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The strengths and weaknesses of gross and histopathological evaluation in hepatocellular carcinoma: a brief review

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Abstract

Careful pathological analysis of hepatocellular carcinoma (HCC) specimens is essential for definitive diagnosis and patient prognostication. Tumor size and focality, gross patterns, macro- and microvascular invasion, degree of histological differentiation and expression of Keratin 19 (K19) are relevant features for risk stratification in this cancer and have been validated by multiple independent cohorts. However, there are important limitations to pathological analyses in HCC. First, liver biopsies are not recommended for diagnosis according to current clinical guidelines. Second, there is limited morphological data from patients at intermediate, advanced and terminal disease stages. Finally, there is little consensus on the evaluation of key histopathological features, notably histological grading (degree of differentiation). Here, we review important morphological aspects of HCC, provide insights to molecular events in relation to phenotypic findings and explore the current limitations to pathological analyses in this cancer.

Hepatocellular carcinoma: general aspects

Hepatocellular carcinoma (HCC) is the major histological subtype of primary liver cancer. It usually develops in a background of cirrhosis secondary to hepatitis B or C viral infection, chronic alcohol consumption, non-alcoholic steatohepatitis and/or other less prevalent risk factors (Llovet et al. 2016; Forner et al. 2018). According to current clinical guidelines, HCC diagnosis is based on imaging exams and needle biopsies for histopathological confirmation are usually restricted to few cases where imaging analyses are inconclusive(Galle et al. 2018; Heimbach et al. 2018).

Imaging analyses coupled to clinical information are also the basis for the Barcelona Clinic Liver Cancer (BCLC) staging system, the most widely adopted in HCC. The BCLC staging confidently predicts patient prognosis and guides therapeutic decisions. Unfortunately, curative-intent treatments including surgical resection, liver transplantation and tumor ablation are restricted to very early or early stage HCC, which correspond to less than 40% of all tumors at diagnosis. Patients with intermediate and advanced HCC benefit from transcatheter arterial chemoembolization (TACE) and systemic therapies (e.g., sorafenib, lenvatinib, regorafenib) respectively, but rarely achieve disease remission; and patients diagnosed at terminal stages should only be referred to supportive care (Llovet et al. 2016; Forner et al. 2018; Villanueva 2019).

Due to the lack of surgical specimens from patients at intermediate, advanced and terminal stages and the guideline restrictions to liver biopsies, most of the pathological data in HCC has been generated from very early and early stage tumors collected from surgical resections or liver transplantations. This remarkable "sampling bias" precludes longitudinal analyses in HCC and a confident evaluation of morphological (and genomic) features that are indeed associated with cancer progression and extrahepatic dissemination. Of note, longitudinal sampling of primary and metastatic thyroid neoplasms was crucial for refining tumor classification and coining the recently-described non-invasive follicular thyroid neoplasm with papillary-like nuclear features(Nikiforov et al. 2016). Despite the sampling limitations in HCC, careful gross and histological evaluation of very early and early stage tumors provide valuable predictive information such as patterns of tumor growth (Hui et al. 2000; Shimada et al. 2001), vascular invasion (Du et al. 2014) and degree of tumor

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differentiation (Han et al. 2013). Our goal here is to review these important macroscopic and histological features of HCC in the context of current pathological guidelines, provide insights to HCC molecular events in relation to histological features and further explore the limitations to pathological analysis in this cancer (and how to potentially overcome those limitations).

Hepatocellular carcinoma: macroscopy

Pathological evaluation of HCC starts with the gross evaluation of the tumor sample. Liver specimens from surgical resections and liver transplantations should first be weighted and measured. Nodules and irregularities on the liver surface should be reported. Thin slices should then be cut perpendicular to the surgical margins (adequately inked) in resection samples and in the long axis in transplant specimens. Each slice should be carefully investigated for essential gross features including:

- Tumor focality: number of suspicious nodules and their location according to liver lobes and segments;
- Size of nodules: each nodule should be individually measured. Specimens with a single nodule < 2.0 cm should be reported as "small HCC", as they usually show lower recurrence rates(International Consensus Group for Hepatocellular NeoplasiaThe International Consensus Group for Hepatocellular Neoplasia 2009; Kikuchi et al. 2009);
- Tumor border: relationship between the nodule and adjacent liver parenchyma;
- Macrovascular invasion: presence of cancer within major vascular structures. Macrovascular invasion is a well-established prognostic factor in HCC (Lee et al. 2014; Noh et al. 2016). This feature, along with tumor focality and size of nodules, is essential for tumor staging: HCC with macrovascular invasion should be categorized as T4 (TNM staging system)(Cancer Protocol Templates. College of American Pathologists 2019).

Small HCC that are less than 2 cm distant from the main tumor should be categorized as "satellite nodules"(Cancer Protocol Templates. College of American Pathologists 2019). These lesions usually arise within the drainage area of the larger nodule and typically represent intrahepatic metastases(Sakon et al. 2002). Other features of intrahepatic metastases include moderate or poor histological differentiation and similar mutation profile compared to the main lesion. Conversely, solitary nodules that are far from the main tumor are typically synchronous in origin. They tend to show lower histological grades (better differentiation) and share less molecular events with the main nodule(Nakashima and Kojiro 2001; Chianchiano et al. 2018; Furuta et al. 2017). The distinction between synchronous tumors and intrahepatic metastases has impact in patient prognosis. Indeed, a systematic review by Yang et al showed that patients with intrahepatic metastases have worse outcome than those with synchronous tumors (Yang et al. 2017).

