Histopathology and Molecular Pathogenesis of Cholangiocarcinoma

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Contents

Abstract

Cholangiocarcinomas (CCs) are primary hepatobiliary carcinomas of increasing importance and with major biological and therapeutic challenges. CCs may occur in any segment along the biliary tract and are presently also classified into two major categories according to their anatomic location. CCs arising from small bile duct and ductules to segmental large bile ducts are designated as intrahepatic or peripheral CC (ICC). Tumors originating from large bile ducts at the hilum (right or left hepatic bile duct or at their junction) or along the extrahepatic biliary tree are designated as extrahepatic bile duct carcinomas (BDC) or extrahepatic CC. Features of cholangiocyte differentiation characterize them; traditionally, they are thought to derive from the malignant transformation of bile duct epithelial cells and histologically classified as adenocarcinoma and rare variants. Recent data emphasized the significant degree of CCs' heterogeneity in terms of their epidemiology and risk factors, pathological and molecular features, pathogenesis, and clinical behaviors and treatment and underlined the role of hepatic stem/progenitor cells as cell origin of a proportion of CC and their possible overlap with the major primary malignant tumor of the liver, namely hepatocellular carcinoma (HCC); precursor lesions and early lesions have been characterized underlining the existence of multistep carcinogenesis process. Overall, these data result in proposal of new histological or molecular classifications that could soon replace current anatomic-based classification and have major impact on establishment of prognosis and on development of novel target treatment approaches.

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1 Introduction

Hepatocellular carcinoma (HCC) and cholangiocarcinoma (CC) are the two major adult hepatic malignant tumors. Although the prevalence of CC disease is increasing worldwide, it remains a rather rare malignant tumor and is far less prevalent than HCC.

Until recently, HCC and CC were believed to derive through dedifferentiation from mature hepatocytes and cholangiocytes, respectively, and were separated one from the other according to this different histogenesis. HCC mostly occurs on a background of advanced chronic liver diseases as cirrhosis, while CC principally arises in the context of a normal liver. In contrast to HCC, only a few high-risk factors for CC have been identified (essentially for extrahepatic bile duct carcinoma), and an established screening system for CC does not exist.

While all this remains mostly true, new insights in regards to combined hepatocellular cholangiocarcinoma (CHC), a histopathologic intermediate tumoral entity between HCC and CC, suggests nowadays-possible histological phenotypic overlap between these two tumors. Recent data on pathogenesis have put forward the fact that hepatic stem/progenitor cells (which can differentiate into either hepatic or biliary cells) may, through maturation arrest phenomenon, give origin to HCC, CHC, or CC, as to a spectrum of liver carcinomas with heterogeneous phenotypic overlaps between these defined entities. Furthermore, if still most CC occurs without underlining liver disease, the incidence of CC is increasing in non-endemic areas of parasitic biliary infection and often in relation to non-biliary chronic liver disease, chronic hepatitis C virus (HCV) infection being a major risk factor for intrahepatic CC. This redefines the boarders of HCC and CC and has extended knowledge of their histogenesis.

Finally, nomenclature of bile duct tumors is still a matter of debate. It has been proposed that the term "cholangiocarcinoma" be limited for intrahepatic peripheral tumors and tumors arising from large bile ducts both at the hilum and along the extrahepatic biliary tree and be designated "bile duct carcinomas." Intrahepatic CC is also assigned as peripheral CC, a term that tends to be discouraged today.

2 Pathology

CCs are currently classified into two major categories according to their anatomic location along the biliary tract:

1. Intrahepatic cholangiocarcinoma (ICC), accounting for 20 % of CC, develops within the hepatic parenchyma and most often appears as a mass without major bile duct obstruction or jaundice.
2. Extrahepatic bile duct carcinoma (EBDC), representing 80 % of CC, encompasses tumors arising from large hepatic hilar bile duct (or Klatskin tumor) to more distal extrahepatic bile ducts but excluding those occurring from ampulla. However, classification of Klatskin tumor is object of some debate. Notably, because a tumor can extend from hilum to the intrahepatic perihilar parenchyma, complicating determination of their anatomic origin, it has been classified in the literature either as intrahepatic or as extrahepatic CC. Moreover, recent studies have highlighted that hilar CC shows similar profile of mucin-producing subtype of ICC [1]. Based on its location and presentation, today's consensus is to classify Klatskin tumor as EBDC but is recurrently considered a form of EBDC separately from the more distal EBDC. Bismuth classification for Klatskin tumor is broadly used to guide surgical treatment [2].

