6 Pathologic Aspects of Hepatocellular Tumors

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ABSTRACT

Hepatocellular tumors are pathologically divided into a limited number of entities such as focal nodular hyperplasia, hepatocellular adenoma, hepatocellular carcinoma and its variants, and hepatoblastoma. Recent advances in immunophenotypic and molecular characterization have led to an increased appreciation of the complexities of these growths. For example, subtypes of hepatocellular adenomas with differing premalignant potentials have been defined, our ability to differentiate hepatocellular carcinoma from high-grade dysplasia continues to improve, and molecular similarities of histologically discordant elements of combined hepatocellular/cholangiocellular carcinoma have been reported. This chapter describes

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pathologic, immunophenotypic, and molecular features of hepatocellular tumors. Continued progress in our understanding of these growths at the cellular and subcellular levels suggests that categorization of these tumors may continue to evolve as additional significant clinicopathologic correlates are discovered.

Key Words: Focal nodular hyperplasia; hepatocellular adenoma; hepatocellular carcinoma; dysplasia; hepatoblastoma; histopathology; immunophenotypic analysis; molecular pathology; tumor staging

1. INTRODUCTION

Hepatocellular tumors are conveniently divided into a limited number of pathologic categories in order to provide a simplified framework allowing rational application of diagnostic and therapeutic procedures. However, such an approach understates the tremendous range of cellular and architectural variation of these tumors, attributable to the wide plasticity of the hepatocyte and its progenitors. This chapter categorizes hepatocellular neoplasias and relevant non-neoplastic growths using established pathologic headings. The ongoing application of molecular techniques to enhance and sometimes transform our understanding of these lesions provides a recurrent theme throughout the discussion. In addition to comprehending the accepted relationships among the various tumors, the reader is challenged to consider alternative relationships that may conceivably mirror the underlying biology in a more accurate fashion. Such examples might involve the presence of mesenchymal metaplasia in lesions as seemingly diverse as hepatoblastoma and mixed hepatocellular carcinoma/cholangiocarcinoma. One may also ask if specific molecular pathways such as β-catenin/Wnt or specific cell types such as bipotential progenitor cells may define subsets of similar tumors that cut across current established morphology-based classifications.

2. FOCAL NODULAR HYPERPLASIA

2.1. Clinical Aspects

Focal nodular hyperplasia (FNH) is a benign mass lesion that arises from a hyperplastic response to locally malformed vasculature and resultant increase in regional blood flow *(1–3)*. FNH can occur in either sex and at any age, although it is most common in women of reproductive age. Estrogen use is not considered to be directly causative but may be associated with lesion growth *(4)*. Rapid growth of FNH in the absence of estrogen use has also been reported *(5)*.

FNH has been associated with other conditions characterized by local vasoformative anomalies such as hepatic hemangiomas or hereditary hemorrhagic telangiectasia *(6)*. Increased frequency of FNH has been reported after anti-neoplastic therapy, where it has been suggested that the increase may relate to vascular injury associated with such treatment *(7)*.

The radiographic appearance of typical FNH is diagnostic and most cases are detected incidentally during abdominal radiographic examination for other conditions. Occasionally it may present as fullness or a mass lesion.

FNH is usually a clinically benign condition and in many cases it can be followed without surgical intervention. Rarely, larger lesions may undergo significant hemorrhage *(8)* and exceptionally, hepatocellular carcinoma has been observed to arise within these hyperplasias *(9)*.

2.2. FNH Macroscopic Aspects

FNH presents as a discrete unencapsulated mass lesion with a lobulated appearance accentuated by bands of fibrosis. These fibrous septa typically radiate from the center of the lesion, where they coalesce into a larger central scar (Fig. 1). This characteristic feature facilitates radiographic diagnosis in most cases. Variations include eccentric scars and multiple smaller fibrous scars. Importantly from a diagnostic perspective, hepatocellular carcinoma may on occasion also contain a central scar and must be distinguished from FNH *(10)*.

A dystrophic vasculature is a ubiquitous feature of FNH and this may be macroscopically detectable in some cases as isolated and enlarged vessels within or at the periphery of the growth. In the recent past, some liver

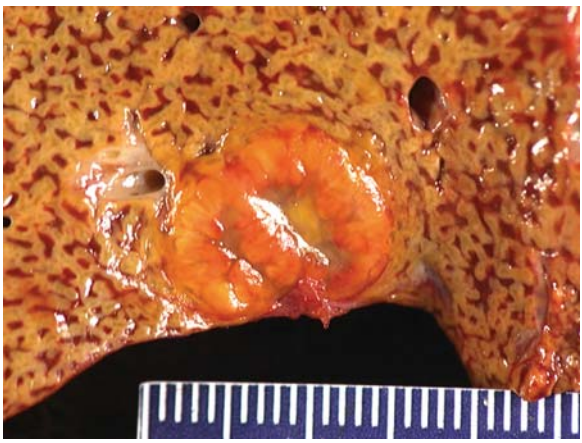

Fig. 1. Focal nodular hyperplasia arising in a noncirrhotic liver. The nodule has centrally depressed areas corresponding to the central fibrous scar. The background liver shows chronic passive congestion.

masses characterized by an excess of vasculature with minimal fibrosis were referred to as telangiectatic FNH; however, clonal studies have unambiguously redefined these tumors as variants of hepatocellular adenomas, and they are discussed in that section (below).

Many but not all FNH are solitary and small. In a recent series, 80% of FNH was under 5 cm, 18% between 5 and 10 cm, and 2% greater than 10 cm in diameter *(11)*. In approximately 20% of cases, multiple FNH coexist. A diagnosis of FNH in one lesion does not ensure that all other lesions are identical, as concurrent hepatocellular carcinoma may also occur in livers harboring FNH *(12, 13)*.

2.3. FNH Microscopic Aspects

The microscopic appearance of FNH is dominated by bland cytology with architectural distortion produced by the central area of fibrosis from which radiate individual fibrous septa that circumscribe complete and incomplete nodules of normal-appearing hepatocytes. When the entire lesion is resected it is not difficult to delineate FNH from the surrounding parenchyma despite both the absence of a pseudocapsule and the bland appearance of hepatocytes.

The fibrous septa contain the dystrophic artery branches that supply the lesion (Fig. 2). These vessels are characterized by asymmetric-appearing muscular layers due to irregularly distributed but benign-appearing areas of muscular hyperplasia throughout their lengths. The recognition of these vessels is of diagnostic importance. Of similar diagnostic import is the

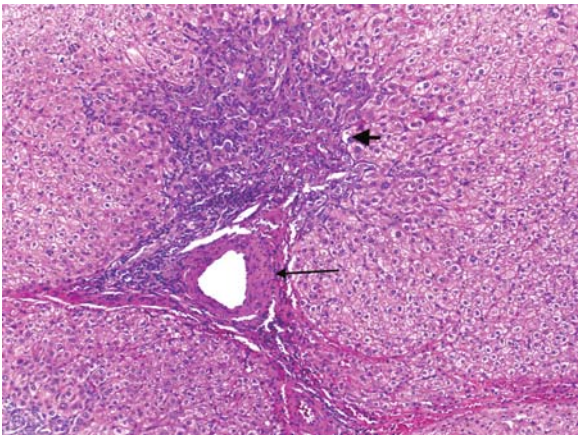

Fig. 2. Focal nodular hyperplasia. A thick walled dystrophic vessel (*thin arrow*) is present within a fibrous area that corresponds to part of the fibrous scar of the lesion. True bile ducts are not present. However, focal cholangiolar proliferation at the interface between fibrous septa and hepatocyte areas may be focally prolific (*thick arrow*) (100×).

absence of accompanying bile ducts in the vicinity of artery branches. On occasion a portal tract may be enveloped within an area of the lesion, but for the most part bile ducts are absent from FNH. In contrast, bile ductular overgrowth is common at the interface between fibrous bands and hepatocyte trabeculae. This may be prolific in some areas and absent in others (Fig. 2), possibly related to microenvironmental differences in blood and bile flow within the lesion. The change is similar to the so-called "biliary interface hepatitis" that occurs with biliary outflow compromise. This similarity extends to the fact that hepatocytes in this area may be swollen due to retained bile salts (cholate stasis). Further, localized increase in copper (and copper binding protein) may occur here and is diagnostically useful as a point in favor of the diagnosis of FNH over other lesions such as hepatocellular adenoma. We have seen rare examples of the latter condition (as well as HCC) producing a positive copper stain, however, and emphasize that the diagnosis must take the entire appearance of the lesion into account.

A needle biopsy may be performed in cases in which the diagnosis is ambiguous by radiographic examination. Several pitfalls may arise in this circumstance. First, if the fibrosis is heavily sampled, a diagnosis of cirrhosis may be entertained. This error may be compounded by the presence of ductular proliferation, in which a biliary etiology might be suggested. Knowledge of the presence of a mass lesion is helpful, and a search for true bile ducts adjacent to artery branches will demonstrate that normal portal tracts are absent. This task can be difficult if some areas do show true ducts. In that case the likelihood that both normal and abnormal areas of liver have been sampled should be considered and an effort to mentally separate these regions undertaken. Examination of the vessels themselves may disclose dystrophic change in some but not other areas and this is a helpful finding.

With knowledge that the biopsy has been performed for diagnosis of a hepatic mass, the differential diagnosis of hepatocellular adenoma often arises, particularly in cases in which ductular proliferation is absent. We find ancillary cytokeratin staining for ductules to occasionally be helpful. In this regard we consider cytokeratin 19 to be more useful than cytokeratin 7, since the latter can often be expressed by hepatocytes adjacent to fibrous regions. Copper stain and search for dystrophic vessels may also be of benefit. Hepatocellular adenomas appear to show a more diffuse distribution of vasculature throughout the hepatocyte regions in contrast to FNH in which the vessels diminish in number and caliber as one leaves the fibrous regions, and occasionally this feature is marked enough to be useful.

It is not always possible to histologically distinguish FNH from hepatocellular adenoma. In some cases clonal or other molecular studies (below) may be of benefit. In other cases clinical circumstances may ultimately dictate whether the lesion is followed by repeat radiographic studies or resected.

3. HEPATOCELLULAR ADENOMA

3.1. Clinical Aspects

Hepatocellular adenoma (HCA) is an uncommon and benign liver tumor arising most frequently in women of childbearing age and with a history of oral contraceptive use. In one early study *(14)*, HCA occurred at a rate of 0.1 per 100,000 women per year when there was no history of oral contraceptives and this rose to 3.4 per 100,000 per year with long-term use of these agents. More recent low-dose formulations do not appear to be associated with this high level of risk. Anabolic steroid use is also associated with hepatocellular adenoma, and an example of this lesion arising in conjunction with growth hormone therapy for Turner's syndrome has been reported *(15)*. Use of the antiseizure medication oxcarbazepine has been associated with HCA in mice and in a single recent clinical case report *(16)*. An association of liver cell adenoma and various genetic metabolic disorders such as glycogen storage diseases types I, III, or IV, galactosemia, and tyrosinemia have been reported. Maturity-onset diabetes of the young, type III (MODY III) and familial adenomatous polyposis are two additional predisposing conditions that have a special relationship with molecular alterations present in HCA and these are considered below.

