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Case Report

Hepatocellular carcinoma of unknown primary: a case report

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Abstract: A previously healthy 46-year-old man was admitted to our hospital for treatment of malignant effusions. An enhanced computed tomography of the chest, abdomen and pelvis along with a magnetic resonance angiography of the abdomen revealed a cirrhotic liver and numerous heterogeneously enhancing nodules on the surface of mesentery and peritoneum, but failed to identify a primary lesion in the liver or other visceral organs. Esophagogastroduodenoscopy, sigmoidoscopy and laparoscopy were performed but none of them could identify a primary lesion or a macroscopic ectopic liver. A nodule was resected for biopsy. Histologically, large polygonal cells with eosinophilic granules and abundant cytoplasm showing moderate atypia were predominantly arranged in a solid pattern and were negative for MOC-31, cytokeratin (CK) 7, CK19, CK20, human chorionic gonadotropin and octamer-binding transcription factor 4. Although the tumor cells showed immunonegativity for hepatocyte paraffin antigen 1, arginase-1, polyclonal carcinoembryonic antigen, CD10 and Villin, expressions of glypican-3, α-fetoprotein, CD34, CK8, CK18 and, human albumin mRNA in situ hybridization were sufficient enough to confirm the diagnosis of hepatocellular carcinoma. To the best of our knowledge, this is the first reported case of hepatocellular carcinoma without a primary in the liver diffusely affecting the mesentery and peritoneum, diagnosed using immunohistochemistry and human albumin mRNA in site hybridization.

Keywords: Cancer of unknown primary, hepatocellular carcinoma, immunohistochemistry, in situ hybridization

Introduction

Cancer of unknown primary (CUP) accounts for 3%-5% of all malignancies and liver/bile duct is the third most frequent origin identified by autopsy. Identifying the origins may be challenging but significant because the similar therapeutic managements to that of known carcinoma are probably effective for this disease. Pathological evaluation, especially a panel of immunohistochemistry (IHC), plays a central role on such conditions. Molecular diagnostic tools, in situ hybridization (ISH) based on mRNA for example, have also shown a perfect performance and should be considered when available. Herein, we report, to the best of our knowledge, the first case of hepatocellular carcinoma without a primary in the liver diffusely affecting the mesentery and peritoneum, diagnosed using a panel of IHC and albumin mRNA ISH.

Case report

A previously healthy 46-year-old man, also a heavy drinker for the past 30 years, presented with a one-month history of progressive abdominal distension, dyspnea and weight loss. He reported no fever, cough, sweating, jaundice, abdominal pain, vomiting or diarrhea. On physical examination, a decreased lung sound on the left-sided chest wall, a massive ascites without signs of peritoneal irritation and several hard and palpable masses on the right lower anterior abdominal wall were noted. Tests for HBV, HCV and autoantibodies were all negative with normal levels of hemoglobin, bilirubin, transaminases, albumin and prothrombin time. But the serum α-fetoprotein (AFP) exceeded the detection limit (1210 ng/ml). Both abdominocentesis and thoracentesis demonstrated malignant effusions. An enhanced computed tomography of the chest, abdomen and pelvis

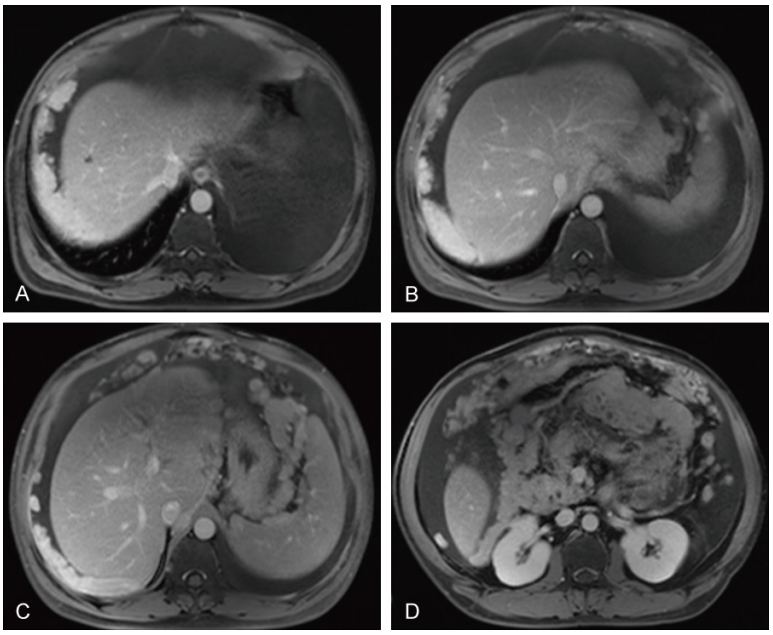

Figure 1. Enhanced MRI of the abdomen, showing the peritoneal and pleural effusions and heterogeneously enhancing nodules.

