Hepatocellular Carcinoma Associated with Hepatitis B Virus

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Contents

16.1 Introduction

Hepatocellular carcinoma (HCC) is ranked as the fifth most common malignant neoplasm in the world [1], and the third most common cause of cancer death worldwide [2]. HCC's global incidence is approximately 600,000 new cases annually, almost 85 % of these in developing countries. In fact, it is the third most commonly diagnosed cancer among males in developing countries and the second leading cause of death among that population. The vast majority of deaths from HCC occur in East Asia, and 50 % are estimated to occur in China alone. Current data indicate that hepatitis B virus (HBV) and hepatitis C virus (HCV) are the most significant hepatocarcinogens for the majority of HCCs in the world [3, 4]. Although less common in developed countries, it is still a major cause of morbidity and mortality. Globally, about 80 % of HCC is considered to be causally associated with chronic infection with HBV [5, 6].

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16.2 Hepatitis B Virus

16.2.1 Background

HBV is a double-stranded DNA virus belonging to the Hepadnaviridae (hepatotropic DNA virus) family, and is classified as hepadnavirus type 1. The intact virus consists of an outer coat component of hepatitis B surface antigen (HBsAg) and an inner core component of hepatitis B core antigen (HBcAg) [7–9]. Hepatitis B e antigen (HBeAg), that is also a product of the C gene, circulates in the blood during periods of high replication [10, 11].

The hepatitis B viral genome is approximately 3200 base pairs in length, is partially double-stranded, and uses a retroviral mode of replication [12, 13]. The viral genome contains genes that code for HBsAg, HBcAg, and DNA polymerase [9, 14]. An additional X gene codes for hepatitis B x antigen (HBx), a protein that is capable of transactivating the transcription of both viral and host genes [15, 16].

HBV predominantly infects hepatocytes, but reservoirs of the virus were found in extra hepatic sites including lymph nodes, bone marrow and circulating lymphocytes, explaining recurrence after liver transplantation [17, 18]. Infection of hepatocytes is by specific binding of the envelope viral protein (specifically the preS1 domain) to a bile salt transporter sodium taurocholate co-transporting polypeptide (NTCP) [19].

Eight genotypes of HBV have been identified (A–H), classified by the subtype-specific antigens on the HBsAg, and their distribution varies geographically. Genotype A is more prevalent in Europe, North America, and Africa, while genotypes B and C are dominant in China and East Asia, where vertical transmission is more common [20, 21]. Genotype D is found most commonly in Europe and

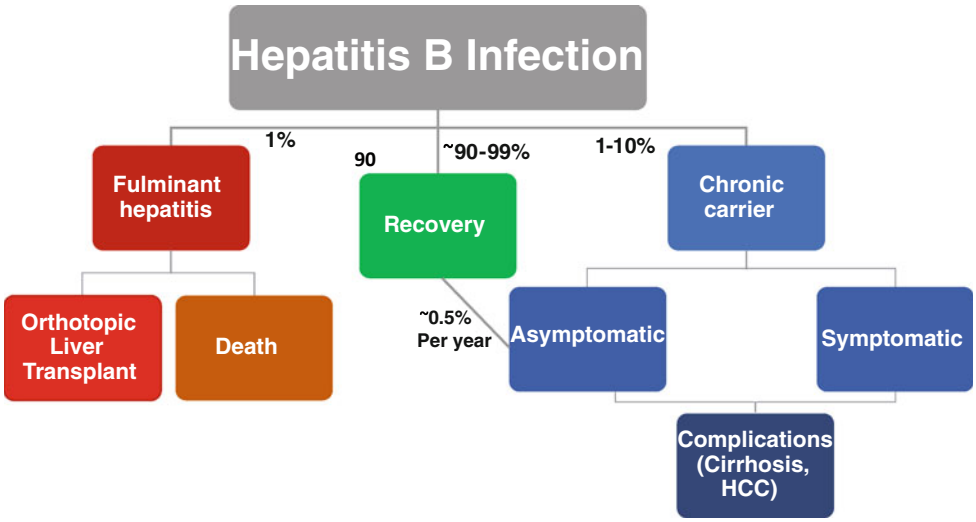

Fig. 16.1 Natural history of HBV infection

Mediterranean countries, genotype E predominates in West Africa, and Genotype F in Central and South America. The specific HBV genotype is associated with clinical characteristics such as disease progression and response to interferon therapy. Genotype B, for instance, appears to be associated with a less rapidly progressive liver disease and a lower likelihood or delayed appearance of HCC [22–25], as opposed to genotype C [26], while patients with genotype A are more likely to respond to interferon therapy [27, 28].

HBV is carried in blood as well as other body fluids. The main routes of transmission are sexual intercourse, perinatal transmission, and parenteral exposure. Perinatal transmission occurs from chronically infected mothers or during acute infection at the third trimester or early postpartum, and is more common in developing countries. The precise mode of perinatal transmission is unknown but most probably occurs at the time of delivery. Risk for infection correlates with viral activity, as 85 % of HBsAg-positive mothers who are HBeAg-positive will transmit the virus to their offspring, whereas mothers who are positive for anti-HBe do so much less frequently (31 %) [29]. Additionally, maternal HBsAg titers correlate with the risk for transmission [30].

The natural history of hepatitis B infection differs by the age of acquisition of the infection. Nearly 90 % of exposed newborns will become chronic carriers, compared to 50 % during infancy and 20 % during early childhood [11, 31–33]. Among healthy adults exposed to HBV infection, 90–99 % have a full recovery, 0.1–1 % develop acute fulminant hepatitis and 1–10 % become chronic carriers (Fig. 16.1). In chronic carriers, the rate of spontaneous HBsAg clearance is approximately 0.5 % per year [34–36].

The natural evolvement of chronic infection can be divided into four phases—immune tolerant phase, characterized by HBeAg (+), high levels of HBV DNA, normal serum aminotransferases and minimal or no inflammation on liver biopsy; immune active/clearance phase, which manifests with elevated serum aminotransferases and active inflammation on liver biopsy; low-replicative phase (inactive carrier), with seroconversion from HBeAg (−) to anti-HBe (+), low serum HBV DNA and normal aminotransferase levels, and finally the HBeAg (−) hepatitis phase (HBV reactivation) which presents high serum HBV DNA and active inflammation [33, 37]. Seroconversion from HBeAg to anti-HBe rates differ by patient age and are approximately 10 % per year for adults but <5 % for patients with perinatally acquired infection [33, 34].

Although chronic HBV carriers have been infected for extended periods of time, most do not have symptoms. Many patients are found to have chronic hepatitis B incidentally during routine screening. Among 139 incidentally identified HBsAg (+) Korean Americans, 11 % were found to have cirrhosis and 42 % to have active hepatitis on complete evaluation including liver imaging studies and liver functions [38].

15–30 % of chronic hepatitis B patients will develop serious sequelae including HCC during their lifetimes. Fortunately, the lengthy interval between the infection and the development of HCC provides an advantage for clinicians to intervene and delay the progression of the disease.

16.2.2 HBV Epidemiology

Hepatitis B is a common infection worldwide, with approximately one third of the world population having serological evidence of past/present infection. At least 350–400 million are chronic HBV carriers worldwide [4] and globally, there are around 4.5 million new infections per year [39].

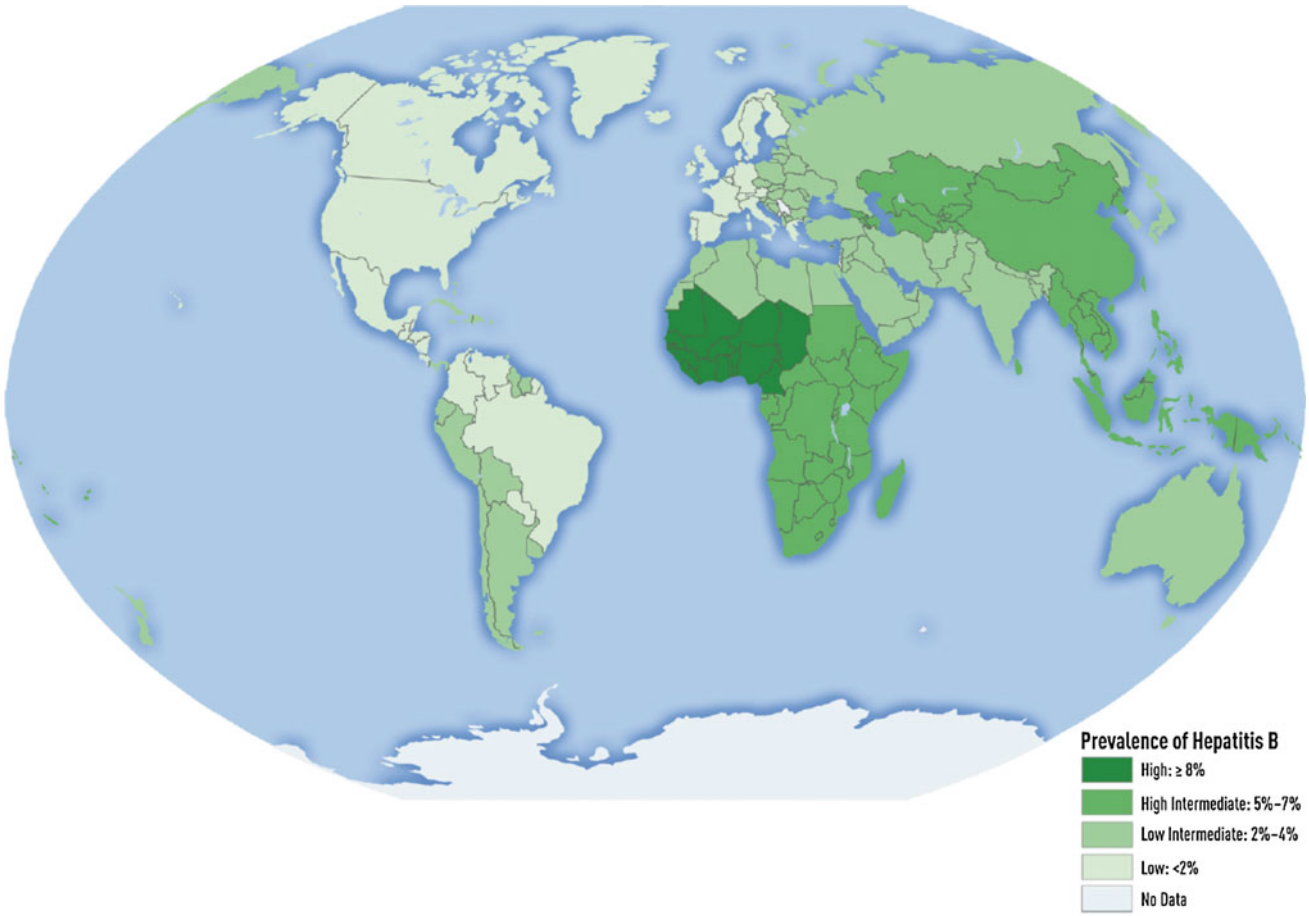

Fig. 16.2 Prevalence of chronic hepatitis B virus infection among adults

The prevalence of hepatitis B differs by geographical region, and countries are divided into high (>8 % prevalence), intermediate (2–7 %) and low (<2 %) endemicity for the virus (Fig. 16.2). Areas considered highly endemic include central and southeast Asia and sub-Saharan Africa. Regions of intermediate prevalence include parts of Southern and Eastern Europe, the Middle East, Japan, the Indian subcontinent, much of the former Soviet Union, and Northern Africa. Regions of low prevalence include North America, Western Europe, certain parts of South America, and Australia [40, 41].

The skewed distribution of HBV infection is most likely due to the different modes of transmission. In the hyperendemic regions, perinatal transmission and horizontal spread among children are the major sources of infection. On the other hand, in the low endemic regions, horizontal transmission through sexual activity among young adults and parenteral exposure are the major modes of transmission [31].

16.2.3 Treatments for HBV

Management of chronic hepatitis B is aimed at HBsAg loss and decrease of active virus replication. Treatment for HBV has come a long way in the past decades, changing the prognosis of chronic hepatitis B carriers. Currently, there are seven FDA-approved antiviral therapies, six of them used routinely (interferon treatment was replaced by pegylated interferon) (see Table 16.1).