Many cases are first detected during abdominal scan *(17)* for low-grade symptoms, feeling of fullness, or other conditions. Intratumoral hemorrhage or rupture with hemoperitoneum may occur, particularly with larger tumors. However, in the series of Toso et al. *(18)*, rupture was seen in HCA as small as 1.7 cm, and these authors recommended resection of all HCA insofar as possible. Immediate management of hemorrhage with or without surgery *(19)* and observation of HCA less than 5 cm in size *(20)* have been emphasized by others and the possibility that HCA may regress if hormonal stimulation is withdrawn has also been noted *(21)*. Malignant transformation is an additional known complication of HCA, and Toso et al. *(18)* documented foci of HCC in 8% of their resected HCA.

3.2. Macroscopic Pathology

Hepatocellular adenoma characteristically appears as a well-circumscribed, nonlobulated lesion within a noncirrhotic liver (Fig. 3). Adenomas can range from 1 to over 30 cm but most are between 5 and 15 cm in diameter. Typically adenomas occur in subcapsular locations and in the right lobe. The tumor may be pedunculated *(22)*. It is usually solitary, but multiple lesions can occasionally be seen, particularly in glycogen storage disease type I and in liver adenomatosis *(23–26)*. The latter by definition consists of 10 or more individual adenomas. An association of adenomatosis with hepatic steatosis has been suggested *(27)*.

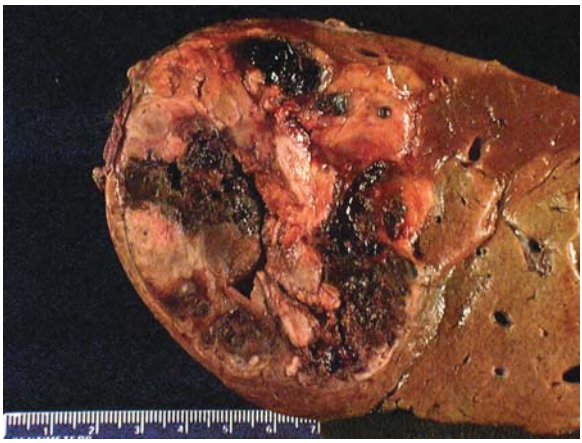

Fig. 3. Hepatocellular adenoma arising in a noncirrhotic liver. This 9.5 cm tumor arose in the noncirrhotic liver of a middle-aged woman with a long history of oral contraceptive use. The dark areas represent hemorrhage that caused pain and led to the discovery of this benign tumor.

Hepatocellular adenomas vary in color from yellow to tan and can be variegated due to a combination of intratumoral hemorrhage, infarction, and fatty changes *(24, 28)*. The tumors are usually unencapsulated.

3.3. Microscopic Pathology

Hepatocellular adenomas contain normal-appearing hepatocytes arranged in a trabecular architecture ranging from one to three cells thick (Fig. 4). There are no portal tracts and therefore the normal hepatic microanatomical relationships are lacking. The hepatocyte nuclei are small, round, and uniform. Nucleoli are inconspicuous. Mitoses are absent or few. Cytoplasm is pale or eosinophilic and marked steatosis may be present. Cholestasis is not uncommon. The normal reticulin pattern is well preserved and Kupffer cells exist in their usual locations. An inflammatory component may be present. Small venous and arterial branches are seen throughout the tumor (Fig. 4). Occasional larger vessels are seen and may also appear as "feeding" vessels adjacent to the tumor. Occasionally the tumoral hepatocytes may contain PAS-positive, diastase-resistant hyaline globules *(29, 30)*, Mallory's hyaline *(31)*, or degenerate-appearing hyperchromatic nuclei *(32)*.

The recent Bordeaux update of liver cell adenoma classification *(1)* has altered our understanding of this lesion and is considered in the next section.

Distinction of hepatocellular adenoma from well-differentiated hepatocellular carcinoma may be difficult or impossible by conventional light microscopy. The clinical context is important in this regard, and the diagnosis of hepatic adenoma outside of the setting of a young woman taking oral

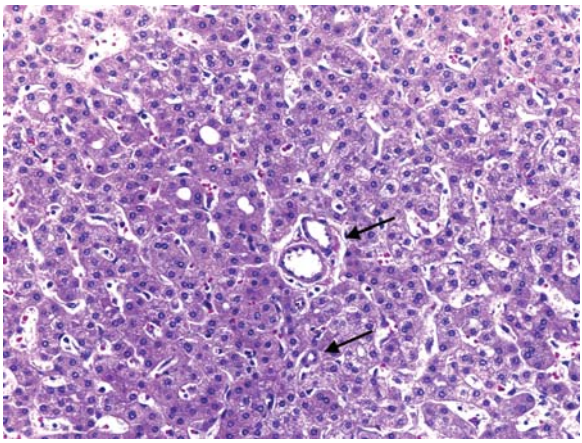

Fig. 4. Hepatocellular adenoma. The tumor is comprised of normal-appearing hepatocytes in an unremarkable trabecular architecture. Isolated artery branches (*arrows*) in the absence of portal tracts do not occur in normal lobules and are consistent with the diagnosis of this lesion as a hepatocellular adenoma (200×).

contraceptives should be viewed with suspicion. Investigations should focus on suspicious-looking areas that are characterized by a clonal appearance (referring to a focus of cells that has a distinctly different look from the surrounding adenoma). This may be due to cytologic differences or to architectural differences such as solid growth or formation of pseudoacini. Micchelli et al. *(33)* extended the earlier finding of Tao et al. *(32)* and noted cytologic atypia as a background change in two of three hepatocellular adenomas harboring foci of hepatocellular carcinoma. This change, demonstrated as enlarged and somewhat hyperchromatic nuclei with underlying intact reticulin architecture, was suggested as a potential risk factor for so-called malignant degeneration of hepatocellular adenoma. However, background atypia was also observed in several other adenomas in which a malignant component was not demonstrated, and the authors concluded that additional studies were necessary to confirm this possible association.

Immunohistochemical and molecular studies are valuable in further characterizing these lesions and are considered next.

3.4. Hepatocellular Adenoma Subtypes and Ancillary Studies

The diagnostic approach to hepatocellular adenomas has been transformed by correlative genotypic and phenotypic studies *(1, 34)* that have led to the recognition of four subgroups with varying risks for malignant transformation. The largest subgroup, comprising between 40 and 50% of adenomas, contains inactivating mutations of the HNF1 alpha gene. In about 85% of cases both mutations are somatic in origin, and in the remaining 15%

one germline and one somatic mutation coexist. Within this latter group are included patients with maturity-onset diabetes of the young type III and a number of patients with a family history of liver adenomatosis. HCA with this mutation characteristically contains significant steatosis and does not show evidence of anaplasia or significant inflammation. Association with hepatocellular carcinoma is estimated at 7% at present. Immunohistochemical absence of liver fatty acid binding protein was associated with this mutation in one study *(34)*.

A minority of HCA, estimated at less than 10%, contains mutations affecting the β-catenin gene. This can be indirectly detected by immunohistochemical demonstration of nuclear translocation of β-catenin. In addition, the products of target genes activated by β-catenin, such as glutamine synthetase, can also be detected *(34)*. These HCA do not usually show the steatosis associated with HNF1α-related tumors but are more likely to contain cellular atypia. These occur more frequently in males, and the association with HCC has been estimated to be approximately 46%.

The remaining HCA do not contain evidence of mutations in either of these genes and likely comprise a heterogeneous group. At present these are subdivided into two categories based on the presence or absence of inflammatory infiltrate. Those with inflammation correspond in part to the previously misnamed telangiectatic focal nodular hyperplasia, now preferably referred to as inflammatory adenoma or telangiectatic adenoma. These lesions have not yet been associated with progression to carcinoma. Immunohistochemical positivity for serum amyloid A2 protein has been suggested as a marker for this variant *(34)*. The second subgroup is comprised of those adenomas without known mutations and without significant inflammation. The association with risk for HCC has been estimated to be approximately 13%.

Demonstration of alpha-fetoprotein positivity is strong evidence in support of hepatocellular carcinoma over adenoma. In our experience, foci of carcinoma may show increased cell cycle activity, highlighted by the proliferation marker Ki-67, in comparison to adjacent adenoma and surrounding liver. Such changes must be interpreted in the context of the overall lesion, i.e., the pathologist must make the interpretation as to whether he or she believes that carcinoma, if found, involves the entire lesion or only a portion of the tumor. Glypican-3 expression favors the diagnosis of hepatocellular carcinoma, as it has not been reported to be expressed in adenomas in several small series *(35, 36)*. Absence of staining does not exclude the possibility of HCC, since the antigen is preferentially expressed on less well-differentiated neoplasms and in one study it was expressed in only 50% of well-differentiated HCC *(35)*. A diffuse, rather than focal, expression of CD34 in tumor-associated vessels is said to favor hepatocellular carcinoma over adenoma *(35)*.

Other immunostains do not add appreciably to the diagnostic information. Estrogen, progesterone, and androgenic steroid receptors have been detected in 26–73% of adenomas in different series *(37, 38)* and may also be seen in hepatocellular carcinoma. Hepatic progenitor cells are identifiable by immunohistochemical means in a considerable proportion of hepatocellular adenomas and support the hypothesis that such cells play a role in the development of hepatic tumors *(39, 20)*. However, their identification does not distinguish benign from malignant tumors.

Comparative genomic hybridization has been suggested as a useful ancillary technique for the distinction of adenoma and carcinoma. Gains and losses of chromosome sites on 1q, 4q, 8p, 8q, 16p, and 17p were found to be the six most frequent alterations in HCC by this approach and detection of one or more of these has been proposed as evidence in support of the diagnosis of carcinoma *(21)*. These authors have updated this technique by utilizing fluorescent in situ hybridization (FISH) to detect quantitative anomalies of chromosomes 1, 6, 7, and 8, thereby distinguishing hepatocellular carcinomas from adenomas and other benign lesions in paraffin-embedded material *(22)*.

