Revista da
ASSOCIAÇÃO MÉDICA BRASILEIRA
www.ramb.org.br

Review article

Hepatocellular carcinoma: epidemiology, biology, diagnosis, and therapies☆

Marcos António Gomes[a], *Denise Gonçalves Priolli*[b], *José Guilherme Tralhão*[c,d], *Maria Filomena Botelho*[a,d,*]

[a] *Service of Biophysics, Instituto Biomédico de Investigação da Luz e Imagem, Coimbra, Portugal*
[b] *School of Medicine, Universidade de São Francisco, Bragança Paulista, SP, Brazil*
[c] *Faculty of Medicine, Department of Surgery, Surgery 3, Hospitais Universitários de Coimbra, Coimbra, Portugal*
[d] *Centro de Investigação em Meio Ambiente, Genética e Oncobiologia, Coimbra, Portugal*

ARTICLE INFO

Article history:
Received 18 June 2012
Accepted 23 March 2013

Keywords:
Hepatocellular carcinoma
Therapy
Diagnosis
Hepatitis B
Hepatitis C
Liver neoplasms

ABSTRACT

Hepatocellular carcinoma is the fifth most common cancer in men and the seventh in women, as is diagnosed in more than half a million individuals worldwide every year. In Portugal, its incidence and mortality rates are low compared to other types of cancers. In Brazil, in the city of São Paulo, according to data released by the Brazilian Unified Health System (Sistema Único de Saúde – SUS), the incidence of primary liver cancer was 2.07/100,000 inhabitants. Although the vast majority of cases (85%) mainly affect developing countries, especially where infection by hepatitis B virus (HBV) is endemic, the incidence in developed countries is increasing. This pathology is associated with several risk factors, not only environmental but also genetic, generating an increasing interest in attaining a better understanding of this disease, which is still associated with very late diagnosis and poor prognosis. Of the available treatments, few patients benefit from their scanty advantages, increasingly stimulating research of new forms of treatment against this disease. This review aimed to briefly but fully identify risk factors, molecular and biochemical pathways, pathophysiology, diagnosis, and possible clinical approaches of hepatocellular carcinoma.

Carcinoma hepatocelular: epidemiologia, biologia, diagnóstico e terapias

Palavras-chave:
Carcinoma hepatocelular
Terapia
Diagnóstico

RESUMO

O carcinoma hepatocelular é o quinto tipo de câncer mais comum em homens e o sétimo em mulheres, diagnosticado todos os anos em mais de meio milhão de pessoas por todo o mundo. Em Portugal, sua incidência e mortalidade são baixas, comparativamente a outros tipos de cânceres. No Brasil, no município de São Paulo, segundo dados divulgados

☆ Study conducted at the Instituto Biomédico de Investigação da Luz e Imagem and at the Centro de Investigação em Meio Ambiente, Genética e Oncobiologia, Coimbra, Portugal.

* *Corresponding author*.
E-mail: filomena@ibili.uc.pt (M.F. Botelho).

2255-4823/$ – see front matter

Hepatite B
Hepatite C
Neoplasias do fígado

pelo Sistema Único de Saúde (SUS), a incidência do câncer primário de fígado foi de 2,07/100.000 habitantes. Apesar de a grande maioria dos casos (85%) afetar principalmente países em desenvolvimento, sobretudo onde a infeção pelo vírus de hepatite B (HVB) é endêmica, a incidência em países desenvolvidos é cada vez maior. Esta patologia está associada a inúmeros fatores de risco não só ambientais, mas também genéticos, os quais, cada vez mais, despertam interesse na procura pelo melhor conhecimento da patologia, muito associada ainda a diagnósticos tardios e maus prognósticos. Dos tratamentos disponíveis, poucos doentes são aqueles que usufruem das suas escassas vantagens, estimulando cada vez mais a pesquisa de novas formas de terapêutica. Esta revisão pretende, de forma breve mas completa, identificar fatores de risco, vias moleculares e bioquímicas, fisiopatologia, diagnóstico e possíveis abordagens clínicas do carcinoma hepatocelular.

Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is the primary liver cancer, that is to say, derived from primary liver cells. As in all cancers, it appears when mutations in the genes of a cell make uncontrolled growth possible. This mutation can be caused by external agents, such as hepatitis virus, or by excessive multiplication of cells, as in chronic regeneration in chronic hepatitis, which increases the risk of replication errors in the genes. HCC is typically aggressive, with a high rate of death after symptom onset (most commonly jaundice and/or ascites). If detected only in the symptomatic phase, the patient has an untreated mean life expectancy of less than one month; even at this stage, the available treatments are limited and ineffective.

HCC represents 70-85% of primary liver cancers, and is the most frequently observed primary malignant liver tumors worldwide,; cholangiocarcinoma, which originates from cholangiocytes, i.e., the epithelial cells lining the bile ducts, constitute 10-15% of primary hepatic malignancies. The remaining 5% are uncommon tumors such as primary liver angiosarcoma, hepatic epithelioid hemangioendothelioma, hemangiopericytoma, or primary hepatic lymphoma.

Annually, HCC is diagnosed in more than half a million people worldwide. The latest figures showed an estimated 748,300 new cases of HCC and 695,900 deaths caused by this disease. In Europe, 60,200 new cases were diagnosed in 2008, making this type of cancer the fifth most common in men and the seventh in women[1,2] (Fig. 1).

In Portugal, the incidence rate of this disease is low compared with other types of cancer, according to data from GLOBOCAN 2008, representing 1.1% of all cancer types. Regarding mortality associated with this type of pathology, it is responsible for 2% of all cancer-related deaths[3]; in the year 2011, according to the National Institute of Statistics of Portugal, 979 deaths due to malignant liver and intrahepatic bile duct neoplasms were recorded, 84 more cases than in the previous year.[4]

The World Health Organization (WHO) indicates HCC as the second leading cause of cancer-related death in humans due to its high incidence in the East, in areas of Africa, and in the Western Pacific. In Brazil, the incidence of HCC is low, while higher in some states such as Espírito Santo and Bahia. In the state of São Paulo, HCC is the fifth in frequency among digestive tract tumors, according to the Brazilian Association of Liver Transplants and Liver Disease Carriers.

Approximately 85% of the cases occur in developing countries, and the highest incidence rates are described in regions where hepatitis B virus (HBV) infection is endemic: Southeast Asia and sub-Saharan Africa. HCC rarely occurs before 40 years of age and reaches its peak at approximately 70 years of age. The prevalence rate of liver cancer among men is two to four times higher than among females.

HCC related to hepatitis C virus (HCV) infection has become the fastest-growing cause of the disease in the United States, contributing to the rising incidence of HCC in that country, which has tripled; the five-year rate survival remained below 12%.

