RECENT RESEARCH PROGRESS IN HEPATOMOCYTOBLAR CARCINOMA

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Abstract: Hepatocellular carcinoma (HCC) is responsible for significant morbidity and mortality throughout the world, and is clearly linked to viral infections. Mass vaccination programs against hepatitis B virus have reduced the incidence of HCC in Taiwanese children, and are likely to yield similar benefits elsewhere. In many countries, a definite increase in the incidence of HCC has been reported, largely attributable to the increasing incidence of hepatitis C virus infection. Although the major viral and environmental risk factors for the development of HCC have been determined, the oncogenic pathways leading to malignant transformation of liver cells have long remained obscure. HCC is also extremely difficult to manage. Although patients at risk can be identified and early detection of HCC is feasible, the current management of HCC is confusing due to the lack of well-designed, randomized clinical trials comparing various treatment modalities. New surgical techniques and postoperative therapies may improve the outcome in some resectable cancers; however, the vast majority of patients have unresectable tumors. Local ablation treatments may shrink or necrose tumors, but the clear benefit of such therapies remains to be seen. Further elucidation of the genetic and molecular features of HCC may lend insight that will lead to the development of innovative strategies to manage this cancer. In this article, the current understanding of HCC with respect to etiologic factors, genetic mechanisms responsible for hepatocarcinogenesis, diagnosis, therapy, and prevention are reviewed.

With an estimated 1 million cases per year, hepatocellular carcinoma (HCC) is the fifth most common cancer in the world; 80% of cases occur in developing countries [1]. Symptomatic HCC usually runs a rapidly progressive course with a low rate of resectability and poor response to non-surgical therapy, and thus has a very poor prognosis [2]. Risk factors associated with the development of HCC include chronic infection with either hepatitis B virus (HBV) or hepatitis C virus (HCV), the presence of cirrhosis, carcinoen exposure especially alfatoxin B1 (AFB1), alcohol abuse, genetic factors, male gender, cigarette smoking, and advanced age [3]. Among these risk factors, chronic hepatitis virus infection, particularly when accompanied by cirrhosis, shows the strongest association with the development of HCC [2–5]. Mass vaccination programs against HBV have reduced the incidence of HCC in Taiwanese children [6] and are likely to yield similar benefits elsewhere. Recently, however, there is evidence that the incidence of HCC is rising in developed countries [7]. This appears to be related to the increasing prevalence and duration of HCV infection. In this article, recent advances in our understanding of HCC are reviewed.
ETIOLOGY

Viruses
Extensive studies have shown a strong epidemiologic association between chronic HBV infection and HCC [2–4, 8]. The increased risk of developing HCC is estimated to be 100-fold for carriers of hepatitis B surface antigen (HBsAg) compared with uninfected populations [9], placing HBV in the first rank among known human carcinogens.

In most Western countries, HCV is a common risk factor for HCC [10, 11]. The relationship between chronic HCV infection and HCC is documented by the presence of HCV markers in a high proportion in patients with HCC and the appearance of HCC in the follow-up of patients with chronic HCV infection. The stronger association between HCC and infection with HCV genotype 1b remains unsettled [12, 13]. Although the relative and attributable HCC risk of HBV and HCV carrier status varies in different countries, these viruses exert a synergistic interaction on HCC [14]. In addition, there is growing evidence to support an increased risk of HCC in patients with chronic HCV infection who are coinfected with occult HBV [15, 16]. In this setting, HCC seems to have a worse prognosis [17]. However, whether the mode of acquisition of HBV infection (vertical vs horizontal) influences the effect of occult HBV infection on the development and pathobiology of HCC remains unknown [18].

Whether hepatitis D virus (HDV) superinfection in HBV carriers accelerates the development of HCC is controversial [19, 20]. A retrospective study suggests that HDV superinfection may increase the risk for HCC threefold in patients with HBV-related cirrhosis [20]. Although GB virus/C hepatitis G virus (GBV-C/HGV) or TT virus (TTV) infection is often found in patients with HCC [21, 22], the effect of these newly identified human viruses on the development of HCC seems unremarkable.

