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HEPATOCELLULAR CARCINOMA: AN OVERVIEW OF CLINICO-PATHOLOGICAL AND MOLECULAR PERSPECTIVES

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ABSTRACT: *Hepatocellular carcinoma (HCC) is a major health problem with a high incidence and mortality all over the world. The current ability to increase the survival of patients with HCC relies upon clinical surveillance, which allows HCC precursors and malignant tumors to be recognized at an earlier stage making cure possible. In this paper we will report the main histopathological criteria to discriminate between HCC precursors and well differentiated HCC, with attention to the role of immunohistochemical markers.*

Over time, many staging and scoring systems have been proposed for the classification and prognosis of patients with HCC. A complete system for HCC classification nowadays should include multifunctional and phenotypic characteristics of the tumor, the general health of the patient and the potential therapeutic approaches available. The second part of the paper is devoted to an overview of the current staging systems for HCC.

Genetic studies of tumor samples and the use of newer molecular approaches such as non coding RNA and genome-wide association studies, according to immunohistochemistry, proteomics and clinical criteria are highlighting the variability that exists among diverse HCC. Identification of new target pathways for the treatment of HCC by more personalized and targeted regimens are the main goals of the current translational medical research. The third part of the paper focuses on the molecular aspects of HCC.

KEY WORDS: *HCC, Pathology, Immunohistochemistry, Staging systems, Molecular analyses.*

INTRODUCTION

Hepatocellular carcinoma (HCC) has an increasing incidence worldwide, and it is the leading cause of death in patients with cirrhosis. The criteria for the diagnosis of Hepatocellular carcinoma (HCC) have been defined at the Barcelona-2000 EASL (European Association fof the Study of the Liver) conference[1]. Not so defined were the staging criteria, a useful tool to the physician to choose the most appropriate treatment. Unlike most other cancers, the HCC staging systems pro-

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TABLE 1. NOMENCLATURE OF SMALL HEPATOCELLULAR NODULES/LESIONS.

Displastic foci
A cluster of hepatocytes with features of early neoplasia (small- and large-cell change or iron-free foci in a siderotic background) measuring less than 0.1 cm

Displastic nodule
Gross features: these nodules have different color, size, texture and bulging from the adiacent liver paranchyma and have distinct margins
Histologic features:
a. *low-grade (LGDN):* mild increase in cell density with a monotonous pattern, no cytologic atypia, detectable portal tracts inside the nodule, large cell change/dysplasia may be present;
b. *high-grade (HGDN):* increased cell density, architectural and cytologic atypia, but insufficient for a diagnosis of HCC, unpaired arteries in most lesions, detectable portal tracts inside the nodule, large cell change/dysplasia and small cell change/dysplasia (most frequent) may be present
Small HCC (≤ 2 cm)
a. *early HCC:* a vaguely nodular lesion with indistinct margins, incomplete/absent capsule, increased cell density (>2x the surrounding non tumoral liver), well differentiated (G1), mild cellular/architectural atypia, scattered intratumoral portal tracts, variable number of unpaired arteries, fatty change, absence of microvascular invasion;
b. *Progressed HCC:* distinctly nodular lesion rimmed by condensed fibrosis/pseudocapsule, well to moderately differentiated (G1/G2), portal tracts are no longer identified, rare steatosis for more complete neoarterialization (unpaired arteries), microvascular invasion is seen in 25% of cases.

posed are not universally accepted, mainly because the staging of the tumor does not take into account the severity of the underlying liver disease that most often is the factor conditioning the prognosis of HCC.

PATHOLOGY

Surveillance and clinical aspects for early detection of HCC

The population groups at risk for HCC have traditionally included those with chronic hepatitis and cirrhosis. Surveillance should be undertaken if the risk of HCC is high enough, if an appropriate treatment is available and if surveillance is cost-effective. The latter parameter seems to be strongly influenced by the incidence of HCC. The American Association for the Study of Liver Disease (AASLD) identified different groups for whom surveillance is recommended, according to specific threshold incidence per year (0.2% for hepatitis B and 1.5-2% for cirrhosis from other causes, for instance)[2].

In non-hepatitic liver, metastases are more common than primary benign and malignant neoplasms. In the hepatitic/cirrhotic liver under surveillance for early HCC detection, the prevalence of malignancy among hepatocellular nodules is largely dependent on the size of the lesion: most of <1 cm lesions are non-malignant whereas the large majority of lesions exceeding 2 cm are HCC. Only a minority of regenerative/dysplastic nodules convert to malignancy (mostly in the group high-grade dysplasia), doing so in a relatively short interval, while 40-60% stabilize and a few definitely disappear during follow-up[3,4].

HCC is fed by a neoarterial supply which is of major importance for its imaging detection. Current international guidelines indicate that nodules greater than 2 cm arising in cirrhotic patients, with typical features for HCC at one dynamic technique, do not require biopsy confirmation. On the contrary, if lesions reveal atypical features, which occurs in 10-15% of the cases[5], biopsy is required. For nodules between 1 and 2 cm, two concordant radiological tests are needed for a non-invasive diagnosis of HCC, otherwise biopsy is recommended and this occurs in about 60–70% of the cases. Thus, most of small incident nodules in cirrhosis, suspicious but not conclusive for HCC, are currently biopsied.

Nomenclature of small hepatocellular nodules

An International Consensus[6] has recently been obtained on the classification of small (≤ 2 cm) hepatocellular nodules: the main definitions and criteria are illustrated in Table 1.

The distinction between this lesion, also termed *HCC of vaguely nodular type or HCC with indistinct margins*, and HGDN often requires additional techniques, as morphology alone is not sufficient. Extensive neovascularization process could be detected by immunohistochemical stain-

ing with endothelial marker CD34 and the Actin Smooth Muscle antigen (SMA) for muscolarized unpaired arteries. Stromal invasion of well differentiated hepatocytes into portal tracts or fibrous septa could be a useful diagnostic clue for malignancy: this histological aspect can be highlighted by immunostaining for cytokeratins CK7/19 depicting the ductular reaction which takes place around non malignant nodules but is absent around HCC. Finally, the overexpression of three specific immunomarkers (Glypican 3 – GPC3; Heat Shock protein 70 – HSP70; Glutamine synthetase – GS) in the neoplastic cell population, has been recognized as able to selectively label small and early HCC as compared to non malignant counterparts[6]. In the appropriate clinico-pathological context, the finding of 2 unequivocal positive immunomarker (out of 3 among GPC3, HSP70, GS) can detect early HCC with a sensitivity of 72% and a specificity of 100%[7].

Role of biopsy sampling in HCC diagnosis

As an increasing number of HCCs are diagnosed without pathologic examination (see paragraph "Surveillance and clinical aspects for early detection of HCC"), pathologists examine fewer cases of HCCs with the typical cytoarchitectural features: most cases are small well-differentiated hepatocellular nodules for which the differential diagnosis between early HCC and precursor lesions should be considered. The first step to characterize hepatocellular nodules in biopsy specimens is to evaluate the adequacy of the specimen itself. The ideal liver biopsy should contain sufficient tissue from both the tumour and the adjacent non-neoplastic liver. If there is no histological evidence of the target lesion, serial or deeper sections should be performed prior to recommending a repeat biopsy or follow up. If there is a suspected target on the biopsy but the lesion is too small for morphological evaluation or accompanied by a marked crushing artifact, an immunohistochemical stain could be performed with the 3-HCC marker panel (GPC3/HSP70/GS), as already stated[6]. The morphological features are recommended to be interpreted in context with the clinico-radiological findings, especially as the gross features of the nodule cannot be evaluated on biopsy specimens.

The replacement of biopsy evaluation as the gold standard for the diagnosis of HCC > 2 cm does not imply that all atypical cases in a cirrhotic liver represent HCC or its precursors: other lesions, such as large regenerative nodules (> 2 cm), may be detected in a cirrhotic liver (e.g. in autoimmune epatitis cirrhosis).