Interferon-α was the first approved treatment for chronic hepatitis B. In IFN responders, a hepatitis-like flare, presumably due to immune activation, accompanies HBeAg seroconversion. Nowadays, pegylated-IFN is used in clinical practice. This drug is given by injection weekly, and is more effective as well as more convenient for patients compared to regular IFN. Despite its proven efficacy it is relatively poorly tolerated because of its side effect profile and mode of administration. HBeAg seroconversion after 1 year is achieved in ~30 % of patients.

Table 16.1 Antiviral agents currently in use for HBV therapy in the U.S.

Drug	FDA approval
Interferon (IFN)	
(regular) IFN-α	1992
Pegylated interferon	2005
Nucleoside analogues (*NUCs*)	
Lamivudine	1998
Adefovir	2002
Entecavir	2005
Telbivudine	2006
Tenofovir	2008

Nucleoside analogues (NUC) are oral medications that suppress viral replication by inhibiting reverse-transcriptase activity. They are well tolerated and effective in viral suppression. HBeAg seroconversion after 1 year is achieved in ~20 %, 30 % after 2 years, and 50 % after 5 years. One of the major problems of NUCs is development of resistance, which can be combatted by adding on or switching to a different NUC.

16.3 HBV and HCC

Half of the world's population lives in the regions of high incidence for HCC, which also coincide with endemic regions for HBV infection. Worldwide, HBV accounts for 54 % of HCC cases, and in Asia and Africa it accounts for 70 % of cases [42].

A large number of epidemiologic studies [43–47] documented the causal association of HCC with HBV. In 1981, the landmark study by Beasley et al. [48] lucidly demonstrated the relationship between HBV and HCC.

During the past decades, the incidence of HCC has decreased in some areas in East Asia such as in Taiwan, Korea and Thailand, as well as other highly endemic areas such as Alaska [49–53]. It is believed that effective HBV vaccination programs have contributed to the reduction (see below). However, the opposite phenomenon was reported in some countries in Europe, North America and Oceania [6, 54]. This increasing incidence of HCC is attributed to HCV and non-alcoholic fatty liver disease (NAFLD)-associated cirrhosis and also the immigration of HBV carriers from endemic regions.

Increasing incidence of HCC in the United States is clearly illustrated by the HCC incidence in California, the state that has the largest Asian American population. As shown in Table 16.2, liver cancer is one of the five most common cancers for Asian American males. This high incidence of HCC among Asian Americans is attributed to the high prevalence rate of HBV infection among them [55]. Although Asian Americans constitute only 4.5 % of the total USA population, they constitute nearly half of the total HBV chronic carriers in the U.S.A [55–57].

Asian immigrants in the U.S.A show high HBV carrier rates reminiscent of their homelands, and accordingly have high risk for sequelae of chronic hepatitis B infection, such as cirrhosis and HCC. In fact, the HBV carrier rate among first generation immigrants is similar to that of people living in their native lands [38, 58, 59].

Currently, the estimated prevalence rate of chronic HBV carriers ranges between 5–13 % among Asian Americans. In contrast, HBV carrier rate for the general U.S. population is less than 0.3 % [60].

Tong and Hwang conducted a prospective study of 207 HBsAg (+) Asian American patients with chronic hepatitis [55]. During an average follow-up period of 3.3 years, eight patients developed HCC; the calculated incidence of HCC in these Asian American patients with chronic hepatitis B was 3865/100,000. This is much higher than those reported in Taiwan by Beasley (495/100,000) [3] and those by Liaw et al. [61] (826/100,000 for all ages and 2768/100,000 for patients older than 35 years of age). Nonetheless, it is important to point out that Beasley followed asymptomatic carriers, and Tong et al. and Liaw et al. followed patients with active hepatitis. Most HCC occur in patients that are between 40 and 60 years of age [5] although there are some exceptions including childhood HCC described in Taiwan [62] and in native Americans in Alaska [53].

16.3.1 Pathogenesis of HCC in HBV

HCC is strongly associated with chronic liver disease, and is uncommon in the absence of inflammation and fibrosis [63]. Hepatic carcinogenesis is a long term, multistage disease process encompassing multiple genetic alterations, including activation of cellular oncogenes and inactivation of tumor suppressor genes [64, 65].

16.3.1.1 Risk Factors for the development of HCC in Chronic HBV carriers

HBV infection carries an increased risk of developing HCC. Several risk factors confer higher risk in HBV carriers (Table 16.3).

Although HBV-associated HCC does not always progress through cirrhosis [66], patients with cirrhosis are at the highest risk for developing HCC. Earlier studies in Japan found that prevalence of overt cirrhosis among patients with HBV-related HCC was 50–60 % [67, 68], but a later study by Yang et al. [69] found that most patients with HBV-related HCC have evidence of cirrhosis (73.4 % when using stringent clinical criteria and 93.8 % using the most inclusive criteria). Interestingly, all Caucasian patients had cirrhosis, compared

Table 16.2 Five most common cancers in males by race/ethnicity, California, 2007–2011

	Rank				
	1	2	3	4	5
Asians					
Laotians	**Liver**	Lung	Colorectal	Prostate	Stomach
Vietnamese	Lung	**Liver**	Prostate	Colorectal	Lymphoma
Chinese	Prostate	Lung	Colorectal	**Liver**	Lymphoma
Korean	Prostate	Colorectal	Lung	Stomach	**Liver**
Filipino	Prostate	Lung	Colorectal	Lymphoma	**Liver**
Non-Asians					
White	Prostate	Lung	Colorectal	Melanoma	Bladder
Hispanic	Prostate	Colorectal	Lung	Lymphoma	Kidney
Black	Prostate	Lung	Colorectal	Kidney	Bladder
American-Indian/Alaska native	Prostate	Lung	Colorectal	**Liver**	Kidney

California Cancer Facts and Figures 2014, American Cancer Society [222]
Bold represents liver cancer

Table 16.3 Risk factors of HCC among HBV carriers

Host-related
Cirrhosis
Age >40 years
High-endemicity areas
Male sex
Serum ferritin >300 ng/ml
Alcoholism
Family history of HBV infection
Viral-related
Genotype
↑ Serum HBV DNA

to a smaller proportion of Asian patients. A systematic review by Fattovich et al. [70] estimated the rates of HCC in East-Asian countries among inactive carriers (HBsAg (+), anti-HBe (+), normal ALT) to be 0.5 per 100 person years, 0.6 per person years in patients with chronic hepatitis but without cirrhosis, and 3.7 per 100 person years in patients with compensated cirrhosis. Importantly, rates were lower in developed countries—0.02 per 100 person years in inactive carriers, 0.3 per person years in patients with chronic hepatitis but without cirrhosis, and 2.2 per 100 person years in patients with compensated cirrhosis.

Earlier age at infection with HBV is associated with higher risk for HCC [71], in correlation with evidence suggesting that it takes 20–40 years to develop HCC from the time of infection [48]. Patients from areas of high HBV endemicity are at higher risk for HCC, presumably because they were likely infected early in life and thus had a longer duration of chronic infection or cirrhosis [63, 70].

Family history of HBV infection has also been found to be a unique risk factor for early onset of HCC [72]. Males have a higher incidence rate for HCC among HBV carriers with a 4:1 male to female ratio [2, 73]. The biologic basis for the gender difference in the risk for HCC is not well understood; however, male hormones [74], differences in body iron storage [75], and additional risks, such as alcohol consumption [76, 77] and smoking [77, 78], have been considered to be contributory factors. Older age is also considered a risk factor, since HCC occurs most commonly later in life [5].

Iron has long been considered a factor contributing to hepatic damage and inflammation via generation of reactive oxygen species (ROS). A sustained serum ferritin level greater than 300 ng/ml was shown to confer a higher risk for HCC. In a longitudinal follow-up study of 249 Korean patients with chronic hepatitis B and cirrhosis, Hann et al. [75] observed that chronic hepatitis B infected males with sustained serum ferritin >300 ng/ml had a 50 % chance of developing HCC compared with 20 % risk for HCC for those with lower serum ferritin levels. Further studies by the same group clearly demonstrated the tumor enhancing effects of iron [79–82].

Multiple studies have shown that increased viral activity [83] and a persistently high level of viral DNA is a strong predictor for HCC development [84–86]. Chen et al. conducted a large-scale longitudinal study of 3653 HBV carriers. During the 12-year follow-up period, 164 persons developed HCC. Their extensive analysis led to the conclusion that the most important risk factor for the development of HCC is an increased serum level of HBV DNA >10,000 copies/ml regardless of the HBeAg status, alanine aminotransferase (ALT) levels or the presence of cirrhosis. The incidence of HCC correlated with serum HBV DNA

level at entry in a dose-response relationship ranging from 108/100,000 person years for an HBV DNA level of <300 copies/ml to 1152/100,000 person years for an HBV DNA level of $\geq$1,000,000 copies/ml.

16.3.1.2 Molecular Biology of HCC associated with HBV

Recent advances in molecular techniques have markedly improved our understanding of HBV-associated hepatic carcinogenesis. The effects of HBV on HCC development can be divided to direct oncogenic mechanisms and indirect effects, via chronic liver inflammation and cirrhosis [87, 88].

Many of the pathogenic mechanisms of the virus are mediated by hepatitis B x protein (HBx), which is a small 154 amino acid protein with transcriptional regulatory activity [89]. The important role of HBx in HBV infectivity was realized when woodchucks injected with HBx-deficient viruses did not develop infection [90, 91]. Furthermore, HBx expression is abundant in the livers of patients with chronic liver disease [92, 93], and expression levels correlate with progression of liver inflammation and cirrhosis [94]. There is accumulating evidence that HBx is important in supporting virus replication and in the pathogenesis of chronic inflammation and HCC [95]. HBx augments viral replication and thus, by maintaining high viral DNA levels it contributes to hepatic carcinogenesis [96].

Hepatitis B and Fibrosis

A salient aspect of chronic liver disease is the development of fibrosis. There is increasing evidence that HBx expressing hepatocytes contribute to pro-fibrotic signaling. For example, HBx has been shown to up-regulate the expression of transforming growth factor beta 1 (TGF-β1) in HBx transgenic mice [97] and in liver cell cultures stably transfected with HBx [98]. In the normal liver, TGF-β1 signals through a group of proteins known as Smads, which inhibit hepatocellular growth and maintain homeostasis [99]. TGF-β1 has long been implicated in promoting fibrosis via activation of hepatic stellate cells, transforming them into myofibroblasts [100]. Additionally, in the presence of HBx, Smad protein transcriptional activity was enhanced, especially in activation of genes involved in extracellular matrix (ECM) production [101]. Besides altering Smad signaling directly, HBx activates other signaling molecules, such as NF-κβ, PI3K, AP-1, and ras/raf/MAPK, among others [102, 103] that override negative growth regulation. Importantly, up-regulated expression of TFG-β1 stimulates expression of platelet derived growth factor (PDGF), constitutively activating β-catenin, which may act as an oncoprotein [104].

Immune Response to HBV Infection and Necro-inflammation

HBV is a non-cytopathic virus [105]. Damage to infected hepatocytes is in large part immune-mediated, mostly via CD8+ cytotoxic T cells [106]. HBV clearance is regulated by the adaptive immune system, with CD4+ and CD8+ T cells mediating immune clearance and B cells supplying protective immunity by generation of neutralizing antibodies [107, 108]. In those patients with chronic infection, immune response is inadequate for viral clearance but still causes liver injury [109]. The factors governing immune clearance versus immune tolerance are not fully understood. One suspected mechanism in chronic infection is faulty modulation of regulatory T cells, which causes down regulation of T cell cytotoxicity [110–112]. Additionally, HBV disrupts toll-like receptor (TLR) signaling, disrupting the response of the innate immune system [113]. Another suggested mechanism of immune-resistance is by up-regulation of URG7 (up-regulated gene, clone 7) via HBx *trans*-activation, which was shown to confer resistance to Fas and tumor necrosis factor alpha (TNFα) mediated apoptosis [114, 115]. Further analysis showed that URG7 blocked apoptotic signals at the level of caspase 8, which is shared by Fas and TNFα signaling pathways.

In the presence of persistent infection and a chronic but ineffective immune response, the liver's unique regeneration ability causes repeated compensatory proliferation, eventually leading to cirrhosis and HCC [116].

