphragm	-	N/A	1600	chronic hepatitis	N/A	N/A	N/A	N/A	N/A	N/A	- (14 months)	
8	1984	M/52		N/A	between stomach, colon and liver	-	N/A	-	cirrhosis	N/A	N/A	N/A	N/A	N/A	N/A	- (16 months)	
9	1985	F/54		N/A	within gall-bladder	N/A	N/A	29,851	-	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
10	1988	M/64		N/A	multiple with-in abdominal cavity	N/A	N/A	117,000	-	N/A	N/A	N/A	N/A	N/A	N/A	liver (40 months)	
11	1989	M/54		N/A	retroperito-neum	-	N/A	465,000	-	N/A	N/A	N/A	N/A	N/A	N/A	- (6 months)	
12	1989	M/70		N/A	r-abdominal wall	N/A	N/A	2,650	-	N/A	N/A	N/A	N/A	N/A	N/A	- (9 months)	
13	1991	M/77		N/A	between diaphragm, stomach and pancreases	-	N/A	1,260	-	N/A	N/A	N/A	N/A	N/A	N/A	N/A	

14	1991	M/67		N/A	stomach submucosa	N/A	N/A	N/A	-	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
15	1992	M/68		N/A	greater omentum adherent to stomach	-	-	12,790	-	N/A	N/A	N/A	N/A	N/A	N/A	- (3 months)	
16	1992	M/59		N/A	left triangular ligament	-	N/A	N/A	-	N/A	N/A	N/A	N/A	N/A	N/A	- (24 months)	
17	1994	M/54		N/A	between the stomach and the diaphragm	-	N/A	1,500	cirrhosis	N/A	N/A	N/A	N/A	N/A	N/A	- (24 months)	
18	1994	M/54		N/A	right chest wall	N/A	N/A	N/A	-	N/A	N/A	N/A	N/A	N/A	N/A	- (12 months)	
19	1994	M/57		N/A	beneath the diaphragm	-	N/A	2,207	-	N/A	N/A	N/A	N/A	N/A	N/A	- (96 months)	
20	1996	M/74		N/A	left chest wall	N/A	+	4,116	-	N/A	N/A	N/A	N/A	N/A	N/A	liver (36 months)	
21	1997	M/67		N/A	r-mesocolon	-	-	>1,000,000	-	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
22	1999	M/64		routine checkup found a mass in the stomach	gastric serosa	-	N/A	4,900	chronic hepatitis	N/A	N/A	N/A	N/A	N/A	N/A	liver (12 months)	
23	1999	F/65	France	abdominal pain/ tumor rupture	between the left lobe and the diaphragm	-	-	N/A	-	N/A	N/A	N/A	N/A	N/A	N/A	- (0 month)	[7]
24	2001	F/66	France	chest wall pain	left chest wall	-	+	N/A	chronic hepatitis	N/A	+	-	N/A	N/A	N/A	- (24 months)	[8]
25	2003	F/43	Korea	abdominal pain	between the spleen and the left diaphragm	+	N/A	2532	-	-	+	N/A	N/A	N/A	N/A	liver (7 months) - (30 months)	[9]
26	2004	F/54	Italy	abdominal pain	gallbladder	-	-	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	- (48 month)	[10]
27		F/34		abdominal pain	subdia-phragm	-	-	1,580	-	N/A	+	+	N/A	N/A	alpha-1-anti-trypsin +	right ovary (3 months) left ovary (15 months) perito-neum (39 months) liver (43 months)	
28		M/62		routine checkup found an elevated serum AFP	between the diaphragm and the spleen	-	-	4,000	-	N/A	N/A	+	N/A	N/A	alpha-1-anti-trypsin +	- (48 months)	
29	2005	F/72	Japan	abdominal pain, fever, jaundice	bile duct	-	-	N/A	-	N/A	N/A	N/A	-	N/A	CK8 +, CK18 +	- (12 months)	[11]
30	2006	M/72	Japan	routine checkup found an intra-pelvic mass	jejuneum	-	-	99,100	-	N/A	+	+	-	N/A	CK8 +, CK18 +, CK20 -	liver (2 months) - (14 months)	[12]