In the setting of non-cirrhotic livers, the possibility of hepatocellular adenoma (HCA) or focal nodular hyperplasia (FNH) should be considered in the differential diagnosis of hepatocellular nodules. HCA is characterized by proliferation of bland-looking hepatocytes. The trabeculae are irregularly and mildly thickened, numerous thin-walled arteries are present without accompanying bile ducts, and pseudoglandular structures can occasionally be seen, especially in β-catenin-activated HCAs. Therefore, the differential diagnosis between HCA and well-differentiated HCC may be challenging on a liver biopsy. It is even more problematic when there is evidence of focal cytologic or architectural atypia and in clinical settings unusual for HCA (atypical age/sex/morphology): terms such as 'atypical HCA-like neoplasms' and 'well-differentiated hepatocellular neoplasm of uncertain malignant potential (HUMP)' have been proposed for similar tumors, which have shown aggressive behavior. GPC3 and HSP70 immunostains may be helpful in distinguishing between HCAs and HCC; whereas GS, also part of the 3-marker HCC panel, is not always helpful in the differential diagnosis between HCC and HCA, as it is diffusely expressed in b-catenin-activated HCAs. The differential diagnosis between FNH and well-differentiated HCC or HCA may be challenging when FNH shows atypical features (e.g. lack of central scar), and the central stellate scar may not be sampled in liver biopsies. Immunohistochemical stains are of invaluable help in this setting: GS is expressed in FNH with a unique 'map-like' staining pattern, which is not showed in HCC or HCA.

Finally, the diagnosis of all the varieties of "combined hepatocellular- cholangiocarcinoma" is very complex on biopsy samples. Immunohistochemistry is usually mandatory to highlight hepatocytic and cholangiocytic differentiation, and mucin stains can be helpful to detect biliary differentiation (see paragraph "Histology – *Recently discovered subtypes*"). However, the diagnosis on liver biopsy may be difficult if the tumour is sampled from an area of typical HCC (or an area of typical cholangiocarcinoma) and the other components are not included in the specimen.

Advanced HCC

Macroscopic morphology

Most HCC are grossly nodular lesion, softer than the background liver. HCC can be unifocal or multifocal. Multifocality is defined as tumor nodules clearly separated by intervening non-neoplas-

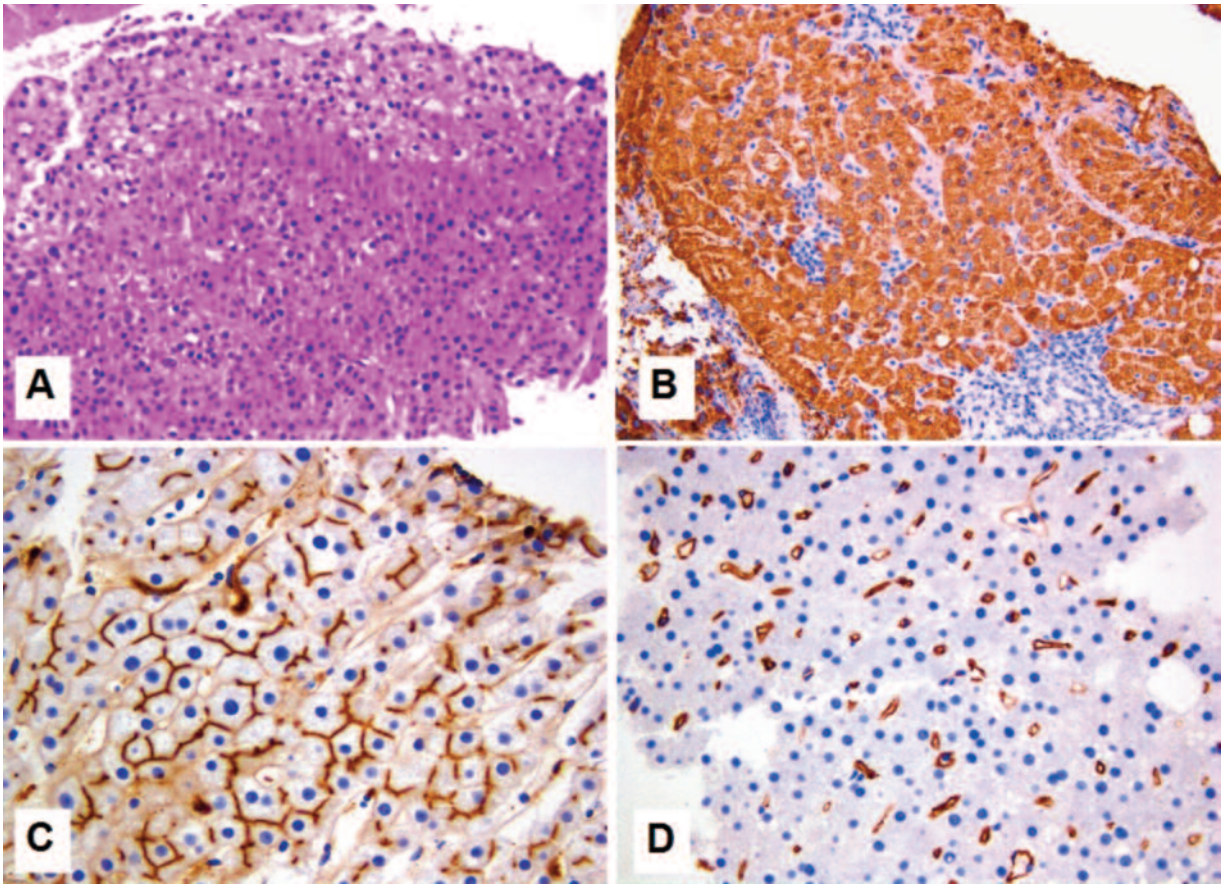

Figure 1. Histological appearance and immunohistochemical staining in HCC. (**A**) trabecular pattern of HCC, composed of cords of variable thickness that are separated by sinusoid-like blood spaces; neoplastic cells tend to resemble normal hepatocytes, maintaining a polygonal shape, with a slightly basophilic cytoplasm and variable degree of nuclear atypia (hematoxylin&eosin). (**B**) Hep Par 1, a specific marker for HCC, yields a diffuse cytoplasmic granular staining pattern in neoplastic hepatocytes, sparing bile duct epithelium and inflammatory cells (Hepatocyte paraffin 1 – Hep Par 1 immunostaining). (**C**) In HCC, pCEA staining reveals a characteristic and specific "chicken-wire fence" canalicular pattern, not observed in adenocarcinomas. The sensitivity of this marker given by the canalicular staining. Monoclonal CEA is not reactive in HCC. (Polyclonal carcinoembryonic antigen – pCEA immunostaining). (**D**) In HCC the stroma is composed of sinusoid-like blood spaces lined by endothelial cells showing changes of "capillarization", as they resemble normal capillaries, with immunoistochemical positivity for CD34 (CD34 immunostaining).

tic liver. Multifocal HCCs can represent either independent tumors arising simultaneously (i.e. multicentric HCC) or intrahepatic metastases from a primary tumor. The latter are more prone to develop early recurrences (usually within 2 years after treatment).

Based on autopsy data from advanced disease, Eggel's gross classification of HCC into nodular, massive, and diffuse types[8] is of limited value in clinical practice, because the majority of surgically resectable HCCs are the nodular type. Based on an analysis of surgically resected HCCs, a new gross classification has been proposed, in which Eggel's nodular type is subclassified into single nodular type, single nodular type with extranodular growth, and contiguous multinodular type. This classification has proven to be of clinical value in predicting patient outcome: single nodular type was demonstrated to be an independent factor for low risk of recurrence and low risk of disease-specific death[9].

Vascular invasion seems to be related to tumor size. Grossly, the distance of the embolized vessels from the main tumor mass, have an independent prognostic significance: vessels distant more than 1 cm from the main tumor mass have a very poor outcome[10].

Histology

HCC features cells that usually resemble normal hepatocytes, whereas the stroma is composed of sinusoid-like blood spaces lined by endothelial cells showing changes of "capillarization", as they resemble normal capillaries, with immunoistochemical positivity for CD34 (Figure 1D), factor VIII antigen or subendothelial laminin. Newly

formed arteries, so-called "unpaired or non-triadal" arteries, supply blood flow for HCC. Portal tracts are not identified within the tumor. The most recent WHO classification has described several architectural patterns:

- Trabecular pattern (most frequent- typical of G1/G2 HCCs), composed of cords of variable thickness that are separated by sinusoid-like blood spaces; such cords become thicker as tumor dedifferentiate (Figure 1A);
- Pseudoglandular/acinar pattern, in which gland-like structures, formed by a single layer of cells, are actually modified, abnormal bile canaliculi formed between tumor cells.
- Compact pattern, a solid appareance, typical of poorly differentiated tumors, with inconspicuous and slit-like blood-spaces.