Epidemiological data regarding the HCC in some countries, such as Brazil and Portugal, are still scarce and dispersed, making the organization and planning of health-promoting activities with an impact on prevention and early diagnosis of this pathology difficult. According to data released by the Brazilian Unified Health System (Sistema Único de Saúde – SUS), the incidence of primary liver cancer in the city of São Paulo, Brazil, was 2.07/100,000 inhabitants. The mean age of patients was 54.7 years, with a male/female ratio of 3.4:1. HBV surface antigen (HBsAg) positivity was 41.6%, anti-HCV positivity was 26.9%, presence of chronic alcoholism was 37%, and cirrhosis was 71.2%.[5]

Estimates made by GLOBOCAN for 2008 suggest that the incidence rate is 3.5/100,000 for males and 1.2/100,000 for females, resulting in 477 new cases per year. Mortality rates are, according to the same source, 3.4/100,000 and 1.1/100,000 for males and females, respectively.

Risk factors

The main risk factors for HCC include infection by HBV and HCV, liver diseases caused by alcohol consumption, aflatoxin exposure, and mainly non-alcoholic fatty liver disease[6] (NAFLD). Less common causes include hereditary

Fig. 1 – Regional variation in incidence rates of hepatocellular carcinoma (standardized age). The incidence rates shown (number of cases per 100,000 inhabitants) are for both genders and all age ranges.[106,107]

hemochromatosis (HH), alpha-1-antitrypsin deficiency, autoimmune hepatitis, certain porphyria types, and Wilson's disease. The distribution of these risk factors among patients with HCC is highly variable and depends on the geographic region, race, and ethnic group.[7]

Most of these risk factors lead to the onset and progression of cirrhosis, which is present in 80-90% of patients with HCC. The cumulative risk at five years for patients with cirrhosis to develop HCC varies between 5% and 30%, depending on the cause, with the greatest risk among those infected with HCV, those with specific risk due to geographic region or ethnic group (17% in USA and 30% in Japan, for instance), and the stage of cirrhosis; patients with decompensated disease present the greatest risk.[8]

Worldwide, approximately 50% of all adult patients with HCC have chronic HBV infection, whereas in children

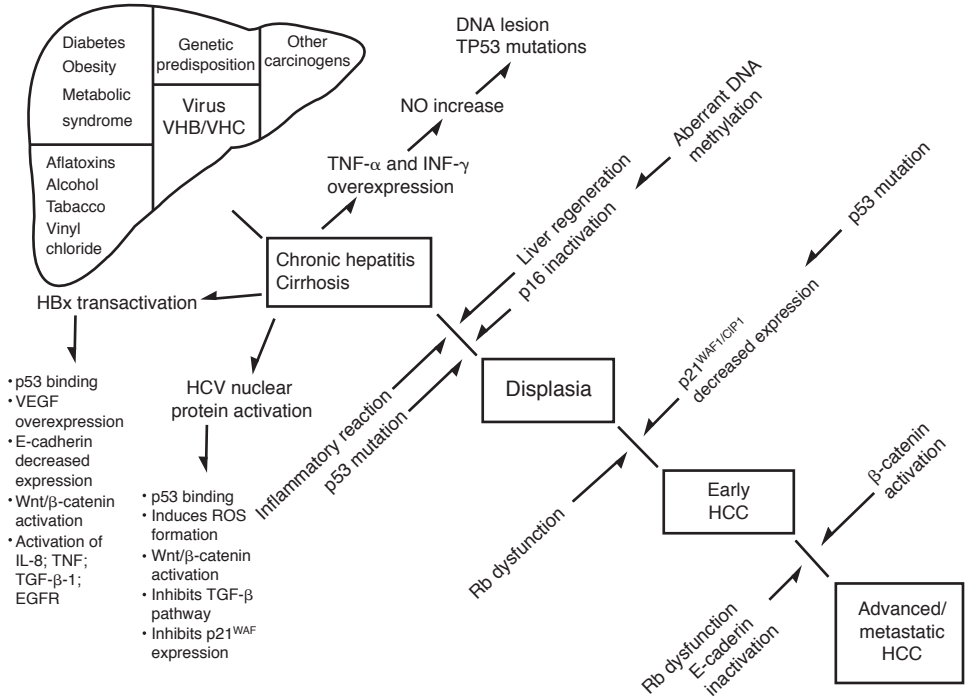

Fig. 2 – Molecular events in the development of hepatocellular carcinoma stages.[39,46-48,50,53-56,58-61,63,65-67,70-74] HCC, hepatocellular carcinoma; HCV, hepatitis C virus; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.

this association occurs in virtually all cases. In endemic areas of Asia and Africa, where HBV infection is vertical, approximately 90% of those infected undergo chronic evolution of the disease, with frequent virus integration into the host DNA.[9,10] The association between HBV and cancer varies greatly depending on the country and the types of laboratory tests used to diagnose the disease[11]; this scenario is changing with the emergence of new diagnostic techniques, especially polymerase chain reaction (PCR).

Although HBV can cause HCC in the absence of cirrhosis, between 70% and 80% of the patients present liver cirrhosis as a consequence of HBV infection. The risk of HCC in individuals with HBV infection is higher in males, the elderly, those with long-term exposure to the virus, family history of HCC, exposure to aflatoxin-type mycotoxins, alcohol or tobacco consumption, HCV or hepatitis delta virus (HDV) co-infection, high levels of HBV viral replication, and/or infection by HBV genotype C.[10,12-15] The estimated risk of HCC is 15-20 times higher among individuals infected with HCV compared with the non-infected; most of the additional risk is limited to those with advanced liver fibrosis or cirrhosis.[16]

An evolutionary molecular analysis study demonstrated that HCV infection occurred in a large number of young adults in Japan in the 1920s, in southern Europe in the 1940s, and in North America in the 1960s and 1970s. These cases in North America occurred as a result of the sharing of contaminated needles among drug addicts and due to blood transfusions[17]; the first case was described in 1989.

HCV infection markers are found in 80-90% of patients with HCC in Japan, 44-66% in Italy, and 30-50% in the United States.[7] Risk factors for HCC among individuals infected with HCV include older age at the time of infection, male gender, co-infection with the human immunodeficiency virus (HIV) or HBV, and probably diabetes or obesity.[18-21] The prolonged and abusive consumption of alcohol, defined as daily ingestion of 40 to 60 g of beverages containing 13.7 g of alcohol is a well-established risk factor for HCC, whether independently, with a 1.5-2.0 times increased risk , or combined with other risk factors, such as infection by HCV, or, to a lesser extent, infection with HBV.[16]

The development of effective screening methods in blood donors regarding the presence of HBV in the 1980s and of HCV in the 1990s drastically reduced the incidence of transfusion-related viral hepatitis, reducing its rates from 33% to 0.3% in the United States.[22] The development of effective prophylactic pre-exposure vaccines against HBV and the combination with hepatitis B immunoglobulins for post-exposure prophylaxis dramatically reduced the incidence of HBV infection.[23,24] In Taiwan, HCC incidence in children after universal vaccination against HBV reduced the number of cases from 0.70 to 0.35 per 100,000 children.[25]