Although not mandatory, it is also good practice to categorize HCC according to macroscopic findings. Egell apud Ishak et al, in the most traditional gross classification of HCC, recommended stratifying this cancer in 1) Nodular form: single or multiple nodules, sharply demarcated; 2) Massive form: large tumor encompassing multiple liver segments or even the whole lobe; 3) Diffuse form: multiple small nodules throughout the liver parenchyma, sometimes mimicking cirrhotic pseudo-lobules (Ishak et al. 2001) (Fig. 1). Egell's classification was proposed in autopsy specimens back in 1901, thus it has limited value for evaluating current HCC clinical specimens, as they are mostly collected from surgical resections of early stage tumors.

Recently, novel macroscopic classifications, mostly derived from Egell's nodular type, have been proposed in surgically resected specimens and are endorsed by The Liver Cancer Study Group of Japan (LCSGJ)(Kanai et al. 1987) and the Korean Liver Cancer Association (KLCA) (Lee et al. 2018) (Table 1). Confluent multinodular and poorly demarcated tumors by the LCSGJ classification tend to show higher incidence of vascular invasion and worse outcome than single nodular tumors (Hui et al. 2000; He et al. 2015). Similarly, multinodular confluent, nodular with peri-nodular expansion and infiltrative HCC by the KLCA gross classification show significantly lower overall- and disease-free survival compared to vaguely nodular and expanding nodular HCC. The three aggressive KLCA gross subtypes are also associated with higher prevalence of vascular invasion, poor histological differentiation and even higher immunohistochemical expression of Keratin 19 (K19) and EpCam (Lee et al. 2018), which are markers of stem-cell properties in HCC (Kawai et al. 2015). Altogether, those studies indicate that careful gross evaluation of HCC specimens may help predict survival and could even suggest innate biological properties in this cancer. However, it will be important to validate those findings in larger and in non-Asian cohorts.

Hepatocellular carcinoma: histology

Histological diagnosis of HCC is often straightforward and is based on architectural and cytological features. Architecturally, HCC presents in three main patterns: trabecular (≥ 3 cells thick), pseudoglandular/acinar or diffuse/solid (World Health Organization 2010; Jain 2014; Manuel Schlageter and Angelo 2014) (Fig. 2). Architectural changes in HCC are usually accompanied by distortion or loss of the reticulin network, which can be appreciated by special stains (Swanson et al. 2015; Shafizadeh and Kakar 2011). Cytologically, HCC cells usually show higher nuclear-to-cytoplasmic ratio, varying degrees of cellular and nuclear

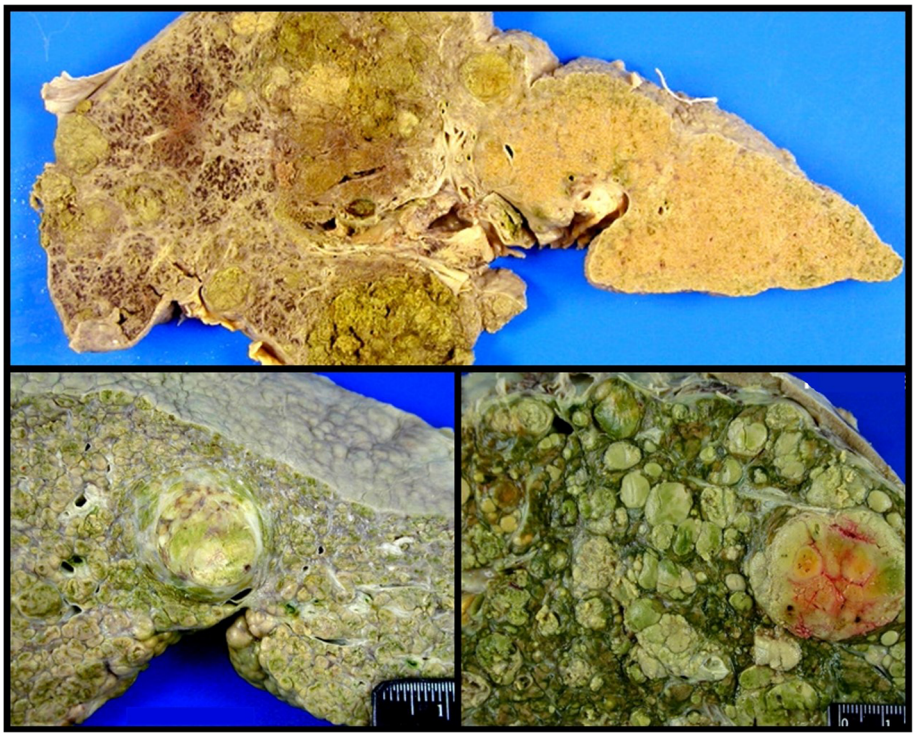

Fig. 1 HCC gross subtypes: massive form (top), nodular form (bottom-left) and diffuse form (bottom-right)

