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Updates in the diagnosis of combined hepatocellular-cholangiocarcinoma

Running title: Updates in the diagnosis of cHCC-CCA

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Conflict of interest

Authors report nothing to disclose

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Abstract

Combined hepatocellular-cholangiocarcinoma (cHCC-CCA) is a rare primary liver carcinoma showing variable degrees of differentiation toward hepatocellular and cholangiocellular carcinoma. Its great heterogeneity in term of morphology, immunophenotype, molecular, radiological and clinical features represents a challenge still to overcome. The multidisciplinary 2018 International Consensus on the nomenclature of cHCC-CCA allowed to review key issues of this entity. Here we review the historical controversies of cHCC-CCA, resume the key elements of the 2018 consensus, now incorporated in the 2019 WHO classification, and propose a short survival guide to help surgical pathologists facing cHCC-CCA in their routine workup.

Keywords: Combined hepatocellular-cholangiocarcinoma, primary liver carcinoma, hepatocellular carcinoma, cholangiocarcinoma, cholangiolocarcinoma.

Historical background

The first description of a combined hepatocellular-cholangiocarcinoma (cHCC-CCA) was given in 1903 by H Gideon Wells¹. In this fortunate report, the author stated that various degrees of transition between the two components were present and he suggested the common embryological development of cholangiocytes and hepatocytes as a possible explanation of his observation. Going through the following sections, the reader may feel a “back to the future” feeling.

It took around 50 years to appreciate from the scientific community a renovated effort to classify these lesions: Allen and Lisa suggested that single tumors showing features of both hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (iCCA) should be considered separately from HCC and iCCA arising at distance in the same liver, or only intermingling at their borders². In 1954, Edmondson and Steiner called these tumors “hepatobiliary cancers” and grouped them with HCC³.

In 1959, Steiner and Higginson described 11 cases of tumors arising in cirrhotic liver, characterized by a proliferation of small ducts in a fibrous stroma, and composed of

relatively monotonous small cells resembling the Hering canal epithelium⁴. Cholangiolocellular carcinoma (CLC) was born. The authors underlined the presence, in some cases, of cells presenting the features of both iCCA and HCC as a proof of concept of the “junctional” potentiality of cholangioles-derived tumors.

In 1985, Goodman et al. proposed a modification to the Lisa and Allen classification, with three different types of cHCC-CCA: a collision, a transitional and a novel type called “fibrolamellar”, defined as a fibrolamellar HCC containing pseudoglands producing mucin⁵.

The 2000 WHO classification tried to put some order in the classification of these tumors. cHCC-CCA, called at that time mixed HCC-CCA, was defined as a rare tumor containing unequivocal elements of both hepatocellular carcinoma and cholangiocarcinoma that are intimately admixed, suggesting pCEA and HepPar1 as markers of hepatocellular differentiation⁶.

The Pandora vase of ancillary tests in the diagnosis of cHCC-CCA was open. In a series of mixed tumors, Tickoo et al. found evidence of a biphenotypic differentiation by detecting in both components the expression of hepatocellular markers such as Albumin mRNA by *in situ* hybridization and AFP and pCEA by immunohistochemistry (IHC), and cholangiocellular IHC markers such as keratins (K) 7 and 19⁷.

Because of these results and the developing evidence for the existence of human hepatobiliary stem cells, the idea of a stem/progenitor cell origin for cHCC-CCA was emphasized and the origin from the common hepatocyte and cholangiocyte progenitor cell stressed in different series⁸⁻¹⁰.

The first direct evidence of this possibility was in a collection of 4 cases of cHCC-CCA ‘with stem cell features’, published in this journal in 2003 showing isolated clusters of K19 positive small cells, with scant cytoplasm and high nucleo/cytoplasmic ration, at the periphery of other tumor cells¹¹.

A series of 13 cases with intermediate morphology between HCC and iCCA mostly showing simultaneous expression of both hepatocytic and cholangiocytic markers seemed to confirm this hypothesis and suggested the existence of a separate histotype, namely “intermediate-cell carcinoma”, originating from transformed hepatic progenitor cells¹².

Then, the concept that CLC could be a form of cHCC-CCA seemed comforted by the publication of a 30 CLC series, all presenting areas resembling HCC. Some morphological and phenotypical homologies observed between these 30 cases and K19-positive hepatocellular carcinoma were interpreted as a proof that CLC originates by the same stem/progenitor cell of HCC¹³.

IHC was also used to yield prognostic information. It has since been shown that approximately 25–30% of HCC diagnosed on routine hematoxylin-eosin (H&E) sections show an expression of biliary markers, such as K7 and/or K19 and this has been correlated to a worse prognosis¹⁴⁻¹⁶.

