Pathology of Hepatocellular Carcinoma

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6.1 Introduction

Hepatocellular carcinoma (HCC) is a primary malignant tumor of the liver consisting of neoplastic hepatocytes. Our understanding of this neoplasm has evolved in recent years and new histological variants and new molecular alterations have been described. By means of new immunohistochemical staining it is now possible to evaluate the response to immunotherapy making the pathologist instrumental in the development of personalized therapies. HCC can affect any age and both sexes. It arises in cirrhotic and non-cirrhotic livers, the former being more frequent. Common risk factors are viral hepatitis C and B, chronic vascular diseases of the liver, alcohol consumption, non-alcoholic steatohepatitis, primary hemochromatosis and malignant transformation of an adenoma. Fibrosis is certainly one of the key factors in the development of HCC, but numerous other factors are yet unknown. The discovery of new molecular signatures in HCC, associated with distinct macroscopic growth patterns, have allowed a molecular classification of this neoplasm.

6.2 Main Gross Pattern of Hepatocellular Carcinoma

The size of HCC can vary greatly, ranging from a diameter of less than 2 cm to very large tumors with a diameter greater than 20 cm that can replace an entire lobe of the liver.

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There are essentially three main macroscopic patterns: nodular, massive, and diffuse or cirrhotomimetic. Related to the hepatectomy specimens, the Liver Cancer Study Group of Japan (LCSGJ) proposed to divide the nodular type into two subclasses: distinctly nodular and vaguely nodular [1]. The former has been further divided by Shimada et al. into simple nodular, simple nodular with extranodular growth (Fig. 6.1) and confluent multi-nodular [2].

- **Early/small HCC** (E-HCC) is defined as a hepatocarcinoma ≤2 cm with distinct margins. The concept of small size emphasizes the diameter of the neoplasm and not an early stage of hepatocarcinogenesis. Macroscopically, they can be single nodular, single nodular with extracapsular growth, confluent multinodular and vaguely nodular [3]. Microscopically, E-HCCs are characterized by a population of well-differentiated neoplastic cells of small or medium hepatocyte-like size; increase in the density of the nuclei (crowding); thin trabecules; pseudoglands with or without bile. E-HCCs can contain poorly differentiated areas that influence the prognosis (nodule-in-nodule). E-HCCs are potentially invasive malignant neoplasms that infiltrate the adjacent parenchyma, invade blood vessels, and metastasize. Compared to the classic HCC they have better differentiation and the disease-free interval is longer with a low recurrence rate. Main risk factors for relapses are the absence of a capsule, vascular microinvasion, and poor cellular differentiation.
- **Nodular HCC** is a well-circumscribed neoplasm of spherical or ovoid shape, characterized by well-defined margins and expansive growth pattern. This HCC often arises in the context of a cirrhotic liver and is often formed by several juxtaposed nodules that can have different colors from yellowish, reddish, to green (Fig. 6.2). This color variation may be due to various factors such as bile content, areas of necrosis and hemorrhages. The texture can range from soft, crumbly, to firm and depends on the extent of necrosis and on stromal reaction.

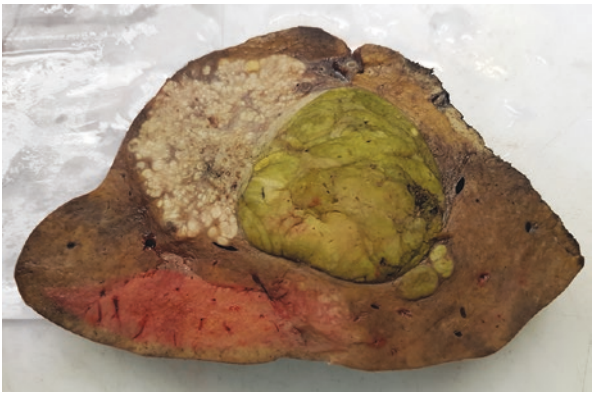

Fig. 6.1 Nodular hepatocellular carcinoma (HCC) with dominant encapsulated nodule with bile accumulation and extracapsular growth with adjacent confluent multinodular HCC

