Molecular Pathology of Liver Tumors

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Hepatocellular Adenoma

Definition

- Hepatocellular adenoma (HCA) is a benign liver neoplasm of hepatocellular differentiation.

Epidemiology

- The incidence is 1–4/100,000 people in Europe and North America, but lower in Asia.
- Most cases (85%) occur in young women.

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Etiology

- Exposure to estrogenic (e.g., contraception >2 years) or androgenic (e.g., bodybuilding) steroids including Klinefelter syndrome or steroid hormone-producing lesions.
- Androgen therapy of Fanconi anemia or acquired aplastic anemia.
- Metabolic disease (e.g., maturity-onset diabetes of the young (MODY) type III, hereditary HNF1α mutation, glycogenosis type 1 (von Gierke disease), or type 3 Forbes disease).
- Familial adenomatosis polyposis coli (adenomatosis polyposis coli (APC) mutation).
- β-thalassemia with iron overload.

Clinical

- Symptoms develop in 90% of patients and include mild chronic or acute abdominal pain due to intratumoral or intraperitoneal hemorrhage.
- Adenoma may present with elevated levels of liver enzymes, or as incidental finding of a liver mass by imaging techniques.
- Intraperitoneal hemorrhage may occur in up to 20–25%; the risk is increased in HCA >5 cm.
- Malignant transformation is rare, but has been repeatedly observed in tumors >6 cm.
- Risk for transformation varies between HCA subtypes (see below) and etiology (higher in glycogenoses and drug anabolic abuse). In case of drug-induced HCA, discontinuation of the drug may result in spontaneous regression.

Histopathology

- HCA shows a solid, clonal growth pattern consisting of liver cell plates, which are up to two cells wide and are arranged in sheets and cords with compression of the sinusoids. HCA typically lacks a capsule (↔progressed hepatocellular carcinoma (HCC)).
- The neoplastic hepatocytes of HCA are usually uniform with regular nuclear:cytoplasmic ratio (↔HCC).
- Cell size is typically mildly increased compared to the normal hepatocytes of the surrounding liver.
- Cytoplasm may be normal or paler compared to normal hepatocytes due to glycogen or fat storage.
- HCA may show some nuclear atypia as well as pseudogland formation with bile plugs within canaliculi (especially in setting of anabolic steroids or glycogenoses), which requires careful differentiation from HCC (see below).
- Hepatocytes of HCA are usually surrounded by a regular reticulin framework.
- HCA (except inflammatory HCA, see below) lack ductular proliferations and portal tracts, but preexisting portal tracts may be entrapped in the periphery of the lesion.
- HCA is supplied by (a few) solitary arteries (e.g., unaccompanied by bile ducts).
- Areas of infarction, hemorrhage, or regression indicate an increased risk of spontaneous rupture.

Differential Diagnosis

- Capillarization of sinusoids (CD34 staining), atypia, pseudogland formation, and mitosis should raise the differential diagnosis of highly differentiated HCC (immunohistochemistry (IHC): glypican 3 (GPC3), heat shock protein 70 (HSP70), glutamine synthetase (GS) positive).
- Presence of ductules and fibrosis require separation of inflammatory HCA from focal nodular hyperplasia (IHC: map-like GS expression) and macroregenerative nodule (comparison to surrounding liver tissue).
- Epithelioid angiomyolipoma may superficially resemble HCA, but is HMB45 and Melan A positive.

Molecular Pathology

- HCAs are heterogeneous, clonal lesions that in up to 20% of cases show a few chromosomal alterations.

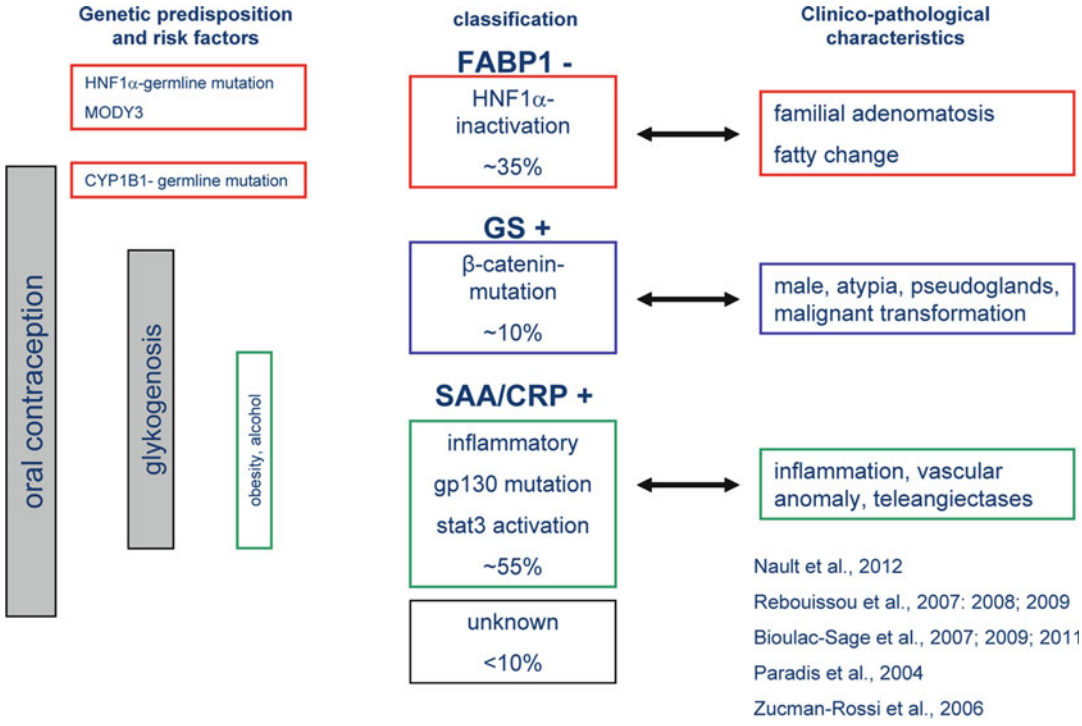


Fig. 3.1 Classification of HCA by genotype and phenotype (material adapted from Rebouissou et al., 2008). *GS* glutamine synthetase; *SAA/CRP* serum amyloid A/c-reacting protein; *L-FABP1* liver-fatty acid binding protein1; *HNF1A* hepatocyte nuclear factor 1-alpha; *CYP1B1* cytochrome P450 family 1, subfamily B, polypeptide 1.

- Genomic aberrations include gains at 1p, 1q, 7p, 11q, 17q, and 20, but the detection of more than two alterations in one tumor favors a different diagnosis (e.g., early HCC).
- The identification of the transcription factor 1 (TCF1) encoded hepatocyte nuclear factor 1α (HNF1α) as a frequently inactivated gene in HCA was the first step to our current understanding of the pathogenesis of HCA, later on complemented by the identification of β-catenin mutations in some HCA and molecular definition of inflammatory HCA.
- Typical histological changes and detection of mutations or their consequences are the basis for our current HCA subtyping. The subtypes described below differ with respect to clinical, genomic, pathological, and radiological features.
- Although epigenetic changes (e.g., aberrant p14(ARF)/p16(INK4a) methylation) have been reported in about 20% of HCA, these features are not included into the current HCA classification that has been independently validated. The genetic predisposition, risk factors, diagnostic marker, and clinicopathological characteristics of HCA subtypes are summarized in Fig. 3.1.