The WHO 2010 Classification

The WHO 2010 Classification recognized a classical type of cHCC-CCA (a tumor containing unequivocal, intimately mixed elements of both HCC and iCCA), and 3 subtypes of cHCC-CCA with stem/progenitor cell features: the typical one (clusters of small K19+ cells intermingled with other tumor cells); the intermediate cell type and the CLC¹⁷. These entities were indicated based on what was available in the literature at that time. However, they were not considered distinctive clinicopathological entities, and it was uncertain whether there were biological differences between them.

This classification was challenged since its publication, particularly on the subtypes with stem/progenitor cell features. The most recurrent criticisms were the following: 1) Stem/progenitor cell features can be observed in many hepatocellular and cholangiocellular carcinomas with prototypical morphology; 2) the 3 putative histotypes with stem/progenitor cell features may coexist; 3) CLC is not always associated with HCC. Moreover, these 3 histotypes were introduced without evidence in support of their clinical relevance and seemed to reflect only a part of the morphological variability observable in primary liver carcinoma (PLC)^{18, 19}.

In particular, the typical histotype with stem/progenitor cell features while introducing the relevant concept of cancer stem cell and tumor heterogeneity, it generated some confusion in the community of pathologists. Because of this issue, the criteria used for selecting these lesions for translation and clinical study were variable, producing conflicting results²⁰.

Moreover, different papers reported that the immunophenotype of PLC is not fixed, but can be modified by environmental condition, questioning the diagnostic role of IHC in cHCC-CCA and introducing the concept of tumor cell transdifferentiation as a possible cause for a combined phenotype, as suggested from animal models²¹⁻²⁴. One clear-cut example was given by the evidence that HCC treated with transarterial chemoembolization (TACE) show increased expression of K19 and other stemness marker²⁵⁻²⁸. An additional point was that Albumin ISH expression, previously reported as a firm hepatocellular marker, could also be detected in morphologically typical iCCA, as demonstrated using modified branched DNA probes²⁹.

The 2018 International Consensus Group on the nomenclature of cHCC-CCA

cHCC-CCA Terminology

To solve these issues an International Consensus Group on the nomenclature of cHCC-CCA was initiated by EM Brunt and ND Theise, composed by an international group of liver pathologists, radiologists, surgeons and clinicians, with the aim to agree on a reliable and reproducible terminology. The conclusions of this consensus group were published in 2018³⁰.

The central statement of the consensus was that PLC represents a spectrum of entities, ranging from two extremes (HCC and iCCA) and encompassing cHCC-CCA, which, on its own, is characterized by a complex morphological and immunophenotypical diversity (Figure 1). Accordingly, all individual tumor in which there are varying degrees of hepatocytic and cholangiocytic cytologies and architectures, either admixed or as contiguous areas is a cHCC-CCA. A direct consequence of this statement is that, as for classical iCCA, CLC should be considered as a cHCC-CCA only when associated with a hepatocellular component (Figure 2).

The consensus also focused to other tumors that were previously separated as unique entity or, included in cHCC-CCA. PLC purely comprised of “intermediate cells,” referred to as intermediate cell carcinoma, was considered as a form of cHCC-CCA, as its cells, neither classic for HCC nor for iCCA, immunophenotypically display variably mixed hepatocytic and cholangiocytic markers at the cellular level (Figure 3).

A series of other entities, listed in Table 1, previously reported as cHCC-CCA by some authors were excluded from this terminology. Authors also suggested abandoning the following terms: mixed hepatobiliary carcinoma, biphenotypic (hepatobiliary) primary liver carcinoma, combined liver cell and bile duct carcinoma, HCC with dual phenotype, HCC with stem/progenitor cell.

The use of immunohistochemistry in cHCC-CCA

Authors of the 2018 consensus stated that the diagnosis of cHCC-CCA and CLC should be based on H&E. They evaluated the use of IHC in cHCC-CCA, reviewing individual biomarkers (Table 2) and underlined that IHC can be a supplement to confirm morphological oriented hypothesis, but should not define the diagnosis on itself.

The use of IHC for identifying “stem/progenitor cell” features/phenotypes in cHCC-CCA was also discussed. Indeed, many of the biomarkers previously suggested for this task, such as EpCAM, NCAM/CD56 and K19 are expressed in cholangiocytes at various stages of development. Thus, their interpretation as cholangiocytic vs “stem/progenitor cell” should primarily rely on the morphological characteristics of the positive cells.