31	2007	M/56	Japan	US found a mass in the pancreas tail during routine checkup	pancreas tail	-	-	N/A	-	-	+	-	-	N/A	CAM5.2 +, CK18 +, COX-2 +, MOC-13 -, TTF-1 -, MUC-1 -, CK20 -, CK8 -	- (36 months)	[13]
32	2007	F/62	Taiwan	abdominal plain	hemidiaphragm	-	-	45,000	-	N/A	N/A	+	N/A	N/A	N/A	- (8 months)	[14]
33	2007	F/81	Taiwan	incidentally felt a mass in abdomen	lower abdomen	-	-	87,500	-	N/A	N/A	N/A	N/A	N/A	N/A	- (0 month)	[15]
34	2007	M/58	Spain	dull back and flank pain	pancreas	N/A	N/A	N/A	-	N/A	+	-	N/A	focally +	alpha-1-antitrypsin +, CEA -, SYN -, CgA -	- (15 months)	[16]
35	2008	M/59	Korea	CT scan found an intra-abdominal mass	between the diaphragm and the spleen	-	-	-	N/A	N/A	+	N/A	N/A	N/A	N/A	- (0 month)	[17]
36	2010	M/46	Netherland	stomach ache	retroperitoneum	N/A	N/A	22,080	-	N/A	N/A	+	+	-	CKpan +, CK18 -, HCG -	- (6 months)	[18]
37	2010	F/59	Japan	routine checkup found an elevated serum AFP	the left triangular ligament of the liver	-	-	2,508	-	+	N/A	N/A	N/A	N/A	N/A	- (18 months)	[19]
38	2010	M/60	India	left flank pain	left suprarenal region	+	N/A	N/A	-	N/A	+	+	N/A	N/A	NSE -, CgA -, LCA -, HCG -, EMA -, S100 -, HMB45 -	multiple metastasis (6 months)	[20]
39	2011	M/64	Japan	X-ray showed multiple lesions on lung and diaphragm	lung and subdiaphragm	-	-	84,865	-	+	N/A	N/A	N/A	N/A	N/A	multiple metastasis (0 month)	[21]
40	2011	M/69	Japan	abdominal pain, hypoglycemia	spleen	N/A	N/A	N/A	-	N/A	+	+	N/A	N/A	PIVKA-II +, IGF-II +	lung (10 months)	[5]
41	2012	M/42	Japan	routine checkup found liver dysfunction	multiple within abdominal cavity	-	-	241	-	N/A	+	N/A	N/A	N/A	N/A	multiple metastasis (0 month)	[22]
42	2013	M/59	Czech	US found a mass	upper pole of the spleen	N/A	N/A	-	-	N/A	N/A	-	N/A	N/A	CEA -, CA199 -	- (0 month)	[23]
43	2014	M/72	Turkey	abdominal pain	retroperitoneum	-	-	>20,000	-	N/A	+	+	-	N/A	CEA +, CK20 -	- (0 month)	[24]
44	2014	F/49	Spain	right hypochondriac pain	gallbladder	-	-	13,785	-	+	N/A	N/A	N/A	N/A	N/A	- (36 months)	[25]
45	2014	M/54	Italy	thoraco-abdominal pain	thoraco-abdominal mass	N/A	N/A	810	-	N/A	N/A	N/A	N/A	N/A	N/A	- (0 month)	[26]
46	2015	M/65	Korea	chest CT found a peritoneal mass	left subphrenic region	-	-	N/A	-	-	+	+	N/A	N/A	CKpan +, vimentin -, SYN -, CgA -, S-100 -, EMA -, CD56 -	- (17 months)	[27]

47	2015	F/64	Asia	atypical abdominal complaints	diaphragm	-	-	200	-	N/A	N/A	N/A	N/A	N/A	N/A	lymph node (24 months) the duodenal wall, descending part of the duodenum (38 months) middle colic artery (46 months) - (48 months)	[28]
48	2016	M/61	China	abdominal pain	gastric antrum	-	-	11,802	-	-	+	+	-	+	EpCAM +,	- (5 months)	present study
49	2016	M/65	China	abdominal pain	between the liver and the spleen	-	-	71	-	-	+	-	-	+	EpCAM +,	- (4 months)	present study

17 patients (60.7%) complained irregular pain of the diseased region. Eleven cases (39.3%) were found during routine check-up or surgery (**Table 2**). Although CELT is usually asymptomatic, it occasionally causes unexpected problem such as tumor rupture and bleeding [7]. Noteworthy, serum AFP levels were elevated in up to 86.1% of available cases (31/36), 29 of which were over 200 ng/ml. It will decrease rapidly and remain in the normal area after the initial operation. The postoperative serum AFP level can also serve as an indicative marker of recurrence or metastasis [6, 9, 10, 28].