This current anatomical-based classification of CC causes notable conflict in accurate assessment of epidemiological background, carcinogenesis, and patients' outcome of CC. ICC and EBDC can be further classified according to their pathology and molecular features (see below).

A significant limitation to exploring risk factors of CC resides in the classification systems that have been used in the literature: (1) Most cancer registries combine CC with other hepatobiliary malignancies; therefore, it is unclear whether CC also includes HCC and gallbladder cancer. (2) When ICC and EBDC are reported separately, sometimes, HCC is included with ICC and gallbladder cancer is included with EBDC. (3) Classification of Klatskin tumor as ICC resulted in an overestimation of the incidence of ICC and an underestimation of EBDC. (4) Most CC studies do not distinguish site (e.g., ductal, hilar, and peripheral) or histology subtype and heterogeneity. Specific risk factors for different types of CC are, therefore, likely to be missed, depending on the distribution of these types in a given study. (5) In studies where the distinction between ICC and EBDC was used, some potential risk factors seem to have a differential effect on CC, depending on the site. The consistent use of a more refined notably histological classification would allow a better understanding of risk factors for CC.

2.1 Intrahepatic Cholangiocarcinoma

ICC is primarily adenocarcinomas with biliary differentiation arising in any segment of the intrahepatic biliary tree, from the peripheral ductules and small portal bile ducts to the perihilar segmental ducts [3]; ICC may also arise from intrahepatic peribiliary glands.

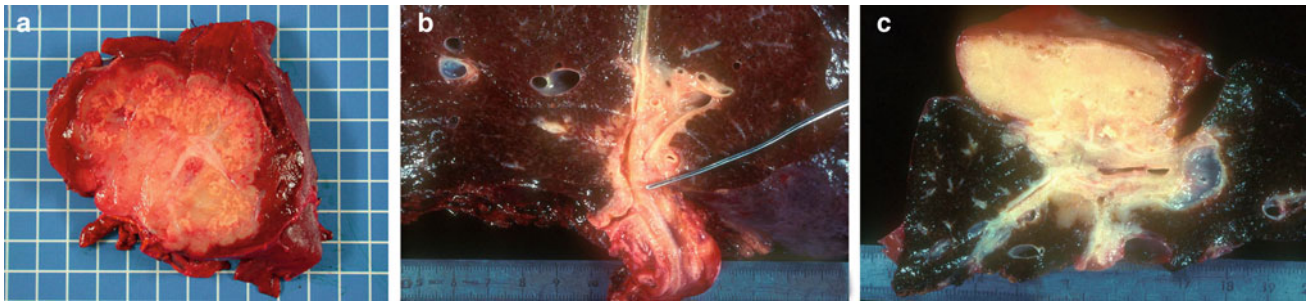

Fig. 1 Different macroscopical types of cholangiocarcinomas. **a** the mass forming type. **b** the periductal infiltrating type. **c** the mixed type

2.1.1 Macroscopic Pattern

According to its macroscopic appearance, the Liver Cancer Study Group of Japan has subdivided ICC into four categories:

1. The mass-forming type (MF), corresponding to a gray-white, well-delimited, firm and solid, non-encapsulated, polylobulated mass within the liver at distance from the hilum and with no connection with a bile duct macroscopically visible (Fig. 1a).
2. The periductal infiltrating type (PI), characterized by a tumoral growth spreading along intrahepatic portal tracts, associated with stenosis of the involved ducts and upstream bile duct obstructive dilatation and cholangitis (Fig. 1b).
3. The intraductal-growing type (IG), defined as a polypoid or papillary tumor mass growing within the lumen of a dilated large bile duct.
4. The mixed pattern (Fig. 1c).

Among these forms, the MF type is the most prevalent gross type, accounting for about 65 % of all ICC types, while PI type and IG type are rare, representing 6 and 4 % of all ICC, respectively. The predominant mixed pattern (around 25 % of ICC) combines to the PI type.

ICC originating from malignant transformation of peripheral ductules and small portal bile ducts usually results in a MF type with no connection with a bile duct macroscopically visible, while perihilar segmental ducts may result in any of the four types and are often associated with intrahepatic biliary fibrosis and cholangitis in the surrounding liver parenchyma. ICC from large intrahepatic bile ducts are often associated with noninvasive intraductal papillary neoplasm (IPN), which may result in mixed pattern, combining a MF to a IG type and extend superficially along the surrounding bile duct epithelium.