The use of molecular studies in cHCC-CCA

At present, few studies have investigated the molecular biology of cHCC-CCA (Table 3). Some of them highlighted the enrichment in stem/progenitor-like signatures, supporting the concept of a stem/progenitor cell origin of cHCC-CCA³¹⁻³³. Other authors presented contrasting results, showing that cHCC-CCA presents a molecular profile more similar to HCC^{34, 35} or to CCA^{31, 36}, or both³⁷. One reason to these discordant results could be identified in differences in the selection of cases and in the methods. Particularly, it is not clear if in cHCC-CCA the molecular profile of unequivocal HCC and CCA areas is similar and derive from a common clonal origin^{33, 38}. Concerning CLC, recent data suggested that its molecular profile is unique and more similar to tumor derived from the bile ducts³². Of notice, it has been suggested that the nontumoral liver background could affect the mutational landscape of cHCC-CCA, which seems closer to HCC in chronic liver disease and to conventional iCCA in absence of hepatitis³⁹. Globally, while adding new interesting insights in the pathophysiology

of cHCC-CCA, these works were not able so far to identify straightforward molecular biomarkers to help the routine diagnosis of these tumors.

Clinical considerations

The studies that aimed to identify the prognosis of cHCC-CCA patients have showed poorer prognosis than HCC, closer to iCCA or in between, eventually being affected by the amount of the iCCA component⁴⁰⁻⁴³. It is thus important to identify these lesions in order to propose a tailored management. To this aim, a radio-pathological workup is needed. Indeed, the imaging features of cHCC-CCA variably overlaps with HCC and iCCA, depending on the amount of each component⁴⁴. While HCC ones can present typical features, without need of further histological confirmation, it is unclear if radiology alone is sufficient, without confirmatory biopsy, for excluding iCCA, or other components of cHCC-CCA. Thus, tumor biopsies can generate important information for the diagnosis and treatment of these lesions, but further studies are necessary to determine how many biopsies are needed, and how they should be targeted^{30, 45}. Of interest also is that distant metastases can be cHCC-CCA or harbor only the HCC or the iCCA components, adding additional complexity to the management of these lesions⁴⁶. After the diagnosis is established, the treatment should be carefully evaluated in accordance with the individual patient condition. In fact, to date, more robust data are needed to define the most appropriate treatment strategy, by comparing resection, transplantation, local ablation and systemic treatments outcome^{30, 47, 48}.

A cHCC-CCA short survival guide for pathologists

The final part of the present paper is aimed to highlight some key steps in the pathological workup of putative combined cHCC-CCA.

At the gross room. All tumoral areas that look differently, and the transition between them, should be carefully sampled. All starts here, if you sample only one component you can miss a potential cHCC-CCA.

At the microscope - morphological appreciation on H&E. The analysis is centred on the morphological appreciation. The following observations can help to rule out cHCC-CCA: 1)

keep in mind that conventional HCC can show variable architectural patterns and degrees of differentiation in the same tumor, including pseudo-glandular and poorly differentiated areas, 2) conventional iCCA looks like any other adenocarcinoma, with abnormal/ abortive glandular structures (in better-differentiated areas) dispersed in a fibrous stroma, 3) reactive ductular reaction is often present at the edge of HCC and should not be considered as a iCCA or CLC component. Their bland looking morphology and peritumoral distribution can help to overcome this issue.

At the microscope - asking for some IHC? The pathologist should be aware that cHCC-CCA encompasses a large spectrum of lesions, both at morphological and immunophenotypical level: all kind of combinations exists (Figure 1). It is advised to analyse IHC stains only in strict correlation with the morphology, as HCC can express cholangiocellular markers (K7 and K19). Notably, K19-positive HCC might show atypical features for HCC including fibrous stroma, more infiltrative growth, and nodal metastasis¹⁶.

For confirming a hepatocellular component, the most specific IHC markers are polyclonal CEA and CD10 that show a canalicular pattern, but their sensitivity is low. Arginase-1 performs better in less differentiated HCC than HepPar1 and AFP is only rarely positive. In any case, use immunostainings that you know better, and check their reliability in your own laboratory⁴⁹. Finally, if IHC is discordant with the first H&E impression, more sampling and more IHC on other slides are advised.

The pathological report. When signing out, conclude to cHCC-CCA only when stringent criteria are respected: overdiagnosis could imply a reduced spectrum of potential treatments (especially vs. HCC). For the histotype, use the terminology “intermediate cell carcinoma” only in homogenous biphenotypic tumors cells and it is up to you whether to mention the presence of stem/progenitor cell features. For staging purposes, the TNM classification should be the same as intrahepatic CCA.

These updates are now incorporated in the most recent (2019) WHO classification of the digestive system tumors⁵⁰.