HNF1α-Inactivated Hepatocellular Adenoma

- HNF1α is a transcription factor involved in hepatocellular differentiation.
- Mutational inactivation of HNF1α is found in 35% of HCA.
- 90% of HNF1α mutations are somatic, the remaining ones are inherited.
- Heterozygous HNF1α germline mutations are responsible for maturity-onset diabetes of the

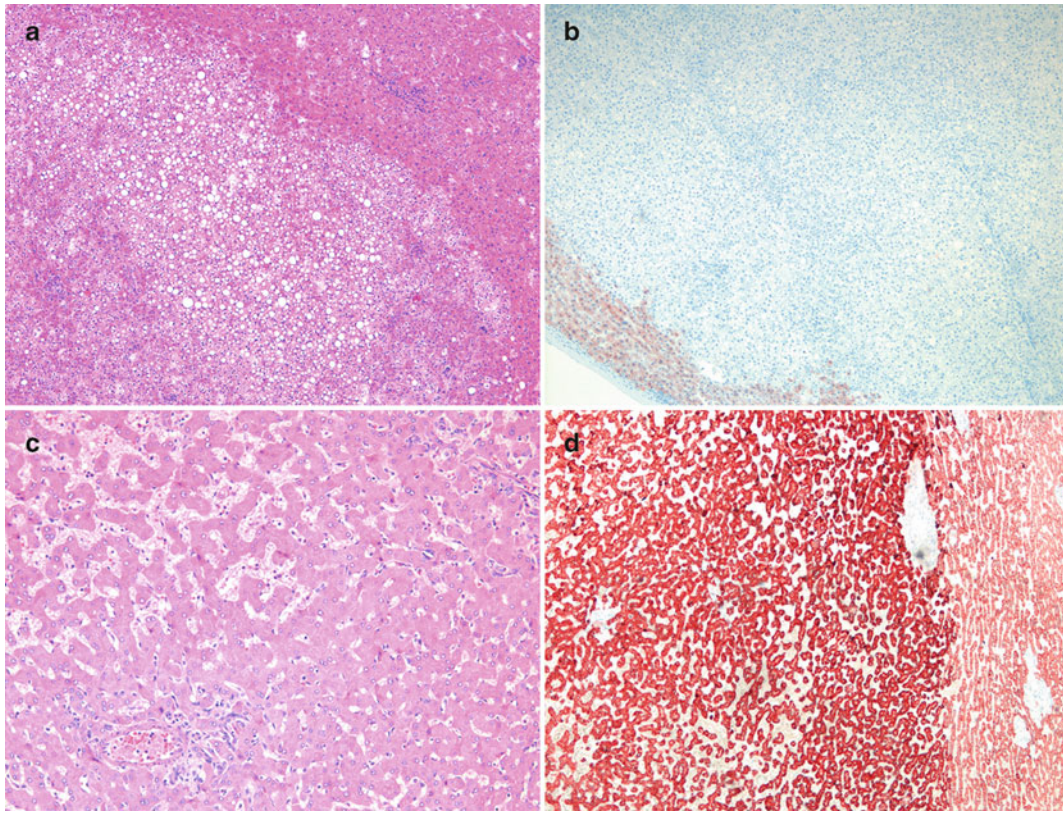


Fig. 3.2 (A+B) HNF1α-inactivated HCA (original magnification 40-fold). (**a**) Note the prominent fatty change and expansive growth. (**b**) The tumor cells show loss of FAPB1 expression. (C+D) Inflammatory HCA. (**c**) Focal sinusoidal dilatation and presence of ductular structures within the tumor (original magnification 100-fold). (**d**) Strong SAA expression of tumor cells in inflammatory HCA (original magnification 40-fold)

young type 3 (MODY3); an autosomal dominant type of diabetes.

- HCA in MODY3 patients carry a second somatic HNF1α-inactivating mutation.
- Germline mutations of CYP1B1 may predispose to the development of somatic HNF1α mutation.
- Histologically, HNF1α-inactivated HCA show severe steatosis, but lack significant inflammatory infiltrates and atypia.
- Liver-fatty acid binding protein (L-FABP) represents an HNF1α target gene. Thus lack of immunohistological L-FABP expression represents as a diagnostic biomarker for HNF1α-inactivated HCAs with the surrounding liver tissue serving as an internal positive control (Fig. 3.2a, b). The down-regulation of L-FABP may contribute to the steatotic phenotype through impaired fatty acid trafficking.
- HNF1α-inactivated HCA occur almost exclusively in women and carry no malignant transformation risk even in the case of adenomatosis.

Inflammatory Hepatocellular Adenoma

- Inflammatory HCA has formerly been termed telangiectatic adenoma or telangiectatic focal nodular hyperplasia; the latter term is now obsolete since the clonal nature of these lesions has been proven. It constitutes the largest subgroup of HCA (55%).
- Histologically inflammatory HCA is characterized by focal or diffuse inflammation and

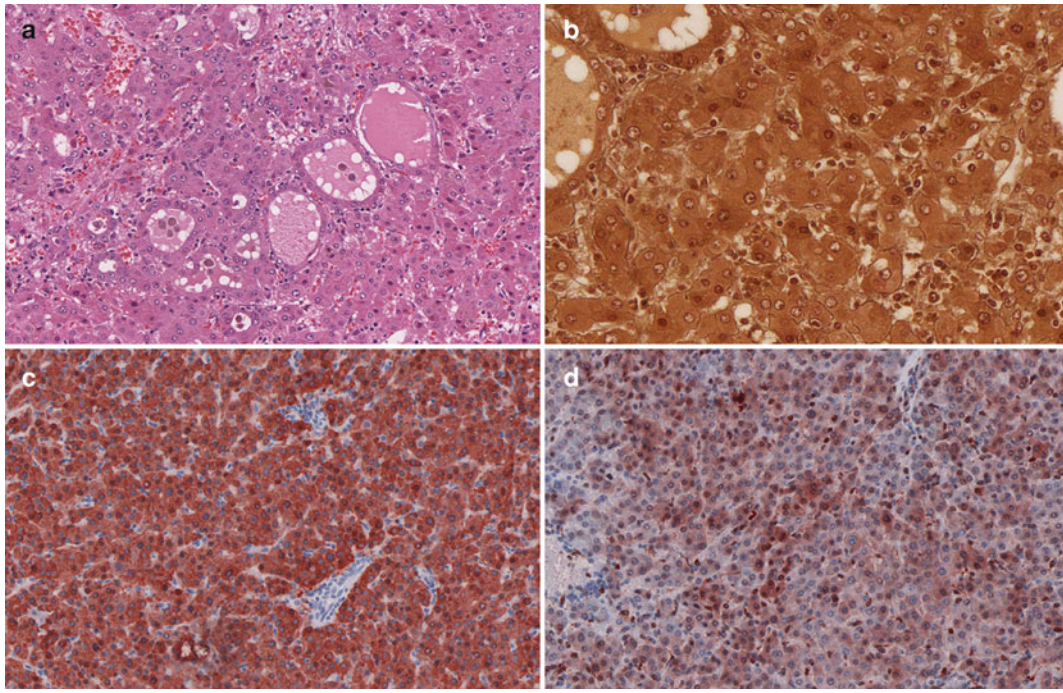


Fig. 3.3 Atypical adenoma detected in a 25-year-old female patient without know liver disease. (**a**) The highly differentiated hepatocellular tumor shows areas of pseudoglandular differentiation (H&E, original magnification 200-fold). (**b**) The reticulin network is lost (modified Gomori stain, original magnification 400-fold). (**c**) Overexpression of glutamine synthetase and (d) HSP70 (original magnification 200-fold)

prominent vascular changes (sinusoidal dilatation, congestion, and thick-walled arteries). A ductular reaction along thin fibrotic septa can be found in inflammatory HCA.

- Increased expression of inflammatory-associated proteins (e.g., serum amyloid A (SAA) and C-reacting protein (CRP)) can be used as immunohistological biomarkers for the differentiation of this subtype (Fig. 3.2c, d).
- Signs of systemic inflammation (e.g., elevated CRP level in serum, increased erythrocyte sedimentation rate) can be found in association with inflammatory HCA.
- About 60% of inflammatory HCA contain mutations in gp130, a coreceptor of IL-6 signaling leading to constitutive activation of IL-6/Stat3 signaling.
- β-catenin and gp130 mutations may occur together indicating that these HCA also carry an increased risk for malignant transformation.
- Inflammatory HCA occurs more likely in women and is associated with obesity and fatty liver disease.