At advanced stages, intrahepatic metastases appear consisting on various sized nodules, which may coalesce; regional lymph nodes as lung metastases may develop.

2.1.2 Microscopic Pattern

Histologically, ICCs were, until recently, classified in classic adenocarcinomas and rare histological variants such as adenosquamous and squamous carcinoma, mucinous carcinoma (often with mucin visible at cut surface and intraductal growth pattern, occasionally associated with intestinal-type goblet cells), signet-ring cell carcinoma, clear cell carcinoma (with abundant cytoplasm), lymphoepithelioma-like carcinoma, and neuroendocrine type or may have sarcomatous area, mimicking a spindle cell sarcoma (Table 1).

Based on recent knowledge in carcinogenesis of CC, notably the existence of cholangiocytes' heterogeneity along the different levels of the biliary tree and the role of hepatic stem cell (HSC), additional histological classifications of ICC have been proposed. These include subdividing ICC in conventional subtype (most often occurring without underlining liver disease) and unconventional subtype (most likely developing on the background of a non-biliary chronic liver disease and cirrhosis) or in mucin-producing or non-producing CC [3–6].

Histologically, conventional ICC is classic mucin-producing adenocarcinomas with biliary features and displays three major (occasionally overlapping) architectural patterns (Fig. 2):

1. Well-differentiated fairly regular tubular glands with lumen of variable size, with or without micropapillary features. Tumoral cells are only slightly atypical and pleomorphic; they look like bile cells with small-to-medium size, cuboidal shape, with small nuclei and nucleoli surrounded by pale and occasionally vacuolated cytoplasm. Occasionally, neutrophil aggregates are intermingled within the tumoral glands.
2. Moderately differentiated, more irregular, and tortuous tubular glands. Tumoral cells are mostly columnar shaped and moderately pleomorphic with a hyperchromatic nuclei and regular mitotic figures, surrounded by an eosinophilic cytoplasm.
3. Poorly marked irregular glands or solid, nested, or cribriform growth pattern, occasionally associated with tumor necrosis. Tumoral cells are small and monomorphic cells with scanty cytoplasm and dark nuclei.

For unconventional ICC, several histological subtypes (both at architectural or cytological level) have been newly defined and subject to intense studies notably on their histogenesis.

Table 1 Classifications of intrahepatic cholangiocarcinoma

Traditional classification	New classification of ICC
Adenocarcinoma	*Adenocarcinoma conventional type*
Well differentiated	Well differentiated
Moderately differentiated	Moderately differentiated
Poorly differentiated	Poorly differentiated
	Adenocarcinoma unconventional type
	Trabecular
	Perihilar
	Ductular
Rare variants	*Rare variants*
Squamous cell type	Squamous cell type
Adenosquamous cell type	Adenosquamous cell type
Mucinous carcinoma	Mucinous carcinoma
Signet-ring cell carcinoma	Signet-ring cell carcinoma
Clear cell carcinoma	Clear cell carcinoma
Sarcomatous carcinoma	Sarcomatous carcinoma

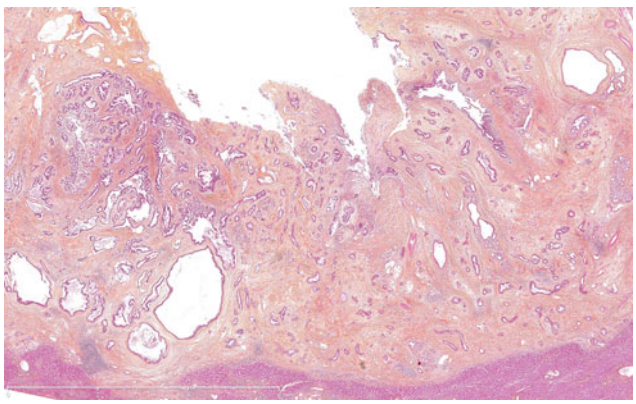

Fig. 2 Microscopical pattern of a well differentiated CC composed of tubular glands and abundant desmoplastic stroma along a large intrahepatic bile duct

