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Original Article

The clinicopathological features of combined primary hepatic adenosquamous-hepatocellular carcinoma

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Abstract: Objective: To explore the pathological features of combined primary hepatic adenosquamous carcinoma (ASC) and hepatocellular carcinoma (HCC). Methods: The clinicopathological data of one case of cASC-HCC was collected, and the features were analyzed through a literature review. Results: The male patient was 59 years old and had suffered from inconsistent, upper abdominal pain without any obvious cause for one year. MRI and B-Mode ultrasound images of the upper abdomen showed abnormal signals in the posterior segment of the right lobe of the liver, measuring 12.2 × 7.7 cm. A right hepatectomy was performed. A gross examination revealed an irregular, gray-white infiltrating growing mass, with a partially grayish-yellow area. The histological morphology and immunohistochemical results showed that the tumor was composed of ASC in the gray-white area, accounting for about 80%, and HCC in the grayish yellow area, accounting for about 20%. Conclusion: cASC-HCC is a rare, malignant tumor with high rates of recurrence and metastasis. It mainly occurs in the right lobe of the liver, especially in older men with a history of hepatitis or intrahepatic cholangiolithiasis. Surgery is the main treatment method.

Keywords: Primary hepatic adenosquamous carcinoma, hepatocellular carcinoma, hepatolithiasis, hepato-cholangiocarcinoma, cholangiocarcinoma

Introduction

Primary intrahepatic adenosquamous carcinoma (ASC) is a rare subtype of cholangiocarcinoma (CCA) according to the *WHO Classification of Digestive System Tumors*, Version 5. Combined adenosquamous-hepatocellular carcinoma (cASC-HCC) is an extremely rare disease defined by the unequivocal presence of both hepatocytic and adenosquamous differentiation within the same tumor. Here we report one case of cASC-HCC, and the clinicopathologic features of this tumor are reviewed in the literature.

Materials and methods

Clinical data collected

In our study, one case diagnosed as cASC-HCC in the liver was obtained from the Department of Pathology, Yantai Yuhuangding Hospital. The clinical data, including the follow-up data, were collected.

Sample process

Tissue samples were immersed in 10% buffered formalin for complete fixation. Subsequently, tissue dehydration and paraffin embedding were carried out, and 3-5 μm sections were cut from tissue blocks for hematoxylin and eosin staining.

Immunohistochemical staining

The EnVision two-step method was adopted by an automatic immunostainer (VENTANA) for immunohistochemical staining and DAB chromogen. Each slice was stained with known positive tissues as the positive control, while negative controls replaced the first antibody with PBS. All the antibodies were bought from the Beijing Zhongshan Jinqiao Biological Technology

Table 1. Results of the immunohistochemical study

Antigen	Antibody/clone	Dilution	HCC	Adenocarcinoma component of ASC	Squamous cell carcinoma component of ASC
α-fetoprotein	EP209	1:100	-	-	-
Hepatocyte	OCH1E5	1:100	+	-	-
Glypian-3	MAXIM001	1:100	+	-	-
P63	4A4	1:100	-	-	+
P40	polyclone	1:100	-	-	+
Cytokeratin19	A53-B/A2.26	1:100	-	+	Focal+
CAM5.2	CAM5.2	1:100	+	+	+
Cytokeratin 8/18	5D3	1:100	+	+	-
Cytokeratin 7	OV-TL12/30	1:100	-	+	-
CEA	Zc23	1:100	-	-	-
CD56	123c.D5	1:100	Focal+	Focal+	Focal+
Chromogranin A	SP12	1:100	-	-	-
Synaptophysin	SP11	1:100	-	-	-
KI-67	MIB-1	1:100	40%	30%	60%

HCC stands for hepatocellular carcinoma; ASC stands for adenosquamous carcinoma.

