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Analysis of Clinicopathological Features of Dual-Phenotype Hepatocellular Carcinoma

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Abstract: *Objective*: To investigate the clinical manifestations, histopathological features, and differential diagnosis of dual-phenotype hepatocellular carcinoma. *Methods*: Histopathological observation and immunohistochemical study were performed on 3 cases of dual-phenotype hepatocellular carcinoma. *Results*: The tissues of 3 cases of dual-phenotype hepatocellular carcinoma showed irregular, unevenly sized nests and trabeculae. One case was dominated by fibrous stroma with indistinct sinusoids, and 2 cases had obvious sinusoids. The cells were large, polygonal, with strong cell adhesion, relatively clear cell boundaries, and abundant and strongly eosinophilic cytoplasm. The cell nuclei exhibited significant pleomorphism and atypia, with thick nuclear membranes, coarse chromatin in clumps, obvious nucleoli, and visible mitoses. *Conclusion*: Dual-phenotype hepatocellular carcinoma is a unique and highly aggressive subtype of primary liver cancer, which is relatively rare and often occurs in patients with hepatitis and cirrhosis, with abdominal pain as the main clinical symptom. It has the biological behaviors of both hepatocellular carcinoma (HCC) and cholangiocarcinoma, and the clinical prognosis is poor.

Keywords: Dual-phenotype hepatocellular carcinoma; Histopathology; Diagnosis

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1. Introduction

Dual-phenotype hepatocellular carcinoma (DPHCC) was first reported in 2011 [1] and represents a unique and highly aggressive subtype of primary liver cancer. It has been clearly defined as an independent pathological type rather than a simple mixture of hepatocellular carcinoma (HCC) and cholangiocellular carcinoma. The incidence of DPHCC accounts for approximately 10.1% of HCC cases [2,3]. DPHCC can be diagnosed when the tumor exhibits a single histological component of HCC while strongly expressing ≥1 HCC marker and at least one cholangiocarcinoma marker in >15% of cancer cells [4]. Although DPHCC shares some histopathological similarities with typical HCC, it also exhibits distinct features and displays biological behaviors characteristic of both HCC and cholangiocellular carcinoma, leading to a poor clinical prognosis. The "Standardized Pathological Diagnosis Guidelines for Primary Liver Cancer (2015 Edition)" has incorporated DPHCC into routine pathological diagnosis [5]. This study retrospectively analyzed three

cases of DPHCC diagnosed at our hospital to explore the key points of diagnosis and differential diagnosis, immunophenotype, relevant treatment methods, and prognostic characteristics.

2. Objects and methods

2.1. Case selection

Three cases of DPHCC were selected from external examination cases in the Department of Tumor Pathology at Harbin Medical University Cancer Hospital between 2018 and 2025. There were two males and one female, with a male-to-female ratio of 2:1. The patients' ages ranged from 37 to 62 years old, with an average age of 50 years old.

2.2. Methods

Histological observation and immunohistochemical analysis were performed on the three DPHCC cases. Specimens were fixed in 10% neutral formalin, routinely dehydrated, embedded in paraffin, sectioned at 4 μm thickness, and stained with HE for slide preparation and immunohistochemical staining. Routine HE staining and light microscopy were used for observation. Immunohistochemical staining was performed using the EnVision method with DAB chromogen and hematoxylin counterstaining. The antibodies used, including CK7, CK19, CK8/18, AFP, PAX-8, Arginase-1, HSP70, CD34, and Glypican-3 kits, were all purchased from Fuzhou Maixin Biotechnology Co., Ltd. CK19, CK7, AFP, CK8/18, and CK20 were expressed in the cytoplasm; Arginase-1 was expressed in the nucleus and cytoplasm; Glypican-3 was expressed in the cytoplasm and cell membrane; Hepatocyte, HSP70, and CD34 were expressed in the cytoplasm; Ki67 was expressed in the nucleus. Positive expression was indicated by brownish-yellow staining in the corresponding areas, with negative and positive controls established simultaneously.

3. Results

3.1. Clinical manifestations

The clinical manifestations of the cases in this group are as follows:

(1) Case 1: The patient presented with intermittent right upper abdominal pain of no obvious cause 15 days prior to admission. An enhanced MR scan at our hospital revealed a space-occupying lesion in liver segments S4/S5, suggesting liver cancer with portal vein tumor thrombus. The patient was admitted with a diagnosis of "liver space-occupying lesion" and was in generally good condition. During surgery, no ascites was observed in the abdominal cavity, and no implant nodules were found on the peritoneum, pelvis, or greater omentum. The liver surface exhibited mixed-type cirrhosis with both large and small nodules, was dark red in color, and had a hard texture. Lesions were palpable in the left medial and right anterior lower segments of the liver, which were hard and consisted of scattered granular multiple tumors fused into a mass measuring approximately 9×7 cm. A tumor thrombus was palpable in the left hepatic duct. The spleen was enlarged, firm, and measured approximately 10×8×6 cm. A liver segment II, III, IV, and V resection was performed.

