Review

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Mechanisms of HBV-related hepatocarcinogenesis

Christine Neuveut, Yu Wei, Marie Annick Buendia*

Oncogenesis and Molecular Virology Unit, Institut Pasteur, Inserm U579, 28 rue du Dr Roux, 75724 Paris cedex 15, France

The hepatitis B virus (HBV) is a small enveloped DNA virus, which primarily infects hepatocytes and causes acute and persistent liver disease. Epidemiological studies have provided overwhelming evidence for a causal role of chronic HBV infection in the development of hepatocellular carcinoma, but the molecular mechanisms underlying virally-induced tumourigenesis remain largely debated. In the absence of a dominant oncogene encoded by the HBV genome, indirect roles have been proposed, including insertional activation of cellular cancer-related genes by HBV DNA integration, induction of genetic instability by viral integration or by the regulatory protein HBx, and long-term effects of viral proteins in enhancing immune-mediated liver disease. Recent genetic studies indicate that HBV-related tumours display a distinctive profile with a high rate of chromosomal alterations and low frequency of β-catenin mutations. This review will discuss the evidence implicating chronic HBV infection as a causal risk factor of primary liver cancer. It will also discuss the molecular mechanisms that are critical for the tumourigenic process due to long lasting infection with HBV.

Introduction

Approximately 2 billion people present evidence of past or present infection with hepatitis B virus (HBV) over the world. With more than 350 million chronic HBV carriers, this virus stands as one of the most common human pathogens and it causes significant public health problems [1]. Despite the availability of an efficacious and safe hepatitis B vaccine [2], chronic HBV infection remains the major aetiological factor of hepatocellular carcinoma (HCC) worldwide with more than one half of HCC patients being chronic carriers [3]. In recent studies conducted in Asia and Northern America, it was estimated that the lifetime risk of developing HCC is increased by 25–37 times in HBsAg carriers compared to non-infected populations [4,5]. Importantly, the first effects of mass vaccination have been detected in Taiwan with significant reduction of HBV-related HCC in vaccinated children [6]. Although high viral load is known to increase the risk of developing HCC [7], the risk remains elevated in HBsAg-negative HBV infection and occult infection [8–10]. In addition, inconsistent geographical variations in HCC mortality and HBV surface antigen (HBsAg) prevalence have been observed in endemic regions, suggesting that other independent or cooperative factors might be implicated. In highly endemic regions, particularly in South Africa and Mainland China, synergistic effects of aflatoxin B1 (AFB1) and HBV infection have been highlighted in several reports [11].

Hepatocellular carcinoma (HCC) is a common malignancy and a leading cause of cancer death worldwide. Recent epidemiological data have demonstrated that liver cancer incidence is continuously rising and will continue to do so for more than a decade, not only in Asia and Africa but also in North America and Europe [3,12]. HCC generally presents with poor prognosis, and no effective treatment is available for most HCC patients because this tumour remains refractory to current chemotherapeutic regimens [13]. Moreover, HCC is frequently diagnosed when advanced stage of the disease precludes local ablative or surgical interventions that could improve patient outcome. In this context, advances in our understanding of the molecular basis of HCC are urgently needed to develop early tumour markers and novel targeted agents with improved therapeutic efficiency [14,15]. Here we review the molecular mechanisms linking chronic hepatitis B to malignant transformation of liver cells.

The HBV life cycle

HBV is the prototype member of a family of small enveloped DNA virus called hepadnaviruses, which infect a restricted number of mammals and birds. These viruses share a narrow host range and preferential tropism for hepatocytes. The genome of hepadnaviruses is a relaxed circular, partially double-stranded DNA genome that replicates via an RNA intermediate [16] (Fig. 1). The HBV genome is around 3.2 kb in length and presents a highly compact genetic organization with 4 overlapping open reading frames (ORFs) that cover the entire genome. The pre-S/S ORF encodes the three viral surface proteins, the pre-C/C ORF encodes the e antigen (HBeAg) and the core antigen (HBcAg), the P ORF encodes the terminal protein (TP) and the viral polymerase that possesses DNA polymerase, reverse transcriptase and RNaseH activities. The X gene encodes a small protein that is essential for virus replication but whose function remains partially understood. Finally, a viral protein termed HBSP has been shown to be encoded by a spliced viral transcript [17].

Keywords: Virus; Cancer; Hepatitis; Hepatocellular carcinoma; Oncogene; Tumour suppressor.

Received 22 September 2009; received in revised form 22 October 2009; accepted 23 October 2009

* Corresponding author. Tel.: +33 145 68 88 66; fax: +33 145 68 89 43.

E-mail address: marie-annick.buendia@pasteur.fr (M.A. Buendia).

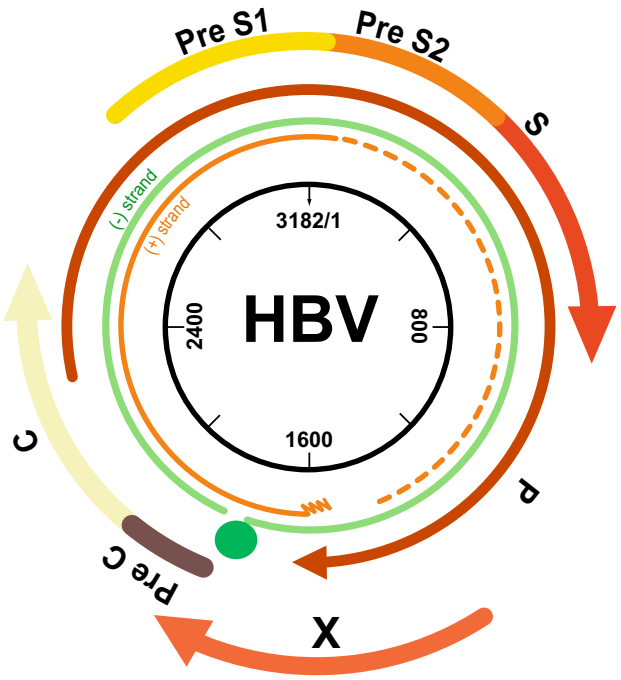

Fig. 1. Schematic representation of the circular, partially double-stranded HBV DNA genome. Both DNA strands of the duplex are held together by base-pairing over 250–300 nucleotides at their 5′ extremities. The unit length-strand (minus strand) is covalently linked to the viral polymerase and bears a 9-nucleotide redundant sequence at its extremities. The complementary strand (plus strand) is linked to a capped oligoribonucleotide at its 5′ end. The plus strand is less than unit length and terminates at different positions resulting in the presence of a single-stranded region of variable length. Two 11-bp repeats, DR1 and DR2, located at the 5′ ends of the minus and plus strands, play a critical role in viral DNA replication. Four open reading frame carried by the minus strand are represented by large arrows.

