



Mini-review

Hepatitis B virus induced hepatocellular carcinoma

I. Chemin*, F. Zoulim

INSERM, U871, 69003 Lyon, France

Université Lyon 1, IFR62 Lyon-Est, 69008 Lyon, France

Hospices Civils de Lyon, Hôtel Dieu, Service d'hépatologie et de gastroentérologie, 69002 Lyon, France

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ABSTRACT

A number of risk factors appear to play a role in Hepatocellular carcinoma (HCC), HBV infection being one of the most important. Chronic inflammation and cytokines are key determinants in the development of fibrosis and liver cell proliferation. HBV DNA integration and/or expression of HBV proteins may have a direct effect on cellular functions. Occult hepatitis B virus infection is characterized by persistence of HBV DNA in hepatitis B surface antigen-negative individuals. There are evidences that occult HBV is a risk factor for the development of HCC and that the potential mechanisms whereby overt HBV might induce tumour formation are mostly maintained.

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1. Introduction

HBV infection is the major risk factor for HCC development worldwide. Intensive research has focused on the role of HBV in hepatocarcinogenesis for the past three decades. Increasing evidence points out to two major HBV-specific mechanisms that contribute to the development of HCC. The first is the integration of the viral genome into the host chromosome causing cis-effects, resulting in loss of tumour suppressor gene functions, and/or activation of tumour-promoting genes. The second mechanism involves the expression of trans-activating factors derived from the HBV genome, which have the potential to influence intracellular signal transduction pathways and alter host gene expression.

Integration of HBV DNA into host cellular DNA is crucial during HBV chronic infection by disrupting or promoting expression of cellular genes that are important in cell growth and differentiation. In addition, expression of HBV proteins may have a direct effect on cellular functions, and some of these gene products can promote malignant

transformation. Several HBV genes have been found in infected tumoural tissues more frequently than others, including truncated pre-S2/S [1], hepatitis B X gene, and a novel spliced transcript of HBV, referred to as the hepatitis B spliced protein [2]. The proteins expressed from these integrated genes have been shown to have intracellular activities that may account for their association with HCC, including effects on cellular growth and apoptosis.

A major player involved in this form of viral transactivation is the X protein (HBx) that display pleiotropic functions and has been implicated in the malignant transformation of chronically-infected liver cells. Moreover, numerous population-based cohort studies and case-control studies have showed that chronic HBV infection authenticated by the presence of hepatitis B surface antigen in serum is associated with elevated risk for HCC development [3].

Finally, some patients with HCC have no detectable hepatitis B surface antigen in serum but do have low levels of HBV DNA in serum and fragments of HBV DNA in genomic cellular DNA (called occult HBV infections). Highest rates of occult HBV infections have been reported in patients with HCC, in particular among HCV carriers [4,5] suggesting that occult HBV worsen the course of HCV infection [6–8]. Prevalence and molecular status of occult

* Corresponding author.

E-mail address: Isabelle.chemin@inserm.fr (I. Chemin).

HBV in patients with HCC has been investigated in a number of studies in patients from different region of the world [9,10,5]. In these HBsAg-negative HCC patients, HBV DNA was detected in tumorous and/or in adjacent non-tumorous liver tissue using polymerase chain reaction (PCR) in almost half of the patients, being anti-HCV positive or not [11]. Some of the patients are positive for anti-HBc antibodies as the only marker of HBV infection, but not all. Covalently closed circular HBV DNA may be detected in the liver of some of these patients indicating the persistence of the template for viral genome transcription and replication. Observational cohort study showed that, among the HBsAg-negative patients with chronic hepatitis C, HCC develops for the most part in carriers of occult HBV.