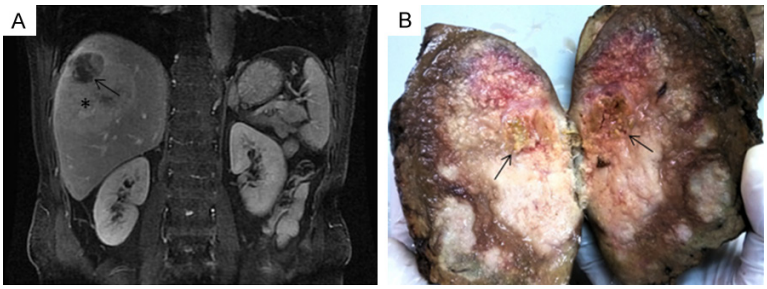

Figure 1. A. MRI showing a significantly enhanced mass in the parenchyma of the liver (* in the area), in which there is a relatively low-enhanced nodule (shown by the arrow). B. The gross examination revealed a gray-white mass of the liver, within a yellow area (shown by the arrow).

Co., Ltd. Information on the antibodies is included in **Table 1**.

Results

Clinical data

The patient was male, 59 years old. He was admitted to our hospital because of "epigastric pain and discomfort for one year, aggravated for one month". The pain was mainly under the xiphoid process and occurred frequently at night. The patient underwent a cholecystectomy in the local hospital 30 years ago because of cholecystolithiasis. He had a history of hepatolithiasis and had no special physical examination. An MRI of the upper abdomen showed a nodular mixed long T1 signal in the posterior segment of the right lobe of the liver. The boundary was not clear. The diffusion sequence showed a slightly high signal intensity, and the size of the focus was about 12.2 × 7.7 cm, while inside the nodule a relatively low density was seen, about 2.5 × 2.2 cm (**Figure 1A**). An enhanced scan showed an abnormal enhancement signal in the shape of a marginal rosette, significantly enhanced heterogeneity in the arterial phase, and low heterogeneity in the vein phase after a 4-5 min delay, leading us to consider the possibility of intrahepatic cholangiocarcinoma. Tumor marker AFP: 20.31 ng/ml (normal: 0.0-7.02 ng/ml), CA19-9: 221.8 U/ml (normal: 0.0-39.0 U/ml). The patient underwent a routine preoperative examination: chest and abdomen CT, abdominal ultrasound, gastrointestinal endoscope examination and PET-CT, and nothing abnormal was detected.

Gross examination

A right hepatectomy was performed. The postoperative specimen (**Figure 1B**) showed a gray-white mass with a size of about 11.5 cm × 10 cm, and a grayish-yellow tubercle area on one side about 2.8 cm × 2 cm, hard and unclear.

Histological findings

A microscopic observation the tumor cells of the yellow region (* of the **Figure 2A** and **2B**) showed a thin trabecular pattern and cytological characteristics such as abundant eosinophilic cytoplasms, central nuclei, prominent nucleoli and large cytoplasmic hyaline eosinophilic globules (**Figure 2C**). The tumor cells of the gray-white mass (# of the **Figure 2A** and **2B**) were arranged like nests (**Figure 2E**), reticulate (**Figure 2F**), glandular (**Figure 2G** and **2H**) structures in the abundant fibrous stroma, and keratinized beads could be seen in the center of some nests, with significant cytological atypia (**Figure 2D**).

Immunohistochemical staining

The immunohistochemical results are listed in the **Table 1**, and some characteristic markers are shown in **Figure 2**.

The immunohistochemical results of the nest structures P40/P63 (**Figure 2J**) were positive. These are typical squamous carcinoma components with a conjugated histologic morphology. The tubular gland structures were P40/P63 negative and the CK7 (**Figure 2K**)/CK19 tested positive, supporting the diagnosis of adenocarcinoma. The tumor cells in the yellow region were the typical HCC components showing the characteristic marker hepatocytes (**Figure 2I**), while the tumor cells in the gray-white area were ASC components. We also found that there were transitional cell regions between the typical HCC and the ASC, where tumor cells with abundant cytoplasms and chromatin powder staining were located in the same nest mass and mistaken for typical squamous cell carcinoma. Immunohistochemical staining does not express either the markers of hepatocytes or the marker of squamous cell carcinoma - P40 ($ of the **Figure 2O**), but P63 is weakly positive ($ of **Figure 2P**). In contrast, it is clear that the P40/p63 of squamous cell carcinoma is strongly positive (& of the **Figure 2O** and **2P**). No glandular tube structure was found in the transitional zone, CK7/CK19 was negative, and CD56 was partially positive. CD56 (**Figure 2L**) has been reported as one of the markers of hepatic progenitor cells in the literature. In this case, focal positivity was found in HCC, ASC, and the transitional area.