Although many steps of hepadnavirus replication have been elucidated (Fig. 2), the initial phases of hepatocyte infection, namely virion attachment, uncoating, and entry remain poorly defined, and a cell-surface receptor has not been identified so far. These limitations may be explained by the low efficiency of HBV infection in few available *in vitro* systems. In the absence of candidate receptor, a less conventional hypothesis has been proposed, in which scavenging liver sinusoidal endothelial cells (LSEC), rather than hepatocytes themselves, mediate the initial uptake of the viral pathogen into the liver [18]. After viral entry, the nucleocapsid is released into the cytoplasm and transported along the microtubules to the nuclear membrane. Nuclear import of the nucleocapsid through the nuclear pore complex appears to be mediated by importins α and β, and it is followed by disintegration of the nucleocapsids and liberation of the HBV genome in the nuclear pore basket [19]. In the nucleus, the relaxed circular, partially duplexed DNA genome is converted into a covalently closed circular molecule: the cccDNA [20–22]. The cccDNA serves as template for transcription of all viral RNAs, including the pregenomic RNA (pgRNA) as well as subgenomic RNAs. This step is critical for amplification of the viral progeny and for maintaining productive infection in the host. Four promoters and two enhancers that are preferentially active in hepatocytes regulate the transcription of viral RNAs, and the HBx protein has been reported to play an essential role in activating HBV transcription [23]. It has been shown that the cccDNA is organized into a mini-chromosome harboring a chromatin-like structure in the nuclei of infected cells [24]. This structure is known to be resistant to anti-viral agents and might be responsible for viral rebound upon withdrawal of anti-viral therapy [25]. Recently, a chromatin immunoprecipitation-based methodology (ChIP/cccDNA assay) has been established to analyze the transcriptional regulation of the cccDNA minichromosome [26]. These studies led to demonstrate that HBV replication is regulated by the acetylation status of the cccDNA-bound H3/H4 histones, and that H3/H4 acetylation status is tightly correlated with the level of viremia in the infected patient.

The regulatory protein HBx has been found to play a key role in HBV transcription and replication. Early studies have shown that HBx stimulates the activity of viral promoters and enhancers, and that the closely related virus WHV deficient for the expression of WHx cannot replicate in the animal host [27,28]. More recently, it was found that the replication of X-deficient HBV genomes was strongly compromised in established cell lines and in the mouse liver, and that HBx provided in "trans" was able to restore HBV replication to wild-type levels [23,29–31]. In most studies, this effect has been attributed to the transactivator activity of nuclear HBx protein.

It is worth noticing that HBV replication does not require a step of viral DNA integration into host chromosomes. In the cytoplasm, the pgRNA is selectively packaged into progeny capsids and reverse-transcribed by the viral polymerase into minus strand DNA followed by the synthesis of relaxed circular DNA (RC-DNA). Capsids containing RC-DNA can be either recycled for intracellular cccDNA amplification [32], or assemble with the viral surface proteins in the endoplasmic reticulum to form the viral particles that will be released from the cell [33,34] (Fig. 2). So far, inspection of the viral genome has not led to identify a dominant viral oncogene, and accordingly, introduction of the HBV genome into primary cells in culture or into transgenic murine models has not provided convincing evidence of cell transformation or liver tumourigenesis.

Natural history of chronic HBV infection

The natural history of chronic hepatitis B infection is a dynamic and complex process that can present a variable course, depending on a balance between viral parameters and host immune response [1]. While 90% of infected newborns and 30% of infected children develop chronic hepatitis B, only 5% of individuals infected at the adult age remain chronically infected, illustrating the primary role of the patient's immune system. Recent consensus statements have schematically classified chronic hepatitis B into five phases: the "immune tolerant phase", the "immune active phase", the "inactive HBV carrier state", the "HBeAg-negative chronic hepatitis B" and finally the "HBsAg-negative phase" [35,36].

Typically, the initial phase of chronic HBV infection is characterized by HBeAg-positivity, high levels of HBV DNA in serum and variable elevation of serum alanine aminotransferase (ALT). However, in a proportion of HBeAg-positive patients, ALT levels are normal and liver damage is minimal despite high levels of HBV replication. This state referred to as the "immune tolerant phase" is frequent after perinatal infections and it can persist for years. The "immune active phase" also referred as "chronic hepatitis B