Direct Oncogenic Effects of HBV and HBV Genome Integration

As mentioned above, patients with chronic hepatitis B are at risk for HCC even in the absence of cirrhosis [66], thus presenting the direct tumorogenic effects of the virus [117].

HBx was shown to localize to mitochondria [118], where it triggers oxidative stress [119] and production of ROS. Generation of ROS causes endoplasmic reticulum stress, which in turn compromises protein-folding ability leading to apoptosis and liver damage.

There is evidence that HBx modulates the integrity of ECM by stimulating expression of selected matrix metalloproteinases and tissue inhibitors of metalloproteinases that are capable of breaking down ECM, thereby promoting metastasis during tumor progression [120–124].

Another direct oncogenic mechanism of HBV affects regulation of cell growth. During chronic infection, fragments of HBV DNA integrate into the human genome at multiple sites [125, 126]. HBV DNA integration can also occur in occult infection [127]. Most of these integrated fragments span the HBx gene of HBV [128, 129].

HCC was shown to harbor mutations that may affect multiple cellular processes. These include inactivating tumor suppressor pathway components, activating oncogenes, and/or blocking DNA repair.

For example, p53 is a tumor suppressor gene that is activated during cellular stress to allow cell cycle arrest and initiation of DNA repair mechanisms [130]. It was shown that HBx binds to and functionally inactivates p53 [131–133], thus blocking p53 dependent transcription coupled repair [132] and

inhibiting nucleotide excision repair [134]. Disruption of cellular repair mechanisms leads to the accumulation of mutations, with those commonly found in HCC including inactivating point mutations of p53 [135, 136] and activating point mutations in β-catenin [137] which then acts as an oncogene in HCC. HBx is also implicated in constitutively activating other genes that appear to contribute to multi-step hepatocarcinogenesis, such as those encoding cyclin D1 [138, 139], URG4 [140] and URG11 [141]. Additionally, HBx down-regulates transcription of $p21^{WAF1/SDI1/CIP1}$ [142], a senescence factor that also inhibits cell cycle progression. Finally, HBx has been shown to overcome RAS oncogene induced senescence [143]. An emerging mechanism in HBV pathogenesis, including carcinogenesis, is by microRNAs (miRNA) [144]. HBV-related HCC cells have decreased expression of miRNAs that are known to regulate genes related to cell death, and increased expression of miRNAs that down-regulate inflammation [145].

HBx also has epigenetic effects on gene expression in liver cells. HBx activates expression of DNA methyltransferase 1 (DNMT1), in addition to other DNMTs, resulting in altered DNA methylation patterns in the chronically infected liver and in HCC [146, 147]. In this context, HBx activation of DNMT1 has been shown to promote hypermethylation of the promoter encoding E-cadherin, effectively suppressing E-cadherin expression [148, 149]. Since E-cadherin is an important cell adhesion molecule, loss of E-cadherin resulted in enhanced cell migration in vitro and enhanced metastasis in vivo, thereby promoting tumor progression.

In a study by Boyault et al. [64] HCC's were classified to 6 groups (G1-6) according to transcriptome analysis. HBV-related HCC's were molecularly distinct from other HCC's, and were classified in groups G1-2. Clinically, G1 tumors were characterized by low HBV DNA levels, high serum α-fetoprotein (AFP), younger age and African origin. Frequent AXIN1 mutations were also seen. These tumors had genes expressed during development. G2 tumors were related to HBV infection with high HBV DNA levels, and frequent local and vascular invasion. Additionally there were frequent mutations in p53. Both G1 and G2 tumors had AKT pathway activation, in G1 via over expression of insulin growth factor 2 (IGF2) and in G2 via PIK3CA mutations.

A later study by Amaddeo et al. [150] further characterized genomics of HBV-related HCC's. A high frequency of p53 mutations was found compared to HCC's of other etiologies. Interestingly, more than 70 % of tumors harbored inactivation mutations in HBx gene, in contrast to non-tumor liver tissues. Among HBV-infected patients with additional risk factors, molecular characteristics were different, suggesting an alteration in carcinogenic mechanisms.

16.4 Prevention of HCC Related to HBV

As hepatitis B infection accounts for more than 50 % of all HCC cases worldwide [42], targeting HBV is an effective way to reduce global HCC burden. The targeted approach to prevent HBV-related HCC is aimed at 3 populations. Primary prevention in uninfected individuals, secondary prevention in chronic hepatitis B infected individuals, and tertiary prevention for HBV carriers who have already developed HCC [151].

16.4.1 Primary Prevention of HCC

Primary prevention of HCC aims to prevent HBV infection altogether among uninfected individuals, thereby reducing the risk for HCC development. This is accomplished by universal vaccination.

The first active vaccine was introduced in the 1980s [152], and was initially offered only to high-risk populations [153]. In 1991 the World Health Organization (WHO) recommended universal vaccinations in all countries [154, 155]. As of 2013, HBV vaccination is part of the vaccination schedule in 183 countries.

The impact of the universal vaccination plans on HCC development was significant [156, 157]. Initially, reduction of prevalence rates was seen in endemic countries, and later the effects on long-term morbidity and mortality were also documented. In Taiwan, where a universal vaccination plan was implemented in 1984, the prevalence of HBsAg among persons younger than 15 years decreased from 9.8 to 0.7 % after 15 years [158]. In Gambia, a study comparing HBV carrier status between vaccinated and unvaccinated 9-year-old children showed prevalence of 0.6 and 10 %, respectively [159]. Studies in China and Korea had similar findings [49, 160]. The benefits of universal vaccination programs were proven also in low or intermediate endemicity regions such as Italy [161].

In accordance with the decrease in chronic HBV carrier status, there has been a decrease in HCC prevalence following implementation of universal vaccination. Studies in Taiwan showed a decrease in incidence of HCC among children born after the implementation of the vaccination program compared to those born before, from 0.7 per 100,000 children to 0.36 [50, 51]. Similar findings were shown in studies in Korea, Thailand and Alaska [49, 52, 53], and in the latter elimination of HCC and acute Hepatitis B were achieved [162].

For unvaccinated patients who are exposed to hepatitis B, post-exposure prophylaxis is implemented using HBIG and the standard active vaccine.

16.4.2 Secondary Prevention of HCC

Secondary prevention of HCC is aimed to prevent HCC in the 400 million patients who are chronic HBV carriers. This is accomplished by effective antiviral treatment to reduce viral replication, thereby reducing the risk for HCC, and by surveillance programs to detect tumors at early stages when curative treatments are optional.

The first important step is to identify all HBV carriers. Current recommendations for groups who should be screened are summarized in Table 16.4 [163, 164].

16.4.2.1 Antiviral therapy

As mentioned above, one of the major risk factors for HCC in HBV carriers is a persistently high viral load. This suggests that treatment aimed to reduce viral load may decrease the risk for long-term sequelae, including HCC.

Current treatments to reduce viral replication include pegylated interferon, nucleotide (zide) (NUC) analogues and combination therapies. Studies that assessed the effect of IFN treatment on prevention of HCC yielded mixed results. Some showed significant reduction of HCC risk with IFN treatment [165–168] while others showed minimal or no effect [169–172]. One long-term, randomized controlled study reported treatment with natural lymphoblastoid interferon-alpha (IFN-αnl), recombinant IFN-α2a and placebo [173]. HCC was detected in 1.5 % of the IFN-αnl group, 3.7 % of the IFN-α2a group and 14.7 % of the control group ($p < 0.05$). In another long-term study that followed 411 chronic hepatitis B patients, of whom 208 were treated with IFN-α and 203 were controls, 4.3 and 1.0 % of patients in the IFN group and controls, respectively, developed complications of cirrhosis and HCC, but without statistically significant differences between the groups ($p = 0.062$) [169]. To overcome the variability in response seen in multiple small studies, a number of meta-analyses were performed. One such meta-analysis in 2001 did not show an effect for IFN in preventing HCC in several European studies [174]. In contrast, a few newer meta-analyses found significant risk reductions with IFN treatment [175–177]. Although some controversy remains, the overall conclusion is in favor of IFN treatment for viral suppression and reduction in HCC risk, in accordance with current treatment guidelines.

Table 16.4 Populations recommended for HBV screening

High and intermediate endemicity regions
All people
Low endemicity
Immigrants or adopted children from intermediate or high-endemicity regions
Household and sexual contacts of HBsAg-positive persons
Persons who have ever injected drugs
Persons with multiple sexual partners or history of sexually transmitted disease
Men who have sex with men
Inmates of correctional facilities
Individuals with chronically elevated ALT or AST
Individuals infected with HCV or HIV
Patients undergoing renal dialysis
All pregnant women
Persons needing immunosuppressive therapy

The majority of studies on NUC treatment have shown beneficial effects in prevention of HCC [178]. Prospective and retrospective studies of large numbers of chronic HBV patients with advanced liver disease have demonstrated that treatment with lamivudine (LAM) both delays disease progression and reduces HCC incidence. In a randomized controlled trial (RCT) by Liaw et al., 651 chronic hepatitis B patients with advanced fibrosis and cirrhosis were randomized to receive antiviral agents, LAM or placebo (2:1). Within 3 years, treatment with LAM not only delayed disease progression but also reduced the incidence of HCC [179].

Case-control studies demonstrated similar beneficial effects. Matsumoto et al. [180] in a retrospective study of 2795 individuals with chronic hepatitis B assessed the effectiveness of LAM in preventing HCC. 657 patients received LAM and the remaining 2138 served as controls. The mean follow-up period was 2.7 years for the LAM group and 5.3 years for the controls. Annual incidence of HCC in the LAM group was 0.4 %/patient/year compared to 2.5 %/patient/year in controls ($p < 0.001$). Yuen et al. [181] compared a group of HBeAg (+) individuals without cirrhosis treated with LAM to controls, with significantly lower rates of HCC and cirrhosis among the LAM-treated participants. In another study by Eun et al. [182] 872 chronic hepatitis B patients treated with LAM were compared to 699 historical controls that were not treated. The annual incidence of HCC was 0.95, 2.18, 5.26, and 4.10 % in patients with sustained viral suppression, viral breakthrough, suboptimal response, and the control group, respectively. A retrospective study from Greece compared 201 LAM-treated patients, of whom 79 of 109 without virological remission received adefovir as rescue therapy, with 209 patients treated with IFN-α and 195 untreated patients [183]. The liver-related survival in LAM-treated patients was significantly better compared with untreated patients or non-sustained responders to IFN-α, and similar compared with IFN-α sustained responders. Beneficial effect of LAM treatment was found in additional studies in Italy [184] and a recent study from Japan [185].

Newer NUCs show even more promising results. A large case-control study from Japan compared the incidence of HCC in entecavir (ETV)-treated patients, LAM-treated patients (with no rescue therapy) and non-treated historical

controls. The cumulative HCC incidence rates in cirrhotic patients at 5 years were 7, 22.2 and 38.9 %, respectively [186]. Additional studies comparing ETV-treated patients to untreated controls showed similar results [187, 188].

In a meta-analysis by Sung et al. [175] five studies comparing NUCs to placebo were analyzed. The use of NUCs (mostly with LAM, with some patients receiving adefovir rescue) reduced HCC development from 11.7 % in controls to 2.5 % in the treatment groups. In subgroup analysis the protective effect applied to patients with cirrhosis (3.9 % in treated patients and 22.4 % in controls), patients without cirrhosis (1.8 % in treated patients and 8 % in controls), and patients with drug resistance (3.3 % in treated patients and 6.4 % in controls).

It is important to stress that although antiviral treatment reduces the incidence of HCC, the risk is not completely eliminated even in the face of adequate viral suppression and undetectable viral DNA [189–191].

16.4.2.2 Surveillance for HCC

Patients with HCC usually present at late stages when curative treatment is not optional. Therefore, surveillance of patients at increased risk for HCC is recommended. Current guidelines recommend screening for HCC in all cirrhotic patients of all etiologies. In addition, non-cirrhotic chronic HBV patients with active hepatitis or family history of HCC should be under surveillance [42].

Surveillance recommendations are based on an RCT by Zhang et al. [192] that compared biannual screening with ultrasonography and AFP to a control group that received no screening. The screened group completed 58.2 % of the screening tests offered. 9 % of patients in the screening group were diagnosed with HCC compared to 7 % in the control group. 46.5 % in the screened group underwent resection versus 7.5 % in the un-screened population. 1-, 3-, and 5-year survival rates were 65.9, 52.6, 46.4 % versus 31.2, 7.2, 0 %, respectively. The benefits of periodic surveillance have been shown in additional studies [193–196].