Table 1 HCC macroscopic classification recommended by the LCSGJ and KLCA

Gross subtypes according to the LCSGJ (as described by Kanai et al and Hui et al)	
Type 1: single nodular (SN)	Single oval or round-shaped nodule with clear boundary
Type 2: single nodular with extra-nodular growth (SNEG)	Similar to type 1, but exhibiting varying degrees of extra-nodular growth
Type 3: confluent multinodular (CMN)	Lobulated tumor comprising clusters of smaller, confluent nodules
Type 4: poorly-demarcated or infiltrative	Tumors with irregular shape and unclear border
Gross subtypes according to the KLCA (according to Jang apud Lee et al)	
Vaguely nodular (VN)	Nodule with indistinct margins. This subtype is usually restricted to early tumors (< 3.0 cm)
Expanding nodular (EN)	Circumscribed nodule with well defined margin
Multinodular confluent (MC)	Clusters of small, confluent nodules forming a uniform tumor
Nodular with peri-nodular extension (NP)	Similar to the EN subtype, but showing small extra-nodular cancer growth (inferior to 50% of the tumor circumference)
Infiltrative (INF)	Tumors with extensive extra-nodular growth (superior to 50% of the tumor circumference)

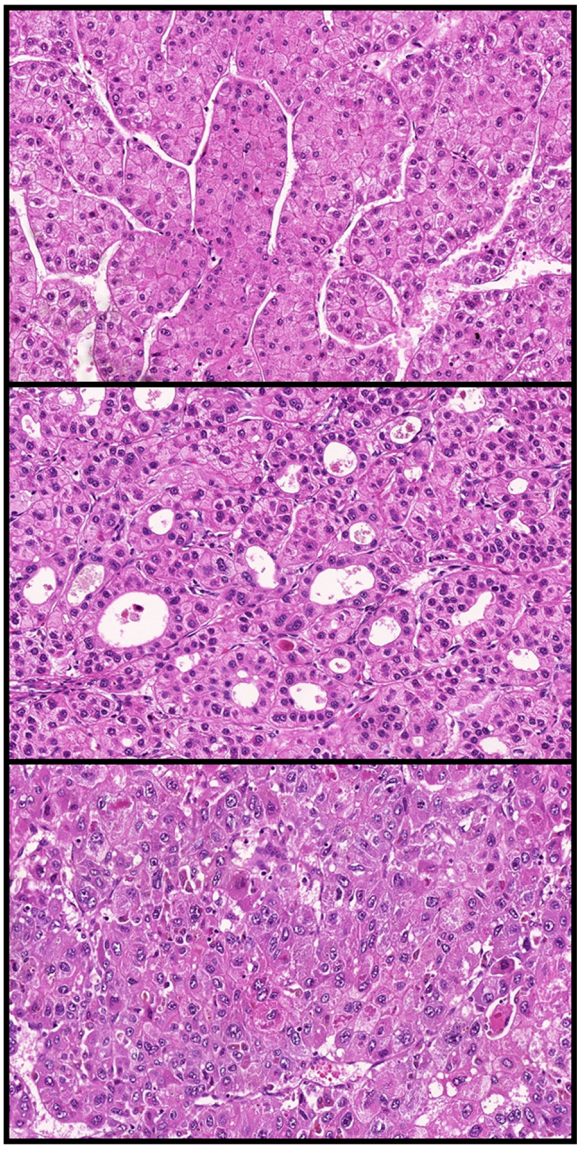

Fig. 2 Architectural patterns in HCC: trabecular, pseudoglandular and solid (from top to bottom)

pleomorphism and, not rare, accumulation of bile, hyaline globules (including Mallory-Denk hyalines), glycogen or fat droplets (Jain 2014; Wee and Sampatanukul 2015). Presence of atypical mitosis and invasion of adjacent liver, stromal components or lympho-vascular structures also indicate the malignant nature of the tumor under investigation and might help in the histological diagnosis (Quaglia 2018; Park et al. 2007).

There are, however, challenging situations for histological confirmation of HCC, most notably on the two extremes: small-vaguely-nodular tumors that show mild cytoarchitectural atypia (well-differentiated HCC) and large tumors that present with highly pleomorphic cells (undifferentiated neoplasms)(International Consensus Group for Hepatocellular NeoplasiaThe International Consensus Group for Hepatocellular Neoplasia 2009; Quaglia 2018; Sherman 2011) (Fig. 3a). In small-vaguely-nodular tumors, subtle features should be carefully investigated. These were highlighted in the pathologic guidelines for early HCC by the International Consensus Group for Hepatocellular Neoplasms, and include "(Llovet et al. 2016) increased cell density more than 2 times that of the surrounding tissue, with an increased nuclear/cytoplasm ratio and irregular thin-trabecular pattern; (Forner et al. 2018) varying numbers of portal tracts within the nodule (intratumoral portal tracts); (Galle et al. 2018) pseudoglandular pattern; (Heimbach et al. 2018) diffuse fatty change; and (Villanueva 2019) varying numbers of unpaired arteries"(International Consensus Group for Hepatocellular NeoplasiaThe International Consensus Group for Hepatocellular Neoplasia 2009). In pleomorphic tumors, clinical and imaging exclusion of cancer of different sites and immunohistochemical demonstration of hepatocellular lineage may be necessary for the definitive diagnosis(Quaglia 2018; Lin and Liu 2014; Chan and Yeh 2010) (Fig. 3b).