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List of abbreviations

iCCA: intrahepatic cholangiocarcinoma

CLC: cholangiocarcinoma

cHCC-CCA: combined hepatocellular-cholangiocarcinoma

HCC: hepatocellular carcinoma

IHC: immunohistochemistry

PLC: primary liver carcinoma

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TABLES

Table 1. cHCC-CCA 2018 International Consensus Group terminology, Yes–No table.

cHCC-CCA	NOT cHCC-CCA
Tumor with intermingled areas of typical HCC and typical iCCA	Distinct (multifocal) HCC and iCCA
Tumor with closed areas of typical HCC and typical iCCA	Collision of HCC and iCCA arising separately in the same liver
PLC purely comprised of “intermediate cells”	Hepatoblastoma or variants (including cholangiocytic or ductal plate components) Pediatric “transitional liver cell tumor” or variants
	Morphologically typical HCC with immunohistochemical expression of K19 or other cholangiocytic or stem/progenitor cell markers
	Morphologically typical iCCA with immunohistochemical expression of hepatocellular or stem/progenitor cell markers, or <i>in situ</i> hybridization markers for hepatocytic differentiation (i.e., albumin)
	Sclerosing/scirrhous HCC
	CLC without HCC component
	Fibrolamellar HCC

Legend: cHCC-CCA: combined hepatocellular-cholangiocarcinoma. HCC: hepatocellular carcinoma. iCCA: intrahepatic cholangiocarcinoma. CLC: cholangiolocellular carcinoma

Table 2. Hepatocellular and cholangiocellular immunohistochemical markers⁴⁹.

	HepPar-1	Arg 1	GPC3	pCEA	CD10	AFP	K7	K19	EpCAM
Hepatocellular	75-85%	85-95%	50-80%	50-80% canalicula r	50-75% canalicul ar	30%	20-30%	10-15%	10-20%
Biliary	Rarely positive	Rarely positiv e	5%	negative	negative	negative	>90%	>75%	>90%

Legend: AFP: alpha fetoprotein. Arg 1: arginase-1. GPC3: glypican 3. HepPar-1: hepatocyte-paraffin 1. K: keratin.

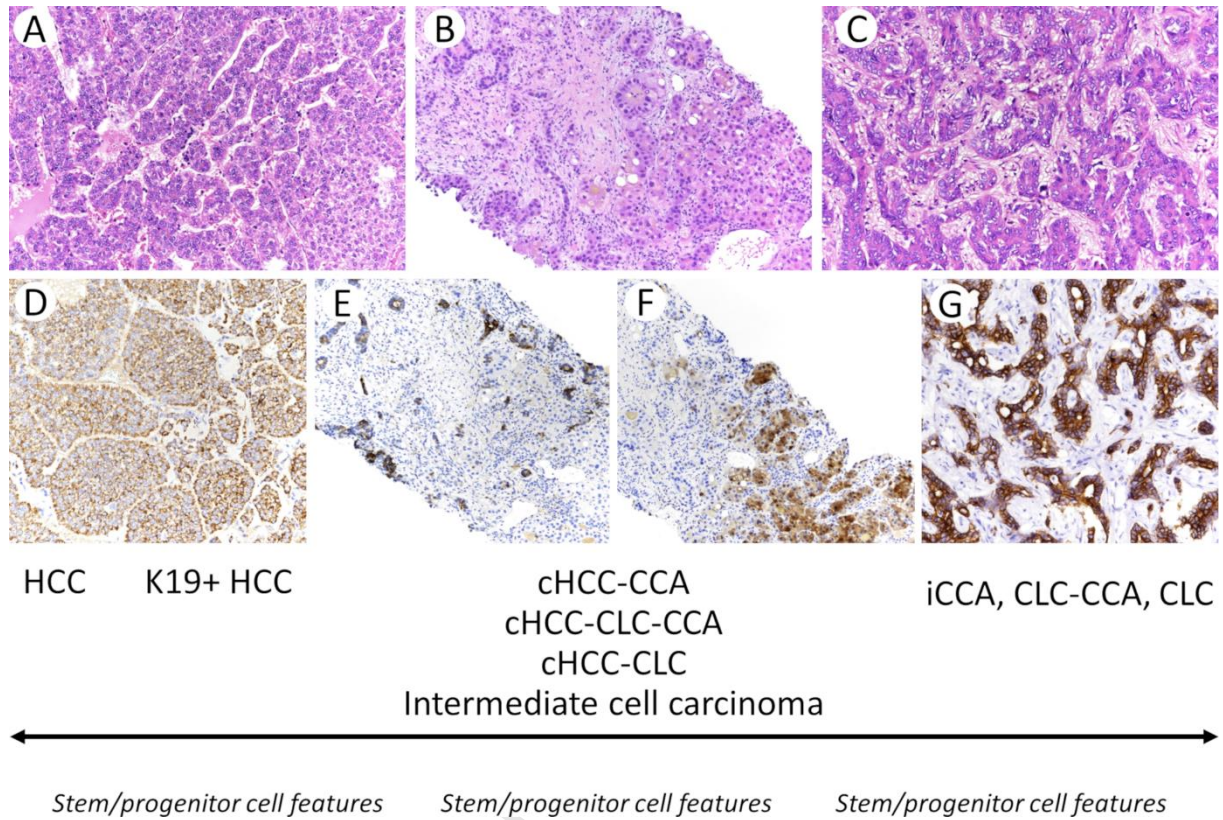
Table 3. Summary of the findings of studies having examined the molecular landscape of cHCC-CCA.

