Review Article

Primary Liver Cancers—Part 1: Histopathology, Differential Diagnoses, and Risk Stratification

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Abstract

Hepatocellular carcinoma (HCC) and cholangiocarcinoma (CC) are the 2 most common primary malignant liver tumors, with hepatocellular and bile ductular differentiation, respectively. This article reviews the key histopathological findings of these 2 primary liver cancers and includes a review of the role of ancillary testing for differential diagnosis, risk stratification according to the American Joint Committee on Cancer (AJCC) staging recommendation, and a review of precancerous lesions. A literature review was conducted to identify articles with information relevant to precancerous precursors, current histopathological classification, ancillary testing, and risk stratification of primary malignant liver tumors. The histomorphology of normal liver, preinvasive precursors, primary malignancies, and morphological variants, and the utilization of ancillary tests for the pathological diagnosis are described. Dysplastic nodules are the preinvasive precursors of HCC, and intraductal papillary neoplasms of bile ducts and biliary intraepithelial neoplasia are the preinvasive precursors of CC. Benign liver nodules including focal nodular hyperplasia and adenomas are included in this review, since some forms of adenomas progress to HCC and often they have to be differentiated from well-differentiated HCC. A number of morphological variants of HCC have been described in the literature, and it is necessary to be aware of them in order to render the correct diagnosis. Risk stratification is still dependent on the AJCC staging system. The diagnosis of primary liver carcinomas is usually straightforward. Application of the appropriate ancillary studies aids in the differential diagnosis of difficult cases. The understanding of the carcinogenesis of these malignancies has improved with the standardization of the pathological classification of preinvasive precursors and studies of the molecular pathogenesis. Risk stratification still depends on pathological staging.

Keywords

hepatocellular carcinoma, cholangiocarcinoma, focal nodular hyperplasia, hepatic adenoma, dysplastic nodule, biliary intraepithelial neoplasia, intraductal papillary neoplasm

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Introduction

Hepatocellular carcinoma (HCC) and cholangiocarcinoma (CC) are the 2 primary malignancies of the liver. Preinvasive precursor lesions include dysplastic nodules (DNs) leading to HCC and biliary intraepithelial neoplasia (BilIN) and intraductal papillary neoplasms of the bile ducts (IPN-B) leading to CC.

Normal Liver

The liver is the largest gland in the human body and weighs on average 1500 g. It is located in the right upper abdomen and

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midabdomen and extends to the left upper abdomen under the diaphragm. The blood supply to the liver comes from the hepatic artery (20%-40%) and the portal vein (60%-80%). Blood flows out of the liver through the hepatic vein into the portal vein.

The liver is regulated by a nervous system containing both afferent and efferent neurons actively involved in numerous biological and physiological processes. The autonomic nervous system of the liver is essential in the maintenance of homeostasis and human metabolism. The afferent branch includes the sensation of nutrition such as lipids, glucose, and a spectrum of metabolites, which are involved in trigging the nervous system to monitor necessary physiological changes. In parallel, the efferent branch is responsible for metabolic regulation, modulation of fibrosis and biliary function, and various associated processes. Subsequently, liver functions as both a sensor and an effector under the influence of neurological signaling.

Microscopically, the liver is composed mostly of hepatocytes with bile duct cells and mesenchymal cells. Figure 1 shows the histology of normal liver and ancillary study staining patterns. Hepatocytes are polygonal cells with eosinophilic granular cytoplasm and round nuclei with 1 or 2 prominent nucleoli. The hepatocytes are arranged in anastomosing plates, usually 1 to 2 cells thick and separated by sinusoids (Figure 1A-B). The sinusoids are lined by endothelial cells and also contain Kupffer cells, which are specialized macrophages. The space of Disse between the sinusoids and the hepatocytes contains stellate cells, members of the myofibroblastic family, causing fibrosis.

The portal triads contain branches of the portal vein, hepatic artery, bile ducts, vagus nerve, and lymphatics (Figure 1C). Blood flows into the liver through the portal tracts and into the sinusoids and exits through the central veins (Figure 1D), which converge to drain into the hepatic vein.

The bile canaliculi are located between the walls of the hepatocytes. These communicate with the canals of Herring, which are lined partly by hepatocytes and partly by bile duct. Bile drains into bile ductules from the canals of Herring, which converge and form larger interlobular bile ducts in the portal triads.

Normal liver will show a normal reticulin framework (Figure 1E). The vascularity will not be increased and focused on portal tracts (Figure 1F). The periodic acid-Schiff-diastase (PASD) stains show the portal tracts (Figure 1G). Glutamine synthetase (GS) is localized to the central vein area (Figure 1H).

Histology of Preinvasive Precursors

Hepatocyte Dysplastic Foci and Nodules

The International Working Party published standardized terminology and criteria for nodular hepatocellular lesions, and this terminology is still used today. Precancerous lesions include dysplastic foci and low- and high-grade DNs, which are detected most often in cirrhotic livers and also livers with chronic, noncirrhotic disease. Dysplastic foci are less than 1 mm in diameter and consist of groups of hepatocytes with dysplasia,[1] now called either large cell or small cell change. Dysplastic nodules may be single or multiple nodules. They are macroscopically larger than the surrounding cirrhotic nodules but less than 15 mm in size. Dysplastic nodules are classified according to international consensus as low grade or high grade, based on histomorphological features.[1]

The most common cytological change found in dysplastic foci is small cell change.[2] These cells are smaller than the adjacent hepatocytes and show an increased nuclear to cytoplasmic ratio (N/C), mild nuclear atypia, hyperchromasia, and cytoplasmic basophilia (Figure 2A-B). This type of dysplasia is considered precancerous, since it harbors molecular alterations involved in carcinogenesis, including chromosomal gains and losses, telomere shortening, and inactivation of cyclin Dependent Kinase Inhibitor 1A *(CDKN1A)*. These areas will also show an increase in proliferation markers compared to adjacent nonneoplastic liver.[3-5] The reticulin stain usually shows a preserved trabecular framework, without decrease in or loss of expression.

Large cell change is seen in the livers of patients with chronic hepatitis B virus (HBV) or hepatitis B virus (HCV) infection or cirrhosis of various etiologies. Pathologically, it is characterized by nuclear and cytoplasmic enlargement, preserved N/C, nuclear pleomorphism, hyperchromasia, and multinucleation. Its exact nature is not clear. In cirrhosis, it usually occurs diffusely and is more likely a degenerative change. In HBV, it appears to be precancerous and is associated with telomere shortening, Cyclin Dependent Kinase Inhibitor 2A (*CDKN2A*)- and *CDKN1A*-regulated checkpoint inactivation, increased DNA damage, and a higher proliferation index.

Low-grade DNs are virtually indistinguishable from macroregenerative nodules and sometimes the 2 terms are used interchangeably. Low-grade DNs may have portal tracts and a bile ductular reaction within the nodule. They have well-defined borders. The cells are fairly uniform in appearance with minimal nuclear atypia, a slight increase in N/C ratio, and no mitoses. Low-grade DNs may also show Mallory bodies, bile stasis, clear cell cytoplasmic change, iron or copper deposits, a slight decrease in cell size, and fatty changes.[6] The architecture is maintained, and the liver plates remain a single-cell thick. Reticulin stain shows a normal framework.

High-grade DNs are characterized by small cell change, hepatic plates up to 3 cells in thickness, and occasional pseudogland formation (Figure 2). Focal decrease in the reticulin could be seen although it may remain normal (Figure 2C). Glypican 3 expression is often negative in the DNs (Figure 2D). These nodules may also contain Mallory bodies, glycogen, fat, clear cell change, rare portal tracts, mitoses, cytoplasmic basophilia, and bile. It is largely accepted that high-grade DN are precursors of HCC. In some cases, it may be impossible to tell a high-grade DN from well-differentiated HCC, especially in needle biopsies. High-grade DNs may develop subnodules of HCC. A distinguishing feature is that DNs do not invade adjacent parenchyma. The cells are fairly uniform in appearance with minimal nuclear atypia, a slight increase in N/C ratio, and no mitoses.[6]

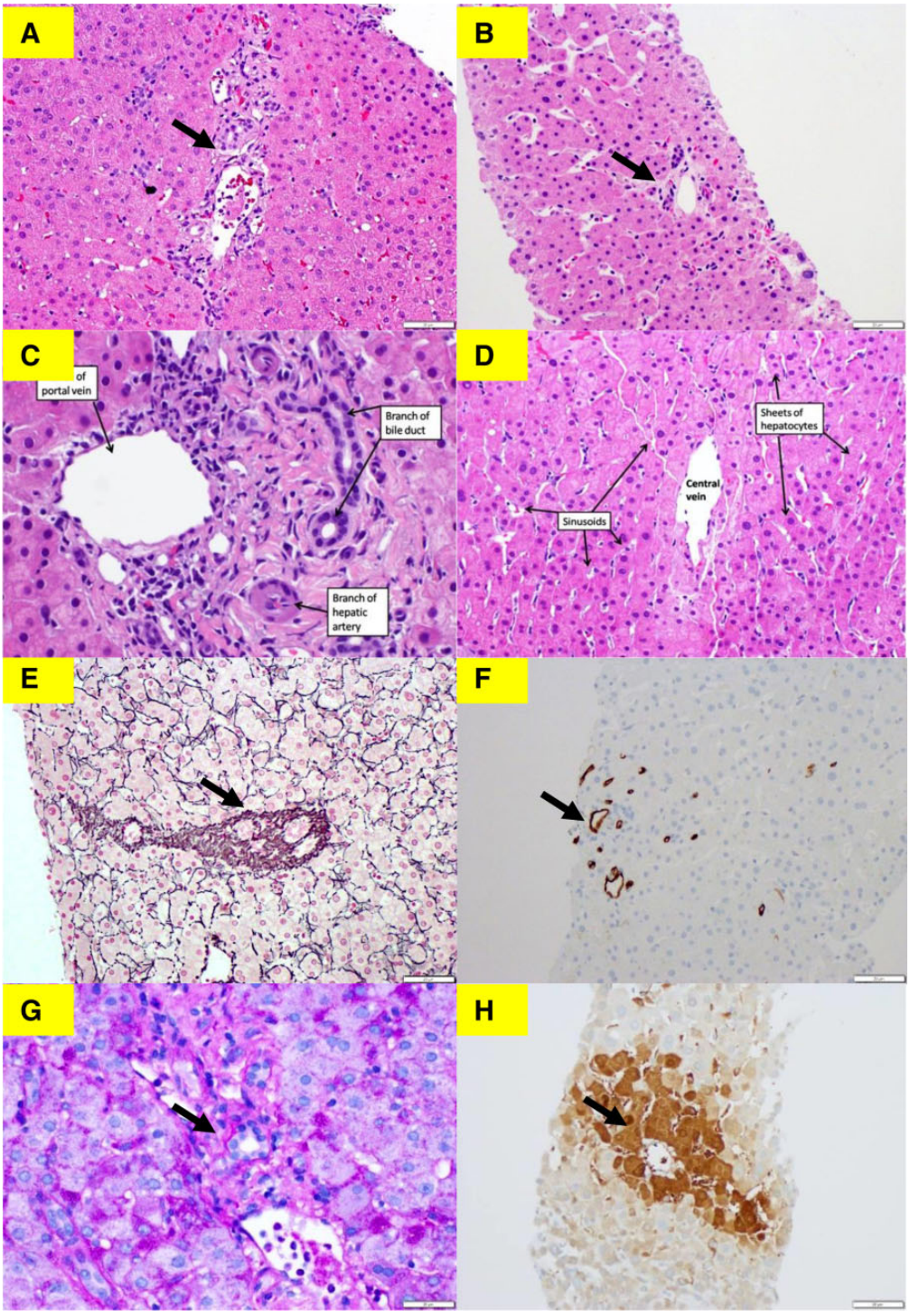

Figure 1. Normal liver. Photomicrograph (original magnification, ×40; hematoxylin–eosin [H&E] stain) of benign hepatic parenchyma obtained by excisional biopsy (A), and needle core biopsy (B). Both show the presence of portal tracts (arrow) and 1- to 2-cell thick hepatic plates. No large vessels, cytologic atypia, mitoses, or necrosis are seen. (C) Portal tract showing bile duct, vein branch, arteriole, nerve, and lymphatic. (D) Liver showing central vein. (E) Photomicrograph of reticulin special stain in benign liver tissue, which highlights 1- to 2-cell thick plates and unremarkable portal tract (arrow). (F) Photomicrograph of CD34 immunostain in benign liver tissue, which demonstrates a restricted pattern of periportal labeling (arrow). (G) Periodic acid-Schiff (PAS)-diastase highlights portal tract and bile duct (arrow). (H) Photomicrograph of glutamine synthetase immunostain in benign liver, with pericentral labeling (arrow); peripheral lobular areas are not labeled.

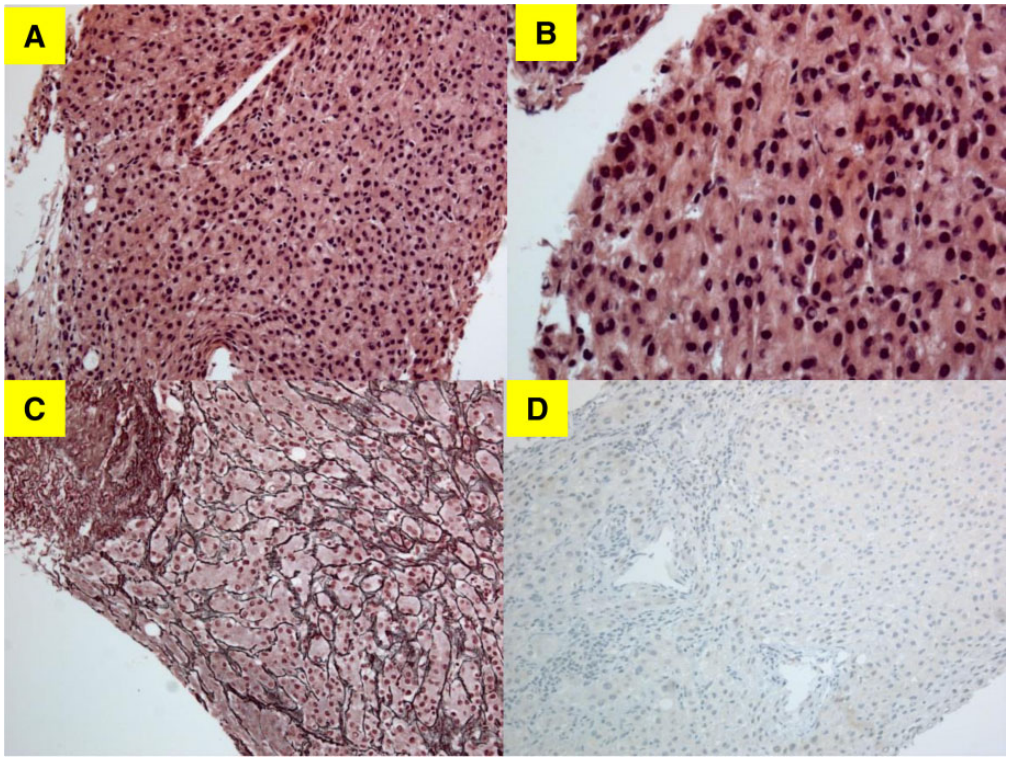

Figure 2. Dysplastic foci and nodule. (A) and (B) Small cell change with a high N/C, and small, hyperchromatic nuclei. H&E, ×20 and ×40. (C) High-grade dysplastic nodule showing retained reticulin framework. Reticulin, 20. (D) Immunohistochemistry for glypican 3 is negative. 20. N/C indicates nuclear to cytoplasmic ratio.

Biliary Intraepithelial Neoplasia and Intraductal Papillary Neoplasia of the Bile Ducts

Biliary intraepithelial neoplasia (BilIN) and intraductal papillary neoplasm of bile duct (IPNB) are precursors of bile duct carcinomas.

Biliary intraepithelial neoplasia. Biliary intraepithelial neoplasia is the preinvasive flat precursor lesion of CC, representing the pathology of multistep cholangiocarcinogenesis.[7] Before 2005, when the term biliary intraepithelial neoplasia was proposed, it was called biliary atypia or dysplasia.[8] Biliary intraepithelial neoplasia is the biliary counterpart of pancreatic intraepithelial neoplasia. In general, it is composed of flat or micropapillary dysplastic epithelium. The terminology applies to lesions in both the intrahepatic and extrahepatic bile duct systems. Biliary intraepithelial neoplasia is classified into 3 grades according to Zen et al.[8]

Biliary intraepithelial neoplasia 1 (low-grade lesion) shows flat or micropapillary architecture with focal area of stratification (up to lower 2/3). The lining cells show basally located nuclei with mild atypia. No mitoses or loss of cellular polarity are noted (Figure 3B).

Biliary intraepithelial neoplasia 2 (intermediate-grade lesion) shows flat, pseudopapillary or micropapillary architecture with luminal surface pseudostratification. The lining cells show moderate nuclear atypia, focal loss of polarity, and rare mitotic figures.

Biliary intraepithelial neoplasia 3 (high-grade lesion, carcinoma in situ) shows pseudopapillary, micropapillary, or flat architecture, and occasionally luminal budding and cribriform formation. The lining cells show severe nuclear atypia with diffuse loss of polarity and increased mitotic figures (Figure 3C).

Gastric foveolar, pyloric, and intestinal metaplasia are infrequently seen in BilIN. They are identified in BilIN-2 and -3 compared to those in BilIN-1.[7,9] Biliary intraepithelial neoplasia is often found at the surgical resection margin of biliary tract adenocarcinomas, but identifying BilIN on frozen section is of no clinical consequence since only invasive cancer will have an impact on patient outcome.[10] Finally, it is important to differentiate BilIN from reactive atypia which is usually associated with acute inflammation, erosion, ulceration, and almost universally, stent effect.