Pathologists should also be aware of important diagnostic pitfalls in liver specimens suspicious for HCC, notably adrenocortical neoplasms and metastases from neuroendocrine tumors. Adrenocortical tumors may directly invade the liver parenchyma or develop in adrenohepatic fusion tissue or in ectopic adrenal gland tissue within the liver. The imaging features of these tumors and HCC are similar, which may lead to misdiagnosis. Histologically, adrenocortical tumors may also show nests or trabeculae of polygonal cells with large eosinophilic cytoplasm and positive staining for Glypican-3, mimicking HCC (Lionti et al. 2018; Park et al. 2017). Clinical history of adrenocortical dysfunction or deregulation, absence of background liver disease, careful macroscopic evaluation and a broad immunohistochemical panel including markers of the adrenal cortex in suspicious cases (e.g., Melan-A, alpha-inhibin, steroidogenic factor-1 (Sangoi et al. 2011)) may aid in the differential diagnosis (Lionti et al. 2018; Park et al. 2017).

Neuroendocrine tumors may also be misdiagnosed as HCC. Liver is the most common metastatic site of neuroendocrine tumors and, not rare, the primary site is unknown (leading to a wrong assumption of primary liver tumor) (Riihimäki et al. 2016). These neoplasms may show hepatocyte-like histology including large, granular and eosinophilic cytoplasm. Furthermore, nuclear features of neuroendocrine differentiation (e.g., salt and pepper nuclei) are not always evident, particularly in small samples (Arista-Nasr et al. 2010). Clinical history of neuroendocrine deregulation (e.g., hormonal imbalances), absence of background liver disease and IHC for neuroendocrine markers including chromogranin A, synaptophysin and

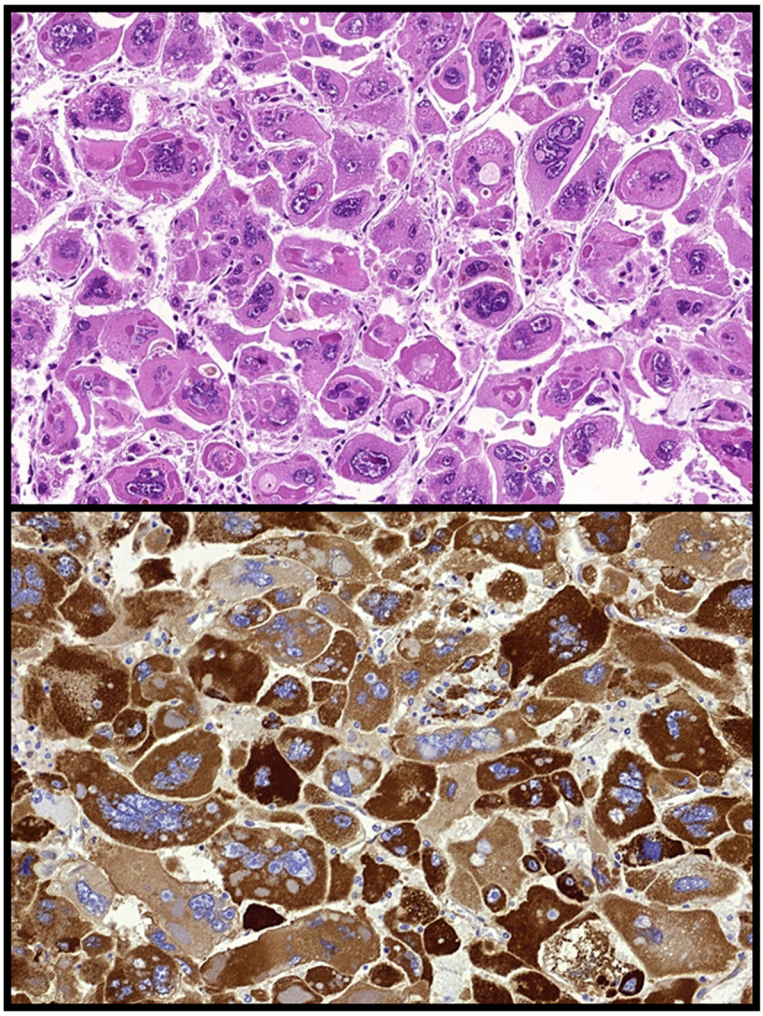

Fig. 3 HCC with high nuclear pleomorphism, but retained expression of HepPar1

NCAM-1are important for the definitive diagnosis (Arista-Nasr et al. 2010; Jin et al. 2016; Sobotka et al. 2019). Of note, these markers are also helpful in cases of combined hepatocellular and neuroendocrine carcinoma. Briefly, these are bi-phenotypic cancers with distinct areas of hepatocellular and neuroendocrine differentiation (Garcia et al. 2006).