The incidence of HCV also decreased after screening of blood donors in the 1990s, but due to the 20-year latency time from the acute infection to onset of cirrhosis and HCC, the impact of the reduction in HCV will not be observed before the year 2015. It is expected that the incidence of cases of HCV-related HCC will continue to decrease. An effective vaccine against HCV infection has not yet been developed due to the high mutation rate of the virus. For patients with chronic HBV or HCV infection, treatment with alpha interferon has been associated with lower incidence of HCC.[26-28]

Several studies performed in Western countries have demonstrated that 30% to 40% of HCC patients had no chronic HBV or HCV infection, suggesting the presence of other causes. Some of these patients had clinical or biochemical alterations compatible with NAFLD, such as obesity or metabolic syndrome. In population-based cohort studies in the United States, Scandinavia, Taiwan, and Japan, HCC was 1.5 to 2.0 times more frequent in obese than in non-obese patients.[29-32] Several case-control studies and some cohort studies have demonstrated that, on average, HCC is twice as likely to develop in individuals with diabetes *mellitus* type 2 than in non-diabetic patients.[33,34] NAFLD, which is present in almost 90% of obese individuals and in almost 70% of individuals with type 2 diabetes *mellitus*, appears to be a possible risk factor for HCC.[35] Due to the limited data establishing a direct association between the progression of hepatic steatosis and HCC, the currently available estimates of risk are not clear. However, due to the high incidence of metabolic syndrome in Western countries, any increase, however small, in the risk of obesity or diabetes can result in a higher number of HCC cases.

The pathogenic mechanisms involved include lipid peroxidation and oxidative stress, production of free radicals as a precursor to tumor onset and progression; chronic activation of the inhibitor of kappa-beta kinase (IKK-β), which leads to inflammation; and hepatic fibrosis due to hyperinsulinemia and hyperglycemia.[36]

Aflatoxins, particularly aflatoxin B1 (AFB-1), are potent liver carcinogens produced by a fungus (*Aspergillus flavus* and *A. parasiticus*), common contaminants of stored grain.[37] Gene mutation caused by AFB-1 at codon 249, exon 7 of the tumor suppressor gene TP53 (GC to TA transversion) was identified and positively correlated with exposure to aflatoxin in a meta-analysis comprising 49 studies.[38,39] In addition, aflatoxins are synergistic with chronic HBV infection for the development of HCC.[11]

Hepatocellular carcinoma molecular pathways

The association between hepatocarcinogenesis molecular pathways and the risk factors associated with this condition is the object of investigation, aiming at adequate, personalized, and effective chemoprevention and treatment of HCC (Fig. 2).

Chronic HBV infection involves three distinct mediation mechanisms in hepatocarcinogenesis. The first mechanism involves integration of viral DNA into the host genome, inducing chromosome instability.[40,41] The second proposed mechanism involves multiple genetic mutations by insertion, resulting in the integration of HBV genome at specific sites that can activate endogenous genes, for instance, RAR (retinoic acid receptor) β, cyclin A, and TRAP1.[42-44] The third mechanism involves modulation of cell proliferation by viral protein expression, particularly HBV X protein (HBx) with 154 amino acids (16.5-kDa), which can transactivate or overexpress a variety of viral and cellular genes.[44,45]

Many studies have associated HBx with the malignant transformation process that occurs in HCC. Studies have

shown that HBx can coactivate the transcription process of some important cell and viral genes, thus coordinating the balance between cell proliferation and apoptosis.[46,47] Cell promoters of genes associated with proliferation, such as interleukin 8 (IL-8), tumor necrosis factor (TNF), transforming growth factor (TGF-β1), and epidermal growth factor receptor (EGFR), as well as transcription factors, are activated with HBx transactivation.[48,49]

HBx also appears to be involved in the activation of signaling cascades involving the Ras/Raf/MAPK pathway, contributing to disregulation of cell cycle checkpoints,[50] as well as to the activation of several oncogenes such as *c-myc*, *c-jun* and *c-fos* in the cytoplasm.[51,52] Studies have also demonstrated two pathways by which HBx can activate the Wnt/β-catenin pathway, in collaboration with the proto-oncogene protein Wnt-1, through the stabilization of cytoplasmic β-catenin in HCC cells,[53] or by activation of the extracellular signal-regulated kinase (Erk), which leads to phosphorylation, and in turn, to the inactivation of kinase-3β of glycogen synthesis (GSK-3β), stabilizing the β-catenin.[54]

Another alternative mechanism of Wnt activation in HCC is by the repression of E-cadherin by HBx in transcription, through the hypermethylation of E-cadherin promoter.[55] These mechanisms demonstrate the association of HBx with decreased expression of E-cadherin and β-catenin accumulation in the cytoplasm and/or nucleus, leading to increased activation of β-catenin.[56] In the normal state, β-catenin is targeted for degradation by casein kinase Iα and GSK-3β.[57] 20% to 90% of all HCC have evidence of β-catenin activation, which has increased interest in the study of this pathway as a therapeutic target.[58-60]

Similarly to what occurs with several proteins codified by virus DNA, HBx can bind to p53, forming protein-protein complexes, and thus inactivating p53-dependent critical activities such as p53-mediated apoptosis,[61] or even repressing TP53 transcription.[62] These modulatory effects of HBx on p53 provide the basis for cell malignant transformation. In addition to this role in apoptosis, HBx can also contribute to tumorigenesis in HCC through the overexpression of the potent angiogenic factor of vascular endothelial growth factor (VEGF), demonstrated in studies under hypoxic conditions.[63,64]

Clinical and epidemiological studies attribute a higher degree of aggressiveness to HCV than to HBV, as there is a higher frequency of cases of HCC in patients with HCV-induced cirrhosis. In contrast to HBV, HCV is an RNA virus that is not integrated in the host genome; nevertheless, several virus-host interactions occur, which are believed to be responsible for the indirect hepatocarcinogenesis of this virus. The core protein of HCV is highly conserved and has been widely studied, as it is believed that it plays an important role in hepatocarcinogenesis through modulation of cell proliferation, apoptosis, and immune response.

This core protein of HCV induces ROS formation through interaction with shock protein Hsp60, in addition to binding to p53, p73, and Rb protein.[47,65] This interaction with tumor suppressor proteins appears to explain the fact that the protein is associated with $p21^{WAF1}$ inhibition, leading to apoptosis inhibition and promoting cell cycle.[47]

The Raf1/MAPK and Wnt/β-catenin pathways are activated by the HCV core protein, while it also inhibits TGF-β pathway and activates TNF-α receptor and NF-κβ pathway.[58,66,67] Studies have also demonstrated that the frequency of the β-catenin gene mutation in HCC of patients with HCV is approximately twice as high when compared to other causes.[68]

It is known that chronic inflammation and infection are often associated with increased risk of cancer. Infections caused by HBV and HCV cause inflammation with production of free radicals, cytokines, and chemokines, resulting in DNA damage, cell proliferation, fibrosis, and angiogenesis. An example of a response to inflammatory stress is the p53 pathway itself.[69] Free radicals, such as reactive oxygen or nitrogen species, can directly damage DNA and proteins, and/or indirectly damage these macromolecules through lipid peroxidation.

