World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i38.4258

World J Gastroenterol 2011 October 14; 17(38): 4258-4270
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

Factors predicting occurrence and prognosis of hepatitis-B-virus-related hepatocellular carcinoma

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Supported by National Natural Science Foundation of China, No. 81025015 and No. 30921006
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Received: May 12, 2011 Revised: July 12, 2011
Accepted: July 19, 2011
Published online: October 14, 2011

Abstract

Primary liver cancer is an important cause of cancer death, and hepatocellular carcinoma (HCC) accounts for 70%-85% of total liver cancer worldwide. Chronic hepatitis B virus (HBV) infection contributes to > 75% of HCC cases. High serum viral load is the most reliable indicator of viral replication in predicting development of HCC. HBV genotype C is closely associated with HCC in cirrhotic patients aged > 50 years, whereas genotype B is associated with development of HCC in non-cirrhotic young patients and postoperative relapse of HCC. Different HBV subgenotypes have distinct patterns of mutations, which are clearly associated with increased risk of HCC. Mutations accumulate during chronic HBV infection and predict occurrence of HCC. Chronic inflammation leads to increased frequency of viral mutation *via* cellular cytidine deaminase induction. Mutations are negatively selected by host immunity, whereas some immuno-escaped HBV mutants are active in hepatocarcinogenesis. Inflammatory pathways contribute to the inflammation-necrosis-regeneration process, ultimately HCC. Their hallmark molecules can predict malignancy in HBV-infected subjects. Continuing inflammation is involved in hepatocarcinogenesis and closely related to recurrence and metastasis. HBV load, genotype C, viral mutations and expression of inflammatory molecules in HBV-related HCC tissues are significantly associated with poor prognosis. Imbalance between intratumoral $CD8^+$ T cells and regulatory T cells or Th1 and Th2 cytokines in peritumoral tissues can predict prognosis of HBV-related HCC. These factors are important for developing active prevention and surveillance of HBV-infected subjects who are more likely to develop HCC, or for tailoring suitable treatment to improve survival or postpone postoperative recurrence of HCC.

Key words: Hepatitis B virus; Hepatocellular carcinoma; Viral load; Genotype; Mutation; Immune cells; Signaling pathway; Cytokine; Prognosis

Peer reviewers: Gloria González Aseguinolaza, BSc, MSc, PhD, Department of Gene Therapy in Hepatology, FIMA, CIMA University of Navarra, Navarra, Spain

Han YF, Zhao J, Ma LY, Yin JH, Chang WJ, Zhang HW, Cao GW. Factors predicting occurrence and prognosis of hepatitis-B-virus-related hepatocellular carcinoma. *World J Gastroenterol* 2011; 17(38): 4258-4270 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i38/4258.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i38.4258

INTRODUCTION

Liver cancer in men is the fifth most frequently diagnosed

cancer worldwide but the second most frequent cause of cancer death. It is the seventh most commonly diagnosed cancer and the sixth leading cause of cancer death in women. Globally, liver cancer rates are more than twice as high in men as in women. An estimated 748 300 new liver cancer cases and 695 900 cancer deaths occurred worldwide in 2008[1]. The highest liver cancer rates are found in East and South-East Asia and in Central and Western Africa. Developing countries contribute more than 80% of cases. About half of these cases and deaths occur in China, due to the endemicity of hepatitis B virus (HBV) infection. Chronic HBV infection accounts for about 60% of the total liver cancer in developing countries and for about 23% of the cancer in developed countries, while hepatitis C virus (HCV) infection accounts for about 33% of the total liver cancer in developing countries and for about 20% in developed countries[2]. Recently, the incidence of liver cancer has been increasing in some western countries due to the increasing prevalence of HCV infection and immigration of people from countries with high endemicity for HBV infection[3]. Among primary liver cancers, hepatocellular carcinoma (HCC) accounts for 70%-85% of the total liver cancer burden worldwide[1]. Chronic HBV infection results in approximately one-third of all cases of liver cirrhosis and more than three-quarters of all cases of HCC worldwide[4]. The relative risks of HCC among people infected with HBV ranges from 5 to 49 in case-control studies and from 7 to 98 in cohort studies, and the incidence of HCC (per 100 000 person/years) among people with chronic HBV infection ranges from 400 to 800 in men and from 120 to 180 in women[5]. Standard HBV vaccination dramatically decreases HCC prevalence among vaccinees aged 6-19 years in Taiwan[6]. In Mainland China, the prevalence of hepatitis B surface antigen (HBsAg) in people born after 1992 has dramatically decreased, possibly due to expanded program of immunization of HBV vaccination, however, the rate in those born before 1992 is around 8%-9%[7,8]. Thus, HCC will remain one of the major public health problems in Mainland China in next 40-50 years.

The patients with HCC usually present late and have mostly developed serious liver cirrhosis. The life expectancy of patients with newly diagnosed HCC is measured in terms of weeks to months with a mortality to incidence ratio close to 1[3]. Therefore, it is critical to clarify some risk factors that can be clinically applied for the prediction of this malignancy in the HBV-infected population. Viral characteristics of HBV and hepatic inflammation status are not only associated with the occurrence, but also with the recurrence and metastasis of HCC after surgical treatment. Prognostic factors for predicting survival probability are invaluable to tailor suitable treatment options.

In this paper, we characterize viral properties of HBV and inflammatory factors that have been recently proven to be significantly associated with the occurrence and the prognosis of HCC, and summarize a group of viral and inflammatory factors (Table 1) that can be of significance for active prevention and surveillance of the HBV-infected subjects who are more likely to develop HCC, and for tailoring suitable treatment options like antiviral treatment to improve the survival or postpone the recurrence of HBV-related HCC.

ROLES OF VIRAL AND INFLAMMATORY FACTORS IN PREDICTING OCCURRENCE OF HBV-ASSOCIATED HCC

HBV replication status and viral variations

Association of viral replication status with HCC: HBV precore region encodes the precore protein, which is processed in the endoplasmic reticulum to produce secreted hepatitis B e antigen (HBeAg). HBeAg expression indicates active viral replication. In community-based HBV-infected subjects, HBeAg-positive rates and viral loads are high in the young, and decrease with age[8]. Continuing high viral load and/or HBeAg expression have been significantly associated with increased risk of HCC in prospective studies[9-11]. With reference to the low serum HBV DNA level ($\leqslant 10^{4.5}$ copies/mL), the hazard ratio (HR) for HCC of the intermediate HBV DNA level ($10^{4.5\text{-}6.5}$ copies/mL) is 1.62 (95% CI: 1.05-2.48) and that of the high HBV DNA level ($> 10^{6.5}$ copies/mL) is 2.73 (95% CI: 1.76-4.25)[12], indicating viral load predicts the risk of developing HCC dose-dependently. In another group of prospective studies, HBV viral load rather than HBeAg has been documented to be independently associated with an increased risk of HCC[13,14]. In cross-sectional case-control studies, high viral load ($\geqslant 10^4$ copies/mL) is independently associated with an increased risk of HCC[15-17]. However, HBeAg expression is not usually high in patients with HCC, and is not significantly associated with an increased risk of HCC in some case-control studies[16,17]. Ongoing high levels of HBV replication in patients seronegative for HBeAg are strongly associated with poor prognosis of patients with chronic hepatitis B (CHB)[18]. Thus, serum viral load is the most reliable indicator in predicating the development of HCC. HBeAg seroconversion reflects viral mutations in the HBV genome, especially in the precore and core promoter regions[19], and this usually happen during the natural course of chronic HBV infection, possibly caused by cytotoxic T-lymphocyte (CTL)-mediated clearance.

Association of HBV genotypes/subgenotypes with HCC: HBV genotypes and subgenotypes have distinct geographical distributions and have been shown to differ with regard to HBeAg seroconversion, clinical outcome, prognosis, and response to antiviral treatment[20-22]. HBV genotypes A, B, D and F are associated with earlier spontaneous seroconversion of HBeAg than genotype C. HBV subgenotype B2 is more likely to cause acute hepatitis B, while subgenotype C2 is more prone to caus-

Table 1 Viral and inflammatory factors associated with the occurrence and prognosis of hepatitis-B-virus-related hepatocellular carcinoma

Factor	Occurrence	Ref.	Prognosis	Ref.
Viral load				
Sera	$\geqslant 10^4$ copies/mL	[9,11,12,15-17]	$> 10^4$ copies/mL (poor survival)	[99,100]
Non-cancerous liver tissues	-	-	$> 3 \times 10^7$ copies/g (poor survival)	[101]
HBV genotype/subgenotype	C2 > B2 C2: cirrhotic patients ⩾ 50 yr B2: non-cirrhotic ones < 50 yr	[15,24-26]	C > B (poor survival) B2 in the young (recurrence)	[15,104]
HBV mutation				
Individual	C1653T, T1753V, T1674C/G A1762T/G1764A, C1766T, T1768A; G1899A, C2002T, A2159G, A2189C, G2203A/T; T53C, preS2 start codon mutation, preS1 deletion, C2964A, A2962G, C3116T, C7A	[17,27-29]	A1762T/G1764A and short stretch (< 100 bp) pre-S deletions in non-cancerous liver tissue of the HCC patients (poor survival)	[101]
Combined	2964C-3116T-preS2 start codon wildtype-7A; 2964C-3116T-7A-76C; 2964A-3116T-7C-76A/T; 2962G-preS2 start codon wildtype-105C-1762T/1764A	[30,31]	-	-
Intratumoral immune cells	M2 macrophage (risk), CD8 + T cells (protective); Imbalance between CD8 + T cells and Treg or between Th1 and Th2	[52-58]	NK and CD8 + T cell (good prognosis); Treg cells (poor prognosis)	[106,107]
Inflammatory pathways and cytokine	Pathway: NF-κB, STAT3, Wnt/β-catenin, TGF-α1, RAF/MEK/ERK, PI3K/AKT/mTOR, p53, VEGF Cytokine: IL-6, TNF-α	[60-81]	IL-2 and IL-15 (good prognosis); G-CSF (poor prognosis); Wnt-1 protein (poor prognosis); LI-cadherin (short survival); ErbB-2 (short survival); Cyclooxygenase-2 (recurrence); Low miR-199-3p (poor survival)	[108-113,116]
Genetic polymorphism of inflammatory molecules	HLA, TGF-β1, IL-β1, IL-18, IL-12, KIF1B-, UBE4B- or PGD-related pathways, NF-κB1, IκBα	[82-88]	VEGF	
ALT	> 45 U/L	[17,28,31]	> 50 U/L (poor survival)	[104]

HBV: Hepatitis-B-virus; HCC: Hepatocellular carcinoma; NF: Nuclear factor; TNF: Tumor necrosis factor; IL: Interleukin; STAT: Signal transducer and activator of transcription; TGF: Transforming growth factor; RAF: Raf protein kinases; PI3K: Phosphatidylinositol-3 kinase; MEK: Mitogen-activated protein kinase; ERK: Extracellular-signal-regulated kinase; mTOR: Mammalian target of rapamycin; AKT: Protein kinase B; HLA: Human leukocyte antigen; ALT: Alanine aminotransferase; VEGF: Vascular endothelial growth factor; G-CSF: Granulocyte-colony stimulating factor; LI: Liver intestine; NK: Natural killer.

ing chronic infection than is subgenotype B2 following an acute course[23]. Chronic infection with genotype C is more likely to cause liver cirrhosis and HCC, as compared with chronic infection with genotype B[24,25]. Chronic infection with genotype C (C2) is closely associated with an increased risk of HCC in the HBV-infected subjects, especially in cirrhotic patients aged > 50 years, whereas infection with genotype B (B2) has been found to be associated with development of HCC in non-cirrhotic young patients and relapse of HCC after surgical treatment[15,26].