One of the markers in HBsAg (-) HCC cases has been the presence of the HBV-X gene expression in HCC since positivity for the HBV-X protein in liver tissue in several studies reached half of the liver tissues specimens [12–14]. In all studies, the significant association of occult HBV with HCC was irrespective of age, sex, and may be contemporary with hepatitis C virus infection. Both integrated viral DNA and covalently closed circular HBV genomes were detected in patients with occult HBV [15,5]. Moreover, the presence of episomal HBV genomes was associated with persistence of viral transcription and replication; there are evidences that occult HBV is a risk factor for the development of HCC and that the potential mechanisms whereby overt HBV might induce tumour formation are mostly maintained in cases of occult infection.

2. HBV replication and pathway towards HCC

2.1. Hepatitis B virus (HBV)

The HBV genome has several unique features; these include: (a) the presence of partially double stranded DNA, (b) circular DNA conformation, (c) dependence on a reverse transcription step in the viral cycle of replication, and (d) persistence of the viral genome in infected cells as either integrated forms, or as episomal form, i.e., cccDNA, the latter being essential for the life cycle as the template for viral RNA transcription. HBV is a partially double stranded DNA virus belonging to the Hepadnavirus family. The viral genome is a relaxed circular DNA molecule of 3.2 kb. It is organized in a compact manner with four partially-overlapping open reading frames (ORF): S, C, P, and X (Fig. 1). Four separate viral promoters drive the expression of several mRNAs which give rise to various viral proteins, with the longest mRNA (pregenomic RNA, 3.5 kb) also serving as the template for viral genome synthesis. HBV shows a high degree of species specificity and infects only humans and higher primates. Other members of the Hepadnavirus family, namely the duck hepatitis virus, woodchuck hepatitis virus and ground squirrel hepatitis virus and others, are also species-specific [16].

2.2. HBV replication

HBV virions infect hepatocytes by binding to cellular surface receptors which remain unknown. After membrane fusion, the viral inner cores are transported into the

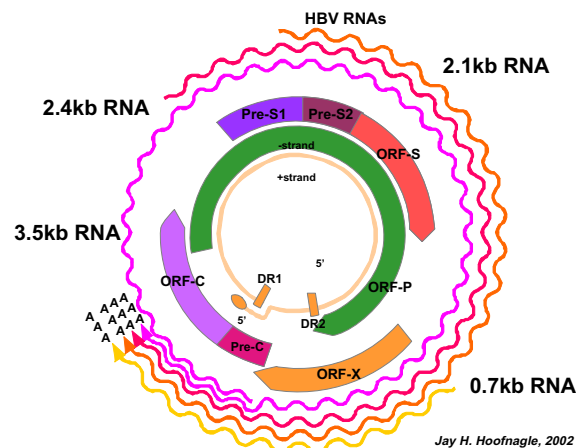


Fig. 1. Hepatitis B viral genome organization, genomic organization, open reading frames and mRNAs.

nucleus, where the viral DNA turns into a covalently closed circular form (cccDNA) (Fig. 2), and is transcribed by the host RNA polymerase II into a series of viral mRNA. All of these transcripts are transported out to the cytoplasm, where they are translated into different viral proteins. Viral inner cores are then assembled in the cytosol, with a single molecule of pregenomic RNA and a viral DNA polymerase packed with core proteins into each particle. The synthesis of the viral genome takes place in newly assembled capsids by reverse transcription of the pregenomic RNA. Most cores are then coated with the viral lipoprotein envelopes by budding into the ER and are exported from the cell as mature virions, while a small portion of core particles are transported back to the nucleus to maintain a stable pool of cccDNA. HBV genome replication requires a reverse transcription step which is error-prone due to the lack of proofreading ability of the viral reverse transcriptase. However, the compactness of the HBV genome prevents large degrees of genetic variability from occurring. As a result, the mutation rate of HBV genome is higher than those of other DNA viruses but lower than those of retroviruses [17].

