Viral Hepatitis

Digestive Diseases

Dig Dis 2013;31:138–151
DOI: 10.1159/000347209

Direct Effects of Hepatitis B Virus-Encoded Proteins and Chronic Infection in Liver Cancer Development

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Key Words
Direct viral factors in hepatocarcinogenesis · Liver cancer · Hepatocellular carcinoma · Chronic hepatitis B infection · Direct role of viral proteins · Hepatocarcinogenesis

Abstract
Hepatocellular carcinoma (HCC) ranks as the third leading cause of cancer-related death worldwide with currently limited treatment options. Chronic hepatitis B virus (HBV) infection accounts for HCC development in more than 50% of cases. The lifetime risk of HBV carriers to develop cirrhosis, liver failure or HCC is estimated to be as high as 15–40%. Although several pathways and triggers contributing to HCC development have been described, many features of hepatocellular carcinogenesis and the attributed direct role of viral factors remain elusive. Host genetic factors, the geographic area and epidemiologic factors, as well as the direct risk related to chronic HBV and hepatitis C virus (HCV) infections, account for geographical and gender differences of HCC prevalence. There is growing evidence that hepatocarcinogenesis is a multistep process. Human HCC is typically preceded by chronic inflammation and apoptotic and non-apoptotic cell death with compensatory liver proliferation. However, we still lack a thorough understanding of the common underlying molecular mechanisms. High levels of HBV replication and chronicity of inflammation are known to independently increase the risk for HCC. A direct carcinogenic role of viral factors is very likely to contribute to liver cancer since HCC is known to also occur in noncirrhotic livers of individuals with an inactive chronic or even with occult HBV infection with no significant histological signs of inflammation or cytopathic effects. Furthermore, synergistic or independent viral risk factors for primary liver cancer development have been described, such as HBV genotype, integration of viral DNA into the host genome and direct effects of viral proteins. A broader understanding of these viral factors in hepatocarcinogenesis might give rise to new diagnostic and therapeutic means in the future. We review the current state of research in liver cancer development and focus on the role of direct viral factors in HBV infection.

Introduction

Worldwide, hepatocellular carcinoma (HCC) is the sixth most prevalent cancer with chronic hepatitis B virus (HBV) infection and subsequent development of liver cirrhosis accounting for more than 50% of HCC cases

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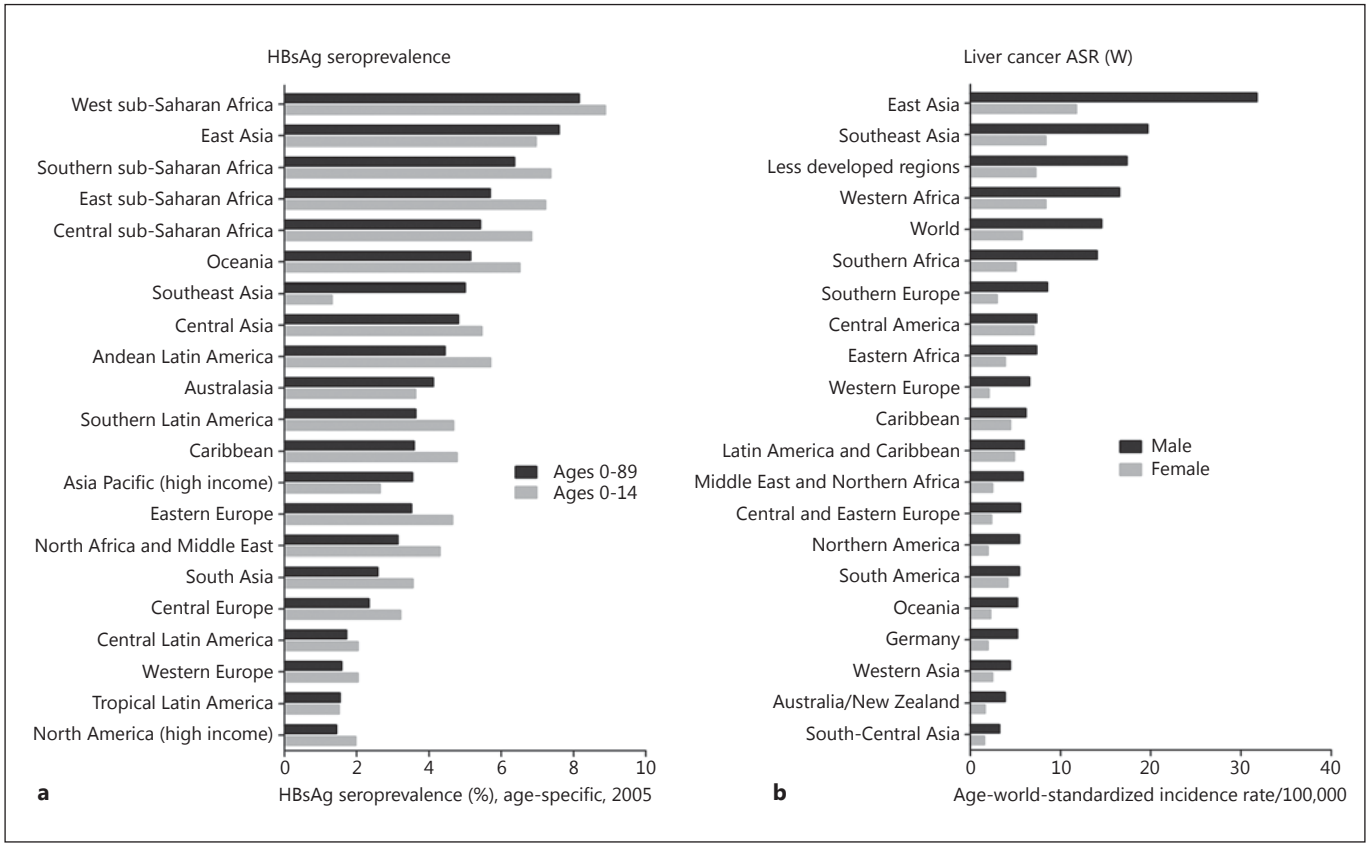

Fig. 1. a Seroprevalence of HBsAg-positive carriers, shown for different geographic regions and age groups (adapted from Ott et al: 2012 [10]). **b** Age-world-standardized incidence rates of liver cancer per 100,000, shown for different regions of the world and for men and women (GLOBOCAN 2008, IARC).

globally [1]. Due to late diagnosis and limited treatment options, HCC is the third most frequent cause of cancer-related death worldwide. In North America, Northern Europe and Australia, the annual incidence of HCC is less than 5/100,000 per year, compared to incidence rates of 10 to >20/100,000 per year in parts of sub-Saharan Africa and Southeast Asia [2]. In some countries in Africa, HCC has even been reported to be the most frequent overall cause for cancer-related death [3]. Up to 80% of the HCC cases worldwide are regarded to be related to HBV or hepatitis C virus (HCV) infection [1, 3].

Epidemiology of HBV Infection

Chronic HBV infection affects about 240 million people worldwide and hepatitis B-related end-stage liver disease and associated HCC are estimated to account for more than 600,000 deaths per year [4, 5]. The lifetime risk of HBV carriers to develop cirrhosis, liver failure or HCC is estimated to be as high as 15–40% [6–9]. The prevalence of HBV infection and the age of individuals infected vary largely in different geographic regions, which at least partially accounts for the different prevalence of HCC (fig. 1a). Furthermore, the implementation of vaccination programs contributes to an even greater variation in epidemiology of the disease [10].

While less than 5% of infected adults develop chronic infection, children have a drastically higher risk to become chronic carriers: approximately 30% with the risk increasing up to 90% when infected perinatally [11]. Therefore, age at infection and race-specific rates in viral spread among different regions as well as diverse distribution of viral genotypes, environmental and host genetic differences strongly account for incidence and outcome of HCC in different populations [1]. The infection route determining the titer of the virus inoculum and the age at the time of infection is of special importance since the risk of

developing a chronic infectious disease drastically decreases with age [12].

There has been substantial progress in understanding the molecular mechanisms and risk factors of primary liver cancer. HBV is a noncytopathic virus, thus the host immune response to the virus and the grade of inflammation determine the severity of liver damage and fibrosis [13]. Subsequent chronic inflammation, cell death and compensatory hepatocyte proliferation, as well as profibrotic effects of an altered cytokine network, are well-known key players in HCC development [14]. HBV usually persists as an episome, but HBV DNA can integrate into host cellular DNA and may drive HCC development by promoting genetic alterations, although the integrate genomes are dead and can no longer drive HBV replication. In addition, expression of HBV proteins and gene products of sporadically truncated HBV genes from integrated HBV DNA may have a direct carcinogenic effect [14, 15]. Thus, an important question remaining is the attributable direct role of HBV proteins in hepatocarcinogenesis. A broader understanding of HBV proteins and sporadically truncated versions like HBV X protein (HBx) or HBV-spliced protein in the pathogenesis of HCC may provide central new insights for diagnostic and therapeutic options.