Reference	Technique	Main Message
<i>Coulouarn C et al. 2012</i> ³¹ <i>Moeini A et al. 2017</i> ³² <i>Wang A et al. 2018</i> ³³	Gene-expression array, WES	Stemness nature
<i>Fujii H et al. 2000</i> ³⁸ <i>Wang A et al. 2018</i> ³³	LOH, WES	Common clonal origin
<i>Sasaki M et al. 2017</i> ³⁷	Sanger sequencing	Variable association of HCC and/or CCA typical mutations
<i>Cazals-Hatem D et al. 2004</i> ³⁶ <i>Coulouarn C et al. 2012</i> ³¹	LOH, sanger sequencing, gene-expression array	Closer to CCA
<i>Liu ZH et al. 2018</i> ³⁴ <i>Joseph NM et al. 2019</i> ³⁵	WES, TDS, RNAseq	Closer to HCC
<i>Fujimoto et al. 2015</i> ³⁹	WGS	Closer to HCC if hepatitis background and to CCA in absence of hepatitis

Legend: WES: whole exome sequencing. WGS: whole genome sequencing. LOH: loss of heterozygosity. TDS: targeted deep sequencing.

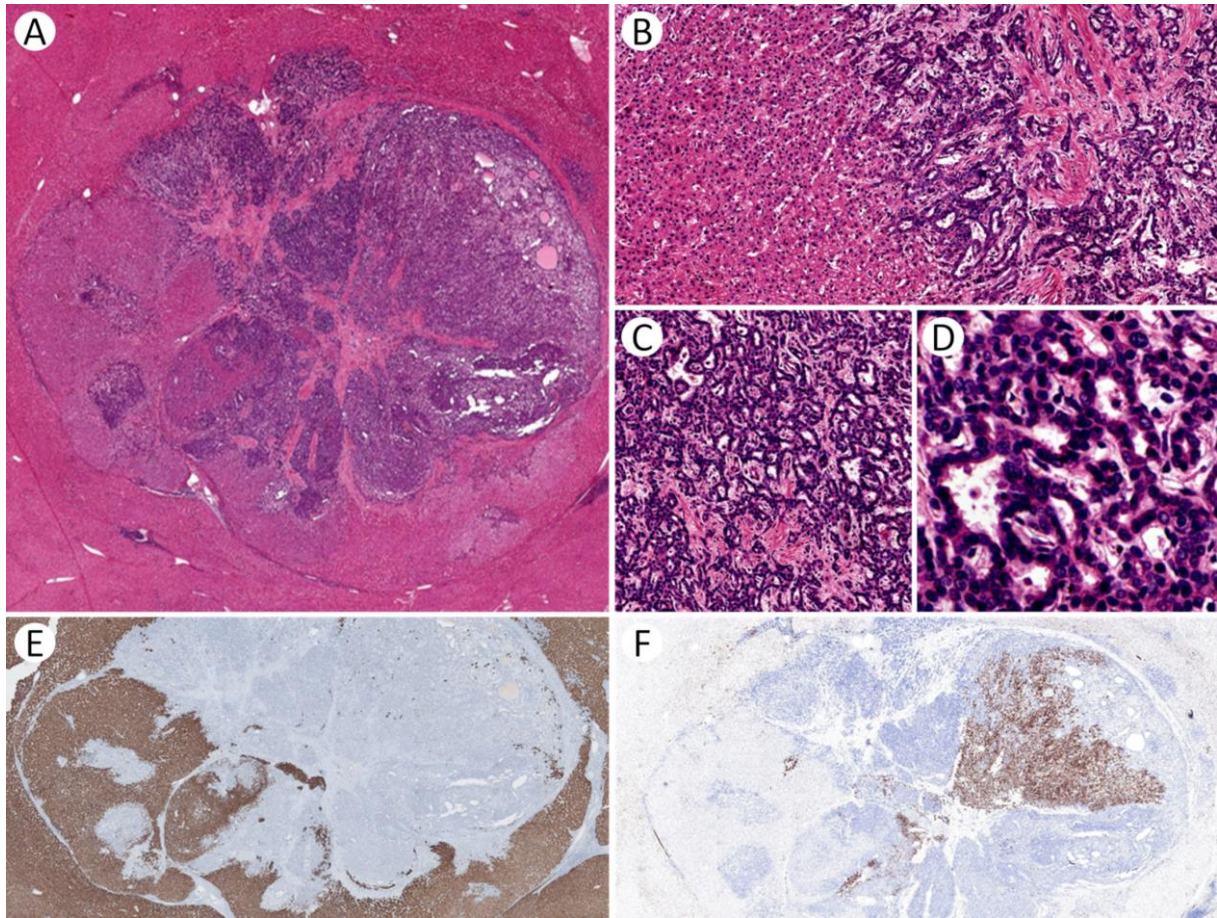
FIGURE LEGENDS

Fig.1. The spectrum of primary liver carcinoma.