Differentiation of hepatocellular adenoma from focal nodular hyperplasia (FNH) also has clinical significance, as FNH is a benign condition that does not have the predisposition to hemorrhage that exists in adenoma, allowing in some cases for a more conservative approach to management *(40)*. (However, it should be noted that rare cases of FNH rupture *(8, 40)* and of hepatocellular carcinoma arising within FNH *(9)* have been recorded.) Magnetic resonance imaging, enhanced CT, scintigraphic findings, and angiography show large peripheral vessels with centripetal flow and are diagnostically useful, but the best method for the differentiation of HA and FNH is surgical biopsy *(41–43)*.

Both FNH and hepatocellular adenoma contain benign-appearing hepatocytes. The presence of fibrous bands with artery branches and peripheral ductular hepatocytes in the absence of true bile ducts is characteristic of FNH. Small vessels are also seen in the lobular portion of FNH, but these derive from the core arteries in the fibrous septa and rapidly diminish in caliber as the distance from the fibrous bands increases. Such a gradient may or may not be apparent in individual adenomas.

4. HEPATOCELLULAR DYSPLASIA

Hepatocellular dysplasia was formally defined by a panel of the International Working Part on the Terminology of Chronic Hepatitis, Hepatic Allograft Rejection, and Nodular Lesions of the Liver in 1995. Lesions were subdivided into dysplastic foci (<1 mm diameter) and dysplastic nodules (≥1 mm diameter) and defined using histologic criteria. These

included variations in nuclear and/or cytoplasmic constituents such that a recognizable cell subpopulation could be distinguished from the surrounding hepatocyte parenchyma. Examples of nuclear changes included alterations in size, at least mild irregularity of nuclear contours, and occasional mitoses. Cytoplasmic changes included basophilia, clear cell change, variation in fat, glycogen, Mallory bodies, or resistance to iron accumulation, any of these features differing from surrounding parenchyma. The net result was often a clone-like population of distinguishable cells with altered nuclear:cytoplasmic ratio. This encompassed a spectrum from mild to severe change, which was arbitrarily divided into low-grade and high-grade forms. The authors realized the inherent difficulty in such an approach and observed that definitive classification, as well as distinction from early HCC, awaited the development of more discriminatory molecular diagnostic tools.

Dysplastic foci have also been subdivided on the basis of cell size into small and large cell types. Large hepatocytes with nuclear variability and prominent nucleoli have been subsequently shown to have a low rate of replication and express p16, prompting the suggestion that it be referred to as large cell "change" rather than dysplasia. In contrast, the small cell variant tends to show a higher proliferative rate than surrounding parenchyma and in one study showed chromosomal changes similar to those of nearby HCC.

4.1. Differential Diagnosis of Hepatocellular Dysplasia

Although our understanding of hepatocellular dysplasia is incomplete, it remains a practical necessity to differentiate these lesions from regenerative nodules at one extreme and hepatocellular carcinoma at the other.

The distinguishing feature of dysplasia is that it leads to the formation of an area in which the hepatocytes differ in a qualitative and/or quantitative fashion from the surrounding parenchyma. Some variables that may lead to this difference are given above. In contrast, regenerative nodules are comprised of normal-appearing hepatocytes and are more likely to contain portal tracts within their substance, without evidence of an aberrant arterial vasculature.

The absence of stromal invasion, which refers to the presence of abnormal hepatocytes directly abutting (without evidence of ductular change) or within portal stroma, has been considered to be the most helpful histologic feature separating dysplasia from HCC, which may exhibit this change.

Di Tommaso et al. *(44)* have recently described the utility of immunohistochemistry in separating hepatocellular dysplasia from early hepatocellular carcinoma. Using an antibody panel consisting of glypican-3, glutamine synthetase, and heat shock protein 70, they found that positivity for any two antibodies yielded a 72% sensitivity and 100% specificity for the diagnosis

of HCC over high-grade dysplasia. All cases of regenerative nodules and low-grade dysplastic nodules were negative for these antibodies. Reference should be made to their illustrations to correctly interpret the qualitative aspects of antibody patterns before applying this to clinical material.

Llovet et al. *(45)* used quantitative real-time RT-PCR to evaluate transcription levels of 55 candidate genes in dysplastic nodules and early hepatocellular carcinomas in patients with underlying hepatitis C virus-associated cirrhosis. They identified a three-gene subset comprised of glypican-3, survivin, and the hyaluronan receptor LYVE-1 that had 95% sensitivity and 94% specificity in distinguishing these two conditions.

5. HEPATOCELLULAR CARCINOMA

5.1. Clinicopathologic Comments

The clinical aspects of hepatocellular carcinoma are dealt with in detail throughout this book and are not repeated here. While the following discussion of hepatocellular carcinoma considers the tumor as a discrete entity, it is emphasized that each HCC likely represents the end result of a number of distinctive and partially overlapping malfunctions of a variety of cellular pathways. Thus, clinically similar HCCs arising in cirrhotic livers caused by alcohol versus infection with hepatitis B or C viruses have likely followed a somewhat different pathogenesis from each other, in addition to differing from HCC arising from a pre-existent hepatocellular adenoma in a noncirrhotic liver of a patient with a history of contraceptive pill use.

Further, we are in a transition period in which progress in molecular analysis is redefining our understanding of disease processes in a stochastic manner. Thus, time-worn descriptive terminology slowly gives way to evolving tumor subclassifications based on distinctive sets of molecular alterations. Which clinicopathologic concepts survive and which are discarded remains to be determined. The two approaches are presented in parallel so that the reader may have an overview of these complementary approaches to tumor pathology.

5.2. Macroscopic Pathology

The majority of hepatocellular carcinomas arise in cirrhotic livers and most frequently involve the right lobe (Fig. 5). The tumors are typically soft, vary in color from gray-green-yellow to light brown, are occasionally bile-stained, and often contain foci of hemorrhage or necrosis. Rarely they may contain a central scar that may mimic focal nodular hyperplasia *(10)*. The tumors can be single or multiple and range from less than 1 cm to over 30 cm in diameter with a tendency toward larger sizes when involving noncirrhotic livers *(46)*.

Fig. 5. Hepatocellular carcinoma (mixed hepatocellular carcinoma and cholangiocarcinoma) arising in a cirrhotic liver. The large and small nodules throughout this liver are consistent with cirrhosis. A hepatocellular carcinoma (*arrow*) is larger and has a different color from the nodules due to bile production. A second white nodule immediately to the left and of similar size was largely necrotic. The small white nodule situated superior to the two larger nodules had features of cholangiocarcinoma. This likely represents a mixed tumor, although molecular analysis was not performed at that time.

A wide variety of macroscopic patterns of tumor growth exist, but these have few clinical correlates. The traditional classification of Eggel *(47)* distinguishes three patterns of hepatocellular carcinomas: multinodular, massive, and diffuse. Multinodular HCC was the most common type in one series. In this pattern multiple tumor nodules are scattered throughout the liver *(46, 48)*. Multinodular HCC is typically associated with cirrhosis *(46)*. In the massive pattern a solitary tumor mass occupies much of the liver and may be associated with smaller satellite nodules. This pattern has been associated with noncirrhotic livers *(46)*. The diffuse pattern is the least common and is characterized by numerous widespread small nodules that mimic cirrhotic nodules; these may virtually replace the liver. In cirrhosis, clinically advanced liver disease has been associated with the diffuse or multinodular patterns of HCC *(48, 49)*. Rarely, HCC may be pedunculated, presumably reflecting an origin within an accessory lobe *(50)*. In one study it was concluded that pedunculated HCC has an unfavorable prognosis if appropriate surgical procedures are not performed during the early stages of development *(51)*.

In more recent macroscopic classifications, hepatocellular carcinomas are further subdivided into two main patterns based on growth characteristics: Expanding or expansive tumors have distinct borders that push aside the adjacent liver, and spreading or infiltrative tumors have poorly defined borders that microscopically invade the adjacent liver *(52, 53)*. Kojiro et al.

(54) applied the terms "distinctly nodular" and "indistinctly nodular" to refer to these growth patterns in small tumors. Small indistinctly nodular tumors were likely to contain both portal and arterial blood supplies, have portal tracts within their substance, and be comprised of uniform, well-differentiated cells. These authors considered the indistinctly nodular form to be the equivalent of carcinoma in situ, and they designated this as early HCC, noting the tendency to categorize such lesions as high-grade dysplasia in Western countries. In contrast, they considered distinctly nodular small HCC to represent an advanced cancer despite its small size.

Kanai et al. *(55, 56)* have additionally subdivided nodular HCC into three subtypes: type 1 is represented by HCC presenting as a single nodule, type 2 is a single nodule with extranodular growth, and type 3 has a contiguous multinodular growth pattern.

Blood groups have been related with macroscopic tumor patterns, with the suggestion that blood group status other than O was an independent risk factor for multinodular pattern HCC in those patients with tumor, and the presence of blood group O was associated with the solitary growth pattern *(46)*.

Portal vein thrombosis occurs in a high proportion of advanced cases *(57)*, and the frequency is lower in small HCC *(58)*. However, it has been proposed that curative resection may be possible, even in the presence of portal vein invasion, if the primary tumor is small, i.e., early stage *(59)*.

Less frequently, HCC may involve the main hepatic veins, the inferior vena cava or right atrium and can even extend into the large bile ducts. The clinical consequences of those involvements include Budd–Chiari syndrome, biliary obstruction, and hemobilia *(60–63)*.

Pathologic staging is a primary determinant of prognosis, and the growth pattern does not add additional information. However, the manner of growth, such as diffuse, may make it less likely that the tumor will be detected at an earlier stage, and, by definition, growth patterns such as diffuse or massive are synonymous with advanced disease and associated poor prognosis *(48, 49)*.

5.3. Staging of Hepatocellular Carcinoma

The International Union against Cancer and the American Joint Committee on Cancer (AJCC/UICC) published the Tumor-Node-Metastasis (TNM) pathologic classification for HCC in 1987 and later modified this in 2002 *(64)*. Most of the revisions were related to categorization of the primary tumor, i.e., T stage. A T1 tumor includes solitary tumors of any size without vascular invasion, and a T2 tumor includes solitary tumors of any size with vascular invasion. Multiple tumors are staged as either T2, in which the size of the largest tumor does not exceed 5 cm, or T3, in which the largest tumor

does exceed 5 cm in diameter. Factors such as bilateral location of tumors, or tumor multifocality versus intrahepatic metastasis of a single tumor, are not taken into account when assessing multiple tumors. Any tumor that involves a major branch of the portal vein (including portal vein and right and left branches) or hepatic vein (including right, left, and middle hepatic vein) is staged as T3. Finally, tumors with direct invasion of adjacent organs (excluding gallbladder) or penetration through the visceral peritoneum are staged as T4. A breakdown of the AJCC TNM Staging and Stage Grouping is provided in Tables 1 and 2.