Increasing evidence indicates that nitric oxide (NO), an important signaling and bioregulation molecule catalyzed by the NOS enzyme family, plays an important role in carcinogenesis.[70,71] In chronic hepatitis, the overexpression of proinflammatory cytokines such as TNF-α, INF-γ, and IL-1, lead to increased concentrations of NO in human hepatocytes.[72] NO can cause DNA damage and induce a stress response through p53 anti-carcinogenic pathway or cause mutations in cancer-related genes, such as TP53.[70]

The mutational inactivation of TP53 has been widely described as one of the molecular mechanisms involved in HCC pathogenesis, especially in geographic areas where exposure to aflatoxin B1 (AFB1) is prominent.[39,73,74] However, and although the G»T mutation in codon 249 of the P53 gene is closely related to the AFB1 exposure, most HCC cases show absence of TP53 mutations, so that this inactivation is associated with other mechanisms, such as interactions with viral proteins.[75]

HCC has a high degree of genetic heterogeneity, suggesting that multiple molecular pathways may be involved in the genesis of hepatocellular cancer subsets. Understanding the molecular and cellular bases of neoplastic transformations that occur in the liver allow for the development of better strategies for the prevention of and/or more effective treatments for HCC.

Symptoms

HCC symptoms are not specific, generally indicate advanced HCC, and are more directly related to liver function impairment. There may be abdominal pain (between 40% and 60%), which may indicate spontaneous bacterial peritonitis; palpable mass in the abdomen on the right (23%); abdominal distension (45%) loss of appetite (45%); jaundice (16%); ascites (26%); weight loss (29%); malaise (60%); andsigns of hepatic encephalopathy, from drowsiness to coma, and gastrointestinal bleeding[76] (7%).

Pathology and diagnosis

The prognosis for patients with HCC is generally dismal. Survival varies from a few weeks to a year, depending on the extent of tumor involvement and other prognostic factors.

HCC may display four degrees of differentiation (according to the classification of Edmondson and Steiner, 1954) and five different histological types:

- sclerosing HCC;
- fibrolamellar carcinoma (which is the most straightforward to undergo surgical resection, thus yielding a better prognosis);
- cholangiocellular carcinoma;
- hepatocholangiocarcinoma;
- hepatoblastoma (more common in children).

Malignant transformation of hepatocytes in HCC is a gradual process associated with genetic mutations, allelic loss, epigenetic alterations, and disruption of cellular and molecular pathways. The phenotypic expression of these alterations may manifest as precursor lesions that accompany HCC, called dysplastic nodules.[77,78] These nodules are distinct from benign regenerative nodules associated with cirrhosis due to their high proliferative index and clonality.

The International Working Party of Terminology of the World Congress of Gastroenterology classified the dysplastic nodules, which are distinct nodular lesions larger than 5 mm in diameter, as low-grade dysplastic nodules (LGDN) and high-grade dysplastic nodules[79] (HGDN). LGDN nodules show only mild dysplasia and do not express any relation to malignancies. HGDN nodules, in turn, are characterized by higher cell density (small-cell lesions) than the surrounding tissues, exhibiting characteristic formations such as "nodule inside nodule", resembling well-differentiated HCC both at radiological and pathological assessment.[80,81]

The distinction between HGDN nodules and early-stage HCC is a challenge yet to be resolved. A panel of three immunohistochemical markers of malignant transformation, such as heat shock protein (HSP) 70, glutamine synthetase, and glypican-3 has been used to differentiate them.[82,83] Additionally, molecular data based on quantitative gene expression profiles with the LYVE-1 gene, E-cadherin, and survivin allow for a reliable diagnosis of early-stage HCC.[84]

The diagnosis of HCC can be attained through imaging tests, tumor markers (blood test), and anatomopathological examination (biopsy). As there is not always an increase in tumor markers and biopsy may not be possible (due to clotting deficiency caused by liver failure, to ascites, or to access difficulty due to tumor location) or recommended (there is a theoretical risk, from anecdotal reports, of tumor dissemination along the needle trajectory), the diagnosis of HCC can be achieved by imaging assessment presenting typical lesion and tumor marker increase, or by the presence of typical image in two different types of imaging assessment.

The guidelines for the diagnosis of HCC[76] suggest that patients with cirrhosis and radiologically-detected mass do not need biopsy confirmation of HCC diagnosis. Criteria for this diagnosis are: lesion of at least 2 cm in diameter with typical vascular patterns in dynamic images obtained from computed tomography (CT), ultrasound with contrast, or magnetic resonance imaging (MRI) showing enhancement in the arterial phase with rapid clearance of contrast in the portal phase.

For lesions 1-2 cm in diameter, at least two dynamic imaging studies are needed to highlight the typical features of HCC. Lesions < 1 cm in diameter should be followed by contrast ultrasound every 3-6 months. These guidelines are supported by the high incidence of regenerative nodules in cirrhotic livers that remain stable for many years, but can undergo malignant transformation to HCC due to changes in vascular supply, with shunting of blood from the portal vein to the hepatic artery and consequent increase in size. A percutaneous biopsy of liver injury in cirrhotic patients increases the risk of bleeding, dissemination of tumor cells, and false negative results due to inaccurate radiological location, particularly with small lesions.

Levels of serum alpha-fetoprotein (AFP), a glycoprotein normally produced by the fetal liver and yolk sac, can be elevated with the development of HCC. AFP levels are particularly indicative if > 500 μg/L[85,86] (normal values 10 to 20 μg/L), although 20% of HCCs are not associated with high serum AFP levels, and moderate increases in AFP can be found in other conditions such as active hepatitis of any etiology (virus-related, drug-related, autoimmune disease), pregnant women carrying fetuses with abnormalities such as spina bifida, and patients with germ-cell or gastric cancer. Serum AFP levels have also been used to enhance the sensitivity of non-contrast ultrasound when screening individuals at high risk for HCC.[87,88]

Staging and treatment

Due to the close association between HCC and cirrhosis, prognosis of individual cases depends on cirrhosis severity and the degree of malignant involvement of HCC. Most staging criteria for HCC prognosis incorporate elements on tumor extent and cirrhosis severity; however, the tumor-node-metastasis (TNM) staging system includes size, multifocality, vascular involvement, and distant metastases, but does not include the characteristics of cirrhosis.[89]

For the prognosis of patients with cirrhosis, the parameters of chronic liver disease evaluation, such as those used in the Child-Pugh classification, have been incorporated to most HCC classification criteria, including the Cancer of the Liver Italian Program score (CLIP), the Japan Integrated Staging Score (JIS), the Chinese University Prognostic Index (CUPI) and the French Prognostic Classification.[90-93]

