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Tumors and Tumor-Like Lesions of the Hepatobiliary Tract

General and Surgical Pathology

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With 880 Figures and 199 Tables

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I dedicate this textbook to my wife, Geneviève, who for many years of preparatory work endured much and offered continued interest, help, and compassion while I was creating this work. She accompanied me with great loyalty in this endeavor. I also dedicate this book to my children, Laxmi and Tristan, who have given so much meaning to my life, and to my venerated late teacher in pathology, Professor Hans Cottier.

Preface

This textbook is designed to be a comprehensive assessment of current knowledge regarding the surgical and general pathology of hepatobiliary tumors and tumor-like lesions. The scope of the book is broad and provides an up-to-date source for the wide-ranging tumor pathology of the entire hepatobiliary tract. In the planning phase of this work, the question had arisen as to the purpose and need of such a book, in the light of numerous excellent monographs and textbooks that have been published in this field during the last years, but a major justification for a new book relates to the rapid change in the role of pathology in the investigation of hepatobiliary tumors and related lesions. Therefore, an update of the dramatic changes that have taken place in the discovery and application of several lines of knowledge referring to hepatobiliary tumor pathology in a broader sense was regarded a worthwhile task. Notwithstanding the impressive increase in diagnostic precision of modern imaging and other, in particular various molecular, methods, tumor biopsy and its morphological interpretation based on complex techniques is still a central diagnostic instrument that serves refined diagnosis and classification, risk stratification, and therapy planning, also in the light of future personalized treatment strategies. A combined approach by using conventional, fine structural, immunohistochemical and hybridization morphological studies, and molecular techniques generated a new concept of the tight correlation between structure and function in tumor pathology, contributing to advanced modes of diagnosis. Apart from information on a given diagnostic tumor entity, differential diagnosis is discussed in depth as a most critical issue.

The main reason for integrating several important issues of general pathology is based on the rapidly evolving and continued changes that are occurring in the disciplines of tumor biology, genomics, and associated molecular features that characterize tumors. The concept of the present work in fact aims at concentrating detailed aspects of surgical pathology needed for diagnosis and the pathogenic mechanisms behind disease in one source with ample color illustrations and a detailed reference corpus.

In the light of refined imaging techniques and other modern diagnostic approaches that can uncover a host of previously undetectable hepatobiliary lesions, a significant part of the textbook is dedicated to an extensive range of tumor-like lesions, pathologies that may, in a clinical-radiological setting, be confounded with true hepatobiliary neoplasms. Part of the tumor-like lesions, including mass-forming infections and infestations, are common entities

world-wide, while others may appear as unexpected or incidental findings, or rare and "exotic" disorders.

This text has been planned to serve hepatopathologists, hepatologists, and others interested and involved in this field, and it is the author's hope that the book is a comprehensive account on the surgical and general pathology of hepatobiliary tumors and their tumor-like mimics. The book is also hoped to be a useful source of information for basic scientists active in the field of liver pathophysiology.

To provide a systematic review of the immense field of hepatobiliary tumors and tumor-like lesions, the textbook has been divided into 39 parts, each covering one to several chapters, in order to assist the reader in locating topics of interest. In what follows, brief overviews on the contents of each chapter are presented.

Part 1 starts off with a series of chapters that supply comprehensive information on tumors characterized by a hepatocyte-derived lineage and its precursors. Chapter ▶ 1 deals with the role of hepatic stem and progenitor cells in hepatocarcinogenic pathways. Hepatocytes were perceived to represent the major cells of origin for numerous neoplasms of the liver, but stem and progenitor cells have been identified as important sources. The chapter addresses issues of hepatic stem cell niches, types of stem/progenitor cells found in these niches, interactions of stem cells with other cells, changes of their microenvironment, and mechanisms involved in a stem cell-cancer sequence. As cancer initiating cells and cancer-associated stem cells can circulate in blood, the significance of cycling clonogenic cells with longevity and remote spread for tumor progression is discussed. Chapter ▶ 2 provides an in-depth description of ordinary hepatocellular carcinoma (HCC), including classification of gross phenotypes, macroscopic growth patterns, pertinent histologic and diagnostic features, and tumor grading. In Chap. ▶ 3, the numerous immunohistochemical features characterizing ordinary HCC are discussed in detail. Chapter ▶ 4 focuses on invasion and metastatic patterns of ordinary HCC. Patterns of macrovascular and microvascular invasion and the features of intrahepatic and remote metastasis are explained and illustrated. This part also provides information on risk factors for metastasis and on the presentation and frequency of extrahepatic organ metastases. In Chap. ▶ 5, secondary changes that develop in HCCs, and in particular the interesting phenomenon of spontaneous tumor regression, are highlighted. Progression and recurrence of HCC are major elements of the tumor's biology of disease. Numerous prognostic factors for the natural course of HCC have been delineated, discussed in detail in Chap. ▶ 6. The issues of Chap. ▶ 7 are the various types of HCC precursor lesions that can develop in cirrhotic livers, including small and large cell change, dysplastic foci, and dysplastic nodules. There is a group of ordinary HCCs characterized by small size at the time point of diagnosis, lesions that are more frequently diagnosed due to improved imaging techniques. These intriguing lesions, specifically their morphology, classification, biology, and relationship to early cancer are dealt with in Chap. ▶ 8. The chapters on neoplasms of the hepatocyte lineage are considerably extended to reflect the growing importance of special types of liver cell cancers in the setting of clinical presentation, detectability by modern imaging techniques,

molecular features, and biology of disease (Chaps. ▶ 9, ▶ 10, ▶ 11, ▶ 12, and ▶ 13). Chapter ▶ 9 deals with clear cell HCC, a group of neoplasms that belong to a growing spectrum of epithelial clear cell tumors of the alimentary tract with a distinct biology of disease. A heterogeneous group of HCCs is characterized by the accumulation of neutral fat, including steatotic HCC and its inflammatory variant, steatohepatitic HCC, tumors that also develop in the setting of nonalcoholic and alcoholic fatty liver disease. Part of these tumors are rich in Mallory-Denk bodies (Chap. ▶ 10). A rare group of HCCs is characterized by the presence of an abundant desmoplastic stroma, similar to cholangiocarcinoma (sclerosing and scirrhous HCCs; Chap. ▶ 11). A further unusual subset of HCCs shows dense infiltrates of mononuclear leukocytes (inflammatory HCCs). One variant of these neoplasms reflects a morphology similar to lymphoepithelial carcinoma, with or without association with EBV virus infection, and another rare variant exhibits a plasmacellular infiltrate and signs of regression (medullary HCC; Chap. ▶ 12). Very rare forms of HCCs are characterized by peliotic change, multinucleated giant cells, chromophobe cells, oxyophilic/oncocytic cells, or cells with a Dubin-Johnson-like pigment (Chap. ▶ 13). An interesting group of liver cell tumors displays the presence of progenitor cells or stem-like cells. Part of these HCCs with progenitor cell features express cytokeratin 19, a feature conferring a more aggressive course (Chap. ▶ 14). A clinically and radiologically intriguing situation is produced by HCCs arising in an ectopic, extrahepatic location (Chap. ▶ 15). HCCs also occur in infancy and childhood (pediatric HCC). It is not yet clarified whether these unusual malignancies are the same or different from their adult counterparts (Chap. ▶ 16). A neoplasm that in several respects mimics HCC is hepatoid carcinoma, which can develop in numerous organs, but is usually manifest in the liver in the form of metastases (Chap. ▶ 17). An intriguing variant of HCC is fibrolamellar HCC, a tumor mainly occurring in younger individuals, showing a biology similar to that of ordinary HCC, and associated with a typical recurrent chimeric transcript (Chap. ▶ 18). A rare group of neoplasms composed of immature hepatocyte progenitors (embryonal and fetal hepatocytes) is formed by the various types and subtypes of hepatoblastoma and related neoplasms (Chap. ▶ 19). The majority of these cancers develops in children younger than 5 years, but rarely also develop in adults. Unusual variants of hepatoblastoma with aberrant differentiation patterns are treated in Chap. ▶ 20, including tumors with a bile duct cell differentiation. The focus of Chap. ▶ 21 relates to the biology and prognostic factors of heptoblastoma, while Chap. ▶ 22 treats risk factors and pathogenic pathways of neoplasms of the hepatoblastoma tumor family. An intriguing neoplasm related to hepatoblastoma and associated with an interesting clinical presentation and unique molecular features, nested stromal epithelial tumor, is the theme of Chap. ▶ 23. A benign tumor of the hepatocyte lineage is hepatocellular adenoma, which has recently been subdivided into several molecular subtypes associated with distinct morphologic patterns. The discussion of this important hepatic tumor and its variants is found in Chaps. ▶ 24 and ▶ 25. A rare group of neoplasms containing hepatocyte-like cells is combined hepatocellular-cholangiocarcinoma, malignancies that display a biphenotypic histologic picture. In contrast to HCC, these highly aggressive

neoplasms occur in cirrhotic and noncirrhotic livers with almost the same frequency (Chap. ▶ 26).

Part 2 of the textbook relates to benign and malignant neoplasms of the cholangiocyte lineage. Cholangiocarcinomas (CC) are divided into two major groups, extrahepatic and intrahepatic bile duct cancers. Among the former, hilar and perihilar CC form a distinct clinicopathologic entity different from CC originating in the mid-region and distal parts of the large bile duct. Intrahepatic CC is a malignancy that can present in three major gross growth patterns and originates from both small or large intrahepatic bile ducts. CC are malignancies of adult patients, but very rarely also develop in the pediatric age group (Chaps. ▶ 27, ▶ 28, ▶ 29, ▶ 30, ▶ 31, and ▶ 32). A distinct group of bile duct neoplasms is formed by intraductal neoplasms, tumors that resemble their pancreatic counterparts. They display a phase of intraluminal, often papillary noninvasive growth and a later high risk of transition into invasive CC (Chap. ▶ 33). In addition to classical forms of CC, there are tumors developing in the setting of hepatobiliary cystic disease, or exhibit distinct differentiation patterns different from those observed in ordinary CC (Chaps. ▶ 34, ▶ 35, ▶ 36, ▶ 37, and ▶ 38). A rare subset of bile duct tumors is characterized by cysts lined by a mucin-producing epithelium, frequently associated with a subepithelial ovarian-like stroma and expression of sex steroid receptors. These mucinous cystic neoplasms (MCN) can undergo a dysplasia-carcinoma sequence (Chap. ▶ 39). Both the intrahepatic and extrahepatic biliary tree can be the site of several types of benign epithelial neoplasms and hamartomas, including tubular and papillary adenomas, peribiliary gland hamartomas, biliary microhamartoma, and neoplasms and hyperplasias of peribiliary glands (Chaps. ▶ 40 and ▶ 41).

Part 3 covers a heterogeneous group of liver tumors derived from other epithelial lineages. Some hepatobiliary carcinomas are characterized by varying proportions of squamous epithelial cells, including squamous cell carcinoma, adenosquamous carcinoma, and mucoepidermoid carcinoma, or acinar cell and adenoid cystic components (Chaps. ▶ 42 and ▶ 43). Rare carcinomas of the biliary tract are undifferentiated neoplasms, such as nonendocrine small cell and spindle cell carcinomas, and carcinomas with rhabdoid features (Chap. ▶ 44). In a small group of hepatobiliary neoplasms, the cells of origin are not yet fully clarified. These lesions mainly comprise hepatic adrenal rest tumor and progenitor cell neoplasms (Chap. ▶ 45).

Part 4 of the textbook refers to mixed epithelial-mesenchymal tumors of the hepatobiliary tract. Chapter ▶ 46 highlights an intriguing group of hepatobiliary malignancies that are composed of a complex mixture of various neoplastic tissue types. These tumors are classified as carcinosarcomas and carcinomas with sarcomatoid features. Few primary hepatic tumors are characterized by the presence of multinucleated, osteoclast-like giant cells with a macrophage/histiocyte lineage (Chap. ▶ 47). Apart from carcinosarcomas, rare hepatic tumors present a mixed cellular phenotype that is still difficult to classify, including malignant mixed tumors, adenosarcomas, stromal tumors, and adult-type mixed hepatoblastomas (Chap. ▶ 48).

Part 5 focuses on the predominant group of hepatobiliary mesenchymal tumors, i.e., vascular tumors. The most important type of primary hepatic

vascular neoplasm is cavernous hemangioma with its various phenotypes and associations with extrahepatic vascular tumors (Chap. ▶ 49). Less common hepatic vascular tumors include several forms of hemangiomatosis, hemangioblastoma, epithelioid hemangioendothelioma, infantile hepatic hemangioma/hemangioendothelioma, kaposiform hemangioendothelioma, angiosarcoma, Kaposi's sarcoma, glomus tumors, myopericytoma, and glomangiopericytoma (Chaps. ▶ 50, ▶ 51, ▶ 52, ▶ 53, ▶ 54, ▶ 55, ▶ 56, and ▶ 57). Unusual vascular malformations, which may be part of complex inborn syndromes, can mimic true vascular neoplasms, such as Klippel-Trenaunay syndrome (Chap. ▶ 58). A second group of reactive vascular lesions that can cause tumor-like hepatic manifestations include bacillary angiomatosis and peliosis hepatis (Chap. ▶ 59).

Part 6 refers to tumors and tumor-like lesions of lymph vessels. In the hepatobiliary tract, these are very rare conditions which include cystic and noncystic lymphangioma, capillary lymphangioma, lymphangiomatosis, hepatic lymphangiectasis, and lymphocele (Chap. ▶ 60).

Part 7 is exclusively dedicated to solitary fibrous tumor and tumors with a hemangiopericytoma-like pattern. These lesions now include at least part of the former hemangiopericytomas and are characterized by a distinct somatic fusion of two genes, NAB2 and STAT6 (Chap. ▶ 61).