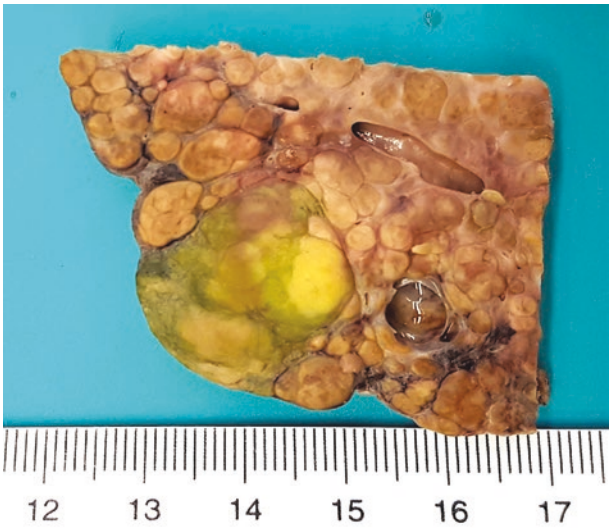

Fig. 6.2 Subcapsular nodular hepatocellular carcinoma in a cirrhotic liver with expanding growth pattern and bile accumulation

- **Massive HCC** causes a marked increase in liver volume that appears to be mostly replaced by large tumor masses that can occupy an entire lobe. On cut surface, massive HCC has a soft consistency and variegated appearance due to the presence of necrosis and hemorrhagic areas.
- **Diffuse or cirrhotomimetic HCC** is characterized by the presence of numerous nodules, similar to each other in size and shape, which can reach several hundred and fill the entire liver. Tumor nodules can be confused with the regenerative nodules of cirrhosis. The presence of numerous satellites around a dominant nodule is not considered a cirrhoticomimetic carcinoma.

In addition, there are at least two other variants represented by the pedunculated type and by the so-called icteric-type HCC.

- **Pedunculated HCC** protrudes from the liver; it is often solitary and can reach a huge diameter. In relation to the presence of a peduncle (hanging lesions) or its absence (sessile forms) it is sometimes sub-classified into type I and type II, respectively. The tumor may also have a long stalk and appear as a free polypoid mass in the abdominal cavity. Sessile forms have a dome-shaped appearance and are covered by the liver capsule (Fig. 6.3).
- **Icteric-type HCC** have the tendency to invade the main bile ducts with occlusion of the lumen and the onset of jaundice. On gross examination these tumors appear nodular with a tumor mass in the lumen of a dilated intrahepatic bile duct. Intraductal growth does not seem to have a different prognosis than other HCCs although the rate of portal venous invasion is higher.

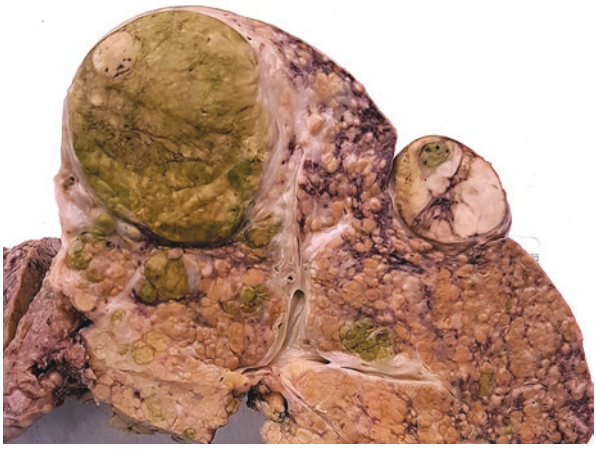

Fig. 6.3 Nodular hepatocellular carcinoma (HCC) in a cirrhotic liver. On the right a lesion that protrudes from the liver surface with a sessile form is visible (pedunculated HCC, sessile type)

6.3 Histology

The microscopic features of HCC reflect its biological complexity and change according to differentiation. Two key aspects of the morphology of these tumors are the absence of portal tracts and the presence of aberrant arteries. HCC shows architectural changes and cytological alterations. The main tumor architectural changes consist in a trabecular, solid or compact, pseudoglandular or acinar, and macrotrabecular (more than 10 cells thick) pattern (Fig. 6.4). Some HCCs show a pelioticlike appearance and many HCCs can have mixed architecture. The tumor stroma is usually not very prominent but cases with stromal reaction (scirrhous HCC, sclerosing HCC, and fibronodular HCC) have been recorded. The stromal reaction plays a decisive role in the growth, spread and even differentiation of the neoplasm. HCCs are highly vascularized neoplasms and show two patterns of microvessels: sinusoidlike and capillary-like microvessels [4] that affect the biological behavior of the neoplasm. E-HCC and distinctly nodular HCC are generally surrounded by a variable thickness fibrous pseudocapsule (FPC) that can be complete or incomplete. FPC influences the biological behavior of the neoplasm [5].