Final diagnosis

Postoperative pathological diagnosis (right half of the liver): Combined primary hepatic adenosquamous carcinoma (accounts for about 80%) and hepatocellular carcinoma (accounts for about 20%); hepatolithiasis. There was no other treatment after the operation. This patient died of intrahepatic recurrence and multiple organ metastasis, including the lungs, the bones of the right shoulder, and the lumbar vertebra after a 6 month follow-up. Combined hepatocellular carcinoma and primary hepatic adenosquamous carcinoma (cHCC-ASC) are extremely rare, and their mechanisms are still not fully understood.

Discussion

According to our PubMed research for "hepatic adenosquamous carcinoma and hepatocellular carcinoma," only two other cases have been recorded in the English-language medical literature. Therefore, we reviewed the three studies (**Table 2**) (including this case) and attempted to identify the clinicopathological features of this tumor [1, 2].

Case 1

A 65-year-old Japanese man was admitted to the hospital with a history of epigastric pain for several months. He had a previous history of cholecystectomy and Vater papillomatosis, for which he surgery 40 years ago because of suppurative cholangitis and Vater papilloma stenosis. Alpha-fetoprotein (AFP) 7.8 ng/ml; carcinoembryonic antigen (CEA) 8.7 ng/ml; carbohydrate antigen 199 (CA19-9) 149.5 ng/ml; hepatitis B surface (HBs) antigen and antibody (-); hepatitis C virus (HCV) antibody (+). An axial CT scan of the abdomen revealed a tumor of 6 cm in diameter located in the left lobe of the liver. There were no metastatic lesions in the liver or the distant organs. A pathological examination of the resected tumor showed that it was squamous cell carcinoma and it partially contained adenocarcinoma. A few months later, the tumor recurred at the edge of the operation. The patient received chemotherapy but died because the recurrent tumor quickly metastasized to his stomach, heart, and lymph nodes. A full autopsy was performed 1 hour and 37 minutes after his death. Tumor recur-