In all of these patterns, cytologic features of HCC tend to resemble those of normal hepatocytes, with variable degree of nuclear atypia (Figure 1A).

Special types of HCC

Fibrolamellar carcinoma: overall, fibrolamellar carcinoma accounts for 0.5-9% of all primary liver cancers, with a peak incidence around 25 years. It typically arises in non-cirrhotic liver and, in two-third of the cases, it occurs in the left lobe, with a central scar. Histologically, fibrolamellar carcinoma has pushing margins and is made up of large polygonal cells, with abundant eosinophilic cytoplasm, large nuclei and nucleoli. The latter, along with lamellar fibrosis, are its distinctive features. Prognosis is more favourable than HCC arising in cirrhosis, but similar to HCC in non-cirrhotic liver.

Scirrhous HCC: about 5% of HCCs showed marked fibrosis along sinusoid-like spaces associated with varying degree of atrophy of neoplastic trabeculae. They typically arise below liver capsule. Similar changes occur after chemotherapy, radiation and transarterial chemoembolization. Due to its morphology and less frequent Hep Par-1 staining (see below) scirrhous HCC must be distinguished from fibrolamellar HCC, cholangiocarcinoma and metastatic adenocarcinoma. Glypican 3 and arginase have yielded a 100% sensitivity to identify scirrhous HCC variant in this context.[11]

Undifferentiated carcinoma: it is a rare primary liver tumor that can be diagnosed as carcinoma on the basis of immunohistochemical findings alone, but cannot be further classified. It shares similar clinical manifestations and imaging data with classic HCC, but prognosis is supposed to be worse.

Lymphoepihelioma-like carcinoma: It is a very rare tumor featuring pleomorphic tumor cells, occasionally organized in a syncytial growth, intermixed with numerous lymphocytes. In some cases, neoplastic cells are positive for Epstein-Barr virus (EBV).

Sarcomatoid HCC: HCC could be partially or fully composed of malignant spindle cells. When such sarcomatoid transformation is prominent, the tumor is defined sarcomatoid HCC. Areas of more typical morphology usually emerge after adequate sampling. Sarcomatoid change is more frequent after chemotherapy and transarterial chemoembolization.

Recently discovered subtypes

HCC with stem/progenitor cell immunophenotype: there is a recently proposed subtype of progressed HCC (mostly G2/G3), not otherwise recognizable by hematoxylin and eosin stain, in which a fraction of tumour cells (>5%) expresses stem/progenitor cell markers such as CK19 (cytokeratin 19), epithelial cell adhesion molecule (EpCAM), CD133, etc. It is still unaddressed whether this phenotype reflects an origin from a stem/progenitor cells or dedifferentiation from mature hepatocytes[12,13]. These tumours have been reported to be more aggressive as compared to usual HCC without stem/progenitor markers, with increased likelihood of recurrence following resection, resistance to chemotherapy, and radiotherapy, and increased likelihood of metastasis[12,13].

Mixed hepatobiliary carcinoma, classical type: mixed hepatobiliary carcinoma, classical type, accounts for about 2.5% of primary liver cancers. The most typical form is combined hepatocellular and cholangiocarcinoma, which has areas of typical HCC and areas of typical cholangiocarcinoma. This definition has not to be applied to collision tumours in which HCC and cholangiocarcinoma arise separately. These tumours can be defined morphologically. Confirmation of hepatocellular differentiation can be supported by immunostaining for hepatocyte specific antigen (Hep Par1), polyclonal anti-carcinoembryonic antigen (pCEA), and CD10. Cholangiocarcinoma components are usually positive for CK7 and CK19. Small areas of intermediate morphology at the interface between HCC and cholangiocarcinoma areas can be detected at careful examination. Therefore, these small tumour cells at the interface suggest a recapitulation of stem/progenitor cells[14]. The prognosis of this subtype has been reported to be poorer as compared to the usual progressed HCC.

Mixed hepatobiliary carcinoma, with stem/progenitor cell phenotype and immunophenotype: recently a rare subtype has been reported, in

which the majority of tumour cells show stem/progenitor like cells or intermediate features between hepatocyte and cholangiocyte. The prognosis of this subtype is uncertain given the absence of large cohorts of patients. The large sized "cholangiolocellular carcinomas" (>4.0 cm) had higher recurrence rates following treatment[15], and the overall survival of intermediate carcinoma was intermediate between those of typical HCCs and typical cholangiocarcinoma, whereas small sized mixed hepatobiliary carcinomas with stem/progenitor cell phenotype and immunophenotype in explanted livers showed no recurrence[16].

Grading

Grading of HCC has relied for many years on Edmondson and Steiner system, which divided HCC into four grades from I to IV on the basis of histological differentiation[17]. Grade I is the best differentiated consisting of small tumor cells, with regular nuclei, arranged in thin trabeculae. Cells in grade II are larger with abnormal nuclei, some hyperchromatism, nucleoli, >N/C ratio and glandular structures may be present. In grade III nuclei are larger and more hyperchromatic than grade II cells and cytoplasm is granular and acidophilic, but less than grade II. In grade IV, tumor cells are much less differentiated with hyperchromatic nuclei and loss of trabecular pattern. Marked anaplasia with giant and pleomorphic cells are evident.

HCC versus metastatic neoplasms

Metastases are by far the most common malignant neoplasms in non-hepatitic/cirrhotic liver. Without antecedent liver disease, HCC seems to account for about 2% of malignant neoplasms in the liver. Because of the wide spectrum of histologic appearance of HCC, the differential diagnosis between HCC and other tumors involving the liver can be challenging. Lung, colon, pancreas and breast are the most common primary sites of hepatic metastases, but malignant tumors from almost any site can metastasize to the liver. Secondary carcinomas may be difficult to assign to their origin but immunohistochemistry may be very helpful to distinguish between primary and metastatic tumors and, among metastases, to identify their primaries[18].

OVERVIEW OF CURRENT HCC STAGING SYSTEMS

The goal of a tumor staging system is to estimate a patient's prognosis, which allows for appropriate therapy to be selected. In HCC the importance to measure the contribution of both the cancer and the impaired liver function – due to metabolic and/or viral conditions at the roots of cancer itself- was early recognized. To be effective and widely used, a staging system has to be reliable, reproducible and simple, using data elements that can be obtained as part of standard clinical practice across a wide range of treatment sites. Most HCC staging systems have identified prognostic factors through multivariate analyses of large cohorts of patients to weight the different variables according to prognostic impact. Once proposed, a classification system must be validated across the spectrum of HCC cohorts.

TNM

The TNM system assesses primary tumor features (T), the presence or absence of nodal involvement (N) and distant metastasis (M). The criteria are developed jointly by the American Joint Committee on Cancer (AJCC) and the International Union for Cancer Control (UICC) and have been lastly updated in 2010, when the Seventh Edition took effect[19]. Recent versions of the TNM staging have been influenced largely by data from patients who underwent curative resections. Different authors proposed that the T-component focused on vascular invasion, tumor number/s and tumor size[20] and found that the key prognostic factors for 5-year survival were major vascular invasion, microvascular invasion and involvement of surrounding tissues[21]. The TNM system is based on histopathology and is applicable in prognosticating survival for the distinct minority of patients who have undergone curative surgery. By itself, the TNM T-stage does not offer guidance on resectability and therefore adds very little discriminatory value to patients' assessment. It has little relevance to patients presenting with advanced disease because of the model's inability to reflect the prognosis of underlying liver disease.