In the past few years, the efficacy of AFP testing as a surveillance tool has been questioned. A population-based observational study among chronic hepatitis B carriers in Alaska showed benefit in survival with AFP screening [197], while a randomized controlled study in China showed earlier diagnosis of HCC, but without a reduction in overall mortality [198]. Another study in China found that only 6–8 % of cases not previously identified by ultrasonography were detected with AFP [199], and a later meta-analysis by Singal et al. [200] found no additional benefit to ultrasonography at all with AFP screening. Thus, it was deemed that AFP testing is suboptimal as a surveillance tool. One of the reasons for limited usefulness is that elevation of AFP levels may reflect viral hepatitis flares as well as HCC development, as was found in a trial among HCV carriers [201]. Another problem is that only 10–20 % of early stage HCCs present with abnormal AFP levels [42].

Due to these reasons, current European and American guidelines recommend periodic ultrasonography alone at 6-month intervals [42, 202]. The European Association for the Study of the Liver (EASL) guidelines recommend considering AFP testing when cost is an issue or ultrasonography is not available. The Asian Pacific Association for the Study of the Liver (APASL) guidelines continue to recommend both AFP screening and ultrasonography [203, 204].

A major obstacle in HCC screening lies with patient adherence. A community-based study in California found that 40 % of patients received poor or no screening, with worse screening in non-cirrhotic patients, possibly due to more regular clinic visits among cirrhotics [205]. A systematic review identified nine studies with a pooled surveillance rate of 18.4 %, with better rates among patients followed-up in gastroenterology clinics compared to primary care clinics (51.7 versus 16.9 %, respectively) [206]. Explanations offered for under-surveillance include lack of provider recommendations and failure to identify at-risk individuals [207]. These issues should be the first targets when attempting to improve patient surveillance.

16.4.3 Tertiary Prevention

When patients with chronic HBV develop HCC, they undergo treatments based on tumor staging. However, without elimination of the virus, new HCCs may develop de novo or recur in one or more sites in the liver. Even after successful curative therapy, the majority of patients (excluding transplanted patients) eventually die of multi-focal intrahepatic HCCs and/or of metastasis [208, 209].

Recurrence of HCC is differentiated to two groups—early recurrence (<2 years after surgery) and late recurrence [210]. Variables associated with early recurrence are microvascular invasion and non-anatomical resection. Variables associated with late recurrence are higher grade of hepatic inflammatory activity, multiple tumors and higher viral load, suggesting de novo mechanisms [211, 212]. And so, tertiary prevention in HBV patients aims to reduce late recurrence by decreasing viral load and inflammation [213].

Several studies examined the effects of IFN treatment on HCC recurrence, with conflicting results [151].

With the arrival of NUCs, the survival of patients that underwent resection of HCC, including those with untreated HBV diagnosed with small HCCs, has significantly improved. A study by Piao et al. [214] compared 30 patients after HCC treatment (by different modalities) that received LAM to 40 matched controls that did not receive antiviral treatment. The LAM-treated patients had improved Child-Pugh scores while no such improvement was seen in the controls. There was no

difference in recurrence of HCC, but a significant improvement was seen in liver function and in mortality due to liver failure in the LAM-treated group. Similar results were seen in a retrospective study by Kuzuya et al. [215]. These studies attributed the longer survival of LAM-treated HCC patients to improvement of liver functions. Other studies demonstrated improved tumor-free survival as well [216–220]. Zhou et al. [221] conducted a meta-analysis to assess the impact of HBV DNA levels and NUC therapy on HCC recurrence after resection. Twenty studies were included, and pooled analysis showed that high viral load was significantly associated with risk of recurrence, poorer disease-free survival, and poorer overall survival. NUC therapy significantly decreased the recurrence risk (RR: 0.69, $p < 0.001$) and improved both disease-free survival (RR: 0.70) and overall survival (RR: 0.46).

16.5 Summary

HBV remains a major cause of liver disease, cirrhosis and HCC, especially in Asia and Africa. Molecular mechanisms implicated in HBV-related HCC include direct viral hepatocarcinogenesis and indirect effects via chronic fibrosis, cirrhosis and inflammation.

For HBV-related HCC, primary prevention of HCC is vaccination for all uninfected individuals. Secondary prevention of HCC focuses on those who are already infected and is aimed at suppression of viral replication, and improving surveillance, thus enabling curative treatments. Future treatments to eradicate the virus in chronic carriers will potentially further reduce the incidence of HBV-induced HCC. Tertiary prevention targets patients with HCC and aims to improve survival and reduce recurrence.

Better understanding of molecular pathways involved in HBV-induced carcinogenesis suggests that we are now on the verge of designing new anti-HBV/HCC drugs that will target these pathways, thus reducing the incidence of this deadly cancer.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61(2):69–90. doi:10.3322/caac.20107.
2. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer. 2010;127(12):2893–917. doi:10.1002/ijc.25516 (Journal International du Cancer).
3. Beasley RP. Hepatitis B virus. The major etiology of hepatocellular carcinoma. Cancer. 1988;61(10):1942–56.
4. European Association for the Study Of the Liver. EASL clinical practice guidelines: management of chronic hepatitis B virus infection. J Hepatol. 2012;57(1):167–85. doi:10.1016/j.jhep.2012.02.010.
5. El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. Gastroenterology. 2012;142(6):1264–73.e1. doi:10.1053/j.gastro.2011.12.061.
6. Mittal S, El-Serag HB. Epidemiology of hepatocellular carcinoma: consider the population. J Clin Gastroenterol. 2013;47(Suppl):S2–6. doi:10.1097/MCG.0b013e3182872f29.
7. Yoffe B, Noonan CA. Hepatitis B virus. New and evolving issues. Dig Dis Sci. 1992;37(1):1–9.
8. Scaglioni PP, Melegari M, Wands JR. Recent advances in the molecular biology of hepatitis B virus. Bailliere's Clin Gastroenterol. 1996;10(2):207–25.
9. Tiollais P, Pourcel C, Dejean A. The hepatitis B virus. Nature. 1985;317(6037):489–95.
10. Yoshizawa H, Itoh Y, Simonetti JP, Takahashi T, Machida A, Miyakawa Y, et al. Demonstration of hepatitis B e antigen in hepatitis B core particles obtained from the nucleus of hepatocytes infected with hepatitis B virus. J Gen Virol. 1979;42(3):513–9. doi:10.1099/0022-1317-42-3-513.
11. Shepard CW, Simard EP, Finelli L, Fiore AE, Bell BP. Hepatitis B virus infection: epidemiology and vaccination. Epidemiol Rev. 2006;28:112–25. doi:10.1093/epirev/mxj009.
12. Summers J, Mason WS. Replication of the genome of a hepatitis B-like virus by reverse transcription of an RNA intermediate. Cell. 1982;29(2):403–15.
13. Beck J, Nassal M. Hepatitis B virus replication. World J Gastroenterol WJG. 2007;13(1):48–64.
14. Tiollais P, Charnay P, Vyas GN. Biology of hepatitis B virus. Science. 1981;213(4506):406–11.
15. Feitelson MA. Hepatitis B virus in hepatocarcinogenesis. J Cell Physiol. 1999;181(2):188–202. doi:10.1002/(SICI)1097-4652(199911)181:2<188:AID-JCP2>3.0.CO;2-7.
16. Martin-Vilchez S, Lara-Pezzi E, Trapero-Marugan M, Moreno-Otero R, Sanz-Cameno P. The molecular and pathophysiological implications of hepatitis B X antigen in chronic hepatitis B virus infection. Rev Med Virol. 2011;21(5):315–29. doi:10.1002/rmv.699.
17. Coffin CS, Mulrooney-Cousins PM, van Marle G, Roberts JP, Michalak TI, Terrault NA. Hepatitis B virus quasispecies in hepatic and extrahepatic viral reservoirs in liver transplant recipients on prophylactic therapy. Liver Transpl. 2011;17(8):955–62. doi:10.1002/lt.22312.
18. Coffin CS, Mulrooney-Cousins PM, Peters MG, van Marle G, Roberts JP, Michalak TI, et al. Molecular characterization of intrahepatic and extrahepatic hepatitis B virus (HBV) reservoirs in patients on suppressive antiviral therapy. J Viral Hepatitis. 2011;18(6):415–23. doi:10.1111/j.1365-2893.2010.01321.x.
19. Yan H, Zhong G, Xu G, He W, Jing Z, Gao Z et al. Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus. eLife. 2012;1:e00049. doi:10.7554/eLife.00049.
20. Kao JH, Chen PJ, Lai MY, Chen DS. Hepatitis B genotypes correlate with clinical outcomes in patients with chronic hepatitis B. Gastroenterology. 2000;118(3):554–9.
21. Norder H, Courouce AM, Coursaget P, Echevarria JM, Lee SD, Mushahwar IK, et al. Genetic diversity of hepatitis B virus strains derived worldwide: genotypes, subgenotypes, and HBsAg subtypes. Intervirology. 2004;47(6):289–309. doi:10.1159/000080872.
22. Chu CJ, Hussain M, Lok AS. Hepatitis B virus genotype B is associated with earlier HBeAg seroconversion compared with hepatitis B virus genotype C. Gastroenterology. 2002;122(7):1756–62.
23. Chu C-M, Liaw Y-F. Genotype C hepatitis B virus infection is associated with a higher risk of reactivation of hepatitis B and