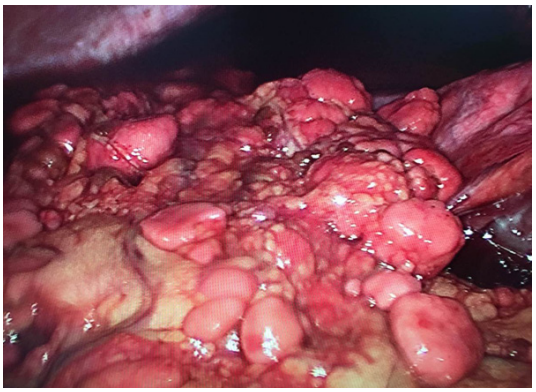

Figure 2. Laparoscopic appearances of the nodules.

along with a magnetic resonance angiography of the abdomen revealed a left-sided massive pleural effusion, a cirrhotic liver, several esophageal varices, a massive ascites and, numerous heterogeneously enhancing nodules diffusely involving the surface of mesentery and peritoneum (**Figure 1** and Supplementary Data), although there were no obvious signs of nodular lesions in the liver or other visceral organs. Neither esophagogastroduodenoscopy nor sigmoidoscopy could locate a primary lesion. Further laparoscopy disclosed no macroscopic ectopic liver except for numerous nodular lesions (**Figure 2**). A nodule was resected for biopsy. Histologically, large polygonal cells with eosinophilic granules and abundant cytoplasm showing moderate atypia were predominantly arranged in a solid pattern (**Figure 3A**) and were negative for hepatocyte paraffin antigen 1 (Hep-Par1), arginase-1 (ARG-1), polyclonal carcinoembryonic antigen (p-CEA), CD10, Villin, MOC-31 (**Figure 3F**), cytokeratin (CK) 7, CK19, CK20, human chorionic gonadotropin (HCG) and octamer-binding transcription factor 4 (OCT4), but showed expression of glypican-3 (GPC-3) (**Figure 3B**), AFP (**Figure 3C**), CD34 (**Figure 3D**), CK8, CK18 (**Figure 3E**) and human albumin mRNA ISH (**Figure 4**). The diagnosis of hepatocellular carcinoma (HCC) of unknown primary was finally established. But he refused chemotherapy and debulking. Herbal preparations were prescribed. Regretfully, his condition rapidly deteriorated and eventually he died of cachexia three months after diagnosis.

Discussion

Cancer of unknown primary (CUP) is a recognized disorder characterized by unknown origin after detail investigations and accounts for 3%-5% of all malignancies [1]. Although the mechanism remains obscure, multiple distinct and poorly understood biology and metastasis-causing genes independent of that of the primary, are suggested to exist to contribute to their unique biology such as early dissemination in the absence of primary tumor, peculiar metastatic pattern and progressive clinical courses. Nonetheless, such tumors may also retain signatures of putative primary origin, allowing the similar diagnostic methods and therapeutic managements to that of known carcinoma to be applicable for this disease [2].

Although there is less agreement on definitive origins in clinical practice, the performance of imaging, endoscopy and serum tumor markers is always unsatisfying in tissue-of-origin identification. Pathological evaluation, especially a