Following histological confirmation, pathologic guidelines recommend reporting degree of differentiation (histological grades) and vascular invasion in HCC (Burt et al. 2018). Different histological grading systems have been described in this cancer (Martins-Filho et al. 2017), but the most widely adopted is that from Edmondson & Steiner (Edmondson and Steiner 1954). According to those authors, HCC should be stratified in four different tiers:

- Grade I: tumor cells show high resemblance to hyperplastic and adenomatous conditions. Diagnosis of grade I HCC is mostly based on the evaluation of other areas of the tumor with more aggressive features. Therefore, pure grade I HCC should be rare.
- Grade II: tumor cells still show high resemblance but have more hyperchromatic and larger nuclei than normal hepatocytes. Pseudoglandular formations are common and their lumen are often filled with bile or protein precipitates.
- Grade III: there is high nuclear to cytoplasmic ratio. Cell cytoplasm is still eosinophilic, but less so than lower grade tumors and normal hepatocytes. Pseudoglandular formations, bile production and protein precipitates are rare.
- Grade IV: tumors show a diffuse or medullary growth pattern and typical hepatocyte trabeculae are not easily identified. Nuclei is large, hyperchromatic and pleomorphic. Cytoplasm is scanty, with few granules. Spindle cells are often detected.

Another commonly used histological grading system in HCC is described in the "WHO Classification of Tumours of the Digestive System" book. The WHO classification also stratifies HCC in 4-tiers: 1) Well-differentiated: tumors with thin trabeculae and frequent acinar structures and minor cytological atypia; 2) Moderately-differentiated: tumors some wider trabeculae (≥ 3 cells thick) and frequent acini, cell cytoplasm is still abundant and nuclei is round with prominent nucleoli; 3) Poorly-differentiated: tumors are commonly solid in architecture and cells show moderate to marked pleomorphism; 4) Undifferentiated: tumors are solid, cells show little residual cytoplasm. Spindle and round-shaped cells are frequent (World Health Organization 2010). Despite the many similarities, there are also subtle (but important) differences between the E&S and the WHO grading systems (previously reviewed here(Martins-Filho et al. 2017)).

HCC has a strong hematogenous tropism and the incidence of vascular invasion is high even in small tumors (Kikuchi et al. 2009). The distinction between macrovascular (assessed macroscopically) and microvascular (assessed histologically) invasion in HCC is required by current pathologic guidelines (Burt et al. 2018) as they reflect different degrees of tumor aggressiveness and distinctively correlate with local recurrence and distant dissemination (Kokudo et al. 2016; Martins-Filho et al. 2019). Moreover, even subtleties such as the caliber and complexity of a compromised microscopic vessel and the distance from that vessel to the tumor seem to impact prognosis in HCC (Roayaie et al. 2009).

Recently, there has been an increasing interest in classifying HCC into different morphological subtypes. These subtypes are defined solely based on histology and often show association with different immunohistochemical stains, molecular events and patient survival (Salomao et al. 2010; Shibahara et al. 2014; Emile et al. 2001; Bannasch et al. 2017; Chagas et al. 2015; Kim et al. 2017; Kim et al. 2009; Haratake and Horie 1991; Liao et al. 2019; Labgaa et al. 2017; Araki et al. 2007; Kohno et al. 2013; Jeon et al. 2019; Calderaro et al. 2017). A detailed characterization of HCC subtypes would require a dedicated review (as performed elsewhere (Torbenson 2017; Calderaro et al. 2019)), but we summarize the main diagnostic criteria and some peculiarities of relevant subtypes in Table 2.

Hepatocellular carcinoma: immunohistochemistry in the clinical routine

The major application of IHC in the context of HCC is to confirm the hepatocellular lineage in clinical samples. The most relevant markers of hepatocellular differentiation include Arginase-1, expressed in 90–95% of the cases of HCC, HepPar1 (80–90%) and Glypican 3 (80%), that usually show granular cytoplasmic staining, and BSEP (75–90%), CD10 (60–80%) and CEAp (75%), that stain the canalicular proteins in the basolateral membrane of hepatocytes (Chan and Yeh 2010; Xiao et al. 2001; Lau et al. 2002; Lin et al. 2004; Nguyen et al. 2015; Fujikura et al. 2016; Chu et al. 2002) (Fig. 4). Despite the high overall expression of these markers in HCC, it is important to note that only Arginase1 and Glypican 3 retain adequate sensitivity in poorly differentiated cases. Therefore, these are the most important markers in the clinical setting, especially considering that histological analyses are sufficient for diagnosis of well and moderately differentiated tumors (Nguyen et al. 2015; Yan et al. 2010).

In poorly differentiated HCC, it is also important to rule out carcinoma from other common tissue sites (Lin and Liu 2014) (e.g., TTF1 for lung, CDX2 for colon). Interestingly, TTF-1 shows strong and diffuse staining in cytoplasmic granules in HCC: this pattern has not been identified in other tumors and such finding may help the diagnosis of HCC even in lung metastasis. Finally, whenever the neoplasm is morphologically undifferentiated, requiring differential diagnosis with non-epithelial tumors, it is necessary to include antibodies anti-Keratins 8 and 18, since some widely used anti-pankeratin antibodies such as AE-1/AE-3 may not react with hepatocytes (van Eyken et al. 1988).