Transmission and Molecular Characteristics of HBV Infection

Human HBV is a member of the Hepadnaviridae family and infects the liver of humans and humanoid primates. It is characterized by very high host and cell tropism, and only infects hepatocytes of humans or humanoid primates. The most important route of transmission worldwide is vertical transmission from infected mothers to their offspring, which is hard to prevent by vaccination programs [16, 17]. After the neonatal period, HBV is predominantly transmitted by percutaneous or mucosal exposure to infected blood.

HBV has a very small DNA genome size (3.2 kb) and a unique replication through reverse transcription [18]. The circular DNA genome of HBV is only partially double stranded. Within the protein capsid, it is transported into the nucleus of hepatocytes after entering the cell. In the nucleus, the partially double-stranded HBV DNA is then converted into a double-stranded covalently closed circular DNA (cccDNA) by the host cell machinery. HBV cccDNA forms a minichromosome and serves as a transcription template for all viral mRNAs. The four overlapping open reading frames of the HBV genome encode for the core protein forming the viral capsid (HBcAg), with its secreted counterpart HBeAg, the small (S), medium (M) and large (L) envelope proteins forming subviral particles (HBsAg), the viral polymerase, which has a reverse-transcriptase and an RNase domain, and HBx [19].

Perinatal infection (vertical transmission) usually occurs shortly before or during birth with the highest transmission rates when the mother is HBsAg- and HBeAg-positive. A 90% risk of developing chronic HBV infection is found if the recommended active and passive vaccination is not applied instantly after birth [20]. Globally, there has been an overall decrease in HBeAg prevalence recently reported, most prominent in young girls in Oceania, South Asia and Southeast Asia (23.3–14% decline). However, in these regions, prevalence is persistently high at 67% among young women and only small decreases have been detected in women of reproductive age [17]. Thus, vertical transmission is rare in Europe and Africa, yet remains a huge health burden in parts of Asia. In a recent Taiwanese study, the risk for chronic HBV infection in children born to HBeAg-positive mothers was shown to be significantly greater than for children with HBeAg-negative mothers chronically infected with HBV (54 vs. 17%, respectively) [21].

Another important cause of chronic HBV infections is horizontal transmission in early childhood. Children who were 1–5 years of age at the time of infection have a risk for persistence of HBV infection of 20–30%. With age this risk drops and resolution of hepatitis B with HBsAg seroclearance occurs in more than 95% of adult patients [22]. In adults, horizontal modes of transmission such as sexual transmission, intravenous drug abuse and health care-associated infections are most prominent in low-risk areas with risk of transition to chronicity reported to be even <1% in immune-competent individuals [16, 23]. Childhood vaccination programs have been able to drastically reduce horizontal HBV infection. Despite inclusion into vaccination programs, however, 1.9–3% of young adult Taiwanese became chronic HBV carriers, with some carrying a vaccine-escape mutant [24, 25]. After vertical transmission, 15% became chronic carriers despite combined active/passive vaccination [24].

In low-prevalence areas such as Western Europe, the United States and Central Latin America, the rate of HBV carriers is about 0.1–2% (fig. 1a). However, within these countries there are differences between areas and ethnic groups. The prevalence of HBV infection within the Unit-

ed States, for example, is higher among African Americans, Hispanics and Asians than in the Caucasian population, and high carrier rates can also be found among the Alaskan Inuit [26]. Intermediate-prevalence areas with rates from 3 to 5%, such as Japan, India and the Mediterranean countries, show a larger rate of HBV infections among young children, which account for most of the chronic infections. An even higher horizontal spread during the first 2 years and often also perinatal transmission with high numbers of resulting chronically infected individuals is found in high-prevalence areas such as East Asia, sub-Saharan Africa, Andean Latin America and Alaska, with rates between 10 and >12% [10].

The Natural Course of HBV Infection

HBV infection may (but does not have to) lead to hepatitis B – a necroinflammatory liver disease. Hepatitis B may manifest with clinical symptoms, such as jaundice, malaise, general fatigue and elevated liver enzymes ranging from asymptomatic infection to fulminant hepatitis. Clinical manifestation, as well as chronicity, depends on various viral and host factors (for review see [13, 22]). Differential diagnoses include hepatotropic viruses such as hepatitis A, C or E viruses, adeno- or herpes viruses, toxins, and autoimmune diseases.

HBVs are prototype noncytopathic viruses [18]. Viral factors for HBV escaping the immune response and causing persistent infection include escape from recognition and induction of regulatory cytokines (stealth strategy), hepatocyte-restricted viral gene expression, and high-level circulating viral antigens, which cause clonal deletion and block the activity of neutralizing antibodies [13]. The stable extrachromosomal transcription template (cccDNA) seems resistant to drug- or cytokine-mediated clearance. In addition, the liver has a unique immunoregulatory function including immunosuppressive mediators, which help to prevent inadvertent organ damage, but also renders the liver an attractive target site for pathogens such as HBV [13]. The correlation between strong HBV-specific T cell responses and virus clearance is well established. Yet, factors responsible for shifting the balance from immune tolerance to immune clearance are hardly understood [27].

The natural course of chronic HBV infection can be divided into specific phases of pathogen host interactions (fig. 2).

A first period with high viremia but low inflammatory activity is particularly found in children and young adults after vertical transmission, with strong positivity for HBeAg and HBV DNA >100,000 copies/ml (20,000 IU/ml). Obvious signs of liver damage are usually lacking and this phase is therefore characterized by a high viremic HBsAg carrier state [28]. These patients show the highest HBV-specific T cell responses [29], although they had wrongly been classified as immunotolerant for a long time.

The second phase is the phase when most infections are diagnosed. Due to detectable liver inflammation, it is referred to as HBeAg-positive chronic hepatitis B. Here the adoptive immune response leads to necroinflammation of the liver and compensatory hepatocyte proliferation. HBV DNA levels, as well as the grade of liver damage, are variable and may fluctuate, and HBeAg may be cleared as anti-HBe levels raise. Liver fibrosis may develop due to necroinflammation ranging from moderate to severe histological manifestation. Progression to liver cirrhosis and HCC is possible. The rate of spontaneous HBsAg clearance ranges from 3 to 12% [23].

Most of the patients (80–90%) reach HBeAg clearance in phase 2 and enter a third phase with seroconversion to anti-HBe ('anti-HBe-positive chronic hepatitis B'). These HBsAg carriers show low viral replication of 10,000 copies/ml (<2,000 IU/ml) and often only minor signs of liver damage, typically with slow disease progression. Some of these patients have already developed liver fibrosis or cirrhosis, which may even progress over time [30, 31]. About 15% relapse to active hepatitis. Most patients, however, remain inactive HBV carriers and 8.1% in 10 years or 40% in 25 years, respectively, may spontaneously clear HBV infection and become HBsAg negative [32].

However, there is also a reactivation phase in which 20–40% of patients may reactivate the infection/disease and show an 'HBeAg-negative/anti-HBe-positive chronic hepatitis B'. Patients have again higher HBV DNA levels >10,000 copies/ml (2,000 IU/ml), ALT elevation and signs of progressive liver damage. These patients have an increasing risk for cirrhosis and HCC. In these cases, additional viral factors, such as HBV genotype and mutations like basal core promoter (BCP) mutation are reported to play an important role [33]. HBeAg-negative chronic hepatitis B cases are reported to represent by far the most common type of hepatitis B, particularly in European, African and Middle East countries of the Mediterranean basin [34]. Even though there have been great improvements in understanding the natural history of chronic hepatitis B infection, new insights are needed for individualized management algorithms [13].