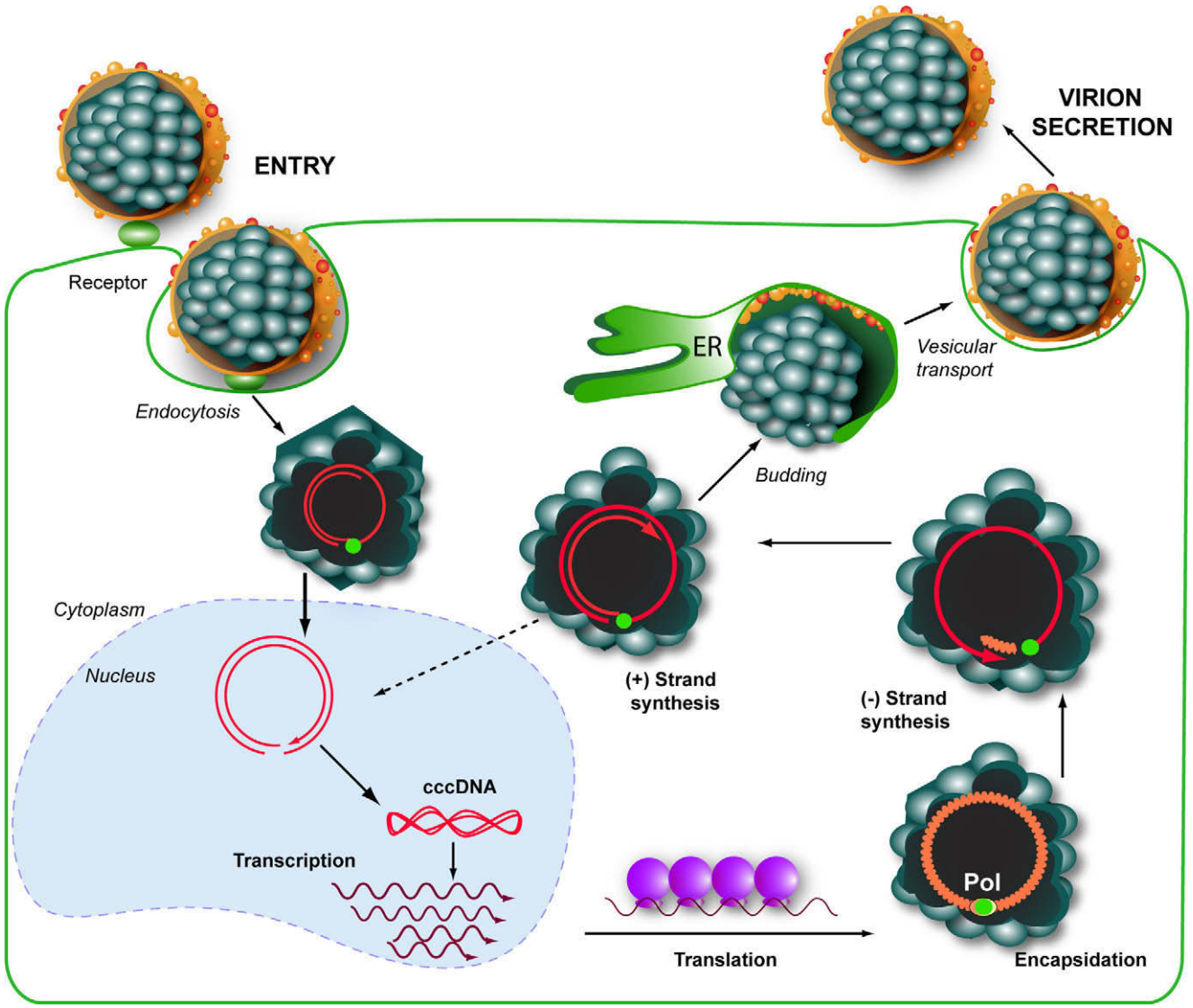

Fig. 2. Schematic representation of the HBV life cycle. HBV infects hepatocytes via an unknown cell-surface receptor. After virion entry and uncoating, nucleocapsids are transported to the cell nucleus where RC-DNA is converted to cccDNA, which is used as template for the transcription of pregenomic and subgenomic RNAs. In the cytoplasm, pgRNA is bound by the viral polymerase and packaged into nucleocapsids, where viral DNA is synthesized. Nucleocapsids can either assemble with envelope proteins in the endoplasmic reticulum, or deliver viral DNA into the nucleus for cccDNA amplification.

phase" is typified by elevated ALT levels, low levels of HBV DNA in serum, and moderate to severe liver necroinflammation that may progress to fibrosis. While this phase can occur several years after experiencing the "immune tolerant phase" in perinatally-infected patients, it can be detected shortly after infection during adulthood. Large cell dysplasia has been frequently found in advanced chronic hepatitis B with increased activity. It involves hepatocytes with senescent features and no sign of proliferative or apoptotic activity, and develops during periods of frequent neoplastic transformation, thus raising the possibility that it might occur as a safeguard against the development of hepatocellular carcinoma [37]. Senescence in tumourigenesis is regarded as a defense mechanism and evasion of senescence is thought to lead to malignancy [38].

Among patients in the "immune active phase", a majority eventually progress into the "inactive HBV carrier phase", characterized by loss of HBeAg, seroconversion to anti-HBe antibodies and decline of serum HBV DNA to barely detectable levels, as well as improvement of fibrosis and inflammation over time [35,39,40]. In prospective studies, this state has been associated with a favorable long-term outcome [41]. However, up to one-third of patients will undergo seroconversion to anti-HBe antibodies and transition to the "HBeAg-negative chronic hepatitis B state", characterized by periodic reactivation associated with fluctuating levels of ALT and HBV DNA, and active hepatitis with variable degree of fibrosis. This transition has been attributed to the occurrence of nucleotide substitutions in the precore and/or the basal core promoter region that preclude expression of the e antigen [42]. Emergence of these HBV variants is associated with active liver disease, and with a high risk of developing cirrhosis and HCC. By contrast, loss of HBsAg in patients in the