Okuda score

The Okuda system is a prognostic score introduced in 1985[22] and incorporates both tumor features as well as the degree of underlying cirrhosis. Okuda and colleagues devised a staging system based on four factors representing advanced disease: tumor occupying greater or less than 50% of the liver, the presence or absence of ascites, and serum albumin and bilirubin levels. Despite the absence of a prospective validation and its crude classification of early stage patients, the Okuda system is still in use. The evolution of imaging

and surveillance make it extraordinary nowadays to discover a tumor when it occupies more than half the liver.

BCLC staging classification

Derived from a single institution experience, the BCLC classification was first published in 1999[23] and is considered the standard HCC system by the American Association of for the Study of Liver Disease (AASLD) and European Association for the Study of the Liver. BCLC considered parameters are: size and extent of the primary tumor, liver function and physiological factors and incorporates the Okuda stage and Child-Pugh score. Each stage has a corresponding treatment schedule, ranging from curative therapies such as resection or transplant for early stage patients to best supportive care for end-stage patients.

CLIP score

Cancer of the Liver Italian Program (CLIP) is a simple scoring system designed by an Italian group with the aim of overcoming the main limitations of the TNM and Okuda. It has been derived from a retrospective cohort study of 435 patients[24] and then externally validated by a randomized trial that prospectively enrolled patients with cirrhosis and HCC. The CLIP includes the liver function according to Child-Pugh score, the morphology of the tumor (uninodular, multinodular, massive), its extension in the liver, the levels of Alpha-fetoprotein and the eventual presence of PVT. This score presents some limits, including the absence of general well-being assessment of the patient, and the inability to identify the early stages, which may benefit from percutaneous or surgical therapies.

Japan Integrated staging (JIS)

In 2003, the The Liver Cancer Study Group of Japan (LCSGJ) proposed the JIS score, which incorporates the LCSGJ's modification of the TNM system and the Child-Pugh score[25]. Arguing that the CLIP score did not provide sufficiently accurate prognostication for the early stage patients commonly diagnosed in Japanese centers due to screening programs and increased awareness of HCC, these investigators emphasized this very favorable group from other early-stage patients. Patients with a JIS score of 0 had a 10-year survival rate of 65% while patients with a CLIP score of 0 had 10-year survival rates of only 23%. It is widely used in Japan but it lacks external validation in Western countries.

Chinese University Prognostic Index (CUPI)

The CUPI[26] was designed in 2002 by the analysis of a cohort of 926 Chinese patients with HCC, adding five prognostic factors (total bilirubin, presence of ascites, alkaline phosphatase, alpha fetoprotein, and asymptomatic disease on presentation) to the TNM, in order to set up 3 classes of risk with highly significant differences in survival. This score was obtained from a monocentric and mono-ethnic cohort of patients, it was not validated in studies involving Western populations and most of the patients had a liver disease secondary to HBV infection. Therefore, the transportability of data of this score could be limited to this specific subset of patients.

Groupe d'Etude et de Traitement du Carcinome Hépatocellulaire (GRETCH)

The French scoring system, proposed by GRETCH in 1999[27], uses objective measures and an estimate of performance status to predict survival. A cohort of 761 consecutive patients across 24 institutions in Europe and Canada were randomly assigned to a training or a validation sample. Five predictors able to affect 1-year survival were identified using univariate analysis with Kaplan-Meier estimates and then included in a Cox proportional hazards model: performance status by Karnofsky score, serum bilirubin, serum alkaline phosphatase, AFP, and presence or absence of portal obstruction by ultrasonography. An advantage of the French classification is that its variables are generally available at the time of initial diagnosis and do not require invasive procedures or sophisticated imaging. The increasing use of cross-sectional imaging as a diagnostic modality could impact the prognostic value of this scoring system by modifying the sensitivity for diagnosis of portal obstruction.

Clinical staging comments

Several studies comparing the predictive power of different models have shown conflicting results, both in the general population and in specific subsets of patients receiving different class of treatments[28]. Currently, there is not an ideal staging and prognostic system for HCC. The BCLC seems to be the most comprehensive, since it integrates information about tumor extension, liver function

and the presence of constitutional symptoms. It also provides prognostic information and guidance to the therapeutic choices, and it has been endorsed by EASL and AASLD as standard for patients with HCC. Anyway, it does not represent a perfect model and still it has several unmet points. First, unlike CLIP, GRETCH and CUPI, the BCLC was not derived from a cohort of HCC patients by a multivariate analysis, and therefore it is not a prognostic model able to predict the mortality of HCC patients, being internally and externally validated just as a staging system. Second, as a classification model, it presents itself some inherent drawbacks. BCLC stage B encompasses a great variety of clinical situations, from four small tumors to near complete replacement of the liver by tumor, provided liver function is preserved and there is no vascular invasion, extrahepatic spread, or compromised performance status, which would upstage to BCLC stage C or D. Consequently, some BCLB-B patients may no longer be eligible for liver directed therapies, and are in practice treated following BCLC-C algorithms. The different conditions included in the BCLC-B stage also introduce the potential for prognostic heterogeneity within clinical research protocols employing BCLC stage for eligibility or stratification.

Third, as a treatment algorithm, the main limitations of the BCLC is represented by its rigidity: some prognostic factors are outlined as contraindications that preclude a therapy, whereas evidence suggests the contrary. Moreover, BCLC algorithm does not provide indications concerning second-line therapies, retreatment choices or combined treatments.

THERAPEUTIC MARKERS FOR HCC

AKT and Wnt/B-catenin pathways

HCC develops in a liver suffering from a chronic disease with addition of multifactorial processes characterized by the accumulation of genetic alterations and progressive chromosomal instability, caused at least in part from chronic inflammatory processes associated to pre-neoplastic stages of the disease. To date, it is not yet possible to identify a unique panel of molecular markers to detect in a uniform and satisfactory way the HCC disease. By analyzing the multitude of genetic profiles originating from HCC of different etiology reported in the literature in the last years, the most important emerging element is the common over-expression and/or genetic alteration of genes involved in cell proliferation and in mitosis. The HCC identified with such "highly proliferative" profiles are characterized by a poorer prognosis

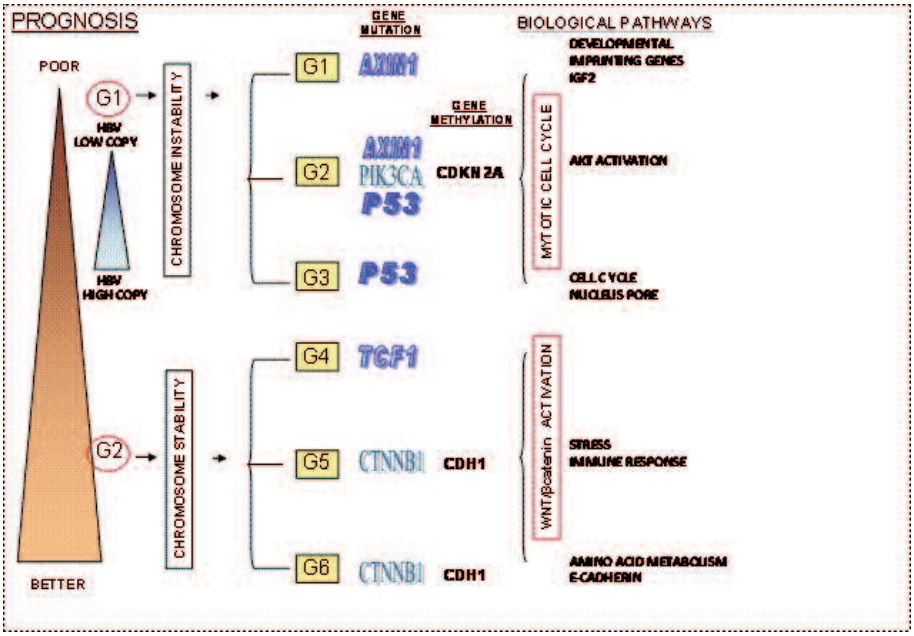

Figure 2. Principal genetic and transcriptomic characteristics of the different G1 to G6 HCC subgroups taken from published data[31]. G1 tumors were associated with low copy number of HBV and overexpression of genes expressed in fetal liver and controlled by parental imprinting. G2 included HCCs infected with a high copy number of HBV and mutations in PIK3CA and TP53. Both G1 and G2 correlated with an activation of the AKT pathway. G3 showed mutation of TP53 and overexpression of genes controlling the cell cycle. G4 showed TCF1-mutated HCC. G5 and G6 were strongly related to β-catenin mutations that lead to Wnt pathway activation; and in particular associated in G6 to E-cadherin expression.