Liver fibrosis or cirrhosis as a long-term histologic consequence of chronic necroinflammation is common-

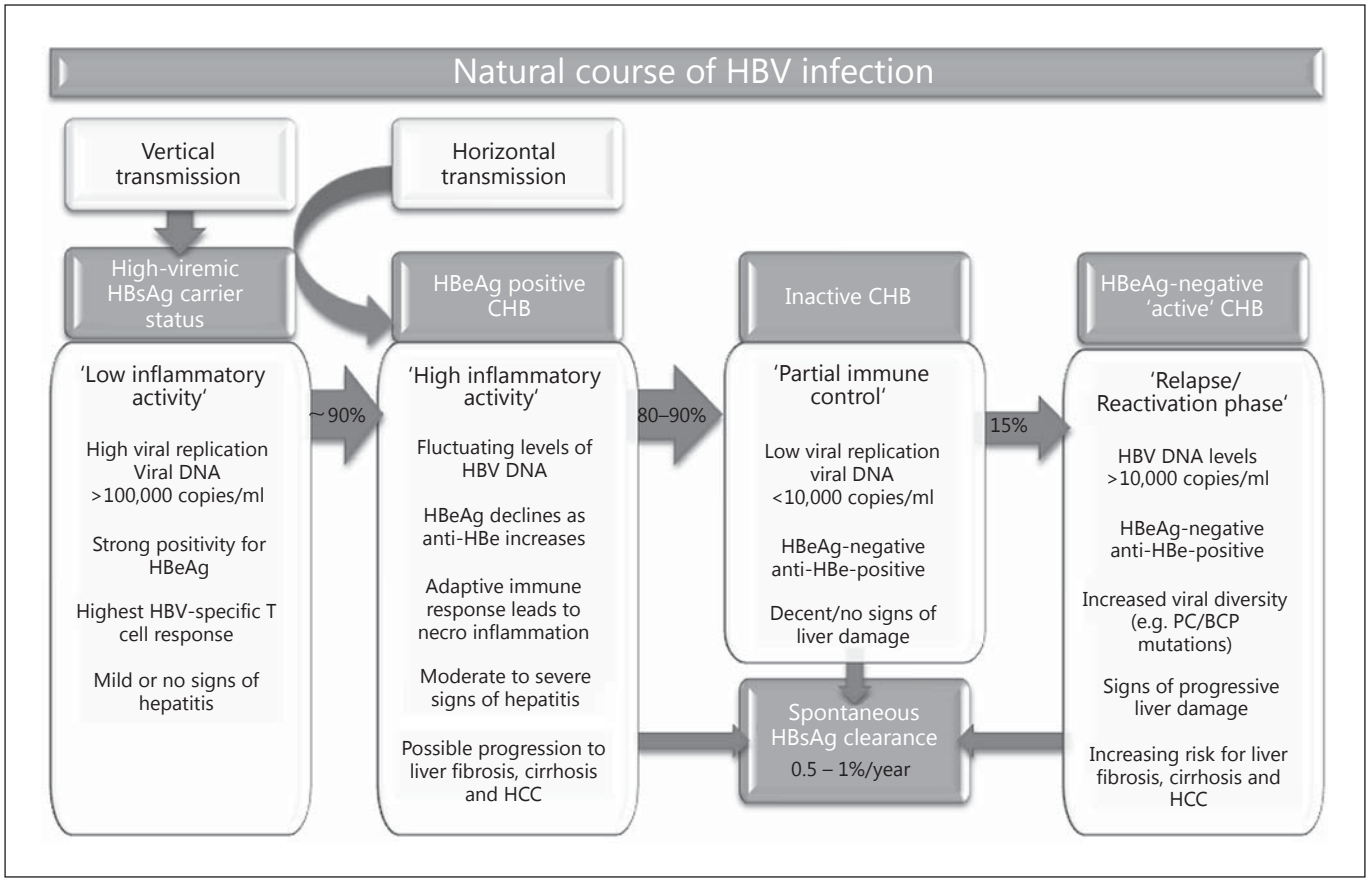

Fig. 2. Natural history of chronic HBV infection. Modified from [11, 34]. CHB = Chronic hepatitis B.

ly found in chronic hepatitis B, with an estimated lifetime risk of approximately 40%. Cirrhosis can be found in 80–90% of liver autopsies bearing HCC worldwide; thus, HCC develops in 10–20% in livers lacking any sign of cirrhosis [35]. Cirrhosis incidence and age of manifestation vary due to clinical status and geographic area. Inactive carriers in Northern Europe and America have a relatively low risk of cirrhosis development (<1/100 person years). In Europe, incidence in patients with HBeAg-positive hepatitis is lower than in HBeAg-negative patients (up to 3.8 as compared to 9.7 per 100 person-years) [36].

Epidemiology and Common Risk Factors for HCC

With 695,900 deaths related to liver cancer, HCC accounts for 70–85% of all liver cancer cases [2]. In North America, Western Europe and Australia, the annual incidence of HCC is less than 4/100,000 per year, compared to incidence rates of up to 50/100,000 per year in parts of sub-Saharan Africa and Southeast Asia (fig. 1b). With 80% of the HCC cases worldwide related to viral hepatitis [1], up to 54.4% are estimated to be attributable to HBV infection. The attributed fraction is lower in developed countries (23.3%) than in developing countries (58.8%) [3], reflecting the variable burden of chronic HBV infection between different areas worldwide.

Besides chronic HBV or HCV infection, other important etiologies for HCC are alcoholic liver disease, nonalcoholic fatty liver disease (NAFLD; developing into nonalcoholic steatohepatitis), certain metabolic liver disease such as hereditary hemochromatosis, α-1-antitrypsin deficiency and porphyria cutanea tarda, autoimmune liver disease such as primary biliary cirrhosis, or autoimmune hepatitis or carcinogen exposure like dietary aflatoxin uptake [37].

High-prevalence areas of chronic HBV infection, such as sub-Saharan Africa and East Asia, show the highest HCC incidence reported (>20 per 100,000 individuals). Low-incidence rates <5 cases per 100,000 individuals can be found in areas like Northern Europe, Northern America and Oceania (fig. 1b) [1]. Cirrhotic patients are reported to have a 5-year cumulative risk for developing HCC of 17% in East Asia and 10% in Western Europe and the United States, and the 5-year liver-related death rate is 15% in Europe and 14% in East Asia [36]. While the HCC rate in HBV high-incidence countries correlates well with HBV infection, in low-risk Western countries such as the United States, alcohol-related cirrhosis and NAFLD are described to account for the majority of HCC [38]. Therefore, HCC incidence is reported to increase in some areas such as the United States and Central Europe due to increasing prevalence of NAFLD and the rise in aged long-term HCV carriers, while it is decreasing in some high-risk areas due to HBV vaccination programs [39].

Table 1. Known risk factors or synergistic effects reported to increase the risk for HCC in patients with chronic HBV infection

Host and viral factors	– High serum virus titer (>10,000 copies/ml) – Age >40 years – Male – Asian or African ancestry – Positive family history of HCC – Genetic diversity, likely to be involved Other examples of host genetic factors: TNFα-238 polymorphism [108], or glutathione S-transferase variants (GSTM1 null genotype) [109]
Environmental factors	– Regular alcohol consumption, alcoholic steatohepatitis – Exposure to aflatoxin or other carcinogens – NAFLD
Active liver disease/ inflammation	Human HCC is typically preceded by chronic inflammation, apoptotic and nonapoptotic cell death with compensatory proliferation [53, 62] Correlating with this risk are: – Stage of liver fibrosis/cirrhosis – Grade of inflammation, ALT elevation – HBV DNA levels above 10,000 copies (2,000 IU/ml) – HBeAg serum levels (in patients with low HBV viral load)
Coinfections	– Coinfections with HDV or HCV – Severe flares of hepatitis in patients coinfected with HIV

Modified from [1, 11, 35, 36].

Role of HBV in HCC Development

Chronic HBV infection remains the most important risk factor for HCC worldwide [2], with the level of viremia being the number one risk factor [40–42]. However, HBsAg seroprevalence in HCC cases varies greatly between areas (from 70% South Korea to 3% Sweden) [1] and thus reflects differences in the prevalence of chronic HBV infection. Synergistic effects that have been reported to increase the risk for HCC in chronic HBV-infected individuals (table 1) can be first divided into demographic factors such as Asian or African ancestry, male sex or advanced age, secondly into environmental factors such as heavy alcohol intake, exposure to aflatoxin or NAFLD, and thirdly into viral factors, such as duration of infection, higher levels of HBV replication, viral coinfections (HBV plus HCV, hepatitis D virus or HIV infection), HBV genotype, integration of viral DNA into the host genome or direct effects of viral proteins [11].

To reveal the individual risk of a chronic HBV carrier for liver damage, progression to cirrhosis and HCC development factors such as genotype C/D, high serum level of HBV DNA, BCP mutation, high serum level of HBsAg, pre-S deletion/truncation or high serum level of HBV DNA have been proposed [42] (table 2).

Several studies have demonstrated that HBV DNA levels above 10^4 to 10^5 copies/ml (i.e. 2,000 or 20,000 IU/ml) show an elevated risk for HCC and increasing HBV DNA levels correlate strongly with an increased risk of progression to cirrhosis independent of HBeAg status and serum alanine transaminase level [40, 41, 43]. A prospective Taiwanese cohort study likewise showed the incidence of HCC to increase with serum HBV DNA level in a dose-response relationship, with serum HBV DNA levels >10^4 copies/ml being a strong risk predictor of HCC independent of HBeAg, serum alanine aminotransferase level and liver cirrhosis. The corresponding cumulative incidence rates for HCC range from 1.3% for an HBV DNA level of <300 copies/ml to 14.9% for an HBV DNA level >1 million copies/ml [40]. This correlation of high viral loads and risk of progression to HCC points to the importance of subsequent inflammation correlated with high phases of viral replication.