Okuda classification

The classification of Okuda (1985) aims to predict the mean survival of patients with HCC not submitted to any type of minimally effective therapy, but submitted to medical treatment or hepatectomy. Based only on four relatively simple and objective parameters (although it is sometimes difficult to determine whether the tumor proportion in relation to the total liver size is greater or less than 50%), tumor stage is defined, along with its estimated prognosis in weeks. The caveat regarding this classification is that the clinical treatments have considerably improved since 1985, particularly with the emergence of new chemotherapeutic agents, improvements in

Table 1 – Targeted therapies in hepatocellular carcinoma.[108-113]

Drug	Drug type	Molecular target	Affected signaling pathway	Approval (EMEA and FDA)
Sorafenib	TC inhibitor	VEGFR, PDGFR, RAF	VEGFR, PDGFR, RAS/MAPK	Approved
Sunitinib	TC inhibitor	VEGFR, PDGFR, c-kit	VEGFR, PDGFR, c-kit	Not approved (undergoing phase II or III trials)
Bevacizumab	Monoclonal antibody to the ligand	VEGFR	VEGFR	
Cetuximab	Monoclonal antibody to the ligand	EGFR	EGFR	
Trastuzumab	Monoclonal antibody to the receptor	Her-2/neu	Her-2/neu	
Erlotinib	TC inhibitor	EGFR	EGFR	
Gefitinib	TC inhibitor	EGFR	EGFR	
Lapatinib	TC inhibitor	Her-2/neu	Her-2/neu	
Rapamycin	ST kinase inhibitor	mTOR	PIK3/Akt/mTOR	
Everolimus	ST kinase inhibitor	mTOR	PIK3/Akt/mTOR	
XL-765	ST kinase inhibitor	PI3K	PIK3/Akt/mTOR	

Akt, protein kinase B; EMEA, European Medicines Agency; FDA, Food and Drug Administration; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; PDGFR, platelet-derived growth factor receptor; PIK3, phosphatidylinositol 3-kinase; ST, serine/threonine; TC, tyrosine kinase; VEGFR, vascular endothelial growth factor receptor.

chemoembolization techniques and percutaneous treatments, and with the aid of new instruments such as argon scalpels and Yag-laser, in addition to the improvement in hepatectomy techniques. The classification remains important regarding the prognosis of untreated patients as a basis for comparison with the currently available treatments.[94,95]

The CLIP classification system encompasses the Child-Pugh classification, which assesses liver function, affected liver fraction (as in the Okuda classification), serum levels of alpha-fetoprotein, and hepatic vessel infiltration by cancer, especially the portal vein. With the obtained score, it is possible to compare the expected life expectancy in similar conditions.[96]

The Barcelona Clinic Liver Cancer (BCLC) is an internationally standardized system recommended by the American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL), and is based solely on clinical parameters.[76,97] Recently (2012) the EASL, together with the European Organization for Research and Treatment of Cancer (EORTC), published a set of clinical guidelines for surveillance, diagnosis, and therapeutic strategies for HCC, thus updating the panel of recommendations proposed in 2001[98] and used to date.[99]

The BCLC system classifies HCC as very early, early, intermediate, advanced, and terminal disease, based on the Child-Pugh criteria, functional status, tumor size, multifocality, and presence of portal vein invasion. Patients with very early and early stages are amenable to curative treatment modalities, including surgical resection, liver transplantation, percutaneous ethanol injection, or radiofrequency ablation.

Patients with intermediate-stage HCC are amenable to treatment by chemoembolization. For patients with advanced-stage HCC, which represent a large number of diagnosed cases, the recommended treatments include several tyrosine kinase inhibitors or participation in phase III and IV clinical trials, considering that the aforementioned treatments are rarely effective. Critically-ill patients are not usually considered for specific treatment and palliative treatment is indicated for symptom control.

Early-stage HCC presents as a small nodule with a diameter < 2 cm and can be classified into two types: small, nodular-type HCC, which is well defined and sometimes encapsulated; or indistinct (vague) nodular-type that is poorly defined and has no clear borders.[100,101] Dysplastic nodules and early HCC are usually asymptomatic and are usually incidental findings on radiographic studies or detected as a result of screening procedures. Early HCC can be indolent or slow-growing (up to one year), until it undergoes rapid tumor progression. In these early stages, HCC gradually progresses with increase in tumor volume, followed by invasion of the portal vein branches, extending to the main portal vein. The portal vein invasion is generally accompanied by a rapid increase in serum concentrations of AFP, even with no alterations in size of the primary liver tumor. The tumor can also directly invade the hepatic vein branches and the intrahepatic inferior vena cava, spreading to the superior vena cava or the right atrium as tumor thrombus. Hepatic artery invasion is much less common.

HCC dissemination occurs primarily through the blood to the lungs, bones, and brain at later stages. These metastatic lesions are typically hypervascular, as is the primary tumor, and predispose to bleeding (intracranial hemorrhage or hemoptysis). Bone metastases can be single or multiple and can produce isolated symptoms such as cranial nerve or peripheral nerve compression.

The standardized assessments for the detection of HCC metastasis are CT with contrast of the skull, chest, abdomen, and pelvis, as well as bone scintigraphy . Dynamic acquisitions with contrast are generally not necessary, but if performed, they can demonstrate arterial hypervascularity of the metastatic lesions similar to that of the primary tumor. Positron emission tomography (PET) has not shown sufficient

reliability to justify its inclusion in routine examinations in HCC staging.[102]

Multiple tumors can arise in cirrhotic liver, particularly with chronic HCV infection, or may represent liver metastases consequent to thrombosis of the portal vein, from the primary tumor and hematogenous spread to the liver.

The clinical and genetic heterogeneity of this disease dictates the fact that there is little effective therapeutic response in HCC. Therapy directed at molecular targets, either genes or their receptors, aims to inactivate activated oncogenes, recover tumor suppressor genes, or any molecule or gene involved in the development of HCC, thereby repairing errors or abnormal functions or biological behavior. Recently, many genome-based molecules have been candidates for targeted therapy. They have been discovered by microarray studies, at the analysis of epigenetic aberrations of the total genome, from high-performance sequencing systems. Some genes or target molecules currently in study are VEGFR, EGFR, DDEFL, VANGL1, WDRPUH, Ephrin-A1, GPC3, and PFTK1, among others.[103,104] Many of these molecules for targeted therapy, such as monoclonal antibodies, small molecules, and antisense molecules are now in phase II and III clinical trials, with promising data at this time. At the moment only sorafenib, a multikinase VEGFR and RAS kinase inhibitor, has been approved by the Food and Drug Administration and by the European Medicines Agency[104,105] (EMEA) (Table 1).

Conflicts of interest

All authors declare to have no conflicts of interest.

REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61:69-90.
2. Ferlay J, Parkin DM, Steliarova-Foucher E. Estimates of cancer incidence and mortality in Europe in 2008. Eur J Cancer. 2010; 46:765-81.
3. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer. 2010; 127:2893-917.
4. INE. Óbitos pela causa tumor maligno do fígado e das vias biliares intra-hepáticas do CID 10: resultados 2002-2011. Lisboa; 2012.
5. Gonçalves CS, Pereira FEL, Gayotto LCC. Hepatocellular carcinoma in Brazil: report of a national survey (Florianópolis, SC, 1995). Rev Inst Med Trop São Paulo. 1997;39:165-70.
6. Baffy G, Brunt EM, Caldwell SH. Hepatocellular carcinoma in non-alcoholic fatty liver disease: an emerging menace. J Hepatol. 2012;56:1384-91.
7. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology. 2007; 132:2557-76.
8. Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. Gastroenterology. 2004;127(5Suppl1):S35-S50.
9. El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. Gastroenterology. 2012;142:1264-73.
10. Ott JJ, Stevens GA, Groeger J, Wiersma ST. Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. Vaccine. 2012;30: 2212-9.
11. Lutwick, L. Relation between aflatoxin, hepatitis-B virus, and hepatocellular carcinoma. Lancet. 1979;313:755-7.
12. Chen CJ, Yang HI, Iloeje UH. Hepatitis B virus DNA levels and outcomes in chronic hepatitis B. Hepatology. 2009;49 (5 Suppl):S72-S84.
13. Yang HI, Yeh SH, Chen PJ, Iloeje UH, Jen CL, Su J, et al. Associations between hepatitis B virus genotype and mutants and the risk of hepatocellular carcinoma. J Natl Cancer Inst. 2008;100:1134-43.
14. Pollicino T, Saitta C, Raimondo G. Hepatocellular carcinoma: the point of view of the hepatitis B virus. Carcinogenesis. 2011;32:1122-32.
15. Fallot G, Neuveut C, Buendia M-A. Diverse roles of hepatitis B virus in liver cancer. Curr Opin Virol. 2012;2:467-73.
16. Donato F, Tagger A, Gelatti U, Parrinello G, Boffetta P, Albertini A, et al. Alcohol and hepatocellular carcinoma: the effect of lifetime intake and hepatitis virus infections in men and women. Am J Epidemiol. 2002;155:323.
17. Tanaka Y, Kurbanov F, Mano S, Orito E, Vargas V, Esteban JI, et al. Molecular tracing of the global hepatitis C virus epidemic predicts regional patterns of hepatocellular carcinoma mortality. Gastroenterology. 2006;130:703-14.
18. Davis GL, Alter MJ, El-Serag H, Poynard T, Jennings LW. Aging of hepatitis C virus (HCV)-infected persons in the United States: a multiple cohort model of HCV prevalence and disease progression. Gastroenterology. 2010;138:513-21.
19. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of US adults. N Engl J Med. 2003;348: 1625-38.
20. Moller H, Mellemgaard A, Lindvig K, Olsen J. Obesity and cancer risk: a Danish record-linkage study. Eur J Cancer. 1994; 30A:344-50.
21. EASL. Clinical practice guidelines: management of hepatitis C virus infection. J Hepatol. 2011;55:245-64.
22. Alter HJ, Houghton M. Hepatitis C virus and eliminating post-transfusion hepatitis. Nature Med. 2000;6:1082-6.
23. Ni Y, Chang MH, Huang LM, Chen HL, Hsu HY, Chiu TY, et al. Hepatitis B virus infection in children and adolescents in a hyperendemic area: 15 years after mass hepatitis B vaccination. Ann Intern Med. 2001;135:796-800.
24. Harpaz R, McMahon BJ, Margolis HS, Shapiro CN, Havron D, Carpenter G, et al. Elimination of new chronic hepatitis B virus infections: results of the Alaska immunization program. J Infect Dis. 2000;181:413-8.
25. Chang MH, Chen CJ, Lai MS, Hsu HM, Wu TC, Kong MS, et al. Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. N Engl J Med. 1997; 336:1855-9.
26. Lin SM, Sheen IS, Chien RN, Chu CM, Liaw YF. Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. Hepatology. 1999;29:971-5.
27. Brunetto M, Oliveri F, Koehler M, Zahm F. Effect of interferon-alpha on progression of cirrhosis to hepatocellular carcinoma: a retrospective cohort study. Lancet. 1998;351: 1535-9.
28. Lin S, Yu M, Lee C, Chien, R, Sheen I. Interferon therapy in HBeAg positive chronic hepatitis reduces progression to cirrhosis and hepatocellular carcinoma. J Hepatol. 2007;46:45-52.
29. Calle E, Rodriguez C. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of US adults. N Engl J Med. 2003;348:1625-8.