HBV genotype
Advances in molecular techniques have resulted in the classification of HBV into seven genotypes, A to G [23, 24]; however, the clinical significance of HBV genotypes has remained largely unknown until recently. It has been suggested that HBV genotype C is associated with the development of cirrhosis and HCC, and genotype B may be associated with the development of HCC in young patients [25]. In addition, HBV genotype C has a lower response rate to interferon (IFN) therapy compared to genotype B [26]. Recent reports from Japan and China also showed that genotype C is associated with more severe liver disease than genotype B [27, 28]. Nevertheless, whether these observations hold true for other HBV genotypes needs to be confirmed in other parts of the world, particularly in Western countries where genotypes A and D are prevalent.

Chemical carcinogens
Aflatoxins are potent animal hepatocarcinogens, and epidemiologic surveys have revealed a significant correlation between aflatoxin ingestion and HCC incidence [3], suggesting that aflatoxins are carcinogenic in humans with particularly high risks, such as individuals with concomitant HBV infection.

Others
Independent and interactive factors significantly related to HCC include cirrhosis, low vegetable intake, low serum retinol concentration, higher concentrations of androgen signaling reflected by higher testosterone concentrations and 20 or fewer CAG repeats in the androgen receptor, cigarette smoking, heavy alcohol consumption, and a history of HCC among intimate family members [3, 29, 30]. Genetic predisposition to the development of HCC in HBV carriers has been studied [31], and N-acetyltransferase (NAT) genotype 2 activity has recently been shown to be critical in smoking-related hepatocarcinogenesis among HBV carriers [32]. These data support a role for tobacco smoke-derived aromatic amines in the etiology of HCC.

CARCINOGENESIS

Viral hepatocarcinogenesis
It is believed that HBV has no direct oncogenic effect on the infected liver cell [33]. However, some mutations in the HBV S and pre-S regions have been shown to be associated with the development of HCC. A transcriptional transactivator function not present in the intact HBV gene is generated by a mutation in the 3’ pre S/S sequence, which is truncated during or after integration into the host genome. The resulting truncated polypeptides might play a role in hepatocarcinogenesis [34]. Recently, a particular HBV X protein mutant has been frequently identified in patients with HCC, indicating that this mutant might represent a strategy of the virus to escape immune surveillance and then contribute to hepatocarcinogenesis [35]. Nevertheless, malignant transformation of liver cells usually occurs after a long period of chronic infection. The long latency of HCC development after initial HBV infection strongly suggests an indirect action of the virus.

So far, the mechanism of hepatocarcinogenesis with HCV remains unclear [8]. HCV RNA does not
Molecular hepatocarcinogenesis

Although major viral and environmental risk factors for HCC development have been unraveled, the oncogenic pathways leading to malignant transformation of liver cells remain obscure. It has been proposed that the development of HCC is a multi-step evolution involving many important and stage-wise genetic changes[8, 33, 35], as in other human cancers. However, unlike the sequential genetic changes in colorectal cancers, most of those in hepatocarcinogenesis remain unknown. The differential involvement of the p53 tumor-suppressor gene in HCC associated with various risk factors has been largely clarified [3]. It is known that p53 can transactivate the transcription of genes that downregulate cellular growth-related genes and become oncogenic as a result of the production of mutant proteins or the loss of expression [36]. Evidence for a crucial role of the reactivation of the beta-catenin pathway, through mutations in the beta-catenin and axin genes, represents a major breakthrough [37]. Beta-catenin mutation may play an important role in the carcinogenesis of a subset of HCC with good prognosis, and mutant and wild-type nuclear beta-catenin proteins may not function equivalently [38, 39].