The cytological alterations of neoplastic hepatocytes are as follows:

- In well-differentiated HCC they appear very similar to normal hepatocytes, and in these cases the diagnosis is often based on nuclear crowding and increase in cytoplasmic basophilia.
- In poorly differentiated HCC the cells are characterized by irregular nuclei, cellular pleomorphism and giant cells with highly atypical nuclei.

In all HCCs numerous inclusions in the cytoplasm of tumor cells are frequent. They include eosinophils globules, hyaline globules, pale bodies and Mallory-Denk bodies (MDBs). Some tumoral hepatocytes can store glycogen or fat and contain bile, the latter specially in biliary canaliculi.

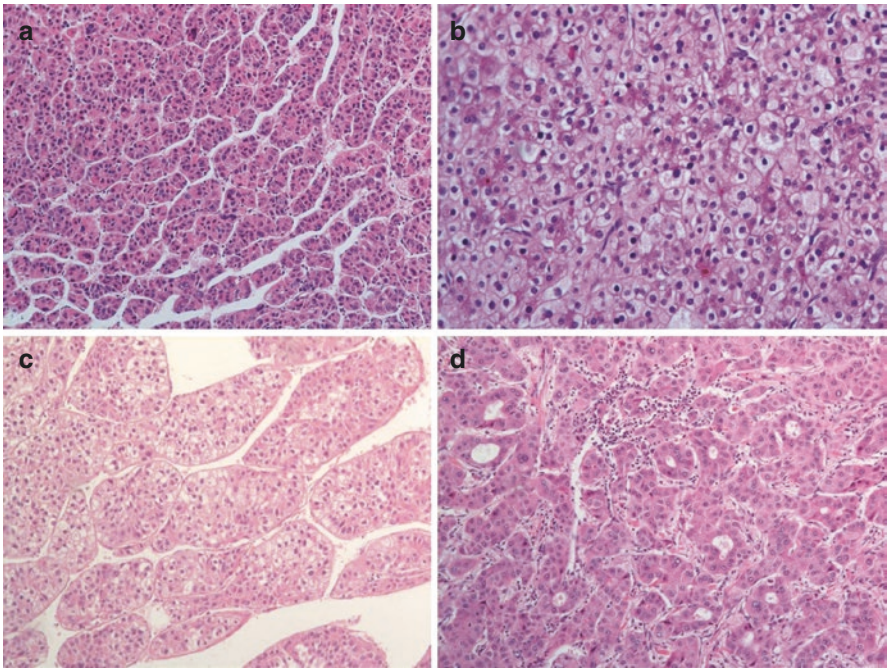

Fig. 6.4 Main histological pattern of hepatocellular carcinoma: (**a**) trabecular; (**b**) solid; (**c**) macrotrabecular; (**d**) pseudoglandular

6.4 Grading, Staging, and Metastases

An important prognostic factor is tumor grade. It predicts patient survival and disease-free interval after HCC resection and liver transplantation. A well-defined, reproducible, and widely accepted grading system has yet to be developed. From a strictly clinical point of view, grading based on architectural and cytological features in a three-tiered system is preferred: well-differentiated, moderately differentiated and poorly differentiated tumors [6], in contrast to the four-tiered grading system by Edmondson and Steiner [7], which is more appropriate for clinical research.

The main factors in HCC staging are: tumor size, multifocality, tumor grade and angiolymphatic invasion. Staging is essential to indicate optimal treatment. The AJCC/UICC (American Joint Committee on Cancer/Union for International Cancer Control) system is very useful for predicting the outcome after surgical resection and transplantation.