Association of HBV mutations with HCC: Different HBV subgenotypes have distinct patterns of mutations. We and others have found that HBV mutations including C1653T, T1753V, A1762T/G1764A, T1674C/G and C1766T/T1768A in the enhancer Ⅱ/basal core promoter (EnhⅡ/BCP) regions; G1899A, C2002T, A2159G, A2189C, and G2203A/T in the precore/core gene; and T53C, preS2 start codon mutation, preS1 deletion, C2964A, A2962G, C3116T, and C7A in the preS region are significantly associated with an increased risk of HCC[17,27-29]. Of the HCC-associated mutations, combined rather than single mutations are significantly associated with the risk of HCC. In the preS region, the frequencies of combined mutations (haplotypic carriages) including 2964C-3116T-preS2 start codon wildtype-7A, 2964C-3116T-7A-76C, and 2964A-3116T-7C-76A/T are significantly higher in the patients with HCC than in those without HCC, whereas the haplotypic carriages with single mutation and other three wild types are inversely associated with HCC[30]. In the preS and EnhⅡ/BCP regions, a haplotypic carriage with 105C and 2962G is significantly more frequent in the patients with HCC than in those without HCC, and the frequency of 2962G-preS2 start codon wild type-105C-1762T/1764A is 47.9% in the patients with HCC and 4.3% in those without HCC[31]. Interestingly, the HBV mutations, either in the preS or in the core promoter region, are significantly associated with HCC, whereas the wild-type nucleotides in these regions are mostly associated with liver cirrhosis[17,28]. HBV mutations can be used as indicators for the prediction of end-stage liver diseases including HCC. Although these mutations and the combinations are specific for HCC to some extent, it will be more practicable if they can predict this malignancy in the HBV-infected subjects before the occurrence of HCC.

Role of viral mutations in prediction of HCC: The majority of patients with chronic HBV infection are believed to be infected *via* perinatal transmission, especially in HBV-endemic areas. The HCC-associated HBV mutants may not transmit *via* mother-to-child transmission because the children whose mothers carry both HBV mutants and wild-type virus are mostly found to be in-

fected with wild-type virus alone[32]. In the early stage of HBV infection, serum viral load is high and HBeAg is frequently positive because immune selection is weak. Immune selection may increase during HBeAg seroconversion. HBV mutations tend to be gradually generated during a chronic immunopathological course after infection. Recent studies have shown that accumulation of HBV mutations in the core region in children with HCC differ from those in HBV-infected children without HCC, and the frequencies of the HCC-associated mutations do not increase with increasing age of HCC patients, in contrast to patients without HCC[17,33]. These data support that the frequencies of HBV mutations might increase to high levels during malignant transformation, no matter how old the HBV-infected subjects are. However, HCC mostly occurs in HBV-infected subjects between 45 and 65 years of age. The HBV mutations mostly experience long-term processes of immune selection. A1762T/G1764A occurs up to 10 years before the onset of HCC and is a valuable indicator of HBV-infected men who are more likely to develop HCC[13,34,35]. The mutations in the BCP region accumulate sequentially during the development of HCC[36]. Some of the mutations including the preS deletion, C1653T, T1753V and A1762T/G1764A accumulate during the course of HBV infection from asymptomatic HBsAg carrier state to cirrhosis or HCC[27]. Thus, these viral mutations might accumulate before the diagnosis of HCC and predict the occurrence of HCC.

Possible mechanisms of viral mutation/selection: The HCC-associated HBV mutations are probably generated *via* an evolutionary process on two aspects: increased frequencies of the viral mutations and directional selection of the mutations by the host immune system. As a result of the spontaneous error rate of viral reverse transcriptase, HBV genome might exhibit higher frequencies of mutations than other DNA viruses. This kind of HBV mutation may be considered as a random mutation. In addition, HBV is highly vulnerable to the editing activity of an endogenous human cytidine deaminase activated by proinflammatory cytokines such as tumor necrosis factor (TNF)-α *via* nuclear factor (NF)-κB activation, especially in late-stage liver diseases in which up to 35% of genomes can be edited[37,38]. The majority of the newly synthesized HBV DNA genomes displayed extensive G-to-A mutations in the presence of APOBEC3C, an important member of cellular cytidine deaminases, contributing to innate anti-HBV host responses[39]. Some of the cytidine deaminases play a role in the carcinogenesis of HCC through the generation of HBVx mutants, providing preneoplastic and neoplastic hepatocytes with a selective clonal growth advantage[40]. The cytidine-deaminase-driven HBV mutations may be classified as "semi-directional mutations". Either random or semi-directional mutations might be responsible for HBV mutagenesis during viral replication and inflammatory processes. Moreover, the direction of the mutations should be negatively selected by host immune responses. Reduction of $CD8^+$ T cell epitopes in HBV is one of the common means to evade immune clearance. HBV accumulates escape mutations via reduction in the epitopes number, eventually leading to the removal of epitopes in HBVx and surface proteins[41,42]. The preS1 and preS2 play an essential role in the interaction with host immune responses because they contain several epitopes for T and/or B cells[43]. The preS deletion is one of the most frequent HCC-associated HBV mutations, even in children with HCC[27,28,30,44]. These mutations might facilitate immune escape of HBV.

Potential function of HBV mutations: Currently, the HCC-associated HBV mutations are mainly found in the preS and Enh Ⅱ/BCP/precore regions. Two functional viral proteins coded by these regions are preS2 and truncated HBVx. HBVx protein may increase the expression of telomerase reverse transcriptase (TERT) and telomerase activity, prolonging the lifespan of hepatocytes and contributing to malignant transformation. A1762T has introduced a translation initiation site ATG at the C-terminal of HBVx protein. HBVx mutations, especially the C-terminal deletions, have been frequently found in tumor tissues of HCC[45]. Most of the C-terminally truncated HBVx proteins lose their inhibitory effects on cell proliferation and transformation, retain their ability to bind to p53, and attenuate DNA repair and p53-mediated apoptosis, which may provide a selective clonal advantage for preneoplastic or neoplastic hepatocytes and contribute to hepatocarcinogenesis[46,47]. HBVx and the preS2 activators exert a tumor-promoter-like function, resulting in positive selection of cells that produce functional regulatory proteins[48]. HBV preS2 also promotes HCC development *via* activation of human TERT[49]. However, the role of the mutated preS2 on hepatocarcinogenesis remains unknown. HBV mutations in the BCP region may alter viral replication ability. *In vitro* transfection of HBV mutants has indicated that high-replication clones with 1762/1764/1766 or 1753/1762/1764/1766 mutations expressed very low levels of HBeAg, whereas high-replication clones with 1753/1762/1764 triple mutations expressed high levels of HBeAg, and both 1762/1764/1766 and 1753/1762/1764/1766 mutations conferred significantly higher viral replication and lower HBeAg expression than 1762/1764 mutation alone[50]. In addition, HBV mutations usually accompany high viral load in patients with HCC[17]. These results indicate that some HBV mutations might promote hepatocarcinogenesis.

Taken together, HBV mutagenesis might be augmented in hepatic inflammatory microenvironment due to induction of activated cytidine deaminases. Host immune selection might differ in determining the distinctive outcomes of chronic HBV infection. Upon negative and incomplete immune selection, the mutations facilitate viral replication in the hepatocytes. The escaped HBV accumulates mutations that might predict

the occurrence of HCC and in turn play an active role in hepatocarcinogenesis.

Inflammatory signaling pathways and hallmark cytokines

The liver is the primary organ in which mammals metabolize nutrients, environmental toxins, and drugs. The liver also comprises enrichment of innate immune cells (such as macrophages, natural killer, natural killer T, and γδT cells), $CD8^+$ cytotoxic T cells, $CD4^+$ T helper cells (such as Th1, Th2, Th17 and Treg) and B cells, playing an important role not only in host defenses against invading microorganisms and tumor transformation, but also in liver injury and repair[51]. Liver injuries caused by HBV are traditionally considered to be immuno-mediated and are mainly due to the activity of HBV-specific T cells. Liver-infiltrating neutrophils, natural killer cells and activated bystander lymphocytes also play important roles in causing HBV-related liver damage. These inflammatory cells release cytokines and chemokines which may favor cancer growth.