Furthermore, HBeAg serum levels have recently been reported as a useful complement for predicting HCC development in patients with low HBV viral load [42]. Be-

Table 2. Proposed direct viral factors in HBV-induced hepatocarcinogenesis

Viral factor	Proposed effects
HBV genotype and mutations	– Increased risk for HCC in genotype C + BCP (A1762T/G1764A) mutation > BCP mutation alone > PC (G1896A) mutation > genotype C alone > genotype B +/– PC mutation [8] – Genotypes A1, F1, G, Ba > A2, Bj [33]
HBV DNA integration	– Chromosomal deletions [73] – Chromosomal translocations – Production of fusion transcripts – Cis-/transactivation (e.g. NTRK2, IRAK2, p42/MAPK1, hTERT) [71]
HBx and truncated X protein	– Antiapoptotic and proapoptotic actions – Silencing of tumor suppressor genes; induction of chromosomal instability [83] – Alteration of p53 binding site selectivity [84] – Cell proliferation through elevation of cytosolic calcium signals [86] – Increased telomerase reverse transcriptase expression [87] – Additionally, natural HBx variants are frequently found in HCC samples [96] – C-terminal truncated HBx correlates with venous invasion – C-terminal truncated HBx activates C-Jun transcription and MMP10 activity [96] – C-terminal truncated HBx promotes EMT [98]
HBSP	– Induction of hepatoma cell aggressiveness, partly by increasing motility and invasion – Activates MAPK and Akt signaling pathway [100, 101]
Pre-S2/ pre-S2 mutants	– Triggers apoptosis through induction of intracellular ROS [70, 103] – Causes aberrant cyclin A expression [106] – PKC-dependent activation of c-Raf-1/Erk2 signaling increases hepatocyte proliferation [104]

EMT = Epithelial-mesenchymal transition; hTERT = human telomerase reverse transcriptase; IRAK2 = IL-1R-associated kinase 2; p42MAPK1 = p42 mitogen-activated protein kinase 1; MMP10 = matrix metalloproteinase 10; NTRK2 = neurotropic tyrosine receptor kinase 2.

sides these easy-to-obtain variables, a liver biopsy might provide further information on the grade of inflammation and fibrosis and therefore help to identify individuals at high risk. However, there is still a high need for noninvasive biomarkers that help identify patients at risk.

Do HBV Genotypes Correlate with Different Risks for HCC?

Eight HBV genotypes and several subgenotypes have been described. Genotypes differ in at least 8% of the whole-genome sequence; subgenotypes differ in their genome sequence to a lesser extent. HBV genotypes show an area-specific distribution with genotype A1 predominantly found in sub-Saharan Africa, A2 in Northern Europe and A3 in Western Africa. The subgenotypes of HBV genotype B1 and 6 (Bj) are common in Japan and the Arctic and B2–5 (bearing a recombination of parts of the core region of genotype C, Ba) in other parts of Asia. Genotype C is usually found in China, Korea and Southeast Asia. Genotype D is predominant in the Mediterranean area, but also common in Eastern Europe, North America and India. Genotype E is found in West Africa, HBV genotype G has been found only in small areas of the world, and the genotypes F and H are found in Alaska and Central and South America [44]. However, migration and an increase in traveling are influencing the distribution of HBV genotypes.

Several studies reported differences in response rates to antiviral therapy due to different genotypes. For example, HBV genotype B seems to be associated with a higher rate of IFN-induced HBeAg clearance as compared to genotype C [45]. Prolonged follow-up of 258 Spanish patients showed sustained biochemical remission, clearance of HBV DNA and clearance of HBsAg to be more likely in genotype A than in genotype D, and death related to liver disease to be more frequent in genotype F than in genotype A [46]. Genotype D was reported to show a better response under antiviral therapy (lamivudine and/or adefovir) as compared to genotype A [47]. In order to identify significant differences in the outcome following the use of nucleos(t)ide analogues, larger prospective studies are needed.

Taking into account the difference in genotype distribution due to the specific geographic location and the different endemic rates in ethnic groups together with different area-specific ways of predominant transmission and rates of synergistic risk factors, it appears difficult to dissect the distinct role of different genotypes in the natural course of HBV-related liver disease.

For instance, genotype A2 is frequently found in Northern Europe and the United States and is associated with a greater chance of resolution of active hepatitis, as well as clearance of HBV DNA and HBsAg with lower risk of HCC. In contrast, genotype A1 is most frequently found in sub-Saharan Africa with a high association with

HCC in young men often anti-HBe-positive and rarely showing cirrhosis [33]. In genotypes B2–5, HCC seems to occur at younger age than in B1 carriers and genotype C was even reported to be an independent risk factor for HCC development in addition to liver cirrhosis [48] (see table 2). Overall, there seems to be more frequently progression to HBV-related liver disease in patients with genotypes A1, C, Ba and F1 than genotypes A2 and Bj [44].

Another important risk factor is HBV genome variation, sometimes accumulated during the natural history of chronic HBV infection. The most frequently reported variants are a double substitution: A1762T and G1764A in the BCP region of HBV and a precore G1896A mutant. Even after adjusting for HBV genotype, BCP was described as an independent risk factor for HCC [8]. The precore mutant has been associated with liver damage and especially with anti-HBe-positive immune active HBV infection, while its role in HCC development seems to be dependent on the genotype [11]. However, further studies focusing on HBV genotyping are needed to help to dissect the course of disease, treatment response and other viral factors from epidemiologic risk factors.

Lessons Learned from Inflammation-Driven HCC Mouse Models and Revealed Molecular Mechanisms

Up-to-date distinct animal models are essential to mimic and understand human disease with respect to molecular, cellular and pathophysiological mechanisms of HCC development in humans. Human HCC is typically preceded by chronic inflammation and apoptotic and nonapoptotic cell death with compensatory proliferation, fibrosis and cirrhosis. This reflects the capacity of the liver to regenerate after damage by proliferation of fully differentiated hepatocytes. For instance, after a 2/3 partial hepatectomy, a sequence of well-orchestrated cellular events is initiated which leads to proliferation of the normally quiescent organ to ultimately restore liver function and size as demonstrated in mouse models [49].

HBV as well as HCV are described to be noncytopathic viruses and liver damage is mainly caused by the influx of immune cells and destruction of infected hepatocytes [50]. The different phases of immune response and viral replication exhibit specific risks for subsequent liver damage (see above). Hence, a sequence of events can be reconstructed in patients who have HCC with an underlying chronic HBV infection: the inability of the patient's immune system to resolve an HBV infection leads to chronic necroinflammation and compensatory proliferation of hepatocytes, which subsequently may develop to liver fibrosis and cirrhosis. Notably, these processes take many years or even decades in the human situation, which makes it almost impossible to reproduce these pathokinetic processes.

These findings are partially reflected in a number of mouse models dissecting the role of chronic inflammation, hepatocyte proliferation, apoptosis and changed cytokine networks in the pathophysiology of HCC development (reviewed in [51]). In chronic HBV infection, immune-mediated liver injury by activated HBV-specific T cells and compensatory liver proliferation drive selection of cellular clones. This was shown to be a main determinant for HCC development in a woodchuck model with chronic woodchuck hepatitis virus inflammation [52]. This as well as the hepatocyte-specific Mcl-1 knockout mouse model [53] reflect the importance of compensatory proliferation and deregulated hepatocyte apoptosis in the course of chronic liver injury and hepatocarcinogenesis.

However, it remains elusive to what extent these mouse models reflect the pathophysiology and the clinical picture of human HCC subtypes and especially HBV-related HCC. Furthermore, a potential successful therapy of such models could indeed be extrapolated to the human situation. For instance, a frequently used and reproducible model for chemical agent-induced hepatocarcinogenesis in mice is the chemical carcinogen diethylnitrosamine (DEN), which induces liver tissue damage, genomic instability and gene mutations after metabolic activation in hepatocytes [54]. However, in DEN-induced carcinogenesis, DNA-damage precedes or is concomitant with acute inflammation. This is contradictory to the most common findings in humans, where HCC develops on the basis of chronic inflammation in the absence of chemical mutagens and DNA damage is believed to be secondary [55]. Moreover, it is not clear whether the molecular and cellular events in DEN-treated mice phenocopy those found in the human situation. However, using this model various signaling pathways have been identified (e.g. Stat3 signaling) that also play an important role in human hepatocarcinogenesis [56–58].