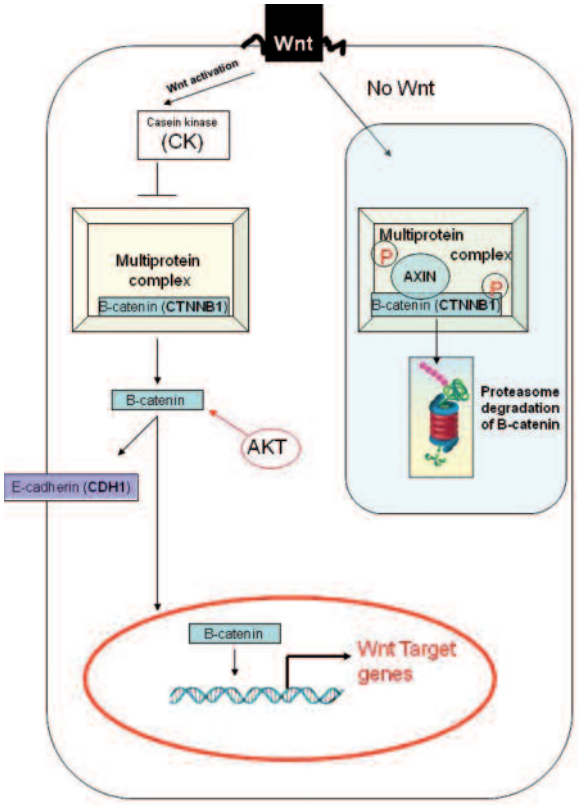

Figure 3. Wnt/β-catenin pathway. **A**) In the presence of a Wnt ligand, Wnt through casein kinase (CK) activity, inhibits the activity of the multiprotein complex (β-catenin-Axin-adenomatous polyposis coli (APC)-glycogen synthase kinase (GSK)-3b), which targets β-catenin by phosphorilation, and thereby indirectly inhibit β-catenin phosphorilation. The overall results culminate an accumulation of β-catenin that in part links to the E-cadherin and in part translocates into the nucleus where it leads to transcription of Wnt target genes. B) Without activation of Wnt the multiprotein complex phosphorilates β-catenin, and this can be now degradated by the proteasome complex.

with a decreased patient survival and regardless of cancer etiology. The more indicative genetic alterations include mutation acquisition or chromosome loss (LOH) leading to an inactivation of p53 and activation of the Wnt/β-catenin pathways mainly throught Axin1 or CTNNB1 gene alterations, activation of RB1, p16 or IGF2 genes or inactivation of IGFR2 receptor by mutation and methylation of the promoter region of the CDKN2A gene. Based on the chromosome instability Laurent-Puig and Zucman-Rossi indicated 2 different HCC classes occurring from a different pathogenetic way[29,30]: a pathogenetic way that lead to poorly differentiated and HBV positive tumors with a high chromosomal instability, mutations of p53 and Axin1 genes; and a second way that leads to well-differentiated tumors, negative for HBV infection and chromosomal instability and with an altered activation of the WNT/B-catenin pathway through CTNNB1 gene alteration (Figure 2). A subsequent study[31] not only confirmed this distinction but further subdivided these 2 groups into 6 groups basing on their transcriptomic profile. Groups 1-3 and groups 4-6, for chromosomal instability and stability, respectively (Fig. 2). A summary of the main pathways, AKT and Wnt/B catenin, involved in HCC are reported in Figures 3 and 4. According with this model it appeared that the "high proliferation index" showed in HCC with a worse prognosis was associated with an increased activity of genes involved in the AKT pathway and suggested that the activation of molecules with tyrosine kinase activity could be a trigger to counteract the high proliferation of tumor cells observed in HCC. Nowadays for advanced-stage HCC the only systemic treatment available is based on the inhibition of multichinase inhibitors, the sorafenib. The prolongation in survival of patients have encouraged to persist the studies in this direction with the development of other drugs targeted to protein kinases and confirmed the predictive value of the molecular signals identified.

p-RPS6 protein and mTOR pathway

However, sorafenib treatment had demonstrated limited benefit and moreover not all patients are responsive. Subsequent studies by using array-based pathway profiling has identified a significant correlation between the phosphorilated form of the RPS6 (p-RPS6) protein, a component of the 40S ribosomal subunit regulating the protein synthesis, and the resistance to sorafenib treatment[32]. Since, p-RPS6 is considered a molecular marker for the activation of mTOR signaling pathway (Figure 5)[33]. These experimental data have led to the use of mTOR inhibitors, such as everolimus, in patients with HCC resistant or not responsive to sorafenib. Partial sucess of sorafenib and everolimus in clinical phase I and II supports the pivotal role of mTOR signaling in HCC and highlights the importance of reliable biomarkers discovery to set best therapeutic options[34]. Identification of p-RSP6 like a predictive marker for response to sorafenib treatment is particularly important since at today several prognostic markers, like Ang2, VEGF, HGF, and IGF-2, were proved unable to predict a response to sorafenib[35].

Expression profiling

Molecular expression profiling has also demonstrated an utility for prognostication of pa-

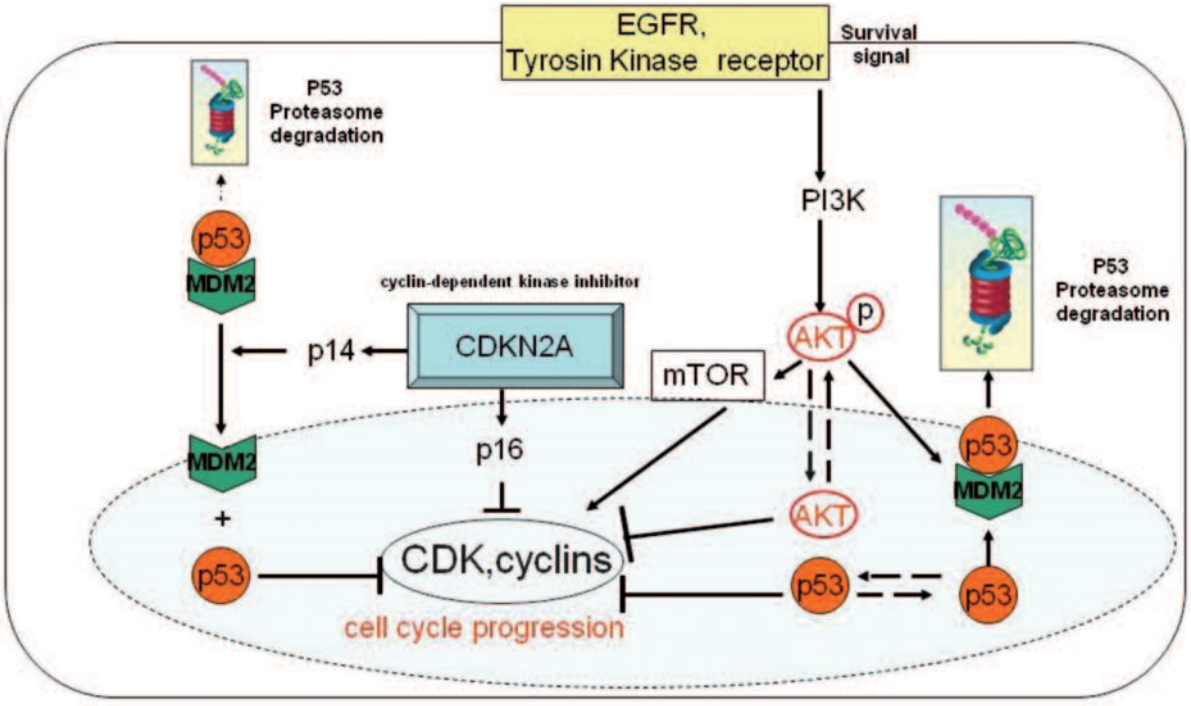

Figure 4. AKT pathway regulates cell cycle proliferation. Binding to tyrosin kinase receptor activates PI3K and then Akt. Activated Akt translocates to the nucleus and shuttles back to the cytoplasm. The transient nuclear Akt phosphorylates cyclin-dependent kinase (CDK) in the nucleus and leads to the temporary CDK relocation to the cytoplasm. When Akt is shuttled back to the cytoplasm, CDK rapidly translocates to the nucleus and helps in the cell cycle progression. AKT phosphorilates MDM2, this results in MDM2 translocation into the nucleus where it binds to P53, and targets it for degradation by the proteasome. Cyclin-dependent kinase inhibitor (CDKN2A) had a role in the cell cycle regulation directly by the tradition of p16 that ihbits cyclin-dependent kinase, and inderectly by producing p16 protein that blocks MDM2 and the consequent degradation of p53.