Intraductal papillary neoplasm of the bile duct. Intraductal papillary neoplasm of bile ducts is a rare tumor arising in intrahepatic or extrahepatic bile ducts. It is considered a mass-forming precursor of invasive carcinoma. The exact etiology and pathogenesis of IPNBs are still unclear, but hepatolithiasis and clonorchiasis are known as the 2 major risk factors.[11] Intraductal papillary neoplasm of bile ducts is usually a single or multiple

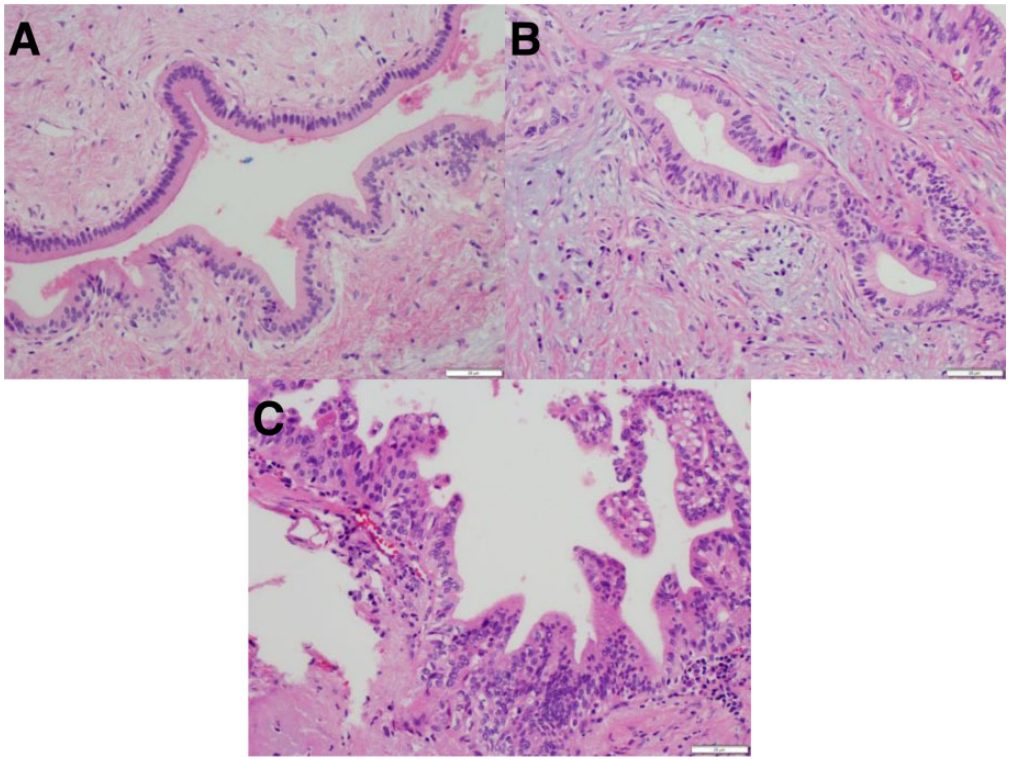

Figure 3. Biliary intraepithelial neoplasia (BilIN). A, Nonneoplastic bile duct lined by columnar epithelium, with basally located nuclei (H&E, ×20). B, BilIN 1. Bile duct lined by pseudostratified columnar cells. The nuclei are slightly enlarged and elongated (H&E, ×20). C, BilIN 3. The cells demonstrate loss of polarity, and extend to the luminal surface. The nuclei are enlarged, with vesicular nuclei and nuclear membrane irregularity (H&E, ×20).

gray tan to yellow, friable polypoid lesions. It is a neoplastic papillary proliferation replacing the normal ductal epithelium (Figure 4A-B). Histologically, it is composed of papillary structures that have fine vascular cores (Figure 4C). The papillae are lined by a spectrum of atypical cells (Figure 4D). It has been reported that it may be associated with foci of invasive carcinoma, which recommends a very careful macroscopic and microscopic examination. According to Wan et al, about 40% to 80% of IPNBs have a component of invasive carcinoma (tubular or mucinous adenocarcinoma).[11]

IPNB is classified in a manner similar to the World Health Organization classification of pancreatic intraductal papillary mucinous neoplasm (IPMN): low-grade, intermediate-grade, and high-grade IPNB, some of which present with an associated invasive carcinoma. There are 4 subtypes of IPNB based on the lining epithelium: pancreatobiliary (the most common type), intestinal (the second most common), gastric, and oncocytic types. Usually there is high-grade dysplasia associated with the pancreatobiliary or intestinal types.[12,13] The pancreatobiliary type is often positive for MUC-1 (and MUC-5AC, cytokeratin [CK]-7, CK-20) but is negative for MUC-2. The intestinal type consistently expresses MUC-2 and MUC-5AC, CK-7, and CK-20) but not MUC-1. The gastric type expresses MUC-5AC, CK-7, and CK-20 but is negative for MUC-1 and MUC-2. The oncocytic type consistently expresses MUC-6, MUC-5AC, CK-7, and CK-20) with focal expression of MUC-1 and/or MUC-2.[11,12,14] Finally, IPNB is considered to be a biliary counterpart of IPMN due to the multiple similar features between both entities; they are both intraductal neoplastic processes, radiologically and grossly identifiable, with intraductal papillary proliferations and are considered precursors of tubular and mucinous adenocarcinoma.

Liver stem cells and the progenitors of hepatocarcinogenesis. Human cancer stem cells possess the capacity to self-renew, to differentiate into multiple malignant cellular lineages, and to proliferate. They are associated with a poorer prognosis because of their greater tumorigenicity and chemoresistance. Recent advances in stem cell biology have enabled our understanding and identification of cancer stem cells in solid tumors as well as putative stem cells in normal solid organs. Recent studies illustrated that stem cells play important roles in the carcinogenesis of various types of cancer, including primary liver tumors.[15,16]

Hepatocellular carcinoma and cholangiocarcinoma are 2 distinct types of liver cancers. It has been generally accepted that the HCC phenotype is derived from hepatocytes and that the cholangiocarcinoma phenotype is derived from cholangiocytes; however, a histopathological intermediate phenotype has

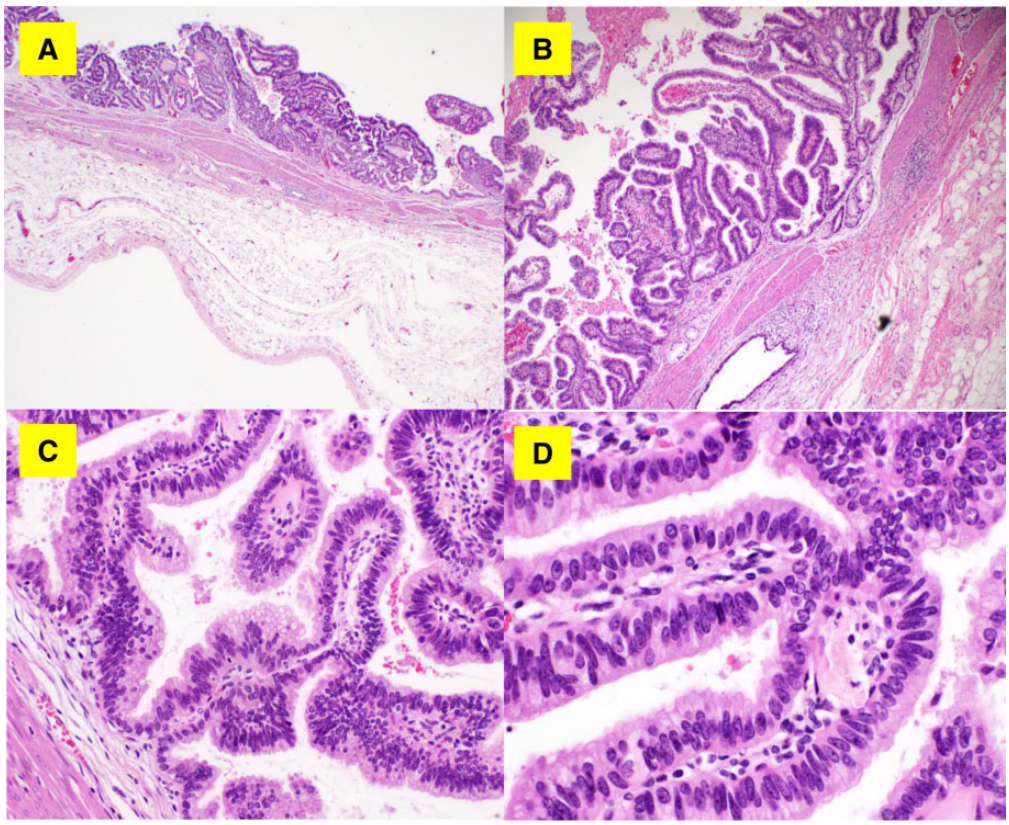

Figure 4. Intraductal papillary neoplasm of the bile duct (IPNB) with moderate dysplasia. This case was adjacent to an invasive carcinoma (not shown here). A, The lumen of the bile duct is completely replaced by a papillary epithelial proliferation (H&E ×4). B. The neoplasm exhibits finger-like, papillary projections (H&E, ×10). C. The papillary fronds are lined by columnar, mucin-containing cells (H&E, ×20). D, The nuclei are enlarged, elongated, and pseudostratified (H&E, ×40).

been recognized, which appears to arise from hepatic progenitor or cholangiocarcinoma stem cells.[15,17]

Hepatic progenitor cells have been identified in HCC and can be identified with EpCam, and CD133, CD90, CD44, and CD13.[18] Cytokeratins 7 and 19 are also recognized as stemness-related markers in HCC, and expression of these proteins in an HCC predicts a worse outcome for the patient. Durnez et al[18] had analyzed 109 cases of HCCs and found that 28% of the tumors contained cells expressing CK-7 or CK-19 or both, with features of liver stem cells. Remarkably, the higher recurrence rate of CK-19-positive tumors after transplantation suggests a worse prognosis for these HCCs compared to CK-19-negative tumors.[18] Gene expression analysis has shown that tumors expressing CK-7 and CK-19 have an expression pattern similar to that of fetal hepatoblasts. In this subset, activation of AP-1 transcription factors appears to play a key role in hepatic carcinogenesis.[15]

Hepatocellular carcinoma expressing stemness-related proteins are more aggressive and have a poorer clinical outcome compared to the conventional HCCs that do not express stemness-related markers. These tumors demonstrate an infiltrative growth pattern, vascular invasion, and more intratumoral fibrous stroma. There is a spectrum of morphological and immunophenotypic features between HCCs with stemness-related marker expression, scirrhous HCCs, and combined hepatocellular–cholangiocarcinoma with stem cell features. Hepatocellular carcinomas with stemness-related marker expression are associated with increased serum α-fetoprotein (AFP) levels and a poor prognosis. The workup of HCC should include markers of stem cellness, such as CK-19, as tumors expressing these markers have increased chemoresistance, earlier recurrence after surgical and/or locoregional treatment, increased invasiveness/metastasis, and poor overall survival.[19,20]

Several studies have also documented the existence of cholangiocarcinoma stem cells; several cell surface antigens such as CD24, EpCAM, CD44, CD133, and others have been shown to label such cholangiocarcinoma stem cells.[17] Of note, elevated expression of stem cell surface markers was associated with more aggressive behavior.[17] To date, reports showing possible signaling pathways in cholangiocarcinoma stem cells are under investigation, whereas distinct and specific pathways are expected to be present in these stem cells compared to other cancer cells that have no stem cell properties.[17]

The multipotent nature of the liver stem cells have been well demonstrated by the recent advancements in stem cell investigations introduced above, especially in the cases of combined hepatocellular–cholangiocarcinomas frequently encountered

clinically. In these combined tumors, the identified progenitor cells merged with HCC components, cholangiocarcinoma components, and the mature-appearing hepatocytes within the same masses.[21] The most likely explanation is that these tumors are of hepatic stem cell origin, supporting the concept that human hepatocarcinogenesis could be due to transformation of progenitor cells and that such a process may lead to the development of certain mixed hepatocellular–cholangiocarcinomas as well as DNs.[21] These liver stem cells have the unique potential to develop into cholangiocarcinoma stem cells through genetic alteration in gene expression profiles; and it has been confirmed that cholangiocarcinoma is of hepatic progenitor cell origin due to the expression of certain stem-specific cell markers.[17] Furthermore, microarray analysis has identified unique gene expression profile between the tumor cells within the same tumor and further demonstrated the so-called several "stemness genes" in the subpopulation of tumor cells.[22] The study further indicated that this minority population of tumor cells possess extreme carcinogenic potential and provide heterogeneity to the cancer stem cell system.[22] These results indicate that both HCC and cholangiocarcinoma may be derived from common bipotent progenitor cells and that the hepatic stem and progenitor cells are able to differentiate into both hepatocytes and cholangiocytes.

Based on the above discussion, it is conceivable that targeting therapies for surface molecular markers or specific signaling pathways of cholangiocarcinoma stem cells may be important in order to improve the clinical outcome of patients with this lethal disease. However, cancer stem cells show overlapping profiles of their markers and signaling pathways with normal tissue cells; therefore, the side effects of targeted therapy remain challenging to predict and could outbalance the clinical benefits. Identification and combating of unique markers exclusively belonging to stem cells are essential to lower the associated toxicity to normal cells and functions and to maximize the clinical benefits. It may be required to combine multiple therapeutic strategies to treat these liver primary tumors.

Hepatocellular Carcinoma

Hepatocellular carcinoma encompasses a group of malignant liver neoplasms that show hepatocellular differentiation.

Histopathology of HCC

Histopathology of HCC, not otherwise specified. In most cases, HCC is at least suspected or recognizable directly on H&E-stained sections, due cytological atypia and architectural abnormalities such as thickened hepatic plates (Figures 5A and B), endothelial lining (Figure 5C), pseudo-glandular configuration (Figure 5D), and lymphovascular involvement by tumor (more often seen in resection material) and the absence of portal tracts and hepatic lobules. Most HCCs grow in trabecular, nested, solid, or pseudoacinar (Figure 5E) growth patterns. Aberrant vessels can be identified within the lobules instead of portal tracts, which is usually a helpful finding in well-differentiated HCC (Figure 5D). Bile production can be seen in fair amount of tumor cells and is pathognomonic for hepatocellular differentiation. Well-differentiated HCCs have abundant finely granular eosinophilic cytoplasm, round nuclei with dispersed chromatin, and prominent nucleoli (Figure 5F). If the biopsy was indeed obtained from a mass in the liver and appears hepatocellular on H&E sections, the differential list includes HCC and its mimickers such as focal nodular hyperplasia (FNH), hepatic adenoma (HA), and DNs.

Similar to benign hepatocytes, the tumor cells in HCC can demonstrate steatotic, clear cell change (Figure 5F), or significant nuclear inclusions (Figure 5F). Most HCCs present with these classic histomorphological characteristics at presentation; a straightforward diagnosis of HCC can usually be made without significant obstacles. As HCC becomes less well differentiated, the amount of cytoplasm generally decreases, the N/C increases, as well as the progression of nuclear atypia. Focal and confluent necrosis is frequently appreciated in resected HCC tumors or in biopsy material of HCCs when ample tissue was sampled. Several variants of HCCs have been recognized in the literature, which are discussed in detail in the following text.

Variants of HCC

Cirrhosis-like HCCs. Cirrhosis-like HCC is an evolving topic; in this type of HCC, tumor masses may mimic multifocal cirrhotic nodules in a patient with known liver cirrhosis, but no true tumor mass can be seen on radiographic or imaging studies. Grossly, cirrhosis-like tumor nodules are identified, with subtle color and texture differences from the background cirrhotic liver parenchyma.[23] Microscopically, tumor appears well to moderately differentiated, frequently with ballooning, Mallory bodies, and cholestasis; tumor cells resemble conventional HCC cells, but a definitive tumor mass is not well appreciated.[23] Cirrhosis-like HCC is an insidious type of HCC and is usually not clinically suspected due to the lack of radiographic findings; subsequently, they are often incidentally identified on a staging protocol and in liver explants. Incidental microscopic findings of HCC in needle cores from routine biopsy procedures are highly suspicious of cirrhosis-like HCC. Cirrhosis-like HCC may also be identified in the vicinity of a dominant nodule of classic HCC.