Previous cytogenetic and subsequent molecular genetic studies have shown interesting findings, including amplification or overexpression of known proto-oncogenes and frequent allelic loss on chromosomes 1p (1p35-36), 4q (4q12-23), and 16q (16q22-23) [3, 8]. Recent extensive allelotype studies and genome-wide analysis of HCC chromosomes using comparative genomic hybridization (CGH), loss of heterozygosity, or spectral karyotyping have resulted in a comprehensive overview of the main genetic abnormalities in HCC, including DNA copy gains and losses [40-43]. CGH has been used to identify the genetic similarities between HBV- and HCV-related HCC [44], which include frequent deletions in 1p (24%), 4q (39%), 6q (41%), 8p (44%), 9p (24%), 11q (24%), 12q (22%), and 13q (39%), as well as common gains in 1q (46%), 6p+ (20%), 8q+ (41%), 11q (27%), and 17q+ (37%). There is no significant difference in the number and type of chromosomal imbalances between HCV- and HBV-infected tumors. These data suggest that both HBV and HCV cause cancer through non-specific inflammatory and regenerative processes.

The cDNA microarray technique allows simultaneous analysis of differential expression of thousands of known human genes and is also utilized to study gene expression in HCC and nontumorous tissues [45-47]. Expression profiling suggests that multiple regulatory pathways are involved in the development of HCC. Nevertheless, the identification of candidate oncogenes and tumor suppressors in the most frequently altered chromosomal regions is still a major challenge, and great insights may come from integrating the signals from different pathways operating at preneoplastic and neoplastic stages. This search might, in time, permit an accurate evaluation of the major targets for therapeutic treatments. Accordingly, the genetic profile and chromosomal allelic imbalance in cirrhotic nodules that define the genetic aberrations in early hepatocarcinogenesis need to be investigated [48, 49]. In one of our studies, we microdissected 180 cirrhotic nodules from seven female HCC patients to study their clonal nature, first by examining the X-chromosome methylation pattern. The allelic imbalance in monoclonal cirrhotic nodules and the corresponding HCCs were further analyzed with microsatellite polymorphic markers. Nearly half of the cirrhotic nodules were monoclonal and already had chromosome aberrations. In addition, the allelic imbalances on 4q, 8p, and Xq may be earlier mutations in hepatocarcinogenesis, whereas the allelic imbalances on 1p, 13q, 16q, and 17p occur later (Figure) [49].

Clonality of HCC

The DNA clonal heterogeneity of HCC has been studied, and multiple HCC and recurrent HCC usually have different clonalities. Recent studies of the clonal analysis of HCC, primary or recurrent, and dysplastic nodules using CGH or the methylation pattern of X-chromosome-linked human androgen receptor gene consistently showed that both methods are useful.
for chromosomal aberration study and tumor clonality analysis [50–52]. In addition, the finding that about half of the micronodules are monoclonal lesions supports the notion that cirrhosis is a preneoplastic lesion.

Hepatocarcinogenesis associated with other factors

The role of irregular regeneration of hepatocytes [53], vascular endothelial growth factor [54], hypermethylation of CpG islands with resulting loss of expression of the 14-3-3 sigma gene [55], or constitutive activation of nuclear factor kappaB [56] in angiogenesis, growth, and development of HCC has been explored. These data may be useful in the design of future interventions for HCC.

DIAGNOSIS

Tumor markers

Although serum alpha-fetoprotein (AFP) is still the most widely used marker for HCC, the sensitivity of AFP is being questioned. The lentil lectin affinity for AFP (L3 fraction of AFP) provides moderately high sensitivity and high specificity in the detection of HCC for persons with high AFP levels, and thus may be a useful adjuvant in a mass survey for HCC. In addition, desγ-carboxy prothrombin, an abnormal form of prothrombin, may serve as a complementary test to serum AFP levels in the diagnosis of HCC, although it is not sensitive enough to detect small HCC [3].

Imaging

Ultrasonography, computerized tomography (CT), spiral CT, and CT with lipiodol have been widely used to image the liver [57]. The clinical utility of these imaging modalities in the diagnosis of HCC depends on their ability to reliably detect small lesions. In addition, CO2; gas-enhanced ultrasonography, color Doppler sonography, duplex pulsed Doppler ultrasonography, leovist-enhanced Doppler sonography, and intraoperative ultrasonography have also been used in clinical practice to detect small liver tumors, differentiate HCC from hemangioma, and identify arterioportal shunting as well as portal vein thrombosis [3, 58]. Recent advances in magnetic resonance imaging (MRI) technology allow more accurate examination of the liver [57].