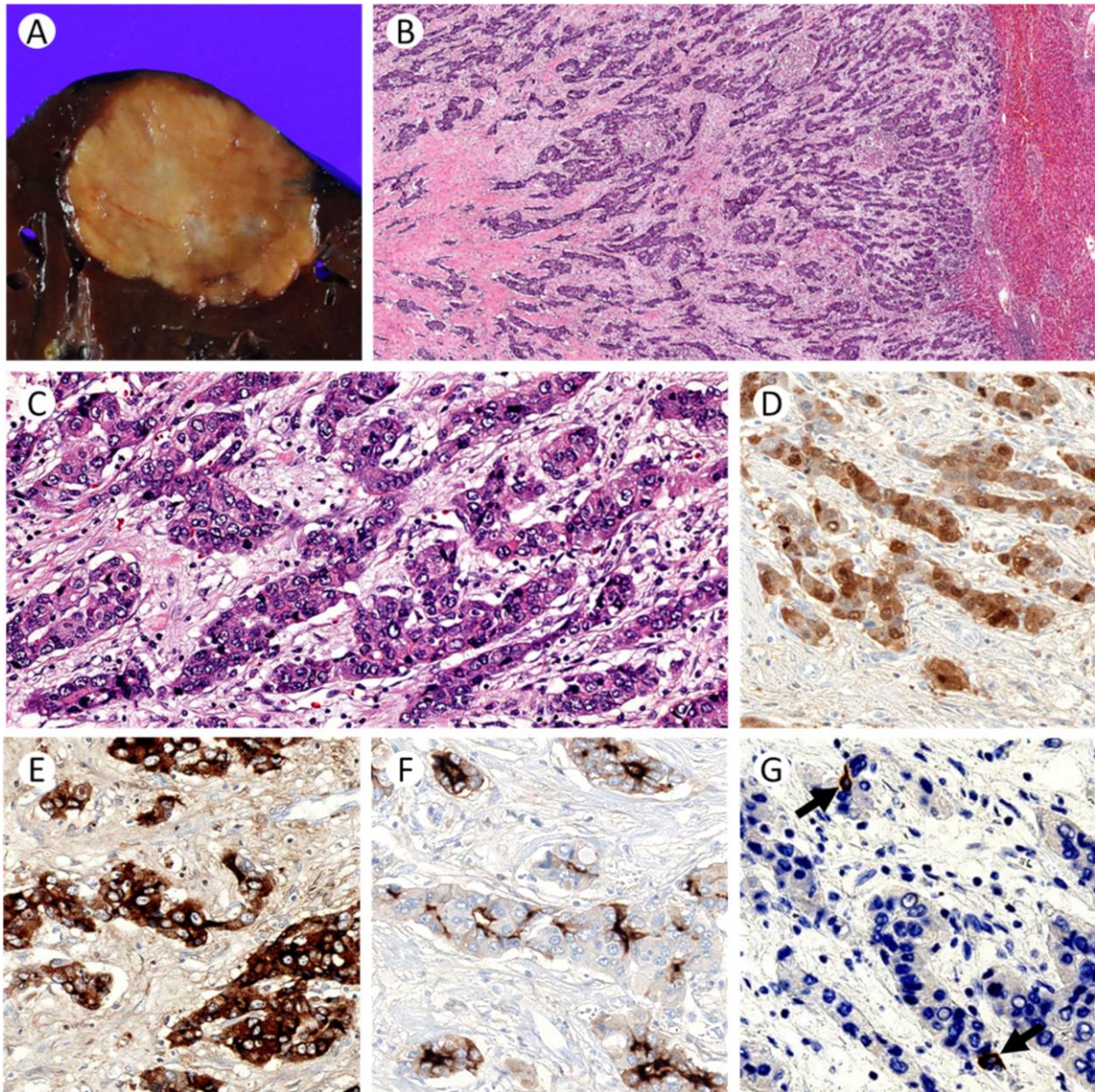
A-C. The morphological spectrum of primary liver carcinoma (PLC) encompasses lesions displaying exclusively trabecular architecture and hepatocellular morphology (A, HCC), lesions with adjacent or intermingled areas of typical HCC and typical iCCA (B, chHCC-CCA) and lesions displaying exclusively glandular architecture (C, iCCA). D. Typical HCC showing diffuse HepPar1 staining. E, F. Typical chHCC-CCA showing areas with K19 (E) and Arginase-1 (F) positive stainings. G. Typical iCCA showing diffuse K7 staining. Are also considered to be combined carcinomas those composed by HCC, CLC and iCCA (chHCC-CLC-CCA), by HCC and CLC (chHCC-CLC) and only by intermediate cells. Stem/progenitor cell features can be observed across the whole PLC spectrum.