The HCC with clear cell change. The HCC with clear cell change is a fairly common variant of HCC that frequently poses a diagnostic challenge, as it shows morphological similarities to a spectrum of adenocarcinoma and epithelioid tumors. Similar to benign hepatocytes, HCC cells may undergo steatosis/fatty change, ballooning, and steatohepatitis-like change with Mallory body formation. The cells show abundant, clear cytoplasm (Figure 6A). Generally, the cytomorphology mimics other neoplasms with clear cell features. The main diagnostic challenge lies in efficient usage of available tissue in performing a panel of special stains and immunohistochemical studies (to be discussed in the later portion of this review) and to

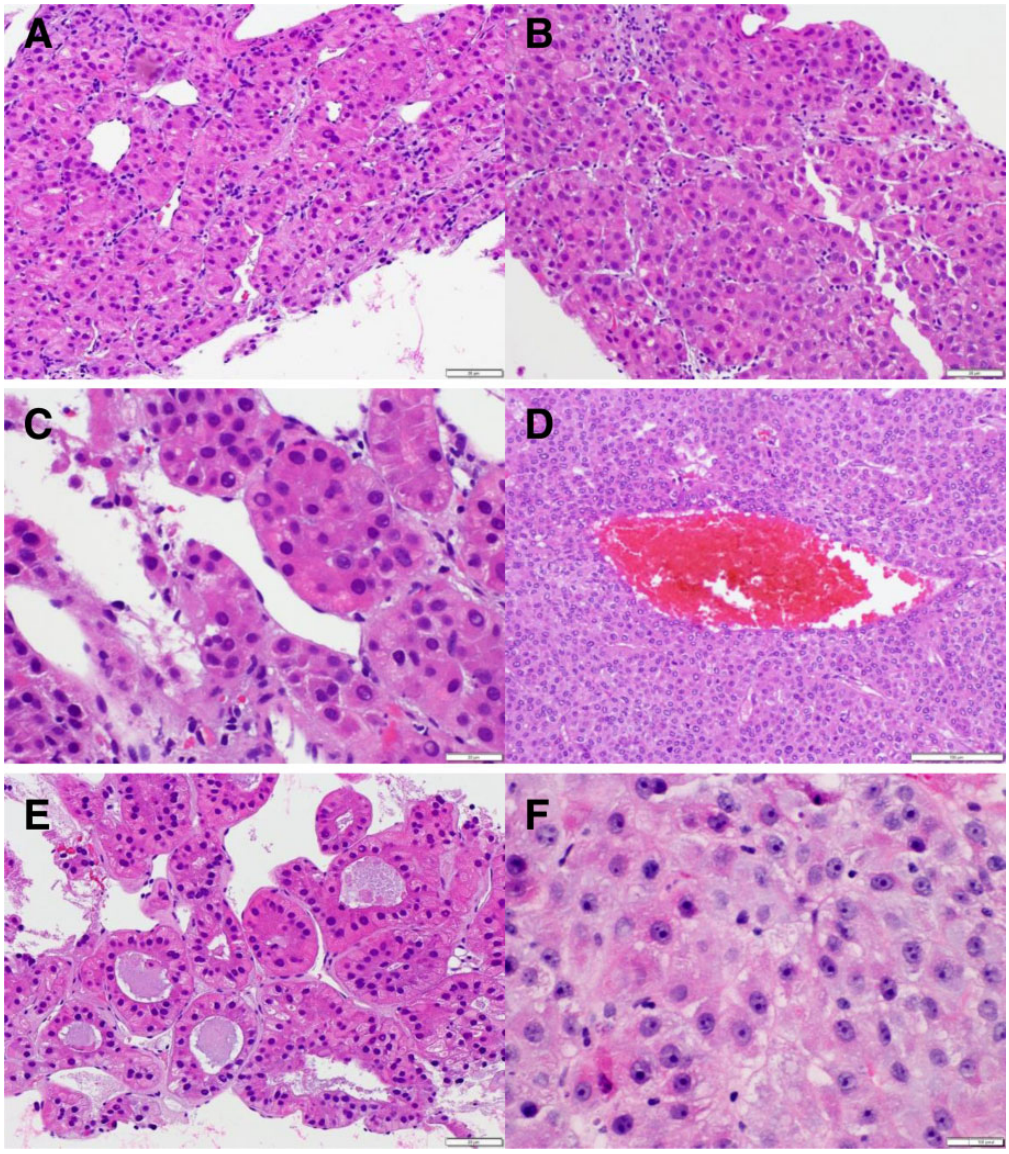

Figure 5. Photomicrograph (H&E stain) of hepatocellular carcinoma. A, Absence of portal tracts and thickened hepatic plates in HCC, ×40. B, Solid and nodular growth pattern, ×100. C, Endothelial wrapping and lining in sinusoidal space in HCC, ×200. D, Unaccompanied vessels. H&E, ×200. E, HCC with a tubular and pseudoglandular pattern, ×100. F, HCC with large nuclei with prominent nucleoli, and steatotic clear change, ×200. HCC indicates hepatocellular carcinoma.

differentiate other types of tumors with clear cell histology, such as metastatic renal cell carcinoma, neuroendocrine neoplasms with prominent clear cells, clear cell melanoma, epithelioid angiomyolipoma, and any other tumors with epithelioid morphology and clear cytoplasm encountered in liver.

Scirrhous HCC. In recent years, scirrhous HCC has been accepted as a unique subtype of HCC, which is distinctive from the better known fibrolamellar carcinoma.[24-27] This type of HCC arises in background cirrhotic liver, accounting for approximately 5% of all HCCs. An interesting fact about this type of HCC is that tumor nodules often develop beneath the liver capsule. Scirrhous HCCs are not usually suspected clinically due to its intriguing radiographic finding that mimics intrahepatic adenocarcinoma, namely, cholangiocarcinoma; the best method for establishing a correct diagnosis is tissue evaluation.[24-27] Microscopically, the characteristics of scirrhous HCC is prominent intratumoral dense fibrosis intermingled with neoplastic hepatocytes arranged in various patterns: trabecular, microtubular, micronodular, and pseudoacinar structures with nests (Figure 6B). Classic HCC-like areas have also been occasionally seen in cases of multifocal scirrhous HCCs.[24-27] The peculiar intratumoral dense fibrosis mimics posttherapeutic changes following neoadjuvant chemoradiation or chemoembolization treatment. Review of the clinical history and correlation with histological and laboratory

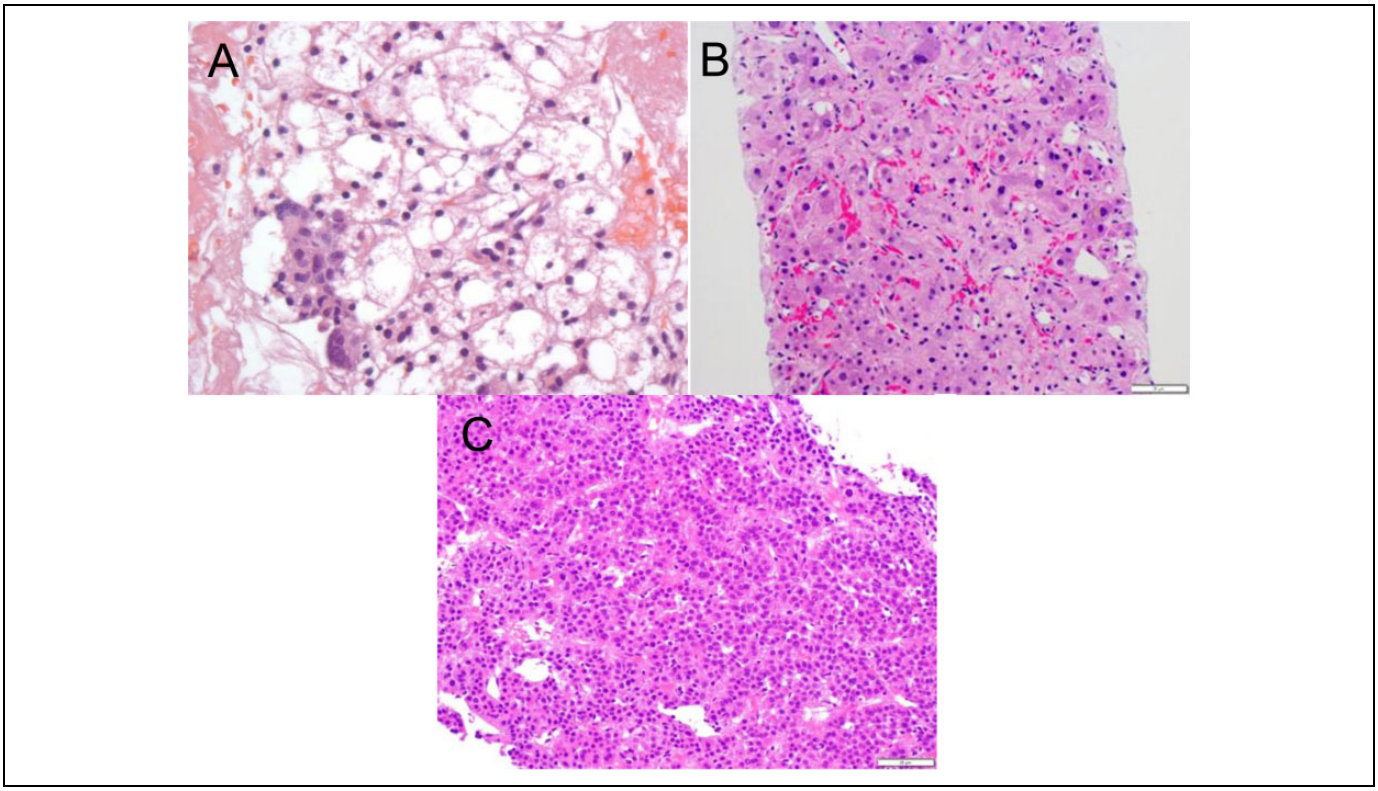

Figure 6. Variants of HCC. A, HCC, clear cell variant. H&E, ×40. B, Scirrhous variant of HCC, ×100. C, Small cell variant HCC. H&E, ×40. HCC indicates hepatocellular carcinoma.

findings is essential in recognizing this rare variant of HCC. Immunohistochemical studies had revealed a significantly higher expression of CK-7 and a significantly lower expression of hepatocyte paraffin 1 (HepPar1) in scirrhous HCC versus conventional HCC.[24,27] Prognostically, there are no significant differences in tumor cell proliferative rate and patient survival have been detected between the patients with scirrhous HCCs and those with classic HCCs.[25,26]

Sarcomatoid HCCs. Sarcomatoid HCC, also named carcinosarcoma and spindle cell carcinoma in the literature, is a rare variant of HCC. The reported prevalence including surgical resection cases has been less than 2%, consisting mostly of individual case reports and small case series.[28-31] The majority of reported sarcomatoid HCC cases contain obvious sarcomatous histology, with or without classic HCC, and with coexisting cholangiocarcinoma elements identified in extremely rare cases.[28-32] Grossly, sarcomatoid HCCs are large tumors, usually with satellite nodules, involving a cirrhotic liver. Microscopically, tumor masses were composed of irregular, polygonal, bizarre, and spindle-shaped malignant epithelioid cells, mixed with various mesenchymal components, such as rhabdomyosarcoma, chondroid sarcoma, osteoclast-like giant cells, and more primitive, hepatoblastoma-like features[33-36]; an unexpected element of cholangiocarcinoma could also be present in this variant of tumors.[32] Upon detailed sectioning, a distinct transition from HCC to sarcomatoid component may be confirmed. Expression of AFP by both HCC-like and sarcomatous cells and elevated levels of AFP in the serum have also been reported in certain cases. Although classic HCC-like tumor cells express E-cadherin but not vimentin, the mesenchymal (sarcomatous) component highly expresses vimentin with a loss of E-cadherin protein.[28,34,37] Nuclear proliferative marker Ki67 was also expressed at higher levels in sarcomatous versus HCC-like tumor cells. Compared to classic HCCs, sarcomatoid HCCs have a more aggressive clinical behavior and are associated with an ominous prognosis.[30,31,38] Optimized management options are currently undetermined.

The HCC with unusual small cell morphology. The authors have seen rare cases of HCC with an unusual small cell morphology, some of which were consultation cases sent to our institute for second opinion or due to a patient's transfer of care; similar cases have also been documented in the literature.[35] The peculiar small cell morphology may cause diagnostic error and adversely impact clinical decision-making. The key problem is distinguishing these carcinomas from neuroendocrine tumors or combined HCC with neuroendocrine differentiation, since their morphological features are suggestive of neuroendocrine differentiation. Some of the cases we observed were misdiagnosed as neuroendocrine tumors at outside institutions due to the fact that the HCC cells lacked bile pigment, grew in solid nests without obvious thickened hepatic plates, and the cells showed a scant to medium amount of slightly eosinophilic,

finely granular to clear cytoplasm, easily mimicking neuroendocrine tumor cells (Figure 6C). Adding to the difficulty when investigating this variant of HCC is that the neoplastic cells might occasionally show patchy, faint nonspecific labeling by synaptophysin or chromogranin, which upon comparison with the positive control, should be interpreted as negative. This subtype will express markers of hepatocellular differentiation, to be discussed in a later section of this review.

This variant of HCC should also be differentiated from HCC with a focal neuroendocrine component or combined neuroendocrine carcinoma and HCC of the liver.[39,40] An immunohistochemically workup will show the HCC component to express markers of hepatocellular differentiation and not express markers of neuroendocrine differentiation. The neuroendocrine component will express markers of neuroendocrine differentiation.[39,40]

Fibrolamellar HCC. Fibrolamellar HCC (FL-HCC) is a rare, yet unique variant of HCC and remains incompletely understood. Fibrolamellar HCC affects youth, adolescents, and elderly patients of both genders. Caucasians are most commonly affected by this particular type of HCC.[41-43] Opposite to what has been known for most HCCs, FL-HCC often occurs in noncirrhotic livers in patients in their mid-20s and almost always in the absence of any known risk factors. A series of reports have stated that patients with FL-HCC have better outcomes than those burdened with classic HCCs, but these interesting findings likely has resulted from the absence of cirrhosis in these relatively young patients, considering cirrhosis as an obvious adverse factor in classic HCC patients.[41-43] The actual outcome of FL-HCC is similar to that of classic HCC arising in noncirrhotic livers. Patients with FL-HCC have a 5-year survival rate of around 50%. Clinically, serum neurotensin[44] and vitamin B12 binding molecules (transcobalamin)[45,46] have been considered laboratory markers for FL-HCC and have been associated with tumor burden. Serum AFP has been additionally studied but was found to be elevated in only a minority of patients with FL-HCC.[47] Radiographically, an occasional central scar may be seen in FL-HCC, a frequent finding classically seen in FNH (a benign entity to be introduced later); however, radiological investigation demonstrated that the FL-HCC scar is often calcified and different from that observed with FNH.

Grossly, FL-HCCs are usually single, firm, and well demarcated; they are generally larger than classic HCCs, with an unusual propensity to metastasize to regional lymph nodes.[47-49] The FL-HCCs often show markedly prominent intratumoral and peritumoral fibrous bands throughout, with a central scar resembling FNH (see later text and discussion), in additional to foci of bile pigment and hemorrhagic necrosis. Although it could occur in both liver lobes, FL-HCC is more commonly seen in the left lobe of the liver for undermined reasons.[48,49]

Microscopically, the following characteristic features should point to the diagnosis of FL-HCC: plump polygonal tumor cells with rich eosinophilic granular cytoplasm (caused by numerous mitochondria), prominent macronucleoli matching the size of lymphocytes, and lastly lamellar fibrosis (Figure 7A). These histological characteristics occur without a clinical presentation of liver cirrhosis or underlying liver disease and appear unique to FL-HCC, as these fibrous bands encircle and surround clusters and nodules of neoplastic hepatocytes (Figure 7B). The nuclei are enlarged with prominent nucleoli (Figure 7C-D). Particularly, FL-HCC cells contain the so-called "pale bodies," which are in fact composed of fibrinogen and albumin (Figure 7E-F); however, these pale bodies are nonspecific, as they can be seen in other types of HCCs, especially in scirrhous HCC. Nonetheless, characteristic fibrous collagen bands, which are thick and homogenous in FL-HCC, are not present in scirrhous HCC or any sclerotic variant of HCC. Moreover, FL-HCC has a higher tendency for regional lymph node metastasis compared to classic HCC and spreads more frequently than classic HCC to the peritoneum, omentum, and lung,[41,47,48,50] with remote metastasis involving left supraclavicular lymph nodes and the abdominal wall seen at our institution.

Steatohepatitic HCC. A recent investigation conducted in India has described a new variant of HCC termed "steatohepatitic hepatocellular carcinoma (SH-HCC)," which has been connected with metabolic risk factors identified in Indian patients.[51] Briefly, 101 cases of HCC within explanted livers from adult patients were inspected for tumor histomorphology in the context of clinically identifiable metabolic risk factors in this study. The authors diagnosed SH variant of HCC in 19 (18.8%) of 101 liver explants. Among SH-HCC cases, 17 were males and 2 were females, ranging from 47 to 65 years (mean age 54.8 years). Of notice, 9 of the 19 SH-HCC cases were associated with HCV, 6 of the 19 were associated with nonalcoholic fatty liver disease (NAFLD), 2 with HBV, 1 with alcoholic liver disease (ALD), and the last 1 with mixed (HBV + HCV + ALD) infection. No obvious difference in the size, location, number of lesions, overall tumor differentiation, and vascular invasion between SH-HCC and conventional HCC cases was observed.[51] In addition to the loss of reticulin, glypican 3 appears to be a good diagnostic tool, as this immunostain performed in 18 cases showed strong cytoplasmic (11 of 13) and focal canalicular (2 of 13) labeling in 13 SH-HCC cases.[51] The authors concluded that a comparison of SH-HCC with non-SH-HCC was statistically significant ($P = .03$) for an SH-HCC association with metabolic risk factors. Due to its rarity, the clinical course of SH-HCC has not been well established, but they appear to have better prognoses when compared to conventional HCCs; among 19 patients with SH-HCC, 16 were alive and disease free at 24 to 72 months following the diagnoses, compared to 76 patients with conventional HCC where 16 patient deaths had been documented.[51]

We encountered similar SH-HCC case(s) in practice, which possessed morphological features mimicking steatohepatitis in the setting of NAFLD. Tumor cells showed macrovesicular steatosis, inflammation, and ballooning degeneration, with frequent Mallory hyaline and globules; microclusters of lymphocytes, plasma cells, and foci of neutrophils were seen in the vicinity of ballooned neoplastic hepatocytes. The clinical course of such cases(s) is under observation.