Positron emission tomography (PET) imaging using 18F-fluorodeoxyglucose uptake to estimate glucose metabolism had a sensitivity of 55% in diagnosing HCC compared to 90% with CT scanning [59]. In addition, well-differentiated and low-grade tumors have lower activity on PET and correspondingly lower PET scores. Thus, PET imaging may help assess tumor differentiation and may be useful in the diagnosis and staging of HCC as an adjunct to CT.

Mass screening for HCC

The utility of screening for HCC is determined by both the sensitivity and specificity of testing, and by the effectiveness of therapy once the disease is detected. In general, patients with cirrhosis may be regularly screened for early detection of tumor. The method most widely used for screening is biannual ultrasonography, accompanied by determination of serum AFP levels. Although screening remains the only realistic approach for improving the treatment of HCC at present [60], its cost-effectiveness remains uncertain. Assessment of the cost-effectiveness of a surveillance program in patients with cirrhosis for the early diagnosis and treatment of HCC found that the overall cost of the surveillance program was US$753,226, the cost per treatable HCC was US$17,934, and the cost per year of life saved was US$112,993 [61]. Thus, a surveillance policy for patients with cirrhosis requires considerable resources but offers little benefit in terms of patient survival. However, the decision of whether to adopt a surveillance policy towards HCC should rely on the prevalence of the disease in the population and on the resources of a particular area.

A recent study from Africa indicated that the arginine-to-serine substitution at codon 249 of the p53 gene, the hotspot mutation in HCC exposed to AFB1, could be detected in cell-free DNA isolated from the plasma of HCC patients [62]. Another study using electrospray ionization mass spectrometry to detect this specific mutation also showed similar results [63]. Thus, this assay may lead to the earlier detection of HCC and serve as a novel screening test in areas endemic for HBV infection and aflatoxin contamination.

Therapy

Current management of HCC is confusing due to the large number of treatment options available. The difficulty of managing a patient with HCC is compounded by the lack of data from well-designed, randomized, controlled clinical trials comparing the various treatment modalities. Nevertheless, many promising therapeutic options are currently available for the treatment of HCC [64].

Surgery

Surgery is generally believed to be the only curative therapy for HCC, although this is not necessarily true [3]. Surgical resection or liver transplantation is often not possible because of the extent of the tumor as well
as the poor condition of the patient. Surgical outcomes were recently compared between 1,000 patients with small HCC (<5 cm) and 1,366 patients with large HCC (>5 cm) [65]. The results showed that patients with small HCC had a higher resection rate, a higher curative resection rate, a lower operative mortality rate, better differentiation of tumor cells, a higher incidence of single nodule tumors, a higher proportion of well-encapsulated tumors, a lower incidence of tumor emboli in the portal vein, and higher survival rates after resection. However, no significant difference was found between survival after minor resection or lobectomy in patients with small HCC. Thus, minor resection instead of lobectomy seems to be the key to increase resectability and decrease operative mortality. A comparison of surgical outcomes for HCC in Western patients with HBV or HCV infection indicated that a much higher proportion of HCC patients with HBV infection than with HCV infection are candidates for resection [66]. The 5-year disease-free survival was significantly higher for HBV patients treated with resection than for those with HCV. In contrast, patients with HCV-related HCC had significantly longer 5-year disease-free survival with transplant when compared to resection. Thus, serious consideration should be given to transplant in patients with resectable HCV-related HCC, especially in those with small HCC.

**Transplantation**

In the early days of orthotopic liver transplantation, the 5-year survival rates for patients with HCC were around 20% [67]. Most deaths after transplantation for HCC are from recurrent tumor, and one of the most frequent sites for recurrence is the transplanted liver. Experience with transplantation has shown that tumor size is a very important variable in predicting recurrence. Tumors smaller than 3 cm are least likely to recur, while those larger than 5 cm probably have an unacceptably high recurrence rate. Currently, features of HCC in patients with cirrhosis that are associated with a 5-year survival rate of 75% after liver transplantation include a solitary tumor of less than 5 cm; three or fewer tumors, each less than 3 cm; and the absence of vascular invasion [68].