Liver immune cells: Macrophages can be divided schematically into two main classes in line with the Th1/Th2 dichotomy: M1 and M2. M1 macrophages (classically activated) originate upon encounter with interferon (IFN)-γ and microbial stimuli and are characterized by interleukin (IL)-12 and IL-23 production and consequent activation of polarized type I T cell response, defending the host from viral infections, fighting against tumors, producing high amounts of inflammatory cytokines, and activating the immune response. Monocytes may differentiate into M2 macrophages upon stimulation with IL-4, IL-10 and IL-13. M2 macrophages are responsible for scavenging debris, angiogenesis, remodeling and repair of wounded/damaged tissues, and promote carcinogenesis and downregulate M1 function and adoptive immunity. Tumor-associated macrophages (TAMs) resemble M2-polarized macrophages[52]. $CD8^+$ T cells recognize viral peptides derived from phagocytosed and proteolytically cleaved HBV proteins, activate and differentiate B cells, and secrete IFN-γ and TNF-α, which inhibit the replication and gene expression of HBV. As discussed before, HBV mutations within T-cell epitopes have been documented during chronic HBV infection. These viral mutations may downregulate T-cell functions, such as proliferation or cytokine secretion, and completely or partially inhibit the immune response against the original epitope. Effective $CD8^+$ T-cell-mediated cytotoxic killing may play a crucial role in the control of cancer development. Although tumor-reactive CTL responses are evident in HCC patients, tumor regression is rarely seen, implying that tumor microenvironment inactivates antitumor effector cells, or induces immune tolerance. Macrophages in liver (also called Kupffer cells) function as M2 and suppress $CD8^+$ T cells in human HCC *via* B7-H1/programmed death-1 interactions and favor HCC growth[53]. Depletion of TAMs in HCC enhances the therapeutic effect of sorafenib on HCC[54]. Treg cells serve as critical gatekeepers in immune homeostasis. Increased Treg cells ($CD4^+CD25^+FoxP3^+$) in HCC may impair the effector function of $CD8^+$ T cells and promote HCC progression[55]. Th17 cells elicit a highly inflammatory immune response. The frequency of IL-17^+ cells is significantly elevated in the patients with chronic liver diseases including viral hepatitis and HCC, and the tumor-derived Th17 cells may promote tumor growth and inflammation, and tumor-activated monocytes secrete a set of key proinflammatory cytokines that trigger proliferation of functional Th17 cells[56,57]. Th17 cells promote angiogenesis, tumor growth and inflammation, while Treg cells appear to have counter-regulatory effects on Th17 cells and can inhibit their function. Therefore, an imbalance between Th17 and Treg cell function may be central in some inflammation-associated malignancies, possibly including HBV-associated HCC. The same is true for the imbalance between Th1 and Th2 functions in the microenvironment of HBV-related HCC[58]. These immune imbalances caused by chronic HBV infection contribute to chronic inflammation, hepatic necrosis and subsequent regeneration, accumulated mutagenesis in hepatocytes and ultimately HCC. Multiple signaling pathways are involved in this inflammation-necrosis-regeneration process and in human HCC development. Hallmark cytokines and related molecules in these pathways might be candidate biomarkers for the prediction of the occurrence of HCC in the HBV-infected population.

NF-κB pathway and hallmark cytokines: NF-κB, a collection of dimeric transcription factors including NF-κB1 (p105 and p50), NF-κB2 (p100 and p52), RelA (p65), RelB and c-Rel, is present in all cells in inactive form. In non-stimulated cells, most NF-κB dimers are retained in the cytoplasm by binding to inhibitory IκB proteins. In response to proinflammatory stimuli, such as TNF-α or IL-1β, the IκB kinase (IKK) complex, composed of the IKKα and IKKβ catalytic subunits and the IKKγ regulatory subunit, is activated, resulting in IκB phosphorylation and eventual ubiquitin-mediated degradation[59], leading to the nuclear entry of freed NF-κB dimmers. Of the two catalytic subunits, IKKβ is the most critical for IκB degradation, forming the core of what is known as the classical NF-κB activation pathway[60]. The classical IKKβ-dependent NF-κB signaling pathway promotes hepatocyte survival in both developing and adult livers. NF-κB activation is often observed in human HCC, particularly following hepatitis. It plays a crucial role in liver inflammatory responses by controlling the expression of an array of growth factors and cytokines. One of the most important NF-κB-dependent cytokines that is produced by activated Kupffer cells is IL-6. IL-6 released by Kupffer cells after NF-κB activation also controls HBV gene expression and replication in hepatocytes at the level of transcription shortly after infection[60]. HBVx protein also stimulates IL-6 expression in hepatocytes *via* a MyD88-dependent pathway[61]. Hepatocyte IKK/NF-κB promotes

HCC development by maintaining liver inflammatory responses[62]. The inflammatory process triggers hepatocyte NF-κB through upregulation of TNF-α in adjacent endothelial and inflammatory cells. NF-κB inhibition through anti-TNF-α treatment or induction of IκB super repressor in later stages of tumor development results in apoptosis of transformed hepatocytes and failure to progress to HCC[63]. Serum levels of IL-6 and TNF-α have been found to be significantly higher in HBV-infected patients with liver cirrhosis and HCC than those without or in accordance with the progress of the disease phases[64,65]. In this pathway, IL-6 and TNF-α are hallmark cytokines whose expression might indicate the risk of HCC in HBV-infected population.

Signal transducer and activator of transcription 3 pathway and hallmark cytokines: Signal transducer and activator of transcription (STAT)3 is inactive in non-stimulated cells, but is rapidly activated by various cytokines and growth factors, such as IL-6 and epithelial growth factor family members, as well as hepatocyte growth factor (HGF)[61]. STAT3 activation requires phosphorylation of a critical tyrosine residue, Tyr705, which mediates its dimerization that is a prerequisite for nucleus entry and DNA binding. The phosphorylation of STAT3 at Tyr705 is most commonly mediated by Janus kinases (JAKs), especially JAK2. C-Jun N-terminal kinase (JNK) plays a dual role in the development of HCC. JNK promotes an inflammatory hepatic environment that supports tumor development, but also functions in hepatocytes to reduce tumor development[66]. Activation of STAT3 also turns on strong negative feedback loops involving suppressor of cytokine signaling 3 (SOCS3). STAT3 is activated in the majority of HCCs with poor prognosis and not in surrounding non-tumor tissue or in normal liver. HBVx expression *in vitro* has a significant inverse correlation with the expression of the highly expressed members of the let-7 miRNA family in HCC patients, while the most highly expressed let-7 family member, let-7a, negatively regulates cellular proliferation partly through targeting STAT3, indicating that HBVx upregulates STAT3[67]. HBVx mutant proteins express an atypical nuclear and perinuclear localization in HCC samples, and the effect of HBVx mutants on STAT/SOCS signaling demonstrates a significant upregulation of STAT3 activation in comparison to wild-type HBVx[68]. These data indicate an active role of HBVx mutants in hepatocarcinogenesis that involves dysregulation of STAT/SOCS signaling. Hepatocyte-specific STAT3 ablation prevents HCC development[69]. Other reasons like obesity-promoted HCC development is dependent on enhanced production of the tumor-promoting cytokines IL-6 and TNF-α, which cause hepatic inflammation and activation of STAT3[70]. The main cause of STAT3 activation in human HCC could simply be the elevated expression of IL-6 and related cytokines, such as TNF-α, IL-11 and IL-23. One of the most critical tumor-promoting cytokines in HCC is IL-6. High serum level of IL-6 predicts future occurrence of HCC in patients with CHB[65].

Wnt/β-catenin signaling pathway: A hallmark of Wnt signaling is the stabilization of cytoplasmic β-catenin. Wnt/β-catenin signaling regulates cytokine-induced human inducible nitric oxide synthase expression through interaction with NF-κB, and plays an important role in the pathophysiology of inflammation-associated carcinogenesis[71]. β-catenin was first identified on the basis of its association with cadherin adhesion molecules, and is widely recognized as a key molecule of the Wnt signaling cascade. Mutations of β-catenin, specifically stabilizing mutations in exon 3, are detected in approximately 30% of primary HCCs, raising the possibility that activation of Wnt/β-catenin signaling contributes to hepatocarcinogenesis[72]. Aberrant activation of the Wnt signaling pathway together with transforming growth factor (TGF)-β has been used for gene expression profiling-based classification of HCC[73]. Wnt/β-catenin signaling is activated relatively early during hepatocyte regeneration, mostly through post-translational modifications. Once activated, β-catenin signaling drives the expression of target genes that are critical for cell cycle progression and contribute to initiation of the regeneration process. Wnt-1 is a survival factor for HCC cells. The blockade of Wnt-1-mediated signaling may offer a potential pathway-specific therapeutic strategy for the treatment of a subgroup of HCC that over-expresses Wnt-1[74]. Wnt/β-catenin signaling is particularly activated by ectopic expression of Wnt-1 in HBV-infected HCC cells. Wnt-1 is necessary but insufficient to activate Wnt/β-catenin signaling in HCC. The enhanced stabilization of β-catenin by HBVx, in addition to Wnt-1, is essential for the activation of Wnt/β-catenin signaling in HCC[75]. However, it should be clarified if HBVx mutant and other HBV mutants play a distinct role in activating this pathway.

TGF-β1 pathway: The balance between death and survival is dysregulated in HCC mainly due to overactivation of antiapoptotic pathways[76]. TGF-β signaling involves both tumor suppression and oncogenesis. TGF-β1 is an important regulatory suppressor factor in hepatocytes, inhibiting proliferation and inducing cell death, however, it may also modulate other pro-tumorigenic processes, such as cell invasion[77]. Elevated TGF-β1 may accelerate hepatic fibrosis through increased TGF-β1-induced proinflammatory signaling pathways in hepatic stellate cells[78]. TGF-β activates TGF-β type I receptor (TβR I) and JNK, which differentially phosphorylate the mediator Smad3 to become C-terminally phosphorylated Smad3 (pSmad3C) and linker-phosphorylated Smad3 (pSmad3L). Reversible shifting of Smad3-mediated signaling between tumor suppression and oncogenesis in HBVx-expressing hepatocytes indicates that TβR I -dependent pSmad3C transmits a tumor-suppressive TGF-β signal, while JNK-dependent pSmad3L promotes cell growth. HBVx shifts hepatocytic TGF-β signaling from

the tumor-suppressive pSmad3C pathway to the oncogenic pSmad3L pathway in early carcinogenesis[79]. Hepatocytic pSmad3L and pSmad3C assessment in HBV-infected liver specimens should prove clinically useful for predicting risk of HCC.

Other inflammatory pathways: Apart from above described pathways, raf protein kinases (RAF)/mitogen-activated protein kinase (MEK)/extracellular-signal-regulated kinase (ERK) pathway, phosphatidylinositol-3 kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway, insulin-like growth factor pathway, HGF/c-Met pathway, the p53 pathway, and growth factor-regulated angiogenic signaling are implicated in hepatocarcinogenesis[76]. These pathways are of interest for a therapeutic perspective, because targeting them may help to reverse, delay or prevent hepatocarcinogenesis[80]. For example, the antitumor effect of sorafenib on HCC can be improved by vertical blockade of RAF/MEK/ERK signaling with CI-1040[81].