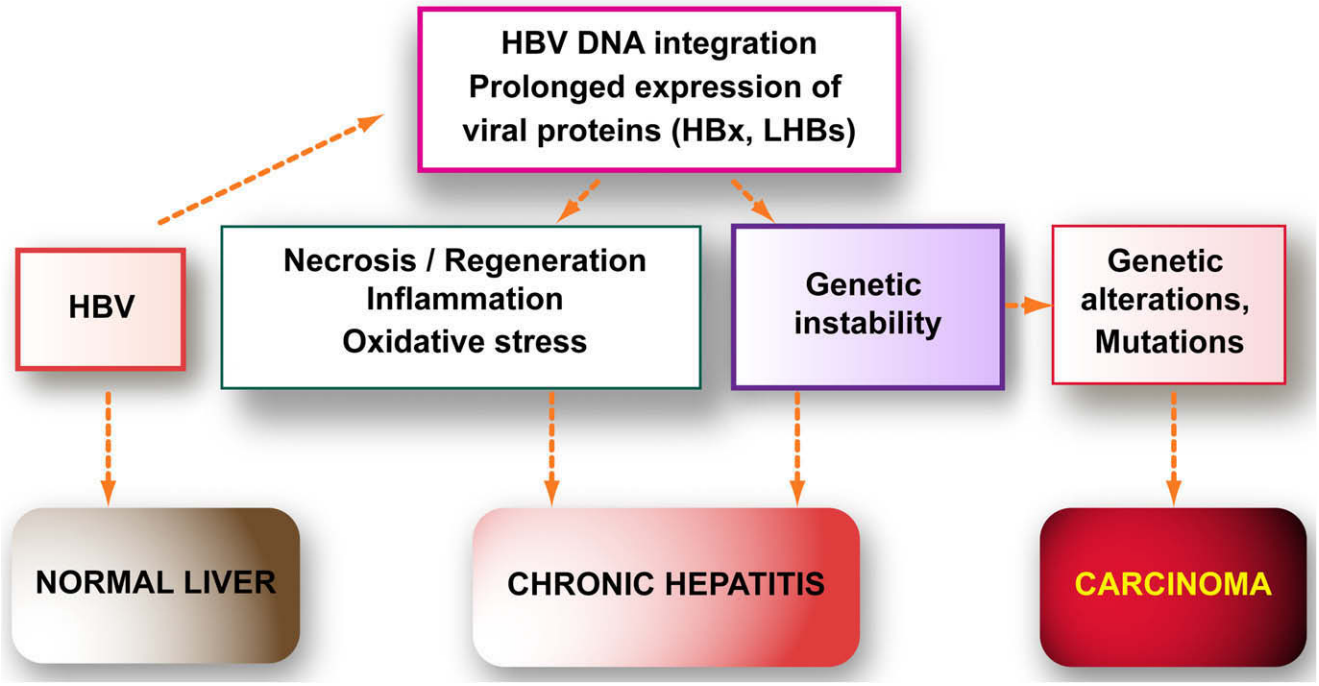

Fig. 3. Chronic HBV infection and hepatocarcinogenesis. HBV DNA integration into the host genome and persistent expression of viral proteins such as HBx and large envelope protein (LHBs) can activate cellular cancer-related genes, induce oxidative stress and genetic instability. In the inflammatory context triggered by host immune responses, the viral functions contribute to ceaseless hepatocyte destruction–regeneration, and provide a favorable ground for emergence of genetic and epigenetic alterations leading to hepatocyte transformation.

"HBsAg-negative phase" has a favorable prognosis, although HCC incidence remains higher than in non-infected populations [43]. This observation may be linked to the finding that persistent traces of HBV DNA are often detectable in the blood for many years after clinical recovery from acute hepatitis, despite the presence of serum antibodies and HBV-specific CTLs [44].

While a majority of liver cancers develop in cirrhotic livers, a significant fraction of HBV-related HCCs occurs in a background of chronic hepatitis B in the absence of liver cirrhosis. The incidence of cirrhosis appears to be about 2-fold higher in HBeAg-negative compared to HBeAg-positive chronic hepatitis [45]. The lower rate of underlying cirrhosis in HBV-related HCCs compared to other aetiologies argues for a more direct role of HBV in the tumoural process. Additionally, distinctive gene expression profiles have been detected in the non-tumoural livers of chronic HBV carriers, such as activated expression of genes implicated in pro-apoptotic, inflammatory and DNA repair responses, suggesting specific pathways triggered by chronic hepatitis B [46,47]. Thus, besides the effects of host immune responses, HBV replication might trigger various signaling cascades in the infected hepatocyte (Fig. 3). Integration of viral, host and environmental parameters with molecular changes might lead to identify novel targeted strategies to improve the management of chronic hepatitis B. Risk scores have been established to estimate the risk of developing HCC in less than 10 years after presentation. Such scores based on age, gender, HBV DNA levels, core promoter mutations and cirrhosis, can be used to identify high-risk patients for treatment and screening of HCC [48].

Molecular mechanisms of hepatocarcinogenesis

With the recent availability of integrative systems biology approaches, global insights into genetic alterations and molecular profiles have been reported in large series of HCCs during the last years. These studies have demonstrated a variety of genetic alterations and marked heterogeneity of gene expression profiles among HCC cases, suggesting that HCC might be one of the most complex and heterogeneous solid tumours in humans [49–51]. Such heterogeneity is in agreement with the multiple aetiologies of HCC and the long period of chronic inflammatory disease that fosters the accumulation of genetic and epigenetic defects. Evidence has been provided for deregulation of various signaling pathways in HCC subsets, such as Wnt/β-catenin signaling [52], and the p14ARF/p53 pathway [53], transforming growth factor beta (TGF-β) signaling, Ras/MAPK signaling and the PTEN/Akt and mTOR pathways [54] (reviewed in [50]). Additionally, altered expression of growth factors such as HGF, IGFs and Amphyregulin, as well as genes involved in angiogenesis may participate in the development and progression of HCC (reviewed in [55]).

Aberrant activation of the Wnt/β-catenin pathway through mutations in either β-catenin or Axin genes has been found in 20–40% of HCC cases, predominantly in HCV-associated tumours and in the absence of viral aetiology [56–58]. Interestingly, HCCs carrying mutant β-catenin display distinctive patterns, with highly differentiated morphology comprising microtrabecular and acinar architectures, low proliferative rate, and frequent intrahepatic cholestasis but no steatosis [59]. This tumour type also displays activated expression of liver metabolic enzymes involved in glutamine synthesis, urea cycle and detoxification

metabolism, which are normally expressed in perivenous hepatocytes [60,61]. Moreover, β-catenin mutation has been correlated with a low rate of genetic instability [56,62].

TGF-β has a complex role in HCC; it is persistently induced during hepatitis and promotes cirrhosis progression by accelerating the deposition of extracellular matrix (reviewed in [63]). Moreover, persistent upregulation of TGF-β in HCC has been suggested to accelerate neoplastic growth [64]. A bipartite role has thus been ascribed to TGF-β, which has tumour suppressor functions at early stages of liver damage, while at neoplastic stages, TGF-β may switch from being a tumour suppressor into being a tumour promoter that induces invasive and metastatic behaviour. Accordingly, blocking TGF-β using a kinase inhibitor of the TGF-β receptor I has an anti-tumoural effect via the regulation of neo-vascularization [65].