β-Catenin-Activated Hepatocellular Adenoma (Atypical Adenoma)

- Activating mutations of β-catenin are present in about 10% of HCA.
- Glutamine synthetase (GS) is a β-catenin target gene.
- Diffuse and strong immunohistological GS overexpression is a marker of β-catenin-activated HCA, whereas nuclear β-catenin staining is less sensitive and may be seen only in the minority of tumor cell nuclei (Fig. 3.3).
- In equivocal cases β-catenin inactivation can be demonstrated through molecular pathological techniques (e.g., demonstration of

β-catenin mutations using DNA from formalin-fixed, paraffin-embedded tissues).
- β-catenin-activated HCA are preferentially associated with male sex, administration of androgenic anabolic steroids, or glycogenoses, and carry an increased risk for malignant transformation (~50%).
- Distinguishing β-catenin-activated HCA from well-differentiated HCC may be difficult and in some cases arbitrary, since both lesions show pseudogland formation and atypia, whereas this subtype is not featured by steatosis and significant inflammation.
- β-catenin-activated HCC is a clear indication for resection.

Unclassified Hepatocellular Adenoma

- About 10% of HCA have neither HNF1α-inactivation, β-catenin-activation, nor gp130 activation and remain molecularly unclassified so far.
- These HCA do not carry an increased risk for malignant transformation.

Diagnosis

- Radiological diagnosis is difficult due to the variable features (especially vascularization) of HCA, but may be possible for HNF1α-inactivated and inflammatory HCA using magnetic resonance imaging.
- In equivocal cases liver biopsy will confirm diagnosis and allow reliable subtyping.

Prognosis and Predictive Factors

- The risk for rupture and intraperitoneal hemorrhage increases when the tumor diameter exceeds 5 cm and may result in hemorrhagic shock and death.
- Pregnancy is considered a risk factor for rupture.
- HCA >6 cm are at risk for malignant transformation, especially when detected in men with metabolic syndrome.
- HCA >5 cm in diameter should be treated by surgery or local intervention (e.g., embolization, radiofrequency ablation) independent of the subtype due to increased risk of rupture.
- Detection of β-catenin-activated HCA is important due to the increased risk of malignant transformation.
- In the case of adenomatosis liver transplantation can be a therapeutic option.

Hepatocellular Carcinoma

Definition

- Malignant primary liver tumor with hepatocellular differentiation.

Epidemiology

- HCC is the sixth most frequent cancer worldwide and the third most frequent cause of cancer-related death.
- HCC accounts for more than 90% of primary liver cancer with approximately 700,000 new case per year worldwide.
- Depending on the region, incidence ranges from <5/100,000 (e.g., in Northern Europe) to more than 15/100,000 (e.g., in the Far East). In all areas males have a significant higher prevalence than females ranging from 2:1 to 4:1.

Etiology

- In more that 80% of all cases a defined etiology causes chronic liver disease, which represents the basis for the development of cirrhosis and HCC. Most prevalent are infections with hepatitis B virus (HBV) and hepatitis C virus (HCV).
- In addition, chronic alcohol abuse, ingestion of mycotoxins (e.g., aflatoxin B1), as well as hereditary metabolic diseases (e.g., haemochromatosis, glycogen storage disease, tyrosinemia)

carry a high risk for the development of HCC while Wilsons disease, α1-AT deficiency, and exposure to chemicals (e.g., vinyl chloride) are low risk factors.

Clinical

- Symptoms either by tumor itself or by advanced stage of underlying chronic liver disease: abdominal pain, weight loss, nausea, ascites, jaundice, or (hepato-)splenomegaly.
- Raised α-fetoprotein (AFP) levels >400 ng/mL or continuous rising AFP >100 ng/mL are indicative of HCC, but AFP elevation is only found in less than 50% of patients and especially early HCCs (see below) are frequently AFP-negative.
- Other serological markers, including lectin-bound AFP, des-gamma carboxythrombin, golgi protein 73, are not universally accepted.

Histopathology

Classical Hepatocellular Carcinoma

- HCC consists of cells that cytologically more or less resemble hepatocytes and that are separated by a sparse stroma consisting mainly of sinusoid-like blood spaces. These are lined by a endothelial cell layer that, in contrast to the specialized normal liver sinusoids, shows a capillarization as determined by immunohistological expression of CD34, factor VIII-related antigen or Annexin A2.
- Progressed HCC is supplied by newly formed unpaired arteries (e.g., not accompanied by bile ducts) and lacks portal triads, but residual portal tracts may be found entrapped at the periphery of a nodule, whereas the blood supply of early HCCs mainly derives from the portal–venous flow.
- Immunohistologically HCC typically (90% of cases) express carbamoyl phosphatase synthetase 1 (as detected by the Hepar1 antibody), but especially poorly differentiated HCCs may be negative.
- A canalicular expression pattern is found for antibodies against polyclonal CEA (pCEA) and CD10. Other markers that support HCC diagnosis include AFP, fibrinogen, cytokeratin (CK) 8 and CK18, whereas CK7 and CK19 are only positive in a fraction of HCCs that potentially evolve from hepatic progenitor cells (see below).
- HCCs are distinguished from benign hepatocellular lesions through both architectural and cytological atypia, although the differences may be subtle in early HCC.
- Architectural atypia includes irregular trabecular growth pattern (more than two-cells-wide cords separated by capillarized sinusoids), pseudoglandular/acinar growth (pseudogland formed due to abnormal and dilated bile canaliculi between tumor cells), and solid growth. These pattern are frequently admixed, especially in less differentiated tumors.
- Cytologically HCC may be hepatoid, pleomorphic, or sarcomatoid (spindle cells), and may show a clear cytoplasm that requires differentiation from other clear cell tumors (e.g., kidney).
- Fatty change is frequently seen, especially in small tumors. Bile plugs may be seen in dilated canaliculi or in areas with pseudoglandular differentiation. Cytoplasmic inclusions are frequently seen and include Mallory–Denk bodies (aggregates of intermediate filaments), globoid bodies (α1-antitrypsin), and pale bodies (fibrinogen).
- Nodule-in-nodule growth (nodule of less differentiated HCC surrounded by a better differentiated component) is indicative of HCC.
- Histological grading is based on tumor differentiation: well-differentiated (<3 cm, mild nuclear atypia, thin trabeculae), moderately differentiated (more than three-cells-wide trabeculae, round nuclei with distinct nucleoli), poorly differentiated (solid growth, nuclear pleomorphism), and undifferentiated (spindle or round shaped tumor cells with sparse cytoplasm).

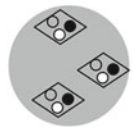
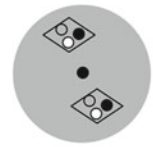
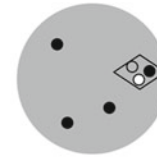
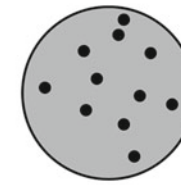
Special Hepatocellular Carcinoma Subtypes

- Fibrolamellar HCC.
  - Fibrolamellar carcinoma (FLC) differs from classical HCC by its clinical, histological, and molecular characteristics.
  - FLC accounts for 1% of all HCC.
  - It occurs mostly in patients younger than 35 years (85% of cases) and women tend to be more frequently affected than men.
  - FLC arises usually in noncirrhotic livers, but etiology and risk factors are not known.
  - Since there is no relation to chronic viral hepatitis, FLC are relatively more frequent in Europe and Asia compared to Asia and Africa.
  - Tumor cells of FLC are large cells with oncocytic cytoplasm (due to innumerable mitochondria), large vesicular nuclei, and large nucleoli. They are arranged in cords separated by (parallel) bands of lamellar fibrotic tissue. As in classical HCC pseudogland formation, pale and Mallory–Denk bodies can be found.
  - CK7 immunostaining is typically seen in FLC (↔ most classical HCCs).
  - There is no significant difference with respect to prognosis between FLC and classical HCC without concomitant cirrhosis, but its prognosis is significantly better than for HCC that developed in a cirrhotic liver.
  - Additional special HCC subtypes include scirrhous HCC (tumor trabeculae separated by marked fibrosis along the sinusoid-like blood spaces), undifferentiated HCC (primary liver tumor with epithelial differentiation lacking further lineage differentiation), lymphoepithelioma-like carcinoma (pleomorphic tumor cells intermixed with dense lymphocytic infiltrates, potentially associated with EBV infection), and sarcomatoid HCC (partially or fully comprised of malignant spindle cells).