The TNM system requires direct pathologic inspection of tumor extent and as such has limited usefulness in some clinical settings. A number of clinical or clinicopathologic staging systems have been proposed as offering more precise prognostic subgrouping and applicability for HCC patients who undergo hepatic resection, transarterial chemoembolization (TACE), or

Table 1
American Joint Committee on Cancer Staging for Intrahepatic Tumors: Definitions of TNM

Primary tumor (T)	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Solitary tumor without vascular invasion
T2	Solitary tumor with vascular invasion *or* multiple tumors none more than 5 cm
T3	Multiple tumors more than 5 cm *or* tumor involving a major branch of the portal or hepatic vein(s)
T4	Tumor(s) with direct invasion of adjacent organs other than the gallbladder *or* with perforation of visceral peritoneum
Regional lymph nodes (N)	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastases
N1	Regional lymph node metastases
Distant metastases (M)	
MX	Distant metastases cannot be assessed
M0	No distant metastases
M1	Distant metastases

Table 2
American Joint Committee on Cancer Staging for Intrahepatic Tumors: Stage Grouping

Stage	*T*	*N*	*M*
I	1	0	0
II	2	0	0
IIIA	3	0	0
IIIB	4	0	0
IIIC	Any	1	0
IV	Any	Any	1

transplantation. Okuda et al. *(65)* developed a three-stage system with prognostic utility and based on tumor size, serum albumin level, presence of ascites and jaundice. The Cancer of the Liver Italian Program (CLIP) system uses the Child–Pugh score, tumor morphology, alpha-fetoprotein level, and portal vein thrombosis as independent predictive survival factors *(66)*. The Barcelona Clinic Liver Cancer (BCLC) Staging System is based on the presence or absence of symptoms, tumor multinodularity, vascular invasion, and extrahepatic spread *(67)*. The Chinese University Prognostic Index (CUPI) is constructed by adding liver function variables (total bilirubin, ascites, alkaline phosphatase, alpha-fetoprotein, and asymptomatic disease on presentation) into the TNM staging system *(68)*. The Prognostic Risk Score is based on vascular invasion (microscopic and macroscopic), lobar distribution, lymph node status, and largest tumor size *(69)*. The Japan Integrated Staging Score *(70)* incorporates a score for Child–Pugh category together with a score for TNM Stage as defined by the Liver Cancer Study Group of Japan. In this approach, the T stage is based on the variables of single versus multiple tumors, tumor size <2 cm, and absence of vascular invasion. HCC fulfilling all three of these criteria are T1, those fulfilling two factors are T2, those fulfilling one factor are T3, and those fulfilling no factors (i.e., multiple tumors, greater than 2 cm with vascular invasion) are considered T4. Final stage also incorporates node and metastasis status. Kudo et al. *(70)* found patient stratification by this approach to be superior to that obtained by the CLIP system.

Other variants, incorporating the Model for End-Stage Liver Disease (MELD) *(71)* criteria into baseline JIS *(72)* or CLIP *(73)* scoring systems, have also been described.

Several reports have compared the efficacies of multiple staging systems in a clinical setting. Cillo et al. *(74)* and Marrero et al. *(75)* found the BCLC staging system to be the best overall approach. In the setting of HCC treated with TACE, Georgiades et al. *(76)* found the nominal Child–Pugh results to

be the most accurate prognostic indicator. In contrast, Cho et al. *(77)* found the CLIP system to excel in this specific patient cohort. Seo et al. *(78)* found the CLIP system to have the best predictive power in a retrospective study.

In the United States at present (mid-2008), liver transplant candidates with either single intrahepatic HCC between 2 and 5 cm or two to three intrahepatic HCC each 3 cm or less in greatest dimension have been granted additional priority (22 points) within the MELD framework for liver transplantation. This approach, based on the Milan criteria proposed by Mazzaferro et al. *(79)*, has been criticized as being too restrictive *(80)*. Conversely, a retrospective study *(81)* of liver transplantation in the United States comparing the 5-year periods before versus after the introduction of the MELD priority exceptions for HCC showed a significantly worse survival for patients with HCC in the 3–5 cm range. Complementary approaches, such as those incorporating loss of heterozygosity analysis, may aid in delineating subgroups of HCC patients most likely to benefit from liver transplantation *(69, 82, 83)*.

5.4. Microscopic Pathology

Hepatocellular carcinomas can contain varied microscopic appearances, most of which recapitulate aspects of normal hepatocyte cytology and architecture. Well-differentiated HCC may be difficult or histologically impossible to distinguish from hepatocellular adenoma *(84–86)* and it may likewise be difficult to precisely establish the interface between tumor and normal liver. In contrast, poorly differentiated examples of HCC may betray only minor evidence of their hepatocellular origin.

The commonest architectural pattern of malignant hepatocytes is an arrangement that caricatures the normal trabecular arrangement of liver lobules (Fig. 6). These neoplastic pseudotrabeculae vary from 2 to over 20 cells in thickness, are irregularly arrayed, generally but not always have a reduced or absent reticulin framework, and are separated by a vascular network lined by endothelial cells and containing isolated arterial/arteriolar branches. In contrast, normal trabeculae are 1–2 cells thick, evenly arranged, bordered by a well-developed reticulin network, and separated by sinusoids without prominent endothelial cells.

Other growth patterns of HCC are variations on this basic theme. A pseudoglandular (pseudoacinar) pattern may result either from dilatation of the bile canaliculi between tumor cells or from central lytic degeneration of solid trabeculae. The gland-like spaces can be empty or contain PAS-positive cellular debris, lipid-laden macrophages, or bile. Complex pseudoglandular formations can result in pseudopapillary structures and give the appearance of "islands" of tumor cells, usually surrounded by a lining of endothelial cells *(87)*. A compact or solid pattern results when malignant cells appose each other closely, rendering sinusoidal or vascular spaces inapparent. It has

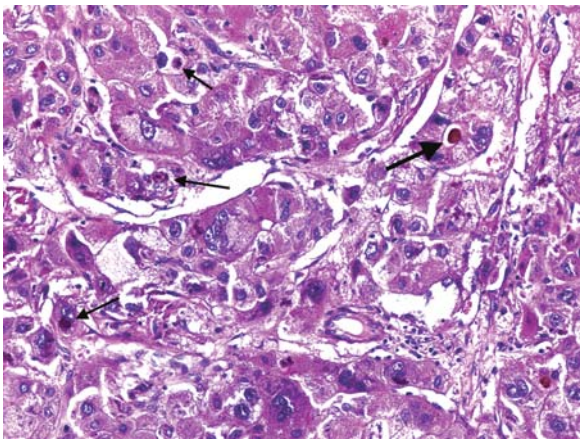

Fig. 6. Hepatocellular carcinoma. The tumor cells grow in distorted cords or trabeculae. Bile production (*large arrow*) and intracytoplasmic Mallory bodies (*small arrows*) are microscopic evidence of hepatocellular differentiation. More commonly, additional techniques are used to establish hepatocyte phenotype.

been suggested that hepatocellular carcinomas with a compact growth pattern have a better prognosis as compared with trabecular and acinar patterns *(88)*.

Tumor cells of HCC generally have more irregular nuclear membranes, coarser and more irregularly distributed heterochromatin, and a slightly higher nuclear:cytoplasmic ratios than do their benign counterparts. Mitotic and apoptotic activity are increased in the tumor cell population. As HCC approaches moderately to poorly differentiated phenotypes, there is a corresponding exaggeration of all of these features, with an increase in cell-to-cell heterogeneity and the emergence of giant and bizarre tumor cells in some cases. Different degrees of differentiation can be seen within a single tumor.

A variety of cytologic modifications may be seen within a given case of HCC. In general these have no prognostic relevance, but they can be useful clues for the diagnostic histopathologist. In some cases clear cells may predominate due to glycogen or lipid accumulation. Macrovesicular steatosis may be diffuse or focal and appears to be a more frequent finding in small HCC.

Bile pigment is noted in about 20% of hepatocellular carcinomas (Fig. 6). Bile within the neoplastic cells or bile canaliculi is an important indicator of hepatocellular origin. Bile is usually evident on routine histology, but on occasion it may be necessary to demonstrate bile canaliculi by polyclonal anti-carcinoembryonic antigen antibody which is cross-reactive with biliary glycoproteins (Fig. 7).

A variety of intracellular inclusions can be identified. Dense eosinophilic globular bodies may be intra- or extracellular. These are usually

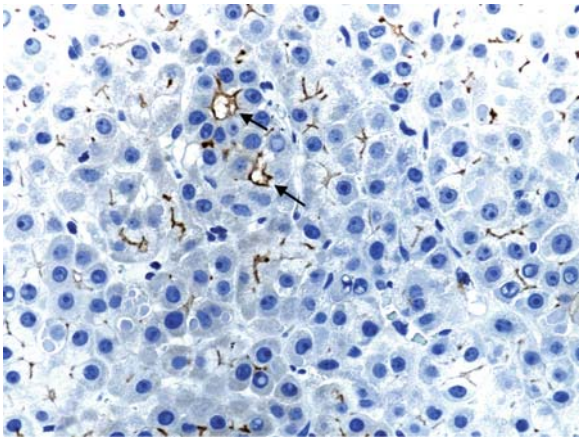

Fig. 7. Polyclonal carcinoembryonic immunostain highlighting bile canaliculi in hepatocellular carcinoma. In this well-differentiated tumor, the dark branch-like structures represent uptake of polyclonal CEA antibody, which cross-reacts with biliary glycoprotein. In some cases canalicular dilatation forms pseudoglandular structures (*arrows*). (polyclonal CEA immunostain with diaminobenzidine, 400×).

PAS-positive and can contain various proteins including alpha-fetoprotein, alpha-1-antitrypsin, alpha-1-antichymotrypsin, albumin, fibrinogen, and/or ferritin. Pale bodies are lightly staining, eosinophilic, intracytoplasmic inclusions that correspond to dilated rough endoplasmic reticulum and contain mainly fibrinogen, probably reflecting defective protein transport *(89)*. Pale bodies may simulate "ground glass" inclusions that are related to hepatitis B virus infection, but unlike true ground glass inclusions, they do not contain viral components *(90, 91)*. It has been suggested that proteins expressed in intracytoplasmic bodies might in some cases contribute to the malignant phenotype, since in one case p62, a phosphotyrosine-independent ligand of p56(lck) and putative signal transducer, was identified as the major component of such inclusions *(92)*. Typical Mallory bodies can be seen in about 20% of hepatocellular carcinomas, regardless of underlying disease *(93)*. Megamitochondria, enlarged lysosomes, myelin deposits, abnormal accumulations of glycogen, and degenerative material are occasionally seen and can be identified ultrastructurally. Copper, copper-related protein, and Dubin–Johnson-like pigment have all been described in tumor cells. The latter may impart a black macroscopic appearance to the tumor *(94)*. Rarely extramedullary hematopoiesis and granulomas can be detected.