Genetic susceptibility of inflammatory factors

The imbalances in inflammation reactions play an important role in HBV-induced hepatocarcinogenesis, therefore, it is necessary to clarify the role of key inflammatory molecule germline mutations, which influence the expression and function of those molecules, on the susceptibility of HCC. Human leukocyte antigen-DR13 polymorphism has been shown to be strongly associated with the clearance of HBV[82]. A study in Japan has evaluated the association of genetic polymorphism in the cytokines TNF-α, IFN-γ, TGF-β1, IL-6 and IL-10 with the risk of HCC in HBV-infected subjects and has found that the risk of HCC was significantly lower in HBV carriers with C/C genotype than in those with T/C or T/T genotype in position +29 of the *TGF-β1* gene, but was not associated with genetic polymorphism of other molecules[83]. It has been demonstrated that in the presence of the IL-1 receptor antagonist *2 allele, the IL-β1-31 polymorphism T/C and T/T genotypes in Taiwan are significantly associated with HBV-related HCC, with adjusted odds ratios (ORs) of 2.93 (95% CI: 1.07-8.07) and 5.76 (95% CI: 1.79-18.53), respectively[84]. A study in Korea has documented that the -148C, +8925G, and +13925C alleles of the *IL-18* gene are associated with HBV-related HCC and the 148G > C single nucleotide polymorphism is functionally important in determining disease outcome[85]. IL-12 polymorphisms have been recently associated with HBV-related HCC in the Chinese population[86]. A recent genome-wide association study (GWAS) in Mainland China has shown that the 1p36.22 locus confers susceptibility to HBV-related HCC, and suggests that KIF1B-, UBE4B- or PGD-related pathways might be involved in the pathogenesis of this malignancy[87]. Our recent data have demonstrated that *NF-κB1* gene promoter NFKB1-94ATTG2 allelic carriage and *IκBα* gene promoter NFKBIA-826T and NFKBIA-881AG allelic carriage, which are related to upregulation of NF-κB function, are significantly associated with an increased risk of HCC in patients infected with genotype C HBV, while the estimated haplotype frequency of NFKBIA promoter -881G-826T-519C is significantly higher in patients with HCC than in HBV-infected subjects without HCC[88]. Problems in current studies on genetic susceptibility of HBV-associated HCC are: (1) GWAS with enough samples has limited coverage, resulting in loss of data; (2) individual case-control studies lack sufficient samples and strict controls; and (3) significant polymorphisms found by GWAS and individual case-control studies have few loci in common. Nevertheless, the contribution of genetic polymorphisms to the occurrence of HCC may be limited because of low ORs obtained both in current GWAS and in case-control studies.

Other parameters

Family history is one of the major risk factors for HCC[89,90]. First-degree relatives of patients with HCC are associated with an increased risk of HBV-related HCC in Taiwan and with HCC in United States independently of HBV and HCV infection. In HBV-endemic regions, family clustering of HCC is, to some extent, related to family clustering of HBV infection *via* perinatal transmission. Apart from HBV and HCV infection, exposure to aflatoxin B1, alcoholic abuse, and diabetes are well-established risk factors for HCC. Regular consumption of coffee or green tea is significantly associated with a decreased risk of HCC in subjects with or without HBV and/or HCV infection[91,92]. Old age, male sex and poor socioeconomic status are also related to the occurrence of HCC in the HBV-infected subjects.

Poor liver function, as indicated by low serum level of albumin and high level of bilirubin, favor HBV-induced hepatocarcinogenesis. Persistent or intermittent increase in serum alanine aminotransferase (ALT) level reflects persistent liver damage. CHB patients with ALT levels 0.5-1 times the upper limit of normal (ULN) and 1-2 × ULN have an increased risk for the development of complications compared with patients with ALT levels < 0.5 × ULN ($P < 0.0001$ for both)[93]. In HBeAg-negative subjects, high viral load is frequently associated with abnormal ALT level, while ALT abnormality is more frequent in those with liver cirrhosis than those without (19.5% *vs* 7.8%, $P = 0.001$)[24]. Abnormal ALT level (> 45 U/L) has been frequently shown to be an independent risk factor for HCC in HBV-infected subjects[17,28,31]. Liver cirrhosis is a well-established important risk factor of HCC[94]. Older age, higher total bilirubin, ALT, and HBV DNA levels, and HBV preS deletion, A1846T and/or T1768A mutations are major independent determinants of progression to cirrhosis in HBeAg-negative patients[17,24,28,95].

Clinical scoring system for prediction of HCC occurrence

A research group in Hong Kong prospectively evaluated 1005 HBV carriers and found that age, albumin, biliru-

bin, HBV DNA, and cirrhosis independently predicted the development of HCC. They used these variables to construct a prediction score ranging from 0 to 44.5 and then validated the score in a different cohort of 424 HBV carriers. In the training cohort, they found cutoff values of 5 and 20 best discriminated HCC risk. In the validation cohort, the 5-year HCC-free survival rates were 98.3%, 90.5% and 78.9% in the low-, medium- and high-risk groups, respectively. HR for HCC in the medium- and high-risk groups was 12.8 and 14.6, respectively. This simple prediction score constructed from routine clinical and laboratory parameters is accurate in predicting HCC development in HBV carriers[96]. To set up a scoring system for the prediction of HCC from CHB patients, 820 CHB patients have been followed up for a mean duration of 76.8 mo in a prospective study. It has been found that male [relative risk (RR) = 2.98], increasing age (RR = 1.07), higher HBV DNA levels (RR = 1.28), core promoter mutations (RR = 3.66), and presence of cirrhosis (RR = 7.31) are independent risks for the development of HCC. A risk score is derived and validated with sensitivity > 84% and specificity > 76% to predict the 5- and 10-year risks for the development of HCC[97]. The two scoring systems can accurately predict HBV carriers and CHB patients who will more likely develop HCC in the near future, and have important implications for treatment allocation and strategic screening for HCC in CHB patients and HBV carriers.

ROLE OF VIRAL AND INFLAMMATORY FACTORS IN PREDICTING PROGNOSIS OF HBV-ASSOCIATED HCC

Viral load

In a study carried out in Japan, a total of 74 patients with HBV-associated HCC who had received either conventional treatment or not were followed up for survival analysis. In multivariate regression analysis, it has been found that serum level of HBV DNA and tumor size at diagnosis are independent and significant prognostic factors ($P = 0.0022$ and $P = 0.0106$, respectively), and a low level of viremia is associated with longer survival ($P = 0.0057$), even in patients seronegative for HBeAg[98]. In another study, a total of 62 HBV-related HCC patients who had achieved complete necrosis with transarterial chemolipiodolization were followed up for analysis of recurrence. Multivariate analysis has established that high viral load (> 10^5 copies/mL) at complete necrosis is among the most important risk factors for post-treatment recurrence[99]. Seventy-two patients who underwent liver resection for HBV-related HCC were followed up. By multivariate analysis, high viral load (> 10^4 copies/mL) (OR = 22.3, $P = 0.001$), α-fetoprotein > 1 mg/mL (OR = 7.4, $P = 0.02$), tumor size > 5 cm (OR = 5.1, $P = 0.02$), and age > 60 years (OR = 4, $P = 0.01$) at the time of tumor resection are independently associated with HCC recurrence after resection. Of those, high viral load (> 10^4 copies/mL) is the most important correctable risk factor[100]. In a Taiwan study, HBV DNA > 3×10^7 copies/g in the non-cancerous liver tissues of patients with HCC was found to be independently associated with shorter overall survival[101]. Lamivudine therapy is beneficial for patients after initial treatment for HBV-related HCC because it contributes to improving remnant liver function, decreasing the risk of liver failure, and increasing the chances of receiving available treatment modalities for recurrent HCC[102]. Treatment of the HBV-infected HCC patients with IFN-α after curative resection efficiently prevents early recurrence and improves overall survival[103]. These data indicate that high viral load is the most reliable factor in predicting poor prognosis of the patients with HBV-related HCC, therefore, antiviral treatment after surgical resection is highly recommended.

HBV genotype and viral mutations

A Taiwan study enrolled 64 patients who underwent liver resection for HBV-related HCC. During a mean follow-up of 26.6 ± 13.2 mo, patients with genotype C had worse disease-free survival rate ($P = 0.028$) than those with genotype B. By univariate analysis, genotype C, ALT > 50 U/L, tumor size ⩾ 5 cm, and microvascular invasion were associated with tumor recurrence. Multivariate analysis indicated that genotype C was a risk factor independently associated with poor prognosis ($P = 0.034$)[104]. Recently, the core gene of HBV isolated from HCC tissues has been found to have fewer mutations compared with those isolated from adjacent non-tumor tissues from the same patients ($P < 0.05$)[105], implying that active immune selection of viral mutation most likely happens in the peritumoral liver tissues. In a study carried out in Taiwan, the association of virological characteristics with the prognosis of the patients was investigated by using the non-cancerous part of surgically removed HBV-associated HCC tissues from 185 patients. All virological and clinicopathological factors were subjected to Cox proportional hazard model analysis to estimate postoperative survival. After adjusting for other confounding factors, multivariate analysis revealed that age older than 50 years, bilirubin > 1.4 mg/dL, and A1762T/G1764A mutation were independently associated with shorter overall survival. Kaplan-Meier survival analysis indicated that in-frame, short stretch (< 100 bp) preS deletions, but not large fragment (> 100 bp) preS deletions, were significantly associated with poorer disease-free ($P = 0.005$) and overall ($P = 0.020$) survival. A hot deletion region located between codons 107 and 141 of the preS sequence was identified for the short stretch preS deletion mutants[101]. The result of viral load in the inflammatory liver tissues seems to be consistent with that of serum viral load. It remains unknown if the mutations of HBV in the tissues are consistent with those in the sera.