1. Trabecular subtype, made of polygonal eosinophilic tumoral cells arranged in thick, occasionally anastomozing trabeculae, mimicking an HCC. They however differ from HCC by indistinct nucleoli, absence of bile production, presence of central fibrosis with sparse tumoral cells, presence of calcification, and immunostaining characteristics.
2. Hilar subtype mimicking typical hilar extrahepatic BDC. Large bile duct shows luminal spread of carcinoma and ulceration surrounded by periductal invasion similar to conventional ICC; peribiliary glands are often invaded. It most likely corresponds to CC originating from large bile ducts that have progressed into MF CC, peribiliary gland carcinoma, or a conventional ICC with secondary involvement of intrahepatic large bile ducts.
3. Intraductal neoplasia of the intrahepatic bile duct
   (a) IPN of bile duct, characterized by a spectrum of lesion ranging from preneoplastic intraductal papillary neoplasm of the bile duct (IPNB) (see below) to well-differentiated papillary, noninvasive and invasive, adenocarcinoma. The invasive component often is a mucinous carcinoma. They typically correspond macroscopically to the intraductal growth type, and intraductal superficial intraepithelial tumoral spread may occur along large and even small bile ducts.
   (b) Intraductal tubular neoplasm of bile duct (ITBN), rare and principally composed of tumor tubular glands, rarely papillary, without mucin, that cast and obstructs the dilated biliary duct.
   (c) Superficial spreading type.
4. Cholangiolocellular carcinoma (CLC), composed of proliferation of very regular and well-differentiated ductular structures within fibrosis, mimicking a ductal plate malformation [7]. It was previously categorized into a subtype of ICC (bile ductular adenocarcinoma) and is still as yet in Japanese literature. Today, because it is thought to originate from the hepatic progenitor cells (HPCs) located in ductules/canals of Hering [8, 9], it is classified in the latest edition (2010) of WHO tumor classification as a subtype of CHC and will be treated in that section (see below).
5. ICC with predominant "ductal plate malformation" pattern characterized irregularly dilated neoplastic glands associated with an important desmoplastic fibrosis.

A general hallmark of ICC is histological heterogeneity (which may be responsible for misclassification at preoperative biopsy because of sampling problem) and an often, abundant desmoplastic fibrosis, variably distributed within the tumor. The center is often more densely fibrotic intermingled sparse tumoral cells, with occasionally focal calcifications. The periphery has more abundant and proliferating tumoral cells that infiltrate the surrounding parenchyma either by compression and infiltration along the sinusoids or by directly replacing hepatocytes in their cords. Portal tract is co-opted within the mass. Portal venules, lymphatic vessels, and intrahepatic nerves are often invaded, already at an early stage.

ICCs are positive at immunohistochemistry for biliary subtype of cytokeratin, namely cytokeratins 7 and 19, but no specific markers still exist in order to distinguish such tumors from HCC or metastases. ICC histological heterogeneity is also underlined by immunohistochemical profiles and gene expression profiling [1].

2.2 Extrahepatic Bile Duct Carcinoma

EBDC is defined as carcinoma arising either (1) from the common hepatic duct (proximal to cystic duct) to right or from left hepatic bile duct (segment assessed as extrahepatic from their junction in hepatic hilum up to the secondary bifurcation). This EBDC is also named hilar or Klatskin tumor or (2) from extrahepatic distal bile duct (segment distal to cystic duct), excluding ampulla of Vater.

2.2.1 Macroscopic Pattern

Distinguishing between perihilar ICC and hilar EBDC relies essentially on macroscopic examination and may be difficult or even arbitrary particularly at advanced stage. Peripheral ICC may have secondarily infiltrated large hilar bile duct, or on the contrary, a hilar EBDC may extend to form a mass with the liver parenchyma. This may explain some discrepancies in the literature notably concerning the epidemiology and incidence of this tumor.

Bismuth subclassification for Klatskin tumors is widely used for surgery. Type I tumor involves the common hepatic duct distal to the biliary confluence; type II tumor involves the biliary confluence; type IIIa tumor involves the biliary confluence plus the right hepatic duct; type IIIb tumor involves the biliary confluence plus the left hepatic duct; and type IV multifocal or tumor involves the confluence and both the right and left hepatic ducts.

According to their macroscopic appearance, BDC may also be further divided into four categories, with, beside the first one, often overlap between the types:

1. The polypoid type (Fig. 3a), with endoluminal mass.
2. The sclerosing (scirrhous) constricting type, the most common, characterized by diffuse bile duct thickening due to extensive tumor infiltration and fibrosis spreading in periductal tissue.
3. The nodular type.
4. The diffusely infiltrating type, spreading linearly along the wall of the bile duct.