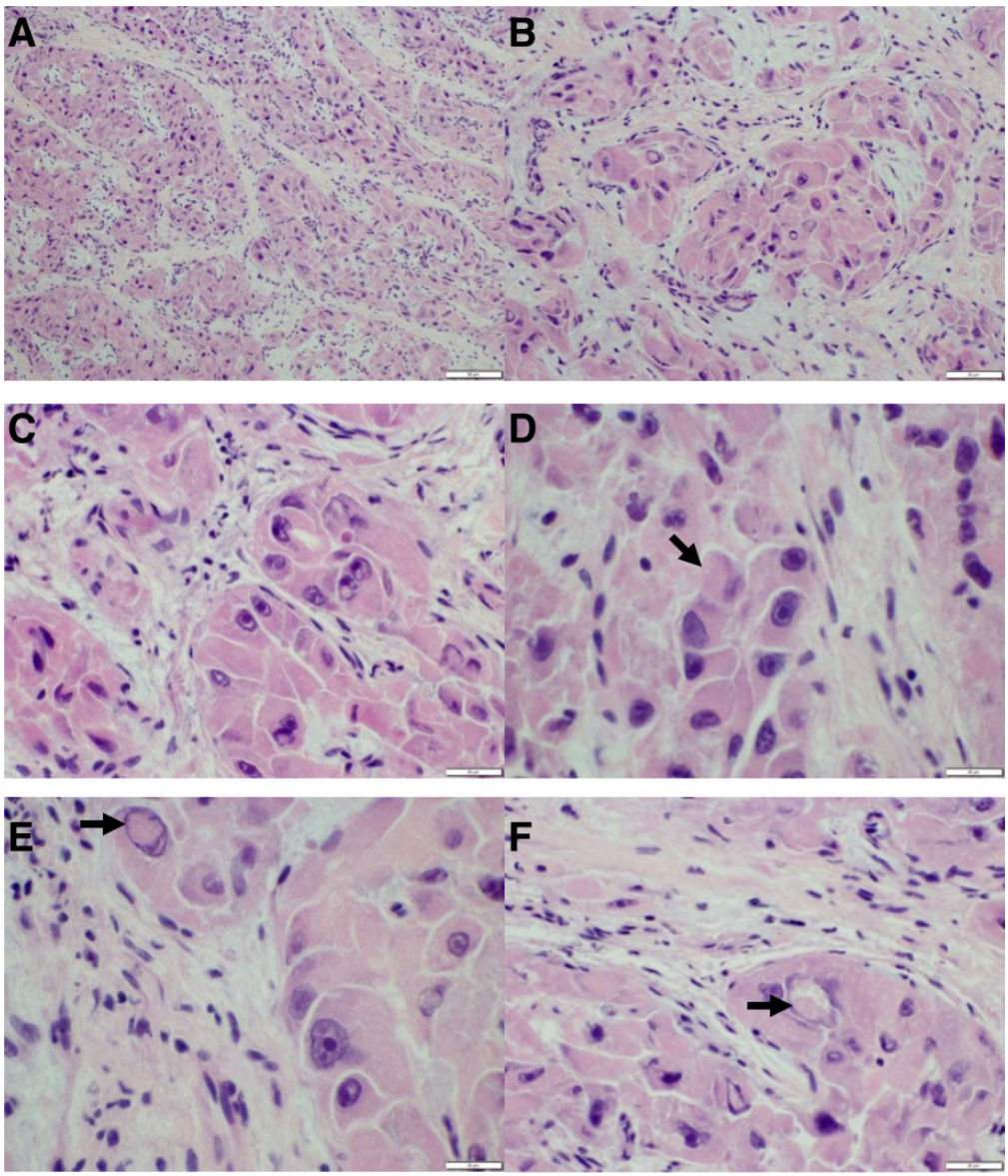

Figure 7. Histomorphology of FL-HCC. (A) and (B) FL-HCC with thick fibrous collagen bands that encircle and surround neoplastic hepatocytes (H&E, ×40 and ×100). (C) and (D) Polygonal FL-HCC cells with abundant eosinophilic cytoplasm, large nuclei, and prominent nucleoli (×200 and ×400; arrow: pale/hyaline body). (E) and (F) Polygonal cells with hyaline bodies, some of which cause nuclear indentation and nuclear pseudo-inclusion (arrows, ×600). FL-HCC indicates fibrolamellar hepatocellular carcinoma.

Ancillary Studies in Diagnosing and Differentiating HCCs

Many cases of liver mass lesions, especially liver core biopsies and wedge specimens, require confirmatory ancillary studies, including immunohistochemistry and special stains. Before selecting any ancillary studies, it is essential to determine whether the biopsy is from a targeted mass or random liver, a lesion is present in the material, the tissue is sufficient for ancillary evaluation, and whether it is hepatocellular or something unexpected or metastatic. The ancillary tools should be chosen, based on the questions.

Multiple special stains and immunohistochemical studies have been described to confirm a diagnosis of HCC, the frequently utilized ones are reticulin, CD34, polyclonal carcinoembryonic antigen (CEA) (P-CEA), CD10, glypican 3 and AFP; HepPar-1 and arginase 1 should be performed when the hepatocellular nature of the tumor is in doubt Arginase 1 shows higher sensitivity and specificity (both approximately 90%) than HepPar-1 and glypican 3 in confirming hepatocytic differentiation.[27,52,53] An investigation conducted by Fujiwara et al summarized the usefulness of Arginase 1, HepPar and glypican 3 in the study of liver tumors. It concluded that arginase 1 is the first choice for confirming or excluding HCC, due to its high sensitivity (80%-95%)

and even higher specificity (95%-100%); especially when combined with HepPar-1 and glypican 3, almost all HCCs, including rare variants, can be diagnosed with confidence. A similar conclusion has been drawn by Radwan et al following comparing arginase 1 and HepPar-1 expression in 50 HCC, 38 metastatic carcinoma, 12 cholangiocarcinoma and 10 benign liver cases.[53,54]

Glutamine synthetase and heat shock protein 70 2 markers used to differentiate benign from malignant liver nodules. In HCC, glutamine synthetase (GS) shows diffuse cytoplasmic staining.[55] In a recent study, GS and HSP 70 were negative in all adenomas. At least one of the 2 markers was positive in 85% of of very well-differentiated HCC. It should be kept in mind that GS also labels FNH (map-like pattern), and could lead to pitfalls initnerpretation.[56,57]

Reticulin is the special stain used most frequently when facing a diagnosis of possible HCC. Reticulin is lost in the majority of HCC tumors; or it may highlight thickened hepatocyte plates, usually not easily appreciated on H&E sections (Figure 8A). In contrast to HCC, the reticulin stain in benign liver, FNH, regenerative nodules, and HAs will highlight the hepatic plates, demonstrating trabecula composed of a single or double layer of hepatocytes (Figure 1E). Alternatively, HCC may show a reduction in the intensity of reticulin staining rather than complete loss, with areas of hepatocytes that do not have direct contact with reticulin fibers. The diagnosis in these cases has to be made by other cytological features, including atypia and proliferative rates. An additional diagnostic pitfall is HCC with steatosis, because benign hepatic tissue with macrovesicular steatosis has focal and patchy reticulin loss, which could mimic HCC. In rare cases of well-differentiated HCC, reticulin stain could be even retained, disfavoring a diagnosis of HCC. Therefore, other essential markers of HCC, as well as cytological atypia and proliferative rates, should be investigated to help characterize the lesion. Radiological studies, combined with laboratory findings are also crucial in helping reach a final diagnosis.

In HCCs, immunostaining for CD34 shows a strong diffuse sinusoidal staining pattern (Figure 8C-D), whereas in benign liver, stains for CD34 highlight the zone 1 (periportal) sinusoids focally (Figure 1F). In HCC with a macrotrabecular growth pattern, a CD34 stain highlights thickened trabeculae, with negative labeling inside the tumor mass (Figure 8C-D). However, occasional HCCs do not show this staining pattern, rendering CD34 less useful versus some of the other available stains.

More than two-thirds of HCC show a canalicular pattern of expression with P-CEA and CD10. Considered as indicators of hepatic differentiation, they are more useful in well-differentiated and moderately differentiated HCCs. Focal and patchy staining patterns for p-CEA (Figure 8E) and CD10 have been seen in nearly one-third of HCCs Staining with P-CEA may be difficult to interpret as sometimes it shows a nonspecific membranous or even cytoplasmic pattern in HCC. It is important to keep in mind that the absence of canalicular staining is usually encountered in poorly differentiated HCCs. The lack of canalicular staining for p-CEA or CD10 does not necessarily exclude a poorly differentiated HCC; it actually implicates additional immunostaining workup and correlation with clinical, radiological, and laboratory findings.

AFP has been the so-called "tumor marker" for HCC several decades, but immunohistochemistry for AFP is positive in only about one-third of HCCs. Due to its low positive rates and the puzzling fact that patients with HCC frequently show normal serum AFP levels, even when tumors are positive for AFP by immunostaining, AFP is not utilized routinely as a first-line choice in the diagnostic workup of HCCs, except in a poorly differentiated HCC. AFP expression is not specific for HCC, as it may also be expressed by intrahepatic CC and metstatic adenocarcinomas from other organs. Hence, a positive AFP immunostain needs to be interpreted in the context of histomorphology, additional immunostains, and clinical findings.

HepPar-1, which recognizes a mitochondrial antigen, is popularly used to confirm hepatocellular differentiation in diagnosing HCCs. Immunohistochemistry will demonstrate coarse granular cytoplasmic staining.[53,58] Approximately 10% of HCCs actually show negative HepPar-1 labeling,[59] especially the poorly differentiated tumors. Therefore, a lack of expression for HepPar-1 does not exclude hepatocytic differentiation. Hepatocyte paraffin 1 expression can be focal instead of diffuse (Figure 8G).

Hepatocyte paraffin 1 also lacks specificity for hepatocytic differentiation, since expression has been reported in multiple other types of carcinomas, such as gastric, esophageal, pulmonary, and colonic adenocarcinomas; hepatoid carcinomas from the stomach and pancreas, and adrenal cortical carcinomas, to name a few.[37,60,61] Arginase immunostain is preferable for the confirmation of hepatocytic differentiation, as it is superior to HepPar-1 in terms of specificity and sensitivity.[52,53]

In parallel, as another frequently used unique marker for HCC, glypican 3 shows positive cytoplasmic labeling in approximately 80% of HCCs, more likely in tumors that arise in cirrhotic livers and in the setting of chronic HBV infection,[62,63] with the background benign liver showing negative staining. Moreover, glypican 3 is usually negative in well-differentiated HCCs.[62] Since glypican 3 is virtually always negative in HAs and in FNH, a positive glypican 3 is highly compatible with HCC. It has been well acknowledged that while HepPar-1 is often positive in well-differentiated HCC, glypican 3 is more so in poorly differentiated tumors.[62] However, like HepPar-1, glypican 3 is not specific for HCCs, as other tumor types might be labeled by it. Furthermore, extra caution is needed for interpreting unexpected glypican 3 positivity associated with significantly inflamed benign hepatocytes,[64] macroregenerative nodules, and DNs.[62] A third problematic issue with glypican 3 immunostaining is that the labeling can be very patchy, especially when dealing with needle biopsy specimens, as up to 50% of the biopsies could be negative for glypican 3 due to limited amount of tissue.[53] It is pivotal to keep in mind that a negative labeling by glypican 3 does not exclude HCC.

Recently, arginase 1 has been identified to be a strong diagnostic tool for identifying HCC,[52,53] which is positive in both

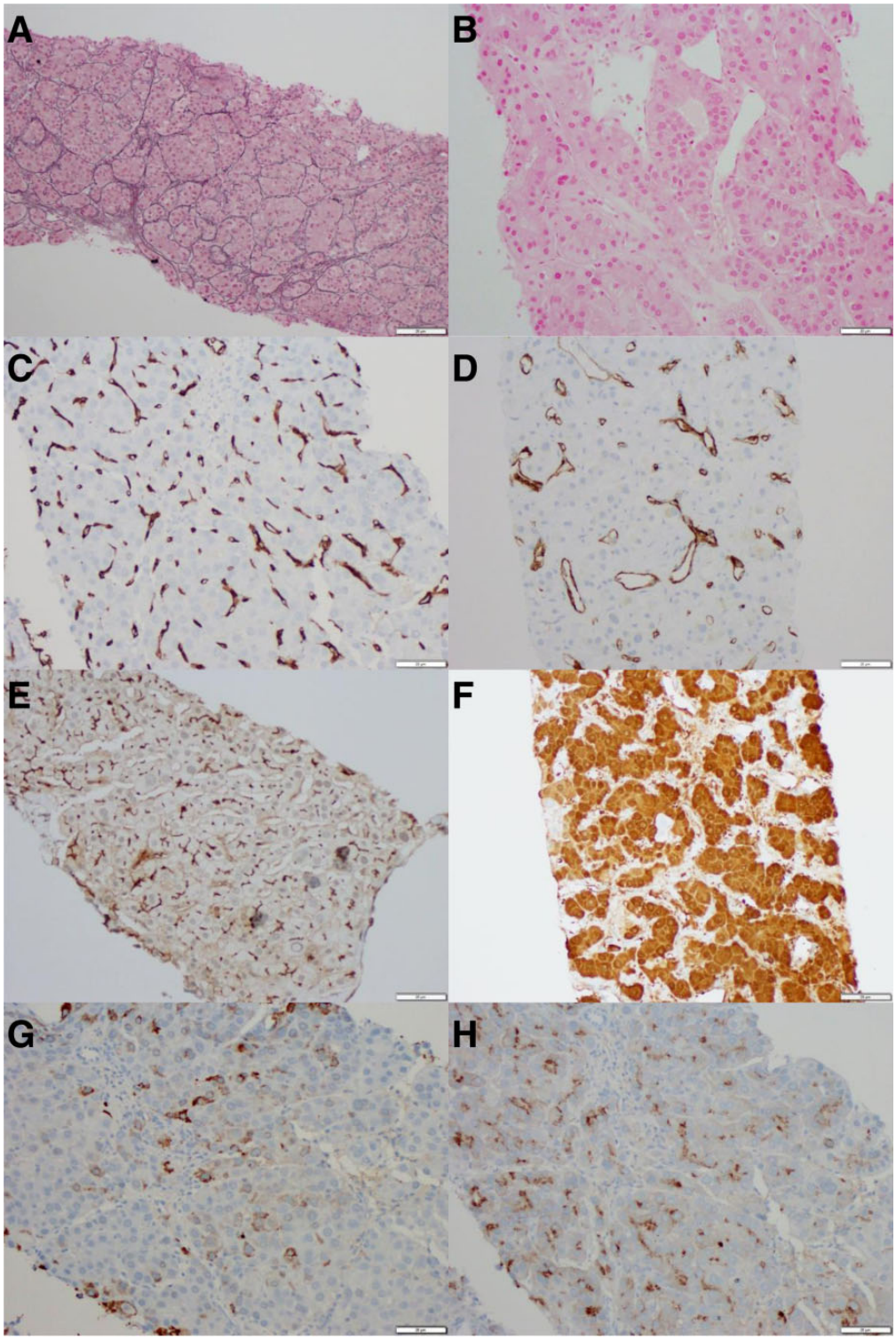

Figure 8. Photomicrograph (special stains and immunostains) of HCC. A, Thickened hepatic plates shown by reticulin, ×40. B, Absence of iron deposition, Prussian blue, ×100. C and D, Diffuse sinusoidal CD34 labeling in HCC, ×100. E, P-CEA labeling with a prominent canalicular pattern, ×100. F, Diffuse glutamine synthetase labeling in HCC, ×100. G, Focal, patchy labeling of heppar 1, ×100. H, glypican 3 immunostain, ×100. HCC indicates hepatocellular carcinoma.

benign and malignant hepatocytes, and has been recognized to possess better sensitivity and specificity than glypican 3 or HepPar-1. A combination of arginase 1, glypican 3, plus reticulin, CD34, and p-CEA are frequently chosen, coupled with the H&E staining probably could satisfy diagnosing almost all HCCs.[53] Finally, a panel of immunostains has been

speculated to be of value in providing prognostic information, as identified for CK-19, whose positivity has been demonstrated in approximately 10% to 15% of HCCs and has been recognized as an indicator of "stemcellness" and a worse prognosis.[65]

Except for FL-HCC, cytokeratin staining is not widely utilized for diagnosing classic HCCs; CAM5.2, CK-8, and CK-18 are expressed in both benign hepatocytes and HCCs.[40] Cytokeratin 7 immunostaining is mostly negative in HCCs but can be positive in HCCs that are cholestatic or that arise in young patients and frequently in FL-HCC.[66] Cytokeratin 20 is generally negative in HCCs. Fibrolamellar HCC tumors express markers of hepatocellular differentiation including arginase, HepPar-1, and p-CEA (canalicular pattern). Cytokeratin 68 shows granular cytoplasmic signal in majority of FL-HCC tumors. The positive rate of glypican 3 in FL-HCC cases is lower than in classic HCCs and are not used as a first-line choice.

Examination of the benign background liver for disease grading and staging is equally important for clinical decision-making. Sections of benign liver parenchyma away from the HCC should be taken. Investigation of hepatitis activity, hepatocellular injury, steatosis and steatohepatitis, and fibrosis need to be undertaken to characterize the background liver. Biopsy tissue cores obtained for the purpose of assessing the nonneoplastic liver should be taken as far away as possible from the HCC or any mass lesion. Also, the margins of resection in a surgical resection specimen should not be used due to thermal artifacts and surgery-related changes.

Benign Hepatocellular Nodules

The 2 other most often encountered hepatocellular lesions in the liver are FNH and HA, both will be discussed in the following text. The difference between HCC (especially well differentiated), FNH, and HA can be clinically and histopathologically challenging. Hepatic adenoma can show similar radiological and imaging features to HCC, due to aberrant arterioles and abundant blood supply, whereas half of the FNH cases don't show radiological or imaging evidence for a scar when the lesions are less than 4 cm in size. An accurate diagnosis is crucial for clinical management and patient outcomes.

Focal Nodular Hyperplasia

Focal nodular hyperplasia is a nonneoplastic, benign reactive nodular lesion frequently encountered clinically, composed of bland hepatocytes and fibrotic septa containing proliferating ductules. Focal nodular hyperplasia develops in noncirrhotic liver parenchyma, likely due to localized shunting of arterial blood flow. Any condition causing blood shunting could induce formation of FNH, as seen in rare cases of HCCs where reactive FNH developed in the vicinity of HCCs due to interference of blood supply. Focal nodular hyperplasia has no malignant potential based on current scientific literature.

Clinically, FNH occur frequently in young and middle-aged women, as either single or multiple lesions, the majority were identified in women between the ages of 20 and 50 years. Focal nodular hyperplasia could also develop in children and teenagers, usually following chemotherapy for other malignancies. FNA may even develop in liver allografts.[67] The precise etiology for FNH development, however, hasn't been determined with certainty, although a potential connection with oral contraception has been proposed. The background liver should not be cirrhotic.