Inflammatory cells and molecules

Inflammatory cells: Immune cells infiltrated into HCC

tissues and adjacent non-cancerous tissues have various roles in conducting inflammatory responses. NK and T cells are present in tumors of HCC patients with longer survival, and exclusively in areas devoid of proliferating tumor cells. NK and $CD8^+$ T cell densities are correlated positively with tumor apoptosis, and negatively with tumor proliferation[106]. On the other hand, higher density of intratumoral Treg cells and lower density of intratumoral $CD8^+$ T cells are independently associated with poor prognosis of HCC. In addition, high Treg cell density is associated with both absence of tumor encapsulation and presence of tumor vascular invasion[107]. Intratumoral balance of regulatory and cytotoxic T cells is a promising independent predictor for recurrence and survival in the HBV-infected patients with HCC.

Th1/Th2-like cytokines: Peritumoral hepatic tissues of patients with HCC have been used for investigating the association of inflammatory cytokines with prognosis in Mainland China. Higher levels of IL-2 and IL-15 in peritumoral liver tissues, but not in tumor tissues, are significantly associated with decreased incidence of recurrence of intrahepatic tumor and prolonged overall survival, whereas peritumoral expression of granulocyte-colony stimulating factor, a Th2-like cytokine, is significantly associated with poor prognosis[108,109]. This reflects that the imbalance of Th1/Th2-like cytokines affects inflammation status, which later determines the malignant phenotype of HCC. Peritumoral levels of Th1/Th2-like cytokines are useful for stratifying patients, even those with early-stage HCC, into subgroups with different prognoses after curative resection. Treatment with Th1 cytokine IFN-α benefits HBV-related HCC patients after curative resection[103], possibly *via* correcting the imbalances.

Other inflammatory molecules: Up-regulation of Wnt-1 protein has been reported in HBV-related HCC tissues. High Wnt-1 expression in HBV-related HCC tissues correlates with enhanced nuclear β-catenin accumulation, diminished membranous E-cadherin expression, and increased HCC recurrence after curative resection[110]. Liver-intestine cadherin (LI-cadherin; CDH-17) is a new member of the cadherin superfamily with distinct structural and functional features. Overexpression of LI-cadherin is well correlated with microvascular invasion in HBV-positive HCC tissues and strongly associated with shorter overall survival as well as higher incidence of tumor recurrence[111]. Thus, LI-cadherin should be a candidate target for HCC intervention. ErbB-2 is strongly upregulated in HBV-infected liver and correlated with HBVx expression. ErbB-2 contributes to the stabilization of β-catenin. Strong ErbB-2 staining in liver tissues of patients with HCC is associated with dysplasia and a shorter survival after tumor diagnosis[112]. Cyclooxygenase-2 is a proinflammatory factor whose expression in non-cancerous liver tissue increases the postoperative recurrence of HCC in patients with HBV-related cirrhosis[113]. These inflammatory molecules may serve as prognostic markers and/or candidate therapeutic targets of HBV-related HCC after surgical treatment.

Signaling-associated miRNAs: miRNAs are a class of small non-coding RNAs that modulate gene function at the post-transcriptional level and act as fine tuners of various processes including cell signaling and apoptosis. miRNAs are associated with different types and stages of cancer, and are involved in liver diseases caused by various factors, including HBV[114]. Some miRNAs are specifically found in HCC tumors and sera of patients. Serum miRNAs including miR-25, miR-375 and let-7f have been recently identified as biomarkers and clearly separate HCC cases from controls, and miR-375 and miR-92a have been identified as HBV-specific[115]. miR-199a/b-3p exhibits its biological activity *via* inhibiting the Raf/MEK/ERK pathway, and is the most consistently decreased miRNA in HCC. Low miR-199-3p expression correlates with poor survival of HCC patients[116]. Current problems of developing miRNAs as diagnostic and prognostic markers are their stability in sera and selection of internal controls. It will be a great challenge to search for miRNA markers, especially for those that target some existing inflammatory signaling pathways involved in the recurrence and metastasis of HBV-associated HCC.

Clinical scoring systems for prediction of HCC prognosis

Clinical scoring systems have been developed not only for the prediction of HCC occurrence, but also for its prognosis. Nathan *et al*[117] have prospectively evaluated survival of HCC patients with small tumors, and have found that tumor size > 2 cm (HR = 1.51), multifocal tumors (HR = 1.51), and vascular invasion (HR = 1.44) remained independent predictors of poor survival (all $P < 0.05$) after adjusting for demographic factors and histological grade. Based on these findings, they developed a prognostic scoring system by allotting 1 point each for these factors. Patients with early HCC could be stratified into three distinct prognostic groups (median and 5-year survival, respectively): 0 point (70 mo, 55%), 1 point (52 mo, 42%), and ≥ 2 points (24 mo, 29%) ($P < 0.001$). However, this scoring system has not been validated using an independent cohort. Hsu *et al*[118] have prospectively investigated the prognostic ability of the five currently used staging systems with 1713 enrolled HCC patients, and have concluded that the CLIP staging system is the best long-term prognostic model for HCC in a cohort of patients with early to advanced stage HCC. Current scoring systems for the prediction of HCC prognosis lack useful viral and inflammatory markers like serum HBV load and expression of inflammatory molecules in tumors and/or non-cancerous liver tissues. Inclusion of viral and inflammatory factors that are closely related to malignant phenotype and poor prognosis will make current scoring systems more accurate in predicting the prognosis of HBV-related HCC after surgical resection.

In summary, continuing inflammation caused by chronic HBV infection is not only involved in hepatocarcinogenesis, but also plays critical roles in the recurrence and metastasis of HCC after surgical treatment. Viral load, genotype/subgenotype, and a subset of viral mutations are major viral factors that may predict the occurrence of HCC. Inflammatory microenvironment may increase the frequency of viral mutation *via* induction of cellular cytidine deaminases, whereas HBV mutations selected by inflammation reaction might in turn promote hepatocarcinogenesis. Imbalance either between peritumoral Th1 and Th2 cytokines or between intratumoral CD8+ T cells and Treg cells contributes, at least partially, to inflammation-necrosis-regeneration response, ultimately HCC. Inflammatory pathways including NF-κB, STAT3, Wnt/β-catenin, and TGF-β1 signaling pathways contribute to the development of HCC, and their hallmark molecules including IL-6, TNF-α and Wnt-1 can predict the occurrence or recurrence of this malignancy. HBV load, viral mutations and imbalance between infiltrating immune cells or between Th1 and Th2 cytokines can predict the prognosis of HBV-related HCC. These factors are of significance for developing active prevention and surveillance of the HBV-infected subjects who are more likely to develop HCC, or tailoring suitable treatment options for HBV-related HCC patients to improve the survival or postpone recurrence after surgical resection.

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 **Parkin DM**. The global health burden of infection-associated cancers in the year 2002. *Int J Cancer* 2006; **118**: 3030-3044
3 **But DY**, Lai CL, Yuen MF. Natural history of hepatitis-related hepatocellular carcinoma. *World J Gastroenterol* 2008; **14**: 1652-1656
4 **Perz JF**, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *J Hepatol* 2006; **45**: 529-538
5 **Nguyen VT**, Law MG, Dore GJ. Hepatitis B-related hepatocellular carcinoma: epidemiological characteristics and disease burden. *J Viral Hepat* 2009; **16**: 453-463
6 **Chang MH**, You SL, Chen CJ, Liu CJ, Lee CM, Lin SM, Chu HC, Wu TC, Yang SS, Kuo HS, Chen DS. Decreased incidence of hepatocellular carcinoma in hepatitis B vaccinees: a 20-year follow-up study. *J Natl Cancer Inst* 2009; **101**: 1348-1355
7 **Liang X**, Bi S, Yang W, Wang L, Cui G, Cui F, Zhang Y, Liu J, Gong X, Chen Y, Wang F, Zheng H, Wang F, Guo J, Jia Z, Ma J, Wang H, Luo H, Li L, Jin S, Hadler SC, Wang Y. Epidemiological serosurvey of hepatitis B in China--declining HBV prevalence due to hepatitis B vaccination. *Vaccine* 2009; **27**: 6550-6557
8 **Yin J**, Zhang H, He Y, Xie J, Liu S, Chang W, Tan X, Gu C, Lu W, Wang H, Bi S, Cui F, Liang X, Schaefer S, Cao G. Distribution and hepatocellular carcinoma-related viral properties of hepatitis B virus genotypes in Mainland China: a community-based study. *Cancer Epidemiol Biomarkers Prev* 2010; **19**: 777-786
9 **Yang HI**, Lu SN, Liaw YF, You SL, Sun CA, Wang LY, Hsiao CK, Chen PJ, Chen DS, Chen CJ. Hepatitis B e antigen and the risk of hepatocellular carcinoma. *N Engl J Med* 2002; **347**: 168-174
10 **Chen CJ**, Yang HI, Su J, Jen CL, You SL, Lu SN, Huang GT, Iloeje UH. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* 2006; **295**: 65-73
11 **Wu CF**, Yu MW, Lin CL, Liu CJ, Shih WL, Tsai KS, Chen CJ. Long-term tracking of hepatitis B viral load and the relationship with risk for hepatocellular carcinoma in men. *Carcinogenesis* 2008; **29**: 106-112
12 **Chan HL**, Tse CH, Mo F, Koh J, Wong VW, Wong GL, Lam Chan S, Yeo W, Sung JJ, Mok TS. High viral load and hepatitis B virus subgenotype ce are associated with increased risk of hepatocellular carcinoma. *J Clin Oncol* 2008; **26**: 177-182
13 **Yang HI**, Yeh SH, Chen PJ, Iloeje UH, Jen CL, Su J, Wang LY, Lu SN, You SL, Chen DS, Liaw YF, Chen CJ. Associations between hepatitis B virus genotype and mutants and the risk of hepatocellular carcinoma. *J Natl Cancer Inst* 2008; **100**: 1134-1143
14 **Tong MJ**, Blatt LM, Kao JH, Cheng JT, Corey WG. Precore/basal core promoter mutants and hepatitis B viral DNA levels as predictors for liver deaths and hepatocellular carcinoma. *World J Gastroenterol* 2006; **12**: 6620-6626
15 **Yin J**, Zhang H, Li C, Gao C, He Y, Zhai Y, Zhang P, Xu L, Tan X, Chen J, Cheng S, Schaefer S, Cao G. Role of hepatitis B virus genotype mixture, subgenotypes C2 and B2 on hepatocellular carcinoma: compared with chronic hepatitis B and asymptomatic carrier state in the same area. *Carcinogenesis* 2008; **29**: 1685-1691
16 **Yuen MF**, Tanaka Y, Shinkai N, Poon RT, But DY, Fong DY, Fung J, Wong DK, Yuen JC, Mizokami M, Lai CL. Risk for hepatocellular carcinoma with respect to hepatitis B virus genotypes B/C, specific mutations of enhancer II/core promoter/precore regions and HBV DNA levels. *Gut* 2008; **57**: 98-102
17 **Yin J**, Xie J, Liu S, Zhang H, Han L, Lu W, Shen Q, Xu G, Dong H, Shen J, Zhang J, Han J, Wang L, Liu Y, Wang F, Zhao J, Zhang Q, Ni W, Wang H, Cao G. Association between the various mutations in viral core promoter region to different stages of hepatitis B, ranging of asymptomatic carrier state to hepatocellular carcinoma. *Am J Gastroenterol* 2011; **106**: 81-92
18 **Fattovich G**, Olivari N, Pasino M, D'Onofrio M, Martone E, Donato F. Long-term outcome of chronic hepatitis B in Caucasian patients: mortality after 25 years. *Gut* 2008; **57**: 84-90
19 **Choi JW**, Ahn SH, Park JY, Chang HY, Kim JK, Baatarkhuu O, Kim do Y, Han KH, Chon CY. Hepatitis B e antigen-negative mutations in the precore and core promoter regions in Korean patients. *J Med Virol* 2009; **81**: 594-601
20 **Schaefer S**. Hepatitis B virus taxonomy and hepatitis B virus genotypes. *World J Gastroenterol* 2007; **13**: 14-21
21 **Cao GW**. Clinical relevance and public health significance of hepatitis B virus genomic variations. *World J Gastroenterol* 2009; **15**: 5761-5769
22 **Zhang Q**, Cao GW. Genotypes, mutations, and viral load of hepatitis B virus and the risk of hepatocellular carcinoma. *Hepat Mon* 2011; **11**: 86-91
23 **Zhang HW**, Yin JH, Li YT, Li CZ, Ren H, Gu CY, Wu HY, Liang XS, Zhang P, Zhao JF, Tan XJ, Lu W, Schaefer S, Cao GW. Risk factors for acute hepatitis B and its progression to chronic hepatitis in Shanghai, China. *Gut* 2008; **57**: 1713-1720
24 **Yin JH**, Zhao J, Zhang HW, Xie JX, Li WP, Xu GZ, Shen J, Dong HJ, Zhang J, Wang L, Han JK, Wang HY, Cao GW. HBV genotype C is independently associated with cirrhosis in community-based population. *World J Gastroenterol* 2010; **16**: 379-383
25 **Chan HL**, Hui AY, Wong ML, Tse AM, Hung LC, Wong VW, Sung JJ. Genotype C hepatitis B virus infection is associated with an increased risk of hepatocellular carcinoma. *Gut* 2004; **53**: 1494-1498