While oncogenic mutations of Ras are rarely seen in HCC, aberrant activation of protein kinases has been implicated in tumour initiation and progression. These kinases include PI3K–AKT, Aurora kinase, and receptor tyrosine kinases of VEGF, EGF, PDGF, Heregulin, and IGF-I [66]. Recent studies have suggested a major role of mitogen-activated protein kinases (MAP kinases) such as p38 and the c-Jun N-terminal kinase (JNK) in driving uncontrolled proliferation and invasion of hepatic tumour cells [67–69]. These factors represent interesting targets for therapeutic intervention, as shown recently by the finding that sorafenib, a multikinase inhibitor, provides substantial survival benefits in patients at advanced HCC stages [70]. Moreover, it has been recently suggested that synergistic effects might be obtained in combination therapy using sorafenib and rapamycin for simultaneous inhibition of the Ras and mTOR pathways [71].

Familial cancer genes with high-penetrance mutations have not been identified so far in HCC. However, recurrent mutations that compromise p53 function have been evidenced in the TP53 gene (17q13.1) in about 25% of primary HCCs [72]. Loss-of-function mutations of the AXIN1 gene (16p13.3) have been detected in 7–10% of cases, leading to the aberrant activation of Wnt signaling and possibly other pathways [73]. Other mutations affecting the RB, CDKN2A, PTEN or CDH1 genes have been found more rarely, and epigenetic silencing mechanisms such as the methylation of promoter sites may be responsible for the inhibition of tumour suppressor functions, as also recently found for the activation of the Ras and Jak/Stat pathways in HCC [74]. Mutations or deletions of other candidates including NF2, Bax, FHIT, LKB1 and LRP1B have been seen at very low frequency (less than 2–4% cases) [75].

The question of whether distinctive molecular mechanisms operate in HBV-related HCCs has been addressed using genetic approaches and microarray technologies for analyzing gene expression profiles. It has been shown that HBV-related HCCs display higher rates of chromosomal alterations than HCCs related to other risk factors [76]. Interestingly, recent studies have evidenced two major routes of hepatocarcinogenesis that differ by the extent of genetic instability, one being predominant in HBV-related tumours [57]. In this work, genetically unstable tumours have been associated with HBV infection, poor differentiation, p53 mutations, and an unfavorable prognosis. Extending these observations, gene expression profiling has revealed that HCC subclasses associated with HBV infection also display an activation of the mitotic cell cycle, deregulated expression of developmental and imprinted genes such as IGF2, as well as activation of the AKT pathway [62]. In different studies, molecular classification of HCC based on gene expression profiles has unveiled the predominant importance of the tumour cell differentiation status for defining molecular subtypes and predicting disease outcome [67,77]. More recently, a meta-analysis integrating transcriptome data from eight independent cohorts has led to the identification of three major molecular subclasses of HCC associated with tumour differentiation and serum alpha-fetoprotein (AFP) levels [78]. Again, in this study, chronic HBV infection was found to be significantly associated with HCC clusters featured by moderate/poor differentiation of tumour cells. Moreover, molecular classification of HBV-related HCCs has been used to predict early recurrence, which is a golden standard to determine the success of curative resection. The group with a high risk of recurrence showed an increased expression of genes involved in cell cycle progression, cell proliferation, migration and motility, and in the Notch signaling pathway [79].

While significant advances can be noted in our understanding of the molecular basis of hepatocarcinogenesis, future studies should be aimed at gaining a comprehensive view of the signaling networks operating in liver cell transformation due to chronic hepatitis B.

Chronic HBV infection and liver oncogenesis

HBV mutants and genotypes

During the progression of chronic hepatitis B from the asymptomatic carrier state to HCC, mutations accumulate in the viral genome in the preS region and in the carboxy-terminal region of the X gene, which overlaps with the enhancer II and the basal core promoter (BCP) [80]. Whether the risk of developing HCC may be influenced by the viral status of the patients, such as HBV genotypes, hepatitis B e antigen (HBeAg) serostatus, and mutations arising during chronic infections are important issues. It has been shown that these parameters play important role, not only in the progression of chronic liver disease, but also in the response to anti-viral therapies [81–83].

While epidemiological studies are usually based on HBsAg positivity, several case-control studies have pointed to HBeAg as a potential predictive marker, with a higher prevalence of HBeAg among patients with HBV-related HCC than among matched HBsAg carriers. In a study of a large cohort of patients, the relative risk of HCC was increased by 6-fold among patients positive for both HBeAg and HBsAg compared with those positive for HBsAg alone [84]. Thus HBeAg may be a useful marker of the risk of cancer, probably because it reflects active HBV DNA replication. The differential oncogenicity of HBV genotypes has been extensively analyzed. Important differences have been observed in the prevalence of serum HBeAg, the levels of HBV DNA, and the occurrence of precore or core promoter mutations between genotypes B and C [85], and between genotypes Aa, Ae, and D [86]. Evaluation of clinical and virological differences between HCC patients infected with genotype B or genotype C in Japan and Taiwan has given rise to divergent data [87,88]. Other studies have concluded that HBV genotype C takes a more aggressive disease course than genotype B in HBeAg-positive patients [89], and HBV genotype C infection (compared to genotype B) has been found as an independent risk factor for HCC development [90]. By contrast, other studies have shown that the prevalence of T1762/A1764 mutation in the basal core promoter increases with the progression of liver disease, and that this mutation is signif-

icantly associated with the development of HCC, both in genotypes B and C [85].

More recently, the notion that HBV genotypes and variants might harbor different oncogenic potential has been strengthened by large-scale studies involving thousands of patients and meta-analysis of compiled data. Statistically significant correlations with HCC occurrence were seen for mutations in the preS region in HBV genotype C, and at positions C1653T in enhancer II as well as T1753V and A1762T/G1764A in the basal core promoter (BCP) in HBV genotype C. These mutations alone or in combination can predict HCC development in 80% of cases [80]. Furthermore, the T1762/A1764 mutation can be detected in plasma up to 8 years before HCC diagnosis; this mutation might therefore be considered as a strong predictive biomarker [91].