Part 8 discusses the complex spectrum of nonvascular mesenchymal tumors of the hepatobiliary tract. All these neoplasm are rare lesions and include fibroblastoid and myofibroblastoid neoplasms, leiomyoma and leiomyosarcomas, rhabdomyosarcomas, lipoma, liposarcoma, myelolipoma, hibernoma, tumors with osteosarcomatous and chondrosarcomatous components, gastrointestinal stromal tumors, benign and malignant nerve sheath tumors, granular cell tumors, synovial sarcoma, and undifferentiated high-grade pleomorphic sarcomas (Chaps. ▶ 62, ▶ 63, ▶ 64, ▶ 65, ▶ 66, ▶ 67, ▶ 68, ▶ 69, ▶ 70, and ▶ 71).

Part 9 summarizes tumors with a mesothelial cell lineage. Primary mesotheliomas of the liver are very rare, but characteristic neoplasms mimic mesothelial tumors in other locations. A unique mesothelial tumor occurring also in the liver is adenomatoid tumor (Chap. ▶ 72).

The theme of Part 10 is a heterogeneous group of neoplasms that are derived from, or related to, perivascular epithelioid cells. The liver is the primary site of various perivascular epithelioid cell tumors or PEComas, all with complex cellular compositions. They include PEComa proper, angiomyolipoma with its various subtypes, clear cell myomelanocytic tumors, clear cell and sugar tumors, and lymphangiomyomatosis (Chap. ▶ 73).

Part 11 presents hepatobiliary tumors of neuroendocrine lineages. Chapter ▶ 74 provides pertinent information regarding extraadrenal paraganglioma, neoplasms that also in the liver range in biology from a benign to frankly malignant behavior. In Chap. ▶ 75, the various types and subtypes of hepatobiliary neuroendocrine tumors are treated, emphasis being placed on novel classifications and grading systems.

In Part 12, a rare group of small tumors that also originate in the liver are discussed. The lesions comprise various small cell blue tumors, such as primitive neuroectodermal tumors (PNET), desmoplastic small round cell tumor, NUT midline carcinoma, and hepatic neuroblastoma (Chap. ▶ 76).

Part 13 refers to the interesting group of primary and secondary melanotic tumors of the hepatobiliary tract. Emphasis is placed on primary and metastatic melanoma, in particular also metastatic ocular melanoma, melanotic progonoma, and manifestations of melanoma of soft parts (Chap. ▶ 77).

Part 14 focuses on hepatic tumors with a rhabdoid cell lineage. Liver and bile duct tumors with rhabdoid cell components are, at least in part, associated with absence of the chromatin remodeling factor SRF5/INI1 and include malignant rhabdoid tumor proper, carcinomas with rhabdoid features, and a subset of small cell hepatoblastoma (Chap. ▶ 78).

In Part 15, primary and metastatic germ cell tumors are treated. Most germ cell tumors occurring in the gonads can occur as primary lesions in the liver, but teratomas, yolk sac tumor, and choriocarcinoma prevail, also in the pediatric age group. The liver is a well-known site of metastatic germ cell tumors and can be the site of growing teratoma syndrome (Chap. ▶ 79).

Part 16 summarizes the pathology of hepatic manifestations of myeloid neoplasms. With the exception of granulocytic sarcomas, these neoplasms cause diffuse infiltration of the liver substance. The conditions discussed comprise polycythemia vera, several types of myeloproliferative syndrome, chronic eosinophilic leukemia and idiopathic hypereosinophilia, mast cell neoplasms, acute leukemias, myeloid neoplasms with a monocytoid lineage, and blastic plasmacytoid dendritic cell neoplasms (Chaps. ▶ 80, ▶ 81, ▶ 82, ▶ 83, ▶ 84, ▶ 85, ▶ 86, and ▶ 87).

Part 17 refers to the complex pathology of hepatobiliary Hodgkin's disease. This disorder causes, on the one hand, tumorous hepatic lesions that can clinically be confounded with liver cancer, but on the other hand also reveals associations with paraneoplastic changes, including vanishing bile duct syndrome (Chap. ▶ 88).

Part 18 covers the large field of hepatobiliary non-Hodgkin's lymphomas, other lymphoproliferative disorders, and neoplasms of dendritic and histiocytic cell systems. Major groups comprise B-cell and T-cell neoplasms that occur in numerous extrahepatic sites, but pseudolymphomas, neoplasms of the Langerhans cell and histiocytic systems, dendritic cell neoplasms, and reactive histiocytic syndromes (such as Rosai-Dorfman syndrome) are also discussed (Chaps. ▶ 89, ▶ 90, ▶ 91, ▶ 92, ▶ 93, ▶ 94, ▶ 95, ▶ 96, ▶ 97, ▶ 98, ▶ 99, ▶ 100, ▶ 101, ▶ 102, and ▶ 103).

Part 19 treats mesenchymal hamartoma of the liver and related neoplasms. Mesenchymal hamartoma and undifferentiated embryonal sarcoma of the liver are typical pediatric hepatic neoplasms, but they have rare counterparts in adult patients (Chaps. ▶ 104 and ▶ 105).

Part 20 is a large complex of chapters that relates to the very important issue of metastatic liver disease. The chapters discuss in detail aspects of gross and microscopic pathology of liver metastases in general, a specific chapter on colorectal cancer metastases, other common and rare metastatic cancers, secondary changes that frequently develop in hepatic metastases, secondary spread of metastatic disease into locoregional lymph nodes, associated liver lesions, growth and regrowth of metastases, and pathogenic features of liver metastasis (Chaps. ▶ 106, ▶ 107, ▶ 108, ▶ 109, ▶ 110, ▶ 111, ▶ 112, ▶ 113, ▶ 114, and ▶ 115).

In Part 21, a small theme of liver tumor pathology is addressed, tumors and tumor-like lesions of hepatic ligaments. Falciform and round hepatic ligaments are the site of rare primary benign and malignant neoplasms, metastases, and various types of cysts (Chap. ▶ 116).

Part 22 contains chapters on reactive nodular hyperplastic hepatocyte lesions of the liver. Focal nodular hyperplasia (FNH) of the liver is an important mass-forming regenerative condition that often develops secondary to localized vascular and circulatory abnormalities of the liver. After hemangiomas, FNH is the second most common benign hepatic tumorous lesion (Chap. ▶ 117). A second important regenerative condition of the liver is nodular regenerative hyperplasia, which is associated with a broad array of causative factors (Chap. ▶ 118).

In Part 23, pseudotumors of the hepatobiliary tract are discussed. Pseudotumors and inflammatory pseudotumors form a heterogeneous group of lesions that share spindle cell proliferations and inflammatory infiltrates of variable density. Inflammatory myofibroblastic tumors is a lesion that contains a subset with neoplastic features and aberrant ALK expression (Chap. ▶ 119).

Part 24 relates to nonneoplastic tumor-like lesions of the liver. The liver is the site of tumor-like ectopias and heterotopias, mass-forming malformations, solitary necrotic nodule, various types of dust-induced nodular lesions, tumor-like lesions caused by gallstones and foreign bodies, pseudotumors consisting of reactive proliferations of hematopoietic cells and macrophages, pyogenic liver abscesses mimicking cancer, numerous hepatic bacterial, fungal, and protozoal infections causing tumor-like hepatic masses (tuberculosis, syphilis, brucellosis, and amebiasis representing prominent examples), tumor-like parasitic lesions (mainly echinococcosis), and liver infarcts (Chaps. ▶ 120, ▶ 121, ▶ 122, ▶ 123, ▶ 124, ▶ 125, ▶ 126, ▶ 127, ▶ 128, ▶ 129, ▶ 130, ▶ 131, ▶ 132, ▶ 133, ▶ 134, ▶ 135, ▶ 136, and ▶ 137).

Part 25 refers to reactive cystic lesions of the liver that may mimic cystic neoplasms. They include simple nonparasitic cysts, ciliated foregut cyst, pancreatitic pseudocysts, and cerebrospinal fluid pseudocysts (Chap. ▶ 138).

Part 26 provides information related to hepatic mass lesions caused by noninfectious granulomas. The main disorder is sarcoidosis that can also cause a complex form of sclerosing bile duct disease with bile duct loss. Blau syndrome and complex inflammatory disorders in part involving deregulated inflammasome function are also discussed (Chap. ▶ 139). A small chapter refers to chronic granulomatous disease (Chap. ▶ 140).

Part 27 presents the hepatic pathology of interesting fibrosclerotic disorders. The conditions include idiopathic retroperitoneal fibrosis and its variants, and the complex spectrum of IgG4-associated systemic sclerosing disease (Chap. ▶ 141).

In Part 28, numerous reactive bile duct alterations that can mimic biliary neoplasms are discussed. Intrahepatic and extrahepatic bile ducts can be involved with inflammatory stenosing polyps, granulomatous cholangitis, follicular cholangitis, oriental cholangitis, xanthogranulomatous cholangitis, bile duct cholesterolosis, sclerosing eosinophilic cholangitis, mechanical and anatomical bile duct alterations, bile duct stenosis caused by congenital

anomalies and acquired disorders of the splanchnic arterial tree and the portal vein, and postcholecystectomy changes (Chaps. ▶ 142, ▶ 143, ▶ 144, ▶ 145, and ▶ 146).

Part 29 addresses the important issue of gallbladder cancer and other tumors and tumor-like lesions of this organ. Ordinary gallbladder carcinoma usually develops in a gallbladder that has undergone secondary changes related to longstanding cholelithiasis and associated inflammations. This neoplasm, an adenocarcinoma, can be associated with epithelial precursor lesions and presents in the form of distinct growth patterns (Chap. ▶ 147). Biology of disease, prognosticators, staging, risk factors, and pathogenic pathways of gallbladder carcinoma are treated in more detail in Chaps. ▶ 148 and ▶ 149. Apart from the common ordinary adenocarcinoma of the gallbladder, several rare variants with other differentiation patterns are recognized, including mucinous, signet ring cell, and squamous cell carcinomas (Chaps. ▶ 150 and ▶ 151). The gallbladder is also the site of rare cystic and mixed neoplasms, such as cystadenoma and cystadenocarcinoma (Chap. ▶ 152). As outlined in Chap. ▶ 153, the gallbladder can give rise to a spectrum of adenomatous, borderline, and dysplastic lesions. Of differential diagnostic importance is the observation of various types of hyperplastic and metaplastic lesions in the gallbladder mucosa (Chap. ▶ 154). Similar to the bile duct system and liver, the gallbladder is a well-known origin of diverse types of neuroendocrine tumors, mesenchymal neoplasms, malignant melanoma, and a wide spectrum of other, however very rare, neoplasms (Chaps. ▶ 155, ▶ 156, and ▶ 157). A broad array of reactive, inflammatory, and noninflammatory alterations of the gallbladder can result in mass lesions that mimic neoplasms, and in particular gallbladder cancer (Chaps. ▶ 158, ▶ 159, ▶ 160, and ▶ 161). A rare group of malignant and benign tumors takes its origin in the cystic duct (Chap. ▶ 162).

Part 30 refers to a heterogeneous group of tumorous and tumor-like peritoneal lesions that may involve the liver surface. They include several primary carcinomas and other malignancies, pseudomyxoma peritonei, gliomatosis peritonei, and various forms of metaplasia, granulomas, endometriosis, and deciduosis (Chap. ▶ 163).

Part 31 is the first part of the textbook referring to aspects of general pathology of hepatobiliary tumors, specifically etiology and pathogenesis of hepatocellular carcinoma (HCC). A first chapter discusses in depth inflammatory and toxic causes, in particular the role of hepatitis virus infections, fatty liver and steatohepatitis, and nutritional and other toxins in hepatocarcinogenic pathways (Chap. ▶ 164). The following chapter (Chap. ▶ 165) discusses HCC that arises in the setting of inborn errors of metabolism, in particular various forms of chronic hepatic iron overload. Chapter ▶ 166 focuses on chromosomal alterations, oncogenes, tumor suppressors, and associated signaling networks that are involved in tumorigenesis, while Chap. ▶ 167 puts emphasis on the roles of transcription factors, regulators of growth and apoptosis, and telomere homeostasis. Finally, Chap. ▶ 168 is an overview on the etiologic and pathogenic significance of epigenetic mechanisms in hepatocarcinogenesis (the epigenome).

Part 32 contains an important chapter on the general pathology of structural and functional nuclear changes in hepatobiliary cancer. In Chap. ▶ 169,

relevant diagnostic and theoretical aspects of nuclear and nucleolar abnormalities, anaplasia, and chromatin alterations are addressed. Numerous types of structural abnormalities of cancer cell nuclei are directly associated with functional disorders of nuclear homeostasis, DNA replication, cell division, and organization of chromatin superstructures during interphase. Abnormal heterochromatin generation, a deranged production of euchromatin strings, malposition of interphase chromosomes, and anomalies of intranuclear chromosome movements are hallmarks of nuclear function failure in cancer cells (Chap. ▶ 170).

Part 33 addresses mitochondrial structure and function in normal and malignant neoplastic cells. Apart from their central role in energy production, mitochondria play a significant role within carcinogenic pathways involving abnormal stress responses and deregulation of cell death pathways. This role has led to the "mitochondrial malignancy theory." Mitochondria hold a central position in apoptosis induction, but they also modulate cell shape and engage in complex interactions with other organelles. Cancer cells exhibit various types of structural abnormalities of mitochondria and may show changes in mitochondrial number, mitochondrial fission, and elimination of this organelle. Part of these alterations are associated with losses and mutations of mitochondrial DNA (Chap. ▶ 171).

The contents of Part 34 pertain to tumor growth and its regulation. Uncontrolled, progressive growth is a key feature of cancers. Net mass increase of tumors not only depends on cell proliferation, but also on cell loss caused by various forms of apoptosis and necrosis and the contribution of nonneoplastic tissues and cells accompanying neoplasms, in particular stroma, blood vessels, and leukocytes. Cell proliferation in liver cell cancer reflects features of normal liver regeneration which is therefore discussed in some detail. The aberrant proliferation of liver cancer cells is related to deranged functions of factors orchestrating cell division, checkpoint regulators, proteins involved in DNA synthesis, proteins of the mitotic apparatus, and the numerous components of the cytoskeleton. Similar to the regenerating liver, growth of liver neoplasm is regulated by numerous growth factors and their receptors, including factors produced by platelets and antagonists of proliferation. Furthermore, hepatobiliary cancers reveal abnormal expression patterns of proteins that control entry into and passage through the cell division cycle, including cyclins and cyclin-dependent kinases. Finally, regulation of tumor growth strongly depends on various epigenetic mechanisms, specifically on complex expression patterns of microRNAs and other RNA classes (Chaps. ▶ 172, ▶ 173, ▶ 174, and ▶ 175).