2.2.2 Microscopic Pattern

Histologically, EBDC is carcinoma with various patterns of differentiation that can occasionally coexist within the same tumor:

1. Adenocarcinoma of biliary type, characterized by tubular glands bordered by cuboidal to columnar tumoral cells, resembling biliary epithelium, with mucosecreting cytoplasm, embedded in a desmoplastic reaction.
2. Adenocarcinoma of foveolar type.
3. Adenocarcinoma of intestinal type, where the tumoral glands share morphological and immunohistochemical characteristics with colonic adenocarcinoma (colonic columnar and goblet cells, positive to CK20 and CDX2).

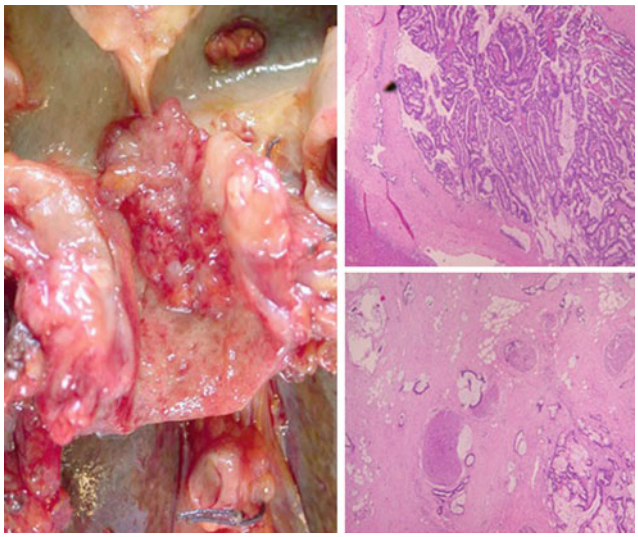

Fig. 3 **a** Macroscopical polipoïd types of extrahepatic bile duct carcinoma. **b** Microscopical papillary pattern of a moderate differentiated carcinoma, with **c** invasive component

4. Squamous cell carcinoma.

Klatskin tumor is characterized either by cords and tubules, or by larger, irregular dilated gland-lined atypical cells with and hyperchromatic nuclei. Extensive portal infiltration, perineural invasion, mucin production, papillary structures, and obvious features of intraductal dysplasia are classically observed.

3 Pathogenesis

Mechanisms implicated in CC carcinogenesis today are not fully established. They are variable, underlined by the differences between ICC and EBDC, as illustrated by geographic and risk factor variations.

Study of the pathogenesis of CC illustrates the major role of infection, chronic epithelial inflammation, and bile stasis in malignant transformation of cholangiocytes. Recent data have broadened the pathogenesis of ICC to the malignant transformation of hepatic progenitor cells (HPC). HPC-derived tumors can show varying hepatocytic and/or cholangiocytic differentiation pattern within the same tumor. Several risk factors and common molecular characteristics have been observed in CC and HCC, underlining the concept of a common origin in a subset of cases of these tumors.

3.1 Precursor Lesions of CC

It is now established that CC develops through multistep carcinogenesis; two types of precursor lesions have today been morphologically identified both in ICC and in EBDC:

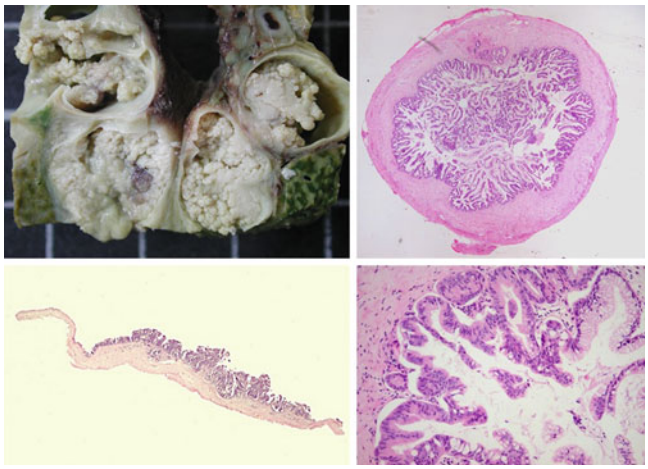

Fig. 4 Macroscopic and microscopical pattern intraductal papillary neoplasm (IPNB)

(1) a flat intraepithelial bilirary neoplasia or Bilin (discernible only at level of microscope) and (2) an intraductal papillary lesion or IPNB (previously named papillomatosis) (discernible on radiologic imaging or at macroscopic examination, Fig. 4) [10]. They usually arise in large intrahepatic, hilar, and extrahepatic bile ducts and are only rarely present in the septal or interlobular bile ducts.