(2) Case 2: Outpatient case with incomplete clinical data.

(3) Case 3: The patient underwent a color ultrasound examination at an external hospital 6 days prior, which revealed multiple liver nodules, diffuse cirrhosis, cholecystitis, multiple gallstones, and splenomegaly.

An enhanced CT scan showed a nodule in liver segment S8, suggesting nodular carcinogenesis and splenomegaly with nodular cirrhosis. During surgery, no ascites was observed in the abdominal cavity, and no implant nodules were found on the peritoneum. The liver surface exhibited mixed-type cirrhosis with both large and small nodules, was dark red in color, and had a hard texture. Imaging revealed a tumor measuring approximately 16 mm in the parenchyma of liver segment S8. The gallbladder contained multiple stones. The spleen was significantly enlarged, and a total splenectomy, cholecystectomy, and ultrasound-guided radiofrequency ablation of the liver tumor were performed.

3.2. Gross examination of specimens

(1) Case 1: The resected specimens included liver segments II, III, IV, and V, along with the gallbladder. The liver measured 103 mm × 60 mm × 50 mm, and a grayish-white rough area measuring 60 mm × 40 mm was visible on the liver capsule. Upon incision, a tumor measuring 60 mm × 60 mm × 30 mm was observed beneath the rough area, which was adherent to the gallbladder.

(2) Case 2: The submitted specimen was a cord-like tissue with a total volume of approximately 2 mm × 2 mm × 1 mm and was grayish-white in color.

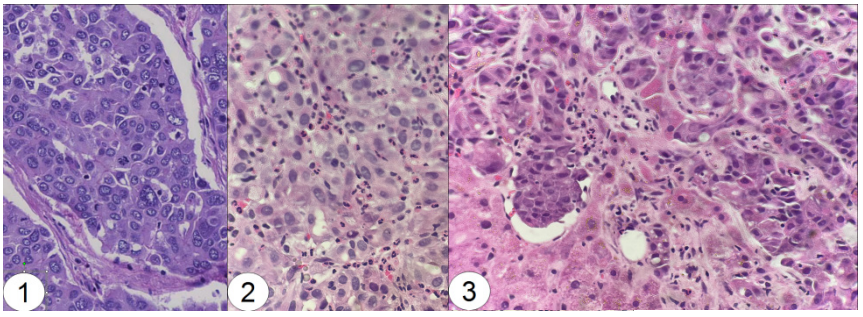
(3) Case 3: The submitted specimen was a cord-like tissue with a total volume of approximately 7 mm × 1 mm × 1 cm and was grayish-white in color.

3.3. Microscopic findings

The cancerous tissue appeared as irregular, variably sized nests and clusters, predominantly composed of sinusoidal stroma. Some cancerous tissues exhibited obvious fibrous stroma, indicating the morphological diversity of DPHCC. The cells were large and polygonal, with strong cell adhesion, clear cell boundaries, abundant cytoplasm, and intense eosinophilia. The nuclei showed significant pleomorphism and atypia, with thick nuclear membranes, coarse chromatin, clumped appearance, distinct nucleoli, and visible mitotic figures. Infiltrative growth was observed at the edge of the tumor tissue into the surrounding normal tissue.

3.4. Immunohistochemistry

All three cases of DPHCC showed diffuse expression of CK7 and CK19. One case expressed AFP, two cases expressed Hepatocyte, and one case expressed HSP70. Arginase-1 was not expressed.



