Focus on hepatocellular carcinoma

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Introduction

Hepatocellular carcinoma (HCC) is the fifth most frequent neoplasm worldwide (>500,000 deaths/year), and in some areas of the world it represents the primary cause of cancer-related death (Parkin et al., 2001). Years ago, HCC was considered a major health problem in Asia and Africa, with minor prevalence in Europe or America. However, current data indicate that its incidence is steadily increasing in the West (Parkin et al., 2001). This has raised the attention paid to its molecular pathogenesis and to aspects related to epidemiology and treatment. This focus summarizes the established knowledge regarding these issues and points out the most active areas of research where strong data are eagerly awaited.

Epidemiology and risk factors

The incidence of HCC has a wide geographical variation due to the large heterogeneity of the penetration of the risk factors within the population (Llovet et al., 2003). Any agent leading to chronic liver injury, and eventually cirrhosis, constitutes an oncogenic agent. The most relevant are hepatitis B (HBV) and hepatitis C (HCV) viruses and alcohol (Table 1). Other causes of hepatocyte damage (iron or copper deposition, non-alcoholic steatohepatitis, or primary biliary cirrhosis), are also risk factors, but their lower prevalence diminishes their overall importance (Llovet et al., 2003).

HBV infection is highly prevalent in undeveloped countries and in some areas of Asia. There, the infection is acquired at birth or early in life, resulting in a very high incidence of HCC in children and adults (Parkin et al., 2001; Llovet et al., 2003). Observational investigations have shown that age of infection, male gender, and active liver damage associated with flares of viral replication are the major characteristics leading to increased HCC risk (Llovet et al., 2003; Evans et al., 2002; Yang et al., 2002). Viral genotypes may play a role, although no strong data are available. Associated alcohol consumption further heightens the risk, which is also enhanced by Aflatoxin B1 (AFB1) intake. This substance is a mycotoxin produced by fungi that contaminate food stored in humid conditions. AFB1 is the most potent oncogenic agent for the liver, and some areas with high HBV infection rate (Philippines, South East Asia) also present a high rate of AFB1 contamination, resulting in the highest HCC prevalence. In some instances, HCC may be related to occult HBV infection that is not detectable by serum markers and is only identified by studying HBV fragments using RT-PCR in tissue samples (Pollicino et al., 2004).

HCV is a blood-transmitted agent and is the major risk factor in Europe, Japan, and America. It overlaps with alcohol intake, which in some areas such as France is still the major oncogenic agent. The rising incidence of HCC in developed countries is likely due to the spread of HCV 20–40 years ago. Since 1990, HCV has been detectable, and blood products are screened. Thus, with proper conditions, transmission of HCV and postprocedure hepatitis (mainly transfusion) has decreased, which should lead to a decrease of HCC incidence. While HBV-related HCC may develop in the absence of cirrhosis or extensive liver fibrosis, the appearance of HCC in patients with chronic HCV infection is usually preceded by the establishment of significant liver damage (Llovet et al., 2003).

Table 1. Incidence of HCC according to geographical area and distribution of risk factors

	Age-adjusted incidence rates		Risk factors			
Geographic area	Male	Female	HCV	HBV	Alcohol	Other
Europe			60%–70%	10%–15%	20%	10%
Western Europe	5.8	1.6				
Southern	9.8	3.4				
Northern	2.6	1.3				
North America			50%–60%	20%	20%	10%
Northern	4.1	1.6				
Southern	4.8	3.6				
Asia and Africa			20%	70%	10%	Aflatoxin
Japan			70%	10%–20%	10%	10%
Eastern Asia	35.4	12.6				
South-Eastern Asia	18.3	5.7				
Middle Africa	24.2	12.9				
World	14.9	5.5				

Modified from Llovet et al., 2003, with permission from *Lancet*.