Both occur more commonly in relation to chronic inflammatory biliary diseases, such as hepatolithiasis, primary sclerosing cholangitis, infestation by liver flukes, as well as congenital biliary diseases. Bilin has been reported in chronic HCV hepatic disease. Bilin more likely progresses to conventional invasive ICC (tubular adenocarcinoma), whereas IPN-B is associated either with colloid carcinoma (mucinous carcinoma) or with conventional ICC.

IPNB is increasingly accepted as the biliary counterpart of intraductal papillary mucinous neoplasm (IPMN) of the pancreas, while intraductal tubular neoplasm of bile duct (ITNB) corresponds to the pancreatic counterpart, intraductal tubular neoplasm of the pancreas. Both biliary and pancreatic ducts derive embryonically from the foregut.

3.2 Risk Factors of Intrahepatic Cholangiocarcinoma

ICC most often occurs without underlining liver disease; nevertheless, it may also develop on the background of a chronic liver disease and cirrhosis. The incidence of ICC is increasing in non-endemic areas of parasitic biliary infection and often in relation to non-biliary chronic liver disease.

Chronic HCV infection is a major risk factor for ICC. Epithelial damage of small intrahepatic bile duct and bile duct dysplasia are observed in chronic HCV infection. However, how HCV is involved in CC carcinogenesis is presently indeterminate. Metabolic diseases such as hemochromatosis and alpha-1-antitrypsin deficiency may also predispose to CC [11, 12].

ICC developing in the context of a non-biliary chronic liver disease is often characterized by ductular morphology, possibly underlining its hepatic progenitor cell origin, and more likely correspond to CHC than conventional ICC.

3.3 Risk Factors of Extrahepatic Bile Duct Carcinoma

Established predisposing factors for CC principally concern EBDCs and are correlated to chronic inflammation of the biliary tract such as primary sclerosing cholangitis in Western countries; liver fluke infestation and hepatolithiasis (recurrent pyogenic cholangitis) in Asian countries; various types of biliary malformations such as choledochal cysts, Caroli disease, congenital hepatic fibrosis, polycystic disease, and von Meyenburg complexes; and Thorotrast exposure.

3.4 Molecular Mechanisms of CC

Molecular mechanisms at the basis of the development of CC are still far from being completely understood. Notably, in the literature, it is recurrently difficult to distinguish specific molecular alterations of ICC versus EBDC, as tumoral location is not always precisely indicated. Molecular data underlining genetic differences in ICC and EBDC are thus rare and today need still to better studied [13].

A variety of mutations in oncogenes, as well as tumor suppressor genes, have been described in ICC. This includes mutations in oncogenes KRAS, BRAF, and EGFR, as in tumor suppressor genes as p53 and bcl-2 (Table 2) [13, 14]. Other particular molecular characteristics of CC reported in few studies are chromosomal aberrations, epigenetic changes, and the process of epithelial-to-mesenchymal transition (EMT) associated with the malignant transformation. Upregulation of different tyrosine kinase receptor-related pathways may also support the use of tyrosine kinase inhibitors as a new therapeutic option [15].

During chronic inflammatory processes, different cytokines such as IL-6, TGF-β, IL-8, and TGF-α released into the biliary microenvironment are responsible for the induction of malignant transformation of cholangiocytes. All of these cytokines produced by cholangiocytes, hepatocytes, and non-parenchymal cells play a fundamental role in the development and growth of biliary tract cancers. In particular, several studies have shown that mitogenic property of IL-6 is mediated by the upregulation of STAT-3, which increases Mcl-1 expression, a key antiapoptotic Bcl-2 family member protein. This suggests a critical role of antiapoptotic signaling

Table 2 Oncogene mutations in ICC

Gene	Abnormalities in ICC
K-ras	Mutated in 2–57 %
P53	Allelic loss or mutation in 1–40 %
BRAF	Activating mutations in 1–22 %
Bcl-2	Expressed in 60–70 %
HER2	Expressed in 30 % (without gene amplification)
EGFR	Expressed in 13 % (without mutation or amplification)
MET	Expressed in 30 % (without amplification)

in biliary malignant transformation [13]. Furthermore, not surprisingly as it has been demonstrated that ICC, combined hepatocellular cholangicarcinoma and poorly differentiated HCC could originate from stem/progenitor cells, some of these tumors share common genomic alterations [16]. This is also supported by the recent data obtained in mice showing that CC may originate from hepatocytes [17].