HBV DNA integration into human chromosomes

HBV DNA integration into human host chromosomes occurs in the infected liver since early stages of natural acute infections [92,93]. Multiple integrations have been detected in chronic hepatitis tissues [94,95], and integrated HBV sequences have been seen in most (about 80%) HBV-related HCCs [96,97]. In the absence of complete genomes in virtually all HBV inserts, these sequences cannot serve as template for viral replication.

HBV DNA integration sites in human HCCs have been localized on almost all chromosomes, suggesting a random distribution throughout the host genome [93,98]. These events could either induce chromosome changes or act *in cis* on the expression or function of nearby cellular genes. For a long time it has been thought that HBV insertional mutagenesis is a rare event, and that simple repetitive elements are hotspots for HBV insertion in the human genome. However, evidence for a direct *cis*-acting promoter insertion mechanism was first provided in two independent HCCs, in which the HBV insertion targeted either the retinoic acid receptor-β (RAR-β) gene or the human cyclin A gene, resulting in tumour-specific chimeric proteins endowed with novel, pro-carcinogenic functions [99–102,103,104]. Thus, the analysis of single HBV insertion sites has allowed identifying new genes that play critical roles in the control of cell growth and differentiation.

In recent studies, the view of random HBV integration has been challenged by large-scale analysis of HBV DNA insertion sites using the Alu-PCR approach. These studies revealed high rates of gene targeting by HBV integration [105,106]. As previously shown for retroviruses [107,108], HBV DNA integration appears to occur frequently in actively transcribed chromosomal regions, within genes or at their immediate vicinity. Recently, sequence analysis of 68 viral–host junctions from 60 HCCs provided evidence for cellular coding regions within several kilobases in 90% of the cases [106]. Moreover, it was shown that HBV integration often targets gene families involved in cell survival, proliferation and immortalization, such as hTERT, PDGF receptor, MLL, calcium signaling-related genes and 60s ribosomal protein genes. Indeed, recurrent viral insertions nearby the hTERT or MLL gene have been reported by different groups [106,109–111]. It can be envisaged that viral insertion induces the first genetic hit in liver tumourigenesis and that different genes targeted by viral integration play important role in hepatocarcinogenesis. While enhanced expression of some cellular target genes upon nearby viral insertion has been documented, the question of whether these genes play a role in tumourigenesis as drivers or represent only bystanders has rarely been addressed. Finally, although integrated viral sequences are defective for replication, they might also contribute "*in trans*" to tumourigenesis through the production of truncated and mutated HBx or preS2/S proteins. These proteins may act on HCC development by disrupting the control of cellular gene expression or by activating oncogenic signaling pathways.

Besides acting by cis- or trans-activation, HBV insertions have been associated with major genetic alterations within the cell genome, including large deletions, duplications and chromosomal translocations [112–116]. The association of HBV integration with large genomic changes might reflect the abrogation of control mechanisms that safeguard chromosomal integrity [49].

The HBx regulatory protein: a viral oncogene?

The HBx protein encoded by the X gene has been designated "viral oncoprotein" by several authors. This protein has been involved in liver cell transformation because of its pleiotropic activities on cell cycle regulation, signaling pathways and DNA repair (reviewed in [117–119]) (Fig. 4). However, evidence for a direct transforming activity of HBx is scarce. HBx has been reported to transform two cell lines immortalized by the simian virus 40 large T antigen (SV40TAg) [120,121]. The viral protein has also been shown to cooperate with Ras in the transformation of primary human fibroblasts, either by activating the phosphatidylinositol-3 kinase and Akt pathway or by overcoming Ras-induced senescence [122,123]. A weak tumourigenicity has been attributed to HBx in TGF-α immortalized murine hepatocytes, but no cooperation could be evidenced with mutated p53 [124]. Other studies describe opposite effects, in which the apoptotic properties of HBx suppress transformation of primary rodent fibroblasts by different oncogenes [125]. One caveat with these observations is that most were made in cell culture with high levels of X gene expression. Indeed, in the chronically infected liver, HBx expression is kept at low, barely detectable levels [126].

Studies of HBx oncogenicity in transgenic mice have yielded divergent results that may be related to different murine genetic backgrounds and various promoters yielding different expression levels of the viral protein. HCC development has been associated with high-level hepatic expression of HBx in a transgenic mouse line generated in the outbred CD-1 background [127]. In most other transgenic lines in different backgrounds, expression of HBx by itself did not lead to HCC development, although slight histopathologic alterations could be observed in the liver [128]. Transgenic expression of HBx in the murine liver activates both proliferation and apoptosis, and sensitizes hepatocytes to oncogenic transformation [129–133]. Consistent with the ability of HBx to deregulate the cell cycle, premature cell cycle entry or impaired regeneration has been observed after partial hepatectomy of the liver of HBx mice from different transgenic lines [134–136].

Among the different activities of HBx, its trans-activation function may play a crucial role in hepatocarcinogenesis because it is involved in the activation of a large number of signaling pathways and cellular genes that are involved in oncogenesis, proliferation, inflammation and immune responses [137–139]. In particular, we have recently shown that HBx interacts with the acetyltransferases CBP/p300, and that this interaction plays a decisive role in the activation of CREB-dependent transcription [140]. The activation of CREB/ATF trans-activation function by