progression to cirrhosis than genotype B: a longitudinal study of hepatitis B e antigen-positive patients with normal aminotransferase levels at baseline. J Hepatol. 2005;43(3):411–7. doi:10.1016/j.jhep.2005.03.018.
24. Sumi H, Yokosuka O, Seki N, Arai M, Imazeki F, Kurihara T, et al. Influence of hepatitis B virus genotypes on the progression of chronic type B liver disease. Hepatology. 2003;37(1):19–26. doi:10.1053/jhep.2003.50036.
25. Yu MW, Yeh SH, Chen PJ, Liaw YF, Lin CL, Liu CJ, et al. Hepatitis B virus genotype and DNA level and hepatocellular carcinoma: a prospective study in men. J Natl Cancer Inst. 2005;97(4):265–72. doi:10.1093/jnci/dji043.
26. Chan HL, Hui AY, Wong ML, Tse AM, Hung LC, Wong VW, et al. Genotype C hepatitis B virus infection is associated with an increased risk of hepatocellular carcinoma. Gut. 2004;53(10):1494–8. doi:10.1136/gut.2003.033324.
27. Fung SK, Lok AS. Hepatitis B virus genotypes: do they play a role in the outcome of HBV infection? Hepatology. 2004;40(4):790–2. doi:10.1002/hep.1840400407.
28. Erhardt A, Blondin D, Hauck K, Sagir A, Kohnle T, Heintges T, et al. Response to interferon alfa is hepatitis B virus genotype dependent: genotype A is more sensitive to interferon than genotype D. Gut. 2005;54(7):1009–13. doi:10.1136/gut.2004.060327.
29. Beasley RP, Trepo C, Stevens CE, Szmuness W. The e antigen and vertical transmission of hepatitis B surface antigen. Am J Epidemiol. 1977;105(2):94–8.
30. Chen CJ, Wang LY, Yu MW. Epidemiology of hepatitis B virus infection in the Asia-Pacific region. J Gastroenterol Hepatol. 2000;15(Suppl):E3–6.
31. Lee WM. Hepatitis B virus infection. N Engl J Med. 1997;337(24):1733–45. doi:10.1056/NEJM199712113372406.
32. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology. 2007;45(2):507–39. doi:10.1002/hep.21513.
33. Yim HJ, Lok AS. Natural history of chronic hepatitis B virus infection: what we knew in 1981 and what we know in 2005. Hepatology. 2006;43(2 Suppl 1):S173–81. doi:10.1002/hep.20956.
34. McMahon BJ, Holck P, Bulkow L, Snowball M. Serologic and clinical outcomes of 1536 Alaska Natives chronically infected with hepatitis B virus. Ann Intern Med. 2001;135(9):759–68.
35. Liaw YF, Sheen IS, Chen TJ, Chu CM, Pao CC. Incidence, determinants and significance of delayed clearance of serum HBsAg in chronic hepatitis B virus infection: a prospective study. Hepatology. 1991;13(4):627–31.
36. McMahon BJ. The natural history of chronic hepatitis B virus infection. Hepatology. 2009;49(5 Suppl):S45–55. doi:10.1002/hep.22898.
37. Jaroszewicz J, Calle Serrano B, Wursthorn K, Deterding K, Schlue J, Raupach R, et al. Hepatitis B surface antigen (HBsAg) levels in the natural history of hepatitis B virus (HBV)-infection: a European perspective. J Hepatol. 2010;52(4):514–22. doi:10.1016/j.jhep.2010.01.014.
38. Hann HW, Hann RS, Maddrey WC. Hepatitis B virus infection in 6,130 unvaccinated Korean-Americans surveyed between 1988 and 1990. Am J Gastroenterol. 2007;102(4):767–72. doi:10.1111/j.1572-0241.2007.01060.x.
39. Franco E, Meleleo C, Serino L, Sorbara D, Zaratti L. Hepatitis A: epidemiology and prevention in developing countries. World J Hepatol. 2012;4(3):68–73. doi:10.4254/wjh.v4.i3.68.
40. Custer B, Sullivan SD, Hazlet TK, Iloeje U, Veenstra DL, Kowdley KV. Global epidemiology of hepatitis B virus. J Clin Gastroenterol. 2004;38(10 Suppl 3):S158–68.
41. Schweitzer A, Horn J, Mikolajczyk RT, Krause G, Ott JJ. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. Lancet. 2015. doi:10.1016/S0140-6736(15)61412-X.
42. European Association for the Study of the Liver, European Organisation for Research, Treatment of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol. 2012;56(4):908–43. doi:10.1016/j.jhep.2011.12.001.
43. Larouze B, Saimot G, Lustbader ED, London WT, Werner BG, Payet M. Host responses to hepatitis-B infection in patients with primary hepatic carcinoma and their families. A case/control study in Senegal, West Africa. Lancet. 1976;2(7985):534–8.
44. Blumberg BS, Larouze B, London WT, Werner B, Hesser JE, Millman I, et al. The relation of infection with the hepatitis B agent to primary hepatic carcinoma. Am J Pathol. 1975;81(3):669–82.
45. Froment A, Larouze B, Feret E, Marinier E, Sow AM, London WT, et al. Hepatitis B infection and the prevention of primary hepatocellular carcinoma: studies in Senegal. Prog Med Virol. 1981;27:133–6 (Fortschritte der medizinischen. Virusforschung Progres en virologie medicale).
46. Hann HW, Kim CY, London WT, Whitford P, Blumberg BS. Hepatitis B virus and primary hepatocellular carcinoma: family studies in Korea. Int J Cancer. 1982;30(1):47–51 (Journal International du Cancer).
47. Kubo Y, Okuda K, Musha H, Nakashima T. Detection of hepatocellular carcinoma during a clinical follow-up of chronic liver disease: observations in 31 patients. Gastroenterology. 1978;74(3):578–82.
48. Beasley RP, Hwang LY, Lin CC, Chien CS. Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22 707 men in Taiwan. Lancet. 1981;2(8256):1129–33.
49. Gwack J, Park SK, Lee EH, Park B, Choi Y, Yoo KY. Hepatitis B vaccination and liver cancer mortality reduction in Korean children and adolescents. Asian Pac J Cancer Prev APJCP. 2011;12(9):2205–8.
50. Chang MH, Chen CJ, Lai MS, Hsu HM, Wu TC, Kong MS, et al. Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. N Engl J Med. 1997;336(26):1855–9. doi:10.1056/NEJM199706263362602 (Taiwan Childhood Hepatoma Study Group).
51. Chang MH, You SL, Chen CJ, Liu CJ, Lee CM, Lin SM, et al. Decreased incidence of hepatocellular carcinoma in hepatitis B vaccinees: a 20-year follow-up study. J Natl Cancer Inst. 2009;101(19):1348–55. doi:10.1093/jnci/djp288.
52. Wichajarn K, Kosalaraksa P, Wiangnon S. Incidence of hepatocellular carcinoma in children in Khon Kaen before and after national hepatitis B vaccine program. Asian Pac J Cancer Prev APJCP. 2008;9(3):507–9.
53. Lanier AP, Holck P, Day GE, Key C. Childhood cancer among Alaska Natives. Pediatrics. 2003;112(5):e396.
54. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology. 2007;132(7):2557–76. doi:10.1053/j.gastro.2007.04.061.
55. Tong MJ, Hwang SJ. Hepatitis B virus infection in Asian Americans. Gastroenterol Clin North Am. 1994;23(3):523–36.
56. Gish RG, Gadano AC. Chronic hepatitis B: current epidemiology in the Americas and implications for management. J Viral Hepatitis. 2006;13(12):787–98. doi:10.1111/j.1365-2893.2006.00787.x.
57. Cohen C, Evans AA, London WT, Block J, Conti M, Block T. Underestimation of chronic hepatitis B virus infection in the United States of America. J Viral Hepatitis. 2008;15(1):12–3. doi:10.1111/j.1365-2893.2007.00888.x.
58. Szmuness W, Stevens CE, Ikram H, Much MI, Harley EJ, Hollinger B. Prevalence of hepatitis B virus infection and hepatocellular carcinoma in Chinese-Americans. J Infect Dis. 1978;137(6):822–9.

59. Franks AL, Berg CJ, Kane MA, Browne BB, Sikes RK, Elsea WR, et al. Hepatitis B virus infection among children born in the United States to Southeast Asian refugees. N Engl J Med. 1989;321(19):1301–5. doi:10.1056/NEJM198911093211905.
60. Kowdley KV, Wang CC, Welch S, Roberts H, Brosgart CL. Prevalence of chronic hepatitis B among foreign-born persons living in the United States by country of origin. Hepatology. 2012;56(2):422–33. doi:10.1002/hep.24804.
61. Liaw YF, Tai DI, Chu CM, Lin DY, Sheen IS, Chen TJ, et al. Early detection of hepatocellular carcinoma in patients with chronic type B hepatitis. A prospective study. Gastroenterology. 1986;90(2):263–7.
62. Wu TC, Tong MJ, Hwang B, Lee SD, Hu MM. Primary hepatocellular carcinoma and hepatitis B infection during childhood. Hepatology. 1987;7(1):46–8.
63. Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. Gastroenterology. 2004;127(5 Suppl 1):S35–50.
64. Boyault S, Rickman DS, de Reynies A, Balabaud C, Rebouissou S, Jeannot E, et al. Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. Hepatology. 2007;45(1):42–52. doi:10.1002/hep.21467.
65. Thorgeirsson SS, Grisham JW. Molecular pathogenesis of human hepatocellular carcinoma. Nat Genet. 2002;31(4):339–46. doi:10.1038/ng0802-339.
66. Shi Y, Wu YH, Wu W, Zhang WJ, Yang J, Chen Z. Association between occult hepatitis B infection and the risk of hepatocellular carcinoma: a meta-analysis. Liver Int. 2012;32(2):231–40. doi:10.1111/j.1478-3231.2011.02481.x (Official Journal of the International Association for the Study of the Liver).
67. Shiratori Y, Shiina S, Imamura M, Kato N, Kanai F, Okudaira T, et al. Characteristic difference of hepatocellular carcinoma between hepatitis B- and C- viral infection in Japan. Hepatology. 1995;22(4 Pt 1):1027–33.
68. Takano S, Yokosuka O, Imazeki F, Tagawa M, Omata M. Incidence of hepatocellular carcinoma in chronic hepatitis B and C: a prospective study of 251 patients. Hepatology. 1995;21(3):650–5.
69. Yang JD, Kim WR, Coelho R, Mettler TA, Benson JT, Sanderson SO, et al. Cirrhosis is present in most patients with hepatitis B and hepatocellular carcinoma. Clin Gastroenterol Hepatol. 2011;9(1):64–70. doi:10.1016/j.cgh.2010.08.019.
70. Fattovich G, Bortolotti F, Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. J Hepatol. 2008;48(2):335–52. doi:10.1016/j.jhep.2007.11.011.
71. Shimakawa Y, Yan HJ, Tsuchiya N, Bottomley C, Hall AJ. Association of early age at establishment of chronic hepatitis B infection with persistent viral replication, liver cirrhosis and hepatocellular carcinoma: a systematic review. PLoS ONE. 2013;8(7):e69430. doi:10.1371/journal.pone.0069430.
72. Li Y, Zhang Z, Shi J, Jin L, Wang L, Xu D, et al. Risk factors for naturally-occurring early-onset hepatocellular carcinoma in patients with HBV-associated liver cirrhosis in China. Int J Clin Exp Med. 2015;8(1):1205–12.
73. Huang YT, Jen CL, Yang HI, Lee MH, Su J, Lu SN, et al. Lifetime risk and sex difference of hepatocellular carcinoma among patients with chronic hepatitis B and C. J Clin Oncol. 2011;29(27):3643–50. doi:10.1200/JCO.2011.36.2335 (Official Journal of the American Society of Clinical Oncology).
74. Yuan JM, Ross RK, Stanczyk FZ, Govindarajan S, Gao YT, Henderson BE, et al. A cohort study of serum testosterone and hepatocellular carcinoma in Shanghai, China. Int J Cancer. 1995;63(4):491–3 (Journal International du Cancer).
75. Hann HW, Kim CY, London WT, Blumberg BS. Increased serum ferritin in chronic liver disease: a risk factor for primary hepatocellular carcinoma. Int J Cancer. 1989;43(3):376–9 (Journal International du Cancer).
76. Ikeda K, Saitoh S, Suzuki Y, Kobayashi M, Tsubota A, Koida I, et al. Disease progression and hepatocellular carcinogenesis in patients with chronic viral hepatitis: a prospective observation of 2215 patients. J Hepatol. 1998;28(6):930–8.
77. Jee SH, Ohrr H, Sull JW, Samet JM. Cigarette smoking, alcohol drinking, hepatitis B, and risk for hepatocellular carcinoma in Korea. J Natl Cancer Inst. 2004;96(24):1851–6. doi:10.1093/jnci/djh334.
78. Chuang SC, Lee YC, Hashibe M, Dai M, Zheng T, Boffetta P. Interaction between cigarette smoking and hepatitis B and C virus infection on the risk of liver cancer: a meta-analysis. Cancer Epidemiol Biomark Prev. 2010;19(5):1261–8. doi:10.1158/1055-9965.EPI-09-1297 (A publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology).
79. Hann HW, Stahlhut MW, Blumberg BS. Iron nutrition and tumor growth: decreased tumor growth in iron-deficient mice. Cancer Res. 1988;48(15):4168–70.
80. Hann HW, Stahlhut MW, Hann CL. Effect of iron and desferoxamine on cell growth and in vitro ferritin synthesis in human hepatoma cell lines. Hepatology. 1990;11(4):566–9.
81. Hann HW, Stahlhut MW, Menduke H. Iron enhances tumor growth. Observation on spontaneous mammary tumors in mice. Cancer. 1991;68(11):2407–10.
82. Hann HW, Stahlhut MW, Rubin R, Maddrey WC. Antitumor effect of deferoxamine on human hepatocellular carcinoma growing in athymic nude mice. Cancer. 1992;70(8):2051–6.
83. Yang HI, Lu SN, Liaw YF, You SL, Sun CA, Wang LY, et al. Hepatitis B e antigen and the risk of hepatocellular carcinoma. N Engl J Med. 2002;347(3):168–74. doi:10.1056/NEJMoa013215.
84. Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA. 2006;295(1):65–73. doi:10.1001/jama.295.1.65.
85. Wu CF, Yu MW, Lin CL, Liu CJ, Shih WL, Tsai KS, et al. Long-term tracking of hepatitis B viral load and the relationship with risk for hepatocellular carcinoma in men. Carcinogenesis. 2008;29(1):106–12. doi:10.1093/carcin/bgm252.
86. Chen JD, Yang HI, Iloeje UH, You SL, Lu SN, Wang LY, et al. Carriers of inactive hepatitis B virus are still at risk for hepatocellular carcinoma and liver-related death. Gastroenterology. 2010;138(5):1747–54. doi:10.1053/j.gastro.2010.01.042.
87. Ringelhan M, O'Connor T, Protzer U, Heikenwalder M. The direct and indirect roles of HBV in liver cancer: prospective markers for HCC screening and potential therapeutic targets. J Pathol. 2015;235(2):355–67. doi:10.1002/path.4434.
88. Arzumanyan A, Reis HM, Feitelson MA. Pathogenic mechanisms in HBV- and HCV-associated hepatocellular carcinoma. Nat Rev Cancer. 2013;13(2):123–35. doi:10.1038/nrc3449.
89. Wei Y, Neuveut C, Tiollais P, Buendia MA. Molecular biology of the hepatitis B virus and role of the X gene. Pathol Biol. 2010;58(4):267–72. doi:10.1016/j.patbio.2010.03.005.
90. Zoulim F, Saputelli J, Seeger C. Woodchuck hepatitis virus X protein is required for viral infection in vivo. J Virol. 1994;68(3):2026–30.
91. Chen HS, Kaneko S, Girones R, Anderson RW, Hornbuckle WE, Tennant BC, et al. The woodchuck hepatitis virus X gene is important for establishment of virus infection in woodchucks. J Virol. 1993;67(3):1218–26.
92. Wang WL, London WT, Lega L, Feitelson MA. HBxAg in the liver from carrier patients with chronic hepatitis and cirrhosis. Hepatology. 1991;14(1):29–37.