Differential Diagnosis

- Well-differentiated HCC have to be differentiated from preneoplastic precursor lesions (so-called dysplastic nodules), HCA, and focal nodular hyperplasia.
- Dysplastic nodules (DN) are usually detected in cirrhotic livers.
- DNs show a clonal growth and mild cytological and structural atypia, but lack definite signs of malignancy, like severe trabecular or cellular atypia and interstitial or vascular invasion.
- They are subdivided into low grade dysplastic nodules (LGDN) and high grade dysplastic nodules (HGDN) depending on the degree of atypia.
- Compared to LGDN cell density and atypia is increased in HGDN (due to small cell change and increased nuclear to cytoplasmic ratio resulting in a nuclear crowding).
- Blood supply in LGDN is usually via the portal vessels, but there is a steady increase of newly formed unpaired arteriolar vessels from LGDN over HGDN to early HCC (Table 3.1).
- Early HCCs show more than twice increased cellular density compared to the surrounding liver tissue, trabecular disarray, pseudogland formation, unpaired arteries, and typically interstitial invasion. Portal tracts may be entrapped at the tumor periphery.
- The process of hepatocarcinogenesis represents a (morphological) continuum and the diagnostic features of a given lesion may not be present in the setting of a liver biopsy.
- Biopsy diagnosis of undefined nodules or minute biopsy specimen may need for additional immunohistological evaluation of transformation associated markers. Such a marker panels has been established and includes glypican 3 (GPC3), glutamine synthetase (GS), and heat shock protein 70 (HSP70).
- The oncofetal protein GPC3 is frequently reactivated in early HCCs. Immunohistological expression of GPC (≥10% of tumor cells) has a reported sensitivity of up to 77% and a specificity of up to 96% for the diagnosis of small HCCs.
- GS is expressed by hepatocytes of the pericentral acinus and in a periseptal localization in fibrosis/cirrhosis. A strong and diffuse staining (≥10% of tumor cells) without a zonal restriction is considered positive for the diagnosis of early HCC. Positive expression of HSP70 (≥10% of tumor cells) was reported in 80% of HCCs, but only occasionally in DN.
- Applying GPC3, GS, and HSP70 as a three-marker panel significantly increased accuracy

Table 3.1 Classification of small nodular lesions in cirrhotic liver according to International Consensus Group for Hepatocellular Neoplasia (2009)

Feature	Low-grade Dysplastic Nodule (LGDN)	High-grade Dysplastic Nodule (HGDN)	well-differentiated HCC	Moderately differentiated HCC
Anatomical change				
Gross appearance			Vaguely nodular	Distinctly nodular
Stromal invasion	(-)	(-)	+/-	+/-
Contrast-enhanced imaging:				
Arterial suppy	Iso-/ hypovascular	Iso-/ hypovascular	Iso-/hypo-/rarely hypervascular	hypervascular
Portal vein supply	+	+	+	-
Clinico-pathological	Premalignant lesion		Early HCC	Progressed HCC

 Intratumoral portal tract ● unpaired artery  Fibrous pseudocapsule

of HCC diagnosis (if at least two of the three markers were positive), particularly when diagnostic interstitial invasion was not seen in a biopsy specimen (Fig. 3.4).

- Further helpful markers include β-catenin, vascular markers (e.g., CD34, CD31, Annexin A2) to detect abnormal capillarization of the liver sinusoids, and CK7 to separate a ductular reaction from an early stroma invasion.

Molecular Pathology

Genomic Instability

- In general, HCC is a chromosomal instable cancer accumulating high numbers of macro- and microimbalances, in part responsible for the activation of oncogenes or inactivation of tumor suppressor genes. The most prominent amplifications of genomic material are present in 1q (57%), 8q (47%), 6p (22%), and 17q (22%), while losses are most prevalent in 8p (38%), 16q (36%), 4q (34%), 17p (32%), and 13q (26%).
- Distinct chromosomal imbalances (gains of 1q21–23 and 8q22–24) precede malignant transformation as they are detectable in a significant number of premalignant lesions.
- Specific chromosomal macroimbalances (e.g., gains of 1q32.1 and losses of 4q21.2–32.33) discriminate between HBV- and HCV-associated HCCs; however, the molecular reason for this observation is unknown, so far.

Epigenetic Changes

- In HCC global DNA hypomethylation has been associated with activation of oncogenes, loss of imprinting, and genomic instability, while hypermethylation of CpG islands located especially in gene regulatory sequences resulted in transcriptional silencing.

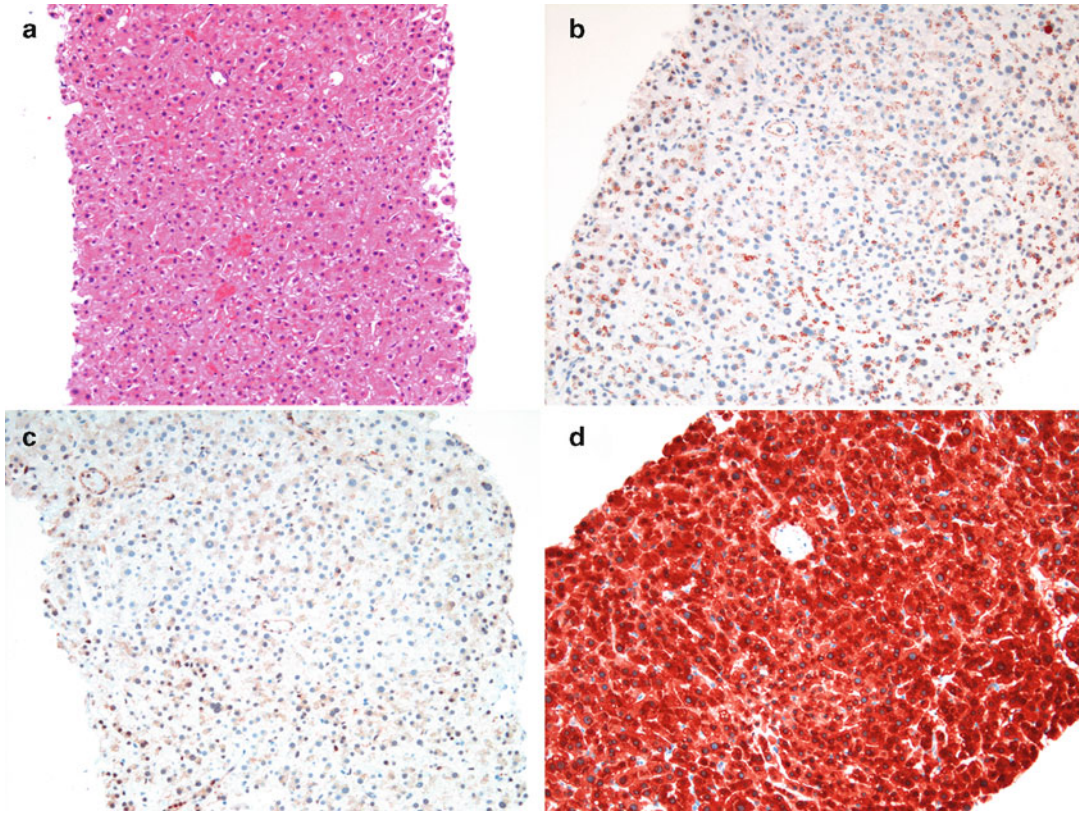


Fig. 3.4 (**a**) Early well-differentiated HCC showing areas of nuclear crowding and mild trabecular disarray. Additionally, a solitary artery can be seen. (**b**) GPC3 is expressed by the majority of tumor cells. (**c**) Predominantly nuclear HSP70 expression in areas with nuclear crowding. (d) Homogeneous overexpression of glutamine synthetase (original magnification 100-fold)

- Polycomb repressive complexes (PRC) target genes are prone to promoter hypermethylation in human HCC, which might be linked to a stem cell like chromatin pattern through de novo methylation in cancer.
- Genomic hypomethylation correlated with genomic instability in HCC while CpG promoter methylation was associated with poor prognosis.
- Methylation changes may occur early in the process of cancer development and CpG island hypermethylation of regulatory regions of tumor-relevant genes is a frequent event accumulating in multistep hepatocarcinogenesis.
- Hypermethylation of CpG islands is associated with the dysregulation of signaling pathway constituents (e.g., RASSF1A), adhesion molecules (e.g., E-cadherin), and cell cycle regulators (e.g., p16/CDKN2/INK4A).