30. Moller H, Mellemgaard A, Lindvig K. Obesity and cancer risk: a Danish record-linkage study. Eur J Cancer. 1994;30A: 344-50.
31. Wolk A, Gridley G, Svensson M. A prospective study of obesity and cancer risk (Sweden). Cancer Causes Control. 2001; 12:13-21.
32. Calle E, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer. 2004;4:579-91.
33. El-serag HB, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. Gastroenterology. 2004;126:460-8.
34. El–Serag HB, Hampel H, Javadi F. The association between diabetes and hepatocellular carcinoma: a systematic review of epidemiologic evidence. Clin Gastroenterol Hepatol. 2006;4: 369-80.
35. Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. Hepatology. 2003;37:1202-19.
36. Edmison J, McCullough AJ. Pathogenesis of non-alcoholic steatohepatitis: human data. Clin Liver Dis. 2007;11:75-104.
37. Peers F, Bosch X, Kaldor J, Linsell A, Pluijmen M. Aflatoxin exposure, hepatitis B virus infection and liver cancer in Swaziland. Int J Cancer. 1987;39:545-53.
38. Hsu I, Metcalf RA, Sun T, Welsh JA, Wang NJ, Harris CC. Mutational hot spot in the p53 gene in human hepatocellular carcinomas. Nature. 1991;350:427-8.
39. Bressac B, Kew M, Wands J, Ozturk M. Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa. Nature. 1991;350:429-31.
40. Minami M, Daimon Y, Mori K, Takashima H, Nakajima T, Itoh Y, et al. Hepatitis B virus-related insertional mutagenesis in chronic hepatitis B patients as an early drastic genetic change leading to hepatocarcinogenesis. Oncogene. 2005;24:4340-8.
41. Shafritz DA, Shouval D, Sherman HI, Hadziyannis SJ, Kew MC. Integration of hepatitis B virus DNA into the genome of liver cells in chronic liver disease and hepatocellular carcinoma. Studies in percutaneous liver biopsies and post-mortem tissue specimens. N Engl J Med. 1981;305:1067-73.
42. Paterlini-Bréchot P, Murakami Y, Saigo K, Chami M, Mugnier C, Lagorce D, et al. Hepatitis B virus-related insertional mutagenesis occurs frequently in human liver cancers and recurrently targets human telomerase gene. Oncogene. 2003; 22:3911-6.
43. Wang J, Chenivesse X, Henglein B, Bréchot C. Hepatitis B virus integration in a cyclin A gene in a hepatocellular carcinoma. Nature. 1990;343:555-7.
44.Gozuacik D, Murakami Y, Saigo K, Chami M, Mugnier C, Lagorce D, et al. Identification of human cancer-related genes by naturally occurring hepatitis B virus DNA tagging. Oncogene. 2001;20:6233-40.
45. Murakami S. Hepatitis B virus X protein: a multifunctional viral regulator. J Gastroenterol. 2001;36:651-60.
46. De Mitri MS, Cassini R, Bernardi M. Hepatitis B virus-related hepatocarcinogenesis: molecular oncogenic potential of clear or occult infections. Eur J Cancer. 2010;46:2178-86.
47. Tsai W-L, Chung RT. Viral hepatocarcinogenesis. Oncogene. 2010;29:2309-24.
48. Andrisani OM, Barnabas S. The transcriptional function of the hepatitis B virus X protein and its role in hepatocarcinogenesis (review). Int J Oncol. 1999;15:373-9.
49. Morris SM, Baek JY, Koszarek A, Kanngurn S, Knoblaugh SE, Grady WM. Transforming growth factor-beta signaling promotes hepatocarcinogenesis induced by p53 loss. Hepatology. 2012;55:121-31.
50. Tarn C, Lee S, Hu Y, Ashendel C, Andrisani OM. Hepatitis B virus X protein differentially activates RAS-RAF-MAPK and JNK pathways in X-transforming versus non-transforming AML12 hepatocytes. J Biol Chem. 2001;276:34671-80.
51. Natoli G, Avantaggiati ML, Chirillo P, Costanzo A, Artini M, Balsano C, et al. Induction of the DNA-binding activity of c-jun/c-fos heterodimers by the hepatitis B virus transactivator pX. Mol Cell Biol. 1994;14:989-98.
52. Benn J, Schneider RJ. Hepatitis B virus HBx protein activates Ras-GTP complex formation and establishes a Ras, Raf, MAP kinase signaling cascade. Proc Natl Acad Sci USA. 1994;91: 10350-4.
53. Cha M-Y, Kim C-M, Park Y-M, Ryu W-S. Hepatitis B virus X protein is essential for the activation of Wnt/beta-catenin signaling in hepatoma cells. Hepatology. 2004;39:1683-93.
54. Ding Q, Xia W, Liu JC, Yang JY, Lee DF, Xia J, et al. Erk associates with and primes GSK-3beta for its inactivation resulting in upregulation of beta-catenin. Mol Cell. 2005;19:159-70.
55. Lee J-O, Kwun HJ, Jung JK, Choi KH, Min DS, Jang KL. Hepatitis B virus X protein represses E-cadherin expression via activation of DNA methyltransferase 1. Oncogene. 2005;24:6617-25.
56. Lian Z, Liu J, Li L, Li X, Clayton M, Wu MC, et al. Enhanced cell survival of Hep3B cells by the hepatitis B x antigen effector, URG11, is associated with upregulation of beta-catenin. Hepatology. 2006;43:415-24.
57. Nelson WJ, Nusse R. Convergence of Wnt, beta-catenin, and cadherin pathways. Science. 2004;303:1483-7.
58. Thompson MD, Monga SPS. WNT/beta-catenin signaling in liver health and disease. Hepatology. 2007;45:1298-305.
59. Luu HH, Zhang R, Haydon RC, Rayburn E, Kang Q, Si W, et al. Wnt/beta-catenin signaling pathway as a novel cancer drug target. Curr Cancer Drug Targets. 2004;4:653-71.
60. Kundu JK, Choi K-Y, Surh Y-J. beta-Catenin-mediated signaling: a novel molecular target for chemoprevention with anti-inflammatory substances. Bioch Biophys Acta. 2006; 1765:14-24.
61. Staib F, Hussain SP, Hofseth LJ, Wang XW, Harris CC. TP53 and liver carcinogenesis. Hum Mutat. 2003;21:201-16.
62. Lee SG, Rho HM. Transcriptional repression of the human p53 gene by hepatitis B viral X protein. Oncogene. 2000;19: 468-71.
63. Moon E-J. Hepatitis B virus X protein induces angiogenesis by stabilizing hypoxia-inducible factor-1alpha. FASEB J. 2004; 18:382-4.
64. Lee SW, Lee YM, Bae SK, Murakami S, Yun Y, Kim KW. Human hepatitis B virus X protein is a possible mediator of hypoxia-induced angiogenesis in hepatocarcinogenesis. Biochem Biophys Res Commun. 2000;268:456-61.
65. Bartosch B, Thimme R, Blum HE, Zoulim F. Hepatitis C virus-induced hepatocarcinogenesis. J Hepatol. 2009;51:810-20.
66. Block TM, Mehta AS, Fimmel CJ, Jordan R. Molecular viral oncology of hepatocellular carcinoma. Oncogene. 2003;22: 5093-107.
67. Chen CM, You LR, Hwang LH, Lee YH. Direct interaction of hepatitis C virus core protein with the cellular lymphotoxin-beta receptor modulates the signal pathway of the lymphotoxin-beta receptor. J Virol. 1997;71:9417-26.
68. Huang H, Fujii H, Sankila A, Mahler-Araújo BM, Matsuda M, Cathomas G, et al. Beta-catenin mutations are frequent in human hepatocellular carcinomas associated with hepatitis C virus infection. Am J Pathol. 1999;155:1795-801.
69. Staib F, Robles AI, Varticovski L, Wang XW, Zeeberg BR, Sirotin M, et al. The p53 tumor suppressor network is a key responder to microenvironmental components of chronic inflammatory stress. Cancer Res. 2005;65:10255-64.