93. Wang WL, London WT, Feitelson MA. Hepatitis B x antigen in hepatitis B virus carrier patients with liver cancer. Cancer Res. 1991;51(18):4971–7.
94. Jin YM, Yun C, Park C, Wang HJ, Cho H. Expression of hepatitis B virus X protein is closely correlated with the high periportal inflammatory activity of liver diseases. J Viral Hepatitis. 2001;8 (5):322–30.
95. Tang H, Oishi N, Kaneko S, Murakami S. Molecular functions and biological roles of hepatitis B virus x protein. Cancer Sci. 2006;97(10):977–83. doi:10.1111/j.1349-7006.2006.00299.x.
96. Tang H, Delgermaa L, Huang F, Oishi N, Liu L, He F, et al. The transcriptional transactivation function of HBx protein is important for its augmentation role in hepatitis B virus replication. J Virol. 2005;79(9):5548–56. doi:10.1128/JVI.79.9.5548-5556.2005.
97. Yoo YD, Ueda H, Park K, Flanders KC, Lee YI, Jay G, et al. Regulation of transforming growth factor-beta 1 expression by the hepatitis B virus (HBV) X transactivator. Role in HBV pathogenesis. J Clin Investig. 1996;97(2):388–95. doi:10.1172/JCI118427.
98. Pan J, Clayton M, Feitelson MA. Hepatitis B virus X antigen promotes transforming growth factor-beta1 (TGF-beta1) activity by up-regulation of TGF-beta1 and down-regulation of alpha2-macroglobulin. J Gen Virol. 2004;85(Pt 2):275–82. doi:10.1099/vir.0.19650-0.
99. Akhurst RJ. TGF-beta antagonists: why suppress a tumor suppressor? J Clin Investig. 2002;109(12):1533–6. doi:10.1172/JCI15970.
100. Gressner AM, Weiskirchen R, Breitkopf K, Dooley S. Roles of TGF-beta in hepatic fibrosis. Front Biosci. 2002;7:d793–807 (A Journal and Virtual Library).
101. Lee DK, Park SH, Yi Y, Choi SG, Lee C, Parks WT, et al. The hepatitis B virus encoded oncoprotein pX amplifies TGF-beta family signaling through direct interaction with Smad4: potential mechanism of hepatitis B virus-induced liver fibrosis. Genes Dev. 2001;15(4):455–66. doi:10.1101/gad.856201.
102. Henkler FF, Koshy R. Hepatitis B virus transcriptional activators: mechanisms and possible role in oncogenesis. J Viral Hepatitis. 1996;3(3):109–21.
103. Feitelson MA, Duan LX. Hepatitis B virus X antigen in the pathogenesis of chronic infections and the development of hepatocellular carcinoma. Am J Pathol. 1997;150(4):1141–57.
104. Fischer AN, Fuchs E, Mikula M, Huber H, Beug H, Mikulits W. PDGF essentially links TGF-beta signaling to nuclear beta-catenin accumulation in hepatocellular carcinoma progression. Oncogene. 2007;26(23):3395–405. doi:10.1038/sj.onc.1210121.
105. Protzer U, Schaller H. Immune escape by hepatitis B viruses. Virus Genes. 2000;21(1–2):27–37.
106. Nakamoto Y, Guidotti LG, Kuhlen CV, Fowler P, Chisari FV. Immune pathogenesis of hepatocellular carcinoma. J Exp Med. 1998;188(2):341–50.
107. Rehermann B, Nascimbeni M. Immunology of hepatitis B virus and hepatitis C virus infection. Nat Rev Immunol. 2005;5(3):215–29. doi:10.1038/nri1573.
108. Ganem D, Prince AM. Hepatitis B virus infection—natural history and clinical consequences. N Engl J Med. 2004;350 (11):1118–29. doi:10.1056/NEJMra031087.
109. Bauer T, Sprinzl M, Protzer U. Immune control of hepatitis B virus. Dig Dis. 2011;29(4):423–33. doi:10.1159/000329809.
110. Stross L, Gunther J, Gasteiger G, Asen T, Graf S, Aichler M, et al. Foxp3+ regulatory T cells protect the liver from immune damage and compromise virus control during acute experimental hepatitis B virus infection in mice. Hepatology. 2012;56(3):873–83. doi:10.1002/hep.25765.
111. Billerbeck E, Bottler T, Thimme R. Regulatory T cells in viral hepatitis. World J Gastroenterol WJG. 2007;13(36):4858–64.
112. Stoop JN, van der Molen RG, Baan CC, van der Laan LJ, Kuipers EJ, Kusters JG, et al. Regulatory T cells contribute to the impaired immune response in patients with chronic hepatitis B virus infection. Hepatology. 2005;41(4):771–8. doi:10.1002/hep.20649.
113. Wu J, Meng Z, Jiang M, Pei R, Trippler M, Broering R, et al. Hepatitis B virus suppresses toll-like receptor-mediated innate immune responses in murine parenchymal and nonparenchymal liver cells. Hepatology. 2009;49(4):1132–40. doi:10.1002/hep.22751.
114. Pan J, Duan LX, Sun BS, Feitelson MA. Hepatitis B virus X protein protects against anti-Fas-mediated apoptosis in human liver cells by inducing NF-kappa B. J Gen Virol. 2001;82(Pt 1):171–82. doi:10.1099/0022-1317-82-1-171.
115. Pan J, Lian Z, Wallett S, Feitelson MA. The hepatitis B x antigen effector, URG7, blocks tumour necrosis factor alpha-mediated apoptosis by activation of phosphoinositol 3-kinase and beta-catenin. J Gen Virol. 2007;88(Pt 12):3275–85. doi:10.1099/vir.0.83214-0.
116. Bouchard MJ, Navas-Martin S. Hepatitis B and C virus hepatocarcinogenesis: lessons learned and future challenges. Cancer Lett. 2011;305(2):123–43. doi:10.1016/j.canlet.2010.11.014.
117. Ringelhan M, Heikenwalder M, Protzer U. Direct effects of hepatitis B virus-encoded proteins and chronic infection in liver cancer development. Dig Dis. 2013;31(1):138–51. doi:10.1159/000347209.
118. Clippinger AJ, Bouchard MJ. Hepatitis B virus HBx protein localizes to mitochondria in primary rat hepatocytes and modulates mitochondrial membrane potential. J Virol. 2008;82 (14):6798–811. doi:10.1128/JVI.00154-08.
119. Cho HK, Cheong KJ, Kim HY, Cheong J. Endoplasmic reticulum stress induced by hepatitis B virus X protein enhances cyclo-oxygenase 2 expression via activating transcription factor 4. Biochem J. 2011;435(2):431–9. doi:10.1042/BJ20102071.
120. Yu FL, Liu HJ, Lee JW, Liao MH, Shih WL. Hepatitis B virus X protein promotes cell migration by inducing matrix metalloproteinase-3. J Hepatol. 2005;42(4):520–7. doi:10.1016/j.jhep.2004.11.031.
121. Lara-Pezzi E, Gomez-Gaviro MV, Galvez BG, Mira E, Iniguez MA, Fresno M, et al. The hepatitis B virus X protein promotes tumor cell invasion by inducing membrane-type matrix metalloproteinase-1 and cyclooxygenase-2 expression. J Clin Investig. 2002;110(12):1831–8. doi:10.1172/JCI15887.
122. Chung TW, Lee YC, Kim CH. Hepatitis B viral HBx induces matrix metalloproteinase-9 gene expression through activation of ERK and PI-3K/AKT pathways: involvement of invasive potential. FASEB J. 2004;18(10):1123–5. doi:10.1096/fj.03-1429fje (Official Publication of the Federation of American Societies for Experimental Biology).
123. Kim JR, Kim CH. Association of a high activity of matrix metalloproteinase-9 to low levels of tissue inhibitors of metalloproteinase-1 and -3 in human hepatitis B-viral hepatoma cells. Int J Biochem Cell Biol. 2004;36(11):2293–306. doi:10.1016/j.biocel.2004.04.022.
124. Han YP. Matrix metalloproteinases, the pros and cons, in liver fibrosis. J Gastroenterol Hepatol. 2006;21(Suppl 3):S88–91. doi:10.1111/j.1440-1746.2006.04586.x.
125. Lupberger J, Hildt E. Hepatitis B virus-induced oncogenesis. World J Gastroenterol WJG. 2007;13(1):74–81.
126. Feitelson MA, Lee J. Hepatitis B virus integration, fragile sites, and hepatocarcinogenesis. Cancer Lett. 2007;252(2):157–70. doi:10.1016/j.canlet.2006.11.010.