Another example for important findings in mouse models, yet producing divergent results, is the role of the transcription factor NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) in hepatocarcinogenesis. The cytokines lymphotoxin (LT) α and β and their receptor (LTβR) are frequently upregulated in HBV- or HCV-induced hepatitis and HCC [59]. Consistently, a study using mice expressing LTαβ in a liver-specific man-

ner (Alb-LTαβ) demonstrated LTαβ-driven development of chronic liver inflammation and HCC. This suggests a causal and procarcinogenic link between constitutive NF-κB activation in hepatocytes during liver cancer development [59]. Similarly, a study using Mdr2-knockout mice, which spontaneously develop cholestatic hepatitis followed by HCC [60], revealed NF-κB- and TNFR1-signaling to be an inflammation-associated tumor promoter.

Paradoxically, an antitumorigenic role of NF-κB had been implied using a hepatocyte-specific IKKβ conditional knockout mouse (a component of the IKK complex necessary to activate NF-κB) in a DEN-induced HCC model [58]. Consistently, an antitumorigenic role was described using hepatocyte-specific deletion of NEMO [I kappa B-kinase (IKK)-subunit NF-κB essential modulator] resulting in spontaneous liver inflammation and HCC development [61]. Nonetheless an NF-κB-independent protumorigenic function of NEMO was described in a model with conditional ablation of TGF-β-activated kinase 1 [62].

Such conflicting results gained from different HCC animal models led to the current hypothesis that homeostasis is crucial for preventing tumorigenesis in the liver. Thus, the same molecule can display an either pro- or antioncologic role in malignant growth due to aberrant gain or loss of function or due to the mouse model used (for review see [55, 63]).

Various different signaling cascades have been described as key players in hepatocarcinogenesis as for instance: several growth factor tyrosine kinase receptors, including epidermal growth factor receptor, insulin-like growth factor receptor and the hepatocyte growth factor receptor MET, as well as downstream signaling pathways, such as RAS/mitogen-activated protein kinase pathway, the PI3K/AKT/mTOR pathway and the Wnt/β-catenin pathway [63–66]. However, novel oncogenic approaches and refined animal models are needed to identify novel tumor drivers as targets and predictors for therapeutic strategies in HCC.

Furthermore, it will be absolutely important to better characterize the in vivo models established to date on molecular, histological and genetic levels, in order to exactly define which murine HCC model reflects to which parts the multiple human HCC subtypes. Recent progress using comprehensive genomic tools started to identify the broad molecular diversity in human HCC and several molecular classifications have been proposed [67]. This complexity is also exemplified by genetic alterations reported to be markedly different in HCC of patients chronically infected with HCV- and HBV-related HCC [15].

Possible Direct Involvement of Viral Factors in Hepatocarcinogenesis

The Role of HBV Integration and Occult HBV Infection in Carcinogenesis

Integration of HBV DNA into the genome of host hepatocytes was already reported over 30 years ago. Although being detrimental for the virus, it is a dynamic process and precedes HCC development [68], but its definite role in the course of hepatocellular carcinogenesis still remains controversial.

HBV has a small, circular DNA genome with overlapping open reading frames and thus it uses its reverse transcription during replication [19]. Viral DNA integration into the cellular DNA disrupts open reading frames and prevents virus replication, but may allow for expression of single HBV proteins. It is believed that a linear double-stranded HBV DNA formed during replication is the preferred substrate for integration [38]. Conditions disrupting host genomic stability, such as oxidative stress, impaired DNA repair or high hepatocyte turnover due to inflammation and/or coinfections are known to increase the dynamic rates of HBV DNA integration [69, 70]. Insertions of HBV DNA into host cell DNA are found in 85–90% of HBV-related HCC cases as single or multiple discrete HBV integrations. These integrations may lead to chromosomal deletions, translocations, the production of fusion transcripts and generalized genomic instability, which might give rise to selection and clonal expansion of a pre-/neoplastic hepatocyte [38]. Besides induction of chromosomal instability, the insertion of viral DNA into the cellular genome might lead to cis-/transactivation, which results in modification of cellular gene expression, possibly inducing malignant transformation. In human HBV-related HCC, there seems to be no specific host genome region or oncogene where integration typically occurs. Although no clear pattern can be identified, several genes important for proliferation, apoptosis and cell signaling were described to be frequently altered due to HBV DNA insertions [15]. For example, integration of HBV DNA into key regulatory cellular genes, such as neurotropic tyrosine receptor kinase 2, IL-1R-associated kinase 2, p42 mitogen-activated protein kinase 1 and even frequently near human telomerase reverse transcriptase are frequently reported [71]. Hepatocytes harboring such insertions might be selected during carcinogenesis and therefore account for the relationship between fragile sites and detected integration events. HBV DNA integration may especially play a role in patients with HBV-induced HCCs occurring in the context of a non cirrhotic liver of chronically HBV-infected children and

young adults [38], or even an occult infection among patients negative for HBsAg.

The association between occult HBV infection (persistence of viral DNA after integration in patients negative for HBsAg) and HCC also remains controversial. A meta-analysis of 16 studies reported a significantly increased risk for HCC in patients with occult HBV infection in comparison with noninfected controls [72]. The current debate on the significance of occult infections in HCC development is ongoing.

A supporting fact might be the substantial risk for HCC in carriers of inactive HBV as compared to individuals not infected with HBV, with age and alcohol intake being independent predictors [31]. However, further population-based studies are needed to provide convincing data for occult infections being an independent risk factor for HCC. The integration of subgenomic HBV DNA fragments into many different locations within the host DNA may promote genetic instability during liver regeneration and genetic instability associated with integration potentially alters the expression of oncogenes, tumor suppressor genes and microRNAs, which may contribute importantly to tumorigenesis [73]. However, it is unclear whether HBV integration initiates or rather is a consequence of transformation [14]. Hence, the distinct role of HBV DNA integration in hepatocarcinogenesis and the attributed risk of occult HBV infection in HCC development remain elusive.

Does the Expression of HBV-Encoded Proteins or Truncated Viral Proteins Sustain Hepatocarcinogenesis?

Several studies have reported procarcinogenic effects of HBV proteins or of their randomly truncated transcripts after integration (table 2).

The Role of HBx in the Development of HBV-Associated HCC and Metastasis

HBx expression was reported to be frequently detectable in HBV-related HCC tissue and, in addition, anti-HBx antibodies are more frequently found in sera of patients who have HCC as compared to chronically HBV-infected controls [74]. This may be explained by the fact that HBs and HBx may still be expressed from integrated, linear double-stranded DNA. Numerous, sometimes contradictory functions of HBx have been described in the development of HBV-associated HCC.

HBx, encoded by the X open reading frame, is a small polypeptide of 154 amino acids in size, usually expressed at low levels during acute and chronic HBV infection. HBx is only expressed by members of the Hepadnaviridea family (with >90% homology) infecting mammalians [75]. HBx can be found in the cytoplasm of infected hepatocytes and at low level in the nucleus. An important role of the HBx protein in HBV transcription and thus DNA replication and HBV infectivity was reported [76]. Moreover, the interaction of HBx with different transcription factors was demonstrated (reviewed in [77] and [78]). The effects of the HBx have been a subject of controversy, including for example pro-proliferative effects [79], induction of cell-cycle arrest [80], prevention [81] and induction of apoptosis [82]. Some of these effects were merely seen in tissue culture or mouse models with nonphysiologically high expression levels of HBx.

The antiapoptotic and proapoptotic effects of HBx seem to be dependent on the status of hepatocyte differentiation [77]. There is recent evidence that HBx exerts some of its effects by inducing regional hypermethylation or global hypomethylation, e.g. by inducing DNA methyltransferase 1 expression. Thereby, it is able to silence tumor suppressor genes and induce chromosomal instability [83]. A second model has been proposed from in vitro studies that links HBx-induced apoptosis to transcriptional regulation of p53. In this model HBx changes the capability of p53 binding to its target genes, which is associated with their aberrant expression [84].

The elevation of cytosolic calcium signals seems to play a possible role for the diverse effects attributed to HBx, including stimulation of cell proliferation and transcription pathways, as well as HBV replication [85]. A recent review on this topic summarizes various studies focusing on these effects and points to an increased mitochondrial calcium uptake, sustained higher cytosolic calcium levels and a stimulating effect on HBV replication [86]. This also affects several intracellular signal pathways and might explain the multitude of reported effects of HBx. Direct interaction of HBx with the endoplasmic reticulum (ER) and mitochondria as well as integration events of the X open reading frame were reported to alter intracellular calcium homeostasis, which might drive early tumor development by constitutively activating src- and ras-signaling [73].