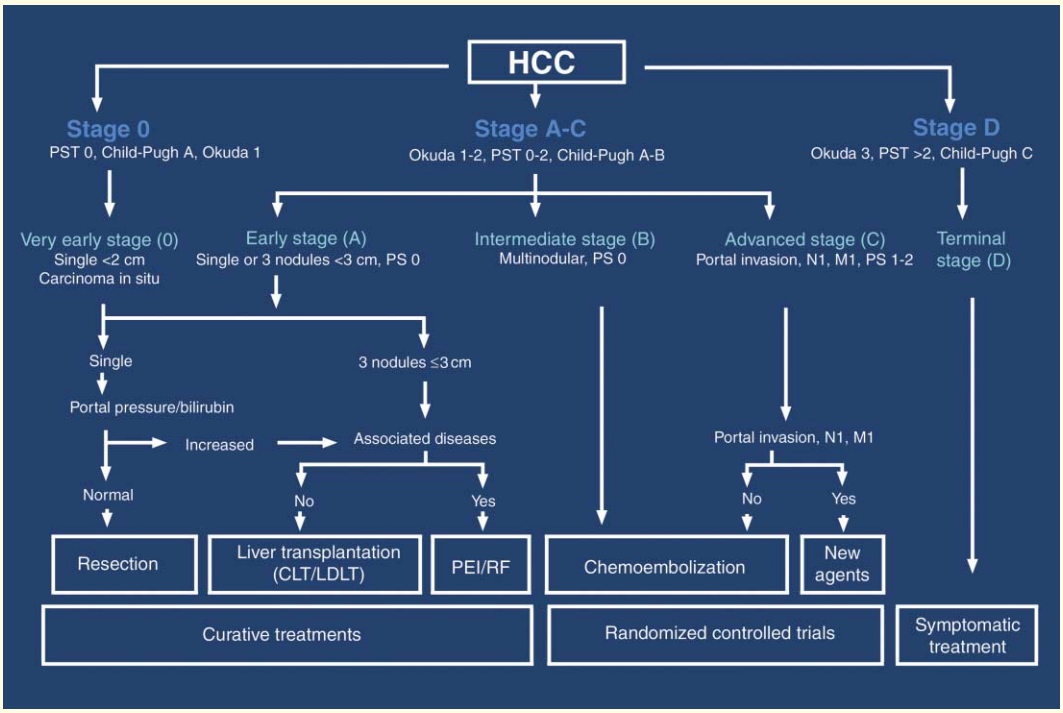

Figure 1. BCLC staging and treatment strategy for patients diagnosed with HCC

HCC, hepatocellular carcinoma; CLT, cadaveric liver transplantation; LDLT, live donor liver transplantation; PEI, percutaneous ethanol injection; RF, radiofrequency. Modified from Llovet et al., 2003, with permission from *Lancet*.

Prevention

As described above, chronic liver damage due to any agent results in an increased HCC risk. Thus, the optimal preventive strategy is to reduce viral transmission and alcoholism. HBV spread can be controlled by vaccination, which, as shown in Taiwan, reduces HBV penetration and HCC incidence (Chang et al., 1997). Thus, this intervention should be implemented worldwide and may turn HCC into the first cancer prevented through vaccination. There is no vaccine for HCV, and prevention of infection should come from proper health care conditions. Finally, public health campaigns should focus on the risks of excessive alcohol intake, as done for smoking.

If primary prevention has not been feasible or successful, the health strategy should aim to eradicate the acquired risk factor, or at least diminish its capacity to induce liver damage and cirrhosis. Antiviral therapy for HBV is available through several agents, but, although viral replication may cease, the virus may integrate in the hepatocyte genome and exert its oncogenicity (Block et al., 2003). Treatment for HCV infection is more effective. The combination of interferon and ribavirin may clear the virus, and thus prevent the long-lasting damage that precedes HCC development (Fried et al., 2002). If cirrhosis is established, there is no proof that any intervention is effective in preventing HCC (Camma et al., 2001). Lowering viral load or alcohol intake may reduce liver disease progression, but the impact in cancer risk is not defined. In fact, the molecular events leading to cancer and the insult/repair phenomena leading to cirrhosis might be coincidental in time, but may not be the same.

Oncogenic mechanisms

The molecular events linked to the development and progression of HCC are not well known. Malignant hepatocytes are the result of sequential changes accumulated in mature hepatocytes or can derive from stem cells. The most accepted hypothesis (Buendia, 2000; Thorgeirsson and Grisham, 2002) describes a step-by-step process in which external stimuli induce genetic alterations in mature hepatocytes leading to cell death, cellular proliferation, and the production of monoclonal populations. These populations harbor dysplastic hepatocytes that evolve to dysplastic nodules (Theise et al., 2002). High-grade dysplastic nodules are considered truly preneoplastic lesions, and may develop into malignant tumors in 30% of cases over a period of 1–5 years (Borzio et al., 2003). Initially, the nodules do not exhibit neovascularization. At a more advanced time point, hepatocytes exhibit a malignant phenotype associated to neovessel formation nourished by the hepatic artery. These early well-differentiated tumors are highly proliferative and become less differentiated when they reach 1–1.5 cm (Nakashima et al., 2003). At this stage, angiogenesis, tissue invasion, and metastasis occur in up to 25% of the cases. Later on, the neoplastic cells become undifferentiated and are able to invade vessels and spread outside the liver, characteristics that define end-stage disease.