127. Saitta C, Tripodi G, Barbera A, Bertuccio A, Smedile A, Ciancio A, et al. Hepatitis B virus (HBV) DNA integration in patients with occult HBV infection and hepatocellular carcinoma. Liver Int. 2015;35(10):2311–7. doi:10.1111/liv.12807 (Official Journal of the International Association for the Study of the Liver).
128. Zahm P, Hofschneider PH, Koshy R. The HBV X-ORF encodes a transactivator: a potential factor in viral hepatocarcinogenesis. Oncogene. 1988;3(2):169–77.
129. Wollersheim M, Debelka U, Hofschneider PH. A transactivating function encoded in the hepatitis B virus X gene is conserved in the integrated state. Oncogene. 1988;3(5):545–52.
130. Hussain SP, Harris CC. p53 biological network: at the crossroads of the cellular-stress response pathway and molecular carcinogenesis. J Nippon Med Sch. 2006;73(2):54–64 (Nippon Ika Daigaku zasshi).
131. Feitelson MA, Zhu M, Duan LX, London WT. Hepatitis B x antigen and p53 are associated in vitro and in liver tissues from patients with primary hepatocellular carcinoma. Oncogene. 1993;8(5):1109–17.
132. Wang XW, Forrester K, Yeh H, Feitelson MA, Gu JR, Harris CC. Hepatitis B virus X protein inhibits p53 sequence-specific DNA binding, transcriptional activity, and association with transcription factor ERCC3. Proc Natl Acad Sci USA. 1994;91(6):2230–4.
133. Ueda H, Ullrich SJ, Gangemi JD, Kappel CA, Ngo L, Feitelson MA, et al. Functional inactivation but not structural mutation of p53 causes liver cancer. Nat Genet. 1995;9(1):41–7. doi:10.1038/ng0195-41.
134. Jia L, Wang XW, Harris CC. Hepatitis B virus X protein inhibits nucleotide excision repair. Int J Cancer. 1999;80(6):875–9 (Journal International du Cancer).
135. Ming L, Thorgeirsson SS, Gail MH, Lu P, Harris CC, Wang N, et al. Dominant role of hepatitis B virus and cofactor role of aflatoxin in hepatocarcinogenesis in Qidong, China. Hepatology. 2002;36(5):1214–20. doi:10.1053/jhep.2002.36366.
136. Hosny G, Farahat N, Tayel H, Hainaut P. Ser-249 TP53 and CTNNB1 mutations in circulating free DNA of Egyptian patients with hepatocellular carcinoma versus chronic liver diseases. Cancer Lett. 2008;264(2):201–8. doi:10.1016/j.canlet.2008.01.031.
137. Austinat M, Dunsch R, Wittekind C, Tannapfel A, Gebhardt R, Gaunitz F. Correlation between beta-catenin mutations and expression of Wnt-signaling target genes in hepatocellular carcinoma. Mol Cancer. 2008;7:21. doi:10.1186/1476-4598-7-21.
138. Park SG, Chung C, Kang H, Kim JY, Jung G. Up-regulation of cyclin D1 by HBx is mediated by NF-kappaB2/BCL3 complex through kappaB site of cyclin D1 promoter. J Biol Chem. 2006;281(42):31770–7. doi:10.1074/jbc.M603194200.
139. Jung JK, Arora P, Pagano JS, Jang KL. Expression of DNA methyltransferase 1 is activated by hepatitis B virus X protein via a regulatory circuit involving the p16INK4a-cyclin D1-CDK 4/6-pRb-E2F1 pathway. Cancer Res. 2007;67(12):5771–8. doi:10.1158/0008-5472.CAN-07-0529.
140. Tufan NL, Lian Z, Liu J, Pan J, Arbuthnot P, Kew M, et al. Hepatitis Bx antigen stimulates expression of a novel cellular gene, URG4, that promotes hepatocellular growth and survival. Neoplasia. 2002;4(4):355–68. doi:10.1038/sj.neo.7900241.
141. Lian Z, Liu J, Li L, Li X, Tufan NL, Clayton M, et al. Upregulated expression of a unique gene by hepatitis B x antigen promotes hepatocellular growth and tumorigenesis. Neoplasia. 2003;5(3):229–44.
142. Noh EJ, Jung HJ, Jeong G, Choi KS, Park HJ, Lee CH, et al. Subcellular localization and transcriptional repressor activity of HBx on p21(WAF1/Cip1) promoter is regulated by ERK-mediated phosphorylation. Biochem Biophys Res Commun. 2004;319(3):738–45. doi:10.1016/j.bbrc.2004.05.047.
143. Oishi N, Shilagardi K, Nakamoto Y, Honda M, Kaneko S, Murakami S. Hepatitis B virus X protein overcomes oncogenic RAS-induced senescence in human immortalized cells. Cancer Sci. 2007;98(10):1540–8. doi:10.1111/j.1349-7006.2007.00579.x.
144. Lamontagne J, Steel LF, Bouchard MJ. Hepatitis B virus and microRNAs: complex interactions affecting hepatitis B virus replication and hepatitis B virus-associated diseases. World J Gastroenterol WJG. 2015;21(24):7375–99. doi:10.3748/wjg.v21.i24.7375.
145. Ura S, Honda M, Yamashita T, Ueda T, Takatori H, Nishino R, et al. Differential microRNA expression between hepatitis B and hepatitis C leading disease progression to hepatocellular carcinoma. Hepatology. 2009;49(4):1098–112. doi:10.1002/hep.22749.
146. Park IY, Sohn BH, Yu E, Suh DJ, Chung YH, Lee JH, et al. Aberrant epigenetic modifications in hepatocarcinogenesis induced by hepatitis B virus X protein. Gastroenterology. 2007;132(4):1476–94. doi:10.1053/j.gastro.2007.01.034.
147. Saito Y, Kanai Y, Sakamoto M, Saito H, Ishii H, Hirohashi S. Expression of mRNA for DNA methyltransferases and methyl-CpG-binding proteins and DNA methylation status on CpG islands and pericentromeric satellite regions during human hepatocarcinogenesis. Hepatology. 2001;33(3):561–8. doi:10.1053/jhep.2001.22507.
148. Lee JO, Kwun HJ, Jung JK, Choi KH, Min DS, Jang KL. Hepatitis B virus X protein represses E-cadherin expression via activation of DNA methyltransferase 1. Oncogene. 2005;24(44):6617–25. doi:10.1038/sj.onc.1208827.
149. Liu J, Lian Z, Han S, Waye MM, Wang H, Wu MC, et al. Downregulation of E-cadherin by hepatitis B virus X antigen in hepatocellullar carcinoma. Oncogene. 2006;25(7):1008–17. doi:10.1038/sj.onc.1209138.
150. Amaddeo G, Cao Q, Ladeiro Y, Imbeaud S, Nault JC, Jaoui D, et al. Integration of tumour and viral genomic characterizations in HBV-related hepatocellular carcinomas. Gut. 2015;64(5):820–9. doi:10.1136/gutjnl-2013-306228.
151. Kim MN, Han KH, Ahn SH. Prevention of hepatocellular carcinoma: beyond hepatitis B vaccination. Semin Oncol. 2015;42(2):316–28. doi:10.1053/j.seminoncol.2014.12.018.
152. Dienstag JL. Toward the control of hepatitis B. N Engl J Med. 1980;303(15):874–6. doi:10.1056/NEJM198010093031509.
153. Mulley AG, Silverstein MD, Dienstag JL. Indications for use of hepatitis B vaccine, based on cost-effectiveness analysis. N Engl J Med. 1982;307(11):644–52. doi:10.1056/NEJM198209093071103.
154. Organization WH. WHO expanded programme on immunisation. Global Advisory Group. Weekly Epidemiol Rec. 1993;1992;3:11–6.
155. Kane M. Global programme for control of hepatitis B infection. Vaccine. 1995;13(Suppl 1):S47–9.
156. Zanetti AR, Van Damme P, Shouval D. The global impact of vaccination against hepatitis B: a historical overview. Vaccine. 2008;26(49):6266–73. doi:10.1016/j.vaccine.2008.09.056.
157. Plymoth A, Viviani S, Hainaut P. Control of hepatocellular carcinoma through hepatitis B vaccination in areas of high endemicity: perspectives for global liver cancer prevention. Cancer Lett. 2009;286(1):15–21. doi:10.1016/j.canlet.2009.08.024.
158. Ni YH, Chang MH, Huang LM, Chen HL, Hsu HY, Chiu TY, et al. Hepatitis B virus infection in children and adolescents in a hyperendemic area: 15 years after mass hepatitis B vaccination. Ann Intern Med. 2001;135(9):796–800.
159. Viviani S, Jack A, Hall AJ, Maine N, Mendy M, Montesano R, et al. Hepatitis B vaccination in infancy in The Gambia: protection against carriage at 9 years of age. Vaccine. 1999;17(23–24):2946–50.
160. Liang X, Bi S, Yang W, Wang L, Cui G, Cui F, et al. Epidemiological serosurvey of hepatitis B in China—declining HBV prevalence due to hepatitis B vaccination. Vaccine. 2009;27(47):6550–7. doi:10.1016/j.vaccine.2009.08.048.

161. Bonanni P, Pesavento G, Bechini A, Tiscione E, Mannelli F, Benucci C, et al. Impact of universal vaccination programmes on the epidemiology of hepatitis B: 10 years of experience in Italy. Vaccine. 2003;21(7–8):685–91.
162. McMahon BJ, Bulkow LR, Singleton RJ, Williams J, Snowball M, Homan C, et al. Elimination of hepatocellular carcinoma and acute hepatitis B in children 25 years after a hepatitis B newborn and catch-up immunization program. Hepatology. 2011;54 (3):801–7. doi:10.1002/hep.24442.
163. Weinbaum CM, Mast EE, Ward JW. Recommendations for identification and public health management of persons with chronic hepatitis B virus infection. Hepatology. 2009;49(5 Suppl): S35–44. doi:10.1002/hep.22882.
164. Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. Hepatology. 2009;50(3):661–2. doi:10.1002/hep.23190.
165. Lin SM, Sheen IS, Chien RN, Chu CM, Liaw YF. Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. Hepatology. 1999;29(3):971–5. doi:10.1002/hep.510290312.
166. Ikeda K, Saitoh S, Suzuki Y, Kobayashi M, Tsubota A, Fukuda M, et al. Interferon decreases hepatocellular carcinogenesis in patients with cirrhosis caused by the hepatitis B virus: a pilot study. Cancer. 1998;82(5):827–35.
167. Tangkijvanich P, Thong-ngam D, Mahachai V, Kladchareon N, Suwangool P, Kullavanijaya P. Long-term effect of interferon therapy on incidence of cirrhosis and hepatocellular carcinoma in Thai patients with chronic hepatitis B. Southeast Asian J Trop Med Public Health. 2001;32(3):452–8.
168. Lin SM, Yu ML, Lee CM, Chien RN, Sheen IS, Chu CM, et al. Interferon therapy in HBeAg positive chronic hepatitis reduces progression to cirrhosis and hepatocellular carcinoma. J Hepatol. 2007;46(1):45–52. doi:10.1016/j.jhep.2006.08.021.
169. Yuen MF, Hui CK, Cheng CC, Wu CH, Lai YP, Lai CL. Long-term follow-up of interferon alfa treatment in Chinese patients with chronic hepatitis B infection: the effect on hepatitis B e antigen seroconversion and the development of cirrhosis-related complications. Hepatology. 2001;34(1):139–45. doi:10.1053/jhep.2001.25273.
170. Krogsgaard K. The long-term effect of treatment with interferon-alpha 2a in chronic hepatitis B. J Viral Hepatitis. 1998;5(6):389–97 (The Long-Term Follow-up Investigator Group. The European Study Group on Viral Hepatitis (EUROHEP). Executive Team on Anti-Viral Treatment).
171. Mazzella G, Saracco G, Festi D, Rosina F, Marchetto S, Jaboli F, et al. Long-term results with interferon therapy in chronic type B hepatitis: a prospective randomized trial. Am J Gastroenterol. 1999;94(8):2246–50. doi:10.1111/j.1572-0241.1999.01300.x.
172. Truong BX, Seo Y, Kato M, Hamano K, Ninomiya T, Katayama M, et al. Long-term follow-up of Japanese patients with chronic hepatitis B treated with interferon-alpha. Int J Mol Med. 2005;16(2):279–84.
173. Lin SM, Tai DI, Chien RN, Sheen IS, Chu CM, Liaw YF. Comparison of long-term effects of lymphoblastoid interferon alpha and recombinant interferon alpha-2a therapy in patients with chronic hepatitis B. J Viral Hepatitis. 2004;11(4):349–57. doi:10.1111/j.1365-2893.2004.00512.x.
174. Camma C, Giunta M, Andreone P, Craxi A. Interferon and prevention of hepatocellular carcinoma in viral cirrhosis: an evidence-based approach. J Hepatol. 2001;34(4):593–602.
175. Sung JJ, Tsoi KK, Wong VW, Li KC, Chan HL. Meta-analysis: treatment of hepatitis B infection reduces risk of hepatocellular carcinoma. Aliment Pharmacol Ther. 2008;28(9):1067–77. doi:10.1111/j.1365-2036.2008.03816.x.
176. Yang YF, Zhao W, Zhong YD, Xia HM, Shen L, Zhang N. Interferon therapy in chronic hepatitis B reduces progression to cirrhosis and hepatocellular carcinoma: a meta-analysis. J Viral Hepatitis. 2009;16(4):265–71. doi:10.1111/j.1365-2893.2009.01070.x.
177. Miyake Y, Kobashi H, Yamamoto K. Meta-analysis: the effect of interferon on development of hepatocellular carcinoma in patients with chronic hepatitis B virus infection. J Gastroenterol. 2009;44 (5):470–5. doi:10.1007/s00535-009-0024-z.
178. Lai CL, Yuen MF. Prevention of hepatitis B virus-related hepatocellular carcinoma with antiviral therapy. Hepatology. 2013;57(1):399–408. doi:10.1002/hep.25937.
179. Liaw YF, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med. 2004;351(15):1521–31. doi:10.1056/NEJMoa033364.
180. Matsumoto A, Tanaka E, Rokuhara A, Kiyosawa K, Kumada H, Omata M, et al. Efficacy of lamivudine for preventing hepatocellular carcinoma in chronic hepatitis B: a multicenter retrospective study of 2795 patients. Hepatol Res. 2005;32(3):173–84. doi:10.1016/j.hepres.2005.02.006 (The Official Journal of the Japan Society of Hepatology).
181. Yuen MF, Seto WK, Chow DH, Tsui K, Wong DK, Ngai VW, et al. Long-term lamivudine therapy reduces the risk of long-term complications of chronic hepatitis B infection even in patients without advanced disease. Antiviral Ther. 2007;12(8):1295–303.
182. Eun JR, Lee HJ, Kim TN, Lee KS. Risk assessment for the development of hepatocellular carcinoma: according to on-treatment viral response during long-term lamivudine therapy in hepatitis B virus-related liver disease. J Hepatol. 2010;53 (1):118–25. doi:10.1016/j.jhep.2010.02.026.
183. Papatheodoridis GV, Dimou E, Dimakopoulos K, Manolakopoulos S, Rapti I, Kitis G, et al. Outcome of hepatitis B e antigen-negative chronic hepatitis B on long-term nucleos(t)ide analog therapy starting with lamivudine. Hepatology. 2005;42 (1):121–9. doi:10.1002/hep.20760.
184. Di Marco V, Marzano A, Lampertico P, Andreone P, Santantonio T, Almasio PL, et al. Clinical outcome of HBeAg-negative chronic hepatitis B in relation to virological response to lamivudine. Hepatology. 2004;40(4):883–91. doi:10.1002/hep.20381.
185. Kurokawa M, Hiramatsu N, Oze T, Yakushijin T, Miyazaki M, Hosui A, et al. Long-term effect of lamivudine treatment on the incidence of hepatocellular carcinoma in patients with hepatitis B virus infection. J Gastroenterol. 2012;47(5):577–85. doi:10.1007/s00535-011-0522-7.
186. Hosaka T, Suzuki F, Kobayashi M, Seko Y, Kawamura Y, Sezaki H, et al. Long-term entecavir treatment reduces hepatocellular carcinoma incidence in patients with hepatitis B virus infection. Hepatology. 2013;58(1):98–107. doi:10.1002/hep.26180.
187. Wong GL, Chan HL, Mak CW, Lee SK, Ip ZM, Lam AT, et al. Entecavir treatment reduces hepatic events and deaths in chronic hepatitis B patients with liver cirrhosis. Hepatology. 2013;58 (5):1537–47. doi:10.1002/hep.26301.
188. Zoutendijk R, Reijnders JG, Zoulim F, Brown A, Mutimer DJ, Deterding K, et al. Virological response to entecavir is associated with a better clinical outcome in chronic hepatitis B patients with cirrhosis. Gut. 2013;62(5):760–5. doi:10.1136/gutjnl-2012-302024.
189. Papatheodoridis GV, Lampertico P, Manolakopoulos S, Lok A. Incidence of hepatocellular carcinoma in chronic hepatitis B patients receiving nucleos(t)ide therapy: a systematic review. J Hepatol. 2010;53(2):348–56. doi:10.1016/j.jhep.2010.02.035.
190. Park YH, Kim BK, Kim JK, Kim HC, Kim DY, Park JY, et al. Long-term outcomes of chronic hepatitis B virus infection in the era of antiviral therapy in Korea. J Gastroenterol Hepatol. 2014;29 (5):1005–11. doi:10.1111/jgh.12478.