Part 35 informs the reader about important aspects of the necrobiology of hepatobiliary cancer. An intricate process to control tumor cell mass is apoptosis, a complex form of tightly controlled cell death. Growth caused by proliferation is also counteracted by necrosis which, in contrast to traditional views, is a controlled process rather than a passive phenomenon. In liver cancer, apoptosis can be assessed by immunohistochemical and molecular methods. In addition to classical apoptosis, necrobiologic processes active in cancer also include various forms of cell death related to, but not identical with, apoptosis. These pathways may play a significant role for future novel

therapies (Chaps. ▶ 176 and ▶ 177). A special chapter is dedicated to the pathophysiology of classical (passive) necrosis in comparison with regulated necrosis (necroptosis). The latter involves a complex signaling platform, the necrosome, a molecular machine that senses ATP depletion and transmits this signal into kinase execution switches (Chap. ▶ 178). An important role in cancer cell biology is played by autophagy, a process involved in the maintenance of cell and tissue homeostasis, control of the protein composition of cells, aging, senescence, and neoplastic transformation. Autophagy is the instrument to eliminate altered proteins, damaged or superfluous organelles, and pathogens, and is a complex system connected with inflammasome function, inflammation, immunogenic cell death, and cell senescence. Special forms of autophagy in cancer cells include mitophagy and nucleophagy (Chap. ▶ 179).

Part 36 covers several important aspects of cancer invasion and metastasis. Invasion and metastatic spread of cancer cells involve a highly complex sequence of events that comprise tumor cell individualization, tumor cell polarization, migration, and the acquisition of a secretory phenotype, with release of histolytic enzymes. It is not yet fully known how these features are acquired by cancer cells in a seemingly concerted fashion. For being able to locomote, cancer cells, similar to leukocytes, must be able to undergo shape change and polarization, a process that requires numerous cytoskeletal components and specific polarity proteins. The invasive process strongly depends on the generation of invadosomes, including podosomes and invadopodia, matrix-degrading adhesive, and actin-dependent dynamic cellular structures or "organelles" that can also extend through endothelial linings and mediate extravasation of tumor cells. An important role for the invasion of carcinomas is the distinct tumor stroma. Stroma is composed of cancer-associated fibroblasts/CAFs, myofibroblasts, mesenchymal stem cells, stellate cells, blood vessel cells, extracellular matrix, and several classes of infiltrating leukocytes. The interaction of stromal cells with cancer cells affects invasive functions and modulates epithelial-mesenchymal transition (Chaps. ▶ 180, ▶ 181, ▶ 182, and ▶ 183). Metastatic spread of cancer cells, preceded by invasion, is a process that depends on the construction of premetastatic niches, the expression of distinct prometastatic genes and metastasis suppressors, on numerous microRNAs, and on the exchange of cellular information through exosomes and other vehicles that transfer signal cargo and extracellular nucleic acids (Chap. ▶ 184).

Part 37 is reserved for a distinct tumor tissue that affects numerous biologic functions of neoplasms, i.e., tumor stroma. Stroma forms a specific microenvironment that critically regulates the development and behavior of malignant neoplasms. Stromal cells interact with tumor cells directly, in part through cell fusion, and via molecular signals, resulting in a complex signal platform that expands in parallel with tumor growth. Stroma regulates tumor growth, differentiation, invasion, and metastatic spread. The various types of leukocytes present in stroma, in particular tumor-associated macrophages, myeloid-derived suppressor cells, lymphocytes, and neutrophils, create a unique inflammatory microenvironment which, through chemokines and other signal substances, significantly affects tumor biology (Chap. ▶ 185).

Part 38 shows how tumor angiogenesis functions and how it is an essential process in many aspects of liver tumor invasion and progression. Angiogenesis, the formation of new tumor blood vessels, is a critical mechanism for the development and progression of hepatobiliary tumors, which are often highly vascular lesions. In contrast to normal tissues, tumor blood vessels often form highly atypical branching patterns, with irregular diameters and abrupt changes from large to small diameters. The cells mediating tumor angiogenesis are endothelial cells and auxiliary cells that modulate the biology of endothelial cells, in particular perivascular cells, stromal cells, and tumor-associated macrophages and other leukocytes. As in normal tissue, initiation and progression of angiogenesis in tumors involve the action of numerous angiogenic factors, but neoplasms also produce several antiangiogenic factors. Tumor angiogenesis is modulated by epigenetic mechanisms, mainly microRNAs expressed by tumor cells and stromal cells (Chap. ▶ 186). This chapter is supplemented by a chapter that addresses basic questions of vasculogenesis, angiogenesis, and lymphangiogenesis (Chap. ▶ 187).

The last part of the present book, Part 39, provides a summary of current systems of tumor staging. As with other cancers, staging of hepatobiliary cancers is critical for prognostication and optimal treatment planning. Staging is a complex task that depends on multiple factors. In recent years, several staging systems have been developed and markedly improved the methods to arrive at optimal risk stratification procedures. Apart from hepatocellular carcinoma, highly reproducible staging systems have been developed for extrahepatic and intrahepatic cholangiocarcinoma and for hepatoblastoma (Chap. ▶ 188).

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About the Author

Professor Arthur Zimmermann is an internationally known specialist in hepatobiliary tumor pathology. Following his training as MD and pathologist at the University of Berne, Switzerland, he worked in basic research for several years, focusing on tumor cell growth regulation, tumor cell locomotion, and cell cycle mutants of cancer cells. In surgical pathology, he analyzed more than 20,000 liver specimens, described new tumor entities, and was an author or coauthor of more than 500 publications in his field of interest. As chapter author, he participated in several well-known books on liver and biliary tract disease, including the 2010 edition of the *WHO Classification of Tumors of the Digestive System*, and was one of the editors of the book, *Pediatric Liver Tumors* (Pediatric Oncology Series, Springer). Professor Zimmermann developed the pathology review center for the multinational SIOPEL pediatric liver cancer treatment studies and was involved in the formulation of the new classification of pediatric liver tumors.

Part I

Tumors of the Hepatocyte Lineage and Its Precursors

Liver Cancer: Stem and Progenitor Cells 1

Contents

A. Zimmermann, *Tumors and Tumor-Like Lesions of the Hepatobiliary Tract*,
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Abstract

Stem cells are involved in hepatocarcinogenic pathways. In the normal liver, hepatic stem cells are self-renewing cells dwelling in distinct compartments termed stem cell niches. These niches are distinct microenvironments which maintain the balance of slow self-renewal of quiescent stem cells and their priming to become hepatocytes and other differentiated cells. Hepatic stem cells possess plasticity, allowing them to undergo lineage diversification. In hepatocarcinogenesis, tumor stem cells mimick certain features of their normal counterparts. Tumor stem cells in hepatocellular carcinoma (HCC) can act as tumor-initiating cells and tumor-propagating cells that express hepatic stem cell markers and show distinct gene expression profiles. Apart from their role in carcinogenic pathways, cancer stem cells in HCC are considered to play an important role in tumor recurrence and metastasis. The expression of stemness-related markers in HCC confers an aggressive phenotype in these neoplasms. Tumor stem cells in HCC modulate several processes, including maintenance of slowly cycling clonogenic cells having longevity, induction of distinct growth features, and tumor progression. Following removal of HCC and other liver cancers, tumor stem cells can remain in the liver and enter the bloodstream to settle in new stem cell niches, including metastatic niches.

Introduction

Stem cells participate in tumorigenic pathways of numerous neoplasms and have therefore anticipated to be involved in hepatocarcinogenesis. Tumor stem cells (cancer stem cells, CSCs) in hepatocellular carcinoma (HCC) drive carcinogenic pathways and maintain a growing cell population in addition to proliferating nonstem cancer cells, but stem cells are also considered to play a significant role in HCC metastasis and recurrence. Furthermore, and similar to normal stem cells, CSCs exhibit the features of plasticity in regard to their lineage fating, an element that plays an important role in tumor heterogeneity and progressive phenotypic change, e.g., in metastases (reviews: Meacham and Morrison 2013; Sukowati and Tiribelli 2013). Recent research on liver cancer stem cells has also been undertaken with the aim of shedding light on novel treatment directions (reviews: Lee et al. 2009; Pang and Poon 2012). Generally, it is proposed that the expression of stemness-related markers in HCC confers an aggressive phenotype to these neoplasms. As in other organs and tissues, hepatic stem cells are self-renewing cells that dwell in distinct compartments termed stem cell niches, specific microenvironments which provide conditions for maintaining a balance of slow self-renewal of the mostly quiescent stem cells and their priming to become differentiated cell lineages. It is assumed that, following removal of HCC and other malignancies, stem cells involved in maintenance of a neoplastic phenotype remain in the liver and can enter the bloodstream to settle in new stem cell niches, including metastatic niches.

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Stem Cells and Progenitor Cells in the Normal Liver: Pacemakers of Hepatic Ontogeny and Regeneration in the Injured Liver

Stem cells are critically involved in liver ontogenesis (reviews: Kamiya et al. 2006; Navarro-Alvarez et al. 2010). After fating of primordial endodermal cells to become liver, hepatic specification produces bipotential hepatic stem cells or hepatoblasts which can differentiate into either hepatocytes or cholangiocytes (reviews: Sangan and Tosh 2010; Parveen et al. 2011; Shin and Monga 2013; Kamiya and Inagaki 2015). Hepatic specification is a highly complex process that not only depends on fated endodermal cells but also on an interaction of several cell types located to endoderm, including the endothelial cell niche which promotes hepatic endoderm expansion and is directly required for liver speciation (Han et al. 2011b). In regard to regeneration of hepatocytes, the liver is in a special situation insofar as most of the cells that repopulate undamaged parenchyma after cell loss (e.g., resection) are the mature hepatocytes themselves, and not stem/progenitor cells, as hepatocytes are in a G0 phase of cycle and can be rapidly recruited into a cell division cycle. Stem cells play a significant role in regeneration of the liver in case of hepatocyte damage, i.e., when metabolic injury and decreased viability precludes a regenerative response of hepatocytes. Two types of hepatic stem cells have been defined, viz. fetal hepatic stem cells and stem cells of the adult liver (oval cells; progenitor cells). Fetal hepatic stem cells can maintain their self-renewal capability in the developing liver (Zheng and Taniguchi 2003). In adult livers, hepatic progenitor cells are present in small numbers and are quiescent stem cells with a low proliferative rate, representing a reserve or "emergency" compartment that is activated in case of marked hepatocyte damage or massive hepatocyte loss (Sharma et al. 2006; Gaudio et al. 2009; "ghosts in the machine"; Darwiche and Petersen 2010). In the normal adult, liver stem cells mainly reside in peripheral parts of portal tracts, in the compartment of canals of von Hering, and ductules (Vessey and de la Hall 2001), but a subset of liver progenitor cells originates from HNF1beta(+) biliary duct cells (Rodrigo-Torres et al. 2014). The progenitor cell response comes in four components, viz. activation/priming, proliferation, migration, and differentiation. In the course of stem cell-mediated regeneration, primed stem cells and their progeny, oval cells in rodents and progenitor cells in humans, form ductular profiles (ductular reaction) that deeply invade the damaged parenchyma and differentiate into focally arranged cell clusters that will generate hepatocytes (reviews: Fausto and Campbell 2003; Vestentoft 2013; Katoonizadeh et al. 2014). These clusters can also form narrow intraparenchymal ductular structures which have been termed parenchymal ductules. The cells forming these ductules have the same immunophenotype as resting stem cells (Papp et al. 2014). Stem cells isolated from fetal liver have the capacity to repopulate up to 10 % of normal liver, while progenitor cell lines from embryonal and adult liver have no significant repopulation activity (review: Dabeva and Shafritz 2003).

Extrahepatic Stem Cells Home to Liver and Liver Cancer and Regulate the Respective Cell Biologies

In addition to resident hepatic stem and progenitor cells, stem cells acting in the liver can also be recruited from extrahepatic organs and tissues. Induced pluripotent stem cells (iPSCs) develop through reprogramming of somatic cells to a pluripotent state by a complex induction process. iPSCs resemble embryonic stem cells/ESCs in several aspects (review: Chiang et al. 2013). iPSCs can, e.g., originate from mesenchymal cells (including mesenchymal stem cells), fibroblasts, adipocytes, and several types of bone marrow derived cells. As iPSCs can undergo tridermal priming/fating and differentiation, they can give rise to several types of hepatic cells. Embryonal

fibroblasts can be reprogrammed by hepatocyte nuclear factor 1 alpha and Foxa3 to bipotential hepatic stem cells (Yu et al. 2013b). Circulating bone marrow and/or adipose tissue-derived hematopoietic and mesenchymal stem cells (MSC) are capable to give rise to hepatic cells enabled to act in hepatic regeneration (Petersen et al. 1999; Grompe 2005; Oh et al. 2007; Guest et al. 2010; Ishikawa et al. 2010; Gong et al. (2013a, b); Stock et al. 2014). MSCs form a heterogeneous population of fibroblastoid cells surrounding blood vessels and are concentrated in the bone marrow and in adipose tissue, from where they can emigrate, circulate in the blood, and home to diverse tissues. Both hematopoietic stem cells and MSCs can home to the liver after hepatocyte loss, populate the remaining liver, and give rise to cells that can regenerate liver. MSCs can also home to tumors, including HCCs, where they exert diverse functions, including contribution to tumor cell development and expansion, generation of metastatic niches, neovascularization, and immunosuppression (review: Reagan and Kaplan 2011). Human fetal hepatic stem/progenitor cells are distinct from, but closely related to, hematopoietic stem cells (Chen et al. 2013d). Bone marrow-derived MSCs homing to liver tumors can, through interaction with tumor cells, increase tumor cell apoptosis and inhibit metastatic spread (Hang and Xia 2014). Homing of hematopoietic stem cells to the liver after hepatectomy requires hepatocyte growth factor (HGF) and stroma-derived factor-1 (SDF-1) (Lehwald et al. 2014), and hepatic differentiation of human adipose tissue-derived stem cells is promoted by distinct microRNAs (Davoodian et al. 2014).