Fig.2. Features of cHCC-CLC.

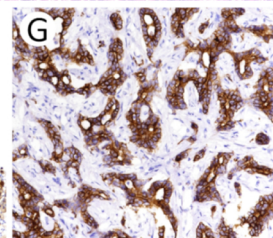
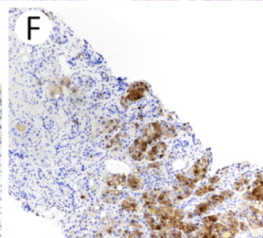
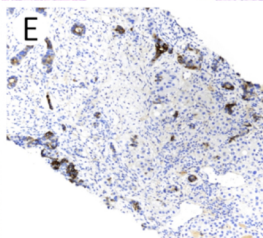
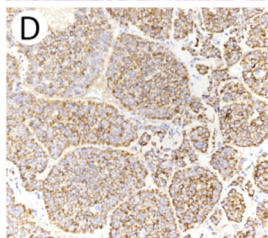
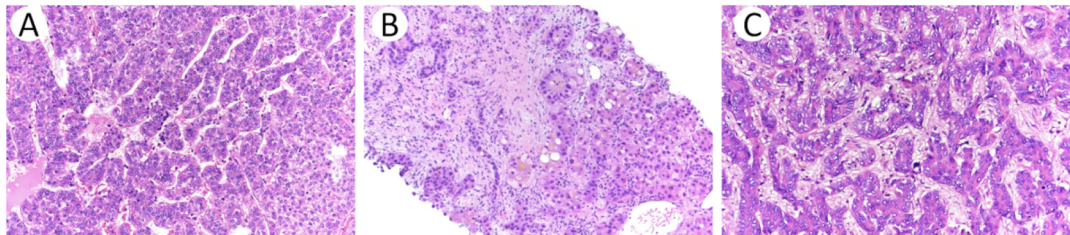


A. A case of cHCC-CLC, displaying at low magnification areas with different morphologies. B-D. At higher magnification, these areas corresponded to HCC (left) and a well-differentiated antler-like proliferation embedded within a fibrous stroma (right) (B), the latter being composed by tubular structures (C), showing monotonous “cholangiolocellular” small cells (D), corresponding to CLC. E, F. By immunohistochemistry, tumor cells stained for HepPar1 in HCC areas (E) and CD56 in CLC areas (F).

Fig.3. Features of intermediate cell carcinoma.



A. A case of intermediate cell carcinoma, macroscopically appearing as a firm, nodular, homogeneous lesion. B. Light microscopy analysis showed a trabecular proliferation embedded within a thick fibrous stroma. C. Tumor cells were of intermediate size, uniform round-to-oval, with scanty cytoplasm and hyperchromatic nuclei. D-F. By immunohistochemistry, all tumor cells stained for Arginase-1 (D) and for Alpha-Fetoprotein (E), as well as for EMA, with a canalicular pattern (F). G. A few K19 positive smaller cells were also present (arrows).