Furthermore, some studies found that HBx increased telomerase reverse transcriptase expression and c-myc-dependent telomerase activity in vitro and in human HCC tissues [87]. Telomerase activation has been implied in immortalization and malignant transformation of cells in vi-

tro [87] and was frequently found in HCC tissue samples [88]. Alternatively, telomerase activation destabilizes chromosomal integrity and limits clonal expansion of tumor cells leading to reduced replication rounds and their decreased probability to form liver cancer. In contrast, telomerase activation was reported to generally increase cancer initiation by limiting regenerative capacity due to critical telomere shortening [89]. Thus, taking into account these publications, the functional role of the HBx protein in telomere shortening within HCC remains unknown.

In a descriptive correlative study of human HCC samples, telomerase positivity was found in 76% of HCC and in 11.8% of chronic hepatitis virus infection patients. However, a significant correlation between chronic HBV infection and of telomere shortening in human HCC tissue samples was not found as compared to other causes of liver cancer [90].

Studies using different HBx transgenic mouse models also produced controversial data. Mice transgenic for HBx under its own transcription enhancer [91] as well as mice transgenic for HBx plus a portion of pre-C-C under the HBV transcriptional enhancer [92] were reported to develop neoplastic nodules at the age of >6–8 months and HCC >11 months, with both showing high levels of HBx expression [91, 92]. In contrast, mice transgenic for HBx under the control of antithrombin regulatory sequence or human α-1-antitrypsin did not show tumorigenesis, indicating no direct carcinogenic effect of HBx but rather only an increased sensitivity to other known carcinogens (DEN) [93, 94]. This observation of an increased sensitivity to carcinogens or oncogene activation by HBx expression is in line with an accelerated HCC development in mice transgenic for the oncogene c-myc and truncated HBx [95]. Therefore, the low level expression of full-length HBx in wild-type HBV infection is unlikely to drive hepatocarcinogenesis independently. This reflects the human situation where HBx is expressed together with other viral proteins and HCC development is rather dependent on inflammation due to chronic HBV infection than on HBx expression.

However, HBx variants could play an important role in mediating mitogenic pathway activation, endogenous gene expression and apoptosis in hepatocarcinogenesis. HBx variants are frequently found in HCC tissues and are encoded by naturally truncated HBV DNA due to integration in genomic host DNA [96]. An increased stability of naturally occurring HBx variants lacking amino acids 134–154 were found to play a role in HCC development, while the C-terminal region spanning amino acids 141–154 decreased HBx stability and amino acids 116–140 were required for mitogenic pathway activation [97]. Truncation of the HBV DNA, particularly at the C-terminal end of HBx, is a common feature found in chronic HBV infection due to random integration of the HBV DNA into the host genome. A recent study from Hong Kong found full-length HBx in 100% of nontumorous liver parts of HBV-positive patients with HCC, but only in 54% of the HCC tumor sites, whereas C-terminal truncated HBx was detected in the remaining 46% of HCC. Natural C-terminal truncated HBx significantly correlated with venous tumor invasion clinically. In vitro data in this study suggested cell invasive potential to be increased through C-Jun transcriptional activity and enhanced transcription of matrix metalloproteinase 10 by a C-terminal truncated version of HBx (24 amino acids at the C-terminal end) [96]. Interestingly, HBx was also reported to promote epithelial-mesenchymal transition by activation of the Twist promoter through activation of STAT3 [98], which might as well link HBx protein expression to invasiveness of HCC.

In conclusion, variation in the observed role of HBx in hepatocarcinogenesis may be due to factors such as amount of expression, hepatocyte differentiation and specific functions of naturally truncated HBx proteins due to HBV DNA integration. More data on interaction partners of HBx in the course of HCC development, tumor aggressiveness and distant metastasis might give novel insights and help to advance diagnostic tools and therapeutic means.

The Spliced HBV Protein

The spliced HBV protein (HBSP) is encoded by a singly spliced pregenomic RNA and anti-HBSP antibodies were reported to show a prevalence of 46% in chronic HBV. Looking for clinical relevance, a hypothesis was suggested that HBSP might play a role in the natural history of HBV infection and may be involved in the pathogenesis and/or persistence of HBV infection [99]. While the effects of HBSP in vitro showed induction of apoptosis without cell-cycle block, another study described expression of HBSP to activate the mitogen-activated protein kinase and Akt signaling pathway, as evidenced by increases in phosphorylation of p38, Jun N-terminal protein kinase, extracellular signal-regulated kinase [100] and Akt [101]. This may promote hepatoma cell aggressiveness, partly by increasing motility and invasion.

The Role of HBV Envelope Protein Expression and Pre-S1/Pre-S2 Mutants in HBV-Related HCC

A second observation of a direct carcinogenic effect of HBV-encoded proteins is the accumulation of HBV surface proteins in the ER. Accumulation of proteins in the

ER is known to trigger apoptosis in case of prolonged and severe stress due to an induction of oxidative stress as a source of intracellular ROS and can thus drive hepatocarcinogenesis [70]. HBV encodes three different envelope proteins (large, middle and small S antigens). Mice overexpressing the large envelope protein in hepatocytes show liver damage and subsequent HCC development [102]. Increased ER stress and compensatory proliferation was also suggested to contribute to HCC development in an HBV transgenic mouse model harboring a mutated preS2 [103].

HBV-encoded proteins of the PreS2 activator family are the large HBV surface protein and C-terminal truncated middle size surface proteins [MHBs(t)] which have a transcriptional activator function. MHBs(t) were shown to trigger PKC-dependent activation of c-Raf-1/Erk2 signaling essential for Ap-1- and NF-κB activation and increase hepatocyte proliferation [104].

A typical histological finding in HBV-infected livers are hepatocytes overloaded with virus protein ('ground glass' hepatocytes), first described by Hadziyannis and Popper in 1973. These 'ground glass' hepatocytes are known to harbor HBsAg, may contain specific pre-S mutants and have been recognized as preneoplastic lesions of HBV-related HCC [105]. The pre-S2 mutants accumulated in ER were reported to induce ER stress, upregulate cyclin A and promote hepatocyte proliferation. Pre-S2 mutants may cause aberrant cyclin A expression and centrosome multiplication through ER stress induction, and thereby function as a factor inducing chromosome instability in HBV-related HCC [106]. Both overexpression and truncation might be facilitated by integration of the HBV DNA into the host genome. Furthermore, findings so far suggest an oncogenic potential of overexpressed or truncated HBV envelope proteins.

HCC Surveillance

Surveillance of individuals at high risk for HCC development is of highest importance. Given the specific risk factors present in HBV infection for primary liver cancer development, special subgroups with sustained risk have been defined for active surveillance. Patients with high HBV DNA concentrations and those with ongoing hepatic inflammatory activity remain at a significantly higher risk for HCC [9, 37]. Diagnosis at early stages offers therapeutic means in which the tumor may be cured by resection, liver transplantation or ablation, and 5-year survival higher than 50% can be achieved [66].

If HCC is diagnosed in a later stage (stages B–D), according to the BCLC staging and treatment strategy, only palliative treatment options are available. In these late stages, chemoembolization can provide survival benefit. In addition, randomized studies found sorafenib provides some survival benefits for individuals with advanced HCC (BCLC stages B–C) [107].

Novel molecular-targeted therapies could bear more effective functions in this chemoresistant cancer [66]. Although the main risk factors for hepatocarcinogenesis are well known, we are still lacking a deeper understanding of the common molecular signaling pathways underlying hepatocarcinogenesis of different etiologies. Therefore, a molecular understanding of the cellular and molecular pathways in the development of different HCC subtypes is desperately needed to clearly define individual risks and to identify novel therapeutic approaches. Therefore, the thorough characterization of distinct animal models mimicking human disease will help to dissect molecular, cellular and pathophysiological mechanisms of HCC development. Furthermore, unraveling the direct role of known viral factors in hepatocarcinogenesis such as genotypes, typical mutations, HBV DNA integration and HBV-encoded proteins is of great importance. Viral factors identified should be tested for potential usage in active surveillance. In addition, novel insights into the role of viral factors in hepatocarcinogenesis will dissect molecular mechanisms in order to clearly identify the individuals at high risk of HBV-related primary liver cancer and might provide the basis for future diagnostic or therapeutic approaches.

Disclosure Statement

M.R. received a clinical leave stipend funded by the German Center for Infection Research (DZIF). The authors declared that no competing interests exist.