The most common sites of HCC metastases are the lung, bone, abdominal lymph nodes and adrenal glands. Peritoneal carcinosis is rare but may be present. Direct invasion of the diaphragm is also possible.

6.5 Immunohistochemistry

Immunohistochemistry (IHC) has to answer two questions: whether the lesion of hepatocellular origin is benign or malignant and whether the neoplasm is an HCC or another malignant tumor. In the non-cirrhotic liver, if the lesion has a clear hepatocellular differentiation, it is necessary to evaluate whether it is a focal nodular hyperplasia, an adenoma or an HCC. In the cirrhotic liver one must differentiate between a macroregenerative nodule, a dysplastic nodule (low or high grade) and HCC. The following immunostains are commonly used for major differentiation diagnosis: glypican-3, alpha-fetoprotein (AFP), HepPar1, arginase-1, polyclonal CEA and CD10 (both canalicular pattern).

6.6 Variants

Several histological subtypes of HCC have been recognized. The subtypes or variants of HCC have a distinct histological morphology, distinct immunohistochemical and molecular markers, a different clinical correlation and a different prognosis [8].

Combined hepatocellular carcinoma-cholangiocarcinoma (cHCC-CCA) is a distinct molecular lesion that can originate in cirrhotic and non-cirrhotic livers and its frequency is estimated at around 2–5% of primary liver tumors. Prognosis is intermediate between HCC and CCA, lymph node metastases are frequent and it has higher risk of recurrence after surgical resection and higher risk of relapse after orthotopic liver transplantation (OLT). A neuroendocrine component in HCC or CCA is very rare. These tumors belong to the group of *mixed neuroendocrine-non-neuroendocrine tumors* (MiNENs). In *clear-cell HCC* we have more than 50% of clear cells. The prognosis is better than non-clear HCCs and the main differential diagnosis is with renal cell carcinoma metastases and other clear-cell tumors.

Other peculiar variants of HCC are *granulocyte-colony-stimulating factor producing HCC*, *lymphocyte-rich HCC*, *scirrhous HCC*, and *steatohepatitic HCC*. The latter subtype often arises in the context of steatohepatitis and shows macrovesicular steatosis, ballooned cells, Mallory-Denk bodies, intratumoral inflammation and fibrosis. The amount of fat needed to qualify a steatohepatitic HCC must be greater than 33%. There seems to be no difference in survival from ordinary HCC.

Fibrolamellar HCC is a distinct subtype that arises in young patients without cirrhosis and no underlying liver disease, with distinct clinical features, as well as unique morphologic, immunohistochemical, and molecular findings. After surgical resection, approximately 55% of cases has intrahepatic recurrence within the first 5 years. Macroscopically, it is a voluminous neoplasm with central scar and calcifications. The neoplastic cells are polygonal, eosinophilic, with macronucleoli, immersed in an abundant collagen stroma with a lamellar appearance. Pale bodies are frequent but not specific. These tumors are HepPar1+ and arginase 1+. Eosinophilic granular cytoplasm is CD68 positive and tumor cells express CK7 and CK19. The *DNAJB1-PRKACA* fusion gene is considered to be pathognomonic [9].

A recently described subtype of HCC is termed *macrotrabecular/massive HCC* (MTM-HCC). MTM-HCC is characterized by large trabeculae with a thickness greater than 10 cells affecting at least 50% of an HCC. It frequently originates in non-cirrhotic livers and it is often associated with high levels of AFP. These tumors are large in size and have frequent angioinvasion with poor prognosis.

Other variants are: *chromophobe HCC*, *fibronodular HCC*, and *myxoid HCC*.

6.7 Differential Diagnosis

The main differential diagnosis of HCC is with benign and malignant neoplasms in cirrhotic and non-cirrhotic livers. In the latter the differential diagnosis is made with focal nodular hyperplasia and adenoma, while in cirrhotic livers with macroregenerative nodules and with low- and high-grade dysplastic nodules. The most suggestive aspects for the diagnosis of HCC are stromal invasion and sinusoid arterialization, well highlighted with immunostain for CD34. The use of a panel of IHC markers (glypican 3, glutamine-synthetase, and HSP70) can help in the differential diagnosis between benign and malignant nodules. A clear positivity of at least two out of three markers strongly supports HCC.

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