Viral genome integration in the host genome is thought to result from the integration of viral double stranded linear DNA in damaged host genome harboring break-points. It is not necessary for viral genome replication. There is no integrase associated enzymatic activity encoded by viral proteins. The process is therefore thought to involve cellular enzymes (topoisomerase I, [18]). The generation of double stranded linear DNA results from spontaneously occurring default in viral genome replication. Therefore, it may be inferred that by blocking viral replication, it may be possible to inhibit the formation of this viral DNA form, thereby preventing the possibility of viral genome integration.

2.3. Pathogenesis and molecular mechanisms of HBV induced HCC (Fig. 3 and Table 1)

Eight HBV genotypes have been identified (A–H) [13,17] based on the variation of the entire genome (>8% of nucle-

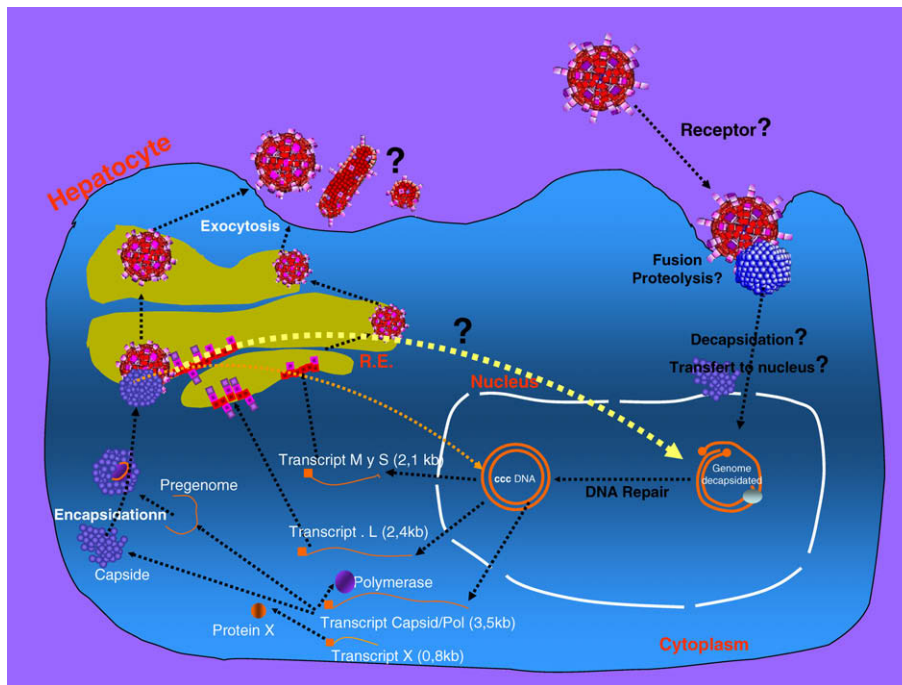


Fig. 2. Hepatitis B life cycle and replication.

otide divergence). HBV strains that belong to different genotypes have distinct geographical distribution, genotype A is mainly found in Northwestern Europe, North America and South Africa; genotypes B and C are prevalent in Eastern and South-Eastern Asia; genotype D is widely distributed from Southern Europe to India; genotype E is restricted to West and South Africa; genotype F and H are found in Central and South America; and genotype G has been reported in France and in the USA [19,17]. There is growing evidence suggesting that viral genotypes may influence the clinical outcomes of HBV infection, including the persistence of the infection, viral replication and HCC risk [20]. For example, genotype B has been reported to faster development of HCC as compared to genotype C in Taiwanese patients, whereas the opposite has been observed in Japan [17]. Moreover, geographical variance of genotype distribution and differences in transmission modes between Asians (perinatal transmission) and Africans (horizontal transmission during early childhood) [20] may also explain the complexity of the clinical observations. In a recent study [21], a significant association between genotype F and the development of HCC among Alaska native people with chronic HBV infection (47 patients with HCC and 1129 patients without HCC were genotyped). Genotype F was found in 68% of patients with HCC, vs. 18% of those without HCC ($P < .001$). For patients with genotype F, the median age at diagnosis of HCC was lower than that for patients with other genotypes (22.5 vs. 60 years, respectively; $P = .002$). Other environmental factors, such as exposure to aflatoxin, alcohol, tobacco, etc. may also increase the risk of HCC development in chronic HBV carriers.