26 **Yu MW**, Yeh SH, Chen PJ, Liaw YF, Lin CL, Liu CJ, Shih WL, Kao JH, Chen DS, Chen CJ. Hepatitis B virus genotype and DNA level and hepatocellular carcinoma: a prospective study in men. *J Natl Cancer Inst* 2005; **97**: 265-272

27 **Liu S**, Zhang H, Gu C, Yin J, He Y, Xie J, Cao G. Associations between hepatitis B virus mutations and the risk of hepatocellular carcinoma: a meta-analysis. *J Natl Cancer Inst* 2009; **101**: 1066-1082

28 **Yin J**, Xie J, Zhang H, Shen Q, Han L, Lu W, Han Y, Li C, Ni W, Wang H, Cao G. Significant association of different preS mutations with hepatitis B-related cirrhosis or hepatocellular carcinoma. *J Gastroenterol* 2010; **45**: 1063-1071

29 **Zhu Y**, Jin Y, Guo X, Bai X, Chen T, Wang J, Qian G, Groopman JD, Gu J, Li J, Tu H. Comparison study on the complete sequence of hepatitis B virus identifies new mutations in core gene associated with hepatocellular carcinoma. *Cancer Epidemiol Biomarkers Prev* 2010; **19**: 2623-2630

30 **Xie JX**, Zhao J, Yin JH, Zhang Q, Pu R, Lu WY, Zhang HW, Wang HY, Cao GW. Association of novel mutations and haplotypes in the preS region of hepatitis B virus with hepatocellular carcinoma. *Front Med China* 2010; **4**: 419-429

31 **Liu S**, Xie J, Yin J, Zhang H, Zhang Q, Pu R, Li C, Ni W, Wang H, Cao G. A matched case-control study of hepatitis B virus mutations in the preS and core promoter regions associated independently with hepatocellular carcinoma. *J Med Virol* 2011; **83**: 45-53

32 **Shen T**, Yan XM, Zou YL, Gao JM, Dong H. Virologic characteristics of hepatitis B virus in patients infected via maternal-fetal transmission. *World J Gastroenterol* 2008; **14**: 5674-5682

33 **Ni YH**, Chang MH, Hsu HY, Tsuei DJ. Different hepatitis B virus core gene mutations in children with chronic infection and hepatocellular carcinoma. *Gut* 2003; **52**: 122-125

34 **Fang ZL**, Sabin CA, Dong BQ, Ge LY, Wei SC, Chen QY, Fang KX, Yang JY, Wang XY, Harrison TJ. HBV A1762T, G1764A mutations are a valuable biomarker for identifying a subset of male HBsAg carriers at extremely high risk of hepatocellular carcinoma: a prospective study. *Am J Gastroenterol* 2008; **103**: 2254-2262

35 **Yuan JM**, Ambinder A, Fan Y, Gao YT, Yu MC, Groopman JD. Prospective evaluation of hepatitis B 1762(T)/1764(A) mutations on hepatocellular carcinoma development in Shanghai, China. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 590-594

36 **Bai X**, Zhu Y, Jin Y, Guo X, Qian G, Chen T, Zhang J, Wang J, Groopman JD, Gu J, Tu H. Temporal acquisition of sequential mutations in the enhancer II and basal core promoter of HBV in individuals at high risk for hepatocellular carcinoma. *Carcinogenesis* 2011; **32**: 63-68

37 **Vartanian JP**, Henry M, Marchio A, Suspène R, Aynaud MM, Guétard D, Cervantes-Gonzalez M, Battiston C, Mazzaferro V, Pineau P, Dejean A, Wain-Hobson S. Massive APOBEC3 editing of hepatitis B viral DNA in cirrhosis. *PLoS Pathog* 2010; **6**: e1000928

38 **Endo Y**, Marusawa H, Kinoshita K, Morisawa T, Sakurai T, Okazaki IM, Watashi K, Shimotohno K, Honjo T, Chiba T. Expression of activation-induced cytidine deaminase in human hepatocytes via NF-kappaB signaling. *Oncogene* 2007; **26**: 5587-5595

39 **Baumert TF**, Rösler C, Malim MH, von Weizsäcker F. Hepatitis B virus DNA is subject to extensive editing by the human deaminase APOBEC3C. *Hepatology* 2007; **46**: 682-689

40 **Xu R**, Zhang X, Zhang W, Fang Y, Zheng S, Yu XF. Association of human APOBEC3 cytidine deaminases with the generation of hepatitis virus B x antigen mutants and hepatocellular carcinoma. *Hepatology* 2007; **46**: 1810-1820

41 **Abbott WG**, Tsai P, Leung E, Trevarton A, Ofanoa M, Hornell J, Gane EJ, Munn SR, Rodrigo AG. Associations between HLA class I alleles and escape mutations in the hepatitis B virus core gene in New Zealand-resident Tongans. *J Virol* 2010; **84**: 621-629

42 **Maman Y**, Blancher A, Benichou J, Yablonka A, Efroni S, Louzoun Y. Immune-induced evolutionary selection focused on a single reading frame in overlapping hepatitis B virus proteins. *J Virol* 2011; **85**: 4558-4566

43 **Kay A**, Zoulim F. Hepatitis B virus genetic variability and evolution. *Virus Res* 2007; **127**: 164-176

44 **Huang HP**, Hsu HY, Chen CL, Ni YH, Wang HY, Tsuei DJ, Chiang CL, Tsai YC, Chen HL, Chang MH. Pre-S2 deletions of hepatitis B virus and hepatocellular carcinoma in children. *Pediatr Res* 2010; **67**: 90-94

45 **Chen GG**, Li MY, Ho RL, Chak EC, Lau WY, Lai PB. Identification of hepatitis B virus X gene mutation in Hong Kong patients with hepatocellular carcinoma. *J Clin Virol* 2005; **34**: 7-12

46 **Kew MC**. Hepatitis B virus x protein in the pathogenesis of hepatitis B virus-induced hepatocellular carcinoma. *J Gastroenterol Hepatol* 2011; **26** Suppl 1: 144-152

47 **Hussain SP**, Schwank J, Staib F, Wang XW, Harris CC. TP53 mutations and hepatocellular carcinoma: insights into the etiology and pathogenesis of liver cancer. *Oncogene* 2007; **26**: 2166-2176

48 **Lupberger J**, Hildt E. Hepatitis B virus-induced oncogenesis. *World J Gastroenterol* 2007; **13**: 74-81

49 **Luan F**, Liu H, Gao L, Liu J, Sun Z, Ju Y, Hou N, Guo C, Liang X, Zhang L, Sun W, Ma C. Hepatitis B virus protein preS2 potentially promotes HCC development via its transcriptional activation of hTERT. *Gut* 2009; **58**: 1528-1537

50 **Parekh S**, Zoulim F, Ahn SH, Tsai A, Li J, Kawai S, Khan N, Trépo C, Wands J, Tong S. Genome replication, virion secretion, and e antigen expression of naturally occurring hepatitis B virus core promoter mutants. *J Virol* 2003; **77**: 6601-6612

51 **Gao B**, Jeong WI, Tian Z. Liver: An organ with predominant innate immunity. *Hepatology* 2008; **47**: 729-736

52 **Solinas G**, Germano G, Mantovani A, Allavena P. Tumor-associated macrophages (TAM) as major players of the cancer-related inflammation. *J Leukoc Biol* 2009; **86**: 1065-1073

53 **Wu K**, Kryczek I, Chen L, Zou W, Welling TH. Kupffer cell suppression of CD8+ T cells in human hepatocellular carcinoma is mediated by B7-H1/programmed death-1 interactions. *Cancer Res* 2009; **69**: 8067-8075