A recent study on a gene expression profile, high-density single-nucleotide polymorphism array, and mutation analyses using formalin-fixed ICC samples has identified two main biological classes of ICC that could result in different treatment approaches [18]. These two main classes are (1) the inflammation class (representing 38 % of ICCs) typified by activation of inflammatory signaling pathways, overexpression of cytokines, and STAT3 activation and (2) the proliferation class (62 %), characterized by activation of oncogenic signaling pathways (including RAS, mitogen-activated protein kinase, and MET), DNA amplifications at 11q13.2, deletions at 14q22.1, mutations in KRAS and BRAF, copy number variations (high-level amplifications in 5 regions, including 1p13 (9 %) and 11q13.2 (4 %), and several focal deletions, such as 9p21.3 (18 %) and 14q22.1 (12 % in coding regions for the SAV1 tumor suppressor) and gene expression signatures previously associated with poor outcomes for patients with HCC.

A major histological characteristic of ICC is abundant desmoplastic fibrosis associated with inflammatory cells, notably macrophages. The role of this cancer microenvironment in particular in promoting cancer growth or in prognosis is under active study.

4 Differential Diagnosis

4.1 Intrahepatic Cholangiocarcinoma

4.1.1 Combined Hepatocellular Cholangiocarcinoma

The latest WHO classification defines combined hepatocellular non cholangiocarcinoma as a tumor composed of definite unequivocal area of both HCC and CC, both closely intermingled. This entity is to be distinguished from collision tumor where both separated HCC and CC coexist within the same liver (either at distance or as close tumors). Macroscopically, combined HCC CC may mimic ICC.

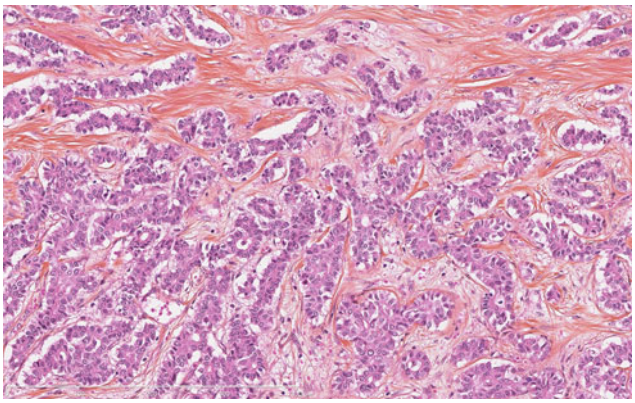

Fig. 5 Microscopical pattern of combined hepatocellular cholangiocarcinoma

CLC, a subtype of CHC, significantly mimics ICC (Fig. 5); it is characterized by proliferation of tumoral cells that look like cholangioles (bile ductules) and share CC immunomarkers such as CK7 and CK19. CLC expresses N-CAM (HPC marker) and albumin mRNA (HCC differentiation marker), helpful for distinguishing CLC from classical well-differentiated ICC. HPC origin of CLC is also emphasized by regular concomitance of CLC with conventional HCC and/or ICC components.

Today, CLC is most likely underdiagnosed, to some extent because when HCC and/or ICC areas are present, the CLC component is often overlooked and, while a specific marker for ICC is currently not available, CK7 expression is used as diagnostic marker of ICC and overdiagnosed ICC.

4.1.2 Hepatocellular Carcinoma

While ICC may have trabecular architecture, HCC may show a pseudoglandular architecture, potentially complicating their histological diagnostic distinctions. However, unlike ICC, HCC fails to show true glands or mucin, while it produces bile, and has often prominent nucleoli. The differential diagnosis is even more problematic when a fibrotic stoma is present in HCC.