Identification of Liver Tumor Stem Cells: Morphology, Stem Cell Markers, and Their Characteristics

Morphology

In the normal and regenerating liver, at least part of the stem or progenitor cells can morphologically be identified (Alison et al. 2001; Forbes et al. 2002; Fausto and Campbell 2003; Strain et al. 2003; Zheng and Taniguchi 2003; Kuhlmann and Peschke 2006; Bird et al. 2008). Stem cells, which in part are located in the lining of bile ductules, from where they can be recruited and mobilized, have morphological features of oval cells, small basophil cells, and transition cells between cholangiocytes and hepatocytes. Recently, it was reported that also ballooned cells forming Mallory-Denk bodies may have features of progenitor cells (French et al. 2013). Numerous investigations provided strong evidence that hepatic cancer stem cells (CSC) can be isolated from HCC (Chiba et al. 2006; Ma et al. 2007; Kamohara et al. 2008; Sell 2008; Sell and Leffert 2008; Lingala et al. 2010; Tomuleasa et al. 2010; review: Ji and Wang 2012).

Markers of Stem/Progenitor Cells

CSC are characterized by several markers, including CD13, CD24, CD44, CD90, CD133, EpCam (CD326), OV6 (Terris et al. 2010), and numerous others (Table 1).

Stem cells or stem-like cells can also be isolated from HCC cell lines in vitro via isolation of the side population (SP) cells, a cell population

Table 1 Markers for hepatocellular carcinoma-related cancer stem cells

CD133 (prominin-1)
OV6
EpCAM (epithelial cell adhesion molecule)
CD24
CD90
CD133
CD176
SALL4 (Sal-like protein 4)
c-Kit
ICAM-1 (intercellular adhesion molecule-1)
NCAM (neural cell adhesion molecule)
Delta-like protein 1
Nestin
Granulin-epithelin precursor (GEP)
BMI1 polycomb product
Doublecortin
CaM kinase-like-1
ABCB5
Tudor domain-containing protein 4

capable to exclude Hoechst 33343 dye owing to the capacity of these cells with sufficient ATP-binding cassettes to transport the Hoechst dye out of the cell, while non-SP cells cannot. Due to this difference, tumor SP cells can then be separated from the other cell populations by use of cell sorting, and the resulting SP cells tested for typical stem cell features. Results of such analyses showed that SP cells contain a CSC cell population (review: Sell and Leffert 2008). However, not all cells of side population cell fractions have the characteristic features of CSCs (Nakayama et al. 2014). HCC-CSCs can also be identified by their molecular makeup. Stem cell features of HCC cells are associated with well-established gene alterations conferring an abnormal growth and apoptosis behavior in HCC, such as TP53 mutations (Woo et al. 2011).

CD133: Prominin 1 is a Crucial Molecule in HCC-CSC Biology

CD133 (prominin 1; PROM1) is a well-known stem cell marker (Mizrak et al. 2008; Wu and Wu 2009; Li 2013) which is involved in cell differentiation and polarity (Fargeas et al. 2011), organ morphogenesis (Anderson et al. 2011), cell surface dynamics (Mak et al. 2011), iron homeostasis of various cells (Bourseau-Guilmain et al. 2011), and interactions between cancer cells and stromal cells (Moriyama et al. 2010; Akita et al. 2013a, b). CD133 is expressed in subsets of liver stem cells (Rountree et al. 2011). These cells display a greater colony-forming efficiency and a higher proliferative output (Ma et al. 2007). Expression of CD133 also characterized part of HCC stem cells (Suetsugu et al. 2006; Ma et al. 2008; Song et al. 2008; Yoshikawa et al. 2009; Suetsugu et al. 2010; Zhang et al. 2011; Tsai et al. 2012; Ma 2013), and CD133+/CD44+ cells in HCC are considered to be true cancer stem/progenitor cells that are involved in tumor maintenance and chemoradioresistance (Zhu et al. 2010; Piao et al. 2012). There is evidence that the presence of CD133+ stem cells in HCCs is related to the etiology of HCC. In an area endemic for HBV virus (Taiwan), CD133 expression in HCC was negatively associated with the presence of HBsAg, implicating a non-HBV viral origin of CD133+ HCC (Yeh et al. 2009). In HCC cell lines, expression of CD133 markedly affects the biological features of the tumor cells. Silencing of CD133 expression impaired in vitro HCC cell proliferation, formation of tumor spheres, colony formation, and in vivo tumorigenicity in immunodeficient mice. In addition, knockdown of CD133 reduced cells in G0/G1 and increased tumor cell apoptosis through modulation of Bcl-2 and Bax (Lan et al. 2013). CD133 expression predicted poor disease-free survival independently of p53 expression (Yeh et al. 2009). Expression of CD133 in HCC cells is a predictor of the effectiveness of S1+ pegylated interferon alpha-2b therapy (Hagiwara et al. 2011). CD133 is also expressed in cholangiocarcinoma and gallbladder carcinoma cells (Shi et al. 2010; Fan et al. 2011; Iwahashi et al. 2013), and this expression is correlated with positive tumor margin status and lymph node metastasis (Leelawat et al. 2011).

Other Stem Cell Markers

A well-known second hepatic stem cell marker, OV6, is also present in HCC-associated CSC. OV6+ HCC stem cells are tumor-initiating cells that possess a high capacity to form tumor spheroids in vitro and give rise to tumors in severe combined immunodeficient (SCID) mice (Yang et al. 2012). CD90 characterizes a stem cell lineage in liver cancer, and cells expressing CD90 express several hundred other genes, including glypican-3 (Ho et al. 2012). A further protein expressed in part of stem and progenitor cells is c-Kit (CD117). In one investigation, c-Kit expression was detected in only 2.3 % of HCC, suggesting that c-Kit is not significantly overexpressed in HCC cells (Becker et al. 2007). In another study, immunoreactivity for c-Kit was detectable in 70 % of HCC with different degrees of intensity, illustrating highly variable results in regard to the expression of this stem cell marker in HCC. However, c-Kit reactivity was also found in 90 % of peritumoral cirrhotic and noncirrhotic liver tissue. Similarly, c-Kit

mRNA was identified in 83 % of HCC and 90–100 % of surrounding tissue (Mansuroglu et al. 2009). Sal-like protein 4/SALL4, a member of a family of zinc finger transcription factors, modifier of somatic cells and regulator of organogenesis and pluripotency, is a stem cell marker. SALL4 is expressed in normal human hepatic stem cells, hepatoblasts, HCC, cholangiocarcinoma, and mixed cholangiocarcinoma but is silenced in the normal adult liver. Its expression correlates with cell and tumor growth and an aggressive HCC phenotype (Yong et al. 2013), whereas its suppression results in slowed tumor growth and tumor cell differentiation (Oikawa et al. 2013). SALL4 is a marker for a progenitor subclass of HCC with an aggressive phenotype (Yong et al. 2013). Epithelial cell adhesion molecule (EpCAM) is expressed in several cancer and HCC stem cells and in tumor-initiating cells (TIC) (Yamashita et al. 2009; Terris et al. 2010; Imrich et al. 2012). In subsets of HCC stem cells, EpCAM is coexpressed with another stem cell marker, CD133, and cells with this phenotype represent tumor-initiating cells (TIC; Chen et al. 2012). In a recent investigation, it was shown that the two CSC markers of HCC, EpCAM and CD90, are independently expressed, in that EpCAM positivity characterized epithelial CSC, while CD90+ CSC had features of vascular endothelial cells, suggesting that more than one type of hepatic CSC may occur together and fulfill different functions (Yamashita et al. 2013). Liver cancer stem cells are also characterized by positivity for the Thomsen-Friedenreich core-1 protein, CD176 (Lin et al. 2011). Stemness features of HCC cells were analyzed by analysis of two stem cell characteristics, low proteasome activity and low intracellular reactive oxygen species/ROS. Isolated HCC with these two features demonstrated asymmetric divisions, tumorigenicity and a metastatic phenotype in immunodeficient mice, upregulated chemokine-related genes, and facilitation of macrophage migration in vitro (Muramatsu et al. 2013), suggesting that HCC stem cells not only affect tumorigenesis as such but also features of spread and interactions with tumor-associated cells such as macrophages. In HCC tumor cell lines kept in culture, a minor cell population expresses intercellular adhesion molecule 1 (ICAM-1). These ICAM-1-positive cells, which were also detected in human HCC and as circulating tumor cells in HCC patients, have greater sphere-forming and tumorigenic properties and represent tumor stem cells (Liu et al. 2013a). A second molecule involved in the regulation of cell adhesion is dysadherin. Dysadherin expression in liver cells characterizes a stem-like cell phenotype (Park et al. 2011). Part of HCC progenitor/stem cells are reactive for the 140 kDa isoform of neural cell adhesion molecule (NCAM) (Tsuchiya et al. 2011). A novel HCC stem cell marker is Delta-like 1 protein (Dlk-1), a surface protein expressed on fetal hepatic stem/progenitor cells but absent from mature hepatocytes in neonatal and adult rodent livers (Yanai et al. 2010). Overall, Dlk-1 is expressed in 20 % of all HCC but is even more frequently detectable in HCC diagnosed in young patients (Nishina 2012). Granulin-epithelin precursor (GEP) is a hepatic oncofetal protein expressed in fetal livers, but not in normal adult livers. Fetal liver cells containing GEP coexpress the stem cell-related signaling molecules beta-catenin, Oct4, Nanog, Sox2, and Dlk-1 and the CSC markers CD133, EpCAM, and ABCB5 (Cheung et al. 2011). The polycomb gene product BMI1 is critically involved in the self-renewal of somatic stem cells and participates in several types of tumorigenesis. BMI1 is expressed in subpopulations of HCC stem cells and plays a role in the maintenance of TICs in HCC (Chiba et al. 2008). HCV virus-induced CSC have a molecular signature containing doublecortin and CaM kinase-like-1 (Ali et al. 2011). A stemness-related molecule in HCC is cytokeratin-19 (CK19) (Lee et al. 2012a). Expression of CK19 in HCC confers increased EMT-related protein and mRNA expression and an invasive, aggressive phenotype (Kim et al. 2011; Lee et al. 2012).

HCC-Associated Stem Cells: Self-Renewing Cells with Various Biological Functions

HCC-associated stem cells promote tumor maintenance and progression.

HCCs can possess various self-renewing tumorigenic cell types, including CSCs sensu strictiori. These cells act as tumor-initiating cells (TICs) and tumor-propagating cells that express numerous morphologic and phenotypic features and distinct gene expression profiles (Colombo et al. 2011). CSCs of HCC express typical hepatic stem cell markers, such as CD133 and CD90, and their priming manifests in the expression of HCC lineage markers, as CD90(+) HCC-CSCs can also express glypican-3 (Ho et al. 2012). HCC-associated CSCs or stem-like cells are present in the tumors themselves, but they also occur in increased numbers in chronic liver disease, e.g., HCV-related liver cirrhosis (Behnke et al. 2013). A distinct property characterizing HCC stem cells is that they can initiate tumor growth in syngeneic or immunocompromised recipients. It is, however, difficult to judge whether such tumor-initiating cells are true CSC or non-CSC tumor cells that can give rise to growing tumors after cell transplantation (review: Sell and Leffert 2008). HCC-associated CSCs differ in several respects from other stem cells. In mouse HCC models, HCC progenitor cells give rise to fully developed cancer only when introduced into a liver showing chronic damage and compensatory hepatocyte regeneration, and tumor growth depends on cytokine stimulation, e.g., IL-6 signaling (He et al. 2013). CSCs occurring in HCC affect and modulate several biological functions, including maintenance of clonogenic cells with low proliferative activity but longevity, induction of distinct growth features (also in vitro; sphere formation, colony formation, anchorage-independent growth), and tumor spread and progression. There is also growing evidence that CSC are involved in the process of hepatocarcinogenesis itself, hepatic progenitor cells exposed to carcinogens progressively accumulating genetic alterations and genomic instability, in part acquiring the features of tumor-initiating cells, and finally showing a stably transformed phenotype (Xu et al. 2010; Kim et al. 2014; Machida et al. 2015). CSCs make part of a hierarchic cancer stem cell model for solid tumors such as HCC (review: Tong et al. 2011).

HCC-associated CSCs are not only involved in the initiation and maintenance of a given tumor but are also operational in HCC recurrence (HCC recurrence-associated stem cells, RASCs), the programming and establishment of metastatic niches (niche-associated stem cells, NASCs), and growth of metastases (metastasis-associated stem cells, MASCs). The pathways leading from the expression of stem cell markers to specific cancer cell behaviors are only partially elucidated. For example, expression of the stem cell marker, CD133, in HCC cells is associated with high capacity for tumorigenicity (Suetsugu et al. 2006; Yin et al. 2007) and confers an aggressive and invasive phenotype to HCC cells through the expression of metalloproteinase-1 (MMP-1) and a disintegrin and metalloproteinase (ADAM9; Kohga et al. (2010). Cytoplasmic expression of CD133 is an important risk factor for overall survival in HCC, specifically for stage III and IVA lesions (Sasaki et al. 2010).

HCC stem cells program epithelial-mesenchymal transition (EMT), a process critical for invasion and spread.