54 **Zhang W**, Zhu XD, Sun HC, Xiong YQ, Zhuang PY, Xu HX, Kong LQ, Wang L, Wu WZ, Tang ZY. Depletion of tumor-associated macrophages enhances the effect of sorafenib in metastatic liver cancer models by antimetastatic and antiangiogenic effects. *Clin Cancer Res* 2010; **16**: 3420-3430

55 **Fu J**, Xu D, Liu Z, Shi M, Zhao P, Fu B, Zhang Z, Yang H, Zhang H, Zhou C, Yao J, Jin L, Wang H, Yang Y, Fu YX, Wang FS. Increased regulatory T cells correlate with CD8 T-cell impairment and poor survival in hepatocellular carcinoma patients. *Gastroenterology* 2007; **132**: 2328-2339

56 **Kuang DM**, Peng C, Zhao Q, Wu Y, Chen MS, Zheng L. Activated monocytes in peritumoral stroma of hepatocellular carcinoma promote expansion of memory T helper 17 cells. *Hepatology* 2010; **51**: 154-164

57 **Littman DR**, Rudensky AY. Th17 and regulatory T cells in mediating and restraining inflammation. *Cell* 2010; **140**: 845-858

58 **Budhu A**, Wang XW. The role of cytokines in hepatocellular carcinoma. *J Leukoc Biol* 2006; **80**: 1197-1213

59 **Sun W**, Tan X, Shi Y, Xu G, Mao R, Gu X, Fan Y, Yu Y, Burlingame S, Zhang H, Rednam SP, Lu X, Zhang T, Fu S, Cao G, Qin J, Yang J. USP11 negatively regulates TNFalpha-induced NF-kappaB activation by targeting on IkappaBalpha. *Cell Signal* 2010; **22**: 386-394

60 **Hösel M**, Quasdorff M, Wiegmann K, Webb D, Zedler U, Broxtermann M, Tedjokusumo R, Esser K, Arzberger S, Kirschning CJ, Langenkamp A, Falk C, Büning H, Rose-John S, Protzer U. Not interferon, but interleukin-6 controls early gene expression in hepatitis B virus infection. *Hepatology*

2009; **50**: 1773-1782
61 **Xiang WQ**, Feng WF, Ke W, Sun Z, Chen Z, Liu W. Hepatitis B virus X protein stimulates IL-6 expression in hepatocytes via a MyD88-dependent pathway. *J Hepatol* 2011; **54**: 26-33
62 **He G**, Karin M. NF-κB and STAT3 - key players in liver inflammation and cancer. *Cell Res* 2011; **21**: 159-168
63 **Pikarsky E**, Porat RM, Stein I, Abramovitch R, Amit S, Kasem S, Gutkovich-Pyest E, Urieli-Shoval S, Galun E, Ben-Neriah Y. NF-kappaB functions as a tumour promoter in inflammation-associated cancer. *Nature* 2004; **431**: 461-466
64 **Song le H**, Binh VQ, Duy DN, Kun JF, Bock TC, Kremsner PG, Luty AJ. Serum cytokine profiles associated with clinical presentation in Vietnamese infected with hepatitis B virus. *J Clin Virol* 2003; **28**: 93-103
65 **Wong VW**, Yu J, Cheng AS, Wong GL, Chan HY, Chu ES, Ng EK, Chan FK, Sung JJ, Chan HL. High serum interleukin-6 level predicts future hepatocellular carcinoma development in patients with chronic hepatitis B. *Int J Cancer* 2009; **124**: 2766-2770
66 **Das M**, Garlick DS, Greiner DL, Davis RJ. The role of JNK in the development of hepatocellular carcinoma. *Genes Dev* 2011; **25**: 634-645
67 **Wang Y**, Lu Y, Toh ST, Sung WK, Tan P, Chow P, Chung AY, Jooi LL, Lee CG. Lethal-7 is down-regulated by the hepatitis B virus x protein and targets signal transducer and activator of transcription 3. *J Hepatol* 2010; **53**: 57-66
68 **Bock CT**, Toan NL, Koeberlein B, Song le H, Chin R, Zentgraf H, Kandolf R, Torresi J. Subcellular mislocalization of mutant hepatitis B X proteins contributes to modulation of STAT/SOCS signaling in hepatocellular carcinoma. *Intervirology* 2008; **51**: 432-443
69 **He G**, Yu GY, Temkin V, Ogata H, Kuntzen C, Sakurai T, Sieghart W, Peck-Radosavljevic M, Leffert HL, Karin M. Hepatocyte IKKbeta/NF-kappaB inhibits tumor promotion and progression by preventing oxidative stress-driven STAT3 activation. *Cancer Cell* 2010; **17**: 286-297
70 **Park EJ**, Lee JH, Yu GY, He G, Ali SR, Holzer RG, Osterreicher CH, Takahashi H, Karin M. Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. *Cell* 2010; **140**: 197-208
71 **Du Q**, Zhang X, Cardinal J, Cao Z, Guo Z, Shao L, Geller DA. Wnt/beta-catenin signaling regulates cytokine-induced human inducible nitric oxide synthase expression by inhibiting nuclear factor-kappaB activation in cancer cells. *Cancer Res* 2009; **69**: 3764-3771
72 **Miyoshi Y**, Iwao K, Nagasawa Y, Aihara T, Sasaki Y, Imaoka S, Murata M, Shimano T, Nakamura Y. Activation of the beta-catenin gene in primary hepatocellular carcinomas by somatic alterations involving exon 3. *Cancer Res* 1998; **58**: 2524-2527
73 **Hoshida Y**, Nijman SM, Kobayashi M, Chan JA, Brunet JP, Chiang DY, Villanueva A, Newell P, Ikeda K, Hashimoto M, Watanabe G, Gabriel S, Friedman SL, Kumada H, Llovet JM, Golub TR. Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. *Cancer Res* 2009; **69**: 7385-7392
74 **Wei W**, Chua MS, Grepper S, So SK. Blockade of Wnt-1 signaling leads to anti-tumor effects in hepatocellular carcinoma cells. *Mol Cancer* 2009; **8**: 76
75 **Cha MY**, Kim CM, Park YM, Ryu WS. Hepatitis B virus X protein is essential for the activation of Wnt/beta-catenin signaling in hepatoma cells. *Hepatology* 2004; **39**: 1683-1693
76 **Fabregat I**. Dysregulation of apoptosis in hepatocellular carcinoma cells. *World J Gastroenterol* 2009; **15**: 513-520
77 **Massagué J**. TGFbeta in Cancer. *Cell* 2008; **134**: 215-230
78 **Ki MR**, Goo MJ, Park JK, Hong IH, Ji AR, Han SY, You SY, Lee EM, Kim AY, Park SJ, Lee HJ, Kim SY, Jeong KS. Helicobacter pylori accelerates hepatic fibrosis by sensitizing transforming growth factor-β1-induced inflammatory signaling. *Lab Invest* 2010; **90**: 1507-1516
79 **Murata M**, Matsuzaki K, Yoshida K, Sekimoto G, Tahashi Y, Mori S, Uemura Y, Sakaida N, Fujisawa J, Seki T, Kobayashi K, Yokote K, Koike K, Okazaki K. Hepatitis B virus X protein shifts human hepatic transforming growth factor (TGF)-beta signaling from tumor suppression to oncogenesis in early chronic hepatitis B. *Hepatology* 2009; **49**: 1203-1217
80 **Whittaker S**, Marais R, Zhu AX. The role of signaling pathways in the development and treatment of hepatocellular carcinoma. *Oncogene* 2010; **29**: 4989-5005
81 **Ou DL**, Shen YC, Liang JD, Liou JY, Yu SL, Fan HH, Wang DS, Lu YS, Hsu C, Cheng AL. Induction of Bim expression contributes to the antitumor synergy between sorafenib and mitogen-activated protein kinase/extracellular signal-regulated kinase kinase inhibitor CI-1040 in hepatocellular carcinoma. *Clin Cancer Res* 2009; **15**: 5820-5828
82 **Kummee P**, Tangkijvanich P, Poovorawan Y, Hirankarn N. Association of HLA-DRB1*13 and TNF-alpha gene polymorphisms with clearance of chronic hepatitis B infection and risk of hepatocellular carcinoma in Thai population. *J Viral Hepat* 2007; **14**: 841-848
83 **Migita K**, Miyazoe S, Maeda Y, Daikoku M, Abiru S, Ueki T, Yano K, Nagaoka S, Matsumoto T, Nakao K, Hamasaki K, Yatsuhashi H, Ishibashi H, Eguchi K. Cytokine gene polymorphisms in Japanese patients with hepatitis B virus infection--association between TGF-beta1 polymorphisms and hepatocellular carcinoma. *J Hepatol* 2005; **42**: 505-510
84 **Chen CC**, Yang SY, Liu CJ, Lin CL, Liaw YF, Lin SM, Lee SD, Chen PJ, Chen CJ, Yu MW. Association of cytokine and DNA repair gene polymorphisms with hepatitis B-related hepatocellular carcinoma. *Int J Epidemiol* 2005; **34**: 1310-1318
85 **Kim YS**, Cheong JY, Cho SW, Lee KM, Hwang JC, Oh B, Kimm K, Lee JA, Park BL, Cheong HS, Shin HD, Kim JH. A functional SNP of the Interleukin-18 gene is associated with the presence of hepatocellular carcinoma in hepatitis B virus-infected patients. *Dig Dis Sci* 2009; **54**: 2722-2728
86 **Liu L**, Xu Y, Liu Z, Chen J, Zhang Y, Zhu J, Liu J, Liu S, Ji G, Shi H, Shen H, Hu Z. IL12 polymorphisms, HBV infection and risk of hepatocellular carcinoma in a high-risk Chinese population. *Int J Cancer* 2011; **128**: 1692-1696
87 **Zhang H**, Zhai Y, Hu Z, Wu C, Qian J, Jia W, Ma F, Huang W, Yu L, Yue W, Wang Z, Li P, Zhang Y, Liang R, Wei Z, Cui Y, Xie W, Cai M, Yu X, Yuan Y, Xia X, Zhang X, Yang H, Qiu W, Yang J, Gong F, Chen M, Shen H, Lin D, Zeng YX, He F, Zhou G. Genome-wide association study identifies 1p36.22 as a new susceptibility locus for hepatocellular carcinoma in chronic hepatitis B virus carriers. *Nat Genet* 2010; **42**: 755-758
88 **He Y**, Zhang H, Yin J, Xie J, Tan X, Liu S, Zhang Q, Li C, Zhao J, Wang H, Cao G. IkappaBalpha gene promoter polymorphisms are associated with hepatocarcinogenesis in patients infected with hepatitis B virus genotype C. *Carcinogenesis* 2009; **30**: 1916-1922
89 **Yu MW**, Chang HC, Liaw YF, Lin SM, Lee SD, Liu CJ, Chen PJ, Hsiao TJ, Lee PH, Chen CJ. Familial risk of hepatocellular carcinoma among chronic hepatitis B carriers and their relatives. *J Natl Cancer Inst* 2000; **92**: 1159-1164
90 **Hassan MM**, Spitz MR, Thomas MB, Curley SA, Patt YZ, Vauthey JN, Glover KY, Kaseb A, Lozano RD, El-Deeb AS, Nguyen NT, Wei SH, Chan W, Abbruzzese JL, Li D. The association of family history of liver cancer with hepatocellular carcinoma: a case-control study in the United States. *J Hepatol* 2009; **50**: 334-341
91 **Bravi F**, Bosetti C, Tavani A, Bagnardi V, Gallus S, Negri E, Franceschi S, La Vecchia C. Coffee drinking and hepatocellular carcinoma risk: a meta-analysis. *Hepatology* 2007; **46**: 430-435
92 **Li Y**, Chang SC, Goldstein BY, Scheider WL, Cai L, You NC, Tarleton HP, Ding B, Zhao J, Wu M, Jiang Q, Yu S, Rao J, Lu QY, Zhang ZF, Mu L. Green tea consumption, inflammation and the risk of primary hepatocellular carcinoma in a Chi-