Histologically, FNH is composed of nodules of cytologically benign hepatocytes separated by thin and thick fibrous bands (Figure 9A) that may coalesce into a larger central scar. In smaller FNHs, the nodularity may be less developed and not easily appreciated at low-power view. The hepatocytes do not show atypia, and a reticulin stain demonstrates a normal 1- to 2-cell thick plates. Both the periphery and the central regions of FNH lesion often show large caliber vessels with thickened muscular walls (Figure 9C-D). The fibrous bands typically have proliferating bile ductules, mostly located at the edges of the fibrous septa (Figure 9E-F). Focal nodular hyperplasias do not have capsules and true portal tracts. A central scar is usually appreciated if the lesion is larger than 4 cm but is only seen in half of the smaller lesions. Because FNH lacks portal tracts and sufficient bile drainage, they often show cholestasis and mild copper accumulation in the areas abutting the fibrous septa. Occasionally FNH shows ballooning and Mallory hyaline or fatty change; features resembling clear cell of the SH variant HCC. The overall architecture and the bland cytology usually allow distinction of these 2 possibilities on H&E staining. In challenging cases, special stains for GS can be resorted.

In normal liver, GS only stains a delicate rim of pericentral hepatocytes surrounding the central veins (Figure 1F). In contrast, staining in FNH will show an irregular, "map-like" pattern (Figure 9G, H); this specific pattern can be extremely helpful in diagnosing FNH.[57] On the other hand, a distinct, diffuse staining pattern can be observed in either HAs or HCCs (Figure 8F). However, most HAs and some HCCs are entirely negative by GS staining. Other useful tools in diagnosing FNH are cytokeratin stains to highlight the proliferating bile ductules, a copper stain to highlight cholestasis, a reticulin stain, and potentially glypican 3 and Ki-67 index to help rule out well-differentiated HCC.[55,57]

It has been noted that less than 50% of the FNH cases could be confidently diagnosed on needle core biopsy. Although this could be due to sampling errors, as multiple passes would increase the rate of definitive diagnosis, a confident diagnosis was often hampered by the absence of essential imaging evidence and laboratory findings. Because a diagnosis of FNH is often made only after correlating with the imaging and histological findings, an experienced pathologist might conclude the findings are consistent with or suggestive of FNH after correlation with available imaging studies. Therefore, immunostaining for GS should be utilized to help clarify these challenging cases.[58] Roncalli

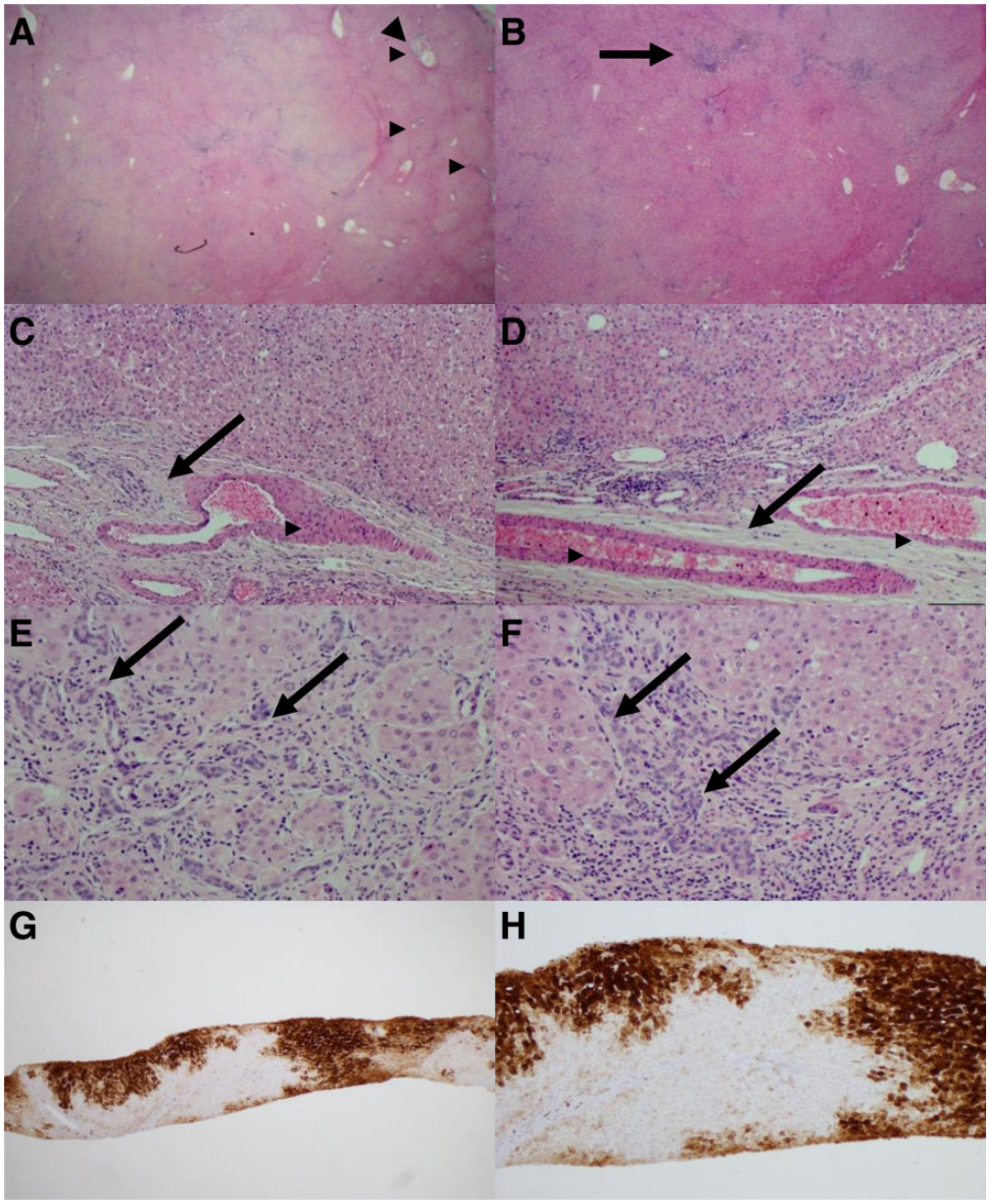

Figure 9. Histopathological features of FNH. (A) and (B) Photomicrograph (×20 and ×40; H&E stain) shows the multinodular contoured lesion with adjacent normal liver with portal tracts (arrow heads, A) and focal inflammatory fibrous bands (arrow, B). (C) and (D) Photomicrograph (H&E stain, ×100) shows a septum (arrow) dividing 2 neighboring nodules (C). The septum contains connective tissue and thick-walled vessels (arrow heads in C and D). (E) and (F), At the interface of the FNH nodule and septa, reactive biliary ductular proliferation is enriched (arrows). (G) and (H), Glutamine synthetase showing a maplkie-pattern. Peroxidase, ×200. FNH indicates focal nodular hyperplasia.

et al reviewed the pathobiology of FNH and HA lesions and proposed a diagnostic algorithm in an effort to increase the diagnostic accuracy of these frequently challenging entities. Basically FNH is a nodular polyclonal tumor-like hepatocytic proliferation that does not undergo hemorrhage or malignant transformation; on the other hand, HAs are a monoclonal proliferation of bland-looking hepatocytes embedded in 1- to 2-cell thick hepatic plates. Nuclear atypia and mitoses could be seen in specific variants. The authors summarized systematically the differential diagnosis between traditional HAs and atypical HAs and well-differentiated HCC.[68] For example, FNH carries nonclonal β-catenin activation without mutations, contributing to hepatocellular hyperplasia and regeneration, and mutations in β-catenin has never been identified in FNHs.[68] In contrast, HAs have been subclassified into different groups based on the status of hepatocyte nuclear factor 1α1 (HNF1A1) β-catenin, and serum amyloid A (SAA), or C-reactive protein (CRP).

Hepatic Adenoma

Hepatic adenomas are a group of hepatocytic neoplasms with well-differentiated morphology that are usually benign. Hepatic adenomas are monoclonal neoplasms with unique molecular signatures and oncogenetic pathways that are distinct from HCCs. Hepatic adenomas are well-known to occur in young females of childbearing age, especially in those with a history of estrogen-based, oral contraceptive pill usage and less often to occur in men, usually with a history of anabolic steroid use. The prevalence of HAs is on the rise due to increased use of imaging modalities, which leads to increased incidental detection. The annual incidence is around 4 per 100 000 per year in developed countries; the female predominance has not been confirmed in Asian patients, seemingly related to lesser use of oral contraceptives in these countries.[69-71] Most HAs are solitary and patients are asymptomatic. The presence of multiple HAs has been termed hepatic adenomatosis.[72] Hepatic adenomas carry a risk of rupture and bleeding, and some subsets of HAs have the potential to undergo malignant transformation.[73] Clinically it is very important to correctly diagnose and manage HAs, especially when HCC is in the differential list.[73] Hepatic adenomas are characterized by a relatively uniform population of hepatocytes arranged in cell plates of3-cell thick. The pattern may be slightly more irregular compared to the adjacent liver. The hepatocytes retain a low N/C. The reticulin remains intact. The cells may have cytoplasmic contents similar to normal hepatocytes. Large arterial vessels, unaccompanied by bile ducts, are prominent. The genotypic type will influence the morphological phenotype. Diagnosis of HA can be challenging in cases with a limited amount of biopsy material as well as due to the overlapping histomorphological features with FNH and well-differentiated HCC.

A molecular pathology-based classification for HA has been established, dividing HAs into subtypes based on their molecular characteristics, as determined by the corresponding immunohistochemical profiles and histomorphological characteristics.[74,75] The established HA subtypes are (1) HAs with mutated, inactive *HNF1A*, (HA-H), (2) HAs with activating mutations in the *CTNNB1* gene encoding β-catenin (HA-B), (3) HA without mutations of *HNF1A* or β-catenin genes but with inflammatory features (HA-I, formerly called telangiectatic FNH), and lastly (4) unclassified HAs that have neither known gene mutations nor a unique histomorphology (HA-U,[74] the histological features of these adenomas are shown in Figure 10A-D, respectively).

Hepatic adenoma with *HNF1A* mutations account for approximately 30% of HAs. The mutations are somatic in most cases; but in some cases, 1 mutation may be germ line. There are differences in presentation and etiology between the somatic and germ line patients. Hepatic adenoma with *HNF1A* mutation with somatic mutations occur mostly in females with a history of oral contraceptive use. Hepatic adenoma with *HNF1A* mutation with germ line mutations also present mostly in females but occur at an earlier age and patients do not typically have a history of oral contraceptive use. The HA-H in these patients are larger on average. Familial hepatic adenomatosis has been well-documented in patients with germ line mutations of *HNF1A* and it is also associated with maturity-onset diabetes mellitus of youth, type 3.[72] Detection of germ line mutations of *HNF1A* in family members of patients with hepatic adenomatosis has been established to identify familial predisposition for the disease.

Both somatic and germ line HA-H are morphologically characterized by significant steatosis, without nuclear atypia or inflammatory features.[74] Expression of liver fatty acid-binding protein (LFABP) is downregulated in HA-H as a consequence of the *HNF1A* mutation,[55] therefore, immunohistochemistry for LFABP serves as a translational marker to identify this subtype of HA, since expression will be lost in HA-H compared to normal liver, in which it is normally expressed.

Hepatic adenomas with activating mutations in the *CTNNB1* gene encoding β-catenin account for 10% to 15% of all HAs. Mutations in this gene are exclusive of *HNF1A* mutations. These occur more often in males than in females. Contributing factors include congenital metabolic disturbances, such as glycogenosis 1 and 3, the consumption of anabolic steroids, or the use of oral contraceptives in females. Morphologically, they are characterized by cytological atypia, peliosis hepatis, pseudoacinar formations, and are less frequently steatotic. In HA-B, two β-catenin target genes, *GLUL* and *GPR49*, were found to be overexpressed 42-fold (ranging from 9 to 87) and 35-fold (ranging from 8 to 57) when compared with nontumor tissues, respectively. *GLUL* encodes GS. Immunohistochemistry for GS and β-catenin is used a translational marker, with strong and diffuse expression for GS and aberrant nuclear and cytoplasmic expression for β-catenin.[74]

HAs with activating mutations in the *CTNNB1* gene encoding β-catenin with mutations in exon 3 of the *CTNNB1* gene encoding β-catenin (HA-B^{ex3}) are associated with a high risk of malignant transformation. Mutations in exons 7 and 8 have also been identified. This type, referred to as the weak β-catenin activation type, has a mild activation of the Wnt/b-catenin pathway and does not have an increased risk of malignant transformation.[76] Immunohistochemcal expression of β-catenin will not usually show nuclear expression, and GS expression is faint and patchy.

Inflammatory adenomas (HA-I) account for over 50% of HAs. These occur in both men and women and may be single or multiple. Obesity, metabolic syndrome, and alcohol consumption are predisposing risk factor. Inflammatory adenomas are characterized by activation of the interleukin 6/Janus kinase (JAK)/Signal transducer and activator of transcription protein (STAT3) pathway, resulting in overexpression of SAA and CRP. This pathway is activated through mutations in *IL6ST* (most common), Fyn Related Src Family Tyrosine Kinase (*FRK*), *JAK1*, *STAT3*, and Guanine nucleotide-binding protein (G9s) subunit alpha isoforms short (*GNAS*).[76] Morphologically, HA-I are usually difficult to distinguish from the adjacent parenchyma. They are characterized by bile ductular proliferations, but the ductular-like proliferations are located in faux portal tracts. The faux portal bands contain small arterioles

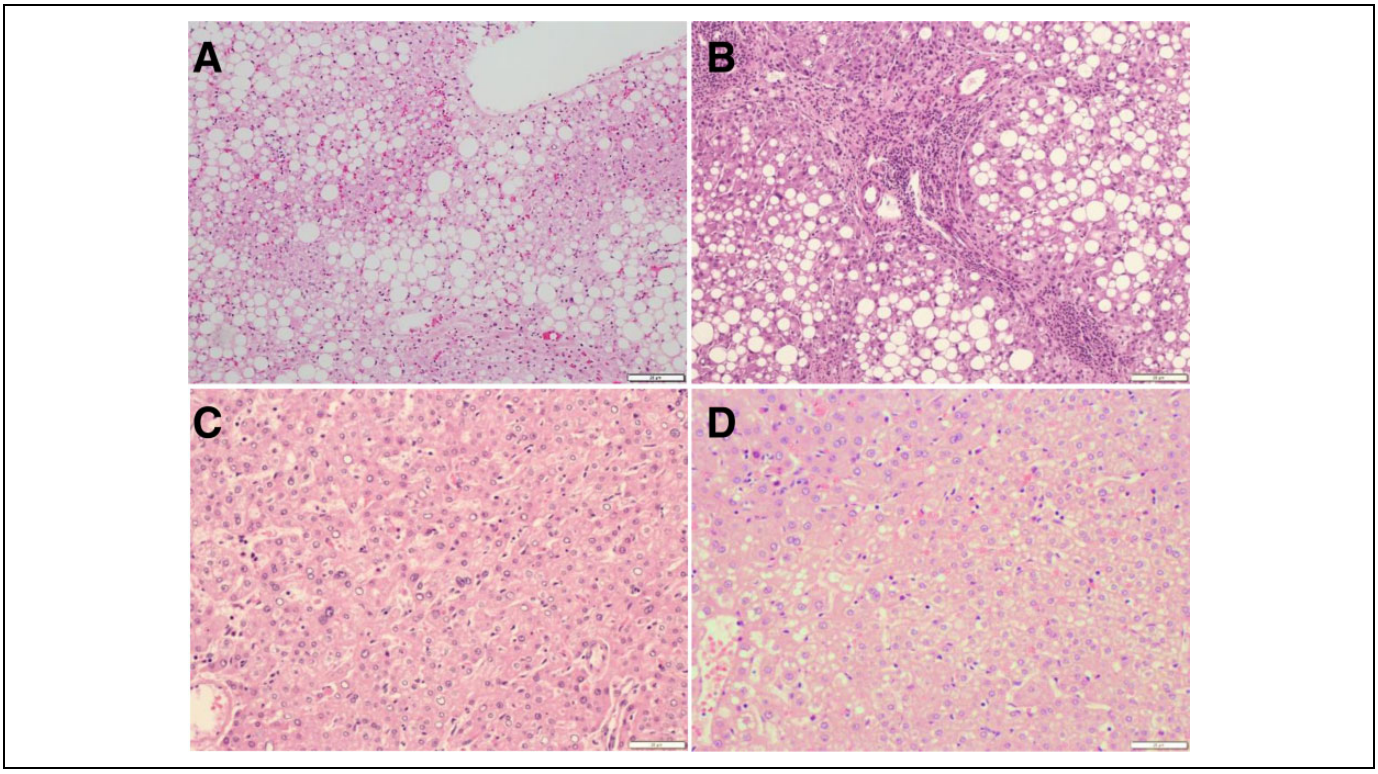

Figure 10. (A) to (D) Type 1 to 4 hepatocellular adenoma: (1) HNF-alpha mutated; (2) inflammatory type; (3) Beta-catenin-mutated; and lastly; (4) nonclassifiable HA. HA indciates hepatic adenoma; HNF, hepatocyte nuclear factor.

with an associated mononuclear cell infiltrate. Immunohistochemistry for SAA and CRP serves as translational marker of this subtype. A subset of HA-I may also have mutations in the *CTNNB1* gene encoding β-catenin, either exon 3 or exons 7 and 8 (HA-IB^{ex3} and HA-IB^{ex7,8}, respectively). These mixed types may be recognized with immunohistochemistry for β-catenin.

It is important not to mix HA-I with FNH. Findings that favor an FNH include the fibrous bands, abnormal thick-walled vessels, and a ductular proliferation that is typically patchy and within the fibrous bands. Finally, attention should be paid to FNH-like changes that develop around any mass lesion in the liver that has interfered with blood flow, including metastatic neoplasms. In these cases, this reactive rim of hepatocytes can be indistinguishable from an ordinary FNH on needle biopsy.