Noncoding RNA/MicroRNA

- miRNA bind complementary sequences in the 3′ end of mRNAs and directly affect promoter activity through binding and or modifying DNA methylation. Therefore miRNAs represent effective posttranscriptional regulators of mammalian gene bioactivity.
- Different stages of hepatocarcinogenesis as well as liver tissues with HBV- or HCV-infection can be differentiated from each other based on their miRNA fingerprints.
- miRNAs such as miR-122a and miR-223 have recurrently been identified by independent screening approaches.

- miR-125b, miR-139, miR-181, and miR-221 are regulators of tumor-relevant proteins and processes in hepatocarcinogenesis.

High and Low Frequent Mutations in Specific Genes

- High frequency mutations in the cell cycle regulator and tumor suppressor gene TP53 can be found in 10–28% of all HCCs. In regions with high aflatoxin B1 exposure, mutations in codon 249 of TP53 can be detected in up to 48% of all HCC cases.
- Stabilizing point mutations and deletions in exon 3 of CTNNB1 (coding for the transcriptional regulator β-catenin) are present in 13–42% of all HCCs. Inactivation mutations lead to an accumulation and nuclear translocation of β-catenin, associated with the transcriptional activation of specific target genes relevant for mitosis and tumor development.
- Loss of heterozygosity at the IGF2R locus (coding for the growth factor receptor IGF2R) has been published for several HCCs and its premalignant lesions, whereas inactivating mutations of the second allele have been described in up to 25% of all cases. Additionally, missense mutations in the extracytoplasmic domain of IGF2R efficiently disrupt receptor/ligand interaction, which may then be followed by increased ligand bioavailability.
- Low frequency mutations (<20% of cases) have been described in AXIN1/2, TCF1/HNFα, PIK3CA, KRAS, p16/CDK2/INK4A, SMAD2/4, RB1, PTEN, ARID1A, ARID1B, and ARID2.

Frequent Aberrant Activation of Signaling Pathways

- High level expression of the insulin-like growth factor (IGF) 2 in up to 40% of all HCCs leads to an activation of the membrane-bound tyrosine kinase receptor IGF1R. Overexpression of IGF2 is predominantly mediated by epigenetic dysregulation of IGF2 gene promoters, IGF-binding proteins, and the presence of IGF2R which directs IGFs to proteasomal degradation. Activation of the IGF-signaling axis supports HCC proliferation, antiapoptosis, and migration.
- Mutations in wingless/β-catenin signaling axis components AXIN1/2 and CTNNB1 increase the nuclear enrichment of β-catenin in up to 40% of all cases. In addition, upregulation of different pathway ligands (e.g., Wnt3/4/5a), receptors (e.g., FZD3/6/7), and pathway modifiers (e.g., PIN1, HDPR1, DKK1) further supports nuclear accumulation of β-catenin. Activation of the Wnt/β-catenin pathway is associated with increased tumor growth and progression.
- The role of TGFβ signaling is controversially discussed, because elevated ligand levels have been detected in serum and urine of HCC patients, while most studies document a reduction of the respective receptors. Moreover, mutations in activating SMADs (SMAD2/4) and overexpression of the antagonistic SMAD7 have been demonstrated. TGFβ signaling may support bifunctional effects; although TGFβ has been suggested to inhibit hepatocyte proliferation, a proinvasive role has been shown, if resistance to its growth inhibitory effects occurs.
- HGF may show higher levels in HCC patients; however, it is not expressed by tumor cells themselves but by stellate cells and myofibroblasts. Its receptor, the tyrosine kinase c-MET, is activated in most HCCs (70%) based on genomic alterations, hypoxia, and growth factor-dependent stimulation. Activation of the HGF/c-MET signaling pathway supports tumor growth and tumor cell invasiveness.
- Several TGFα/EGF ligand family members are highly expressed in up to 80% of HCCs (e.g., TGFα, heparin-binding EGF) and may stimulate the group of EGF tyrosine kinase receptors. Furthermore, overexpression of these receptors (EGFR/HER1, HER2, HER3, and HER4) has been demonstrated in most HCCs (e.g., EGFR/HER1 in up to 70%). Stimulation of the TGFα/EGF signaling axis supports HCC cell proliferation
- Constitutive activation of growth factor pathways occurs at different levels: increased ligand and/or receptor bioavailability, mutational inactivation or constitutive activation of pathway constituents, and aberrant activity/expression of cytoplasmic downstream effectors.

Diagnosis

- Besides serum AFP level noninvasive HCC diagnosis is based on contrast-enhanced (CE) imaging studies.
- The standard techniques are CE computer tomography (CT), magnetic resonance tomography (MRT), and sonography.
- Typical findings in progressed HCCs are hypervascularity during arterial imaging phase followed by a rapid washout during portal venous phase.
- Early HCC cannot reliably diagnosed using these imaging techniques, since solitary arteries responsible for the blood flow characteristics of progressed HCC are rare in these lesions.
- New techniques like dynamic CE sonography with Kupffer phase imaging or MRT using hepatocyte-specific contrast media like gadolinium-ethoxybenzyl diethylenetriamine pentaacetic acid (Gd-EOB) may improve the noninvasive differentiation of early HCC from dysplastic nodules.
- Liver biopsy is the diagnostic technique with highest specificity and high sensitivity and is recommended in all suspicious lesions (especially less than 2 cm) without diagnostic features.
- Needle-tract seeding is a specific complication following biopsy of HCC and has been observed in 2.7% of cases.
- Since the risk for needle-tract implantation increases concurrent with the number of needle passes the use of guiding needles may significantly reduce this complication and needle track resection during surgery has been recommended.

Prognosis and Predictive Factors

- The overall 5-year survival rate of HCC patients with symptomatic HCC is less than 5%.
- Portal vein thrombosis is associated with worse outcome.
- The recurrence rate within 5 years after curative resection (e.g., partial hepatectomy) is 70% and 5-year survival after resection is 25–74%.
- Liver transplantation results in a 5-year survival rate of 75% in selected patients (e.g. (extended) Milan criteria).
- Local ablative treatment (e.g., transarterial chemoembolization, radiofrequency ablation, ethanol injection) can be used for local tumor control and bridging for liver transplantation.
- Molecular classification of HCC (predominantly based on genomic and transcriptomic analyses) revealed genetic signatures that correlate with differentiation, tumor size, prognosis, vascular invasion, and metastatic potential.
- Expression of e.g., FAS, AFP, Aurora kinase B, and the mitotic index as well as TP53 mutation and a progenitor cell phenotype (e.g., CK19) are known negative predictive factors.

Intrahepatic Cholangiocarcinoma

Definition

- Primary malignant epithelial liver tumor with biliary differentiation. Presumed to arise from epithelium of intrahepatic bile ducts (proximal of bile ducts of second order; more distal tumors (Klatskin tumors) are considered separately with extrahepatic cholangiocarcinoma).

Epidemiology

- Second most frequent malignant liver tumor (5–15% but variable depending on geographic region) but much less frequent compared to HCC.
- Highest incidence in Southeast Asia (liver fluke infestation); age-standardized incidence in Thailand: 88/100,000 males; 37/100,000 females.
- In US higher incidence in African–American population.
- Usually older patients, slight male predominance.
- Incidence increasing, even in nonendemic regions.

Etiology

- In most cases unknown.
- Defined etiologies for more central/hilar types are liver fluke infestation (primarily opisthorchiasis and clonorchiasis), hepatolithiasis (7%; about 50% of ICC associated with hepatolithiasis), primary sclerosing cholangitis (5–15%; highest incidence in Northern Europe), and biliary tree malformations.
- Defined etiologies for peripheral type are similar to HCC and include all causes of nonbiliary cirrhosis, especially viral etiology (HBV, HCV (1,000-fold increased risk in HCV cirrhosis in Japan)).