In a research setting, many IHC markers have been associated with poor prognosis in HCC including K7, K19, EpCam, CD44, p53, SALL4 and Vimentin, among others (Liao et al. 2019; Durnez et al. 2006; Lai et al. 2015; Endo and Terada 2000; Alves et al. 2004). Among those, only K19 has been validated by several cohorts from different Cancer Centers and deserves special attention in the clinical routine. Indeed, expression of K19 in ≥5% of the cancer cells in HCC specimens is associated with higher prevalence of vascular invasion, resistance to sorafenib, local recurrence, extra-hepatic dissemination and lower overall and disease-free survival. In the non-neoplastic liver, K19 is expressed in biliary cells but also in hepatocyte precursors and hepatic progenitor cells. Therefore, K19-positive HCC represents a subset of tumors with stem-like properties and higher proliferative capacity(Martins-Filho et al. 2019; Durnez et al. 2006; Govaere et al. 2014; Fatourou et al. 2015; Takano et al. 2016).

Hepatocellular carcinoma: molecular events in relation to pathologic features

Multiple studies have explored molecular events in HCC including somatic mutations, transcriptomic signatures, methylation profiles and microRNA changes (Zucman-Rossi et al. 2015; Hoshida et al. 2008; Wahid et al. 2017). In this topic, we will focus on some studies that have

Table 2 HCC histological subtypes

Histological subtype	Diagnostic criteria and clinical associations
Steatohepatitic HCC	Presence of steatohepatitic features such as cell ballooning and steatosis in more than 50% of the cancer cells. This subtype is enriched in patients with metabolic syndrome, diabetes and non-alcoholic steatohepatitis. Prognosis is similar or better than usual HCC(Salomao et al. 2010; Shibahara et al. 2014)
Clear cell HCC	Usual criterion for diagnosis is presence of cytoplasmic clearing due to glycogen and/or lipid accumulation in 50% of the cancer cells. This cutoff might be lower in other cohorts. Exclusion of renal cell carcinoma is mandatory and based on clinical information and immunohistochemistry (clear cell HCC tend to retain expression of hepatocellular markers)(Emile et al. 2001; Bannasch et al. 2017)
Fibrolamellar carcinoma	Presence of prominent intratumor fibrosis. Tumor cells have a polygonal shape and usually depict large, eosinophilic cytoplasm. Fibrolamellar carcinoma usually develops in younger patients with no clinical history of liver disease(Chagas et al. 2015; Kim et al. 2017). Some sources consider fibrolamellar carcinoma an HCC variant while others consider it a unique form of primary liver cancer(Cancer Protocol Templates. College of American Pathologists 2019; World Health Organization 2010; Chagas et al. 2015; Kim et al. 2017).
Scirrhous HCC	Presence of intratumor fibrosis in more than 50% of the tumor area. Fibrolamellar carcinoma is an important differential diagnosis, but it usually develops in younger patients with no background liver injury. Scirrhous HCC develops in older patients with cirrhosis(Kim et al. 2017; Kim et al. 2009)
Sarcomatoid HCC	Presence of multiple spindle cells in the specimen. The minimum cutoff of spindle cells for diagnosis is not established, but the high proliferative nature of these cells usually makes them the dominant population in the specimen. Expression of Vimentin is frequent(Haratake and Horie 1991; Liao et al. 2019)
Lymphoepithelioma-like HCC	Defined as poorly differentiated tumors with syncytial sheets of inflammatory cells (mostly lymphocytes). Immunohistochemistry might be useful for confirming the epithelial nature and the hepatocellular lineage of this subtype(Labgaa et al. 2017)
Granulocyte-colony-stimulating factor producing HCC	Massive presence of neutrophils in the tumor sinusoids. Tumors are usually poorly- to undifferentiated and may show sarcomatoid changes. This subtype tends to occur in older patients and is associated with poor prognosis(Araki et al. 2007; Kohno et al. 2013).
Macrotrabecular massive HCC	Tumors show high prevalence of macrotrabecular architecture (> 6 cells thick); a recent publication suggest a 30% cutoff for diagnosis(Jeon et al. 2019). This subtype is constantly associated with vascular invasion and presence of *TP53* mutations. Prognosis is worse than usual HCC(Calderaro et al. 2017).

correlated relevant molecular events to specific pathologic features in HCC.