Epithelial-mesenchymal transition (EMT) is a process that occurs in many epithelial lineages and tumors derived thereof, characterized by the acquisition of a mesenchymal phenotype. The reverse process, mesenchymal-epithelial transition (MET) is less well known. EMT is driven by a distinct set of non-tissue-specific master transcriptional regulators (review: Cicchini et al. 2014). HCC stem cells are involved in the programming of EMT, a phenomenon which is crucial to invasion and spread of cancer cells and their interactions with the tissue microenvironment. On the other hand EMT creates a niche in which CSCs can settle and undergo differentiation. In this niche, with its features as an inflammatory microenvironment, mesenchymal stem cells can settle and accelerate HCC metastasis via induction of EMT (Jing et al. 2012). In cancers, EMT is chiefly characterized by disruption of intercellular contacts and connections leading to individualization of cells, enhancement of cell motility, and achievement of a primitive cell type capable to integrate into novel microenvironments (Guarino et al. 2007). In human HCC stem-like cells, the EMT promoter Twist2 induces self-renewal of these cells in a CD24-dependent manner (Liu et al. 2014). An EMT phenotype is

induced in hepatic oval cells by downregulation of microRNA-200a via targeting the beta-catenin signaling pathway (Liu et al. 2013b), which in turn affects the expression of cadherin, an important modulator of EMT. MicroRNA-200 upregulates vasohibin2 which in turn induced EMT and promotes HCC transformation (Xue et al. 2014). HCC cells that express the stem cell marker CD133 display more invasive characteristics by upregulation of invasion-associated genes and EMT-associated genes (Na et al. 2011). The relationship between EMT and a more invasive phenotype of cancers is linked to a distinct E-cadherin adhesion molecule repressor interactome in EMT (Hugo et al. 2011). Loss of E-cadherin, a typical feature of EMT, is associated with the acquisition of stem cell signatures and of cell with increased invasiveness (Nakagawa et al. 2014). NANOG, a major transcription factor essential for stem cell self-renewal, regulates self-renewal of CSC through the insulin-like growth factor pathway in human HCC (Shan et al. 2012) and promotes HCC invasion by inducing MEM, via activation of NODAL and CRIPTO-1 to promote SMAD3 phosphorylation and SNAIL expression. Interestingly, NANOG is preferentially expressed at the tumor edge, where invasion is most active (Sun et al. 2013a), suggesting that tumor stem cells may not be randomly distributed in liver cancer, but may rather populate distinct tumor areas. EMT can also be induced by hepatocyte growth factor secreted by hepatic stellate cells (HSCs), associated with upregulation of cancer stem cell-like properties in HCC cells (Yu et al. 2013a). The function of stem cell in the setting of EMT is modulated by tumor-associated macrophages (TAMs). In HCCs, TAMs promote cancer stem cell-like properties through TGF-beta1-induced EMT (Fan et al. 2014).

Stem Cells in HCC: Significance for Invasion, Spread, Stroma Formation, and Angiogenesis

A meta-analysis of HCC demonstrated that the presence of cancer stem cells was significantly associated with poor histological grade and elevated serum AFP levels, but there was no correlation between the presence of cancer stem cells and tumor size, tumor stage, or chronic liver disease/cirrhosis. Cancer stem cells in HCC were significantly associated with poor survival, including overall survival and disease-free survival, suggesting that the analysis of stem cells has an impact on the prognostication of HCC biology (Ma et al. 2013). HCC harboring CD133+ cells showed an aggressive, invasive phenotype, complicated by bile duct tumor invasion and thrombi (Yu et al. 2011). The reason why CSCs in HCC can significantly affect tumor biology and prognosis is related to several CSC-modulated mechanisms involved in tumor growth, invasion spread, and generation of a tumor-specific microenvironment. CSCs interact with and modulate the function of bone marrow-derived stromal stem cells (BMSCs), stem cells that are recruited to form tumor stroma and that are prone to undergo progressive changes similar to that of their cancerous partner cells, including telomere attrition due to telomerase failure (review: Saeed and Iqtedar 2013). CSCs contribute to neovascularization and angiogenesis through the production of proangiogenic factors, transdifferentiation of CSCs into endothelial and myoid cells, and formation of non-endothelium-lined channels called vasculogenic mimicry (reviews: Yu et al. 2010a, b; Barajas et al. 2007; Ping and Bian 2011; Zhu et al. 2012a, b). High expression levels of hepatic stem cell markers are related to tumor angiogenesis and poor prognosis in HCC. HCC with higher levels of CSC displayed higher levels of vascular endothelial growth factor and had an angiogenic pattern characteristic for aggressive tumors (Ho et al. 2006; Yang et al. 2010). Part of angiogenic effects exerted by hepatic CSC are linked to the angiogenic effects of CD133 (Akita et al. 2013a). Expression of CD133 in liver tumor-initiating cells (TIC) promotes tumor angiogenesis, growth, and cell renewal via a neurotensin / interleukin-8/CXCL1 signaling pathway, causing activation of p-ERK1/2 and RAF-1, components of the mitogen-activated protein kinase/MAPK pathway (Tang et al. 2012). A second stem cell factor that affects invasive properties of HCC cells is EpCAM. There is evidence that HCC growth

and invasiveness is dependent on a subset of EpCAM+ stem cells (Terris et al. 2010). In addition to the proangiogenic effect of CSCs, neovascularization /angiogenesis in malignant neoplasms is also accomplished by homing of angiogenic circulating progenitor cells to tumors. These cells are either angiogenic stem cells already primed for endothelial differentiation or homing MSCs that will upon their settling in the tumor be fated to become angiogenic cells (review: Melero-Martin and Dudley 2011). On the other hand, endothelial progenitor cells, through their angiogenic modulation of the tumor niche, affect HCC progression. These cells promote HCC intrahepatic metastasis via monocyte chemotactic protein-1 induction of microRNA-21 (Shih et al. 2014).

Cancer Stem Cells and Vasculogenic Mimicry

Vasculogenic mimicry (VM) was described in 1999 based on the detection of vascular channel formation by human melanoma cells (Maniotis et al. 1999). The term, VM, denotes a phenotype resembling the pattern of embryonic vasculogenic networks and describes the plasticity of cancer cells and their precursors forming de novo vessel-like channels lined by cancerous cells, whereby these channels contribute to perfusion and transport fluid from leaky vessels (Frenkel et al. 2008). Like this, the pseudovascular structures in VM contribute to neovascularization of rapidly growing malignancies in a mode that is independent of classical angiogenesis and vasculogenesis. The development of fluid-transporting spaces in VM facilitates metastatic spread (reviews: Folberg et al. 2000; Folberg and Maniotis 2004; Ping and Bian 2011; Kirschmann et al. 2012; Liu et al. 2012; Seftor et al. 2012). In cancers, two main patterns of VM are recognized, i.e., tubular VM and patterned matrix VM. In tubular VM, fluid-containing spaces are surrounded by tumor cells, while in patterned matrix VM laminin-reactive loops with PAS-positive laminas surrounding packets of tumor cells are seen (laminin-rich looping VM). Mainly in patterned matrix VM epithelial-mesenchymal transition (EMT) induced by Twist1 plays a significant pathogenic role whereby cancer cells can acquire a mesenchyme-like phenotype and may even become endothelial-like cells. In EMT-induced VM, CSCs are important actors, whereby EMT itself is potent for the acquisition and maintenance of stem-like features of cancer cells (Fan et al. 2013). Twist1 involved in EMT can stimulate cancer cell migration (Matsuo et al. 2009) and via this effect facilitates VM which requires tumor cell movements. In hypoxia-induced VM, Bcl-2 can induce a major endothelial adhesion molecule in tumor cells, i.e., VE-cadherin (Zhao et al. 2012). However, VM is resistant to antiangiogenic therapies (Fan and Sun 2010). Apart from factors regulating EMT, several other molecules are active in the promotion of VM, including the PI3-K signaling pathway, matrix metalloproteinases, laminin-5-gamma chain, protein tyrosine kinases, epithelial cell kinase (ECK), focal adhesion kinase, Eph receptors, Nodal, Notch4, hypoxia-inducible factor, galectin-3, bone morphogenetic proteins, cAMP signaling, cyclooxygenase-2, inhibitor of DNA binding 2/Id2, autophagy-related proteins, and VEGF (Hess et al. 2001; Hess et al. 2007; Sun et al. 2007; Lissitzky et al. 2009; Su et al. 2009; Fan and Sun 2010; Hardy et al. 2010; McAllister et al. 2010; Paulis et al. 2010; Che et al. 2011; Han et al. 2011a; Sun et al. 2012; Vartanian et al. 2013; Ding et al. 2014).

Both tubular and patterned matrix VM occur in HCC and is correlated with aggressiveness and poor clinical prognosis (Sun et al. 2006; Zhao et al. 2006; Guzman et al. 2007). Patterned matrix VM in HCC was associated with larger tumor, vascular invasion, high tumor grade, and late stage (Liu et al. 2011b). Similar to other cancers, VM induced by HCC cells is related to the induction of EMT, in that HCC cells in VM express the EMT inducer, Twist1 (Sun et al. 2010). Via EMT, Twist1 directly induces VM in hypoxic HCC cells (Ma et al. 2011) and promotes a metastatic phenotype (Lee et al. 2006). EMT-associated VM induced by Twist1 is stimulated by Bcl-2, which binds to and activates Twist1 under hypoxic conditions (Sun et al. 2011). Various factors present in EMT microenvironments are involved in

VM. The extracellular protease ADAMTS1 is a critical enzyme for cancer cells to acquire endothelial-like properties (Casal et al. 2010), and VM in HCCs is associated with the expression of osteopontin and matrix metalloproteinase-2 (Liu et al. 2011d). In the course of EMT-induced VM, poorly differentiated HCC cells can become CD31 positive and therefore phenotypically resemble endothelial cells (Zhao et al. 2007).

HCC-associated CSCs play a role VM. In the process of VM, a stem cell-like phenotype is involved, whereby cells forming the vascular channels express stemness markers, including CD133 (Yao et al. 2011; Lai et al. 2012; Valyi-Nagy et al. 2012). VM channel formation in HCCs is associated with EMT, dedifferentiation of tumor cells, and increased expression of stemness genes (Lirdprapamongkol et al. 2012). Stem cell-induced VM is particularly prevalent in EMT that develops in hypoxia. Expression of Slug, a potent inducer of EMT, in HCCs promotes the biogenesis and maintenance of a CSC subpopulation that is capable to produce VM (Sun et al. 2013b). EMT itself promotes the formation of HCC cells with stem-like properties via Twist1-Bmi1 signaling, Bmi1 belonging to the polycomb repressive complex 1 (Wu and Yang 2011), and this connection may favor EMT-induced VM mediated by cancer stem cells.

CSCs in Tumor Recurrence and Metastatic Spread: RASCs, NASCs, and MASCs

Tumor recurrence is traditionally viewed as a process that originates from remaining cancer cells that have not been radically removed and/or were resistant to chemo- or radiotherapy. However, within the population of recurrent HCC cells, cells with stem-like features were identified (Yi and Nan 2008; Xu et al. 2010a, b), and it is assumed that such cells contribute to recurrence and significantly modify the recurrence phenotype and pattern. For hepatic recurrence, recurrence-associated stem cells (RASCs) may either represent (a) surviving HCC-CSCs capable to resume growth following therapy, (b) novel stem cell species arising from the recurrent tumor, or (c) stem cells derived from an extrahepatic site and homing to the recurrent tumor with its new microenvironment. Tumor stem cells and their fully malignant offspring play a critical role in the biogenesis of metastatic niches. In human HCCs, expression of the two stem cell markers CD90 and EpCAM was higher in the early recurrence group (Guo et al. 2014). As niche-associated stem cells (NASCs), CSCs undergo a complex crosstalk with normal cells in the prometastatic niche, in that they secrete factors that affect stromagenesis, recruitment of tumor-associated macrophages (TAMs) and tumor-associated endothelial cells (TECs), and angiogenesis (Gupta et al. 2014). These factors include the TGF-beta/Smad system, PDGF, VEGF, and IL-6. Similar to the complex function of the portal fibroblast, which provided a niche for controlled cholangiocyte turnover (Wells 2014), prometastatic niches contain a fibroblastoid population that interacts with NASCs, with a mutual regulation of the cells involved. Part of fibroblastoid cells and angiogenic cells constituting the prometastatic niche are derived from mesenchymal stem cells (MSCs) that circulate in the blood and home to the tumor microenvironment (Wang and Chen 2013), where they interact with tumor cells and NASCs. The mode of this interaction is not yet well known, but MSCs rarely fuse with HCC cells in murine models (Li et al. 2013). HCC cells and their stromal component themselves modulate the migratory behavior of MSCs (Garcia et al. 2011). As the tumor microenvironment is often hypoxic and/or deficient in nutrients, niche-associated tumor cells have developed strategies to circumvent this stress situation. CD133(+) HCC-CSCs showed higher survival, less apoptosis, and higher clonogenicity under hypoxic and nutrient deprivation stress, in part associated with increased autophagy (Song et al. 2013). CD133 is involved in the regulation of autophagy and glucose uptake (Chen et al. 2013d). Following their exit from a primary or recurrent tumor, or a metastatic niche, RASCS and NASCs enter circulation and can become metastasis-associated stem cells or MASCs. There is evidence that circulating HCC stem cell-like cells capable to generate metastatic recurrence (MASCs) express certain markers that allow their identification, such as the stem cell marker CD90 (Yamashita et al. 2013), the oncofetal

marker Lin28B (Cheng et al. 2013), and the G protein-coupled receptor 87/GPR87 (Yan et al. 2013). Expression of CD133 in liver CSC plays a critical role in hematogenous metastasis of HCC (Hou et al. 2012; Chow et al. 2013). CD133-positive CSCs play a role in repeated pulmonary recurrence of HCC (Toshima et al. 2013). In regard to prognostication, the various HCC stem cell markers differ in several respects. While expression of CD133 was more frequently found in small tumors in cirrhotic livers and early stage disease, expression of EpCAM characterized small tumors and poor differentiation and was an independent prognosticator at all stages (Chan et al. 2014). Can circulating CSCs rehome to primary cancers or metastatic niches and modulate the features of their tumor of origin?

Tumor Stem Cells in Nontumorous Liver Tissue

CSC are readily detectable in HCC tissue itself by means of their distinct marker profile, but cells with similar or the same features also occur in the nonneoplastic parenchyma of livers harboring HCC. Cell lines producing colonies in soft agar and showing anchorage-independent growth were isolated form liver resections with HCC, and these cells revealed the marker profile of CSC (Zhang et al. 2010).