The probability of developing persistent infection increases sharply as the age at infection decreases, probably due to the immaturity of the immune system in children and infants. In case of vertical transmission, HBeAg may cross the placenta and induce an HBV-specific immune tolerance in the baby. Among infants less than 6 months old, about 90% of HBV infections develop into persistent infections [22]. A majority (>90%) of primary infections in adults results in complete recovery mainly due to the HBV-specific T cell response of the host. This immune response is generally attenuated in adults that develop chronic infection [22,23].

The association between chronic tendency of HBV infection and the age of infection appears to contribute to the geographical variation of HCC incidence. Indeed, in HBV endemic areas, infections mainly occur during infancy or early childhood (1–5 years old) through perinatal transmission or through close contact between young children. In non-endemic areas, however, infections are acquired mainly during adolescence or adulthood through sexual contacts or transfusions [24]. Therefore, a majority of infections in endemic areas develops into persistent infections, leading to higher risk for HCC development.

2.3.1. Chronic inflammation and cirrhosis

A potential element of HBV-related hepatocarcinogenesis is the dynamic course of chronic hepatitis B. It is characterized by periodic down-regulations of viral titers accompanied by immune-mediated liver injuries known as “flares”. Such periodic injuries result in repeating cycles of death and proliferation of hepatocytes [25,22].

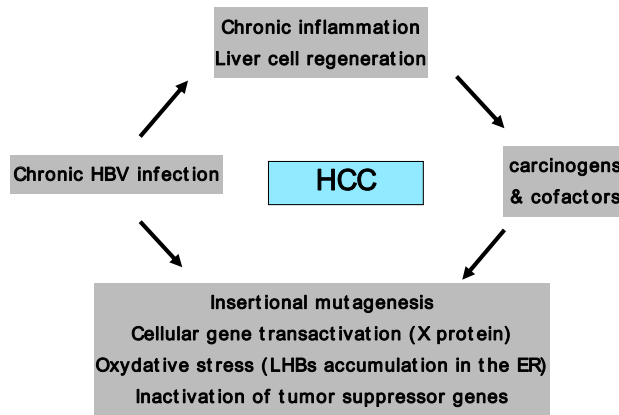


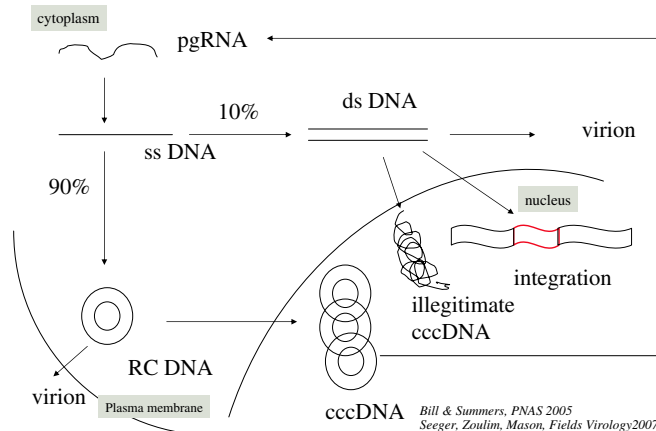
Fig. 3. Role of HBV in liver cancer-pathogenesis.

Table 1
Main factors involved in HBV induced HCC.

Viral factors	Cellular factors	Environmental factors
Insertional mutagenesis	Chronic inflammation	Carcinogens (aflatoxin B1)
Cellular gene transactivation (X protein)	Liver cell regeneration	Alcohol, tobacco
Oxydative stress (LHBs accumulation in the ER)	Epigenetic changes	HCV co-infection
Inactivation of tumour suppressor genes		

Liver injuries caused by chronic hepatitis B are considered to be immuno-mediated and are mainly due to the activity of HBV-specific T cells. However, some data suggests important roles of non-specific chemokine-mediated infiltration of neutrophils, nature killer cells and activated bystander lymphocytes in causing HBV-related liver damage [22]. These inflammatory cells release cytokines and chemokines which may favor cancer growth [26]. High hepatocyte proliferation rate is a major risk factor for hepatocellular carcinoma development in the cirrhotic liver [27].