Kupffer cells are present but quantitatively reduced in hepatocellular carcinomas, with more prominent decreases noted in larger and less well-differentiated tumors *(95)*. However, small, well-differentiated HCC may contain Kupffer cells in nearly normal numbers. Reduced Kupffer cell

function and cytokine production have been suggested as possible augmenters of HCC progression in an experimental animal model *(96)*.

The stroma of HCC is usually scanty. In some cases there can be a fibrous background and differentiation from other forms of adenocarcinoma may become problematic.

Tumor nodules are frequently surrounded by distinct fibrous capsules, and septum formation can be observed during the development of HCC. The capsule consists primarily of Type III collagen with Type I collagen facing the tumor in well-developed examples *(97–99)*. Small HCCs have a higher proportion of well-encapsulated tumors. The capsule and septa are mainly formed by alpha-smooth muscle actin-positive mesenchymal cells and can result from interactions between tumor and host liver parenchyma. It is thought by some that the capsule is a manifestation of host defense that can interfere with the growth and invasiveness of HCC *(97, 99)*. It has been suggested that tumor infiltration of the peritumoral capsule or of the surrounding parenchyma might correlate with a higher frequency of portal vein invasion and intrahepatic metastases *(48)*.

A four-tiered histologic grading system was originally devised by Edmondson and Steiner *(100)*, with Grades I–IV denoting progressive loss of differentiation. Tumor grades have been shown to correlate with the gross morphology, DNA content, proliferation markers, metastases, and AFP production but grading is a weak independent prognostic predictor *(101–103)*.

In our practice, about 15–20% of HCC behave in an aggressive fashion, despite small size. It is therefore incumbent upon the pathologist to assess each tumor for degree of differentiation and search for vascular invasion, regardless of tumor size. Whether such lesions have specific and early genetic or epigenetic changes that define such behavior remains to be determined.

5.5. Immunocytochemical Markers of Hepatocellular Carcinoma

A wide variety of antigens are detectable within HCC cells, and one recent textbook lists 109 such markers *(104)*. Some of these are of use in dissecting the various pathways of neoplastic progression that may occur in these tumors. Only a subset of markers has routine diagnostic applications and those are briefly considered herein.

Detection of alpha-fetoprotein expression is a classical approach to the diagnosis of HCC. The specificity of AFP is as high as 97%, but its sensitivity is low. Expression is often patchy and weak, and it has been suggested that AFP positivity correlates with size and differentiation of the tumor; small, well-differentiated HCCs are less positive than poorly differentiated ones.

This association apparently also extends to a lectin-reactive fraction of AFP (AFP-L3) that is currently used as a serum marker of HCC. Several studies have shown that serum AFP-L3-positive HCC patients have less well-differentiated tumors than do patients negative for this marker *(105, 106)*.

A number of other antibodies have long been used in the routine diagnostic evaluation of hepatocellular phenotype. Detection of biliary glycoprotein by the use of cross-reactive polyclonal anti-carcinoembryonic antigen (CEA) antibody highlights a bile canalicular pattern in 60–90% of HCC and was estimated in one series to be 79% sensitive and 97% specific for these tumors *(110)*. Adenocarcinomas and cholangiocarcinomas can show cytoplasmic staining with these antibodies, a pattern that is less common in HCC. Further, these other tumors can also react with the more specific monoclonal anti-CEA antibodies, a result that is only rarely seen with HCC when appropriate clones are used.

A canalicular pattern of staining in benign and malignant hepatocytes can also be demonstrated with antibody to CD10 (neprilysin) *(137, 138)*. In one study this antibody showed 68% sensitivity and 100% specificity for the differential diagnosis of HCC, although it did not distinguish it from normal liver parenchyma *(137)*.

HepPar 1 is a monoclonal antibody that detects the urea cycle enzyme carbamoyl phosphate synthetase 1 *(107)*. It decorates both benign and neoplastic liver cells and is not absolutely specific for the hepatocyte phenotype, as it may rarely be expressed in other cell and tumor types *(108, 109)*. However, in one study HepPar1 had 82% sensitivity and 90% specificity for the detection of hepatocellular carcinomas *(110)*. When it is used as a part of a diagnostic panel its diagnostic accuracy is enhanced *(110–113)*. HepPar-1 is more likely to be expressed in well-differentiated as opposed to poorly differentiated tumors.

For the differentiation of hepatocellular carcinoma from cholangiocarcinoma and metastatic carcinomas, particularly those of colorectal origin, immunostaining for individual cytokeratins is reportedly helpful. Normal adult liver cells contain cytokeratins 8 and 18 as defined in Moll's catalogue, and bile duct epithelial cells contain cytokeratins 7 and 19. At least in our experience this approach is less helpful than the use of other markers, since (a) hepatocytes can express CK7 when there is nearby fibrosis and this is particularly relevant with the scirrhous variant of HCC; (b) some HCC also express CK19, which is interpreted as showing a bipotential phenotype, although the tumor is still recognized as HCC; and (c) we have experienced significant artifactual staining with antibody to CK8. Of perhaps more utility is the use of cytokeratin antibodies to differentiate tumors of hepatocellular origin from colorectal adenocarcinoma. The latter are most often cytokeratin 20^{+} 7^{-}, a pattern rarely seen in either HCC or cholangiocarcinoma *(139)*.

Glypicans are a family of six heparin sulfate proteoglycans that are mainly expressed in a stage- and tissue-specific manner during development *(114)*. One form, glypican-3, is highly transcribed in hepatocellular carcinoma *(115)* and can serve as a marker for this tumor. Its use as part of a panel in the differentiation of HCC from hepatocellular dysplasia was considered above. It is not specific for HCC, with expression seen in about half of the cases of squamous cell lung carcinomas, liposarcomas, and nonseminomatous germ cell tumors *(116)* and in approximately 80% of melanomas *(117)*. In contrast to HepPar-1, glypican 3 is more sensitive in the detection of poorly differentiated as opposed to well-differentiated HCC *(116)*. Care in the use of this diagnostic marker is indicated, as it has been reported to be positive in 16% of preneoplastic nodular liver lesions *(116)* and also in 25 of 30 cases of benign liver tissue with prominent inflammation related to hepatitis C virus infection *(118)*.

β-Catenin translocation to the nucleus as a result of mutations or other aberrations of the β-catenin pathway is detectable in a minority of HCC, as is the expression of target gene products such as glutamine synthetase *(119)*. However, since these markers can also be expressed in a subset of hepatocellular adenomas, the diagnostic utility of these antibodies is somewhat limited. The possible prognostic significance of these markers remains unsettled at present.

Epithelial glycoprotein-2 is a cell surface molecule present on many carcinomas but absent on HCC *(140)*. The glycoprotein is detected by the monoclonal antibody MOC-31 and a positive staining result with this antibody would suggest a tumor other than HCC *(94)*.

Serum des-carboxy-prothrombin, also known as protein induced by vitamin K absence II (PIVKA-II) is useful as a marker of HCC. Immunohistochemical detection of this protein within the cytoplasm of HCC tumor cells was documented *(120)* and the authors suggested that it may prove useful in separating small HCC from examples of adenomatous hyperplasia. A separate study found an association with immunohistochemical detection of PIVKA-II within HCC and the presence of vascular invasion or higher tumor stage, suggesting its utility as a prognostic as well as a diagnostic marker *(121)*.

Gotoh et al. showed overexpression of osteopontin in HCC by quantitative PCR and immunohistochemistry *(122)*. This secreted glycoprotein is an organic component of the bone matrix, but is secreted by a number of other cell types. Osteopontin expression in HCC was associated with infiltration into the tumor capsule *(122)*, early tumor recurrence, metastasis, and lower survival *(123)*. Elevated serum levels of osteopontin had similar significance and were considered superior in one study to measurement of AFP or PIVKA-II *(124)*. Zhang et al. *(125)* showed that preoperative plasma osteopontin levels was an independent prognostic indicator of both overall and disease-free survival in a multivariate model.

Other potential prognostic immunohistochemical markers, such as galectin-3 *(126)*, survivin *(127)*, the stem cell markers CD133 *(128)* or EpCAM *(129)*, Aurora kinase B *(130)*, WT-1 *(131)*, histone deacetylase 1 *(132)*, phospho-ERK1/2 *(133)*, the transcription factor Twist *(134)*, mortalin (heat shock 70-kDa protein 9) *(135)*, the polycomb group oncogene Bmi-1 *(136)*, among others, are under active investigation at present.

Morphometric image analysis has been used to aid in the differential diagnosis of benign versus malignant hepatocellular lesions *(141–143)*. In one case, correlation of nuclear features with a specific loss of heterozygosity on 17p13 was reported *(144)*. Clinical application of these techniques, although promising, remains limited and the introduction of a more user-friendly technical infrastructure in the near future seems likely.

5.6. Molecular Pathology

The underlying molecular biology of HCC is covered elsewhere in this book and is not considered here. Likewise, specific cellular pathways of diagnostic pathologic importance for dysplastic lesions and hepatocellular adenomas are discussed above. Here we are concerned with the application of ancillary studies that may shed light on the behavior or prognosis of HCC beyond that discernible by the diagnostic histopathologist (in addition to potential prognostic markers already mentioned). Despite the impressive number of studies and the resultant large strides in understanding over the past decade, such approaches must still be considered to be early in evolution. These studies will eventually generate a comprehensive picture of HCC at the cellular and subcellular level which will in some cases confirm, and in other cases likely overthrow, our current concepts of this disease.

In the simplest hypothetical construct, cancer can be considered to represent an imbalance between cellular growth and cellular death. Thus, inappropriate activation of cell proliferation pathways and inhibition of apoptotic pathways could each tip the balance in favor of the tumor. Early studies of cellular proliferative markers, including S-phase fraction *(102)*, quantitation of silver staining nucleolar organizing regions (AgNORs) *(145)*, and immunohistochemical assessment of cell cycle proliferation antigens Ki67/MIB-1 or PCNA/cyclin *(145, 146)* all showed an inverse association with patient survival. Similar correlations extend to individual components of the cell cycle machinery. Overexpression of cyclin A and cyclin D1 was inversely associated with disease-free survival in some *(147, 148)* but not all *(149)* studies.

The application of microarray studies has upheld and expanded these studies. Lee et al. *(150)* examined cDNA derived from 91 HCC by unsupervised hierarchical clustering supplemented by additional analytic

procedures. They found two major subclasses of tumors that were strongly associated with patient survival, and increased translation of genes associated with cell proliferation was the strongest predictor of decreased survival.