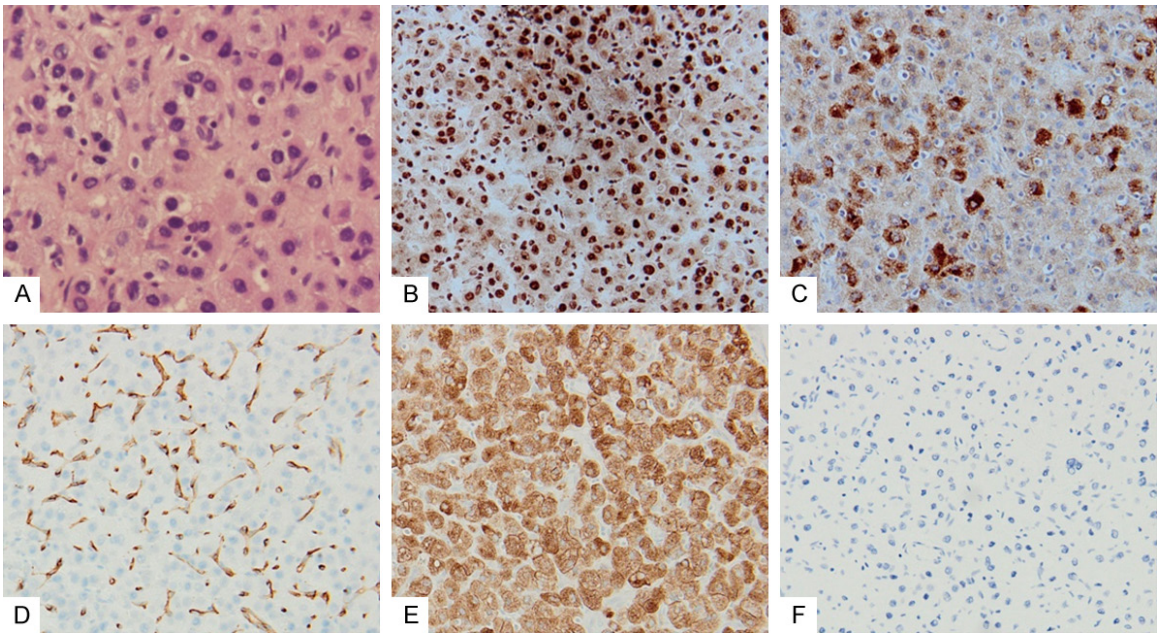

Figure 3. A. Biopsy from one nodule. HE (×200). B. GPC-3 (×100). C. AFP (×100). D. CD34 (×100). E. CK18 (×100). F. MOC-31 (×100).

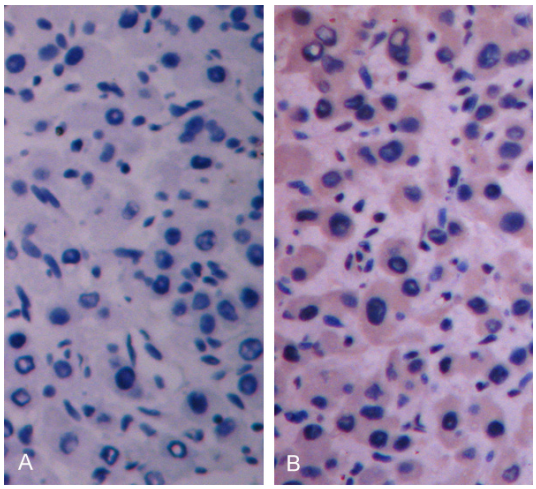

Figure 4. Detection of albumin mRNA in formaldehyde-fixed, paraffin-embedded tissue of the nodule with a digoxigenin-labeled oligonucleotide antisense probe. Note the diffuse and strong hybridization signals in cytoplasm (B) and the much weaker ones in the negative control (A).

panel of immunohistochemistry (IHC), plays a central role on such conditions [1-3]. Adequatetumor tissue and proper process are essential, and a stepwise pathologic algorithm is recommended to predict the primary site efficiently and accurately. First, whether there is a lesion based on clinical context and cell morphology, and if so, is it malignant? Second, what broad type the cancer is, carcinoma, melanoma, lymphoma, or sarcoma? If not distinguishable on morphology alone, then first-line IHC panel including AE1/3 (epithelial marker), S100 (melanocytic marker) and CLA (lymphoid marker) should be used. Third, if carcinoma, whether the subtype is adenocarcinoma, germcell tumor or solid organ tumors? In this case, tumor cell morphology and representative IHC markers play a significant role. Finally, a panel of specific IHC markers was applied to predict the primary site and to avoid errors. Recently, molecular diagnostic tools, ISH based on mRNA for example, have also shown a perfect performance, but are always unavailable [3].