**Chemoembolization**

Transcatheter arterial chemoembolization (TACE) for HCC is performed by giving intra-arterial substances to occlude the vessel in the hope of obtaining high tumor drug levels in a hypoxic environment [3]. The analysis of long-term effects of lipiodol-transcatheter arterial embolization (Lp-TAE) combined with cisplatin (CDDP) or doxorubicin (ADM) on unresectable HCC showed that CDDP-Lp-TAE may improve the prognosis of unresectable HCC more than ADM-Lp-TAE, which may be attributable to the fact that CDDP-Lp-TAE treatment could be repeated more often than ADM-Lp-TAE [69].

Using the beta-coefficients of serum AFP level (>400 ng/mL), tumor size (>50%), and Child-Pugh score, a prognostic index has been proposed to predict the survival of patients treated with TACE [70]. This index could be used to decide which patients with unresectable HCC should receive this therapy. TACE as an adjunct to ablation or resection therapy may play an increasing role in palliating or down-staging a patient with advanced HCC [71, 72].

**Ablation therapies**

Several ablative therapies, including percutaneous ethanol or acetic acid injection, radiofrequency (RF) ablation, and cryosurgery have been introduced for the treatment of small HCC, and may yield a survival benefit [73–75]. Among these therapies, RF ablation may also be an effective and relatively simple procedure in the treatment of medium or large HCC [76]. However, RF ablation with a cooled-tip needle has been reported to be associated with a higher risk of tumor seeding [77].

**Systemic chemotherapy and combination therapy**

HCC is well known to be resistant to chemotherapy. Until recently, the response rate to chemotherapy alone was less than 20%, and complete response was extremely rare. A randomized controlled trial indicated that IFN is not well tolerated in patients with cirrhosis and advanced HCC, and its administration provides no benefit in terms of tumor regression and survival [78]. However, a pattern of effective therapy has been emerging over the past 5 years. This involves combination therapy to increase the operative rates in patients with unresectable disease, and postoperative adjuvant therapy to decrease the high relapse rate that is so characteristic of HCC. The combination therapy involves cytotoxic drugs and IFN. Adjuvant systemic therapy of HCC has not yet been widely tested, but success with locoregional lipiodol iodine 131 is proof of principle [79]. The coming decade should see a significant improvement in the outcomes of patients with HCC as multimodality treatment becomes more widely investigated and practiced.

**Other therapies**

It has been suggested that adoptive immunotherapy is a safe and feasible treatment to lower recurrence and improve recurrence-free outcomes after surgery for HCC [80]; however, further studies are needed to
confirm these findings. In addition, gene therapy targeting HCC has recently been vigorously studied [81, 82]. The AFP regulatory sequences are among the best known tumor-specific transcriptional regulators. A number of groups have demonstrated that a variety of genes can be expressed in an HCC-specific manner under the control of the AFP regulatory sequences in vitro and in vivo [83]. It would appear that, with the development of a suitable delivery system, HCC-directed gene therapy using the AFP regulatory sequences holds promise.

**Prognostic System**

The prognosis of patients with cirrhosis and HCC usually depends on both residual liver function and tumor extension. A new scoring system, the Cancer of the Liver Italian Program (CLIP) score, has recently been proposed, which includes the parameters involved in the Child-Pugh stage, macroscopic tumor morphology, AFP levels, and the presence or absence of portal vein thrombosis. The discriminatory ability of the CLIP score was compared with those of the Okuda and TNM staging systems and the Child-Pugh classification in a group of cirrhotic patients with HCC [84]. The results showed that the CLIP score is able to predict survival better than the Okuda or TNM staging system. The predictive capacity of the CLIP score was also confirmed in the subgroup of patients undergoing chemoembolization. Thus, the new CLIP score may represent a useful tool in treatment planning by improving baseline prognostic evaluation of patients with HCC; it can be used in prospective therapeutic trials as a stratification variable to reduce the variability of results owing to patient selection [85].