Inhibition of apoptosis might be expected to stabilize a tumor population and serve as a negative prognostic indicator. In this regard Garcia et al. *(151)* used multivariate analysis to determine that a high level of immunohistochemical staining for the pro-apoptotic Bax protein was associated with a 31.9-month median survival whereas patients with weak or absent staining had 6.6-month median survival. Conversely, those patients with strong intratumoral expression of the antiapoptotic bcl-x had only a 5.8-month median survival, which increased to 32.7 months with strong expression. Nuclear expression of the antiapoptotic protein survivin was also associated with nuclear grade, microvascular invasion, proliferative rate, and local tumor recurrence as well as decreased survival in one study *(152)*. Lee et al. *(150)* also found a number of antiapoptotic molecules to be overexpressed in their poor survival group using a microarray approach. Similarly, telomerase activation serves to short-circuit normal cell senescence and subsequent cell death, and this protein is frequently activated in HCC *(153)*. High levels of telomerase activity are associated with recurrence following hepatectomy as well as decreased survival *(154, 155)*.

Disruption of cell cycle checkpoint proteins may facilitate genomic instability and the generation of tumor subclones with enhanced malignant behavior. The p53 tumor suppressor gene has been extensively studied in this regard *(156–165)* (reviewed in *(166)*). Immunohistochemical detection of p53 should be combined with p21 immunostaining to differentiate functional (p21 positive) from mutant (p21 negative) p53 expression. Additionally, some p53 mutations result in protein dysfunction without extended half-life and would therefore result in false-negative results by immunodetection. For these reasons, DNA mutation analysis is preferred. Mutations of p53 have generally been associated with disease recurrence and decreased survival *(166)*. P53 overexpression has also been associated with nuclear β-catenin expression and downregulation of E-cadherin in some studies *(167)* but not others *(168)*. Protein p73, which is an analogue of p53, also can induce apoptosis and in one immunohistochemical study was detectable in 32% of 193 HCC and found to be a correlate of poor prognosis *(103)*.

Aberrant retinoblastoma gene protein expression, including both absence and overexpression, was associated with poorly differentiated tumors and metastases in one study *(159)* and was felt to be a marker of advanced disease. Similar results were reported by this group for loss of the INK4 cyclin-dependent kinase (CDK) inhibitor p16 *(160)*. Inactivation of the INK4 CDK inhibitor p15 detected by promoter methylation-specific PCR was found in 64% of tumors in one study *(169)* and was associated with recurrence or

metastatic disease. This assay was also used to detect circulating tumor cells and the authors concluded that it might prove useful for both diagnostic and monitoring purposes.

Genomic instability may also manifest as increased aneuploidy and this has been associated with the degree of histologic differentiation *(101)* and decreased survival *(170)*. Markers of microsatellite instability have also been examined and in some studies have been associated with reduced disease-free survival *(171)*.

Composite markers of genetic alterations have been applied in a clinical setting. Marsh et al. *(83)* analyzed loss of heterozygosity at multiple loci to generate a fractional allelic loss index. Although this could not be used as a stand-alone assay due to the variability in the number of informative markers for a given tumor, these investigators were able to incorporate this information into a previously developed neural network model to accurately predict tumor recurrence in 81 of 81 evaluable patients. This approach, as well as comparative genomic hybridization *(172)*, has also found utility in distinguishing multiple independent primary HCC from intrahepatic metastases in some cases.

Microarray studies have generated a plethora of HCC-related data that must be integrated and simplified for clinical use. As examples, molecular signatures associated with intrahepatic versus extrahepatic metastasis *(173)*, vascular invasion *(174)*, clinical outcome including delineation of possible progenitor cell tumors *(175, 176)*, and recurrence following transplantation *(177)* represent some early results along these lines. Iizuka et al. *(178)* have recently presented a high-level overview of HCC-related microarray studies with a focus on current problems and challenges. The availability of high-throughput analysis of single nucleotide polymorphisms (SNP) will add an additional dimension to our ability to define HCC behavior. For example, SNP associated with high levels of alpha-fetoprotein production *(179)*, an adverse prognostic indicator, may eventually form part of a panel allowing a detailed clinicopathologic assessment of HCC. Such an approach will need to take into account the underlying etiologic factors, i.e., hepatitis B or C virus, aflatoxin, alcohol, as well as the presence or absence of cirrhosis, at a minimum.

6. PATHOLOGIC VARIANTS OF HEPATOCELLULAR CARCINOMA

6.1. Fibrolamellar Carcinoma

Fibrolamellar hepatocellular carcinoma (FL-HCC), also known as oncocytic hepatocellular carcinoma or polygonal cell-type hepatocellular carcinoma with fibrous stroma, is separable from ordinary hepatocellular

carcinoma on the basis of macroscopic, histologic, ultrastructural, and molecular features *(180)*. This distinctive variant of HCC is seen predominantly in young patients (90% under 35 years of age) without cirrhosis *(181)*. In a recent study using data from the Surveillance, Epidemiology, and End Results (SEER) program, El-Serag et al. *(182)* found this variant to comprise 13.4% of all primary liver cancers in patients under 40 years of age and 0.85% above this age. There appears to be a predominance in whites *(182)*, with relative rarity in Asia *(183)*, although it may be becoming more commonly recognized in that geographic area *(184)*. No sex predilection is known.

The clinical presentation is typically vague, with components of abdominal pain, malaise, and weight loss *(180)*. Less common presentations include biliary obstruction *(180)*, thrombophlebitis *(185)*, or massive bilateral metastatic spread to the ovaries (Krukenberg tumor) *(186)*.

The tumors are solitary in 90% of cases, ranging on average from 9 to 14 cm at time of presentation *(180)*. This neoplasm is unique among hepatocellular tumors in that the majority arise in the smaller left hepatic lobe *(104)*. The fibrous component of FL-HCC often forms a central scar that can be demonstrated by radiological techniques *(187, 188)*. The fibrous component also provides increased firmness to the tumor in comparison to typical HCC and may also be the site of calcification. The pattern of fibrous scar formation may superficially mimic that seen in focal nodular hyperplasia. It had been previously suggested that fibrolamellar HCC and focal nodular hyperplasia may be pathogenetically related, but most investigators do not subscribe to that concept *(189)*.

Microscopically, there is usually a compact architectural growth pattern but trabecular or acinar patterns can also be observed. The neoplastic cells are larger than normal hepatocytes (Fig. 8), polygonal in shape, and possess granular, eosinophilic cytoplasm, a so-called "oncocytic" appearance, due in fact to numerous swollen mitochondria *(190)*. Nuclei are vesicular, rounded, and have prominent nucleoli, the latter being a characteristic feature of this tumor. Mitoses are usually sparse; pleomorphism and multinucleation are infrequent. Tumor cells contain pale bodies that are reactive for fibrinogen and hyaline globular inclusion bodies may be present *(191)*. Intracellular bile production, fat, glycogen, copper and copper-associated protein can be detected *(192)*. In some tumors mucin production can be detected. Pseudoacinus formation may be seen, but the typical small glandular pattern associated with cholangiocarcinoma is not part of the normal spectrum of fibrolamellar HCC. Nevertheless, rare cases exist of fibrolamellar HCC combined with cholangiocarcinoma *(193)* or more typical HCC *(194)*. Clear cell changes have been described in a case of otherwise typical fibrolamellar HCC *(195)*.

Tumor cells are positive for HepPar-1 *(196, 197)* and hepatocyte cytokeratins 8 and 18 and may also contain biliary cytokeratins 7 and 19 *(180,*

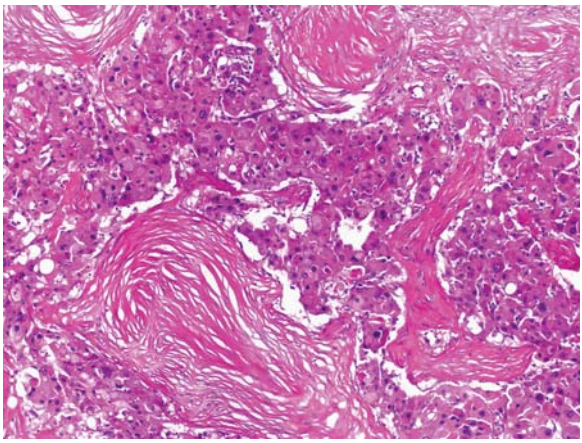

Fig. 8. Fibrolamellar hepatocellular carcinoma. In this variant, malignant cells contain plentiful cytoplasm and the tumor characteristically contains lamellated or layered areas of fibrosis (100×).

198). The tumor cells are usually reactive with antibodies to polyclonal CEA, alpha-1-antitrypsin, ferritin, and C-reactive protein. Alpha-fetoprotein is present in only occasional cases *(190, 199)*, and prominent AFP positivity, particularly when combined with elevated serum levels, suggests that a search for areas of more typical HCC should be undertaken *(200)*. Glypican-3 immunopositivity was seen in 64% of fibrolamellar HCC in one small series *(36)*, and in some cases uptake was patchy.

A prominent collagenous fibrous stroma that is arranged in thin parallel bands (lamellae) is a characteristic feature of fibrolamellar HCC (Fig. 8), but may be sparse or even absent in some tumors. The collagen is predominantly composed of types I, III, and V *(201)*. It has been suggested that lamellar fibrosis might be due to the production of collagen by stromal cells which in turn are stimulated by transforming growth factor-β (TGF-β) produced by tumor cells *(202)*.

Wilkens et al. *(203)* applied comparative genomic hybridization (CGH) to a series of HCC and found 1q amplification in one of two fibrolamellar HCC, with no changes in the other tumor. A separate study *(204)* also using CGH suggested that 4q+, 9p–, 16p–, and Xq– were more typical of fibrolamellar HCC than of other types of hepatocellular tumors. Fibrolamellar HCC is also marked by an absence of molecular alterations commonly found in other forms of HCC. These include an absence of TP53 mutations *(205)*, absence of β-catenin gene mutations *(206)*, and lack of survivin overexpression in fibrolamellar HCC in separate studies *(207)*. Fibrolamellar HCCs also show less promoter methylation than do HCC arising in cirrhotic livers *(208)*. However, 80–100% of fibrolamellar HCC in this study did show methylation of the CDH1 (e-cadherin) and RASSF1A (Ras association domain family 1

isoform A) genes *(208)*. The product of this latter gene is thought to act as a tumor suppressor by modulating a number of apoptotic and cell cycle checkpoint pathways *(209)*. Overexpression of the MAP kinase and phosphatidylinositol 3 kinase pathways in fibrolamellar HCC was detected in a separate DNA microarray study *(210)*. A number of other changes, including overexpression of the neurotensin gene, were also observed. This study again pointed to chromosome 1q as a significant locus for genetic alterations in this tumor.