Immunohistochemically, ICC fails to express the specific hepatocytic markers as HepPar1 and AFP as glypican-3 or CK8 and 18 (commonly expressed by HCC), while, contrary to HCC, they show diffuse cytoplasmic labeling with polyclonal CEA, monoclonal CEA, and CA19–9.

A focal expression of CK7 and of CK19 is observed in 15 % and 10–27 % of HCC, respectively. HCC expressing HPC or ductular markers like CK19 has a more aggressive clinical course. CK19 expression in HCC highlights a

subcategory of HCC with stemness features and is a significant predictor of worse overall survival and of early postoperative recurrence in these patients [19, 20].

4.1.3 Scirrhous HCC

HCC with large area (>50 % of area) of fibrosis (scirrhous pattern) may be misdiagnosed as an ICC both on diagnostic imaging and on gross appearance [21]. Macroscopically, it is often white-grayish, solid, and well demarcated but no encapsulated subcapsular mass with area of stellate-shaped fibrosis. Histologically, non-scirrhous HCCs are characterized as a diffuse band of fibrosis along sinusoid-like blood spaces intermingled with tumoral cell trabeculae of varying grades of thickness. Key element for diagnosis is the tumor cell morphology that is no different from regular HCC. Immunohistochemistry studies illustrated a significantly higher expression of cytokeratin 7 (>60 % of cases) and a significantly lower expression of hepatocyte paraffin 1 in scirrhous HCC than in ordinary HCC, underlining a peculiar histogenesis of this HCC variants, occurring probably also as CLC from stem/progenitor cells and the limited use of immunohistochemistry for the differential diagnosis of ICC.

4.1.4 Metastases

Diagnosis of ICC is established by exclusion of metastatic adenocarcinoma. The basic immunohistochemical panel combining CK7, CK20, CDX-2, TTF-1, ER, PR, BRST-2, and PSA selected according to the clinical setting contribute to rule out hepatic metastases from common primary sites including colon, lung, breast, and prostate. ICC is usually diffusely positive for CK7, while negative or slightly positive for CK20 and other previously cited markers. However, metastatic carcinoma from gallbladder, pancreas, or upper gastrointestinal tract can be distinguished neither morphologically nor by immunomarkers from ICC.

4.1.5 Bile Duct Adenoma

Bile duct adenoma (BDA) may be object of incidental finding, often confused with peripheral ICC. Being benign, it is not a true neoplasm and is currently regarded as a peribiliary gland hamartoma or a localized reactive ductular proliferation due to previous unknown injury. It is usually subcapsular and measures from 1 to 20 mm and is macroscopically firm, gray-white, tan or yellow, and well circumscribed but non-encapsulated round most often solitary mass. Histologically, benign, non-cystic ductules and variable degrees of inflammation and fibrosis characterize BDA. The immunophenotype of these ductules was similar to that of interlobular bile ducts. The absence of bile and cystic changes and lack of association with polycystic disease of the liver and kidneys are the main features distinguishing BDA from von Meyenburg complex.

4.2 Extrahepatic Bile Duct Carcinoma

4.2.1 Endobiliary Metastases

Intraepithelial spread along bile ducts of colorectal adenocarcinoma is a recognized behavior of hepatic metastases [22]. Morphologically, this pattern closely resembled high-grade dysplasia (i.e., carcinoma in situ) of the extrahepatic and intrahepatic bile ducts. A definite diagnosis of metastatic carcinoma is established by medical history, thorough evaluation of the morphologic features and histologic comparison with the primary colon cancer.

4.2.2 Rare Variants

Neuroma, granular cell tumor, endocrine tumor are rare variants of endobiliary tumors.

5 Conclusion

CC is a heterogeneous malignancy that consists of two different anatomically distinguishable categories (namely ICC and EBDC), according to several macroscopic and histologic subtypes. CC is characterized by a poor prognosis and a limited response to conventional anticancer therapies. Currently, there is limited understanding of the pathogenesis of this cancer. A universal consensus on the anatomical definition of intrahepatic CC versus extrahepatic bile duct cancer will facilitate improvement in the design of future clinical trials. Recent studies have made significant progress in the clarification of the intracellular pathways and molecular mechanisms involved in ICC pathogenesis. Cholestasis and chronic inflammation trigger genomic and epigenetic damage, therefore leading to a malignant transformation of cholangiocytes. Advances in the complete characterization of the molecular abnormality involved in the pathogenesis will help to better classify CC and develop new specific molecular targets for therapies.

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