Cells with Stem Cell-Like Features in Special Variants of Hepatocellular Carcinoma and in Precursor Lesions

Tumor stem cells seem to be involved in pathogenesis of scirrhous HCC. In this variant of HCC, small neoplastic cells were reactive for cytokeratin 7 and ATP-binding cassette transporter G2. Part of these cells (termed type 1 cells) stained for cytokeratin 19, neural cell adhesion molecule (NCAM), and epithelial cell adhesion molecule, while another subset (termed type 2 cells) did not show this immunophenotype. In type 3 scirrhous HCC, no small tumor cells with stem cell features were present. It was suggested that type 1 and type 2 cells in scirrhous HCC correspond to a side population of cultured cells (Fujii et al. 2008). Apart from conventional HCC, features of stemness are also present in dysplastic hepatocytes (Lingala et al. 2010), fibrolamellar hepatocellular carcinoma (Zenali et al. 2010), and hepatoblastomas (Akita et al. 2013).

Stem Cells in Hepatoblastomas

Hepatoblastomas (HBs) have been shown to contain cells with stem-like features. Hepatoblasts, a putative normal counterpart of HB, show a pattern of proteins shared by hepatic stem cells, i.e., CK19, human epithelial antigen-125/HEA-125, and OV-6 (Crosby et al. 1998). This has in particular been observed in HBs of low differentiation, such as small cell undifferentiated HB (see the respective chapter). In subsets of HB, expression of CK19, Oct-3/4, and EpCAM was observed (Yun et al. 2013). HB cells can express proteins that are present in human fetal liver, including delta-like protein/DLK, a membrane protein of unknown function (Dezso et al. 2008; Yun et al. 2013).

Biogenesis of HCC-Associated Stem Cells

Mechanisms of Stem Cell Formation in the Liver and Liver Neoplasms

Recent findings showed that several mechanisms can elicit tumor stem cells and stem cell signatures in normal liver, regenerating liver, and HCCs. They comprise the expression of transcription factors (Sox2, c-Myc, LIN28, NANOG), several signaling pathways (TGF-beta, Wnt/beta-catenin signaling), factors modulating the cancer microenvironment (Notch signaling), microRNAs, and differential DNA methylation patterns (reviews: Zheng et al. 2013; Bogaerts et al. 2014; Dang et al. 2014). Expression of transforming growth factor beta play an important role in signaling pathways taking place in hepatic and intestinal stem cell niches (review: Majumdar et al. 2012).

Chronic and constant TGF-beta stimulation results in the generation of hepatoma-initiating cells derived from hepatic progenitor cells (Wu et al. 2012), and TGF-beta secreted by HCC cells mediates the crosstalk between cancer cells and the tumor microenvironment (Gupta et al. 2014).

How are the distinct growth and survival features of CSCs regulated, in particular their critical capacity for self-renewal? Overall, the expression of stemness-related proteins by HCC cells is related to increased telomere lengths, increased expression of hTERT and shelterin complex proteins, and increased chromosomal/genomic instability in comparison with conventional HCC (Idrissi et al. 2013). Maintenance of telomeres is compromised in HCCs, and it is expected that a derangement of the telomere platform may be an early event in hepatocarcinogenesis, eventually already involving CSCs (Ye et al. 2010). Specifically, there is evidence that abnormal stem cells may lack telomerase, an enzyme responsible for telomere extension (reviews: Gardano et al. 2013; Saeed and Iqtedar 2013). Telomeres are the terminal elements at the ends of linear chromosomes and are critically involved in the prevention of exonucleolytic degradation, inter- and intrachromosomal fusion, and subsequent chromosomal instability (Ye et al. 2010; Calado and Dumitriu 2013). Telomere integrity is critically required for the unlimited replicative potential of cancer cells. The stability of telomeres depends on the binding of the six-protein subunit complex shelterin to telomeric repetitive DNA sequences (Palm and de Lange 2008; Xin et al. 2008). Further proteins playing a role in telomere stability are the sirtuins (Rodriguez et al. 2013). Telomerase elongates telomeres and is recruited by homeobox telomere-bending protein1/HOT1 (Kappel et al. 2013). Furthermore, the subunit telomerase reverse transcriptase (TERT) has additional functions, including transcriptional regulation and metabolic reprogramming (Low and Tergaonkar 2013). All telomeric proteins involved in telomere function form, together with the telomere proper, the telosome (Liu et al. 2004; Folini et al. 2009).

Epigenetic mechanisms play a central role in the generation of hepatic stem cells and side population cells occurring in HCC. Side population cells of HCC have a high rate of DNA hypermethylation as compared with their corresponding non-side population cells, and the differentially methylated genes in side population cells were involved in numerous signaling pathways (Zhai et al. 2013). Among stem cell molecules regulated by epigenetic mechanisms is CD133, this regulation pathway involving TGF-beta1 which inhibits DNA methyltransferase 1 and DNA methyltransferase 3beta expression and subsequent demethylation of promoter-1 (You et al. 2010). CD133 expression is also promoted by hypoxia, in that hypoxia-inducible factors activate CD133 promoter via ETS family transcription factors (Ohnishi et al. 2013). Certain transcription factors and transcription repressors are preferentially active in stem and progenitor cells and affect epigenetic mechanisms. BORIS (brother of regulator of imprinted sites) is a protein encoded by the CTCFL gene, is a paralog of the transcription suppressor CTCF (Tiffen et al. 2013), and is an ubiquitous 11-zinc finger protein with highly versatile functions, including transcriptional silencing and organization of epigenetically controlled chromatin insulators that regulate imprinted genes in the soma both in normal and cancerous tissues (Klenova et al. 2002; Loukinov et al. 2002; Rosa-Garrido et al. 2012). As BORIS expression in HCC is correlated with expression of the stem cell marker CD90, BORIS may itself be a property of hepatic and HCC-associated stem cells (Chen et al. 2013b).

Pathways from Primed Stem Cells to Differentiated Cells

In parallel to normal stem cells it may be anticipated that CSC can undergo priming to more differentiated neoplastic cells. This is an important study field, because it potentially opens pathways for novel treatment strategies. One factor involved in the switch turning CSC to differentiated cells is the Hippo pathway. Hippo signaling

has a dual regulation of Hippo in liver tumor suppression as well as a transition of oval stem cells to fully differentiated hepatocytes and hepatocyte-like cells in tumors (Zheng et al. 2011). A second crucial pathway involved in the phenotypic shift of CSC to a more differentiated offspring is the WNT/beta-catenin-signaling pathway (Yang and Poon 2008). Expression of beta-catenin is pertinent in hepatic oval cell activation and differentiation (Nejak-Bowen and Monga 2011), and it is expected that the same mechanism is operational in HCC and related neoplasms. As discussed in the respective chapter, immature cells forming undifferentiated clusters in hepatoblastomas are markedly reactive for beta-catenin and seem to give rise to more differentiated but rapidly proliferating offspring surrounding the stem-like cell clusters (Zimmermann 2005).

Regulation of Stem Cells by microRNAs and Other Noncoding RNAs

The function of HCC-associated stem cells is itself regulated by several factors and signaling pathways, including microRNAs (reviews: Chai and Ma 2013; Qi et al. 2013; Chen and Verfaillie 2014). In HCCs and HCC cell lines several and in part specific microRNAs have been identified (Xu et al. 2013). miRNA-130b was shown to promote liver tumor-initiating cell growth and self-renewal through induction of nuclear protein 1 by tumor p53 (Ma et al. 2010). In isolated subsets of side population cells, silencing of microRNA-21 reduced migration and invasion of cancer cells, while overexpression of mRNA-21 drastically inhibited the expression of PTEN, RECK, and PDCD4 proteins (Zhou et al. 2013). MicroRNA-142-3p regulates the expression of the functional hepatic cancer stem cell marker CD133 (Chai et al. 2014). microRNA-181 is highly expressed in embryonic liver tissue and in isolated hepatic stem cells. Specifically, inhibition of microRNA-181 resulted in reduction in EpCAM+ HCC cells and tumor-initiating capacity (Ji et al. 2009). microRNA-150 inhibits human CD133+ liver cancer stem cells through negative regulation of the transcription factor cMyb, associated with downregulation of the cell cycle regulator cyclin D1 and the cell survival regulator Bcl-2 (Zhang et al. 2012). micro-612 suppresses the stemness of HCC CSCs via the Wnt/beta-catenin-signaling pathway (Tang et al. 2014). The tumorigenicity of cancer stem-like cells derived from HCC is regulated by microRNA-145, probably through modulation of the downstream target, the stem cell-related gene Oct4 (Jia et al. 2012; Wang et al. 2013). microRNA-148a inhibits EMT and several CSC properties and by this impairs metastasis of HCC (Yan et al. 2013). Lin-41, a stem cell-specific E3 ligase and a target of the tumor suppressor microRNA let-7 mediates ubiquitinylation and degradation of the microRNA pathway protein Ago2. Lin-41 is overexpressed in HCC, where it is involved in growth control through regulating the RISC complex proteins Ago1 and Ago2 to inhibit microRNA-mediated gene silencing and promoting the expression of oncogenic proteins (Chen et al. 2013a). Lin-41 expression in HCC confers an aggressive phenotype associated with early recurrence and poor survival, suggesting that Li41 is involved in HCC CSC. Hepatic stem cell biology is also affected by long noncoding RNAs. Oncofetal long noncoding RNA PVT1 promotes proliferation and stem cell-like properties of HCC cells by stabilizing NOP2 (Wang et al. 2014).

Modulation of Stem Cell Biogenesis and Function by Microvesicles and Exosomes: Can Cancer Stem Cells "Infect" Other Cells and Transmit Them a Malignant Phenotype?

Mesenchymal stem cells (MSC) as a potential source of hepatic cells possess a complex stem cell secretome that can be transferred to other cell systems through exosomal pathways (Bruno et al. 2013; Kupcova 2013; Xiong et al. 2013). Microvesicles of MSC can serve as trophic shuttles for diverse cell types, including stem cells (Aliotta et al. 2012; Mokarizadeh et al. 2013) and act as a complex paracrine information system within cell interactomes (Quesenberry and

Aliotta 2010), e.g., in regeneration and tissue repair (Camussi et al. 2010; Anthony and Shiels 2013) and the induction of epithelial-mesenchymal transition (Garnier et al. 2013). Via this mechanism, stem cell properties are maintained, while loss of the pathway may result in differentiation of stem cells (Bauer et al. 2011). Specifically, microvesicles released from stem cells confer a stem cell-like phenotype to other stem cells and damaged cells, evoking stem cell maintenance and self-regenerative programs (Herrera et al. 2010; Quesenberry et al. 2012; Camussi et al. 2013), but they may also transfer stem cell-like features to neoplastic cells being in close contact with them (Muralidharan-Chari et al. 2010; Lee et al. 2011; Pap 2011).

A critical property of stem cell microvesicles and exosomes is their genetic information cargo, i.e., they are loaded with microRNAs and mRNAs, genetic information that can be transferred among cells through microvesicle release (Yuan et al. 2009; Deregibus et al. 2010; Lee et al. 2012b). The molecular mechanisms by which genetic information such as microRNAs are loaded into microvesicles/exosomes are not yet well known, but distinct RNA-related zipcode-like sequences seem to act as sentinel sequences for these processes (Bolukbasi et al. 2012). Multipotent mesenchymal stem cells can affect and modulate their partners and other cells through CD133/prominin-1, contained in lipid rafts of these cells, by release of microvesicles. Similarly, cancer cells possess exosomes containing CD133 and numerous other proteins and microRNAs that are involved in metastatic pathways (Rappa et al. 2013). Human liver stem cell-derived microvesicles inhibit HCC growth in immunodeficient mice by releasing antitumor microRNAs (Fonsato et al. 2012). Informational cargo transported and transmitted by exosomes also modulates processes that are associated with and required by stem cell-driven cancer growth and spread. Endothelial progenitor cells located within tumors produce microvesicles that activate an angiogenic program in endothelial cells by horizontal transfer of specific mRNAs (Deregibus et al. 2007).

Systemic and Hepatic Mesenchymal Stem Cells and Stem-Like Cells: Modulation of HCC Biology

In murine models, there is evidence that HCC may originate from genetically mutated MSCs (Gong et al. 2013). MSC interact with HCC cells and modulate their behavior and affect interactions of HCC cells with stromal and vascular cells. In the nude mouse tumor transplantation model, BMSCs could promote the growth of small vessels and increased microvascular density (Gong et al. 2013a, b; Kupcova 2013), and in another murine HCC model, hematogenic MSC could modulate their microenvironment via secreted cytokines that promoted tumor initiation, growth, and homing to tumor sites (Gong et al. 2013). Hepatic stellate cells (HSC) and their precursors are specific mesenchymal cells of the liver that exert a profound impact on growth, differentiation, and morphogenesis of several other hepatic cell systems and neoplasms derived thereof (review: Yin et al. 2013).

Dormant Cancer Stem Cells

Dormant cancer stem cells (D-CSC) are defined as cells that remain intact after apparent eradication of cancer, including HCC, and are capable to maintain a transformed phenotype and to generate recurrent cancer. In one study, such D-CSC were detected in human HCC cell population grown in immunodeficient murine hosts after chemotherapy in the form of human AFP+/CD13+/PCNA cells (Martin-Padura et al. 2012).

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Hepatocellular Carcinoma (Ordinary Hepatocellular Carcinoma)

2

ICD-O code 8170/3

Contents

A. Zimmermann, *Tumors and Tumor-Like Lesions of the Hepatobiliary Tract*,
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Abstract

Hepatocellular carcinoma (HCC) is a malignant neoplasm composed of cells with a hepatocellular differentiation. Most HCCs exhibit a trabecular and/or acinar histologic pattern (ordinary or classical HCC), but there also exist numerous variants with a different morphology and a fibrolamellar variant treated in a separate chapter. HCC is a frequent malignancy and is estimated to be the fifth most common malignancy in males worldwide. Most of the tumors arise in patients with liver cirrhosis caused by various etiologies, hepatitis virus infections, and toxic agents playing a central role. HCC displays specific macroscopic growth patterns. Eggel divided the neoplasms in nodular, massive, and diffuse types, nodular HCC being the most common. This classification has subsequently been extended and refined. HCC frequently invades large and small hepatic vessels and bile ducts, causing distinct clinical disorders. Histologically, hepatocyte-like cells arranged in large plates with an abnormal reticulin pattern and loss of Kupffer cells are the hallmarks of trabecular HCC, whereas pseudoglandular structures are found in other tumors. The grading of HCC has been standardized and is an important prognosticator. Morphological classifications of HCC are currently extended by the use of molecular signatures of these neoplasms.