tients with HCC. To this an HCC five-gene score (HN1, RAN, RAMP3, KRT19 and TAF9) rigorously validated has been strongly proposed as prognostic markers for survival of patients after liver resection[36]. These genes are not functionally related, but for the first time, they represent a useful tool to stratify patients for various therapeutic decisions. In particular HCC with KRT19 expression could be used to identifie a subtype of HCC originated from a common progenitor between hepatocytes and cholangiocytes and thus perhaps to be managed differently[13].

Epigenetics

With the advent of new techniques like the genome-sequencing, epigenetics and proteomics analyses, new perspectives regarding prognostic markers are now appearing. Genome-sequencing had highlighted that about 5% of all HCC had not mutation in any protein kinase genes, thus suggesting that any kinase inhibitors, as sorafenib, would be effective in these cases. In the remaining 95% of cases mutations of CTNNB1 (11–32%) and AXIN1 (15%), and alteration of genes involved in cell cycle regulation such as p53 (18-35%) and CDKN2A (7-10%) were confirmed as previously found[37-40]. In addition, by studying noncoding RNA, new insights in HCC tumorigenesis has been found. According to their size, noncoding RNAs are divided into two groups: small (<200 nucleotides, miRNA) and long (>200 nucleotides, LncRNA). On the basis of overall miRNA profiles and integrating them with gene expression analysis and assessment of cellular pathway activation previously reported, a classification of HCC based on miRNA profiles has been proposed[36]. Moreover, it was found that lncRNA-UFC1 by interacting with b-catenin promotes the tumor progression when overexpressed[41]. Thus, lncRNA-UFC1 has been identified as both a prognostic marker and a potential new therapeutic target associated with the WNT/□-catenin pathway in HCC.

Epigenetic studies in HCC emphatisizes an important role of chromatin remodelling proteins in hepatocarcinogenesis. These studies are interesting because they shifted HCC therapeutic targets from coding tumor sequences of AKT and

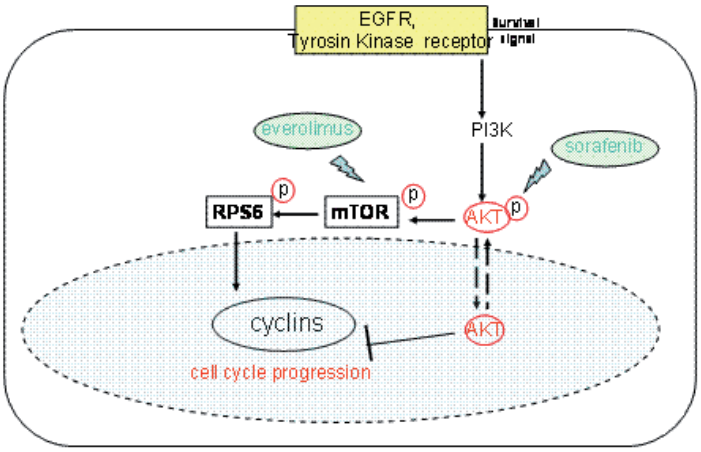

Figure 5. RPS6 is considered a molecular marker for the activation of mTOR signaling pathway and a marker for everolimus treatment. HCC who did not respond to sorafenib showed a significant correlation between the phosphorilated form of the RPS6 protein, a component of the 40S ribosomal subunit regulating the protein synthesis, and the resistance to treatment. AKT and mTOR pathways were both involved on cell cyle progression deregulated in HCC, thus sorafenib and everolimus have being proposed in advanced HCC treatment.

WNT/β-catenin pathways towards the area of chromatin-regulatory enzymes[42-49]. Telomerase (TERT) is a RNA polymerase that added telomere repeat sequences at the end of chromosomes and it is normally repressed in postnatal somatic cells. Telomeres shorten progressively with each round of cell division up to the death of the cell. A worst prognostic factor and aggressive behavior of HCC had been associated with long telomeres and increased telomerase activity[42]. Moreover, HBV genome integration was recurrently found into the promoter region of the TERT gene, and this correlated with the increased TERT activity[43]. Genes involved in chromatin remodeling are the histone methyltransferase (MLL), human AT-rich interaction domain (ARID) family of genes, SWI/SNF complexe[39,40,48,49]. Key proteins that mediate epigenetic signalling through the acetylation and methylation of histones and through chromatin remodelling represent a new frontier for the development of future therapeutics[46,47]. The clinical characteristics of tumors harboring these types of gene mutation suggest that their inactivation may be associated with an aggressive tumor phenotype and reinforce the link between cancer genome defects and epigenetic alterations in liver tumorigenesis.

Metabolomics

HCC metabolite profiling (metabolomics), the global view of aminoacids end products of cellular processes, is another promising new approach to underlying patients classification by using a rapid sensitive measure of cellular phenotype. Integration of both proteomics and genomics data will lead to a more accurate and precise known of cellular processes, which will move to the identification of key biomarkers for a personalized prognostic classification of HCC. A preliminar study of Budhu and col. by using a complex integrated metabolic and transcriptome analyses highlighted metabolites coding someenzymes that catalyzed the byosinthesis of monounsatures fatty acid with the HCC proliferation[50]. They hypothesed that a reduction in lipid bioavailibity, which affect the liver microenvironement may promote the HCC aggressiveness, since expression of these metabolites were significantly and independently associated with survival times and HCC recurrence. This study is interesting because it shifts the focus from tumor cells to the microenvironment. Accordantly, an analog study by combining HCC pathway classification with metabolomic results showed that HCC tumor with a good prognosis harbored a diminished concentration of certain statured lipids, consistent with an up-regulation of lipid catabolism in HCC[51].

Altogether, these data (Table 2) appear to be promising, but it must be remembered that genome-sequencing and genomic expression require further investigation to establish the real clinical utility of these assays. Moreover, further studies are needed to elaborate how disrupted histone and chromatin regulators cooperate with alterations in known signaling pathways, such as AKT, Wnt/b-catenin, and gene expression alteration in HCC progression

CONCLUSIONS

There is increasing need for reliable pathological classifications of HCC based on morphological and immnophenotypic grounds. New pathological entities are constantly recognized and defined by using multiparametric characteri-

TABLE 2. CONTRIBUTION TO THE DEFINITION OF SOME AMONG THE KEY MARKERS FOR HCC.

Type of analysis	Main results	Comments
Genomics studies	AKT and Wnt/β-catenin pathways	The main pathways involved in HCC
Molecular expression profiling	five-gene score	Expression of unrelated genes associated with the prognosis
Epigenetics studies	TERT and chromatin remodeling	A new therapeutic target based on epigenetic fators
	lncRNA-UFC1 related to Wnt/β-catenin pathways	An epigenetic factor associated to the Wnt/β-catenin pathway
	miRNA for HCC classification	A classification of HCC based on miRNA
Proteomics studies	up-regulation of lipid catabolism	Markers associated with the tumor microenvironement

zation. Data generated by massive genome sequencing offers new possibilities for better taxonomy and advanced therapeutic approaches. Results could be integrated with transcriptomic, methylome analysis, proteomic, and metabolomic in order to capture the full complexity of liver cancer and heterogenous patient's response. Over the past years, several therapeutic trials of newer tirosin kinase targeting pathways have been developed, but, in this category of sistemic drug, sorafenib remains the only drug currently approved to treat advanced HCC. Genetic studies of tumor samples and the use of newer molecular approaches such as non coding RNA and genome-wide association studies, according to immunohistochemistry, proteomics and clinical criteria are highlighting the variability that exists among diverse HCC. Identification of new target pathways for the treatment of HCC by more personalized and targeted regimens are the main goals of the current translational medical research.