In 2007, Pentheroudakis G et al. [4] analysed 884 CUP patients who underwent autopsy. A primary lesion was identified in 644 patients of them and the most frequent primary sites were lung (27%), followed by pancreas (24%) and liver/bile duct (8%). But to our knowledge, HCC of unknown primary was rarely reported. In fact, extrahepatic HCC without an intrahepatic primary should be interpreted carefully because hepatoid adenocarcinoma (HAC) or HCC originated from ectopic liver can mimic HCC morphologically and biologically [5-7]. HAC is a rare special type of extrahepatic adenocarcinoma that predominantly occurs in stomach with pathological presentation mimicking HCC.

Table 1. Special biomarkers for the detection of hepatocellular differentiation

IHC/ISH stains	Sensitivity	Other tumors commonly positive	Poorly differentiated HCCs
Hep-Par1 [3, 7, 11]	55%-99%, patchy staining in 20% of HCC	Lung, colon, esophageal, and gastric carcinoma, HAC	≤50%
ARG-1 [11, 12]	85.7%-100%	Rare	85.70%
GPC-3 [7, 8, 11, 12]	64%-90%, well-differentiated HCC can be negative.	Nonseminomatous germ cells tumors, squamous cell carcinoma lung, liposarcoma, melanoma, high grade dysplastic nodules, HAC	57%-83%
CD34 [11]	Diffuse sinusoid type, 20%-40%	Focal hyperplasia, hepatic adenomas	Not available
AFP [7, 8, 11, 12]	8.2%-50%	Germ cell tumors, HAC	Negative
p-CEA, CD10 [3, 8, 12]	Canalicular type, 60%-90%	None	25%
Villin [8]	Canalicular type, 20%	None	Not available
mRNA ISH [12, 16]	>95%	HAC	>95%

IHC stains such as MOC-31, CK7, CK19 and CK20 that are generally positive in adenocarcinoma but not in HCC are useful to discern between HAC and HCC [3, 7, 8]. Recently, Abbas SH et al. [9] reported a HCC case that metastasized to the pelvis and vertebrae in which imaging of the liver did not show any primary lesion and the hepatoid tumor cells expressed GPC-3 and Hep-Par1. Nonetheless, MOC-31, CK7, CK19 and CK20 should have been used to rule out HAC.

Ectopic liver is a rare developmental abnormality, mostly found incidentally in various sites near the mother liver including gall bladder, hepatic ligament, omentum, posterior peritoneal cavity, or the thorax. In the absence of complete vascular and ductal system, ectopic liver is vulnerable to carcinogenic factors including viral hepatitis infection or liver cirrhosis and may develop HCC [6]. As for our patient, the tumor nodules mainly located in the right subdiaphragm around the liver. Although laparoscopy disclosed no macroscopic ectopic liver, microscopic ectopic HCC in the right subdiaphram may serve as the origin. Another explanation may be that intrahepatic or superficial microhepatocellular carcinoma has been destroyed by the immune system or regressed spontaneously while the metastatic HCC keeps developing [10].

Special biomarkers for the detection of hepatocellular differentiation were summarized in **Table 1**. As for our patient, in the absence of intrahepatic lesions, immunonegativity for Hep-Par1 and ARG-1, two of the most sensitive markers of hepatocellular differentiation (the reported sensitivities for arginase-1 in well, moderately, and poorly differentiated HCC were 100%, 96.2%, and 85.7%, respectively, and Hep-Par 1 were 100%, 83%, and 46.4%, respectively) [11] might miss the diagnosis of HCC. Whereas negative MOC-31, CK7, CK19, CK20, HCG and OCT4 also lessened the likelihoods of HCC mimickers including HAC, germ cell tumor and neuroendocrine tumor [3, 7]. Noteworthily, serum AFP increased markedly and the liver is cirrhotic. Furthermore, positive AFP, GPC-3, CD34 and albumin mRNA ISH, a new highly sensitive and specific markers of HCC (both sensitivity and specificity are >95%) [12] along with the tumor cell morphology were sufficient enough to establish the final diagnosis. Just like the peculiar patterns of IHC staining, the rapidly progressive course consisted with the features of CUP, because widespread peritoneal metastasis always results from advanced HCC, percutaneous needle biopsy, surgical operation or ruptured HCC [13-15].