The Cancer Genome Atlas (TCGA) Research Network recently published a comprehensive analysis of the main genomic/epigenomic alterations in HCC based on the integration of multiple molecular platforms. The TCGA consortium performed clustering analysis of DNA copy number, DNA methylation, mRNA, miRNA and protein array data and generated three HCC molecular subtypes associated to specific demographic, mutation and pathologic data. The first cluster (iClust1) showed overexpression of proliferation markers (such as *MYBL2*, *PLK1* and *MKI67*) and low prevalence of *CTNNB1* and *TERT*-promoter mutations. It showed a higher prevalence of younger, female and Asian patients. Tumors were often poorly differentiated (49%) and macrovascular invasion was a common finding (10%). Not surprising, this cluster was associated with poor prognosis in independent datasets. iClust2 and iClust3 showed high frequency of *TERT*-promoter and *CTNNB1* mutations and enrichment for *HNF1A* mutations and *CDKN2A* (p16) silencing by hypermethylation. In iClust2, only 17% of the tumors were poorly differentiated and macro- and microvascular invasion were not common (not detected in 85% of the cases). Tumors in iClust3 showed a high frequency of *TP53* mutations (45% vs. 25% in the other clusters) and deep deletions in 17p. One-third of the tumors were poorly-differentiated and the frequency of macro- and microvascular invasion was 6 and 33%, respectively(Wheeler and Roberts 2017).

Further associations between molecular and clinicopathologic features were explored by Calderaro et al. Those authors showed that HCC histological subtypes are associated to specific underlying molecular features. For instance, scirrhous HCC is associated with TSC1/TSC2 mutations and epithelial-mesenchyme transition and stem-like properties, whereas steatohepatitic HCC shows frequent JAK/STAT activation and are *TP53*, *CTNNB1* and *TERT*-promoter wild type(Calderaro et al. 2017). The authors also validated important associations between HCC-driver genes *TP53* and *CTNNB1* and pathological findings. In fact, *CTNNB1*-mutated HCC were usually large tumors, albeit well-differentiated, with microtrabecular or pseudoglandular architecture. These tumors also lacked a strong inflammatory

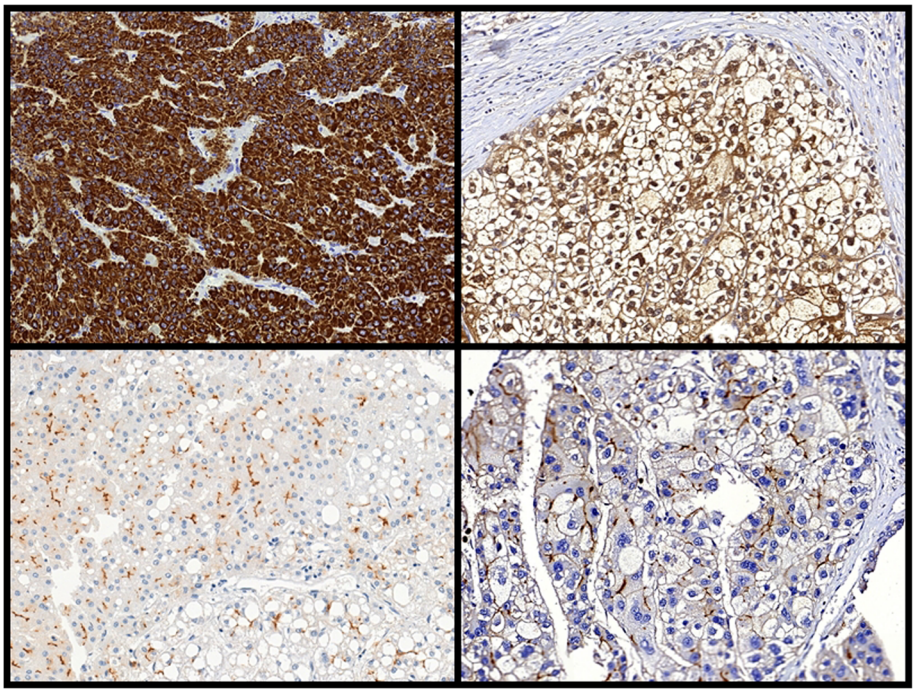

Fig. 4 IHC markers in HCC: HepPar1 (top-left), Arginase-1 (top-right), CD10 (bottom-left) and CEAp (bottom-right)

component, validating results from other study that suggested an immune-exclusion nature of *CTNNB1* mutations in HCC (Sia et al. 2017). Conversely, *TP53* mutants were solid, poorly-differentiated tumors, with higher frequency of pleomorphic cells and constant vascular invasion(Calderaro et al. 2017).

Altogether, results from these studies suggest that molecular events related to HCC development and progression shape the cancer cell morphology and impact stromal components (e.g., fibroblasts and inflammatory cells). Most importantly, these tumor and environmental changes can be inferred by histological analyses. An important and common aspect of these studies is the presence of expert liver pathologists carefully choosing the criteria and guiding the pathological analyses that ultimately revealed these remarkable morpho-molecular associations.