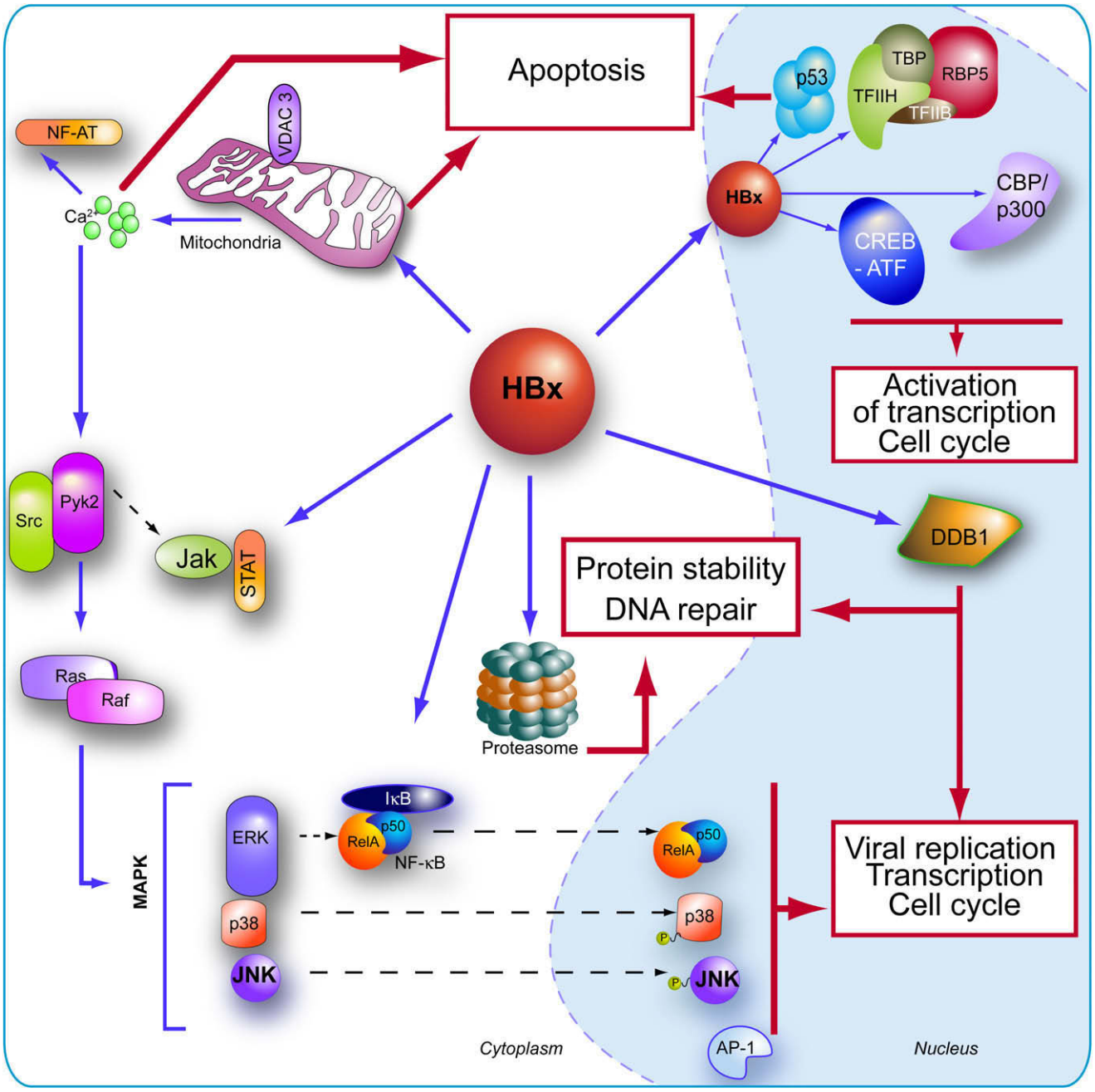

Fig. 4. Multiple biological activities of HBx. HBx binds various transcription factors, coactivators and components of the basal transcription machinery and participates directly in the control of viral and cellular transcription. HBx can also regulate transcription indirectly by acting on cellular signaling pathways such as NF-κB, MAPKs and JAK/STAT. Moreover, HBx may play a role in protein degradation and in apoptosis via its interaction with proteasome subunits, mitochondrial proteins, DDB1 and p53.

HBx appears double since HBx has been shown to increase CREB/ATF DNA-binding affinity as well as to enhance the recruitment of CBP/p300 to CREB/ATF bound to cellular DNA [140,141] (Fig. 5). The modulation of CREB/ATF activity by HBx represents an important aspect of HBx activities since the CREB/ATF family members play an essential role in liver metabolism and proliferation, and CREB has been implicated in hepatocarcinogenesis [142]. Moreover, the coactivators CBP/p300 are known to bind and activate a large variety of cellular transcription factors [143]. Some of these factors, such as c-Jun, c-Fos and NF-κB are also activated by HBx, and the interaction between HBx and CBP/p300 could explain, at least partially, the broad activity of HBx on transcription.

Another reported activity of HBx targets centrosome dynamics and mitotic spindle formation through the binding of HBx with different cellular partners implicated in centrosome formation. HBx has been shown to bind and partially inactivate BubR1, an effector of multiple mitotic kinases that specifies microtubule attachments and checkpoint functions [144]. HBx also interacts with HBXIP, a major regulator of centrosome duplication,

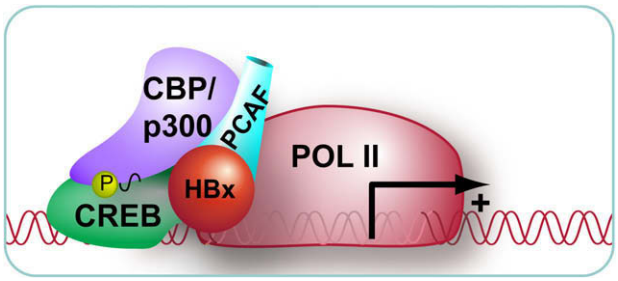

Fig. 5. Modulation of CREB transcriptional activity by HBx. HBx interacts with the acetyltransferases CBP/p300 and PCAF, and favors recruitment of these coactivators onto CREB-responsive promoters, leading to the activation of gene expression.

required for bipolar spindle formation and cytokinesis [145]. Interestingly, recent data have linked DNA re-replication induced by HBx to partial polyploidy, known to be associated with cancer pathogenesis [146]. Although the precise HBx activities that interfere with the mitotic cell cycle remain to be determined, these data provide a strong link between HBx expression and chromosomal instability in HBV-related carcinogenesis.