nese population. *Cancer Epidemiol* 2011; **35**: 362-368
93 **Yuen MF**, Yuan HJ, Wong DK, Yuen JC, Wong WM, Chan AO, Wong BC, Lai KC, Lai CL. Prognostic determinants for chronic hepatitis B in Asians: therapeutic implications. *Gut* 2005; **54**: 1610-1614
94 **Ishikawa T**. Clinical features of hepatitis B virus-related hepatocellular carcinoma. *World J Gastroenterol* 2010; **16**: 2463-2467
95 **Chen CH**, Hung CH, Lee CM, Hu TH, Wang JH, Wang JC, Lu SN, Changchien CS. Pre-S deletion and complex mutations of hepatitis B virus related to advanced liver disease in HBeAg-negative patients. *Gastroenterology* 2007; **133**: 1466-1474
96 **Wong VW**, Chan SL, Mo F, Chan TC, Loong HH, Wong GL, Lui YY, Chan AT, Sung JJ, Yeo W, Chan HL, Mok TS. Clinical scoring system to predict hepatocellular carcinoma in chronic hepatitis B carriers. *J Clin Oncol* 2010; **28**: 1660-1665
97 **Yuen MF**, Tanaka Y, Fong DY, Fung J, Wong DK, Yuen JC, But DY, Chan AO, Wong BC, Mizokami M, Lai CL. Independent risk factors and predictive score for the development of hepatocellular carcinoma in chronic hepatitis B. *J Hepatol* 2009; **50**: 80-88
98 **Ohkubo K**, Kato Y, Ichikawa T, Kajiya Y, Takeda Y, Higashi S, Hamasaki K, Nakao K, Nakata K, Eguchi K. Viral load is a significant prognostic factor for hepatitis B virus-associated hepatocellular carcinoma. *Cancer* 2002; **94**: 2663-2668
99 **Jang JW**, Choi JY, Bae SH, Yoon SK, Woo HY, Chang UI, Kim CW, Nam SW, Cho SH, Yang JM, Lee CD. The impact of hepatitis B viral load on recurrence after complete necrosis in patients with hepatocellular carcinoma who receive transarterial chemolipiodolization: implications for viral suppression to reduce the risk of cancer recurrence. *Cancer* 2007; **110**: 1760-1767
100 **Hung IF**, Poon RT, Lai CL, Fung J, Fan ST, Yuen MF. Recurrence of hepatitis B-related hepatocellular carcinoma is associated with high viral load at the time of resection. *Am J Gastroenterol* 2008; **103**: 1663-1673
101 **Yeh CT**, So M, Ng J, Yang HW, Chang ML, Lai MW, Chen TC, Lin CY, Yeh TS, Lee WC. Hepatitis B virus-DNA level and basal core promoter A1762T/G1764A mutation in liver tissue independently predict postoperative survival in hepatocellular carcinoma. *Hepatology* 2010; **52**: 1922-1933
102 **Kuzuya T**, Katano Y, Kumada T, Toyoda H, Nakano I, Hirooka Y, Itoh A, Ishigami M, Hayashi K, Honda T, Goto H. Efficacy of antiviral therapy with lamivudine after initial treatment for hepatitis B virus-related hepatocellular carcinoma. *J Gastroenterol Hepatol* 2007; **22**: 1929-1935
103 **Qu LS**, Jin F, Huang XW, Shen XZ. Interferon-α therapy after curative resection prevents early recurrence and improves survival in patients with hepatitis B virus-related hepatocellular carcinoma. *J Surg Oncol* 2010; **102**: 796-801
104 **Liang TJ**, Mok KT, Liu SI, Huang SF, Chou NH, Tsai CC, Chen IS, Yeh MH, Chen YC, Wang BW. Hepatitis B genotype C correlated with poor surgical outcomes for hepatocellular carcinoma. *J Am Coll Surg* 2010; **211**: 580-586
105 **Zhu Y,** Jin Y, Cai X, Bai X, Chen M, Chen T, Wang J, Qian G, Gu J, Li J, Tu H. Hepatitis B virus core protein variations differ in tumor and adjacent nontumor tissues from patients with hepatocellular carcinoma. *Intervirology* 2011 Feb 16; Epub ahead of print
106 **Chew V**, Tow C, Teo M, Wong HL, Chan J, Gehring A, Loh M, Bolze A, Quek R, Lee VK, Lee KH, Abastado JP, Toh HC, Nardin A. Inflammatory tumour microenvironment is associated with superior survival in hepatocellular carcinoma patients. *J Hepatol* 2010; **52**: 370-379
107 **Gao Q**, Qiu SJ, Fan J, Zhou J, Wang XY, Xiao YS, Xu Y, Li YW, Tang ZY. Intratumoral balance of regulatory and cytotoxic T cells is associated with prognosis of hepatocellular carcinoma after resection. *J Clin Oncol* 2007; **25**: 2586-2593
108 **Zhou H**, Huang H, Shi J, Zhao Y, Dong Q, Jia H, Liu Y, Ye Q, Sun H, Zhu X, Fu L, Guo K, Gao D, Sun J, Yan Z, Ren N, Tang Z, Qin L. Prognostic value of interleukin 2 and interleukin 15 in peritumoral hepatic tissues for patients with hepatitis B-related hepatocellular carcinoma after curative resection. *Gut* 2010; **59**: 1699-1708
109 **Zhu XD**, Zhang JB, Zhuang PY, Zhu HG, Zhang W, Xiong YQ, Wu WZ, Wang L, Tang ZY, Sun HC. High expression of macrophage colony-stimulating factor in peritumoral liver tissue is associated with poor survival after curative resection of hepatocellular carcinoma. *J Clin Oncol* 2008; **26**: 2707-2716
110 **Lee HH**, Uen YH, Tian YF, Sun CS, Sheu MJ, Kuo HT, Koay LB, Lin CY, Tzeng CC, Cheng CJ, Tang LY, Tsai SL, Wang AH. Wnt-1 protein as a prognostic biomarker for hepatitis B-related and hepatitis C-related hepatocellular carcinoma after surgery. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 1562-1569
111 **Ding ZB**, Shi YH, Zhou J, Shi GM, Ke AW, Qiu SJ, Wang XY, Dai Z, Xu Y, Fan J. Liver-intestine cadherin predicts microvascular invasion and poor prognosis of hepatitis B virus-positive hepatocellular carcinoma. *Cancer* 2009; **115**: 4753-4765
112 **Liu J**, Ahiekpor A, Li L, Li X, Arbuthnot P, Kew M, Feitelson MA. Increased expression of ErbB-2 in liver is associated with hepatitis B x antigen and shorter survival in patients with liver cancer. *Int J Cancer* 2009; **125**: 1894-1901
113 **He YF**, Jin J, Wei W, Chang Y, Hu B, Ji CS, Jia WD, Wang XQ, Chen K, Chen J. Overexpression of cyclooxygenase-2 in noncancerous liver tissue increases the postoperative recurrence of hepatocellular carcinoma in patients with hepatitis B virus-related cirrhosis. *Can J Gastroenterol* 2010; **24**: 435-440
114 **Bala S**, Marcos M, Szabo G. Emerging role of microRNAs in liver diseases. *World J Gastroenterol* 2009; **15**: 5633-5640
115 **Li LM**, Hu ZB, Zhou ZX, Chen X, Liu FY, Zhang JF, Shen HB, Zhang CY, Zen K. Serum microRNA profiles serve as novel biomarkers for HBV infection and diagnosis of HBV-positive hepatocarcinoma. *Cancer Res* 2010; **70**: 9798-9807
116 **Hou J**, Lin L, Zhou W, Wang Z, Ding G, Dong Q, Qin L, Wu X, Zheng Y, Yang Y, Tian W, Zhang Q, Wang C, Zhang Q, Zhuang SM, Zheng L, Liang A, Tao W, Cao X. Identification of miRNomes in human liver and hepatocellular carcinoma reveals miR-199a/b-3p as therapeutic target for hepatocellular carcinoma. *Cancer Cell* 2011; **19**: 232-243
117 **Nathan H**, Schulick RD, Choti MA, Pawlik TM. Predictors of survival after resection of early hepatocellular carcinoma. *Ann Surg* 2009; **249**: 799-805
118 **Hsu CY**, Hsia CY, Huang YH, Su CW, Lin HC, Lee PC, Loong CC, Chiang JH, Huo TI, Lee SD. Selecting an optimal staging system for hepatocellular carcinoma: comparison of 5 currently used prognostic models. *Cancer* 2010; **116**: 3006-3014

S- Editor Tian L **L- Editor** Kerr C **E- Editor** Li JY