Chronic inflammation also results in increased production of ROS which can cause DNA damage and leads to gene mutations [26,28]. Increased intracellular ROS levels can also activate several signal transduction pathways that regulate proliferation, differentiation and apoptosis, including the MAP-kinase/AP-1 and NF-kappaB pathways [28]. Inflammation-induced oxidative stress and influx of Kupper cells can promote the activation of stellate cells. The latter are the main producers of extracellular matrix in the liver [29]. Their persistent activation can finally lead to cirrhosis, which is characterized by the co-existence of regenerative nodules, irreversible fibrosis and severe liver injury [25,29]. Cirrhosis is an important predisposing state for HCC development. About 80–90% of HCCs of all etiological backgrounds arise in cirrhotic livers. In HBV endemic areas, infected patients with cirrhosis have an approximately 3-fold higher risk for HCC than those with only chronic hepatitis B, and a 16-fold higher risk than asymptomatic carriers [30]. The incidence of HCC development is approximately 3–5% per year in HBV associated cirrhosis. The long-term persistence of occult might also exerce an indirect oncogenic role by chronically sustaining a mild necroinflammation contributing to cirrhosis and HCC development [31–33].



Bill & Summers, PNAS 2005
Seeger, Zoulim, Mason, Fields Virology2007

Fig. 4. Viral genome persistence.

2.3.2. Integration of viral DNA into the host genome (Fig. 4)

The extent of which HBV genome insertions contribute to hepatocarcinogenesis is still controversial. Integration events are thought to precede the development of the tumour, since they are also found in individuals with chronic hepatitis and are even observed during the acute stage of infection [34]. The integrations of HBV DNA into the genome of host hepatocytes are found in 85–90% of HBV-related HCCs [35].

Viral genome insertions are shown to be facilitated by cellular conditions that disrupt host genomic stability or increase cellular DNA replication, such as exposure to oxidative stress or DNA-damaging agents, deficiency of the DNA repair machinery, co-infection with other viruses and chronic liver injuries that increase hepatocyte turnover [35]. Unlike the woodchuck hepatitis B virus (WHV), which preferentially inserts its DNA genome into *c-Myc* or *N-Myc* genes in the woodchuck genome, no specific genes in humans have been identified so far to be the preferential target for HBV insertion [35]. However, the insertion itself can induce general genomic instability in host cells, leading to chromosome deletions and transpositions of viral and flanking host sequence between chromosomes [34]. Indeed, in HCC cells and liver tissues from HCC patients, a series of cancer-relevant cellular genes has been shown to be altered by HBV DNA insertion, in particular those regulating cellular immortalization (*hTERT*), proliferation (*MAPK1*, *cycline A*), and viability (*TNF receptor-associated protein 1*), suggesting that hepatocytes harboring these insertions are preferentially selected during hepatocarcinogenesis [35]. The rearrangement of viral and host DNA sequences during integration can lead to the production of altered protein products whose functions may render hepatocytes prone to transformation. For example, a C-terminal truncated form of the X protein resulting from a deletion of inserted viral DNA has been reported to enhance oncogene-induced transformation, whereas the wild-type full-length protein suppresses such transformation [36]. HBx mutations have been frequently described among occult HBV infections [12,37].

Taken together, though the insertion of HBV DNA into the host genome might be a random event, that contributes to the formation of an unstable environment, and occasionally induces genomic alterations that facilitate hepatocyte transformation even in the case of occult HBV infections. Therefore, HBV integration may contribute, at least in part, to the development of most of HBV induced HCCs.