In a recent report, Nault et al analyzed the expression of 20 genes and sequenced exon regions of the 8 genes, *HNF1A*, *IL6ST*, *CTNNB1*, *FRK*, *STAT3*, *GNAS*, *JAK1*, and Telomerase Reverse Transcriptase (*TERT*) , in 607 samples of 533 HAs from 411 patients, collected from 28 centers mainly in France from a 14-year span.[76] Based on their molecular data, they classified HA into 8 subgroups, including a novel subgroup, representing 4% of HA previously classified as HA-U.[76] This subgroup was characterized by activation of the sonic hedgehog signaling pathway (HA-SH) and was associated with obesity and bleeding. This pathway is driven by structural rearrangements of Inhibin Beta E Subunit *(INHBE)* producing a highly expressed INHBE_-Glioma-associated oncogene family zinc finger 1 (GLI1) fusion protein leading to the constitutive activation of the sonic hedgehog pathway. This subtype is exclusive of all other subtypes. Immunohistochemistry for the protein Prostaglandin D2 synthase (PTGDS) serves as a translational marker.

Studies of intra- and intertumoral genetic heterogeneity showed a molecular subtype field effect. Nault et al[76] correlated their molecular data with risk factors for HA development, the risk of bleeding, and malignant transformation. The cumulative intake of Oral contraceptives (OCP), obesity, and alcohol intake contributed to estrogen exposure. Body mass intake also contributed. Patients with higher estrogen exposure or high BMI were more likely to develop HA-I or HA-SH. Patients with HA-IB^{ex3} were more likely to have had androgen exposure. The frequency of histological hemorrhage was higher in HA-IB^{ex7,8} and SA-SH. Features associated with malignant transformation included *TERT* promoter mutations, *CTNNB1* exon 3 mutations, a unique nodule at imaging, high alcohol intake, fibrosis in nontumoral liver, and diabetes type 2. In patients with multiple HA, the largest HA tended to be associated with *CTNNB1* exon 3 mutations; thus, image-guided biopsies in patients with adenomatosis can be directed at the largest nodule. Based on these findings, the molecular profile can be used to guide resection in female patients. In male patients, the baseline risk of malignant transformation is higher (41% in females vs 65% in males), and, therefore, this risk stratification is not as useful.[76]

The final subset has no recognized mutations and no specific morphological features. This subset is referred to as HA-U. This genotypic–phenotypic classification and risk factors for HCC are shown in Figure 11.

Atypical Adenoma

Despite all the investigations discussed so far, atypical yet well-differentiated hepatocellular lesions keep posing diagnostic challenges. In these unusual cases, the utilization of immunohistochemical markers discussed earlier, glypican 3, Glutamine synthetase (GS), and Heat shock protein 70 (HSP70), could serve as useful tools to help reach the correct diagnoses. In making a definite diagnosis, the appropriate approach to these entities is unexceptionally clinical and radiological, as HA but not FNH is usually associated with young to middle-aged female, history of oral contraceptive usage, frequently metabolic syndrome (diabetic, hyperlipidemia, obesity), inflammatory syndrome, and possibly alcohol assumption. All these traits require detailed and systematic investigation. It has been recognized that sporadic adenomas unrelated to certain clinical traits/diseases are rare. And it has been proposed that for those cases with definitely equivocal pathological features, and those that are frequently associated with incomplete or an absence of radiological and laboratory information, the term of well-differentiated hepatocellular neoplasm of uncertain malignant potential instead of atypical hepatocellular neoplasm or atypical HA should be used.[68]

Histopathological Staging and Risk Stratification of HCC

The prognosis of patients with HCC is influenced by tumor size, tumor number, and the presence of angiolymphatic invasion (HCC staging). The combination of these factors is key in predicting the clinical course and patient outcomes. Gross finding of large vessel invasion has a worse prognosis than small vessel invasion, which is recognized only on microscopy examination. Tumor differentiation also influences prognosis, as do morphologic variants discussed in the following texts. After all, the most critical prognostic factor in HCC is the resectability. Other prognostic indicators include age (younger patients better), gender (women usually better), the activity, and the stage of background liver disease(s).

TNM and Anatomic Stage/Prognostic Groupings

The TNM staging system of the American Joint Committee on Cancer (AJCC) and the International Union against Cancer (UICC) has been widely accepted for staging and risk stratification of HCC. The T classification depends on the number of tumor nodules, the size of the largest nodule, and the presence or absence of blood vessel invasion. Vascular invasion includes either grossly appreciated or microscopically identified tumor involvement of vessel spaces. Portal vein invasion by HCC is an important adverse prognostic factor and should be reported. The eighth edition of the AJCC is to be implemented on January 1, 2018.[77]

Based on AJCC/UICC convention, the designation "T" represents a primary HCC that has not been treated previously. The symbol "p" represents the pathologic classification of the TNM, as opposed to the clinical classification; the scale of symbol p is based on gross findings and microscopic examination. pT entails a resection of the primary HCC or biopsy with sufficient material for evaluating the highest pT category; pN entails removal of lymph nodes adequate to validate metastatic disease involving lymph nodes, and pM indicates microscopic examination and findings on potential metastatic diseases (distant lesions). On the other hand, clinical classification (cTNM) is usually performed and determined by the referring clinician prior to any treatment, frequently during initial evaluation of the patient or when pathologic classification is not possible, such as when the pathology material has not become available for pathologist's review.

For identification of special cases of pTNM classifications, the "m" suffix and "y," "r," and "a" prefixes are used. The m suffix indicates the presence of multiple primary tumors in a single site, recorded as pT(m)NM. The y prefix indicates that the tumor classification is performed during or after initial multimodality therapy (ie, neoadjuvant chemotherapy, radiation therapy, or chemoradiation therapy). The cTNM or pTNM category is identified by a y prefix. The ycTNM or ypTNM categorizes the extent of actual tumor at the time of that examination. The r prefix indicates a recurrent tumor when staged after a documented disease-free interval and is identified by the r prefix. Finally, the a prefix designates the tumor stage determined at autopsy: aTNM. The primary tumor classification for HCC is shown in Table 1. Lymph node status and distant metastases are also prognostic indicators.

A complete pathologic staging is almost always carried out on surgically resected primary HCC. Accurate pathologic staging depends on pathologic documentation of the anatomic extent of tumor and whether the primary tumor has been completely excised. In rare cases, when a biopsied or aspirated tumor becomes nonresectable due to any reason (such as when the patient declines surgery, it is technically infeasible, or clinically not indicated), or if the highest T and N categories, or the M1 category of the metastatic disease has been confirmed microscopically, the abovementioned criteria for pathologic classification and staging have been satisfied without surgical resection of the primary tumor.

Cholangiocarcinoma

Cholangiocarcinoma is a malignant adenocarcinoma with evidence of biliary differentiation. Based on the location, cholangiocarcinomas are divided into intrahepatic and extrahepatic groups. Klatskin tumor, or the so called "hilar cholangiocarcinoma," is an extrahepatic tumor arising in the right or left hepatic duct or at their junction. Tumors arising from the intrahepatic hepatic ducts include intrahepatic and perihilar cholangiocarcinomas. In practice, the precise origin of some larger tumors can be challenging as there is no clear evidence whether they are intrahepatic or extrahepatic, but fortunately all these tumors show histomorphology of biliary differentiation. The global incidence of intrahepatic cholangiocarcinomas, but not extrahepatic types, shows an increasing trend. Although the exact etiology has not been well established, cholangiocarcinomas are associated with chronic inflammatory conditions of the biliary tract and are also associated with chronic bile stasis. Based on the current understanding, risk factors for

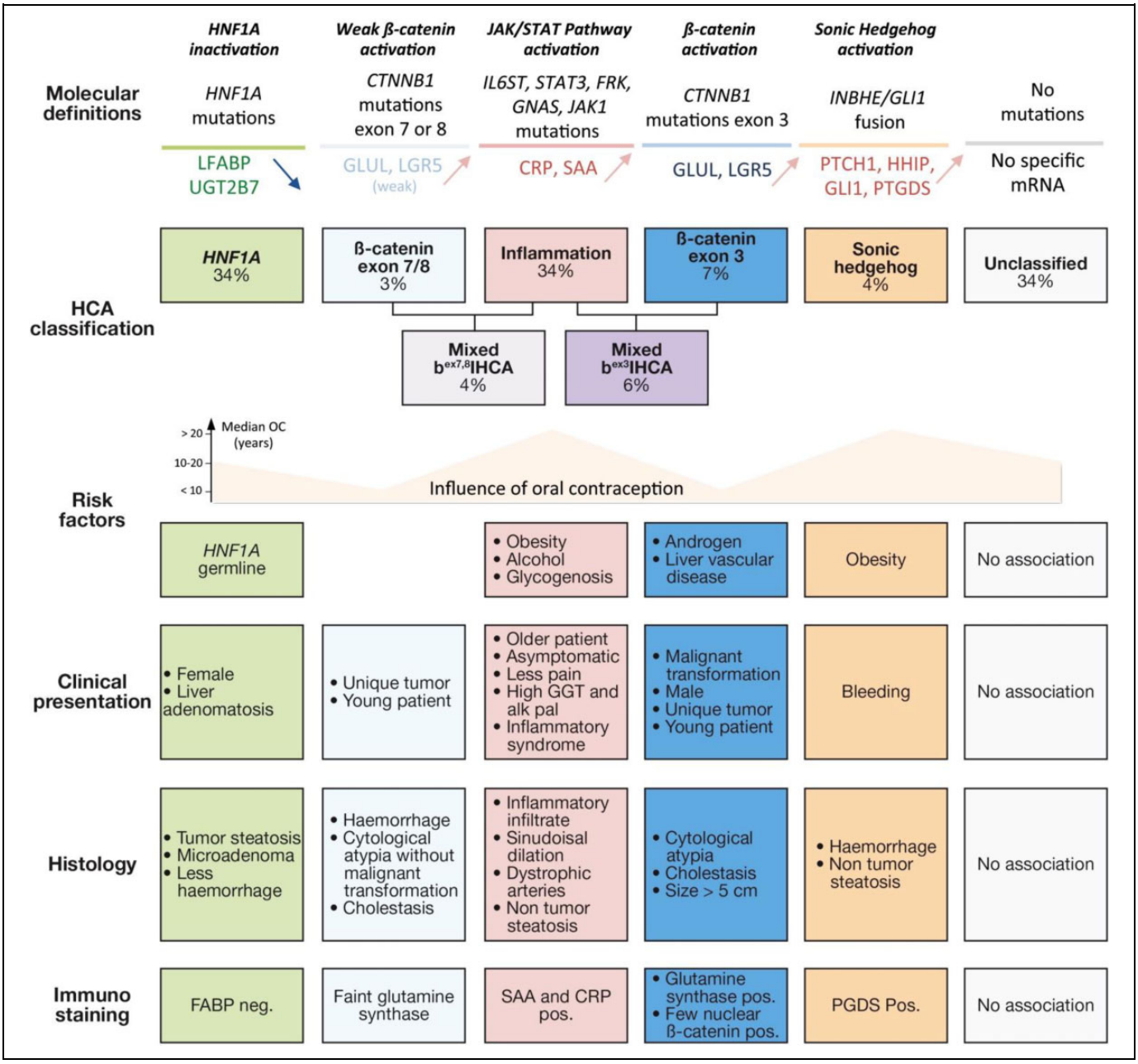

Figure 11. A new nosology of hepatic adenomas. Reprinted from Gastroenterology, 152(4), Nault JC, Couchy G, Balabaud C, et al. Molecular Classification of Hepatocellular Adenoma Associates With Risk Factors, Bleeding, and Malignant Transformation, 880–894, 2017, with permission from Elsevier.

cholangiocarcinoma include chronic viral hepatitis (type C and B), metabolic syndrome, obesity and alcohol usage.[78] In addition, parasitic fluke infections, hepatolithiasis and primary sclerosing cholangitis (PSC) are other identified risk factors.[78]

Precursor lesions leading to cholangiocarcinoma include high-grade BilIN, discussed earlier in this review. Of notice, high-grade BilIN-3 lesions are more frequent in cirrhotic livers, usually involving the medium- and large-sized intrahepatic branches of the biliary tree, especially in the setting of chronic hepatitis C and alcohol abuse–related chronic liver disease.[79]

Histopathology

Compared to other types of cancer involving liver, cholangiocarcinomas are the ones that elicit a marked desmoplastic fibrotic reaction. It has been well recognized that cholangiocarcinomas demonstrate a wide spectrum of growth patterns (Figure 12A-F). Tumors can be composed of irregular cystic, branching/glandular tubular structures or as irregular aggregates of infiltrating glands (Figure 12A-B). Cholangiocarcinomas do not always show easily identifiable tubular contours; instead, tumor cells often grow in a nodular pattern or

Table 1. The T Categories for Hepatocellular Carcinoma.

T Category	T Criteria
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Solitary tumor $\leq$2 cm, or >2 cm with vascular invasion
T1a	Solitary tumor $\leq$2 cm
T1b	Solitary tumor >2cm with vascular invasion
T2	Solitary tumor with vascular invasion; or multiple tumors, none >5 cm in greatest dimension
T3	Multiple tumors, at least one of which is >5 cm
T4	Single tumor or multiple tumors of any size, involving a major branch of the portal vein or hepatic vein, or tumor with direct invasion of adjacent organs than the gallbladder or with perforation of the visceral peritoneum

form sheets of solid nests that mimic neuroendocrine neoplasms and/or HCC (Figure 12C). Other tumors may show copious mucin components, resembling mucinous adenocarcinoma or colloid tumors (Figure 12D). It should be kept in mind that for any cholangiocarcinoma, it is common to see a variety of the abovementioned histomorphological features in the same lesion. Focal features of sarcomatoid or clear cell morphology are not uncommon.

Cholangiocarcinoma tumor cells frequently extend along the portal tracts by growing within the connective tissue, without directly invading into the bile ducts in the vicinity (Figure 13A), even though this pattern of tumor invasion can be seen in not only cholangiocarcinoma but also other types of tumors, including metastatic carcinomas. Also, tumor cells can colonize and extend along the bile ducts and intermingle with native benign bile ducts and adjacent reactive ductules (Figure 13A). It should always be kept in mind that cholangiocarcinomas are neurotropic, and tumor cells frequently approach nerve tracts, with frequent perineural and intraneural involvement, as shown in Figure 13B. In practice, this pattern can be very challenging to recognize, especially during frozen section evaluation; more so when the primary tumor is small and when there is no clinical evidence of metastatic disease. Another malignant behavior of cholangiocarcinomas is lymphovascular invasion, as shown in Figure 13C. Astute microscopic examination and extensive tumor sectioning is essential in reaching such a diagnosis. The usual intraluminal "dirty" necrosis, frequently associated with colorectal primary adenocarcinomas, are not typically seen in cholangiocarcinomas.

Ancillary Studies

The pathological diagnosis of primary intrahepatic cholangiocarcinoma still mostly remains one of exclusion, because of a lack of specific markers. However, a novel RNA platform using in-situ hybridization for albumin RNA has shown specificity for primary liver cancers, including CC.[80] However, since this test is not widely available, diagnosis of primary intrahepatic cholangiocarcinoma still requires exclusion of all other possibilities by analyzing histomorphology, immunoprofiles, imaging studies, as well as clinical evidence.

By immunostaining, cholangiocarcinomas are strongly positive for CK-7 and CAM5.2 and show cytoplasmic labeling by p-CEA. Mouse monoclonal antibody that recognizes human EPCAM on cell membrane (MOC31) is positive in around 90% of cases, whereas CK-19 is positive in 70% to 80% of cases.[59] Interestingly, there have been findings to suggest that peripheral cholangiocarcinomas are more likely to be CK-7 positive but CK-20 negative, whereas central counterparts tend to be positive for both CK-7 and CK-20.[81] CD56 also is reported to be positive in peripheral cholangiocarcinomas, which does not necessarily mean there is neuroendocrine differentiation, if other neuroendocrine markers are negative.[82] One particularly useful immunostain marker for confirming cholangiocarcinoma is S100P,[83] which is negative in benign biliary epithelium but usually positive in cholangiocarcinomas, especially when combined with U3 small nucleolar ribonucleoprotein protein (IMP3) and a protein that in humans is encoded by the von Hippel -Lindau tumor suppressor gene (pVHL).[84] In comparison, HCCs, including all the variants, are predominantly positive in the described pattern for the immunostain markers introduced earlier, including arginase, HepPar-1, glypican 3, CD34, CD10, and p-CEA; HCCs are also positive for AE1/3 and CAM5.2 in almost all cases but remain negative mostly for CK-7 and CK-20. In contrast, cholangiocarcinomas are mostly negative for the markers for HCC, except for p-CEA, which could be positive in various adenocarcinomas, irrespective of the organs of origin. Rarely, HepPar-1 could be positive in cholangiocarcinoma cases, with foci of labeling, but given the negativity by other HCC markers and adenocarcinoma histomorphology and immunoprofiles, a diagnosis of cholangiocarcinoma should be beyond the question; this occasional cellular labeling is not specific. For combined HCC-CC, please refer to the later section.

In addition to HCC, a metastatic process from another organ or site is also frequently suspected when facing a potential cholangiocarcinoma, such as tumors originating in gastrointestinal luminal sites, especially those from colorectal regions. It should be made clear to the entire clinical team that there are no specific markers that can be dependable for diagnosing cholangiocarcinoma. Cholangiocarcinomas are usually positive for CK-7, and sometimes for CK-20, similar to upper gastrointestinal tract and lung adenocarcinomas. A positive Thyroid Transcription Factor 1 (TTF-1) or napsin-A immunostain would strongly favor pulmonary origin; however, occasional lung tumors are negative for TTF-1 or napsin-A by immunostaining; these cases require detailed histomorphological evaluation and systemic radiological and clinical correlation to narrow down the most likely origin of the tumor. Likewise, the positive CK-20 labeling and/or focal CDX-2 immunoreactivity does not necessarily indicate gastrointestinal tract or colorectal origin. Clinical history, endoscopic investigation, and imaging studies are all pivotal in reaching the correct conclusion.