Conflict of Interests:

The Authors declare that they have no conflict of interests.

REFERENCES

1. Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodés J; EASL Panel of Experts on HCC. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. J Hepatol 2001; 35: 421-430.
2. Sherman M. Modern approach to hepatocellular carcinoma. Curr Gastroenterol Rep 2011; 13: 49-55.
3. Borzio M, Fargion S, Borzio F, Fracanzani AL, Croce AM, Stroffolini T, Oldani S, Cotichini R, Roncalli M. Impact of large regenerative, low grade and high grade dysplastic nodules in hepatocellular carcinoma development. J Hepatol 2003; 39: 208-214.
4. Kobayashi M, Ikeda K, Hosaka T, Sezaki H, Someya T, Akuta N, Suzuki F, Suzuki Y, Saitoh S, Arase Y, Kumada H. Dysplastic nodules frequently develop into hepatocellular carcinoma in patients with chronic viral hepatitis and cirrhosis. Cancer 2006; 106: 636-647.
5. Forner A, Vilana R, Ayuso C, Bianchi L, Solé M, Ayuso JR, Boix L, Sala M, Varela M, Llovet JM, Brú C, Bruix J. Diagnosis of hepatic nodules 20 mm or smaller in cirrhosis: prospective validation of the noninvasive diagnostic criteria for hepatocellular carcinoma. Hepatology 2008; 47: 97-104.
6. International Consensus Group for Hepatocellular Neoplasia. Pathologic diagnosis of early hepatocellular carcinoma: a report of the International Consensus Group for Hepatocellular Neoplasia. Hepatology 2009; 49: 658-664.
7. Di Tommaso L, Franchi G, Park YN, Fiamengo B, Destro A, Morenghi E, Montorsi M, Torzilli G, Tommasini M, Terracciano L, Tornillo L, Vecchione R, Roncalli M. Diagnostic value of HSP70, glypican 3 and glutamine synthetase in hepatocellular nodules in cirrhosis. Hepatology 2007; 45: 725-734.
8. Eggel H. Ueber das primare Carcinom der Leber (in German). Beitz Pathol Anat 1901; 30: 506-604.
9. Hui AM, Takayama T, Sano K, Kubota K, Akahane M, Ohtomo K, Makuuchi M. Predictive value of gross classification of hepatocellular carcinoma on recurrence and survival after hepatectomy. J Hepatol 2000; 33: 975-979.
10. Roayaie S, Blume IN, Thung SN, Guido M, Fiel MI, Hiotis S, Labow DM, Llovet JM, Schwartz ME. A system of classifying microvascular invasion to predict outcome after resection in patients with hepatocellular carcinoma. Gastroenterology 2009; 137: 850-855.
11. Krings G, Ramachandran R, Jain D, Wu TT, Yeh MM, Torbenson M, Kakar S. Immunohistochemical pitfalls and the importance of glypican 3 and arginase inthe diagnosis of scirrhous hepatocellular carcinoma. Modern Pathology 2013; 26: 782-791.
12. Durnez A, Verslype C, Nevens F, Fevery J, Aerts R, Pirenne J, Lesaffre E, Libbrecht L, Desmet V, Roskams T. The clinicopathological and prognostic relevance of cytokeratin 7 and 19 expression in hepatocellular carcinoma. A possible progenitor cell origin. Histopathology 2006; 49: 138-151.
13. Lee JS, Heo J, Libbrecht L, Chu IS, Kaposi-Novak P, Calvisi DF, Mikaelyan A, Roberts LR, Demetris AJ, Sun Z, Nevens

F, Roskams T, Thorgeirsson SS. A novel prognostic subtype of human hepatocellular carcinoma derived from hepatic progenitor cells. Nat Med 2006; 12: 410-416.
14. Roncalli M, Park YN, Di Tommaso L. Histopathological classification of hepatocellular carcinoma. Dig Liver Dis 2010; 42S: S228-S234.
15. Komuta M, Spee B, Vander Borght S, De Vos R, Verslype C, Aerts R, Yano H, Suzuki T, Matsuda M, Fujii H, Desmet VJ, Kojiro M, Roskams T. Clinicopathological study on cholangiolocellular carcinoma suggesting hepatic progenitor cell origin. Hepatology 2008; 47: 1544-1556.
16. Fujii T, Zen Y, Harada K, Niwa H, Masuda S, Kaizaki Y, Watanabe K, Kawashima A, Nakanuma Y. Participation of liver cancer stem/progenitor cells in tumorigenesis of scirrhous hepatocellular carcinoma-human and cell culture study. Hum Pathol 2008; 39: 1185-1196.
17. Edmondson HA, Steiner PE. Primary carcinoma of the liver: a study of 100 cases among 48,900 necropsies. Cancer 1954; 7: 462-503.
18. Kakar S, Gown AM, Goodman ZD, Ferrell LD. Best practices in diagnostic immunohistochemistry: hepatocellular carcinoma versus metastatic neoplasms. Arch Pathol Lab Med 2007; 131: 1648-1654.
19. Sobin LH, Gospodariwicz M, Wittekind C, eds. TNM classification of malignant tumors. 7th ed. Hoboken, NJ: Wiley-Blackwell, 2009.
20. Vauthey JN, Lauwers GY, Esnaola NF, Do KA, Belghiti J, Mirza N, Curley SA, Ellis LM, Regimbeau JM, Rashid A, Cleary KR, Nagorney DM. Simplified staging for hepatocellular carcinoma. J Clin Oncol 2002; 20: 1527-1536.
21. Poon RT, Fan ST. Evaluation of the new AJCC/UICC staging system for hepatocellular carcinoma after hepatic resection in Chinese patients. Surg Oncol Clin N Am 2003; 12: 35-50.
22. Okuda K, Ohtsuki T, Obata H, Tomimatsu M, Okazaki N, Hasegawa H, Nakajima Y, Ohnishi K. Natural history of hepatocellular carcinoma and prognosis in relation to treatment. Study of 850 patients. Cancer 1985; 56: 918-928.
23. Llovet JM, Brú C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. Semin Liver Dis 1999; 19: 329-338.
24. The Cancer of the Liver Italian Program (CLIP) investigators. A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients. Hepatology 1998; 28: 751-755.
25. Kudo M, Chung H, Osaki Y. Prognostic staging system for hepatocellular carcinoma (CLIP score): its value and limitations, and a proposal for a new staging system, the Japan Integrated Staging Score (JIS score). J Gastroenterol 2003; 38: 207-215.
26. Leung TW, Tang AM, Zee B, Lau WY, Lai PB, Leung KL, Lau JT, Yu SC, Johnson PJ. Construction of the Chinese University Prognostic Index for hepatocellular carcinoma and comparison with the TNM staging system, the Okuda staging system, and the Cancer of the Liver Italian Program staging system: a study based on 926 patients. Cancer 2002; 94: 1760-1769.
27. Chevret S, Trinchet JC, Mathieu D, Rached AA, Beaugrand M, Chastang C. A new prognostic classification for predicting survival in patients with hepatocellular carcinoma. Groupe d'Etude et de Traitement du Carcinome Hépatocellulaire. J Hepatol 1999; 31: 133-141.
28. Maida M, Orlando E, Cammà C, Cabibbo G. Staging systems of hepatocellular carcinoma: A review of literature. World J Gastroenterol 2014; 20: 4141-4150.
29. Zucman-Rossi J, Jeannot E, Nhieu JT, Scoazec JY, Guettier C, Rebouissou S, Bacq Y, Leteurtre E, Paradis V, Michalak S, Wendum D, Chiche L, Fabre M, Mellottee L, Laurent C, Partensky C, Castaing D, Zafrani ES, Laurent-Puig P, Balabaud C, Bioulac-Sage P. Genotype-phenotype correlation in hepatocellular adenoma: new classification and relationship with HCC. Hepatology 2006; 43: 515-524.
30. Laurent-Puig P, Legoix P, Bluteau O, Belghiti J, Franco D, Binot F, Monges G, Thomas G, Bioulac-Sage P, Zucman-Rossi J. Genetic alterations associated with hepatocellular carcinomas define distinct pathways of hepatocarcinogenesis. Gastroenterology 2001; 120: 1763-1773.
31. Boyault S, Rickman DS, de Reyniès A, Balabaud C, Rebouissou S, Jeannot E, Hérault A, Saric J, Belghiti J, Franco D, Bioulac-Sage P, Laurent-Puig P, Zucman-Rossi J. Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. Hepatology 2007; 45: 42-52.
32. Masuda M, Chen WY, Miyanaga A, Nakamura Y, Kawasaki K, Sakuma T, Ono M, Chen CL, Honda K, Yamada T. Alternative mTOR signal activation in sorafenib-resistant hepatocellular carcinoma cells revealed by array-based pathway profiling. Mol Cell Proteomics 2014; 13: 1429-1438.
33. Steelman LS, Chappell WH, Abrams SL, Kempf RC, Long J, Laidler P, Mijatovic S, Maksimovic-Ivanic D, Stivala F, Mazzarino MC, Donia M, Fagone P, Malaponte G, Nicoletti F, Libra M, Milella M, Tafuri A, Bonati A, Bäsecke J, Cocco L, Evangelisti C, Martelli AM, Montalto G, Cervello M, McCubrey JA. Roles of the Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR pathways in controlling growth and sensitivity to therapy-implications for cancer and aging. Aging (Albany NY) 2011; 3: 192-222.
34. Bhoori S, Toffanin S, Sposito C, Germini A, Pellegrinelli A, Lampis A, Mazzaferro V. Personalized molecular targeted therapy in advanced, recurrent hepatocellular carcinoma after liver transplantation: a proof of principle. J Hepatol 2010; 52: 771-775.
35. Llovet JM, Peña CE, Lathia CD, Shan M, Meinhardt G, Bruix J; SHARP Investigators Study Group. Plasma biomarkers as predictors of outcome in patients with advanced hepatocellular carcinoma. Clin Cancer Res 2012; 18: 2290-2300.
36. Nault JC, De Reyniès A, Villanueva A, Calderaro J, Rebouissou S, Couchy G, Decaens T, Franco D, Imbeaud S, Rousseau F, Azoulay D, Saric J, Blanc JF, Balabaud C, Bioulac-Sage P, Laurent A, Laurent-Puig P, Llovet JM, Zucman-Rossi J. A hepatocellular carcinoma 5-gene score associated with survival of patients after liver resection. Gastroenterology 2013; 145: 176-187.
37. Guichard C, Amaddeo G, Imbeaud S, Ladeiro Y, Pelletier L, Maad IB, Calderaro J, Bioulac-Sage P, Letexier M, Degos F, Clément B, Balabaud C, Chevet E, Laurent A, Couchy G, Letouzé E, Calvo F, Zucman-Rossi J.Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. Nat Genet 2012; 44: 694-698.
38. Fujimoto A, Totoki Y, Abe T, Boroevich KA, Hosoda F, Nguyen HH, Aoki M, Hosono N, Kubo M, Miya F, Arai Y, Takahashi H, Shirakihara T, Nagasaki M, Shibuya T, Nakano K, Watanabe-Makino K, Tanaka H, Nakamura H, Kusuda J, Ojima H, Shimada K, Okusaka T, Ueno M, Shigekawa Y, Kawakami Y, Arihiro K, Ohdan H, Gotoh K, Ishikawa O, Ariizumi S, Yamamoto M, Yamada T, Chayama K, Kosuge T, Yamaue H, Kamatani N, Miyano S, Nakagama H, Nakamura Y, Tsunoda T, Shibata T, Nakagawa H. Whole-genome sequencing of liver cancers identifies etiological influences on mutation patterns and recurrent mutations in chromatin regulators. Nat Genet 2012; 44: 760-764.
39. Huang J, Deng Q, Wang Q, Li KY, Dai JH, Li N, Zhu ZD, Zhou B, Liu XY, Liu RF, Fei QL, Chen H, Cai B, Zhou B, Xi-