References

1 El-Serag HB: Epidemiology of viral hepatitis and hepatocellular carcinoma. Gastroenterology 2012;142:1264–1273.e1.
2 Jemal A, et al: Global cancer statistics. CA Cancer J Clin 2011;61:69–90.
3 Parkin DM: The global health burden of infection-associated cancers in the year 2002. Int J Cancer 2006;118:3030–3044.
4 Kim DY, Han KH: Epidemiology and surveillance of hepatocellular carcinoma. Liver Cancer 2012;1:2–14.
5 World Health Organisation: (2012) Hepatitis B. Fact Sheet No. 204. Geneva, WHO, 2012.
6 Fattovich G, et al: Long-term outcome of chronic hepatitis B in Caucasian patients: mortality after 25 years. Gut 2008;57:84–90.

7 Chen CJ, Yang HI: Natural history of chronic hepatitis B REVEALed. J Gastroenterol Hepatol 2011;26:628–638.
8 Yang HI, et al; Associations between hepatitis B virus genotype and mutants and the risk of hepatocellular carcinoma. J Natl Cancer Inst 2008;100:1134–1143.
9 Kim BK, Han KH, Ahn SH: Prevention of hepatocellular carcinoma in patients with chronic hepatitis B virus infection. Oncology 2011;81(suppl 1):41–49.
10 Ott JJ, et al: Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. Vaccine 2012;30:2212–2219.
11 McMahon BJ: The natural history of chronic hepatitis B virus infection. Hepatology 2009; 49(5 suppl):S45–S55.
12 Asabe S, et al: The size of the viral inoculum contributes to the outcome of hepatitis B virus infection. J Virol 2009;83:9652–9662.
13 Protzer U, Maini MK, Knolle PA: Living in the liver: hepatic infections. Nat Rev Immunol 2012;12:201–213.
14 Bouchard MJ, Navas-Martin S: Hepatitis B and C virus hepatocarcinogenesis: lessons learned and future challenges. Cancer Lett 2011;305:123–143.
15 Brechot C: Pathogenesis of hepatitis B virus-related hepatocellular carcinoma: old and new paradigms. Gastroenterology 2004; 127:S56–S61.
16 Mast EE, Alter MJ, Margolis HS: Strategies to prevent and control hepatitis B and C virus infections: a global perspective. Vaccine 1999; 17:1730–1733.
17 Ott JJ, Stevens GA, Wiersma ST: The risk of perinatal hepatitis B virus transmission: hepatitis B e antigen (HBeAg) prevalence estimates for all world regions. BMC Infect Dis 2012;12:131.
18 Protzer U, Schaller H: Immune escape by hepatitis B viruses. Virus Genes 2000;21:27–37.
19 Quasdorff M, Protzer U: Control of hepatitis B virus at the level of transcription. J Viral Hepat 2010;17:527–536.
20 Mast EE, et al: A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP) part 1: immunization of infants, children, and adolescents. MMWR Recomm Rep 2005;54:1–31.
21 Chen HL, et al: Effects of maternal screening and universal immunization to prevent mother-to-infant transmission of HBV. Gastroenterology 2012;142:773–781.e2.
22 Liaw YF, Chu CM: Hepatitis B virus infection. Lancet 2009;373:582–592.
23 Chu CM, et al: Natural history of hepatitis B e antigen to antibody seroconversion in patients with normal serum aminotransferase levels. Am J Med 2004;116:829–834.
24 Wu TW, Lin HH, Wang LY: Chronic hepatitis B infection in adolescents who had received primary infantile vaccination. Hepatology 2013;57:37–45.
25 Lai MW, et al: Increased seroprevalence of HBV DNA with mutations in the s gene among individuals greater than 18 years old after complete vaccination. Gastroenterology 2012;143:400–407.
26 Kim WR: Epidemiology of hepatitis B in the United States. Hepatology 2009;49(5 suppl): S28–S34.
27 Bauer T, Sprinzl M, Protzer U: Immune control of hepatitis B virus. Dig Dis 2011;29:423–433.
28 Lai CL, et al: Viral hepatitis B. Lancet 2003; 362:2089–2094.
29 Kennedy PT, et al: Preserved T-cell function in children and young adults with immune-tolerant chronic hepatitis B. Gastroenterology 2012;143:637–645.
30 Lesmana CR, et al: Significant hepatic histopathology in chronic hepatitis B patients with serum ALT less than twice ULN and high HBV-DNA levels in Indonesia. J Dig Dis 2011;12:476–480.
31 Chen JD, et al: Carriers of inactive hepatitis B virus are still at risk for hepatocellular carcinoma and liver-related death. Gastroenterology 2010;138:1747–1754.
32 Chu CM, Liaw YF: HBsAg seroclearance in asymptomatic carriers of high endemic areas: appreciably high rates during a long-term follow-up. Hepatology 2007;45:1187–1192.
33 McMahon BJ: The influence of hepatitis B virus genotype and subgenotype on the natural history of chronic hepatitis B. Hepatol Int 2009;3:334–342.
34 Hadziyannis SJ: Natural history of chronic hepatitis B in Euro-Mediterranean and African countries. J Hepatol 2011;55:183–191.
35 Fattovich G, et al: Hepatocellular carcinoma in cirrhosis: incidence and risk factors. Gastroenterology 2004;127(5 suppl 1):S35–S50.
36 Fattovich G, Bortolotti F, Donato F: Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. J Hepatol 2008;48:335–352.
37 Bruix J, Sherman M, Practice Guidelines Committee, American Association for the Study of Liver Diseases: Management of hepatocellular carcinoma. Hepatology 2005;42: 1208–1236.
38 Bonilla Guerrero R, Roberts LR: The role of hepatitis B virus integrations in the pathogenesis of human hepatocellular carcinoma. J Hepatol 2005;42:760–777.
39 Bosetti C, et al: Trends in mortality from hepatocellular carcinoma in Europe, 1980–2004. Hepatology 2008;48:137–145.
40 Chen CJ, et al: Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 2006;295: 65–73.
41 Iloeje UH, et al: Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology 2006;130:678–686.
42 Lin CL, Kao JH: Risk stratification for hepatitis B virus related hepatocellular carcinoma. J Gastroenterol Hepatol 2013;28:10–17.
43 Yu MW, et al: Hepatitis B virus genotype and DNA level and hepatocellular carcinoma: a prospective study in men. J Natl Cancer Inst 2005;97:265–272.
44 Tanwar S, Dusheiko G: Is there any value to hepatitis B virus genotype analysis? Curr Gastroenterol Rep 2012;14:37–46.
45 Wai CT, et al: HBV genotype B is associated with better response to interferon therapy in HBeAg(+) chronic hepatitis than genotype C. Hepatology 2002;36:1425–1430.
46 Sanchez-Tapias JM, et al: Influence of hepatitis B virus genotype on the long-term outcome of chronic hepatitis B in western patients. Gastroenterology 2002;123:1848–1856.
47 Jardi R, et al: Analysis of hepatitis B genotype changes in chronic hepatitis B infection: influence of antiviral therapy. J Hepatol 2008; 49:695–701.
48 Chan HL, et al: Genotype C hepatitis B virus infection is associated with an increased risk of hepatocellular carcinoma. Gut 2004;53: 1494–1498.
49 Ringelhan M, Schmid RM, Geisler F: The NF-kappaB subunit RelA/p65 is dispensable for successful liver regeneration after partial hepatectomy in mice. PLoS One 2012;7:e46469.
50 Guidotti LG, Chisari FV: Immunobiology and pathogenesis of viral hepatitis. Annu Rev Pathol 2006;1:23–61.
51 Fausto N, Campbell JS: Mouse models of hepatocellular carcinoma. Semin Liver Dis 2010;30:87–98.
52 Summers J, Mason WS: Residual integrated viral DNA after hepadnavirus clearance by nucleoside analog therapy. Proc Natl Acad Sci USA 2004;101:638–460.
53 Weber A, et al: Hepatocyte-specific deletion of the antiapoptotic protein myeloid cell leukemia-1 triggers proliferation and hepatocarcinogenesis in mice. Hepatology 2010;51: 1226–1236.
54 Aleksic K, et al: Evolution of genomic instability in diethylnitrosamine-induced hepatocarcinogenesis in mice. Hepatology 2011;53: 895–904.
55 Vucur M, et al: Mouse models of hepatocarcinogenesis: what can we learn for the prevention of human hepatocellular carcinoma? Oncotarget 2010;1:373–378.
56 Maeda S, Karin M: Oncogene at last – c-Jun promotes liver cancer in mice. Cancer Cell 2003;3:102–104.
57 Naugler WE, et al: Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. Science 2007;317: 121–124.
58 Maeda S, et al: IKKbeta couples hepatocyte death to cytokine-driven compensatory proliferation that promotes chemical hepatocarcinogenesis. Cell 2005;121:977–990.
59 Haybaeck J, et al: A lymphotoxin-driven pathway to hepatocellular carcinoma. Cancer Cell 2009;16:295–308.
60 Pikarsky E, et al: NF-kappaB functions as a tumour promoter in inflammation-associated cancer. Nature 2004;431:461–466.