In conclusion, peculiar patterns of IHC staining and metastasis as well as progressive clinical course are the common features of CUP. Pathological evaluation, especially a panel of IHC, plays a central role in tissue-of-origin identification in CUP and molecular diagnostic tools should be considered when available. To our knowledge, this is the first reported case of hepatocellular carcinoma without a primary in the liver diffusely affecting the mesentery and peritoneum.

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Disclosure of conflict of interest

None.

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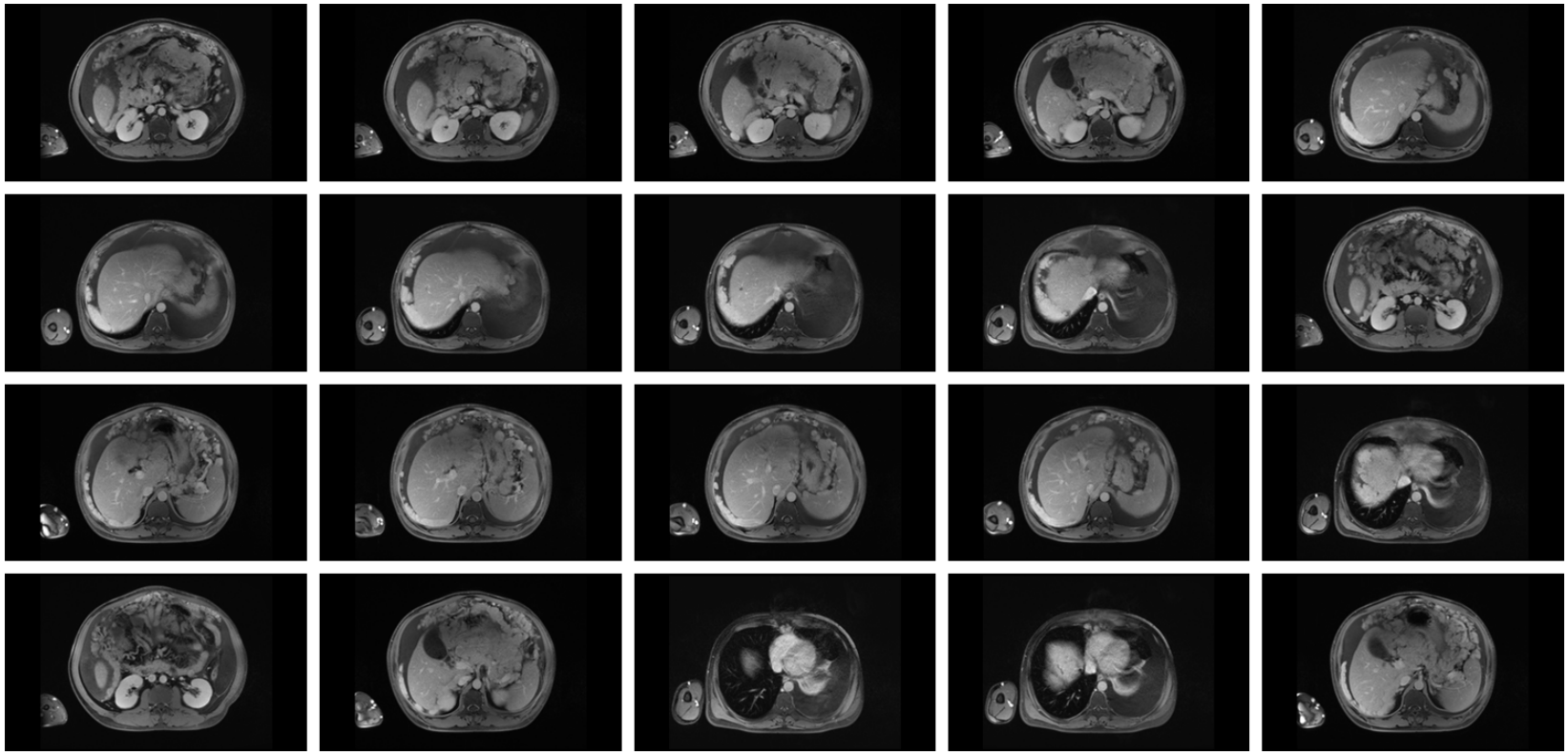

Supplementary Data. Enhanced MRI of the abdomen.