2.3.3. Role of hepatitis B virus proteins

In addition to the integration of viral genome into host DNA, another direct role of HBV in hepatocarcinogenesis involves the long-term expression of viral proteins such as surface proteins and the X protein (HBx). This is supported by the observation that in a large portion of HCCs, viral DNA sequences encoding these proteins are found to be integrated in the host genome [34].

2.3.3.1. HBV surface proteins. The oncogenic potential of HBV surface proteins have been suggested by experiments showing that a truncated form of the large surface protein

can increase hepatocyte proliferation by upregulating *cyclin A* expression, and that a truncated M surface protein can specifically activate *c-Raf-1/Erk2* signaling and increase hepatocyte proliferation [38].

Moreover, HBV surface proteins may contribute to HCC development by accumulating in the endoplasmic reticulum (ER) and inducing ER stress. ER stress is defined by the imbalance between the quantity of unfolded or misfolded proteins that enters the ER, and the capacity of the ER machineries to handle the folding of these proteins. Such imbalance activates intracellular signaling pathways that are often referred to as unfolded protein response (UPR) [39]. Activation of UPR can lead to various biological outcomes depending on the intensity and duration of ER stress. Under mild and temporary ER stress, UPR lowers the ER burden by attenuating protein synthesis and increasing protein degradation, as well as by increasing the expression of chaperones that refold misfolded proteins [39,40]. In the case of severe or prolonged ER stress, UPR triggers apoptosis through yet poorly understood mechanisms [39]. An important side effect of UPR relevant to carcinogenesis is the induction of oxidative stress. UPR has been shown to increase the production of intracellular ROS through upregulating the oxidative folding machinery [41,42]. The latter is responsible for oxidizing thiol groups to form disulfide bonds, and has been shown to be an important source of intracellular ROS [43]. UPR-mediated upregulation of ROS has been reported to contribute to ER stress induced apoptosis [41]. Moreover, the oxidative stress caused by increased ROS can lead to various oncogenic consequences, such as DNA damage, activation of mitogenic response and the deregulation of signaling pathways that control proliferation, cell survival and cell death [44].

During viral infection, the ER serves as an essential organelle for viral replication and maturation. Recent evidence suggests that infection by many types of virus, including HBV and HCV, can induce ER stress due to the large amount of unfolded and misfolded viral proteins synthesized in infected cells [40]. In human liver tissues with chronic HBV infection, cytoplasmic accumulation of HBsAg is often observed and has long been associated with the formation of “ground-glass” hepatocytes [45,46]. These hepatocytes often demonstrate ER proliferation and distortion [46,47]. Several studies have showed that ground-glass hepatocytes contain mutations in the pre-S region of the HBV genome, which lead to the overproduction of the large surface protein that blocks the secretion of all forms of surface proteins from the ER [48,49]. Accumulation of these viral proteins in the ER triggers ER stress, leading to the induction of oxidative stress and DNA damage, and to the predisposition of hepatocytes to transformation [50]. The above scenario has been reproduced in transgenic mice containing the entire HBV envelope coding region. Four transgenic mice lineages presenting progressively higher intrahepatic concentration of HBsAg were studied. Similar to human chronic HBV infection, these mice also demonstrate ground-glass alteration in their hepatocytes due to the ER accumulation of the large surface protein. Such accumulation results in a series of oncogenic events, including liver dysplasia, severe prolonged

liver damage, regenerative hyperplasia and nodule formation and incidence of HCC was directly related to the amount of HBsAg accumulated in the hepatocytes [51,52]. Moreover, increased levels of oxidative DNA damage has been observed in the liver of these mice [53]. These observations, together with data obtained using human HCC cells described above, support the notion that HBV surface proteins harbor oncogenic effects, and that such effects are mainly mediated by the induction of ER stress and the consequent elevation of intracellular ROS levels.