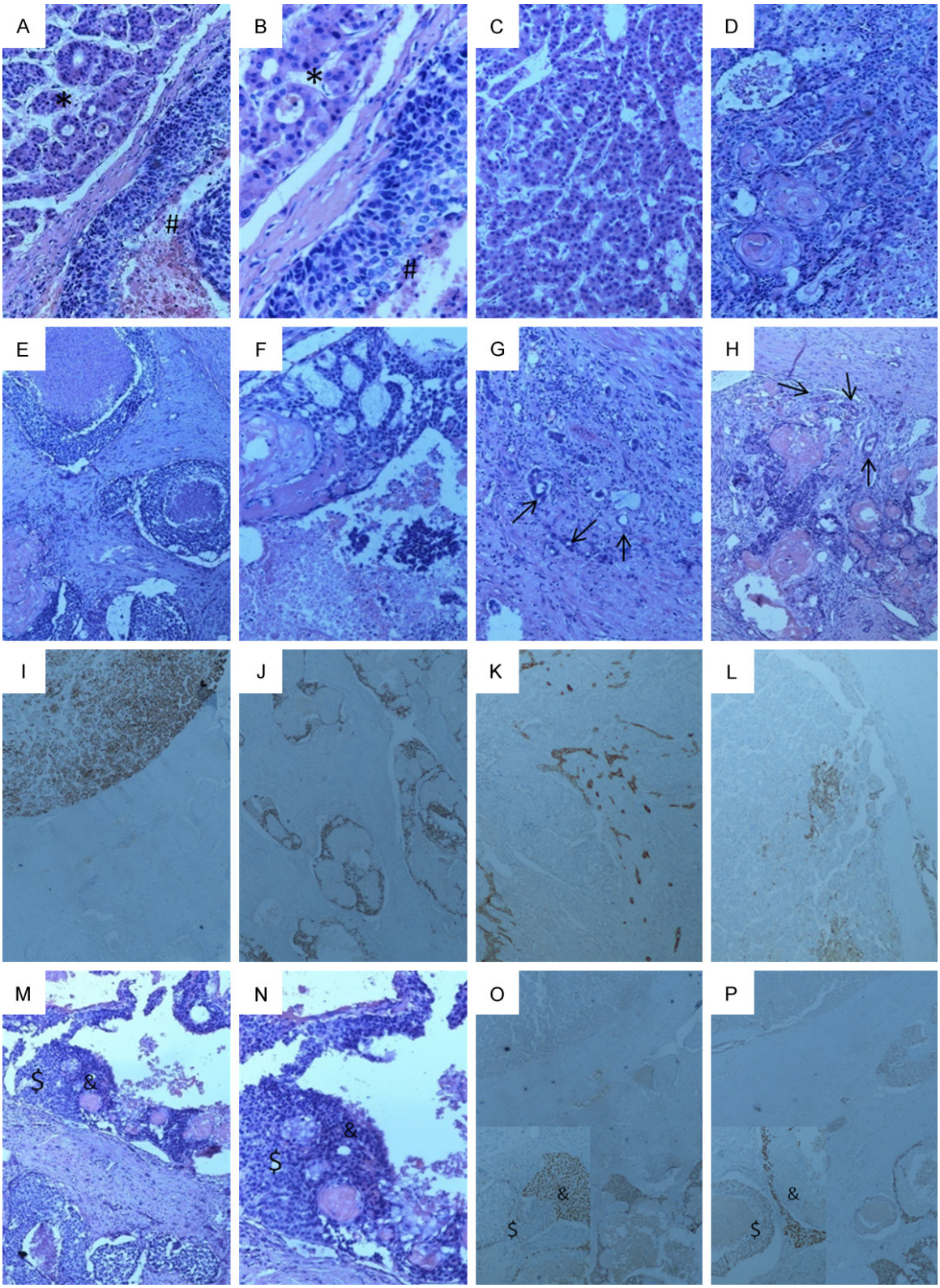

Figure 2. (A) The gray-white area and the gray-yellow area can be seen under the microscope, hepatocellular carcinoma in the lower left corner (#), squamous cell carcinoma in the upper right corner (*). (B) is a magnification of (A, C) trabecular or a pseudoglandular structure in the hepatocellular carcinoma. (D) The image shows typical squamous cell carcinoma, and keratinized beads can be seen in the centers of cells. (E and F) show a nest-like growth pattern and ethmoidal structure, with central necrosis. (G and H) show adenocarcinoma differentiation

where well-formed glandular tubules are rarely seen (arrow) and most of the ill-formed glandular structures may be present in a desmoplastic stroma with the athpical epithelium. Some characteristic immunohistochemical markers Hepa (I), p63 (J), and CK7 (K) respectively showed positive staining in hepatocellular carcinoma, squamous cell carcinoma, and adenocarcinoma. CD56 (L) was localized positive in all three components mentioned above. (M) The transitional carcinoma ($) and squamous carcinoma (&) are shown in (M and N) (magnification figure). P40 (O) and p63 (P) respectively show negative and weakly positive cells in the transitional carcinoma cells ($), and CK7 is all negative (no picture is shown). P40 (O) and p63 (P) were strongly positive in typical squamous cell carcinoma (&), and the figure has all the high magnifications in the lower left corner.

Table 2. Summary of the three cases of combined primary hepatic adenosquamous carcinoma and hepatocellular carcinoma in the literature

No	Age/sex	Clinical presentation	History	Hepatitis surface antigen	Location/color/size	outcome
1	65/m	epigastralgia	Cholangitis, Vater's papilla	HBV (-), HCV (+)	S6, 8 cm, gray-white; S7,3 cm, yellow	Multiple organic metastasis, died after two years
2	76/m	epigastralgia	Alcoholic liver disease	HBV (-), HCV (-)	Left lobe, 4.5 cm, gray-white; caudate lobe, 2.5 cm, yellow	Lymphatic metastasis after three months
3 (this case)	59/m	epigastralgia	Liver nodular cirrhosis	HBV (-), HCV (-)	S7/S8, 11.5 cm, gray-white/ yellow	Multiple organic metastasis, died after six months