Clinical

- Nonspecific and depend on location and stage of disease as well as secondary consequences (e.g., cholangitis). General symptoms of malignant tumor disease may be present.
- Symptoms of preexisting/predisposing disease may dominate and mask tumor-related symptoms (e.g., PSC).
- Tumor-dependent hepatomegaly, portal hypertension, ascites are rarely or never present.
- Peripheral mass-forming tumors may grow to large size undetected; tumors close to the hilum may exhibit signs of biliary obstruction.

Histopathology

- Almost exclusively adenocarcinomas.
- Histologically no definitive distinction from other carcinomas of the pancreatobiliary system possible.
- Mainly tubular growth pattern; but basically all adenocarcinoma variants may occur, although at very low frequency; not infrequently tumors arising from the large bile ducts may show an intraductal papillary component; tumor cell anaplasia and sarcomatoid dedifferentiation may occur.
- Tendency to invade along portal tracts, infiltrate lymphatic vessels and perineural sheets (of large portal tracts) (preferentially periductal infiltrating or mixed types).
- Premalignant lesions identified
  - Biliary intraepithelial neoplasia (BilIN (1–3)) preferentially for periductal infiltrating type.
  - Intraductal papillary neoplasia of the bile duct (IPN-B; rare) for intraductal growing type (pancreatobiliary, intestinal, gastric, and oncocytic types).
  - Mucinous cystic neoplasia (MCN; rare; only females) for hepatobiliary cystadenocarcinoma.
- Grading follows standard UICC criteria.

Differential Diagnosis

- Differential diagnosis is aided by immunohistology for pancreatobiliary markers (e.g., CK7, CK19, Ca19-9) and markers for metastasis differentiation in question; ICC cannot be safely distinguished immunohistologically from other carcinomas of the pancreatobiliary system.
- Differential diagnostic problems may include:
  - Benign fibroinflammatory lesions in case of highly differentiated tubular adenocarcinoma (histology, proliferation marker).
  - Mixed hepatocellular–cholangiocarcinoma (for peripheral, mass-forming tumor) demonstration of hepatocellular differentiation (histology; e.g., Hepar1).
  - Metastases of extrahepatic adenocarcinomas.
  - Benign biliary lesions: bile duct adenoma, biliary adenofibroma, von Meyenburg complex, biliary cysts.
  - Premalignant lesions (BilIN, IPN-B, MCN) have to be evaluated meticulously for invasive growth.
- Cytological or biopsy diagnosis in PSC (dominant strictures) is difficult with a sensitivity of about 60–70%.

Molecular Pathology

- The value of most molecular analyses is restricted by the lack of consideration of the different cholangiocarcinoma topographies and types.

- Multiple pathogenetically relevant changes have been identified.
- Chronic inflammation is thought to contribute to cholangiocarcinogenesis; stroma cells as well as cholangiocytes may secrete cytokines (IL-6, IL-8, TGFß, TNFα). Cytokines may induce iNOS leading to increased formation of reactive oxygen species.
- Autocrine IL-6-mediated stimulation leading to enhanced tumor cell survival and promoter methylation of several oncogenes; activated JAK/STAT signaling was correlated with resistance to apoptosis.
- Resistance of cholangiocarcinoma cells to TGFß-mediated growth inhibition (by TGFßR- or SMAD4-mutations) may promote fibrous tissue deposition, neoangiogenesis (via VEGF), and tumor progression.
- EGFR-activation correlated with tumor recurrence and poorer prognosis; also HER2 expression is described to correlate to ICC progression; intracellular mechanisms may involve activation of RAS-RAF-MAPK or COX2; also activation of the HGF-MET axis has been described.
- Dysregulation of wnt-associated signaling (loss of membranous E-cadherin and ß-catenin expression) associated with invasiveness, poor differentiation, and potentially neoangiogenesis (via VEGF).
- Telomerase activation is present in almost all cholangiocarcinomas.
- Activation of the inducible COX2 is frequent in cholangiocarcinoma and has been linked to stimulation of proliferation in vitro and may reduce TRAIL- and FAS-mediated apoptosis.
- Antiapoptotic proteins, such as BCL2 and MCL1 can be highly expressed in cholangiocarcinoma cells; MCL1 overexpression may be a consequence of miR-29 downregulation; furthermore some data suggest NOS-, Notch-, and COX2-mediated antiapoptotic effects.
- Constitutive activation of RAS-RAF-MAPK signaling has been reported at varying frequency. KRAS mutations (20–50%; codon 12>13) were reported to correlate with perineural infiltration and poor prognosis. BRAF mutations are also described in CC.
- Alterations in tumor suppressor genes encompass p53 (20–80%) and p21 mutations, together with MDM2 upregulation leading to functional in activation of p53 in the vast majority of cholangiocarcinomas; furthermore, APC and DPC4 deletions and mutations or hypermethylation (up to 80%) in the promoter region of p16, leading to its downregulation, occur in cholangiocarcinoma.
- CDNK2A inactivation occurs in PSC-related cholangiocarcinoma; enhanced CDNK2A promoter methylation and suppression in hepatolithiasis-associated cholangiocarcinoma.
- Microsatellite instability appears to be infrequent.

Diagnosis

- Diagnosis is based on standard morphology aided by immunohistology.
- Diagnosis of resection specimen includes typing, staging (TNM criteria including vascular and perineural infiltration and resection margin status), grading, and analysis of the nontumorous liver tissue.
- Since in biopsy differentiation from other tumors of the pancreatobiliary system is not definitively possible by histology alone and differentiation from metastatic lesions of other tumors may require additional immunohistological analyses, clinical and imaging information regarding potential extrahepatic tumor manifestations is mandatory.

Prognosis and Predictive Factors

- Intrahepatic and extrahepatic metastases, macroscopic vascular invasion, advanced TNM stage, and noncurative resection are negative prognostic factors.
- Macroscopic type is of prognostic relevance after operation (5-year survival for mass-forming type: 40%; for intraductal growing type: 70%; for periductal infiltrating type: <5%).
- Grading is not an unequivocal prognostic factor; (partial) squamous or sarcomatoid

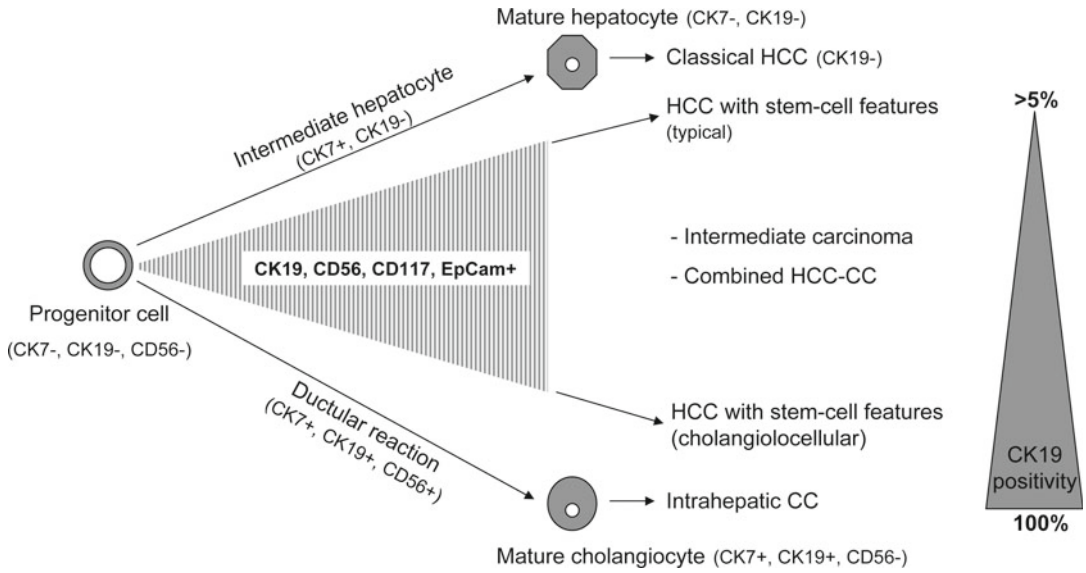


Fig. 3.5 Schematic representation of the possible histogenesis of hepatocellular carcinoma, mixed hepatocellular–cholangiocarcinoma, and cholangiocarcinoma (material adapted from Komuta et al., 2008). *CC* cholangiocarcinoma; *CK* cytokeratin; *EpCam* epithelial cell adhesion molecule; *HCC* hepatocellular carcinoma

differentiation has been correlated with poorer prognosis; mucin typing (MUC5AC vs. MUC2) has been correlated with prognosis but independency has not been demonstrated so far.
- There is no established predictive marker for targeted therapy so far; KRAS mutation status may have potential to predict response to EGFR-targeted therapy.