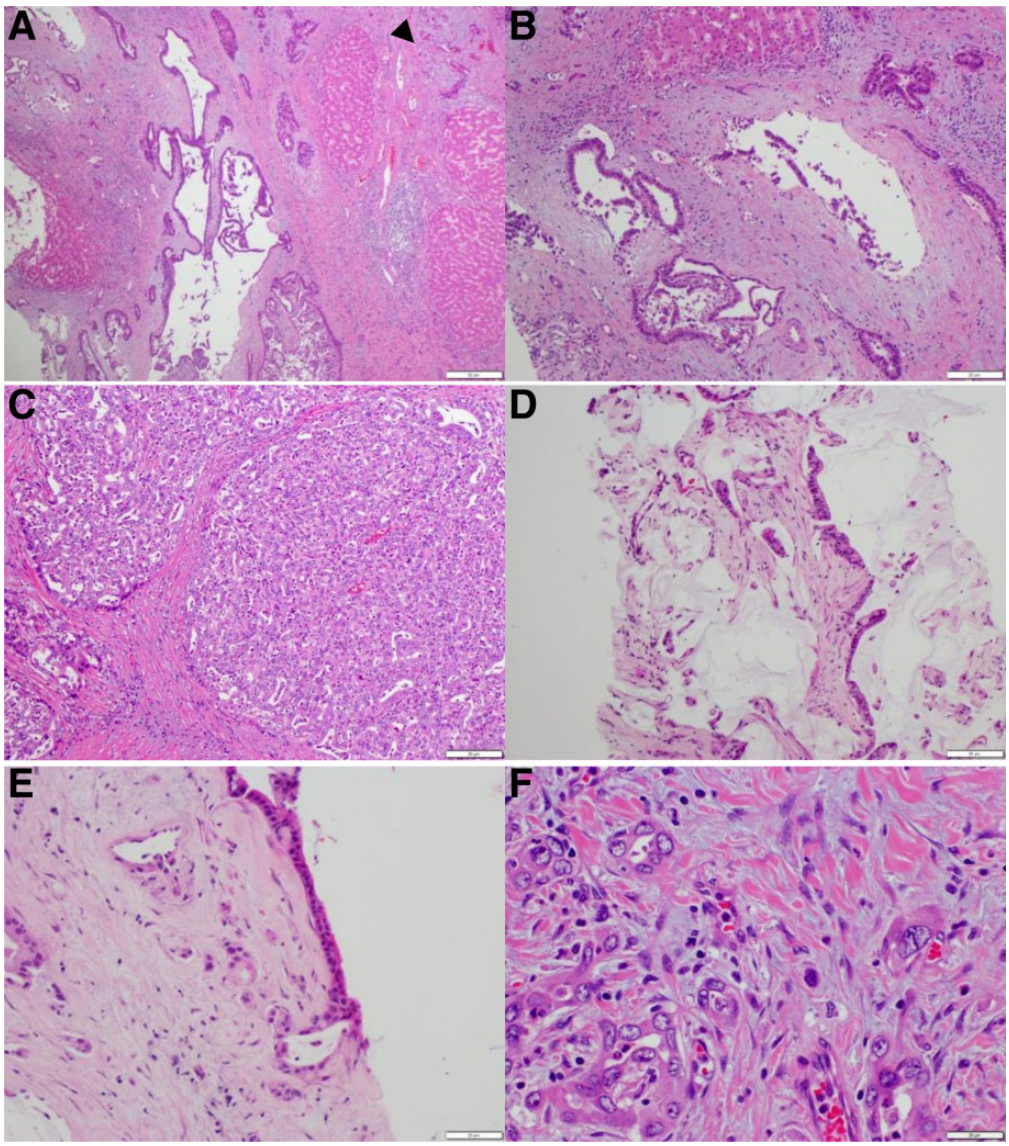

Figure 12. Histopathological features of intrahepatic cholangiocarcinoma (ICC). (A) and (B), Photomicrograph (×20 and ×40; H&E stain) shows an ICC with cystic glandular structures and adjacent benign liver. (C) Photomicrograph (H&E stain, ×40) shows a solid, nodular pattern of ICC. (D) Photomicrograph (H&E stain, ×40) shows mucinous adenocarcinoma morphology of ICC. (E) Photomicrograph (H&E stain, ×40) shows a well-differentiated IHCC. (F) Photomicrograph (H&E stain, ×100) shows a moderately to poorly differentiated ICC.

Recently, as already mentioned, modified branched DNA probes for albumin RNA were developed for in-situ hybridization of albumin. This probe showed a sensitivity of 99% for detecting intrahepatic cholangiocarcinoma and 100% sensitivity for detecting HCC.[80] Carcinomas arising at other sites, including extrahepatic cholagniocarcinomas, tested negative.

Histopathological Staging and Risk Stratification of Cholangiocarcinoma

TNM and anatomic stage/prognostic groupings. The TNM staging system of the AJCC and the UICC applies to all primary carcinomas of the intrahepatic bile ducts and combined hepatocellular–cholangiocarcinoma. It does not apply to hepatic sarcomas or to metastatic tumors of the liver. Pathologic staging is usually performed after surgical resection of the primary tumor. Pathologic staging depends on pathologic documentation of the anatomic extent of disease, whether the primary tumor has been completely removed. If a biopsied tumor is not resected for any reason and if the highest T and N categories or the M1 category of the tumor can be confirmed microscopically, the criteria for pathologic classification and staging have been satisfied without complete removal of the primary cancer. The TNM descriptors are as already reported for HCC. The AJCC eight edition classifies the T category according to vascular invasion, extent of invasion, and the number of

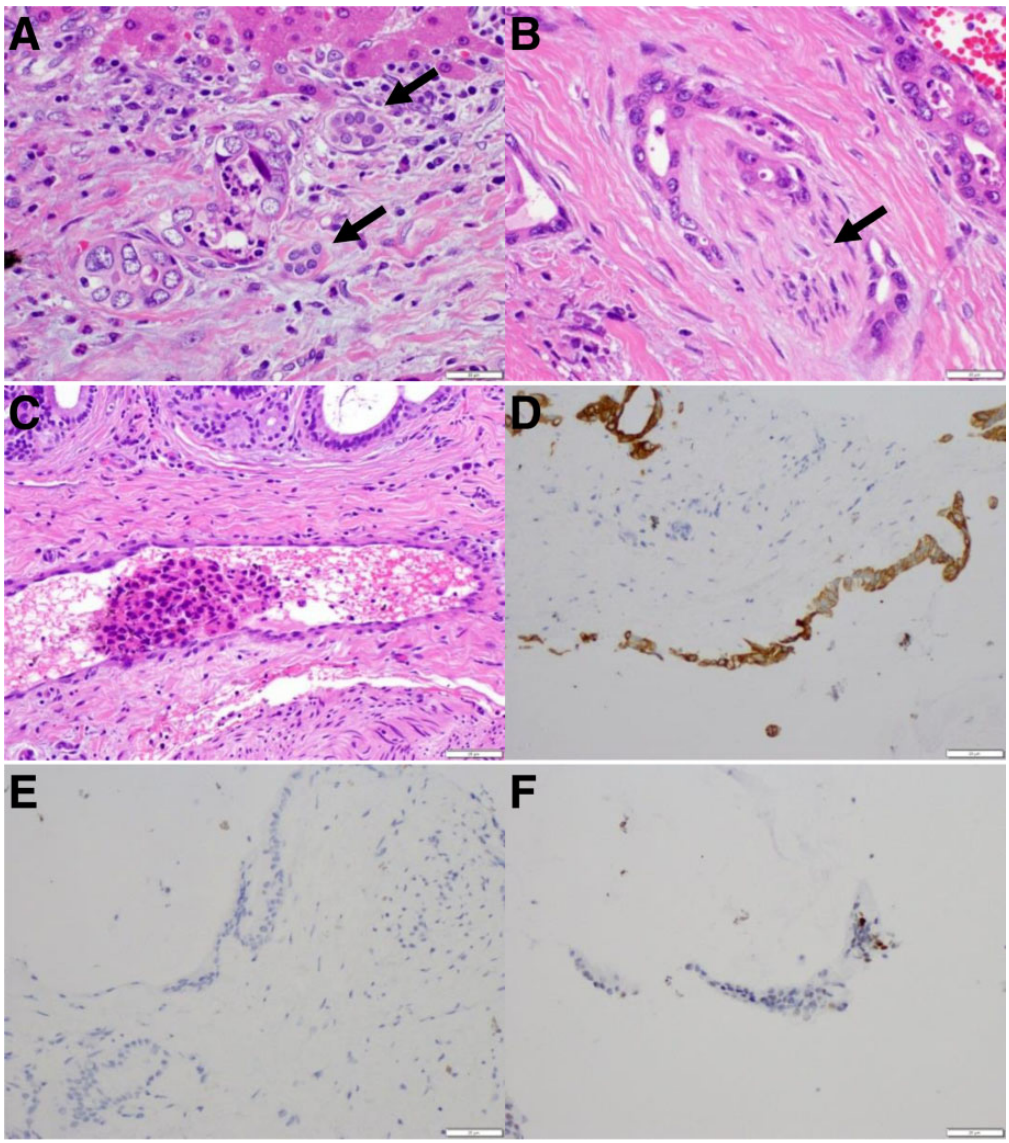

Figure 13. Highly invasive behavior and immunohistochemistry of intrahepatic cholangiocarcinoma (IHCC). A, Photomicrograph (×100; H&E stain) shows IHCC gland invading into benign liver, with adjacent reactive bile ducts (arrows for benign ducts). B, IHCC with intraneural and perineural invasion; arrow: nerve tract (×100, H&E stain). (C) Photomicrograph (H&E stain, ×40) shows a vascular space containing tumor embolus. (D) CK-7 immunostain, (×100) shows uniform strong labeling in IHCC tumor cells, in contrast to entirely negative CK-20 (E, ×100). (F) Photomicrograph (×100) shows occasional nuclear labeling by Caudal Type Homeobox 2, a nuclear transcription factor in intestinal type epithelium (CDX-2). CK indicates cytokeratin.

tumors. The T category for intrahepatic cholagniocarcinoma is shown in Table 2.[77]

Combined HCC and Cholangiocarcinoma

Combined HCC and cholangiocarcinoma, also named mixed HCC and cholangiocarcinoma, or biphenotypic HCC, previously called "collision tumor," is a unique tumor that is composed of 2 histopathologically distinct components: a part of the tumor looks and stains like conventional HCC, whereas the other part looks and stains like classical cholangiocarcinoma. Based on the literature, these combined tumors account for approximately 2% to 3% of all HCCs.[85] The 2 components are intimately intermingled or in direct contact at least with each other in the same lesion, with a subtle transition zone identified in some cases. The HCC component is histologically and immunophenotypically similar to single HCCs, whereas the cholangiocarcinoma portion demonstrates unequivocal H&E findings of adenocarcinoma, with glandular and tubular structure, micronodular and nested pattern, intracellular and extracellular mucin production (can be confirmed with either mucicarmine or PAS-diastase histochemical stains) as well as immunophenotypes similar to any given intrahepatic cholangiocarcinoma.[86] Diagnostically, the HCC portion of this

Table 2. The T Categories for Intrahepatic Cholangiocarcinoma.

T Category	T Criteria
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ (intraductal tumor)
T1	Solitary tumor $\leq$5 cm without vascular invasion
T1a	Solitary tumor without vascular invasion, $\leq$5 cm or >5 cm
T1b	Solitary tumor >5 cm without vascular invasion
T2	Solitary tumor with vascular invasion or multiple tumors, with or without vascular invasion
T3	Tumor perforating the visceral peritoneum
T4	Tumor involving the local extrahepatic structures by direct invasion

combined tumor follows the same histological and immunophenotypical patterns, as discussed above, including sarcomatoid HCC.[87] The investigation of the cholangiocarcinoma portion has additionally been discussed in the aforementioned text with conventional intrahepatic cholangiocarcinoma.

Differential Diagnoses of Cholangiocarcinoma Versus Metastatic Tumor From Other Organ/Sites

Frequently, making a diagnosis of cholangiocarcinoma especially the intrahepatic ones, instead of a metastatic adenocarcinoma from another organ or site, can be difficult and challenging, more so when there are none or limited clinical and radiological information available for analysis. The clinical, histological, and molecular information are all essential for decision-making and management planning.

Clinically, cholangiocarcinomas are associated with chronic biliary inflammation and regeneration, as frequently seen in PSC, fluke infestation, chronic hepatitis C and hepatitis B viral infection, profession-related chronic exposure to chemicals, and pancreatobiliary malfunction. On the other hand, history of colorectal, gastrointestinal, or gynecological malignancies as well their stages are helpful in determining the possibility of dealing with a metastatic tumor. It is very important to compare the histomorphology of the primaries and the adenocarcinoma in the liver to see whether there is any similarities; unfortunately in the setting of a comprehensive cancer center, it is not unusual to face a newly identified intrahepatic adenocarcinoma with no or minimal history and no previous cases for morphological comparison.

Histologically, cholangiocarcinoma follows a stepwise carcinogenesis process through a precursor lesion: BilIN, ranging from low to high grade (carcinoma in situ). The presence of these precursor lesions in the vicinity of an intrahepatic adenocarcinoma, combined with marked desmoplastic reaction and high-grade cytological atypia, is highly suggestive of cholangiocarcinoma, instead of metastatic colorectal adenocarcinoma; the latter usually demonstrates palisading, pencil-like nuclei and intraluminal "dirty" necrosis. However, a frequent obstacle encountered in practice is the small size of the biopsy material, which inevitably restricts the differential list and limits reaching a definitive diagnosis due to small areas of useful diagnostic material. In practice, immunohistochemical studies (such as cytokeratin-7 (CK-7), cytokeratin-19 (CK-19), cytokeratin-19 (CK-20), CDX-2, and carbohydrate antigen 19-9 (CA-19-9), in an attempt to tell one potential primary source from another one, these immunophenotypes have been discussed in the earlier sections in this review. However, none of these immunophenotypes are specific for telling cholangiocarcinoma; they are just frequently seen from pancreatobiliary and cholangiolar primaries. Subsequently, it is extremely important to correlate radiological, clinical, and histopathological findings in order to draw a correct conclusion.

Molecular wise, in recent years, it has been recognized that a spectrum of genetic alterations are responsible for the initiation, progression, and prognosis of cholangiocarcinoma; the main culprits include Kirsten rat sarcoma virus oncogene *(KRAS)*, tumor protein p53 *(TP53)*, a tumor suppressor gene, mothers Against DPP Homolog 1 gene *(SMAD)* mutation, *BRAF*, and *INK4a*, the gene encoding the p16 protein,[88-90] while genetic and epigenetic alterations both cause the activation of oncogenes and/or loss of tumor suppressor functions.[91-93] However, these genetic alterations cannot be used to predict site of origin, as these alterations have also been observed in colorectal adenocarcinoma or malignancies from other sites. For example, *KRAS* mutation is also a frequent event observed in gastrointestinal adenocarcinomas, especially those from colorectal regions. The only exception is loss of SMAD expression, which has been detected exclusively in about 50% of pancreatobiliary adenocarcinomas.

To summarize briefly, in order to decide whether an intrahepatic adenocarcinoma is a primary cholangiocarcinoma or a metastatic process, morphological and ancillary test results need to be utilized in combination with the clinical and radiological findings to reach the most likely conclusion regarding the true nature of the tumor.

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References

1. International Working Party. Terminology of nodular hepatocellular lesions. *Hepatology*. 1995;22(3):983-993.
2. Watanabe S, Okita K, Harada T, et al. Morphologic studies of the liver cell dysplasia. *Cancer*. 1983;51(12):2197-2205.
3. Adachi E, Hashimoto H, Tsuneyoshi M. Proliferating cell nuclear antigen in hepatocellular carcinoma and small cell liver dysplasia. *Cancer*. 1993;72(10):2902-2909.