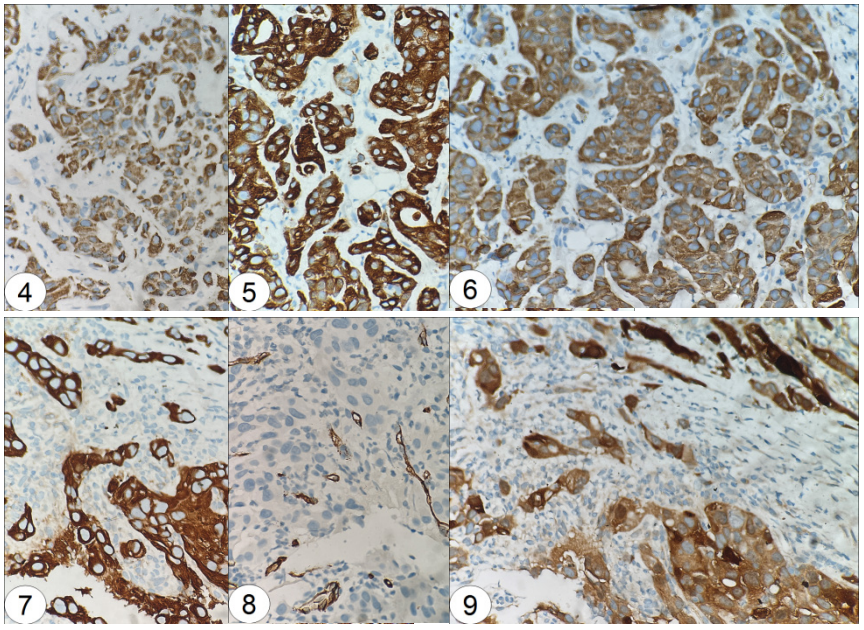


Figure 1. Tumor cells are arranged in nests and clusters with sparse interstitial fibers; **Figure 2.** Diffuse, ill-defined nests and clusters; **Figure 3.** Tumor tissue exhibits infiltrative growth into surrounding normal liver tissue; **Figure 4.** Diffuse cytoplasmic expression of Hepatocyte; **Figure 5.** Diffuse cytoplasmic expression of CK7; **Figure 6.** Diffuse cytoplasmic expression of CK19; **Figure 7.** Diffuse cytoplasmic expression of CK8/18; **Figure 8.** Interstitial sinusoids are not prominent; **Figure 9.** Diffuse cytoplasmic expression of HSP70.

4. Discussion

4.1. Clinical characteristics and pathogenesis

In China, the high prevalence of hepatitis leads to a relatively high incidence of hepatocellular carcinoma (HCC), which is characterized by high malignancy, recurrence, and mortality rates. Liver cancer ranks fifth in incidence and second in mortality among the most common malignancies in China [6]. The postoperative recurrence rate is as high as 70%, with most recurrences occurring within two years after surgery [2]. Clinically, DPHCC presents similarly to ordinary HCC without specific symptoms, commonly including right upper abdominal discomfort, pain, weight loss, and fatigue. Serum alpha-fetoprotein (AFP) levels are typically significantly elevated in patients. Some patients may also exhibit elevated CA19-9 levels, suggesting the presence of cholangiocellular differentiation components. On CT or MRI, DPHCC often manifests as atypical liver lesions, lacking the typical "fast-in, fast-out" enhancement pattern of HCC. Instead, it may show inhomogeneous arterial enhancement and persistent enhancement in the portal or delayed phases, resembling cholangiocarcinoma. The vast majority of HCC cases occur based on viral hepatitis and cirrhosis, with a strong correlation with hepatitis B virus infection (especially in Asia). Similar to conventional HCC, DPHCC predominantly affects middle-aged and elderly males. Therefore, when histopathological classification is challenging, especially with small biopsy specimens, clinical data, including AFP and CA19-9 levels, play a crucial role in accurate diagnosis. The incidence of DPHCC is higher in males than in females [7], which aligns with our data. DPHCC is a novel subtype of HCC first reported in 2011, accounting for approximately 10.1% of all HCC cases [3,4], although some reports suggest an incidence of 1–5%. Based on the number of cases retrieved from our hospital, the incidence is closer to

the latter estimate. The World Health Organization classifies primary liver cancer into three categories: HCC, intrahepatic cholangiocarcinoma (ICC), and mixed hepatocellular-cholangiocarcinoma [8]. DPHCC is a new subtype separated from within the HCC category. The pathogenesis of DPHCC remains unclear, with two main hypotheses proposed. The first hypothesis suggests that DPHCC originates from hepatic progenitor cells (HPCs), which possess bidirectional differentiation potential into hepatocytes and cholangiocytes and serve as liver reserve cells [9]. This indicates their stem cell-like properties: Lee JS et al. [10] pioneeringly defined liver cancer expressing cholangiocellular markers (such as CK19) and stem cell markers (such as EpCAM) as a "hepatic progenitor cell-derived" subtype in 2006, revealing the cellular origin basis of its malignant biological behavior. Since DPHCC tumor cells can simultaneously express HCC and cholangiocellular markers, this hypothesis suggests that DPHCC tumor cells may originate from HPCs with bidirectional differentiation potential. The second hypothesis proposes dedifferentiation of HCC. Previous studies have found that although CK19 can be expressed in some HCC cells, it is negative in HCC precancerous lesions. Cells expressing CK19 are mature hepatocytes that gradually dedifferentiate during malignant transformation rather than originating from HPCs [11].

4.2. Histopathology and immunohistochemistry

DPHCC shares some histopathological similarities with typical HCC. Well-differentiated DPHCC cells may form glandular, pseudoglandular, or delicate trabecular patterns; moderately differentiated DPHCC may exhibit relatively thick trabeculae; and poorly differentiated DPHCC may present as solid nests or sheets with indistinct trabecular structures. Hepatic sinusoid-like vascular networks are visible around the tumor, with minimal fibrous interstitium.