Combined Hepatocellular–Cholangiocarcinoma

Definition

- Malignant primary liver carcinoma with unequivocal and intimately mixed (↔collision tumor) components of hepatocellular and cholangiocellular differentiation.

Epidemiology

- Combined hepatocellular–cholangiocarcinoma (HCC–CC) represents about 1–5% of all primary liver tumors.
- Whether age, sex, geographic distribution, and etiological risk factors are similar to classical HCC or CC varies significantly between different studies.

Histopathology

- Typically intimately mixed areas of classical HCC and CC are seen.
- Immunohistochemistry can support the dual differentiation (e.g., HCC: Hepar1, CD10, pCEA, (AFP) and CC: CK7, CK19 positive, but HCC areas with progenitor cell features may also be positive).
- A mixed phenotype can be found at the interface of both regions, which has to be separated from HCC–CC with stem cell features.
- HCC–CC with stem cell features (Fig. 3.5) may show nests of mature hepatocytes with peripheral clusters of small cells positive for CK7, CK19, CD56, CD117, and/or epithelial cell adhesion molecule (EpCam); intermediate small ovoid cells with scant cytoplasm (positive for Hepar1 or AFP and CK7, CK19, or CD117) surrounded by a desmoplastic

stroma that may contain ill-defined glands; or cholangiocellular differentiation with small (CK19, CD56, CD117, and EpCam positive) cells with mild atypia growing in a so-called antler-like pattern that consists of cord-like anastomosing tubuli arranged in a fibrous stroma. The latter pattern is considered to reflect the cholangioles of the canal of Hering.

Differential Diagnosis

- The differential diagnosis includes HCC, CC, and a collision tumor of HCC and CC depending on the tissue (biopsy) available for diagnosis.

Molecular Pathology

- Genomic alterations in HCC–CC include loss of heterozygosity (LOH) at 4q, 8p, 16q, and 17p, which are at least partially identical between HCC and CC areas in most cases.
- HCC–CC may show genomic alterations that are more common in classical CC than in HCC (e.g., LOH of 3p and 14q).
- In contrast to HCC, β-catenin mutations have not been described in HCC–CC, whereas TP53 mutations (25–30%) and RB1 deletions may be present.

Diagnosis

- Definite noninvasive diagnosis by imaging is impossible due to the similarities with either classical HCC or CC, but HCC typical imaging combined with elevation of both AFP and carbohydrate antigen 19–9 (CA19-9) may be indicative.
- Radiological imaging aids in the detection of liver cirrhosis, the extent of intrahepatic disease, vascular involvement, and extrahepatic spread.
- Diagnosis may be incidentally made on liver biopsy specimen, but most diagnoses evolve from histological evaluation of surgical specimen.

Prognosis and Predictive Factors

- The prognosis of HCC–CC is worse than HCC and similar to CC with repeatedly reported 5-year survival rates of less than 25%.
- HCC–CC tends to behave like HCC with respect to vascular invasion and like CC with respect to lymph node metastasis
- In noncirrhotic patients partial hepatectomy with hilar lymph node dissection is the best treatment option, whereas in cirrhotic patients the functional reserve of the remaining liver has to be considered before surgery.
- Although the present data are based on experience of single cases, liver transplantation seems no choice due to the short survival time compared to classical HCC.
- Local ablative therapy (e.g., radiofrequency ablation) may be a treatment option for local recurrences.
- Predictive factors have to be evaluated through long-term followup of larger cohorts.

Hepatoblastoma

Definition

- Primary malignant blastomatous liver tumor with hepatic precursor cell differentiation that may show various combinations of several, variably mature epithelial and mesenchymal lineages.

Epidemiology

- Hepatoblastoma (HB) is rare (incidence 1/1,000,000), but represents the most frequent liver tumor in children and occurs slightly more often in males.
- 80–90% of HBs are detected before the age of 5 years, the median age at diagnosis is 1 year.
- Most cases arise sporadic, but the incidence is up to 2,000-fold increased in kindreds with

familial adenomatous polyposis (FAP) and Beckwith–Wiedemann syndrome (BWS; imprinting defect with overexpression of IGF2).

Etiology

- HB is associated with premature birth and low birth weight.
- HB can be associated with congenital diseases and syndromes (e.g., BWS, FAP monosomy 7, trisomy 9, 18 and 21, Acardia syndrome, Goldenhar syndrome, neurofibromatosis type 1, and Prader–Willi syndrome).

Clinical

- Symptoms are typically an enlarged abdomen that may be accompanied by anorexia or weight loss.
- Other unspecific symptoms like nausea, abdominal discomfort or pain may be present, whereas jaundice is rare (<5%).
- HB is associated with paraneoplastic hematological syndromes (e.g., anemia, thrombocytosis).
- Pulmonary metastases are present in 10–20% of patients at diagnosis. Other sites of metastases are skeleton, brain, ovaries, and eyes.
- Marked AFP elevation is found in 90% of cases, the remaining (AFP-negative) cases show a more aggressive course and are associated with a high risk histology (see below).
- In most cases (up to 85%) a single mass is detected in imaging studies.
- Up to 50% of HBs show areas of calcification or ossification.
- Preoperative chemotherapy is able to reduce tumor size in >80% of cases.

Histopathology

- HBs display a variety of morphological differentiation and growth pattern that may be present to a variable extent.
- HBs are classified into pure epithelial type, a mixed epithelial and mesenchymal (MEM) type (with/without teratoid features), and hepatoblastoma, not otherwise specified.
- Wholly epithelial type HB can be subtyped into fetal, mixed fetal and embryonal, macrotrabecular, and small cell undifferentiated (SCUD).
- Fetal subtype HB account for nearly 1/3 of all HBs and the neoplastic cells resemble hepatocytes of the developing liver. Due to variable glycogen and lipid content the cytoplasm of these hepatoblasts shows a typical light and dark pattern at scanning magnification. The nuclei are small and round with fine chromatin distribution. Immunohistologically fetal HBs show a variable AFP and a membranous β-catenin expression.
- Mixed fetal and embryonal HB accounts for 1/5 of cases and areas with embryonal differentiation resemble hepatoblasts of gestational weeks 6–8. The hepatoblasts lack visible glycogen or lipids and have a scant, dark granular cytoplasm and a large nucleus with coarse chromatin. β-catenin is expressed at the cell membrane and in less differentiated areas nuclear staining is seen.
- Macrotrabecular HB is rare and shows wide trabeculae (>6 cells) as the predominant growth pattern. The trabeculae may be composed of embryonal, fetal, and/or hepatocyte-like cells. Macrotrabecular HB (MT) can be divided in MT1 (exclusively hepatocyte-like cells, more aggressive course) and MT2 (fetal/embryonal cells).
- SCUD HBs are composed entirely of noncohesive sheets of so-called small blue cells without discernible cytoplasm. SCUD HBs are very rare and represent the least differentiated subtype that is not associated with AFP elevation. The SCUD phenotype, even if expressed focally, is an adverse prognostic factor (Fig. 3.6).
- MEM type HBs account for 45% of cases and consist of neoplastic epithelial and neoplastic mesenchymal components. About 80% of MEM type HBs do not show teratoid features, whereas the remaining cases display complex heterologous tissues of all three germ layers (e.g., endodermal, neuroectodermal, complex