Limitations and perspectives to pathological analyses in hepatocellular carcinoma

As mentioned before, there are relevant limitations to pathological analysis in HCC. First, needle-biopsies and histological diagnosis are not recommended by current clinical guidelines and, for that reason, are not the standard practice in most services(Forner et al. 2018; Galle et al. 2018; Heimbach et al. 2018). The lack of biopsies precludes histological confirmation of HCC and the investigation of predictive factors such as tumor differentiation and expression of K19. Also, as suggested by Torbenson and Schirmacher, limited exposure to biopsies leads to "atrophy by neglect", i.e., underexposure to such specimens lowers the diagnostic performance of pathologists and even reduces the interest of trainees to the important field of liver pathology(Torbenson and Schirmacher 2015). Currently, the main arguments against biopsies are the high accuracy of imaging exams in the diagnosis of HCC and the unignorable risk of clinical complications such as chronic pain, bleeding (sometimes quite severe) and tumor seeding in the needle trajectory (Seeff et al. 2010; Schölmerich and Schacherer 2004). However, as procedures get safer, these complications tend to be better handled. Indeed, at the Toronto General Hospital, liver biopsies have been the standard practice for the diagnosis of HCC for several years and, consequently, the incidence of complications has reduced to an extent that it does not significantly impact survival. Furthermore, the Toronto group uses tumor differentiation to expand the current eligibility criteria for liver transplantation in patients with HCC, with fantastic results. For instance, patients with well-differentiated HCC, even if they have large tumors (beyond early-stage), are considered for surgical treatments and show prolonged survival (DuBay et al. 2011; Sapisochin et al. 2016). Other groups have also shown that expression of K19 in biopsy specimens predicts for resistance to sorafenib and poor outcome in patients with advanced HCC (Govaere et al. 2014; van Malenstein et al. 2012).

Second, and excepting the Toronto group, most of the current pathologic data derives from surgical specimens collected from patients at very early or early stage disease. In other words, very few studies explored morphological (and molecular) features of HCC at later stages, less so in extra-hepatic sites. Considering that cancer is a dynamic disease, it is fair to speculate that late-stage tumors are enriched for morpho-molecular features that might be overlooked if solely analyzed in earlier stages (Walter et al. 2012). Autopsy studies, particularly in an academic service, offer a valuable opportunity to overcome this sampling limitation. Indeed, we have recently published the evaluation of 230 HCC nodules from 88 patients who underwent autopsy, including 20 patients with extra-hepatic spread. In our cohort, metastatic nodules showed higher prevalence of poor histological differentiation and increased expression of K19 and EpCam compared to primary tumors. We also showed a strong predilection of HCC for lung dissemination: 21/36 (58%) of the metastatic nodules were collected from the lungs. Conversely, only 4/36 (11%) came from lymph nodes (Martins-Filho et al. 2019). This result slightly contrasts a previous imaging study that showed that 41% of the patients with metastatic HCC had lymph node involvement (Katyal et al. 2000). Our hypothesis for this difference is that, in some of the patient from the imaging study, HCC could have de- or transdifferentiated and transformed into combined hepatocellular-cholangiocarcinoma. These tumors show a more unpredictable dissemination pattern (De Vito et al. 2017). Based on these findings, we strongly advocate for a combination of both imaging and pathological analyses for a confident prediction of the preferred patterns of distant dissemination in HCC.

Finally, another important limitation to pathological analysis in HCC is the little consensus on the evaluation of histological grades in this cancer. This was ratified by a systematic review from our group showing that different grading classifications are used in the literature, with sometimes inaccurate criteria and/or grading tiers (Martins-Filho et al. 2017). Although some other HCC classifications have been described, we and other authors still recommend the use of the Edmondson and Steiner system, with four tiers, to histologically grade HCC. In fact, when adequately used, this grading classification shows strong predictive value in this cancer (Han et al. 2013; Martins-Filho et al. 2017). Alternatively, Edmondson and Steiner's grades I and II can be combined as well-differentiated HCC, following the recommendations by the International Collaboration on Cancer Reporting (ICCR) (Burt et al. 2018).

Recently, some of the international leaders in clinical hepatology have recognized that "Tumor biopsies may help to reliably distinguish HCC from other tumors, mostly cholangiocarcinoma as well as to identify the patient populations who most benefit from target-driven HCC treatments, in order to improve the success rate of experimental therapies" (Rimassa et al. 2017). In other words, liver biopsies might increase the diagnostic accuracy of HCC and improve patient selection for biomarker-oriented clinical trials. For instance, Rimassa et al has used liver biopsies for patient stratification in a recent HCC trial. Most notably, the authors conclude their analyses stating that "although this METIV-HCC trial was negative, the study shows the feasibility of doing integral tissue biomarker studies in patients with advanced hepatocellular carcinoma"(Rimassa et al. 2018). Such statements illustrate a positive change in opinion by clinicians concerning liver biopsies in HCC. This could lead to increased sampling and improved pathological characterization of these tumors in the clinical setting.

Conclusions

We expect that the present review has shown convincing evidences that, despite the important limitations to pathological analysis described here, careful gross and histological evaluation of HCC specimens remain very useful for diagnostic confirmation and patient prognostication. Pathological findings also often correlate with immunohistochemical markers, transcriptomic signatures and mutation data and could be important features in the screening of patients for future molecular-oriented therapies in this cancer.

Abbreviations

BCLC: Barcelona Clinic Liver Cancer; HCC: Hepatocellular carcinoma; IHC: Immunohistochemistry; K19: keratin 19; K7: keratin 7; KLCA: Korean Liver Cancer Association; LCSGJ: Liver Cancer Study Group of Japan; TACE: Transcatheter arterial chemoembolization; TCGA: The Cancer Genome Atlas

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Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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