2.3.3.2. HBV X protein. HBx is shown to interfere with numerous cellular activities, in particular, proliferation, apoptosis, senescence and DNA repair. However, controversial or even opposite effects of HBx on the same cellular function are often reported, depending on the experimental models used [54].

HBV X protein (HBx) is a small protein of 154 amino acids, and is encoded by the X ORF which is highly conserved among mammalian Hepadnaviruses. HBx has been shown to be required for infectivity *in vivo* in the woodchuck model, and in some experimental conditions it may enhance viral DNA replication in tissue culture. Contradictory observations have been reported concerning the intrinsic tumorigenic effect of HBx *in vivo*, with HCC developing in some, but not all of transgenic mice carrying the X gene. However, in these mice, HBx was shown to increase HCC development in the presence of c-Myc expression or upon exposure to low levels of carcinogens, suggesting that the viral protein may assist hepatocarcinogenesis in the context of a cancer-prone environment [54]. It was shown to activate moderately *in trans* a variety of cellular gene expression. Furthermore, HBx was reported to inhibit apoptosis induced by p53, TNF, Fas, or TGF- β , while it was shown to promote cell death by inducing mitochondrial aggregation and cytochrome *c* release, or by sensitizing cells to TNF-mediated killing. Several groups also reported its interaction with DNA repair proteins (UVDB1). HBx was observed to increase proliferation by inducing G0–G1 transition, and stimulating cell cycle progression in differentiated hepatocytes, whereas it causes S phase arrests in dedifferentiated cells. HBx may also play a major role in the epigenetic control of viral and host gene expression.

3. Selection of hepatocytes during chronic infection

Selection and expansion of cellular clones is one of the major determinants in HCC development. This aspect has been studied in detailed in the woodchuck model. In the study by Summers and Mason [55] WHV chronically-infected woodchucks received 30 weeks of nucleoside analog therapy (clevudine). Viral covalently closed circular DNA declined 20- to 100-fold. Integrated viral DNA showed no discernable decrease over the course of treatment. Clearance of cccDNA did not involve the replacement of the infected hepatocyte population with uninfected progenitors. Uninfected hepatocytes in the treated liver derived from the infected hepatocyte population. The frequency of integrated DNA in chronically-infected woodchucks was 1 or 2

orders of magnitude higher than that in transiently infected woodchucks. It was also shown that integration and other genomic damage accumulated over the duration of infection. Noteworthy, genetic changes from this host genetic damage remained in the liver even while viral genome replication was cleared. All results argued for early antiviral intervention in chronic hepatitis, to stop viral genome replication at an early stage and prevent subsequent viral genome integration and accumulation of genetic damage.

Another important aspect of HCC biology is the hepatocyte turnover, whose role was studied in the same animal model. Increased hepatocyte turnover rate may favor the accumulation of mutations in the host genome, including in cancer-related genes, and therefore may facilitate the transformation of hepatocytes. The study from Zhu et al. [56] set out to quantify the extent of liver turnover by measuring the clonal proliferation of hepatocytes by using integrated viral DNA as a genetic marker for individual hepatocyte lineages. Liver tissue from woodchucks with chronic woodchuck hepatitis virus (WHV) infection was assayed for randomly integrated viral DNA by using inverse PCR. Serial endpoint dilution of viral–cell junction fragments into 96-well plates, followed by nested PCR and DNA sequencing, was used to determine the copy number of specific viral cell junctions as a measure of the clonal distribution of infected cell subpopulations. The results indicated that the livers contained a minimum of 100,000 clones of >1000 cells containing integrated DNA, representing at least 0.2% of the hepatocyte population of the liver. Because cells with integrated WHV DNA comprised only 1–2% of total liver cells, it was hypothesized that the total number of clones far exceeds this estimate, with as much as one-half of the liver derived from high copy clones of >1000 cells. It may be inferred that these clones have a strong selective growth or survival advantage. The results provide evidence for a large amount of hepatocyte proliferation and selection having occurred during the period of chronic WHV infection in these animals.