CELT is prone to metastasize. Fourteen of forty-five (31.1%) available cases developed metastatic lesions. Mother liver was the most common involved site (7, 50%). The close relationship between the supportive vascular systems of ELT and mother liver may potentially account for the underlying mechanisms [29]. CELT clearly distinct from primary HCC in their more favorable prognosis. By the end of the published studies, no patient died of CELT. Surgical resection has been proposed as the first choice for patients with CELT. However, percutaneous biopsy is not recommended because of the risks of tumor rupture and peritoneal spread.

It is urgent to differentiate CELT from other AFP-producing tumors, including metastatic testis or ovary cancer, HCC and hepatoid adenocarcinoma, since the prognosis and therapeutic approaches differ essentially. However, the differential diagnosis is always difficult. If the malignant tumor derives from testis or ovary, the histological pattern of biopsy tissue should be an important clue. Metastatic HCC is unlikely for several reasons. First, imaging results showed no tumor in the mother liver at the time of the preoperative diagnosis. Second, most of metastatic lesions are found after the onset of clinical symptoms of primary tumors. Third, the locations of CELT, including pancreas [13, 16], chest wall [6, 8] and spleen [5], are always uncommon for HCC metastasis. Fourth, the majority of patients with CELT had no typical risk factors for HCC such as virus infection and alcohol/drug abuse. Only three patients (3/36, 8.3%) were infected with HBV [6, 9, 30] and two patients (2/23, 8.7%) suffered from chronic HCV hepatitis [6, 21]. Meanwhile, more than 80% of patients who develop HCC have cirrhosis [31]. In the reported cases, however, cirrhosis had been detected in only seven mother livers (7/47, 14.9%) and in none of ELT (**Table 2**).

CELT arising in stomach and pancreas must be distinguished from hepatoid adenocarcinoma, which is a rapidly growing malignant tumor associated with a high propensity for vascular invasion and metastasis. They share numerous clinicopathological characteristics in addition to elevated serum AFP level [32, 33]. Two key points have been proposed as diagnostic clues for hepatoid adenocarcinoma. First, foci of conventional gastric or pancreatic adenocarcinoma can be observed near the hepatoid adenocarcinoma. Second, malignant cells of hepatoid adenocarcinoma usually exhibit focal positivity for Hep Par 1 [33]. The expression pattern of Hep Par 1 in the presented two cases was consistent with previous studies showing diffuse-strong reactivity. It is now clear that there is not a sharp line of demarcation between hepatoid adenocarcinoma and CELT. The tumor cells of hepatoid adenocarcinoma can secret bile and express Albumin, a unique marker of liver cells [34].

Accumulating evidence indicate that the defective vascular supply and biliary drainage system contribute to the pathogenesis of CELT [14]. However, the exact molecular mechanisms are still under debate. We hypothesized that HPC may serve as a cellular resource of CELT. Hepatocytes of ELT are capable of bile production [22]. But the abnormal bile duct system may result in cholestasis and induce chronic injury. Chronic inflammation, steatosis and aberrant deposition of extracellular matrix have been confirmed in ELT or non-tumoral tissue adjacent to CELT [23, 35]. The regenerative capacity of hepatocytes is overwhelmed during this process [36]. Consequently, HPCs originate in or near the canal of Hering (COH) and proliferate to compensate the loss of parenchymal cells [36].

However, the genomic changes may increase susceptibility of HPCs to carcinogens and finally lead to the malignant transformation of HPCs [37]. Thus, CELT may exhibit both hepatocellular and cholangiocellular carcinoma features. Histologically, the trabecular and pseudoglandular pattern were observed in several reported cases [10, 16, 19, 20]. Immunohistochemical study demonstrated that the malignant cells showed simultaneously positivity for typical

markers of hepatocyte (Hep Par1 and/or AFP), cholangiocyte (CK7 and/or CK19) [16, 18]. Our results further demonstrated that the Hep Par1 +/CK19 + cells displayed membrane positivity for EpCAM, a typical HPC marker. P53 has long been known for its role in the regulation of cell cycle. In the present study, the majority of malignant cells expressed mutant p53 indicating its important role in this process. Although we provided preliminary evidence for the involvement of HPCs in the carcinogenesis of CELT, our study did have some potential limitations, especially the lack of available cases. Further researches are needed to explore the exact mechanisms.

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Disclosure of conflict of interest

None.

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