**Prevention**

**Primary prevention**

A safe and effective vaccine to prevent HBV infection is available and a mass vaccination program against HBV in Taiwan has been shown to reduce the incidence of HCC in children [6], especially in boys [86], which may also be seen in young adults soon. An 80 to 85% decrease in HCC in all Taiwanese adults in 30 to 40 years is anticipated [3]. Given estimates that approximately 70% of HCC in developing countries is attributable to HBV, mass vaccination could prevent more than 500,000 cases per year in these areas. Unfortunately, development of a vaccine against HCV is more problematic due to the genetic heterogeneity of the virus [8]. Nevertheless, with 24% of HCC in developing countries attributable to HCV (approximately 93,000 cases per yr) [11], prevention of HCV infection would make a major contribution to cancer prevention. Until effective immunoprophylaxis is available, interruption of transmission routes such as implementation of blood donor screening for anti-HCV, adequate sterilization of surgical instruments or the use of disposable medical instruments, and avoidance of sharing personal tools remains the mainstay to prevent HCV infection [87].

**Secondary prevention**

The currently recommended therapy for chronic hepatitis B is a 4- to 6-month course of IFN-α in doses of 5 to 10 million units three times a week. This regimen results in sustained clearance of serum HBV DNA and hepatitis B e antigen (HBeAg) in approximately 25 to 40% of patients, and a loss of HBsAg in 10% of Western patients [88]. Long-term follow-up of patients who respond to IFN-α treatment with clearance of HBeAg indicates that the majority ultimately clear HBsAg as well and have continued remission of liver disease; this is usually associated with improved clinical outcomes [89, 90]. However, whether IFN reduces the risk of HCC in patients with cirrhosis remains controversial [90–92]. Recently, several oral nucleoside analogues that have potent activity against HBV replication have been developed. Among them, lamivudine (3-thiacytidine) can induce marked reduction of serum HBV DNA levels and improvement in serum aminotransferase activities and hepatic histology [93]. However, whether these therapeutic effects persist and lower the incidence of cirrhosis or even HCC needs further long-term observation.

For chronic hepatitis C, the currently recommended 6- to 12-month course of combination therapy with IFN-α and ribavirin can clear HCV RNA and normalize serum aminotransferase levels as well as liver histology in approximately 40 to 50% of patients [94, 95]. Sustained responses are associated with marked improvements in hepatic histology, and long-term studies indicate that the majority of patients remain free of virus in serum and liver, suggesting a ‘cure’ of the infection [96]. Recent studies have indicated that patients with chronic hepatitis C have a significantly lower risk of HCC and mortality if treated with IFN than those who are not treated [92, 97]. The search for other antiviral compounds is in progress, and a novel combination of long-acting IFN (pegylated IFN) with ribavirin has shown to be more effective in clearing the virus, with an overall sustained virologic response rate of 60% [98].

Curcumin, a food additive widely used as a spice and coloring agent, has been found to possess chemopreventive effects against several cancers. The
chemopreventive effect of curcumin on murine hepatocarcinogenesis was recently reported [99]. The feasibility of using curcumin in the chemoprevention of HCC in high-risk individuals needs to be further explored.

**CONCLUSION**

In summary, HCC is a human cancer that has been linked to viral infections, and universal vaccination programs against HBV have reduced the incidence of HCC in Taiwanese children. The major viral and environmental risk factors for the development of HCC have been identified; however, the oncogenic pathways leading to malignant transformation of liver cells have yet to be fully described. Although at-risk groups can now be defined and early detection of HCC is feasible, the current management of HCC is confusing because of the lack of data from well-designed, randomized clinical trials comparing various treatment modalities. Further elucidation of the genetic and molecular mechanisms of HCC will shed much light on innovative strategies to prevent and manage this dreadful cancer.

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**REFERENCES**