191. Papatheodoridis GV, Manolakopoulos S, Touloumi G, Vourli G, Raptopoulou-Gigi M, Vafiadis-Zoumbouli I, et al. Virological suppression does not prevent the development of hepatocellular carcinoma in HBeAg-negative chronic hepatitis B patients with cirrhosis receiving oral antiviral(s) starting with lamivudine monotherapy: results of the nationwide HEPNET. Greece cohort study. Gut. 2011;60(8):1109–16. doi:10.1136/gut.2010.221846.
192. Zhang BH, Yang BH, Tang ZY. Randomized controlled trial of screening for hepatocellular carcinoma. J Cancer Res Clin Oncol. 2004;130(7):417–22. doi:10.1007/s00432-004-0552-0.
193. Han KH, Kim DY, Park JY, Ahn SH, Kim J, Kim SU, et al. Survival of hepatocellular carcinoma patients may be improved in surveillance interval not more than 6 months compared with more than 6 months: a 15-year prospective study. J Clin Gastroenterol. 2013;47(6):538–44. doi:10.1097/MCG.0b013e3182755c13.
194. Trevisani F, Santi V, Gramenzi A, Di Nolfo MA, Del Poggio P, Benvegnu L et al. Surveillance for early diagnosis of hepatocellular carcinoma: is it effective in intermediate/advanced cirrhosis? Am J Gastroenterol. 2007;102(11):2448–57; quiz 58. doi:10.1111/j.1572-0241.2007.01395.x.
195. Thompson Coon J, Rogers G, Hewson P, Wright D, Anderson R, Cramp M et al. Surveillance of cirrhosis for hepatocellular carcinoma: systematic review and economic analysis. Health Technol Assess. 2007;11(34):1–206.
196. Trevisani F, Cantarini MC, Labate AM, De Notariis S, Rapaccini G, Farinati F, et al. Surveillance for hepatocellular carcinoma in elderly Italian patients with cirrhosis: effects on cancer staging and patient survival. Am J Gastroenterol. 2004;99(8):1470–6. doi:10.1111/j.1572-0241.2004.30137.x.
197. McMahon BJ, Bulkow L, Harpster A, Snowball M, Lanier A, Sacco F, et al. Screening for hepatocellular carcinoma in Alaska natives infected with chronic hepatitis B: a 16-year population-based study. Hepatology. 2000;32(4 Pt 1):842–6. doi:10.1053/jhep.2000.17914.
198. Chen JG, Parkin DM, Chen QG, Lu JH, Shen QJ, Zhang BC, et al. Screening for liver cancer: results of a randomised controlled trial in Qidong, China. J Med Screen. 2003;10(4):204–9. doi:10.1258/096914103771773320.
199. Zhang B, Yang B. Combined alpha fetoprotein testing and ultrasonography as a screening test for primary liver cancer. J Med Screen. 1999;6(2):108–10.
200. Singal A, Volk ML, Waljee A, Salgia R, Higgins P, Rogers MA, et al. Meta-analysis: surveillance with ultrasound for early-stage hepatocellular carcinoma in patients with cirrhosis. Aliment Pharmacol Ther. 2009;30(1):37–47. doi:10.1111/j.1365-2036.2009.04014.x.
201. Di Bisceglie AM, Sterling RK, Chung RT, Everhart JE, Dienstag JL, Bonkovsky HL, et al. Serum alpha-fetoprotein levels in patients with advanced hepatitis C: results from the HALT-C Trial. J Hepatol. 2005;43(3):434–41.
202. Bruix J, Sherman M, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. Hepatology. 2011;53(3):1020–2. doi:10.1002/hep.24199.
203. Omata M, Lesmana LA, Tateishi R, Chen PJ, Lin SM, Yoshida H, et al. Asian Pacific Association for the Study of the Liver consensus recommendations on hepatocellular carcinoma. Hep Int. 2010;4(2):439–74. doi:10.1007/s12072-010-9165-7.
204. Asia-Pacific Working Party on Prevention of Hepatocellular C. Prevention of hepatocellular carcinoma in the Asia-Pacific region: consensus statements. J Gastroenterol Hepatol. 2010;25(4):657–63. doi:10.1111/j.1440-1746.2009.06167.x.
205. Wong CR, Garcia RT, Trinh HN, Lam KD, Ha NB, Nguyen HA, et al. Adherence to screening for hepatocellular carcinoma among patients with cirrhosis or chronic hepatitis B in a community setting. Dig Dis Sci. 2009;54(12):2712–21. doi:10.1007/s10620-009-1015-x.
206. Singal AG, Yopp A, Skinner CS, Packer M, Lee WM, Tiro JA. Utilization of hepatocellular carcinoma surveillance among American patients: a systematic review. J Gen Intern Med. 2012;27(7):861–7. doi:10.1007/s11606-011-1952-x.
207. Singal AG, Tiro JA, Gupta S. Improving hepatocellular carcinoma screening: applying lessons from colorectal cancer screening. Clin Gastroenterol Hepatol. 2013;11(5):472–7. doi:10.1016/j.cgh.2012.11.010 (The Official Clinical Practice Journal of the American Gastroenterological Association).
208. Ou DP, Yang LY, Huang GW, Tao YM, Ding X, Chang ZG. Clinical analysis of the risk factors for recurrence of HCC and its relationship with HBV. World J Gastroenterol WJG. 2005;11(14):2061–6.
209. Kumada T, Nakano S, Takeda I, Sugiyama K, Osada T, Kiriyama S, et al. Patterns of recurrence after initial treatment in patients with small hepatocellular carcinoma. Hepatology. 1997;25(1):87–92. doi:10.1053/jhep.1997.v25.pm0008985270.
210. Imamura H, Matsuyama Y, Tanaka E, Ohkubo T, Hasegawa K, Miyagawa S, et al. Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy. J Hepatol. 2003;38(2):200–7.
211. Wu JC, Huang YH, Chau GY, Su CW, Lai CR, Lee PC, et al. Risk factors for early and late recurrence in hepatitis B-related hepatocellular carcinoma. J Hepatol. 2009;51(5):890–7. doi:10.1016/j.jhep.2009.07.009.
212. Kim BK, Park JY, Kim DY, Kim JK, Kim KS, Choi JS, et al. Persistent hepatitis B viral replication affects recurrence of hepatocellular carcinoma after curative resection. Liver Int. 2008;28(3):393–401. doi:10.1111/j.1478-3231.2007.01625.x (Official Journal of the International Association for the Study of the Liver).
213. Llovet JM, Bruix J. Novel advancements in the management of hepatocellular carcinoma in 2008. J Hepatol. 2008;48(Suppl 1):S20–37. doi:10.1016/j.jhep.2008.01.022.
214. Piao CY, Fujioka S, Iwasaki Y, Fujio K, Kaneyoshi T, Araki Y, et al. Lamivudine treatment in patients with HBV-related hepatocellular carcinoma–using an untreated, matched control cohort. Acta Med Okayama. 2005;59(5):217–24.
215. Kuzuya T, Katano Y, Kumada T, Toyoda H, Nakano I, Hirooka Y, et al. Efficacy of antiviral therapy with lamivudine after initial treatment for hepatitis B virus-related hepatocellular carcinoma. J Gastroenterol Hepatol. 2007;22(11):1929–35. doi:10.1111/j.1440-1746.2006.04707.x.
216. Kubo S, Tanaka H, Takemura S, Yamamoto S, Hai S, Ichikawa T, et al. Effects of lamivudine on outcome after liver resection for hepatocellular carcinoma in patients with active replication of hepatitis B virus. Hepatol Res. 2007;37(2):94–100. doi:10.1111/j.1872-034X.2007.00013.x (The Official Journal of the Japan Society of Hepatology).
217. Urata Y, Kubo S, Takemura S, Uenishi T, Kodai S, Shinkawa H, et al. Effects of antiviral therapy on long-term outcome after liver resection for hepatitis B virus-related hepatocellular carcinoma. J Hepato Biliary Pancreat Sci. 2012;19(6):685–96. doi:10.1007/s00534-011-0489-z.
218. Wu CY, Chen YJ, Ho HJ, Hsu YC, Kuo KN, Wu MS, et al. Association between nucleoside analogues and risk of hepatitis B virus-related hepatocellular carcinoma recurrence following liver resection. JAMA. 2012;308(18):1906–14.
219. Yin J, Li N, Han Y, Xue J, Deng Y, Shi J, et al. Effect of antiviral treatment with nucleotide/nucleoside analogs on postoperative prognosis of hepatitis B virus-related hepatocellular carcinoma: a two-stage longitudinal clinical study. J Clin Oncol. 2013;31

(29):3647–55. doi:10.1200/JCO.2012.48.5896 (Official Journal of the American Society of Clinical Oncology).
220. Hann HW, Coben R, Brown D, Needleman L, Rosato E, Min A, et al. A long-term study of the effects of antiviral therapy on survival of patients with HBV-associated hepatocellular carcinoma (HCC) following local tumor ablation. Cancer Med. 2014;3(2):390–6. doi:10.1002/cam4.197.
221. Zhou Y, Zhang Z, Zhao Y, Wu L, Li B. Antiviral therapy decreases recurrence of hepatitis B virus-related hepatocellular carcinoma after curative resection: a meta-analysis. World J Surg. 2014;38(9):2395–402. doi:10.1007/s00268-014-2586-z.
222. Society AC. California Cancer Facts & Figures. 2014. http://www.ccrcal.org/.