Several investigators have searched for a specific genetic damage at different evolutionary stages (Buendia, 2000; Thorgeirsson and Grisham, 2002) (Table 2). However, no homogeneous pattern has been defined. Few alterations have been characterized in preneoplastic lesions and early HCC. Loss of heterozygosity at 1p (1p36-p34) and in *M6F/IGF2R* are early events in hepatocarcinogenesis, along with telomere shortening. More recently, some genes (*SP70*, *Glypican-3*) or gene sets (Smith et al., 2003; Paradis et al., 2003) have been

Table 2. Chromosomal abnormalities in human HCC and genes affected

Chromosome	Numerical aberrations	Structural aberrations	Loss of heterozygosity	Genes involved
1		del 1p22, del 1p32-p36	1p, 1p35-36	*L-myc*
3				*β catenin*
4	–		4p, 4q, 4q32, 4q11-23	*AFP/Alb/α-FGF*
5	–	t5;9	5p, 5q, 5q35-qter	*APC (5q21)*
6	–	del 6(q13-qter)	6q26-q27	*M6P/IGF-IIR*
7	+	t17;7 (p13;p14)		
8	–	inv 8(q10)	8q	*c-myc (8q24)*
9	–	t5;9, inv 9(p12:q12)		*p16 INK4A & p19 ARF (9p21)*
10	–		10q	*PTEN (10q23)*
11	–,+	dup 11p15, del 11(p13-p14), del 11p11, t11;22	11p, 11p13-p15.1	*WT-1, IGF-II, Cyclin D (11q13)*
12	–			
13	–,+		13q, 13q12, 13q12-q31	*Rb-1 (13q-14)*
15	–,+			
16	–	del 16q	16p, 16q22-24	*Axin-1 (16p13.3), E cadherin (16q22.1), SOCS-1 (16p13.13)*
17	–	t17;7 (p13;p14), t17;18(q25;q11), t17;X, del 17(p12)	17p, 17p13, 17p13-pter	*p53 (17p13)*
18	–,+	t17;18 (q25;q11)		*SMAD2, SMAD4 (18q21)*
20	+			
21	–,+			
22		t22;?	22q, 22q11-12	
X	–	t17:X		

proposed as early HCC markers, although these results need further confirmation.

In advanced stages, allelic alterations involve all the chromosomes, the most specific being found in chromosomes 1, 4, 8, 16, and 17, but with none of them affecting more than 60% of cases. Few studies link genetic abnormalities with gene expression. Downregulation of *p53* is present in 30%–40% of cases. The G to T mutation at codon 249 of the *p53* gene defines genetic damage due to aflatoxin (Staib et al., 2003).

Recent studies have suggested that the pathway leading to HCC may differ according to etiology. HBV-related HCC would exhibit a more intense genetic instability with several chromosome changes. By contrast, non-HBV related HCC would present less instability, but more frequently suffer β *catenin* mutations and activation of the wnt signaling pathway with development of large nondisseminated tumors (Hsu et al., 2000; Laurent Puig et al., 2001). Lately, microarray investigations have allowed the identification of a large number of genes with increased or reduced expression, but the meaning of these findings is still unclear. In most cases, the genes are related to cell cycle regulation, inflammation, apoptosis, and collagen deposition. In fact, a myriad of investigations have described overexpression or repression of genes involved in the above mentioned processes and even linked some of them to prognosis and evolution after treatment. Particularly, some genes have been related to tumor progression (*p16*, *SOCS-1*, *PEG10*) or tumor dissemination and metastasis (*nm23-H1*, *osteopontin*, *ARHC* [*Rho C*], *KAI1*, and *MMP14*). However, none of the findings has turned into a well-established marker in clinical practice. Ongoing investigations joining tumors of different origin, stage, and evolution should finally identify the most relevant abnormalities and differentiate those that are the cause of the tumor and its evolution from those that are the effect of the ongoing changes.