HCC K19+ HCC

cHCC-CCA
 cHCC-CLC-CCA
 cHCC-CLC

iCCA, CLC-CCA, CLC

Intermediate cell carcinoma



Stem/progenitor cell features

Stem/progenitor cell features

Stem/progenitor cell features

Figure 1

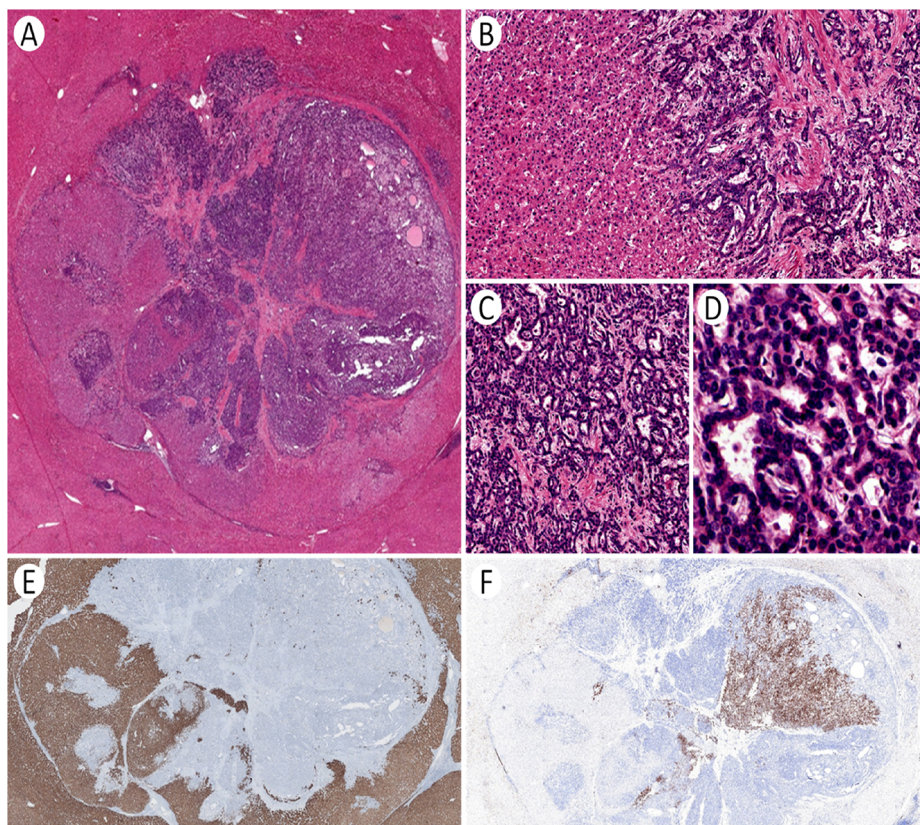


Figure 2

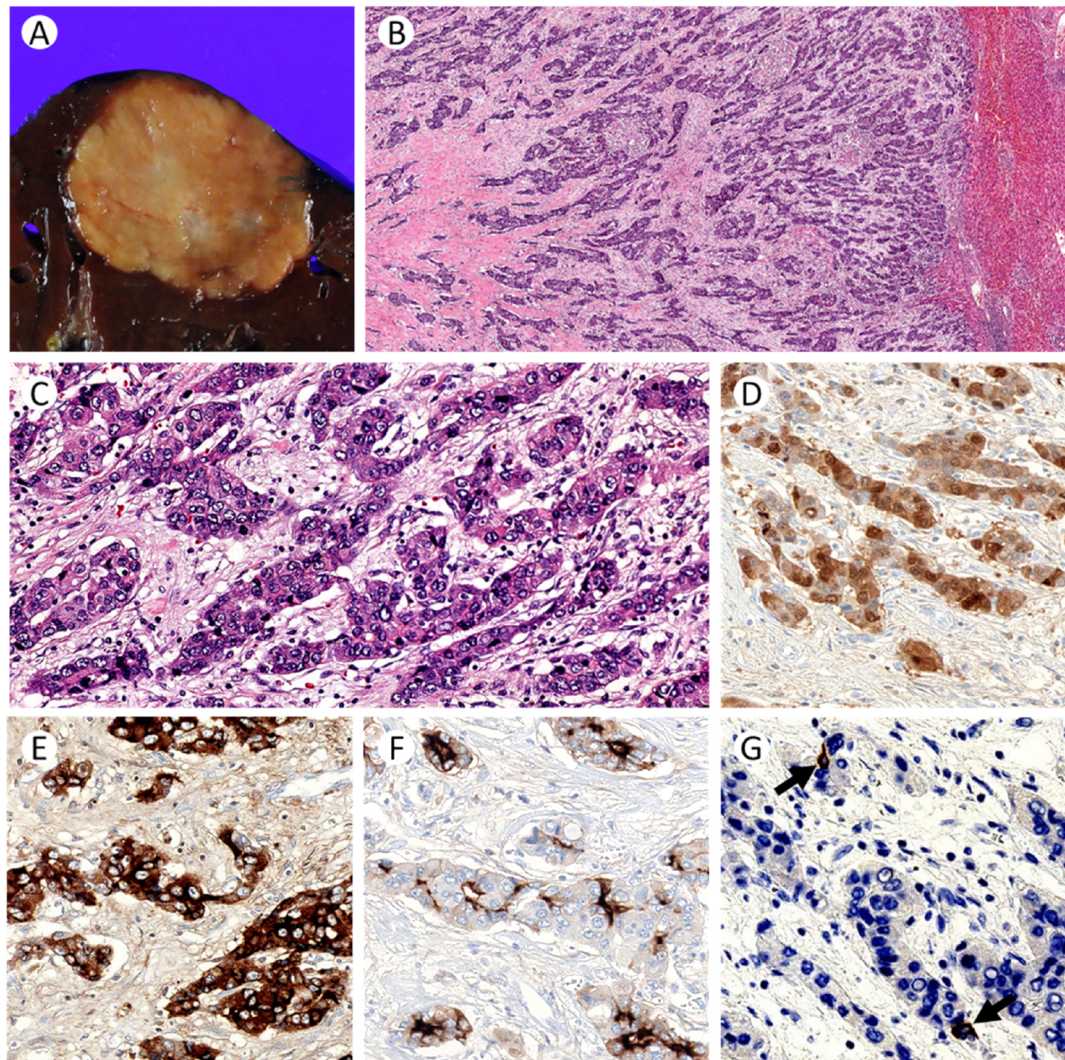


Figure 3