4. Marchio A, Terris B, Meddeb M, et al. Chromosomal abnormalities in liver cell dysplasia detected by comparative genomic hybridisation. *Mol Pathol*. 2001;54(4):270-274.
5. Plentz RR, Park YN, Lechel A, et al. Telomere shortening and inactivation of cell cycle checkpoints characterize human hepatocarcinogenesis. *Hepatology*. 2007;45(4):968-976.
6. Ferrell LD, Crawford JM, Dhillon AP, Scheuer PJ, Nakanuma Y. Proposal for standardized criteria for the diagnosis of benign, borderline, and malignant hepatocellular lesions arising in chronic advanced liver disease. *Am J Surg Pathol*. 1993;17(11):1113-1123.
7. Sato Y, Harada K, Sasaki M, Nakanuma Y. Histological characterization of biliary intraepithelial neoplasia with respect to pancreatic intraepithelial neoplasia. *Int J Hepatol*. 2014;2014: 678260.
8. Zen Y, Adsay NV, Bardadin K, et al. Biliary intraepithelial neoplasia: an international interobserver agreement study and proposal for diagnostic criteria. *Mod Pathol*. 2007;20(6):701-709.
9. Aishima S, Kubo Y, Tanaka Y, Oda Y. Histological features of precancerous and early cancerous lesions of biliary tract carcinoma. *J Hepatobiliary Pancreat Sci*. 2014;21(7):448-452.
10. Matthaei H, Lingohr P, Strasser A, et al. Biliary intraepithelial neoplasia (BilIN) is frequently found in surgical margins of biliary tract cancer resection specimens but has no clinical implications. *Virchows Arch*. 2015;466(2):133-141.
11. Wan XS, Xu YY, Qian JY, et al. Intraductal papillary neoplasm of the bile duct. *World J Gastroenterol*. 2013;19(46):8595-8604.
12. Rocha FG, Lee H, Katabi N, et al. Intraductal papillary neoplasm of the bile duct: a biliary equivalent to intraductal papillary mucinous neoplasm of the pancreas? *Hepatology*. 2012;56(4):1352-1360.
13. Ohtsuka M, Shimizu H, Kato A, et al. Intraductal papillary neoplasms of the bile duct. *Int J Hepatol*. 2014;2014:459091.
14. Sclabas GM, Barton JG, Smyrk TC, et al. Frequency of subtypes of biliary intraductal papillary mucinous neoplasm and their MUC1, MUC2, and DPC4 expression patterns differ from pancreatic intraductal papillary mucinous neoplasm. *J Am Coll Surg*. 2012;214(1):27-32.
15. Lee JS, Heo J, Libbrecht L, et al. A novel prognostic subtype of human hepatocellular carcinoma derived from hepatic progenitor cells. *Nat Med*. 2006;12(4):410-416.
16. Oishi N, Kumar MR, Roessler S, et al. Transcriptomic profiling reveals hepatic stem-like gene signatures and interplay of miR-200c and epithelial-mesenchymal transition in intrahepatic cholangiocarcinoma. *Hepatology*. 2012;56(5):1792-1803.
17. Kokuryo T, Yokoyama Y, Nagino M. Recent advances in cancer stem cell research for cholangiocarcinoma. *J Hepatobiliary Pancreat Sci*. 2012;19(6):606-613.
18. Durnez A, Verslype C, Nevens F, et al. The clinicopathological and prognostic relevance of cytokeratin 7 and 19 expression in hepatocellular carcinoma. A possible progenitor cell origin. *Histopathology*. 2006;49(2):138-151.
19. Kim H, Park YN. Hepatocellular carcinomas expressing 'stemness'-related markers: clinicopathological characteristics. *Dig Dis*. 2014;32(6):778-785.
20. Kim H, Choi GH, Na DC, et al. Human hepatocellular carcinomas with "stemness"-related marker expression: keratin 19 expression and a poor prognosis. *Hepatology*. 2011;54(5):1707-1717.
21. Theise ND, Yao JL, Harada K, et al. Hepatic 'stem cell' malignancies in adults: four cases. *Histopathology*. 2003;43(3):263-271.
22. Chiba T, Kita K, Zheng YW, et al. Side population purified from hepatocellular carcinoma cells harbors cancer stem cell-like properties. *Hepatology*. 2006;44(1):240-251.
23. Jakate S, Yabes A, Giusto D, et al. Diffuse cirrhosis-like hepatocellular carcinoma: a clinically and radiographically undetected variant mimicking cirrhosis. *Am J Surg Pathol*. 2010;34(7):935-941.
24. Matsuura S, Aishima S, Taguchi K, et al. 'Scirrhous' type hepatocellular carcinomas: a special reference to expression of cytokeratin 7 and hepatocyte paraffin 1. *Histopathology*. 2005;47(4):382-390.
25. Kurogi M, Nakashima O, Miyaaki H, Fujimoto M, Kojiro M. Clinicopathological study of scirrhous hepatocellular carcinoma. *J Gastroenterol Hepatol*. 2006;21(9):1470-1477.
26. Lee JH, Choi MS, Gwak GY, et al. Clinicopathologic characteristics and long-term prognosis of scirrhous hepatocellular carcinoma. *Dig Dis Sci*. 2012;57(6):1698-1707.
27. Krings G, Ramachandran R, Jain D, et al. Immunohistochemical pitfalls and the importance of glypican 3 and arginase in the diagnosis of scirrhous hepatocellular carcinoma. *Mod Pathol*. 2013;26(6):782-791.
28. Haratake J, Horie A. An immunohistochemical study of sarcomatoid liver carcinomas. *Cancer*. 1991;68(1):93-97.
29. Inoue T, Kudo M, Minami Y, Chung H, Fukunaga T, Kawasaki T. Case of rapidly progressed sarcomatoid hepatocellular carcinoma in a young female without risk factor. *Liver Int*. 2007;27(10): 1428-1430.
30. Lao XM, Chen DY, Zhang YQ, et al. Primary carcinosarcoma of the liver: clinicopathologic features of 5 cases and a review of the literature. *Am J Surg Pathol*. 2007;31(6):817-826.
31. Koo HR, Park MS, Kim MJ, et al. Radiological and clinical features of sarcomatoid hepatocellular carcinoma in 11 cases. *J Comput Assist Tomogr*. 2008;32(5):745-749.
32. Papotti M, Sambataro D, Marchesa P, Negro F. A combined hepatocellular/cholangiocellular carcinoma with sarcomatoid features. *Liver*. 1997;17(1):47-52.
33. Akasofu M, Kawahara E, Kaji K, Nakanishi I. Sarcomatoid hepatocellular-carcinoma showing rhabdomyoblastic differentiation in the adult cirrhotic liver. *Virchows Arch*. 1999;434(6):511-515.
34. Fu Y, Kobayashi S, Kushida Y, et al. Sarcomatoid hepatocellular carcinoma with chondroid variant: case report with immunohistochemical findings. *Pathol Int*. 2000;50(11):919-922.
35. Cho MS, Lee SN, Sung SH, Han WS. Sarcomatoid hepatocellular carcinoma with hepatoblastoma-like features in an adult. *Pathol Int*. 2004;54(6):446-450.
36. Dahm HH. Immunohistochemical evaluation of a sarcomatoid hepatocellular carcinoma with osteoclast-like giant cells. *Diagn Pathol*. 2015;10:40.
37. Chu PG, Ishizawa S, Wu E, Weiss LM. Hepatocyte antigen as a marker of hepatocellular carcinoma: an immunohistochemical comparison to carcinoembryonic antigen, CD10, and alpha-fetoprotein. *Am J Surg Pathol*. 2002;26(8):978-988.
38. Pua U, Low SC, Tan YM, Lim KH. Combined hepatocellular and cholangiocarcinoma with sarcomatoid transformation: radiologic-pathologic correlation of a case. *Hepatol Int*. 2009; 3(4):587-592.

39. Yamaguchi R, Nakashima O, Ogata T, Hanada K, Kumabe T, Kojiro M. Hepatocellular carcinoma with an unusual neuroendocrine component. *Pathol Int*. 2004;54(11):861-865.
40. Han CP, Hsu JD, Koo CL, Yang SF. Antibody to cytokeratin (CK8/CK18) is not derived from CAM5.2 clone, and anticytokeratin CAM5.2 (Becton Dickinson) is not synonymous with the antibody (CK8/CK18). *Hum Pathol*. 2010;41(4):616-617; author reply 617.
41. Ichikawa T, Federle MP, Grazioli L, Madariaga J, Nalesnik M, Marsh W. Fibrolamellar hepatocellular carcinoma: imaging and pathologic findings in 31 recent cases. *Radiology*. 1999;213(2): 352-361.
42. Katzenstein HM, Krailo MD, Malogolowkin MH, et al. Fibrolamellar hepatocellular carcinoma in children and adolescents. *Cancer*. 2003;97(8):2006-2012.
43. Stipa F, Yoon SS, Liau KH, et al. Outcome of patients with fibrolamellar hepatocellular carcinoma. *Cancer*. 2006;106(6): 1331-1338.
44. Ehrenfried JA, Zhou Z, Thompson JC, Evers BM. Expression of the neurotensin gene in fetal human liver and fibrolamellar carcinoma. *Ann Surg*. 1994;220(4):484-489; discussion 489-491.
45. Simonsen K, Rode A, Nicoll A, et al. Vitamin B(1)(2) and its binding proteins in hepatocellular carcinoma and chronic liver diseases. *Scand J Gastroenterol*. 2014;49(9):1096-1102.
46. Allan BJ, Wang B, Davis JS, et al. A review of 218 pediatric cases of hepatocellular carcinoma. *J Pediatr Surg*. 2014;49(1):166-171; discussion 171.
47. Lim II, Farber BA, LaQuaglia MP. Advances in fibrolamellar hepatocellular carcinoma: a review. *Eur J Pediatr Surg*. 2014; 24(6):461-466.
48. El-Serag HB, Davila JA. Is fibrolamellar carcinoma different from hepatocellular carcinoma? A US population-based study. *Hepatology*. 2004;39(3):798-803.
49. Torbenson M. Fibrolamellar carcinoma: 2012 update. *Scientifica (Cairo)*. 2012;2012:743790.
50. Moreno-Luna LE, Arrieta O, Garcia-Leiva J, et al. Clinical and pathologic factors associated with survival in young adult patients with fibrolamellar hepatocarcinoma. *BMC cancer*. 2005;5:142.
51. Jain D, Nayak NC, Kumaran V, Saigal S. Steatohepatitic hepatocellular carcinoma, a morphologic indicator of associated metabolic risk factors: a study from India. *Arch Pathol Lab Med*. 2013; 137(7):961-966.
52. Yan BC, Gong C, Song J, et al. Arginase-1: a new immunohistochemical marker of hepatocytes and hepatocellular neoplasms. *Am J Surg Pathol*. 2010;34(8):1147-1154.
53. Fujiwara M, Kwok S, Yano H, Pai RK. Arginase-1 is a more sensitive marker of hepatic differentiation than HepPar-1 and glypican-3 in fine-needle aspiration biopsies. *Cancer Cytopathol*. 2012;120(4):230-237.
54. Radwan NA, Ahmed NS. The diagnostic value of arginase-1 immunostaining in differentiating hepatocellular carcinoma from metastatic carcinoma and cholangiocarcinoma as compared to HepPar-1. *Diagn Pathol*. 2012;7(149):1-12.
55. Nguyen TB, Roncalli M, Di Tommaso L, Kakar S. Combined use of heat-shock protein 70 and glutamine synthetase is useful in the distinction of typical hepatocellular adenoma from atypical hepatocellular neoplasms and well-differentiated hepatocellular carcinoma. *Mod Pathol*. 2016 Mar;29(3):283-292.
56. Bioulac-Sage P, Balabaud C, Zucman-Rossi J. Subtype classification of hepatocellular adenoma. *Dig Surg*. 2010;27(1):39-45.
57. Bioulac-Sage P, Cubel G, Taouji S, et al. Immunohistochemical markers on needle biopsies are helpful for the diagnosis of focal nodular hyperplasia and hepatocellular adenoma subtypes. *Am J Surg Pathol*. 2012;36(11):1691-1699.
58. Wennerberg AE, Nalesnik MA, Coleman WB. Hepatocyte paraffin 1: a monoclonal antibody that reacts with hepatocytes and can be used for differential diagnosis of hepatic tumors. *Am J Pathol*. 1993;143(4):1050-1054.
59. Chan ES, Yeh MM. The use of immunohistochemistry in liver tumors. *Clin Liver Dis*. 2010;14(4):687-703.
60. Fan Z, van de Rijn M, Montgomery K, Rouse RV. Hep par 1 antibody stain for the differential diagnosis of hepatocellular carcinoma: 676 tumors tested using tissue microarrays and conventional tissue sections. *Mod Pathol*. 2003;16(2): 137-144.
61. Kakar S, Muir T, Murphy LM, Lloyd RV, Burgart LJ. Immunoreactivity of Hep Par 1 in hepatic and extrahepatic tumors and its correlation with albumin in situ hybridization in hepatocellular carcinoma. *Am J Clin Pathol*. 2003;119(3):361-366.
62. Shafizadeh N, Ferrell LD, Kakar S. Utility and limitations of glypican-3 expression for the diagnosis of hepatocellular carcinoma at both ends of the differentiation spectrum. *Mod Pathol*. 2008;21(8):1011-1018.
63. Yan B, Wei JJ, Qian YM, et al. Expression and clinicopathologic significance of glypican 3 in hepatocellular carcinoma. *Ann Diagn Pathol*. 2011;15(3):162-169.
64. Abdul-Al HM, Makhlouf HR, Wang G, Goodman ZD. Glypican-3 expression in benign liver tissue with active hepatitis C: implications for the diagnosis of hepatocellular carcinoma. *Hum Pathol*. 2008;39(2):209-212.
65. Uenishi T, Kubo S, Yamamoto T, et al. Cytokeratin 19 expression in hepatocellular carcinoma predicts early postoperative recurrence. *Cancer Sci*. 2003;94(10):851-857.
66. Klein WM, Molmenti EP, Colombani PM, et al. Primary liver carcinoma arising in people younger than 30 years. *Am J Clin Pathol*. 2005;124(4):512-518.
67. Ra SH, Kaplan JB, Lassman CR. Focal nodular hyperplasia after orthotopic liver transplantation. Liver transplantation. *Liver Transpl*. 2010;16(1):98-103.
68. Roncalli M, Sciarra A, Tommaso LD. Benign hepatocellular nodules of healthy liver: focal nodular hyperplasia and hepatocellular adenoma. *Clin Mol Hepatol*. 2016;22(2):199-211.
69. Barthelmes L, Tait IS. Liver cell adenoma and liver cell adenomatosis. *HPB (Oxford)*. 2005;7(3):186-196.
70. Lin H, van den Esschert J, Liu C, van Gulik TM. Systematic review of hepatocellular adenoma in China and other regions. *J Gastroenterol Hepatol*. 2011;26(1):28-35.
71. Sasaki M, Nakanuma Y. Overview of hepatocellular adenoma in Japan. *Int J Hepatol*. 2012;2012:648131.
72. Greaves WO, Bhattacharya B. Hepatic adenomatosis. *Arch Pathol Lab Med*. 2008;132(12):1951-1955.

73. Farges O, Dokmak S. Malignant transformation of liver adenoma: an analysis of the literature. *Dig Surg*. 2010;27(1):32-38.
74. Zucman-Rossi J, Jeannot E, Nhieu JT, et al. Genotype-phenotype correlation in hepatocellular adenoma: new classification and relationship with HCC. *Hepatology*. 2006;43(3):515-524.
75. Bioulac-Sage P, Rebouissou S, Thomas C, et al. Hepatocellular adenoma subtype classification using molecular markers and immunohistochemistry. *Hepatology*. 2007;46(3):740-748.
76. Nault JC, Couchy G, Balabaud C, et al. Molecular classification of hepatocellular adenoma associates with risk factors, bleeding, and malignant transformation. *Gastroenterology*. 2017;152(4): 880-894 e886.
77. *AJCC Cancer Staging Manual*. Amin MB. 8th ed. Springer; 2017.
78. Palmer WC, Patel T. Are common factors involved in the pathogenesis of primary liver cancers? A meta-analysis of risk factors for intrahepatic cholangiocarcinoma. *J Hepatol*. 2012;57(1):69-76.
79. Torbenson M, Yeh MM, Abraham SC. Bile duct dysplasia in the setting of chronic hepatitis C and alcohol cirrhosis. *Am J Surg Pathol*. 2007;31(9):1410-1413.
80. Ferrone CR, Ting DT, Shahid M, et al. The ability to diagnose intrahepatic cholangiocarcinoma definitively using novel branched DNA-enhanced albumin RNA in situ hybridization technology. *Ann Surg Oncol*. 2016;23(1):290-296.
81. Rullier A, Le Bail B, Fawaz R, Blanc JF, Saric J, Bioulac-Sage P. Cytokeratin 7 and 20 expression in cholangiocarcinomas varies along the biliary tract but still differs from that in colorectal carcinoma metastasis. *Am J Surg Pathol*. 2000;24(6):870-876.
82. Kozaka K, Sasaki M, Fujii T, et al. A subgroup of intrahepatic cholangiocarcinoma with an infiltrating replacement growth pattern and a resemblance to reactive proliferating bile ductules: 'bile ductular carcinoma'. *Histopathology*. 2007;51(3):390-400.
83. Hamada S, Satoh K, Hirota M, et al. Calcium-binding protein S100P is a novel diagnostic marker of cholangiocarcinoma. *Cancer Sci*. 2011;102(1):150-156.
84. Schmidt MT, Himmelfarb EA, Shafi H, Lin F, Xu H, Wang HL. Use of IMP3, S100P, and pVHL immunopanel to aid in the interpretation of bile duct biopsies with atypical histology or suspicious for malignancy. *Appl Immunohistochem Mol Morphol*. 2012;20(5):478-487.
85. Yeh MM. Pathology of combined hepatocellular-cholangiocarcinoma. *J Gastroenterol Hepatol*. 2010;25(9):1485-1492.
86. Radwan NA, Ahmed NS. The diagnostic value of arginase-1 immunostaining in differentiating hepatocellular carcinoma from metastatic carcinoma and cholangiocarcinoma as compared to HepPar-1. *Diagn Pathol*. 2012;7:149.
87. Chin S, Kim Z. Sarcomatoid combined hepatocellular-cholangiocarcinoma: a case report and review of literature. *Int J Clin Exp Pathol*. 2014;7(11):8290-8294.
88. Hsu M, Sasaki M, Igarashi S, Sato Y, Nakanuma Y. KRAS and GNAS mutations and p53 overexpression in biliary intraepithelial neoplasia and intrahepatic cholangiocarcinomas. *Cancer*. 2013; 119(9):1669-1674.
89. Robertson S, Hyder O, Dodson R, et al. The frequency of KRAS and BRAF mutations in intrahepatic cholangiocarcinomas and their correlation with clinical outcome. *Hum Pathol*. 2013; 44(12):2768-2773.
90. Jang S, Chun SM, Hong SM, et al. High throughput molecular profiling reveals differential mutation patterns in intrahepatic cholangiocarcinomas arising in chronic advanced liver diseases. *Mod Pathol*. 2014;27(5):731-739.
91. Tischoff I, Wittekind C, Tannapfel A. Role of epigenetic alterations in cholangiocarcinoma. *J Hepatobiliary Pancreat Surg*. 2006;13(4):274-279.
92. Isomoto H. Epigenetic alterations associated with cholangiocarcinoma (review). *Oncol Rep*. 2009;22(2):227-232.
93. Hamilton JP. Epigenetic mechanisms involved in the pathogenesis of hepatobiliary malignancies. *Epigenomics*. 2010;2(2): 233-243.