Early detection, diagnosis confirmation, and staging

If prevention is not effective, the only strategy to decrease cancer-related mortality is early detection, allowing effective therapy. HCC accomplishes the requirements to become the target of surveillance: it is a frequent disease with relevant morbidity and mortality that has a well-defined population at risk—patients with chronic liver disease. This population can be screened by a sensitive tool (ultrasonography [US]) that is easily acceptable, and effective treatment options are available (Llovet et al., 2003).

There are no large randomized clinical trials (RCTs) comparing surveillance versus no surveillance, but based on results from cohort investigations, regular US examination has become a routine clinical practice in cirrhotics. Almost 50% of HCC detected under surveillance are amenable to effective therapy that improves their outcome, but the main concern is the cost-effectiveness of this intervention. If applied to all individuals with chronic liver disease, the cost per year of life saved will largely exceed the conventional $50,000 USD cutoff (Bolondi et al., 2001). Thus,

Table 3. Incidence of HCC according to risk factors and degree of liver disease

HBV chronic infection	Noncirrhotic HBV carriers : 0.4%–0.6%/year Cirrhotic patients: 2.6%–6% /year
HCV chronic infection	Noncirrhotic HCV individuals: <0.1%/year Cirrhotic patients: 3%–8%/year
Genetic hemochromatosis	Cirrhosis: 5%/year

surveillance has to be restricted to subjects at high risk that would be potentially cured if diagnosed with HCC. Identification of high-risk individuals is therefore a critical point. Table 3 depicts the incidence of HCC in HBV and HCV infected subjects according to the status of the liver. The risk is significant when cirrhosis is established, whereas healthy carriers or patients with chronic hepatitis exhibit a moderate risk that will never allow cost-effective surveillance. HCC risk can also be stratified among cirrhotics. Male sex and increased α-fetoprotein (AFP) concentration are the strongest predictors. Other parameters such as dysplasia, increased proliferation, or irregular regeneration need further validation. Clearly, the major advancement will come from the identification of the genetic events that prompt the development of a distinct hepatic nodule that ultimately will evolve into HCC. Most HCC are initiated as a minute nodule in which cells strongly retain the phenotype of benign hepatocytes. Dysplastic changes and neoangionesis mark the transition to well-differentiated HCC and overt malignancy. These events occur when the nodules measure between 1 and 2 cm in diameter and herald the ability to proliferate, invade, and disseminate. At this stage, the tumors are easily detected by imaging techniques (US, computed tomography, magnetic resonance) that base their diagnostic capacity in the recognition of an increased arterial vascularization (Burrel et al., 2003). This allows for diagnosis without needing a positive biopsy (Llovet et al., 2003), but at earlier stages, when vessel formation is absent, biopsy is the sole diagnostic tool. Unfortunately, at early stages, the cells retain their differentiation and biopsy is seldom confirmatory. Accordingly, current research aims to identify the molecular changes that govern transition along the depicted phases and allow an accurate molecular diagnosis and definition of the evolutionary stage. This will also depict the therapeutic targets to prevent the evolution into overt malignancy, and as a whole, these data may ultimately establish a molecular classification of HCC.

Treatment

The therapeutic strategy for HCC patients has to take into account that in the vast majority of individuals, the tumor develops in a diseased liver with a variable degree of function derangement. Thereby, treatment should have a limited impact in liver function. On the other hand, if the degree of liver function impairment is severe, it will determine by itself a very dismal outcome of the patient, irrespective of the treatment success, which would be absolutely irrelevant. Figure 1 depicts the tumor staging and treatment strategy that is currently applied in the Barcelona Clinic Liver Cancer (BCLC) group. It links patient evaluation with treatment indication, and thus is intended to guide clinical practice. Unfortunately, as discussed above, there are no definitive molecular tools to characterize the biology of HCC, and most treatment decisions are still based on parameters such as size and tumor number, as defined by imaging techniques.

Patients diagnosed at an early stage benefit from effective treatments such as surgical resection, transplantation, or percutaneous ablation. Unfortunately, these benefit 30%–40% of the patients currently evaluated in referral centers in the West, and hence the vast majority are candidates to palliative treatments that in most instances have never proven to have a positive effect in survival (Llovet et al., 2003).