Introduction

Hepatocellular carcinoma (HCC) is a malignant neoplasm composed of cells with a hepatocellular differentiation. The majority of HCC shows trabecular and/or pseudoglandular/acinar patterns that consist of a hepatocyte-like cell lineage (ordinary, "classical," or "standard" HCC). In addition, there exist numerous variants of ordinary HCC and a distinct entity, fibrolamellar hepatocellular carcinoma, treated in separate chapters.

Eggel (1901) was the first to clearly state that primary liver cell cancer is derived from hepatocytes. Apart from the morphologic resemblance between hepatocytes and hepatoid cells in moderately to well-differentiated HCC, a crucial argument of Eggel was his discovery of bile depositions in 6 of the 117 histologically examined tumors that he compiled from the literature, by adding a case of his own. Identification of bile in and between tumor cells still is a hallmark for the diagnosis of HCC.

Epidemiology

Liver cancer is a frequent malignancy worldwide with an incidence that is particularly high in all low-resource regions of the world, except Northern Africa and Western Asia. It is estimated that 82 % of HCC occur in developing countries (Leong and Leong 2005; Chuang et al. 2009). Estimates from the year 2004 show that HCC is the fifth most common malignancy in males and the eighth in females worldwide (Table 1).

About 564,000 new cases are diagnosed per year, with 398,000 in men and 166,000 in females, illustrating that HCC is two to four times more common in men than in women (reviews: Bosch et al. 2004, 2005). The yearly incidence comprises between 2.5 % and 7 % of patients with liver cirrhosis (Montalto et al. 2002). The highest reported incidence of 100 cases per 100,000 of population annually had been reported from Mozambique, due to high prevalence of HBV infection followed by cirrhosis, aflatoxin

Table 1 Age-adjusted incidence of HCC per 100,000 inhabitants (Llovet et al. 2003; modified)

Area	Age-adjusted incidence (males/females)
Worldwide	14.9/5.5
East Asia	35.4/12.6
Middle Africa	24.2/12.9
Southeast Asia	18.3/5.7
Southern Europe	9.8/3.4
Western Europe	5.8/1.6
South America	4.8/3.6
North America	4.1/1.6
Northern Europe	2.6/1.3

and alcohol toxicity, and possible iron overload. The peak age of onset of HCC is continuously rising worldwide, and there is evidence that the clinical features in the elderly differ from those in younger individuals. In a study of 622 patients with HCC, including 91 patients 70 years old or older, the proportion of females increased, and tumor sizes at diagnosis were smaller in the elderly than in younger patients, whereas clinical stage taking liver function into consideration was similar in the two age groups (Nomura et al. 1994). Whereas an increase in incidence has been noted in certain regions, the incidence of HCC has, e.g., been static over recent decades in the Asia Pacific region (Yuen et al. 2009).

HCC is closely associated with liver cirrhosis (Siegenbeek van Heukelom 1894; Kew and Popper 1984; Gall 1960; Fattovich et al. 2004), the main causes of this chronic liver disease being hepatitis virus infections, alcoholic liver disease, and nonalcoholic fatty liver disease (NAFLD). The development of HCC in a background of cirrhosis varies as a function of underlying disease. Particularly high incidences of HCC in cirrhotic liver are found in HCV infection and hereditary hemochromatosis. HCV infection was found to have the highest HCC incidence in cirrhotic patients, with a 5-year cumulative incidence of 17 % in Western countries and 30 % in Japan. In the cirrhotic stage of hemochromatosis, the 5-year cumulative incidence was 21 %. Lower values are observed in other important causes of cirrhosis, i.e., HBV infection, alcoholic liver disease, and various forms of biliary cirrhosis. In high endemic areas of HBV infection, the 5-year cumulative incidence of HCC is 15 %, while it is 10 % in Western countries. Overall, alcoholic liver cirrhosis has a 5-year cumulative HCC risk of 8 % (review: Fattovich et al. 2004). Male gender and age more than 50 years are risk factors for HCC in cirrhotic patients. A subset of HCC develops in the absence of cirrhosis (Tabarin et al. 1987; Giarelli et al. 1991). In an autopsy study from Japan, non-cirrhotic cases among 618 HCC patients amounted to 10.7 % (Okuda et al. 1989). In Western countries, HCC in non-fibrotic livers was associated with younger age and female sex (Bège et al. 2007).

It seems that the earliest true primary HCC was a case reported in Frerich's series in 1861, followed by a bona fide example of HCC in a cirrhotic liver with invasion of the inferior vena cava, reported in 1876 (Weigert 1876). It is striking to note that in early works on the incidence of HCC in Europe, relatively few cases were found in a clinical setting or in autopsy series in comparison with metastatic liver disease. In his work on liver tumors, Thöle (1913) cites that von Hansemann (a pathologist, assistant of Virchow, who formulated the concept of anaplasia) found 225 metastases and 6 HCC among 258 malignancies observed until 1889 and that Ahlenstiel detected 95 hepatic malignancies in 6,000 autopsies, of which 90 were metastases and 5 primary liver cancer. Similarly, Mau identified, in 1901, 246 hepatic malignancies among 8,587 autopsies performed in Hamburg, Germany, including 242 metastases and 4 primary liver cancers. Riesenfeld (1868) diagnosed liver cancer in 2.3 % of autopsies performed in Berlin between 1864 and 1868. Among 24,400 consecutive autopsies performed at Bellevue Hospital, New York, from 1906 to 1936, 62 cases of primary hepatic carcinoma were identified (Gustafson 1937). Between 1850 and 1950, many publications were written on single cases and small series, and numerous later studies and reviews have then addressed issues of occurrence patterns and epidemiology of HCC.

Selected References Anthony 1973; Sabourin 1881; Okuda et al. 1985; Okuda 1990; Dominguez-Malagon and Gaytan-Graham 2001; El-Serag 2002; Llovet et al. 2003; Bosch et al. 2004, 2005; Srivatanakul et al. 2004; Lee et al. 2009; McGlynn and London 2011.

Clinical Features

General Features

In early HCC, many patients are asymptomatic. In the absence of distinct complications, symptoms and signs in symptomatic patients are nonspecific and in part related to underlying chronic liver

disease, in particular hepatic cirrhosis. They include upper abdominal discomfort or pain, effects of hepatomegaly, malaise, anorexia, weight loss, and anemia (Berman 1959). In one European study, malaise (85 %), weight loss (78 %), anorexia (67 %), and hepatomegaly (84 %) were common findings at presentation (Kaczynski et al. 2005). In advanced disease, signs of hepatic failure and jaundice may ensue. In patients with underlying cirrhosis, sequelae of portal hypertension are often the leading clinical signs. Serologically, several markers for HCC and HCC recurrence have been identified, but AFP and its variants remains the most frequently used tumor marker for the diagnosis of HCC (Vogel et al. 1974; Gonzalez and Keeffe 2011; Nakao and Ichikawa 2013). However, a significant proportion of HCC patients do not show elevated serum AFP levels (low-AFP HCC; Carr et al. 2010). Serum AFP is employed for monitoring patients' response to therapy. Specifically, monitoring of *Lens culinaris* agglutinin-reactive AFP (AFP-L3, the fucosylated variant of AFP) is useful for early detection of recurrent HCC (Okuda 2000; Giannelli and Antonaci 2006). Elevated serum AFP is not only a bystander, but the protein exerts distinct functions. HCC patients with high or very high AFP serum levels show a higher mortality rate, probably related to a tumor growth-promoting activity of AFP (Li et al. 2011). It was earlier reported that AFP has immunosuppressive properties and induces spontaneous T and B lymphocyte responses in HCC patients. These responses are related to distinct immunogenic epitopes of the AFP molecule (review: Bei and Mizejewski 2011). AFP is also capable to activate monocytes and to augment phagocytic capacity of both monocytes and granulocytes (Kong et al. 2012), but abolishes the activation of natural killer cells by inhibiting the function of dendritic cells (Yamamoto et al. 2011).

Assessment of des-gamma-carboxy prothrombin (PIVKA-II), an abnormal prothrombin secreted by HCC, by sensitive assays has an important place in the diagnosis and follow-up of HCC (Takikawa et al. 1992; Bertino et al. 2012). In one study of 116 HCC, PIVKA-II was detected in serum in 54.3 % of patients and the concentration showed a positive correlation with tumor size (Takikawa et al. 1992). Further serum markers for HCC include glypican-3 and Golgi protein 73 (Giannelli and Antonaci 2006; Tian et al. 2011). Serum carcinoembryonic antigen (CEA) is raised in part of HCC patients (Macnab et al. 1978). For small or early HCC, reliable serum markers have not yet been established (Talwalkar and Gores 2004).

Paraneoplastic Syndromes

Hepatocellular carcinomas can be associated with a wide array of paraneoplastic syndromes (Table 2).

Complications

HCC may cause several types of complications. Rupture of HCC is a particularly severe

Table 2 Paraneoplastic syndromes associated with hepatocellular carcinoma

Hematologic paraneoplastic syndromes
Erythrocytosis (polyglobulism, erythremia)
Thrombocytosis
Leukemoid reactions (paraneoplastic neutrocytosis)
Peripheral hypereosinophilia
Paraneoplastic dermatological syndromes (paraneoplastic dermadromes)
Prurigo
Papuloerythroderma
Lichen planus
Lichen myxedematosus
Psoriasiform dermatoses (psoriasis guttata)
Pemphigus-like disorders
Lupus erythematosus-like syndromes
Acanthosis nigricans
Acquired perforating dermatoses
Papuloerythroderma of Ofuji
Acrokeratosis paraneoplastica (Bazex syndrome)
Disseminated porokeratosis
Pityriasis rotunda
Acquired porphyria

(*continued*)

Table 2 (continued)

Neurological paraneoplastic syndromes
Necrotizing leukoencephalopathy
Demyelinating polyradiculoneuropathy
Encephaloradiculopathy
Cerebellar ataxia
Necrotizing myelopathy
Demyelinating polyneuropathy
Peripheral neuropathy
Retinopathy
Autoimmune paraneoplastic syndromes
Dermatomyositis/polymyositis
Autoimmune arthritis/polyarthritis
Myasthenia gravis and other muscle weaknesses
Autoimmune thyroid disease
Behçet's disease
Metabolic disorders
Hypoglycemia
Hypercalcemia
Hypophosphatemia
Paraneoplastic hyperlipidemia and hypercholesterolemia
Hormonal syndromes
Paraneoplastic hyperthyroxinemia
Inappropriate ADH secretion
Coagulation disorders
Acquired von Willebrand disease
Cryofibrinogenemia
Combined paraneoplastic syndromes
Fever of unknown origin (FUO)

complication of this neoplasm and bears a high mortality. Rupture may occur spontaneously or following blunt abdominal trauma (Ong and Taw 1972; Chearanai et al. 1983; Van Landingham et al. (1985); Clarkston et al. (1988); Kanematsu et al. (1992); Chen et al. (1996); Castells et al. (2001); Chedid et al. 2001; Polat et al. 2005; Lai and Lau 2006; Kim et al. 2008). Spontaneous rupture of HCC can also occur in pediatric HCC (Nejmeddine et al. 2010). Localization of tumor to the left lobe and expansion of HCC outside the liver surface are risk factors for tumor rupture (Chen et al. 1995). Spontaneous rupture of HCC with hemoperitoneum is a rare condition in Western countries (Pombo et al. 1991; Chedid et al. 2001), while over 10 % of patients with HCC may experience tumor rupture in certain regions of Africa and Asia (Nagasue and Inokushi 1979). Spontaneous rupture of HCC was found to be the cause of death in only 3 % of patients in one study of 530 HCC patients (Kaczynski et al. 2005). Disruption of the tumor surface and a tear in a parasitic feeding artery are typical causes of HCC rupture (Chen et al. 1995; Kim et al. 2008). Vascular injuries that favor spontaneous rupture include degradation of collagen in the vascular wall (Zhu et al. 2001, 2002) and immune complex-induced damage of elastic lamellae (Zhu et al. 2004, 2006). Risk factors for HCC rupture included underlying disease such as hypertension and cirrhosis, a tumor size exceeding 5 cm, tumor protrusion from the liver surface, vascular thrombus, and extrahepatic invasion (Miyoshi et al. 2011; Zhu et al. 2012). HCC rupture can cause massive hemoperitoneum (Gibbons and Bell 1963). Parts of HCC present with jaundice, which is a negative prognosticator. In a minority of jaundiced patients, hyperbilirubinemia is due to biliary obstruction caused by the tumor, a situation outlined in more detail below. In the majority of patients, however, jaundice is caused by hepatic insufficiency caused by tumor and/or associated liver cirrhosis. Among 530 HCC patients with clinically detectable jaundice, 481 had jaundice due to hepatic insufficiency and 49 patients had obstructive jaundice. Patients with hepatic insufficiency had extremely poor prognosis, and 90 % of them died within 10 weeks of first presentation (Lau et al. 1997).

Imaging Features

Imaging features of HCC have been described in detail in numerous publications and are not specifically dealt with in this chapter (Okuda 1980a; Vermess et al. 1985; Stevens et al. 1996; Lee et al. 2011). Diagnostic imaging of HCC has recently undergone marked progress, mainly due to the introduction of the ultrasound contrast agent Sonazoid used in contrast-enhanced US (CEUS) and MR techniques employing a liver-specific gadolinium MR contrast agent (Ogawa et al. 2006; Xu et al. 2011; Tanaka et al. 2014; review: Kudo 2011).