61 Luedde T, et al: Deletion of NEMO/IKKgamma in liver parenchymal cells causes steatohepatitis and hepatocellular carcinoma. Cancer Cell 2007;11:119–132.
62 Bettermann K, et al: TAK1 suppresses a NEMO-dependent but NF-kappaB-independent pathway to liver cancer. Cancer Cell 2010;17:481–496.
63 Feng GS: Conflicting roles of molecules in hepatocarcinogenesis: paradigm or paradox. Cancer Cell 2012;21:150–154.
64 Zender L, et al: Cancer gene discovery in hepatocellular carcinoma. J Hepatol 2010;52:921–929.
65 Feitelson MA: Parallel epigenetic and genetic changes in the pathogenesis of hepatitis virus-associated hepatocellular carcinoma. Cancer Lett 2006;239:10–20.
66 Forner A, Llovet JM, Bruix J: Hepatocellular carcinoma. Lancet 2012;379:1245–1255.
67 Zucman-Rossi J: Molecular classification of hepatocellular carcinoma. Dig Liver Dis 2010;42(suppl 3):S235–S241.
68 Shafritz DA, et al: Integration of hepatitis B virus DNA into the genome of liver cells in chronic liver disease and hepatocellular carcinoma. Studies in percutaneous liver biopsies and post-mortem tissue specimens. N Engl J Med 1981;305:1067–1073.
69 Kremsdorf D, et al: Hepatitis B virus-related hepatocellular carcinoma: paradigms for viral-related human carcinogenesis. Oncogene 2006;25:3823–3833.
70 Chemin I, Zoulim F: Hepatitis B virus induced hepatocellular carcinoma. Cancer Lett 2009;286:52–59.
71 Paterlini-Brechot P, et al: Hepatitis B virus-related insertional mutagenesis occurs frequently in human liver cancers and recurrently targets human telomerase gene. Oncogene 2003;22:3911–3916.
72 Shi Y, et al: Association between occult hepatitis B infection and the risk of hepatocellular carcinoma: a meta-analysis. Liver Int 2012;32:231–240.
73 Feitelson MA, Lee J: Hepatitis B virus integration, fragile sites, and hepatocarcinogenesis. Cancer Lett 2007;252:157–170.
74 Hwang GY, et al: Detection of the hepatitis B virus X protein (HBx) antigen and anti-HBx antibodies in cases of human hepatocellular carcinoma. J Clin Microbiol 2003;41:5598–5603.
75 Misra KP, Mukherji A, Kumar V: The conserved amino-terminal region (amino acids 1–20) of the hepatitis B virus X protein shows a transrepression function. Virus Res 2004;105:157–165.
76 Lucifora J, et al: Hepatitis B virus X protein is essential to initiate and maintain virus replication after infection. J Hepatol 2011;55:996–1003.
77 Bouchard MJ, Schneider RJ: The enigmatic X gene of hepatitis B virus. J Virol 2004;78:12725–12734.
78 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.
79 Madden CR, Slagle BL: Stimulation of cellular proliferation by hepatitis B virus X protein. Dis Markers 2001;17:153–157.
80 Bouchard M, et al: Hepatitis B virus HBx protein activation of cyclin A-cyclin-dependent kinase 2 complexes and G1 transit via a Src kinase pathway. J Virol 2001;75:4247–457.
81 Elmore LW, et al: Hepatitis B virus X protein and p53 tumor suppressor interactions in the modulation of apoptosis. Proc Natl Acad Sci USA 1997;94:14707–1412.
82 Su F, Schneider RJ: Hepatitis B virus HBx protein sensitizes cells to apoptotic killing by tumor necrosis factor alpha. Proc Natl Acad Sci USA 1997;94:8744–8749.
83 Jung JK, et al: 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;67:5771–5778.
84 Chan C, et al: Altered binding site selection of p53 transcription cassettes by hepatitis B virus X protein. Mol Cell Biol 2013;33:485–497.
85 Bouchard MJ, Wang LH, Schneider RJ: Calcium signaling by HBx protein in hepatitis B virus DNA replication. Science 2001;294:2376–2378.
86 Yang B, Bouchard MJ: The hepatitis B virus X protein elevates cytosolic calcium signals by modulating mitochondrial calcium uptake. J Virol 2012;86:313–327.
87 Zhang X, et al: Effects of hepatitis B virus X protein on human telomerase reverse transcriptase expression and activity in hepatoma cells. J Lab Clin Med 2005;145:98–104.
88 Yokota T, et al: Telomere length variation and maintenance in hepatocarcinogenesis. Cancer 2003;98:110–118.
89 Satyanarayana A, Rudolph KL: Telomeres and telomerase: distinctive roles in liver regeneration, cirrhosis and carcinogenesis; in Dufour JF, et al (eds): Signaling Pathways in Liver Diseases. Berlin, Springer, 2005, pp 333–339.
90 Saini N, et al: Telomerase activity, telomere length and human telomerase reverse transcriptase expression in hepatocellular carcinoma is independent of hepatitis virus status. Liver Int 2009;29:1162–1170.
91 Kim CM, et al: HBx gene of hepatitis B virus induces liver cancer in transgenic mice. Nature 1991;351:317–320.
92 Yu DY, et al: Incidence of hepatocellular carcinoma in transgenic mice expressing the hepatitis B virus X-protein. J Hepatol 1999;31:123–132.
93 Terradillos O, et al: The hepatitis B virus X gene potentiates c-myc-induced liver oncogenesis in transgenic mice. Oncogene 1997;14:395–404.
94 Slagle BL, et al: Increased sensitivity to the hepatocarcinogen diethylnitrosamine in transgenic mice carrying the hepatitis B virus X gene. Mol Carcinog 1996;15:261–269.
95 Lakhtakia R, et al: Hepatocellular carcinoma in a hepatitis B 'x' transgenic mouse model: a sequential pathological evaluation. J Gastroenterol Hepatol 2003;18:80–91.
96 Sze KM, et al: C-terminal truncated HBx is associated with metastasis and enhances invasiveness via C-Jun/ MMP10 activation in hepatocellular carcinoma. Hepatology 2013;57:131–139.
97 Li H, et al: The mitogenic function of hepatitis B virus X protein resides within amino acids 51 to 140 and is modulated by N- and C-terminal regulatory regions. J Virol 2006;80:10554–10564.
98 Teng J, et al: HBx-dependent activation of Twist mediates STAT3 control of epithelium-mesenchymal transition of liver cells. J Cell Biochem 2012, E-pub ahead of print.
99 Soussan P, et al: The expression of hepatitis B spliced protein (HBSP) encoded by a spliced hepatitis B virus RNA is associated with viral replication and liver fibrosis. J Hepatol 2003;38:343–348.
100 Voehringer D, et al: Break of T cell ignorance to a viral antigen in the liver induces hepatitis. J Immunol 2000;165:2415–2422.
101 Chen WN, et al: Interaction of the hepatitis B spliced protein with cathepsin B promotes hepatoma cell migration and invasion. J Virol 2012;86:13533–13541.
102 Chisari FV, et al: A transgenic mouse model of the chronic hepatitis B surface antigen carrier state. Science 1985;230:1157–1160.
103 Na B, et al: Transgenic expression of entire hepatitis B virus in mice induces hepatocarcinogenesis independent of chronic liver injury. PLoS One 2011;6:e26240.
104 Hildt E, et al: The PreS2 activator MHBs(t) of hepatitis B virus activates c-raf-1/Erk2 signaling in transgenic mice. EMBO J 2002;21:525–535.
105 Su IJ, et al: Ground glass hepatocytes contain pre-S mutants and represent preneoplastic lesions in chronic hepatitis B virus infection. J Gastroenterol Hepatol 2008;23:1169–1174.
106 Wang LH, et al: Aberrant cyclin A expression and centrosome overduplication induced by hepatitis B virus pre-S2 mutants and its implication in hepatocarcinogenesis. Carcinogenesis 2012;33:466–472.
107 de Lope CR, et al: Management of HCC. J Hepatol, 2012;56(suppl 1):S75–S87.
108 Yang Y, et al: The TNF-alpha, IL-1B and IL-10 polymorphisms and risk for hepatocellular carcinoma: a meta-analysis. J Cancer Res Clin Oncol 2011;137:947–952.
109 Wang B, et al: Null genotypes of GSTM1 and GSTT1 contribute to hepatocellular carcinoma risk: evidence from an updated meta-analysis. J Hepatol 2010;53:508–518.