rence was seen at the resection margin of the original liver, which was gray-white and hard, with a size of 8 cm × 7 cm × 6 cm. The tumor invaded the stomach, diaphragm, the left adrenal gland, the pericardium, and the heart, and the peripancreatic lymph nodes had extensive metastasis. At the same time, a gray-white mass with a diameter of 5 mm was seen in the right lobe of the liver, located in S6; a gray-yellow mass was found not far away, measuring about 3 cm in diameter, located in S7, with common bile duct dilatation, but no stones were found. No primary cancer was found in the other organs. Under the microscope, the main tumors have two different histological forms, squamous cell carcinoma and adenocarcinoma. S6 shows the same histological features, and S7 shows the typical histological features of moderately differentiated hepatocellular carcinoma. The liver tissue outside the tumor showed chronic active hepatitis with fibrosis and interlobular bile duct dilatation.

Case 2

A 76-year-old male patient was transferred to the hospital for treatment of a liver tumor. The patient's chief complaint was epigastric pain. He had a history of chronic liver disease secondary to alcoholism and consumed more than 50 grams of alcohol a day. Alpha-fetoprotein 363.2 ng/ml (normal: 10 ng/ml), carbohydrate antigen 19-9 285 U/ml (normal: 37 U/ml), the patient was negative for hepatitis B virus surface antigens and hepatitis C virus antibodies. A dynamic CT showed 2 cases of intrahepatic tumors. The first case was a low-density tumor with a diameter of 4 cm and an irregular enhancement at the edge of the left lateral area. The other was 2.5 cm in diameter and was located in the caudate leaf. Magnetic resonance and cholangiopancreatography showed intrahepatic bile duct dilatation with multiple cystic lesions. A left hemihepatectomy with a caudate lobectomy was performed. The section on the left side of the tumor was gray-white with a soft texture, a size of 4.5 cm × 0.9 cm, a clear edge, and no fibrous capsule. The caudate lobe showed a yellow solid tumor, the size of which was 2.4 cm × 0.9 cm × 1.8 cm. A microscopic examination showed that alcoholic hepatitis caused scattered inflammatory cell infiltration in the hepatocytes. The left tumor showed two components of moderately differentiated tubular adenocarcinoma and highly differentiated squamous cell carcinoma. This part accounts for more than 80% of tumors. The composition of primary carcinoma in situ in cystadenoma is very similar to adenocarcinoma. In addition, there is a transitional zone between adenocarcinoma and squamous cell carcinoma. The tumor invades the blood vessels, biliary tract and lymphatic vessels. Microcystic lesions can be seen around the biliary tract. There was no continuity between peribiliary cyst and the adenosquamous carcinoma and cystadenoma. The manifestations of caudate lobe tumor are consistent with the typical characteristics of moderately differentiated hepatocellular carcinoma. About 3 months after the operation, an abdominal CT showed enlarged lymph nodes in

the abdominal paraaortic lesions. PET also detected an increase in the 18F-FDG uptake in the enlarged lymph nodes (maximum standard uptake value: 9.5). The patient received systemic chemotherapy including gemcitabine and cisplatin.

The most common primary epithelial tumors of the liver are HCC and CCA. Some articles have reported that cHCC-CCA occurs in the liver with the same tumor by the unequivocal presence of both hepatocytic and cholangiocytic differentiation entities [3]. It is a rare tumor with a poor prognosis, and its incidence makes up 1.0%-4.7% of all primary hepatic tumors [4]. This will be soon renamed Hepato-cholangiocarcinoma. Liver ASC was first reported by Barr et al. in 1975 and has special histological features. Its general shape is mostly a single tumor nodule, and it is gray-white, moderately hard, and there are more cysts in the center. The etiology is also controversial. Most researchers found that the patient often has a history of hepatitis, cirrhosis, or liver abscess [5]. It is generally believed that it is related to hepatic teratoma, hepatolithiasis, and simple benign non-parasitic hepatic cysts, which may be caused by the occurrence of squamous cell metaplasia from the intrahepatic bile duct or the cyst wall lined with cuboidal or columnar epithelia under the stimulation of chronic inflammation, followed by the development of atypical hyperplasia, leading to the occurrence of adenosquamous carcinoma [6]. The origin of hepatic ASC is not clear, and some scholars believe that intrahepatic bile duct cells are stem cells with multipotential differentiation, and adenosquamous carcinogenesis occurs gradually under the action of the carcinogenic factors such as inflammatory stimulation and biliary tract infection [7]. Some studies indicate that squamous cell carcinoma arising in a ciliated hepatic foregut cyst (CHFC) [8].CHFC is a very rare disease of liver cystic abnormalities and may be liver diverticulum primordium residues or nearby intestine independent bud formation, similar to bronchial and esophageal cysts [9]. CHFC has long been considered a benign tumor lesion, but there are three cases of malignancy. Microscopically, the columnar epithelia have undergone squamous metaplasia, and they proliferate abnormally, eventually becoming cancerous [10, 11].