Pathology

Introduction

As outlined in the paragraph on classification, HCCs are characterized by several distinct macroscopic patterns. The preparation and documentation of specimens for the identification of gross features has been standardized (Ruby 2000), as has the reporting of tissues removed (Dabbs et al. 2004; Association of Directors of Anatomic and Surgical Pathology 2005). Similarly, the definitions and nomenclature of nodular liver cell lesions has been standardized in a consensus panel (International Working Party 1995). In this paragraph, the morphology of advanced HCC is discussed, while the pathology of early and small HCC (Wanless 2007) is discussed in a separate chapter.

Selected References Rindfleisch 1878; Eggel 1901; Wegelin 1905; Rosenberg and Ochsner 1948; Edmondson and Steiner 1954; Edmondson 1955; Berman 1959; Patton and Horn 1964; Foster and Berman 1977; Mori et al. 1980; Anthony 1999; Kai et al. 2012.

Both the macroscopic growth patterns and the histopathologic presentation of HCC vary as a function of geography and genetic background, and between individual patients (Okuda et al. 1984; Okuda 1997). There are certain geographic specificities in regard to the presentation of diffuse and other variants of HCC and the type of underlying liver disease. This issue has been outlined by Okuda (1997) who emphasized that there are significant global variations of clinicopathologic features of HCCs, based on comparative studies of patients from Japan, Los Angeles, and Pretoria in South Africa (Okuda et al. 1984). For example, most patients from Japan show HCCs growing in an expanding fashion, with formation of a fibrous capsule, while capsule formation was very uncommon in South African Blacks. Depending on its gross growth patterns, primary HCC can involve one liver lobe or both liver lobes, whereby bilobar involvement had been found more frequently in HCC not associated with cirrhosis (Ho et al. 1981).

Classifications

Clinical Classifications

Several classifications of HCC are based on clinical and staging data (Llovet et al. 2003; review: Llovet 2007). The Barcelona Clinic Liver Cancer (BCLC) classification is an important classification for the clinical management of HCC and design of trials, endorsed by EASL and AASLD guidelines. It links stage stratification with a recommended therapy strategy. Other systems include the Cancer of the Liver Italian Program (CLIP) investigators, the Chinese University Prognostic Index (CUPI), and French staging systems, forming scoring systems predicting outcome in patients with advanced-stage disease.

Classifications Based on Macroscopic Presentation and Growth Patterns

Virchow separated primary from secondary liver cancer. However, in the thesis of Riesenfeld performed under Virchow's guidance (1868), gallbladder cancer was still regarded as a primary liver cancer. A fundamental step into the direction of modern pathology classifications of HCCs was the work of Eggel (1901) who divided liver cancers into nodular, massive, and diffuse forms. This and subsequent classifications are discussed in detail in the paragraph on tumor macroscopy.

Originally, primary HCC was divided into two major macroscopic groups, i.e., nodular carcinoma formed of discrete nodules of varying size and massive carcinoma, consisting of one large tumor mass. Hanot and Gilbert (1888) divided HCCs into three forms, i.e., nodular cancer ("cancer nodulaire," several to numerous nodules of varying size), massive cancer ("cancer massif," one large tumor mass), and cancer with cirrhosis, i.e., they proposed a classification based on tumor morphology on the one hand and underlying liver disease on the other hand. This system had

intrinsic inconsistencies of logic and was, therefore, applicable only with difficulty or not at all, because also nodular and massive cancers of course develop in cirrhotic livers. Already Eggel (1901) put his finger on the fact that Hanot and Gilbert themselves allocated one cancer in cirrhosis each to a nodular and a massive tumor.

A major step ahead is based on the novel classification of Eggel (1901), as further outlined below. Early comments on this classification were reviewed by Wegelin (1905). Today we know that HCCs show a characteristic spectrum of macroscopic growth patterns, comprising usual and unusual gross appearances (review: Kai et al. 2012). The macroscopic growth of HCCs can be classified according to several criteria (Table 3).

Table 3 Classification of HCC growth patterns

Eggel's classification (1901):
Nodular HCC
Massive HCC
Diffuse HCC
Kaufmann's classification (1958)
Large massive node
HCC in cirrhotic liver
(a) Multiple nodules
(b) Diffuse growth
(c) Combination of nodules and growth
Okuda classification (1984)
Expanding
Spreading
Multifocal
Surgical classification:
Invading lesions ("invaders")
Expanding lesions ("pushers")
Pedunculated lesions ("hangers")
Classification of the Liver Cancer Study Group of Japan (LCSGJ):
Nodular HCC (distinctly nodular type)
Simple nodular (SN) type
Simple nodular type with extranodular growth (SNEG)
Confluent multinodular type (CMN)
Nodular HCC
Small nodular type with indistinct margins (vaguely nodular type)
Classification according to size:
Small HCC
Solitary large HCC

In Eggel's investigations, nodular HCCs accounted for 64.6 %, massive for 23 %, and diffuse cancers for 12.4 %. Herxheimer in Germany (1930) arrived at similar figures (65 %, 28 %, and 7 %, respectively). In rare instances, a mixture of all three patterns is present. Eggel noted that mainly the massive form of HCC resulted in marked hepatomegaly, but hepatomegaly was in fact found in most of Eggel's original cases. He described a patient with a liver weight of 7 kg, but Herxheimer (1930) cites an observation by Bruzelius and Schwink reporting a liver cancer causing a liver weight of 14 kg. In some of the cases, the macroscopic presentation is dominated by liver cirrhosis, and tumor is only detectable on cut sections.

Eggel (1901) divided HCCs into two histologic groups (carcinoma solidum and carcinoma adenomatosum) and classified the macroscopic growth patterns, also based on the concepts of Hanot and Gilbert, into three patterns, i.e., nodular, massive, and diffuse. Eggel's classification of HCCs, proposed more than 100 years ago (Eggel 1901), was widely used, however, mainly for autopsy studies and less for surgical cases. The nodular type is grossly characterized by single or multiple discrete nodular neoplasms with a clear demarcation. Massive HCCs present as a large mass that almost completely replaces the left or right liver lobe. The diffuse type of HCC is characterized by a diffuse infiltration of the liver by numerous small to minute cancer nodules that macroscopically somewhat resemble cirrhotic nodules. The nodular form is the most common and is, based on data in the literature, present in 64.6 % of cases, while the massive form was found in 23 % and the diffuse form in 12.4 %. The nodular category of Eggel is sometimes applicable to surgical specimens with some difficulties only. For the adequate classification of HCC in hepatectomy specimens, the Liver Cancer Study Group of Japan proposed to divide nodular HCCs in subclasses. In 1984, Okuda and coworkers proposed a simple classification based on gross anatomical features of HCC from three disparate geographic areas, common major patterns being expanding, spreading, and multifocal (Okuda et al. 1984). Growth patterns were used as a stage

classification, e.g., the expansive-invasive-disseminative growth staging classification (Zhu et al. 2013).

Molecular Classifications

Based on the continuously growing recognition of distinct molecular signatures found in HCC, attempts to classify HCC according to molecular features have been and are undertaken (Boyault et al. 2007; Katoh et al. 2007). Katoh and coworkers (2007) found that HCC is composed of several genetically homogeneous subclasses, each having characteristic genetic alterations. Molecular signatures are associated with distinct macroscopic growth patterns. For example, EpCAM was predominantly expressed in the confluent multinodular (CM) type of HCC which is an aggressive phenotype with poor outcome (Murakata et al. 2011). In the setting of an integrative transcriptome analysis, three robust HCC subclasses termed S1, S2, and S3 have been identified, and each of these molecular subclasses correlated with clinical parameters, including tumor size, extent of cellular differentiation, and serum AFP levels. The S1 subclass reflected aberrant activation of the WNT signaling pathway, S2 proliferation, as well as MYC and AKT activation, and S3 was associated with hepatocyte differentiation (Hoshida et al. 2009).

Macroscopic Pathology of Main Gross Patterns of HCC

Size of Hepatocellular Carcinoma

The size of HCC at diagnosis varies markedly, ranging from tumors defined as early or small HCC (see the respective chapter) to very large lesions exceeding 20 cm in diameter and replacing most of the liver. Multinodular tumors with a liver weight exceeding 10 kg have been reported (Cooper et al. 1935), suggesting that, similar to other organs, HCC can grow to enormous size without leading to death through metastatic disease. HCC occurring in normal livers were, at least in part of analyses, significantly larger in average tumor size than those occurring in cirrhotic livers (Kishi et al. 1983).

Nodular HCCs

Nodular cancer in HCCs forms well-circumscribed, more or less spherical masses, mostly in a cirrhotic liver. These nodules most commonly either form simple and firm to friable masses of variable color or are closely grouped or form irregular nodular masses (Berman 1959). The color as seen on cut sections is yellowish, yellow-green, frankly green (in tumors with marked bile accumulation; "green hepatomas"), or gray to reddish. The green color of bile-rich lesions typically becomes more strong after formalin fixation. In cases where nodular cancers form groups or clusters of nodules, the color may vary from one nodule to the other, reflecting variations in differentiation (e.g., bile production) caused by progressive genomic instability or variations in regressive phenomena. Such differences can sometimes be found within one and the same nodule. Frequently, bile-storing green HCC nodules stand out against the non-bile-stained background of the cirrhotic liver. Large nodules can contains extensive hemorrhage, sometimes leaving a dark red-blue nodular lesion resembling a hemangioma (Figs. 1, 2, 3, 4, 5, 6, 7, 8, and 9). Clustered lesions form HCCs of the confluent multinodular type. In some cases, numerous nodules are present, and this presentation may be difficult to distinguish from multiple intrahepatic metastatic spread (Fig. 10). Small nodules are usually more firm than large one. The variable consistency depends on the ratio between preserved or necrotic epithelial cells versus stroma, the latter inducing firmness of the lesions when present in significant amounts. Large nodules are often soft and friable, whereby the centers are softer than the periphery owing to central necrosis. When cutting through large nodules, a creamy matter of necrotic tissue typically sticks to the blade of the knife. Macroscopically, nodular lesions tend to show an expanding growth pattern and therefore display a sharp border, but there also

Fig. 1 Nodular hepatocellular carcinoma in a cirrhotic liver. The tumor mass displays an expanding growth pattern and shows a rather homogeneous cut surface (formalin-fixed resection specimen)

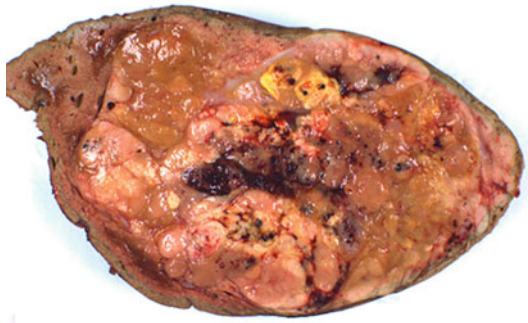

Fig. 2 Nodular hepatocellular carcinoma with an expanding growth pattern in a non-cirrhotic liver. The heterogeneous cut surface shows central hemorrhage and few *yellow* areas with necrosis and fatty change

lesions with a blurred border due to a macroscopically invasive phenotype. Mainly in large tumors, central necrosis, sometimes with cyst formation, is often impressive.

Small HCCs with a diameter of less than about 2 cm are classified into two major types, i.e., small nodular type with indistinct margins (SN-IM, vaguely nodular type) and the distinctly nodular type (SN-DN; Kojiro and Nakashima 1999). SN-IM may, due to its blurred margins, be difficult to distinguish from surrounding cirrhotic nodules and/or nodular precursor lesions. SN-IM are histologically often well-differentiated lesions with so-called replacing growth at the periphery. The tumors can contain portal tracts in markedly reduced numbers. These portal tracts are deformed by invasive growth of HCC, the so-called stromal

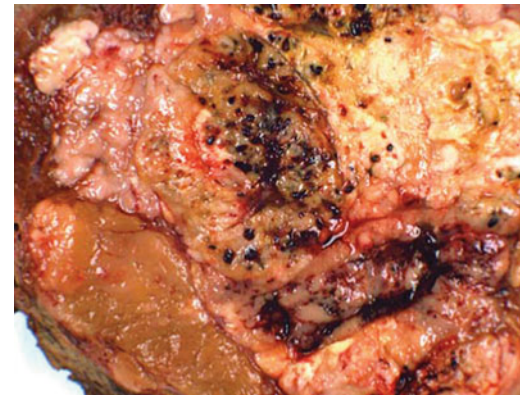

Fig. 3 Cut surface of a nodular hepatocellular carcinoma at higher magnification. This tumor is highly heterogeneous: the *greenish* part to the *left lower corner* exhibits bile accumulation, while the *whitish-pink* parts showed less differentiated neoplastic tissue. This tumor heterogeneity can cause bioptic sampling errors in regard to typing and grading. Extensive necrosis and hemorrhage are observed (*center* and *upper right corner*)

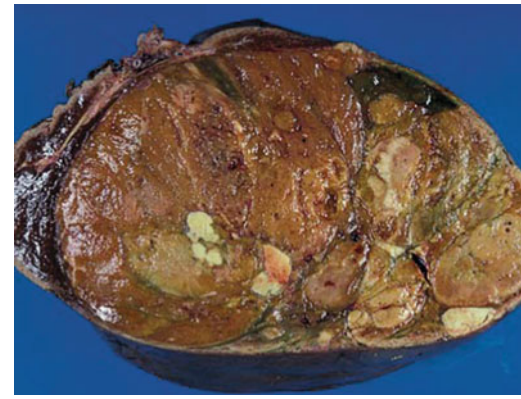

Fig. 4 Large hepatocellular carcinoma, nodular type with multinodular interior composition. The cut surface is characterized by *brown* and *greenish* areas (bile accumulation) and several *white* necrotic foci. In this non-fixed specimen, tumor tissue bulges from the cut surface

invasion, which is a very important diagnostic element. In some studies, these lesions have been termed, early HCC (Kojiro 2007), a lesion further discussed in a separate chapter.

As seen in Table 3, Japanese investigators have subdivided nodular HCCs into three subcategories, in order to cope with imaging findings surgically resected HCCs (The Liver Cancer Study Group of Japan (LCSGJ) 2003). In this