ao HS, Qin LX, Han ZG. Exome sequencing of hepatitis B virus-associated hepatocellular carcinoma. Nat Genet 2012; 44: 1117-1121.
40. Nault JC, Zucman-Rossi J.Genetics of hepatocellular carcinoma: the next generation. J Hepatol 2014; 60: 224-226.
41. Collins JF.Long noncoding RNAs and hepatocellular carcinoma. Gastroenterology 2015; 148: 291-294.
42. Nault JC, Mallet M, Pilati C, Calderaro J, Bioulac-Sage P, Laurent C, Laurent A, Cherqui D, Balabaud C, Zucman-Rossi J. High frequency of telomerase reverse-transcriptase promoter somatic mutations in hepatocellular carcinoma and preneoplastic lesions. Nat Commun 2013; 4: 2218.
43. Cleary SP, Jeck WR, Zhao X, Chen K, Selitsky SR, Savich GL, Tan TX, Wu MC, Getz G, Lawrence MS, Parker JS, Li J, Powers S, Kim H, Fischer S, Guindi M, Ghanekar A, Chiang DY. Identification of driver genes in hepatocellular carcinoma by exome sequencing. Hepatology 2013; 58: 1693-1702.
44. Oh BK, Kim H, Park YN, Yoo JE, Choi J, Kim KS, Lee JJ, Park C. High telomerase activity and long telomeres in advanced hepatocellular carcinomas with poor prognosis. Lab Invest 2008; 88: 144-152.
45. Kim H, Yoo JE, Cho JY, Oh BK, Yoon YS, Han HS, Lee HS, Jang JJ, Jeong SH, Kim JW, Park YN. Telomere length, TERT and shelterin complex proteins in hepatocellular carcinomas expressing "stemness"-related markers. J Hepatol 2013; 59: 746-752.
46. Toh ST, Jin Y, Liu L, Wang J, Babrzadeh F, Gharizadeh B, Ronaghi M, Toh HC, Chow PK, Chung AY, Ooi LL, Lee CG. Deep sequencing of the hepatitis B virus in hepatocellular carcinoma patients reveals enriched integration events, structural alterations and sequence variations. Carcinogenesis 2013; 34: 787-798.
47. Sung WK, Zheng H, Li S, Chen R, Liu X, Li Y, Lee NP, Lee WH, Ariyaratne PN, Tennakoon C, Mulawadi FH, Wong KF, Liu AM, Poon RT, Fan ST, Chan KL, Gong Z, Hu Y, Lin Z, Wang G, Zhang Q, Barber TD, Chou WC, Aggarwal A, Hao K, Zhou W, Zhang C, Hardwick J, Buser C, Xu J, Kan Z, Dai H, Mao M, Reinhard C, Wang J, Luk JM. Genome-wide survey of recurrent HBV integration in hepatocellular carcinoma. Nat Genet 2012; 44: 765-769.
48. Arrowsmith CH, Bountra C, Fish PV, Lee K, Schapira M. Epigenetic protein families: a new frontier for drug discovery. Nat Rev Drug Discov 2012; 11: 384-400.
49. Lin C, Song W, Bi X, Zhao J, Huang Z, Li Z, Zhou J, Cai J, Zhao H. Recent advances in the ARID family: focusing on roles in human cancer. Onco Targets Ther 2014; 7: 315-324.
50. Budhu A, Roessler S, Zhao X, Yu Z, Forgues M, Ji J, Karoly E, Qin LX, Ye QH, Jia HL, Fan J, Sun HC, Tang ZY, Wang XW. Integrated metabolite and gene expression profiles identify lipid biomarkers associated with progression of hepatocellular carcinoma and patient outcomes. Gastroenterology 2013; 144: 1066-1075.
51. Beyoglu D, Imbeaud S, Maurhofer O, Bioulac-Sage P, Zucman-Rossi J, Dufour JF, Idle JR. Tissue metabolomics of hepatocellular carcinoma: tumor energy metabolism and the role of transcriptomic classification. Hepatology 2013; 58: 229-238.