Tumor cells are typically polygonal or cuboidal with eosinophilic or clear cytoplasm, well-defined borders, abundant cytoplasm, and strong eosinophilia. Nuclear atypia ranges from moderate to severe, with common mitotic figures. In glandular regions, cells may appear columnar, resembling cholangiocytes. The tumor interstitium may be rich in fibrous connective tissue, similar to the "desmoplastic" reaction seen in cholangiocarcinoma, which differs from ordinary HCC. Accurate diagnosis of DPHCC requires immunohistochemical detection. When a tumor has a single HCC cellular component and strong positivity for one or more hepatocyte markers is observed in more than 15% of tumor cells, along with strong positivity for one or more cholangiocellular markers, DPHCC can be diagnosed. HepPar-1 typically shows strong, diffuse cytoplasmic granular staining, representing one of the most specific hepatocyte markers. Arginase-1 exhibits nuclear and cytoplasmic positivity with high sensitivity and specificity. AFP may be positive but is usually focal. Cholangiocellular markers (at least two positives), with CK7 and CK19 being the most commonly used combination.

4.3. Treatment and prognosis

Literature universally indicates that dual-phenotype liver cancer represents a "bottleneck" in liver cancer treatment, showing resistance to existing therapeutic approaches. Therefore, accurate pathological diagnosis and classification are crucial for prognosis assessment and the exploration of new treatment strategies. Currently, the primary treatment for DPHCC patients is complete surgical resection, supplemented by immunotherapy, targeted therapy, and chemotherapy when necessary. The overall survival and recurrence-free survival of DPHCC patients are lower than those of ordinary HCC patients [3]. According to studies by

Kim H et al. and several similar investigations, CK19 positivity (especially when co-expressed with CK7) is an independent risk factor for poor prognosis in liver cancer patients, closely associated with high tumor aggressiveness, vascular invasion, and chemotherapy resistance. Overall, the prognosis is extremely poor. Numerous studies confirm that the prognosis of dual-phenotype liver cancer is significantly worse than that of ordinary HCC and combined hepatocellular-cholangiocarcinoma. Its aggressive behavior is characterized by higher early recurrence rates, higher lymph node metastasis rates, and a greater tendency for distant metastasis. Patients' overall survival is significantly shortened.

5. Differential diagnosis

5.1. Combined hepatocellular-cholangiocarcinoma

In combined carcinomas, hepatocellular and cholangiocellular components are spatially separated and coexist, containing distinct histological components of HCC and cholangiocarcinoma, each expressing their respective immunomarkers [12,13]. In contrast, dual-phenotype liver cancer consists of a single tumor cell population with dual hepatocellular and cholangiocellular phenotypes, reflecting tumor cell dedifferentiation and stem cell-like properties.

5.2. Classic hepatocellular

Carcinoma with CK19 Expression Approximately 10-30% of ordinary HCCs may focally express CK19 ("stem cell-related subtype"), but they typically do not express or only express CK7 in a minority of cells. Dual-phenotype liver cancer requires co-expression of CK7 and CK19.

5.3. Intrahepatic cholangiocarcinoma

Cholangiocarcinoma expresses CK7 and CK19 but does not express HepPar-1 and Arginase-1. Occasionally, weak positivity or false positivity for HepPar-1 may occur, but Arginase-1 exhibits extremely high specificity.

5.4. Metastatic hepatoid adenocarcinoma

Metastatic hepatoid adenocarcinoma is a rare, poorly differentiated adenocarcinoma with histological and immunohistochemical similarities to HCC. Although its histological and immunophenotypic features overlap with DPHCC, metastatic hepatoid adenocarcinoma originates from organs outside the liver and typically expresses SALL4, allowing differentiation from DPHCC [14].

6. Conclusion

Dual-phenotype hepatocellular carcinoma is a unique and highly aggressive subtype of primary liver cancer. According to the 2019 WHO Classification of Tumours of the Digestive System, it is clearly defined as an independent pathological type rather than a simple mixture of HCC and cholangiocarcinoma. Its most significant feature is the simultaneous expression of HCC and cholangiocarcinoma immunophenotypes by the same tumor cell population. Compared to ordinary HCC, DPHCC exhibits higher malignancy and worse prognosis. It is associated with higher rates of microvascular invasion, intrahepatic and extrahepatic metastasis, and postoperative recurrence. The classification of DPHCC is of great significance for precise treatment and prognosis assessment in HCC patients. Due to the limited extent of current research on

DPHCC, misdiagnosis or missed diagnosis often occurs in clinical practice.

Disclosure statement

The authors declare no conflict of interest.

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