All these results obtained in animal models suggest that viral genome integration and expansion of hepatocyte clones may occur even if antiviral therapy is successful to control viral replication when started at a late stage of infection.

4. Clinical correlates between HBV replication and HCC

4.1. Correlation between viral load and HCC

4.1.1. Serum HBV DNA level as a predictor of hepatocellular carcinoma (HCC)

Several large cohort studies from China, Taiwan, and Senegal reported that high serum hepatitis B virus (HBV) DNA levels at the time of enrollment were associated with an increased risk of cirrhosis and HCC. Therefore, it was suggested that Serum HBV DNA level, and not only liver disease activity, might be used as an indication for antiviral therapy. In a recent article, Chen and colleagues [57] reported that among 3653 hepatitis B surface antigen

(HBsAg) carriers, 164 had HCC after a mean follow-up of 11.4 years. There was a dose–response relationship between serum HBV DNA level at entry and subsequent HCC development. The authors concluded that high serum HBV DNA levels ($>10^4$ copies/mL) were a strong predictor of HCC independent of hepatitis B e antigen (HBeAg), serum aminotransferase levels, and the presence of cirrhosis. Furthermore, a subanalysis showed that spontaneous decline of viremia levels from levels higher than 10^5 copies/mL to levels below 10^4 copies/mL was associated with a reduced risk of HCC development by comparison with patients who maintained high viremia levels. Therefore, the authors suggested that effective control of HBV replication with antiviral therapy may lower the risk of HCC. However, this remains to be demonstrated by prospective clinical studies.

Other studies showed an association between persisting high viral load and the risk of HCC. In one study, the role of both viral load and genotypes was investigated. It was shown that Genotype C HBV was associated with an increased risk of HCC compared with other HBV genotypes (adjusted OR = 5.11, 95% CI = 3.20–8.18). Furthermore, Genotype C HBV was associated with increased viral load, and associations of HBV genotype and viral load with HCC risk were additive [58]. *This suggests that viral load and genotype determination may be important factors to consider regarding screening program for the detection of HCC and treatment indication.*

5. Conclusions

During the past decade, many important information was generated regarding the molecular mechanism of HBV induced HCC and the clinical correlates of HCC development. However, many questions remains to be answered in terms of clinical management of chronic HBV infections to improve the prevention of HCC development: (1) Can the correlations between high viral load and HCC risk be generalized to all HBV carriers whatever their HBeAg status, ALT levels, and stage of chronic hepatitis. (2) Another important clinical issue is whether a single time point assessment can predict the prognosis of HBV carriers? (3) Finally, the major clinical question is whether antiviral therapy can prevent HCC? The best evidence that it may was derived from a prospective, randomized, placebo-controlled trial of lamivudine [59], in which treatment was associated with a decrease in HCC ($P = .047$), but the difference was not significant ($P = .052$) when prevalent cases of HCC (those diagnosed during the first year of the trial) were excluded. Other studies of antiviral therapy in cirrhotic patients did not show a benefit in terms of HCC development. This may argue for an earlier antiviral intervention, before the development of cirrhosis, to prevent HCC development. The demonstration of the benefit in HCC prevention will require long-term prospective studies of antiviral therapy in chronic HBV carriers.

However, results of experimental studies suggest that early treatment intervention is necessary to prevent liver cell damage and decrease viral genome integration. With the availability of more potent antivirals with a high genet-

ic barrier to resistance, one might consider to propose antiviral treatment even in patients with moderate liver disease, especially in case of long-term infection acquired vertically.

Another important finding from recent studies is that viral genome integration persists despite antiviral induced viral suppression and cccDNA clearance. This was shown to be associated with expansion of cellular clones not expressing viral antigens. Therefore, screening of HCC remains mandatory even in patients with sustained viral suppression induced by antiviral therapy to detect small size HCCs for which curative treatments can be proposed.

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