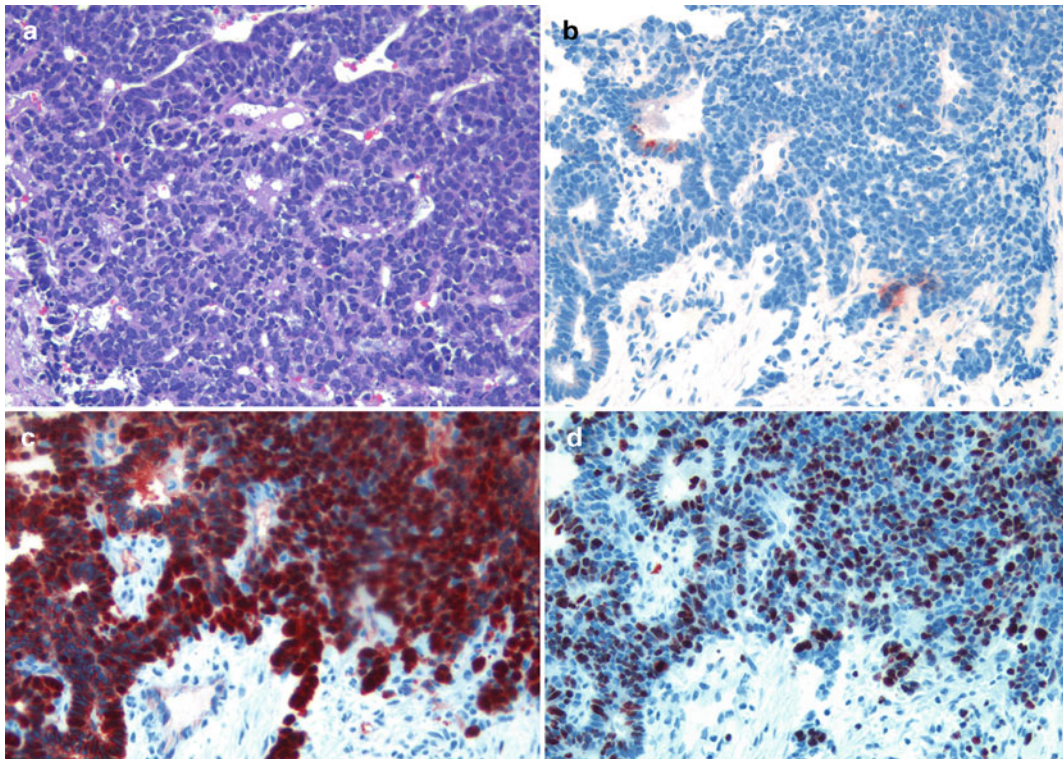


Fig. 3.6 Small cell undifferentiated hepatoblastoma. (**a**) Small cell blue tumor arranged in solid sheets. (**b**) AFP expression is only seen in individual tumor cells. (**c**) Strong nuclear and cytoplasmic β-catenin expression. (**d**) Ki67 stains demonstrate high proliferative activity (original magnification 200-fold)

mesenchymal). Osteoblast-like cells adjacent to osteoid may be epithelial-derived as indicated by CK8 and AFP expression and blending with epithelial components.

- Independent of the subtype neoadjuvant chemotherapy may result in fibrosis, hemorrhage, and necrosis, whereas squamous metaplasia may be seen in residual viable tumor areas. Other treatment-induced changes include ballooning, fatty change, nuclear pleomorphism, and foreign body reaction.
- Special variants include HB with cholangiocellular components, transitional liver cell tumor (TLCT, intermediate between hepatoblast and hepatocyte, giant cells, very high serum AFP), and the calcifying nested stromal epithelial tumor.

Differential Diagnosis

- HB must be differentiated from infantile hemangioendothelioma, mesenchymal hamartoma, or undifferentiated embryonal sarcoma that occur in the same age group.
- Rhabdoid features may occur in SCUD HB and then require differentiation from a primary rhabdoid tumor of the liver, which shows no immunohistological expression of SMARCB1/INI1.

Molecular Pathology

- Although no consistent pattern of chromosomal anomalies has been detected, low number alterations that include gains of 1q, 2, 4q,

Table 3.2 Molecular findings in hepatoblastoma (Material adapted from Zimmermann and Saxena, 2010)

Microsatellite instability	17/21 cases (81%)
TGFβ signaling	Upregulation
PPARα	Upregulation
Adipocytokine signaling	Upregulation
Extracellular matrix-receptor interaction	Upregulation
Apoptosis	Downregulation
WNT signaling	Upregulation
β-catenin	Mutation, deletions in exon 3 and 4
AXIN1	Mutations
APC	Mutations (in cases associated with familial adenomatosis polyposis)
Cell cycle dysregulation	
PLK1	Upregulation
CDKN2A	Promoter methylation
CDKN2B	Loss of expression
DLK1	Upregulation
Dysregulation of IGF-signaling	
PLAG1	Overexpression
11p15 gene cluster	Imprinting errors (BWS)

7, 8q, 10, 12q, 17q, 20, Xp, and Xq as well as losses of 1p, 2q, 4q, and 11p have been commonly reported.

- Gains of 2q, 8q, and 20q have been associated with a poor outcome.
- LOH of the maternally imprinted allele at 11p15 is nearly pathognomonic for patients with BWS. This region encodes p57/kip2, IGF2, and H19.
- Alterations in the APC/ β-catenin pathway play an important role in the pathogenesis of HB as demonstrated by the association with FAP, the presence of APC mutations in sporadic HBs, and the detection of nuclear β-catenin accumulation due to deletions in exons 3 and 4 in about 70–80% of cases.
- Besides the most commonly affected β-catenin and IGF-signaling pathways altered gene expression is found for components of several other pathways (Table 3.2).
- The molecular changes correlate in part with the histological differentiation (Table 3.3), whereas others, like p53 mutation and mismatch repair defects, do not.

Table 3.3 Genotype–phenotype correlation in histological subtypes of hepatoblastoma (Material adapted from Zimmerman and Saxena, 2010)

Histological subtype	Molecular alterations
Small cell undifferentiated hepatoblastoma	Overexpression of MAPK pathways
	Loss of CDKN1B
	FOXG1 overexpression
	Downregulation of Notch signaling (HES1)
	Downregulation of GS
Fetal hepatoblastoma	Large deletion in exons 3 and 4 of β-catenin
Mesenchymal hepatoblastoma	Upregulation of pathways responsible for extracellular matrix-receptor interaction

Table 3.4 Staging of hepatoblastoma according to the Children's Oncology Group

Stage	Criterion
I	Tumor completely resected without microscopic residual disease
II	Presence of microscopic residual disease
	Pre- or intraoperative tumor rupture
III	Unresectable tumor
	Resectable tumor with grossly visible residual disease
	Nodal metastasis
IV	Distant metastasis

Diagnosis

- HB diagnosis is reached via imaging studies in combination with serum AFP level and biopsy.

Prognosis and Predictive Factors

- The Children's Oncology Group staging system (Table 3.4) is used to assess completeness of resection, the most important prognostic factor.
- The PRETEXT (pretreatment extent of disease) system is used for staging before initiating therapy and correlates with overall and event-free survival.

- High PRETEXT stage, low serum AFP, vascular invasion, and certain histological subtypes (SCUD, rhabdoid features, TLCT) predict aggressive behavior, whereas low stage and purely fetal morphology are favorable prognostic factors.
- HB is usually chemosensitive and thus preoperative chemotherapy allows downstaging and enables secondary resection, but this approach has been questioned since about 40% of HBs are primarily resectable and those with pure fetal histology and low mitotic rate do not require additional chemotherapy.
- Liver transplantation is the only treatment option for unresectable HBs and results in 10-year survival rates of 66–78%.
- The only contraindication for transplantation of HBs is the persistence of extrahepatic disease unresponsive to chemotherapy.
- Polo-like kinase 1 (PLK1) expression has negative prognostic value independent of β-catenin mutation, age, stage, and histology.
- Hypermethylation of the RASSF1A promoter is associated with less response to preoperative chemotherapy.

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