70. Fukumura D, Kashiwagi S, Jain RK. The role of nitric oxide in tumour progression. Nat Rev Cancer. 2006; 6:521-34.
71. Lala PK, Chakraborty C. Role of nitric oxide in carcinogenesis and tumour progression. Lancet Oncol. 2001;2:149-56.
72. De Vera ME, Shapiro RA, Nussler AK, Mudgett JS, Simmons RL, Morris SM, et al. Transcriptional regulation of human inducible nitric oxide synthase (NOS2) gene by cytokines: initial analysis of the human NOS2 promoter. Proc Natl Acad Sci USA. 1996;93:1054-9.
73. Blonski W, Kotlyar DS, Forde KA. Non-viral causes of hepatocellular carcinoma. World J Gastroenterol. 2010;16: 3603-15.
74. Ming L, Franchi G, Park YN, Fiamengo B, Destro A, Morenghi E, et al. Dominant role of hepatitis B virus and cofactor role of aflatoxin in hepatocarcinogenesis in Qidong, China. Hepatology. 2002;36:1214-20.
75. Aguilar F, Harris CC, Sun T, Hollstein M, Cerutti P. Geographic variation of p53 mutational profile in nonmalignant human liver. Science. 1994;264:1317-9.
76. Bruix J, Sherman M. Management of hepatocellular carcinoma. Hepatology. 2005;42:1208-36.
77. Theise ND. Macroregenerative (dysplastic) nodules and hepatocarcinogenesis: theoretical and clinical considerations. Sem Liver Dis. 1995;15:360-71.
78. Theise ND, Schwartz M, Miller C, Thung SN. Macroregenerative nodules and hepatocellular carcinoma in forty-four sequential adult liver explants with cirrhosis. Hepatology. 1992;16: 949-55.
79. Wanless IR. Terminology of nodular hepatocellular lesions. Hepatology. 22:983-93.
80. Kojiro M, Roskams T. Early hepatocellular carcinoma and dysplastic nodules. Semin Liver Dis. 2005;25:133-42.
81. Roncalli M, Park Y, Di Tommaso L. Histopathological classification of hepatocellular carcinoma. Dig Liver Dis. 2010; 42(Suppl 1):S228-S34.
82. Suriawinata A, Thung SN. Molecular signature of early hepatocellular carcinoma. Oncology. 2010;78(Suppl 1):36-9.
83. Di Tommaso L, Franchi G, Park YN, Fiamengo B, Destro A, Morenghi E, et al. Diagnostic value of HSP70, glypican 3, and glutamine synthetase in hepatocellular nodules in cirrhosis. Hepatology. 2007;45:725-34.
84. Llovet JM, Chen Y, Wurmbach E, Roayaie S, Fiel MI, Schwartz M, et al. A molecular signature to discriminate dysplastic nodules from early hepatocellular carcinoma in HCV cirrhosis. Gastroenterology. 2006;131:1758-67.
85. Wu JT. Serum alpha-fetoprotein and its lectin reactivity in liver diseases: a review. Ann Clin Lab Sci. 1990;20:98-105.
86. Chan SL, Chan ATC, Yeo W. Role of alpha-fetoprotein in hepatocellular carcinoma: prognostication, treatment monitoring or both? Future Oncol. 2009;5:889-99.
87. Singal A, Wolk ML, Waljee A, Salgia R, Higgins P, Rogers MA, et al. Meta-analysis: surveillance with ultrasound for early-stage hepatocellular carcinoma in patients with cirrhosis. Aliment Pharmacol Ther. 2009;30:37-47.
88. Sherman M, Peltekian KM, Lee C. Screening for hepatocellular carcinoma in chronic carriers of hepatitis B virus: incidence and prevalence of hepatocellular carcinoma in a North American urban population. Hepatology. 1995.22:432-8.
89. Vauthey J-N, Lauwers GY, Esnaola NF, Do KA, Belghiti J, Mirza N, et al. Simplified staging for hepatocellular carcinoma. J Clin Oncol. 2002;20:1527-36.
90. Daniele B, Annunziata M, Barletta E, Tinessa V, Di Maio M. Cancer of the Liver Italian Program (CLIP) score for staging hepatocellular carcinoma. Hepatol Res. 2007;37(Suppl 2): S206-9.
91. CLIP Prospective validation of the CLIP score: a new prognostic system for patients with cirrhosis and hepatocellular carcinoma. Hepatology. 2000;31:840-5.
92. 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: 207-15.
93. Leung TWT, Tang AM, Zee B, Lau WY, Lai PB, Leung KL, et al. 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;:92:1760-9.
94. Okuda K, Ohtsuki T, Obata H, Tomimatsu M, Okazaki N, Hasegawa H, et al. Natural history of hepatocellular carcinoma and prognosis in relation to treatment study of 850 patients. Cancer. 1985;56:918-28.
95. Okuda K. Natural history of hepatocellular carcinoma including fibrolamellar and hepato-cholangiocarcinoma variants. J Gastroenterol Hepatol. 2002;17:401-5.
96. A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients: the Cancer of the Liver Italian Program (CLIP) investigators. Hepatology. 1998;28: 751-5.
97. Llovet JM, Brú C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. Semin Liver Dis. 1999;19:329-38.
98. Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, et al. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. J Hepatol. 2011;35:421-30.
99. European Association for the Study of the Liver & European Organisation for Research and Treatment of Cancer EASL-EORTC Clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol. 2012;56:908-4.
100. International Concensus Group for Hepatocellular Neoplasia. Pathologic diagnosis of early hepatocellular carcinoma: a report of the international consensus group for hepatocellular neoplasia. Hepatology. 2009;49:658-64.
101. Kojiro M. Diagnostic discrepancy of early hepatocellular carcinoma between Japan and West. Hepatol Res. 2007;37 (Suppl 2):S121-4.
102. Wolfort RM, Papillion PW, Turnage RH, Lillien DL, Ramaswamy MR, Zibari GB. Role of FDG-PET in the evaluation and staging of hepatocellular carcinoma with comparison of tumor size, AFP level, and histologic grade. Int Surg. 2010;95:67-75.
103. Llovet JM, Bruix J. Molecular targeted therapies in hepatocellular carcinoma. Hepatology. 2008;48:1312-27.
104. Midorikawa Y, Sugiyama Y, Aburatani H. Molecular targets for liver cancer therapy: from screening of target genes to clinical trials. Hepatol Res. 2010;40:49-60.
105. Thomas M. Molecular targeted therapy for hepatocellular carcinoma. J Gastroenterol. 2009;44(Suppl 1):136-41.
106. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Cancer incidence and mortality worldwide: IARC CancerBase No. 10. GLOBOCAN 2008 v1.2, Lyon, France: International Agency for Research on Cancer; 2010. Lancet Oncol. 2012;13(6): 607-15.
107. Bray F, Ren J, Masuyer E, Ferlay J. Estimates of global cancer prevalence in 2008 for 27 sites in the adult population. Int J Cancer. 2013;132:1133-45.
108. Zender L, Kubicka S. Molecular pathogenesis and targeted therapy of hepatocellular carcinoma. Onkologie. 2008;31:555-5.

109. Thomas MB, Chadha R, Glover K, Wang X, Morris J, Brown T, et al. Phase 2 study of erlotinib in patients with unresectable hepatocellular carcinoma. Cancer. 2007;110:1059-67.
110. Zhu AX, Stuart K, Blaszkowsky LS, Muzikansky A, Reitberg DP, Clark JW, et al. Phase 2 study of cetuximab in patients with advanced hepatocellular carcinoma. Cancer. 2007;110:581-9.
111. Liu L, Cao Y, Chen C, Zhang X, McNabola A, Wilkie D, et al. Sorafenib blocks the RAF/MEK/ERK pathway, inhibits tumor angiogenesis, and induces tumor cell apoptosis in hepatocellular carcinoma model PLC/PRF/5. Cancer Res. 2006; 66:11851-8.
112. Faivre S, Raymond E, Boucher E, Douillard J, Lim HY, Kim JS, et al. Safety and efficacy of sunitinib in patients with advanced hepatocellular carcinoma: an open-label, multicentre, phase II study. Lancet Oncol. 2009;10:794-800.
113. Papaetis GS, Syrigos KN. Sunitinib: a multitargeted receptor tyrosine kinase inhibitor in the era of molecular cancer therapies. BioDrugs. 2009;23:377-89.