Pure fibrolamellar HCC has a better prognosis than typical HCC primarily because it often presents as a surgically resectable lesion. For this reason, aggressive surgical management has been advocated for this tumor *(211–214)*. Resectability is an important prognostic variable *(215, 216)*, and Katzenstain et al. *(217)* concluded that resectability, not the fibrolamellar pattern, is the primary prognostic criterion, with patients presenting with an initially resectable lesion having a good prognosis regardless of histologic subtype.

6.2. Clear Cell Hepatocellular Carcinoma

Clear cell hepatocellular carcinoma is comprised of malignant hepatocytes, the large majority of which contain a clear or empty-appearing cytoplasm reflecting the accumulation of intracellular glycogen or lipid *(218, 219)*. The tumor typically arises in a background of cirrhotic liver, although it has rarely been reported in a noncirrhotic setting *(220)*. Liu et al. *(221)* found an association of clear cell change with hepatitis C virus infection in an Asian series, and individual associations with non-alcoholic steatohepatitis in a diabetic patient *(218)*, hypoglycemia, and hypercholesterolemia *(222)* have also been reported. One study *(223)* uncovered an example of clear cell HCC with a histologic appearance similar to that of chromophobe renal cell carcinoma. Since this tumor had significant microsatellite instability in contrast to the remainder of clear cell HCC in that series, the authors concluded that clear cell HCC represents a heterogeneous category of tumor. Orsatti et al. *(224)* also pointed to subtypes within this category. They showed that nondiploid clear cell tumors in their series were more pleomorphic and had a higher mitotic rate than diploid clear cell HCC and suggested that differences between these subgroups might account in part for differing opinions regarding the behavior of clear cell HCC.

One source of diagnostic difficulty lies in the possible histologic confusion with other tumors that may present as clear cell neoplasms, in particular renal cell carcinoma and adrenal cortical tumors. Immunohistochemical studies may be of aid in defining a hepatocellular phenotype of these lesions *(225)*.

Several series *(223, 226)* found no difference in overall clinical behavior between clear cell and typical HCC. In contrast, Liu et al. *(221)* reported

higher survival in clear cell versus common type HCC. They ascribed these differences to the more frequent presence of a tumor capsule and a lower rate of vascular invasion in the clear cell tumors. Jeon et al. *(227)* report the remarkable case of an elderly male who experienced spontaneous regression of a large clear cell HCC with metastases.

6.3. Scirrhous (Sclerosing) Hepatocellular Carcinoma

Scirrhous hepatocellular carcinoma is a rare variant of HCC that usually occurs in older age groups. It is reportedly associated with hypercalcemia in cases occurring in the United States but not in those reported from Japan *(228)*. Parathyroid hormone-related protein was detected by immunohistochemical means in tumor cells of one case and this was suggested as the cause of tumor-associated hypercalcemia *(229)*. The margin is often ill-defined on CT scan *(230)*. Macroscopically, the mass is usually large, firm, and gray-white in color. The characteristic histological features of the sclerosing hepatocellular carcinoma are nonlamellar, extensive fibrosis (Fig. 9) that extends from the sinusoidal areas *(231)* and a pseudoacinar formation of the tumor cells. Tumor capsule formation is seen in about 30% of cases *(230)* or less *(232)*, and in one series vascular involvement was more common than in typical HCC *(230)*. Origin within a dysplastic nodule has been described *(231)*.

The hepatocellular component of the tumor shows higher expression of cytokeratin 7 and lower expression of HepPar-1 than ordinary HCC

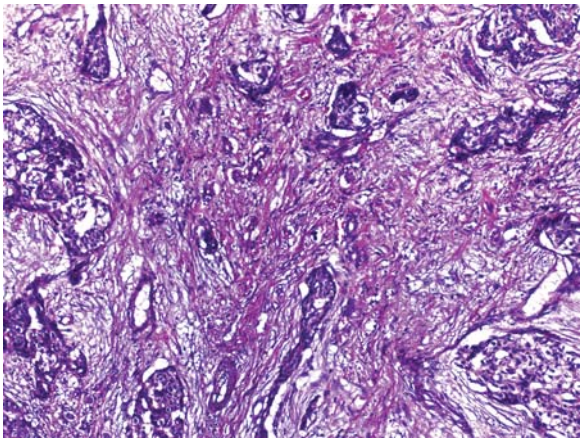

Fig. 9. Scirrhous hepatocellular carcinoma. In this variant, there is typically a diffuse fibrous background that simulates the pattern associated with cholangiocarcinoma. The malignant cells do not have the large appearance of the fibrolamellar variant, and ancillary techniques are usually required to identify them as having hepatocellular lineage (100×).

(233, 234). Frequent alpha-smooth muscle actin-positive-activated stellate cells have been described within this variant *(232)* and may contribute to the stromal changes. The sclerotic stroma, together with the occasional pseudoglandular pattern assumed by the tumor cells, may lead the diagnostic histopathologist to an incorrect diagnosis of cholangiocarcinoma. Okamura et al. *(235)* demonstrated that the stroma of scirrhous HCC lacks laminin-5 expression and shows only low levels of tenascin-C, both of which are highly expressed in cholangiocarcinoma. Further, stromal cells of scirrhous HCC are strongly alpha-smooth muscle actin positive, whereas those of cholangiocarcinoma reportedly have a more prominent glial fibrillary acidic protein-positive population *(235)*. Presence of intracellular mucin would also favor cholangiocarcinoma (or metastatic adenocarcinoma).

No significant clinical differences in the behavior of scirrhous HCC relative to ordinary HCC are known *(230)*.

6.4. Combined Hepatocellular/Cholangiocellular Carcinoma

Combined hepatocellular/cholangiocellular carcinoma is the least common type of primary epithelial liver cancer, accounting for approximately 2% of such tumors with reported frequencies ranging from 0.4 to 14.2% *(236)*. The World Health Organization defines this tumor as one that contains unequivocal elements of both hepatocellular carcinoma and cholangiocarcinoma that are intimately admixed, while also stipulating that this tumor be distinguished from synchronous intrahepatic hepatocellular carcinoma and cholangiocarcinoma that may also coexist adjacent to each other *(237, 238)*. Acceptable features of a hepatocellular component include the presence of bile, positivity for polyclonal carcinoembryonic antigen in a canalicular pattern, and/or demonstration of other hepatocyte marker such as alpha-fetoprotein *(239)* or HepPar-1. Demonstration of neutral epithelial mucin or cytokeratin 19 (and somewhat less specifically, cytokeratin 7) would suffice for demonstration of a biliary component.

Serum markers may mimic the mixed nature of the tumor, and concomitant elevations of AFP and CA19-9 may occur *(240)*. There are some purported differences in clinicopathologic features related to geographic area *(236)*. In Asian series, these tumors have been more often associated with underlying chronic liver disease and hepatitis B virus infection, whereas in Western series, more examples occur in the absence of chronic liver disease. This has practical implications, as patients without cirrhosis are more likely to qualify as resection candidates.

The tumors morphologically consist of mixed populations of hepatocytes, neoplastic cholangiolar cells, and small undifferentiated intermediate or oval-like cells on the basis of both light and electron microscopy *(241)*.

Characteristically, areas of trabecular hepatocellular carcinoma are mixed with varying numbers of bile duct-type cells (Fig. 10a). Generally the central areas are typical of hepatocellular carcinoma and the peripheral cells resemble biliary-type cells. In other cases there may be distinctive nodules of differing appearance, and in yet other examples the two histologic phenotypes may be finely mixed *(242)*. There is a variable component of stromal fibrosis and mixed neutrophilic and lymphocytic inflammation that is usually related to the cholangiolar component *(243)*. A proportion of combined hepatocellular/cholangiocellular carcinomas can be associated with a sarcomatoid component *(241, 244)* (Fig. 10b).

Opinions regarding the pathogenesis of combined HCC/CC ascribe it variously to metaplasia of pre-existent HCC into cholangiocarcinoma *(242)* or to a bipotential progenitor cell capable of giving rise to both components *(245)*.

The two components of HCC/CC do share a number of features. Imai et al. *(246)* showed similar p53 and RB-1 locus mutations in both hepatocellular and cholangiocellular components of mixed HCC/CC in some patients. A cell line derived from a human HCC/CC showed features of one or the other cell component dependent on growth conditions *(247)*. Gil-Benso et al. *(248)* were able to derive in vitro rat hepatocellular, cholangiocellular, and oval type cell lines from a single founder cell line derived from a rat HCC/CC. These lines showed similar molecular genetic alterations.

Immunophenotypic analysis of HCC/CC also discloses a subpopulation of cells corresponding to intermediate- or small-sized cells that contain

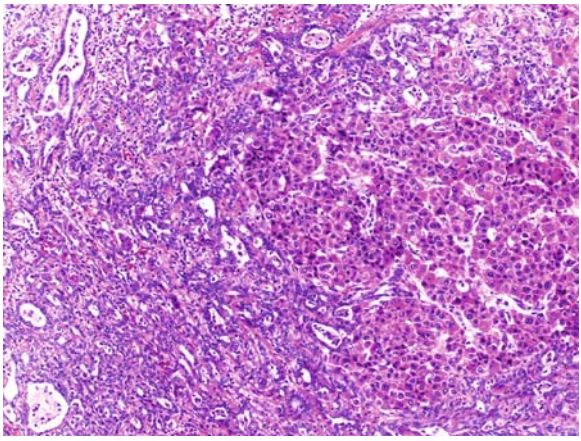

Fig. 10a. Mixed hepatocellular and cholangiocarcinoma. This tumor shows solid areas of cells resembling hepatocytes on the right side of the photograph, whereas smaller cells with significant gland formation largely populate the left side. The immunophenotype of these areas also varied between hepatocellular and biliary (100×).

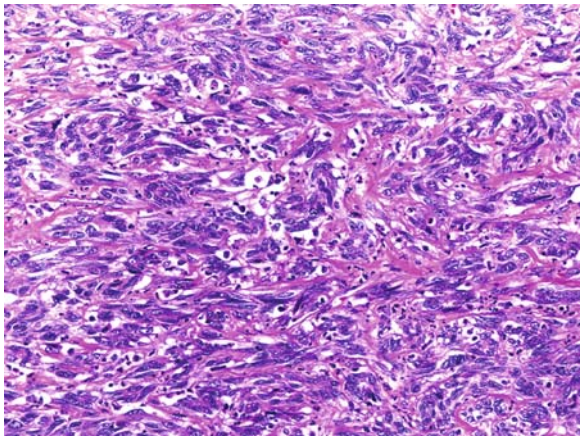

Fig. 10b. Sarcomatoid change in mixed hepatocellular/cholangiocarcinoma. This is a separate area of the tumor shown in Fig. 10a. In this region the neoplastic cells have a spindled or "streaming" appearance that is usually found in sarcomas. These cells expressed both vimentin and cytokeratins, supporting the concept that they arose by a form of metaplasia or tumor progression (or "dedifferentiation") from the epithelial elements in the tumor.

biphenotypic markers. Zhang et al. *(245)* found that these cells coexpress HepPar-1 and CK19 by double immunofluorescence studies and also found similar results using a combination of OV-6 and c-kit. (The presence of c-kit in 83% of their tumors also led them to suggest investigation of Gleevec therapy in these tumors.) They interpreted these cells as putative progenitor cells. These cells are not diagnostic for combined HCC/CC, as similar cells have been described in dysplastic foci.