Surgical resection

Surgical resection is for most authors the first option in patients with solitary tumors with well-preserved liver function and normal portal pressure. Patients fitting into this profile will tolerate the resection of a hepatic segment, and their liver will not be damaged by the cytokine release and ischemia reperfusion injury related to surgery. Survival of optimal candidates may exceed 60%–70% at 5 years, the main drawback being the high rate of disease recurrence during followup (>50% at 3 years) (Llovet et al., 2003). Recurrence might be due to tumor dissemination or to de novo tumor formation. Presence of vascular invasion or additional nodules are powerful predictors of recurrence related to dissemination, but future molecular analysis should be able to give a more accurate prognosis than conventional pathology. p53 protein dysfunction and reduced expression of p27 or nm23-H1, among others, have been related to higher recurrence rate, but more data are needed prior to integrating any gene signature into risk assessment. Acyclic retinoids, interferon, adoptive immunotherapy, and internal radiation may have some preventive effect, but their efficacy requires extensive investigation (Llovet et al., 2003).

Liver transplantation

Liver transplantation is the treatment of choice for patients with early HCC in decompensated cirrhosis. Patients with solitary tumors $\leq$5cm or with up to 3 nodules $\leq$3 cm achieve outcomes identical to patients transplanted because of end stage cirrhosis without malignancy (70% at 5 years), and the recurrence rate is less than with surgery (Llovet et al., 2003). This difference might be used to support transplantation as the first therapeutic option, but transplant has several drawbacks. The shortage of donors prompts a waiting time between enlistment and transplantation. During this time, the tumor may progress and impede the procedure. Hence, the intention-to-treat survival is significantly reduced. Live donation may partially ameliorate the shortage of donors, but still we will have HCC patients waiting for a liver during several months. Predictors of tumor progression are not available, and this is another field where molecular profiling should provide major help. Graft rejection and viral reinfection are also major unsolved issues after transplant. HCV reinfection of the graft is the rule, and at 5 years followup almost 50% of them will present with cirrhosis again (Prieto et al., 1999). Development of better antiviral therapies and tools to prevent collagen deposition are needed.

Percutaneous ablation

Percutaneous ablation is the third treatment option that may provide long-term cure. The most frequent techniques to ablate tumors by physical means are ethanol injection and radiofrequency. Both are done under image guidance, and their maximal activity is achieved in nodules <3 cm, when complete response rates account for 80% of cases (Llovet et al., 2003). The main drawback is again the high rate of disease recurrence. If occurring in the surroundings of the treated area, it reflects failure to provide adequate local control. This lack of local control is not an issue with surgical resection, which eliminates the tumor and the surrounding tissue, containing the local tumor spread. Thus, percutaneous ablation is an effective option, but is usually indicated if surgery is unfeasible.

Palliative therapy

Palliation aims to reduce symptoms related to cancer progression and improve survival. Hence, only those treatments that have consistently been shown to achieve this goal should be considered effective. Arterial chemoembolization and tamoxifen administration have undergone such robust investigation, but only the former has proved survival benefits. Chemoembolization consists of the selective injection of chemotherapy into the hepatic artery followed by the obstruction of the vessels feeding the tumor. This combined maneuver results in extensive tumor necrosis with

slower tumor progression. However, during followup, the vascularization is recovered, and despite repeated therapy, the neoplasm progresses and leads to death. Meta-analysis of all published RCTs indicates that this option significantly improves survival (Llovet et al., 2003). However, it benefits a narrow range of nonsurgical patients who, being still asymptomatic, do not present associated liver decompensation and/or invasion of the portal vein or extrahepatic spread (30% of patients with nonsurgical disease). Hence, those with slightly advanced liver disease presenting symptoms, vascular invasion, or extrahepatic spread are excluded, and there is no effective therapy for them. Tamoxifen administration aiming to blockade estrogen receptors is ineffective, and the same stands for chemotherapy as the potential, effects are prevented by the overexpression of the multidrug resistance gene. Doxorubicin or cisplatinum have some marginal activity, but lack any impact in survival (Llovet et al., 2003). Radiation has antineoplastic capacity, but the hepatocytes are also radiosensitive, and thus, extensive tumors can not be safely treated. Immune control through activated lymphocytes is explored by several groups, and the same applies for gene therapy that up to now is mostly restricted to laboratory animals. Several biomodulators and new agents aiming to modulate cell cycle, vessel formation, and matrix invasion are currently being explored, but have not yet reached conventional clinical practice.

Conclusion

HCC is one of the major cancer killers that may benefit from several health interventions and research actions. The identification of the main risk factors allows the implementation of primary prevention, while the recognition of the population at risk permits effective surveillance to achieve early detection and effective therapy. Current investigations should define the molecular pathways leading to cancer and define the targets for new preventive and therapeutic strategies.

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