As liver ASC was a rare subtype of intrahepatic CCA in the WHO 2010 classification, our case, which has a mixed growth of HCC and primary ASC, should be subsumed into the classification of combined hepatocellular-cholangiocarcinoma (cHCC-CCA), an extremely rare disease. A malignant transformation of the liver progenitor cells occurs, and the cells differentiate completely and incompletely to HCC and ASC. The collision tumor, arising independently and separately in the same entity without an intimate relationship, is not categorized as cASC-HCC. This example of combined ASC and HCC is a quite rare case. Presumably it involves hepatic progenitor cells of divergent differentiation directions [12].

This type of tumor is mostly located in the right lobe of the liver, includes two different colors of gross examination, with no capsule or cystic lumen in the center. The serum AFP and CEA were not high in the laboratory tests, and the ca19-9 level was abnormally high. B-ultrasound or CT often show a low-density shadow or cyst-like changes in the liver. The CT and MRI findings are not easily differentiated from biliary cell carcinoma or hepatocellular carcinoma [13]. The symptoms and imaging manifestations of mixed hepatocellular carcinoma include lack of specificity, and the diagnosis often depends on the pathological examination, and the immunohistochemistry must distinguish between primary adenosquamous carcinoma and a metastatic tumor.

The main disease outcomes are intrahepatic recurrence and lymph node, lung and bone metastases. The factors affecting the prognosis of the surgical treatment of mixed hepatocellular carcinoma are slightly different in different published studies. They mainly include the degree of cell differentiation, macrovascular invasion, positive incisal margins, lymph node metastasis, tumor locations, tumor size, number of tumors, increased components of cholangiocarcinoma, and elevated tumor markers [14]. This study shows that the history of hepatolithiasis and the invasion of the liver capsule are adverse factors affecting the prognosis, and postoperative transcatheter arterial chemoembolization treatment helps prolong patients' overall survival times. Tumors invading the liver capsule have the advantages of late TNM stages, easy recurrence, and metastasis, which are also unfavorable prognostic factors in hepatocellular carcinoma, and some long-term alcoholics also suffer from hepatitis B virus infections, severe liver cirrhosis, and

low liver reserve capacity, which can also lead to tumor recurrence [15].

Surgical resection is the main treatment for mixed liver cancer. Lymph node dissection should be performed routinely in patients with suspected mixed liver cancer or hilar lymph node enlargement during the operation. The efficacy of systemic chemotherapy and liver transplantation are still controversial, but they may benefit some patients. In recent years, it has been reported that local treatments, such as microwave ablation, can benefit patients with primary liver cancer, but there is still a lack of clinical research specifically for mixed liver cancer.

A greater understanding of the clinical characteristics and prognostic factors of mixed liver cancer needs to be developed by studying additional cases. In a word, it is still difficult to diagnose liver cASC-HCC before the operation. Clinical experience should be constantly relied on to strive for early diagnoses and early treatment. Especially for elderly patients, liver ASC should be highly suspected if it conforms to the relevant characteristics. Strengthening the research in this area and seeking related treatment targets may help to improve the diagnosis and treatment of the disease. At present, there is a lack of genetic and molecular research, so we look forward to more in-depth study on this disease.

Disclosure of conflict of interest

None.

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