Aishima et al. *(243)* examined a series of small (<3 cm) HCC/CC and found that those in which the biliary component coexpressed CK19 and mucin had worse survival and more frequent tumor recurrence than did those without these two markers.

Related studies raise the possibility that a limited form of biphenotypic expression may be more widespread than commonly appreciated. Dumez et al. found that 28% of 107 otherwise typical hepatocellular carcinomas expressed CK7 and/or CK19. Those expressing the biliary marker CK19, but not those expressing CK7, had a higher recurrence rate.

6.5. Sarcomatoid Hepatocellular Carcinoma

Sarcomatoid hepatocellular carcinoma is a rare variant of HCC that may contain spindle-shaped cells with features of any of a variety of sarcomas *(249, 250)*, including fibrosarcoma, leiomyosarcoma, rhabdomyosarcoma, osteosarcoma, and others. Oscteoclast-like or anaplastic giant cells may also

be seen, with the former cell type thought to represent benign reactive histiocytes *(251)*. A malignant hepatocellular component is present, and rarely this may take the form of hepatoblastoma *(252)*. The sarcomatoid component is considered to represent a form of tumor progression, or "dedifferentiation" of the epithelial component, as attested to by the demonstration of hepatocyte keratin subtypes or alpha-fetoprotein positivity reported in the sarcomatous elements in some cases *(249, 253)*. Haratake et al. *(253)* suggest the keratin 8 positivity in the sarcomatoid element may be diagnostically helpful in distinguishing these tumors from true intrahepatic sarcomas.

Park et al. *(254)* examined expression of the transcription factor SRF (serum response factor, c-fos serum element-binding transcription factor) in HCC. This protein regulates expression of a number of genes and is thought to play an important role in mesoderm development during embryogenesis *(255)*. SRF expression was found to be prominently expressed in high-grade HCC, especially sarcomatoid HCC. They proposed that this protein activated genes that contributed to acquisition of a mesenchymal phenotype, thereby contributing to tumor progression.

Sarcomatoid change can also occur in mixed hepatocellular–cholangiocellular tumors as noted above (Fig. 10b) *(241, 244)*, and the relationship between those tumors and sarcomatoid HCC is currently undefined.

Given the rarity of this variant, most conclusions regarding survival are based on single case reports or small series and appear to follow the course expected of a high-grade malignancy.

7. HEPATOBLASTOMA

7.1. Clinical Aspects

Hepatoblastoma is the most common primary liver tumor of infancy and childhood. It arises most frequently during the first 5 years of life and may rarely be diagnosed in the fetus *(256)*. Rare cases are reported in adults *(257–259)*. The male:female ratio for hepatoblastoma is approximately 3:1 and the tumor can be associated with several congenital abnormalities, including hemihypertrophy, Beckwith–Wiedemann syndrome, familial colonic polyposis, cardiac and renal malformations, Noonan syndrome *(260)*, and glycogen storage disease type IA *(261–265)*. There is no known relationship with liver cirrhosis.

Clinically, a rapidly enlarging upper quadrant mass, vomiting, and/or fever are frequent presenting signs and symptoms. Serum alpha-fetoprotein is elevated in approximately 90% of patients. In infants and children with a primary liver tumor, low levels of AFP suggest the presence of either well-differentiated or immature hepatoblastoma or fibrolamellar hepatocellular

carcinoma. In occasional cases, HCG production may occur and may be sufficient to cause virilization *(266)*.

7.2. Macroscopic Pathology

Macroscopically, the tumor usually presents as a single, well-circumscribed, large mass up to 25 cm. The gross tumor appearance may be heterogeneous due to any combination of necrosis, hemorrhage, calcification, and cystic degeneration. The presence of a mesenchymal component in some tumors may also contribute to this variability.

7.3. Microscopic Pathology and Ancillary Studies

There are several histologic patterns that segregate into pure epithelial type and mixed epithelial–mesenchymal type. The epithelial type is further categorized based on the appearance of the cells into fetal, embryonal, small cell undifferentiated, or macrotrabecular patterns. These patterns may occur alone or in combination.

Fetal type cells bear a resemblance to normal fetal liver cells with granular cytoplasm, round to oval centrally placed nuclei and single small nucleoli. Mitoses are scant. The cytoplasm may contain fat and glycogen. They may assemble in irregular cords that are two cells in thickness and contain bile canaliculi and sinusoids *(265)*. Embryonal type cells are small and elongated with hyperchromatic nuclei and scant cytoplasm. Mitoses can easily be detected and foci of necrosis can also be present. The cells are arranged in ribbons, cords, or rosettes *(267)*. Fetal- and embryonal-type hepatoblastomas in particular commonly show foci of extramedullary hematopoiesis *(268)*. The small cell undifferentiated variant is comprised of small, round, and loosely arranged cells that are histologically similar to those of other pediatric "small blue round cell tumors" *(199, 208, 209, 269–271)*. Enlarged, bizarre cells may also occur and mucoid stroma can be associated with the small cell variant *(272)*. The macrotrabecular pattern differs in that this term refers to architecture, not cell appearance, and consists of thick columns, or trabeculae, of fetal or embryonal cells or of cells resembling those of typical HCC.

Mixed-type hepatoblastomas combine the epithelial elements listed above with a metasplastic mesenchymal component that characteristically has a spindled, undifferentiated appearance. Osteoid is also frequently present. Other components such as cartilage, bone, striated muscle, neural tissue, respiratory or intestinal type epithelial cells, and other mature tissues may occur in some tumors and this combination of tissues gives rise to what has been termed teratoid hepatoblastoma *(273)*.

Hepatoblastomas typically express AFP in epithelial cells, especially in fetal and embryonal variants. Other markers of hepatocellular phenotype, such as HepPar-1 *(108, 110, 274)* and glypican-3 *(275)*, are also expressed. Hepatocyte cytokeratins 8 and 18 are expressed; in addition, cytokeratin 7 expression may occur in small epithelial cells in association with albumin expression, suggesting a stem-like or bipotential cell population *(276)*. Fiegel et al. *(277)* examined a series of hepatoblastomas for expression of stem cell and hepatic or biliary lineage markers and concluded that a stem-like population of cells existed within duct-like structures in the tumors. Phenotypic plasticity may play a role in the development of mesenchymal components of these tumors, and this is reflected in immunophenotype. For example, HCG positivity can be detected in giant cells *(278)*, and vimentin is positive in anaplastic cells and osteoid. It should also be noted that the mesenchymal elements generally retain cytokeratin expression, which belies their epithelial origin. From a practical diagnostic perspective, such variability may present difficulties when a diagnosis must be rendered on a small sample such as a needle biopsy. Ramsay et al. *(279)* observed that such samples could focally express antigens such as CD99, CD56, desmin, or PGP9.5 that are usually associated with other pediatric neoplasms such as small round cell tumors.

Similar to HCC, hepatoblastomas may show β-catenin activation. Curia et al. *(280)* showed mutations in this gene in 19% of sporadic hepatoblastomas in their series, but also demonstrated nuclear accumulation of this protein in 67%, suggesting separate alterations in this pathway in individual cases. This group also found p53 mutations in 24% of cases, and evidence of microsatellite instability in 81% of tumors in their series. They were unable to associate these findings with specific histologic subtypes. A discrepancy between the low frequency of detectable β-catenin gene mutations and the ubiquitous accumulation of this protein was also observed in the study of Yamaoka et al. *(281)*.

Intranuclear accumulation of β-catenin was also observed in both pre- and post-treatment biopsies of hepatoblastomas in another study *(282)*. In contrast, aberrant cytoplasmic localization of the hepatocyte growth factor receptor Met, present in pretreatment biopsies, showed a decreased uptake following treatment in 85% of cases. This led the authors to suggest that Met might have a significant role to play in the pathogenesis of this tumor *(282)*.

Hepatoblastomas with embryonal and/or small cell components show significantly higher expression of the FOXG1 (human forkhead box G1) protein than do purely fetal–epithelial-type tumors *(283)*. This protein, which is one component of a large family of transcription factors with diverse actions *(284)*, may be associated with repression of TGFβ-1-induced p21 expression, and these authors suggested that it may contribute to the undifferentiated state in hepatoblastomas.

Delta-like protein (DLK/Pref-1) is a membrane protein expressed in normal hepatoblasts *(285)* and it has found recent use as a marker to define and isolate these progenitor cells *(286)*. Deszo et al. found expression of this marker in 100% of 31 hepatoblastomas by immunohistochemical staining and recommended it as a potential marker for these tumors. In the global microarray gene expression study of Luo et al. *(115)*, DLK was one of several genes with prominent increased expression in a subset of hepatoblastomas relative to HCC. Other overexpressed genes included mitogen-inducible gene 6 (Mig6) and TGFβ-1. IGF2 was also overexpressed in a subset of hepatoblastomas relative to HCC. In vitro studies support the concept that this can act as a growth factor for hepatoblastoma via the IGF-I receptor and PI3 kinase, and this pathway may be a good target for molecular therapy *(287)*.

7.4. Staging and Prognosis

In contrast to staging of HCC, staging of hepatoblastoma incorporates the results of surgery. Postsurgical Stage I disease implies complete tumor resection, Stage II includes those patients with postsurgical microscopic residual disease, tumor spill, or rupture at surgery. Stage III patients have unresectable tumor or gross residual tumor or positive lymph nodes and Stage IV is defined by the presence of distant metastases. The U.S. National Cancer Institute estimates the present cure rate at over 90% for Stages I and II, 60% for Stage III, and approximately 20% for Stage IV. Austin et al. *(288)* recently reviewed the United Network for Organ Sharing (UNOS) database and found that liver transplantation for unresectable hepatoblastomas was associated with 66% actuarial 10-year survival, with 54% of deaths related to recurrent or metastatic disease.

In addition to stage, a low serum alpha-fetoprotein level is viewed as a poor prognostic indicator. In the series of D'Antiga et al. *(289)*, patients with multifocal hepatoblastoma in association with AFP <100 ng/ml survived only with transplantation. De Ioris et al. *(290)*. found 9 of 15 patients with serum AFP below this level and with evaluable histology had a small cell undifferentiated epithelial component, and the overall 2-year survival in their patients with low AFP level was 24%.

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