Conclusions and perspectives

Consistent with the multifactorial aetiology of HCC and the long latency period of tumour formation, a large variety of oncogenes, growth factors and tumour suppressors have been implicated in human hepatocarcinogenesis [49,51]. The question of whether HBV and other aetiological factors and co-factors may trigger different tumourigenic pathways can now be addressed, owing to the development of novel technologies for genome-wide scans of genetic alterations and gene expression, and to new technologies in proteomic analysis. Increased frequency of p53 mutations in HCC has been associated with AFB1 exposure and chronic HBV infection, while HBV-related tumours display high chromosomal instability and low rate of β-catenin mutation. So far, studies have mainly been focused on tumours; it will therefore be important to analyze gene expression and proteomic changes in large series of samples from chronic hepatitis B at different stages in order to identify suitable prognostic markers and therapeutic targets. Besides genomic alterations, epigenetic factors like methylation-associated gene silencing and altered expression of microRNAs may play an important role in the deregulation of cellular functions leading to malignant transformation, and they warrant attention. Better understanding of the complex role of HBV in liver tumourigenesis will undoubtedly contribute to improve the management of liver diseases induced by chronic hepatitis B.

Acknowledgements

We apologize to the authors whose excellent works have not been quoted in this review paper due to space restriction. This work was supported by grants from the Institut Pasteur, the Institut National de la Santé et de la Recherche Médicale (Inserm), the Agence Nationale de Recherche sur le SIDA (ANRS), the Association pour la Recherche sur le Cancer (ARC) and the Ligue Nationale contre le Cancer (LNCC).

References

[1] Ganem D, Prince AM. Hepatitis B virus infection – natural history and clinical consequences. N Engl J Med 2004;350:1118–1129.
[2] Zanetti AR, Van Damme P, Shouval D. The global impact of vaccination against hepatitis B: a historical overview. Vaccine 2008;26:6266–6273.
[3] Parkin DM. The global health burden of infection-associated cancers in the year 2002. Int J Cancer 2006;118:3030–3044.
[4] Hassan MM, Hwang LY, Hatten CJ, Swaim M, Li D, Abbruzzese JL, et al. Risk factors for hepatocellular carcinoma: synergism of alcohol with viral hepatitis and diabetes mellitus. Hepatology 2002;36:1206–1213.
[5] Sun CA, Wu DM, Lin CC, Lu SN, You SL, Wang LY, et al. Incidence and cofactors of hepatitis C virus-related hepatocellular carcinoma: a prospective study of 12,008 men in Taiwan. Am J Epidemiol 2003;157:674–682.
[6] Lee CL, Hsieh KS, Ko YC. Trends in the incidence of hepatocellular carcinoma in boys and girls in Taiwan after large-scale hepatitis B vaccination. Cancer Epidemiol Biomarkers Prev 2003;12:57–59.
[7] Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 2006;295:65–73.
[8] Paterlini P, Driss F, Pisi E, Franco D, Berthelot P, Bréchot C. Persistence of hepatitis B and hepatitis C viral genomes in primary liver cancers from HBsAg negative patients: a study of a low endemic area. Hepatology 1993;17:20–29.
[9] Pollicino T, Squadrito G, Cerenzia G, Cacciola I, Raffa G, Craxi A, et al. Hepatitis B virus maintains its pro-oncogenic properties in the case of occult HBV infection. Gastroenterology 2004;126:102–110.
[10] Ikeda K, Kobayashi M, Someya T, Saitoh S, Hosaka T, Akuta N, et al. Occult hepatitis B virus infection increases hepatocellular carcinogenesis by eight times in patients with non-B, non-C liver cirrhosis: a cohort study. J Viral Hepat 2009;16:437–443.
[11] Ming L, Thorgeirsson SS, Gail MH, Lu P, Harris CC, Wang N, et al. Dominant role of hepatitis B virus and cofactor role of aflatoxin in hepatocarcinogenesis in Qidong, China. Hepatology 2002;36:1214–1220.
[12] El-Serag HB. Hepatocellular carcinoma: recent trends in the United States. Gastroenterology 2004;127:S27–S34.
[13] Bruix J, Boix L, Sala M, Llovet JM. Focus on hepatocellular carcinoma. Cancer Cell 2004;5:215–219.
[14] Bruix J, Hessheimer AJ, Forner A, Boix L, Vilana R, Llovet JM. New aspects of diagnosis and therapy of hepatocellular carcinoma. Oncogene 2006;25:3848–3856.
[15] Avila MA, Berasain C, Sangro B, Prieto J. New therapies for hepatocellular carcinoma. Oncogene 2006;25:3866–3884.
[16] Summers J, Mason WS. Replication of the genome of a hepatitis B – like virus by reverse transcription of an RNA intermediate. Cell 1982;29:403–415.
[17] Soussan P, Garreau F, Zylberberg H, Ferray C, Brechot C, Kremsdorf D. In vivo expression of a new hepatitis B virus protein encoded by a spliced RNA. J Clin Invest 2000;105:55–60.
[18] Breiner KM, Schaller H, Knolle PA. Endothelial cell-mediated uptake of a hepatitis B virus: a new concept of liver targeting of hepatotropic microorganisms. Hepatology 2001;34:803–808.
[19] Rabe B, Vlachou A, Panté N, Helenius A, Kann M. Nuclear import of hepatitis B virus capsids and release of the viral genome. Proc Natl Acad Sci USA 2003;100:9849–9854.
[20] Beck J, Nassal M. Hepatitis B virus replication. World J Gastroenterol 2007;13:48–64.
[21] Mason WS, Seal G, Summers J. Virus of Pekin ducks with structural and biological relatedness to human hepatitis B virus. J Virol 1980;36:829–836.
[22] Weiser B, Ganem D, Seeger C, Varmus HE. Closed circular viral DNA and asymmetrical heterogeneous forms in livers from animals infected with ground squirrel hepatitis virus. J Virol 1983;48:1–9.
[23] Tang H, Delgermaa L, Huang F, Oishi N, Liu L, He F, et al. The transcriptional transactivation function of HBx protein is important for its augmentation role in hepatitis B virus replication. J Virol 2005;79:5548–5556.
[24] Bock CT, Schranz P, Schroder CH, Zentgraf H. Hepatitis B virus genome is organized into nucleosomes in the nucleus of the infected cell. Virus Genes 1994;8:215–229.
[25] Lewin S, Walters T, Locarnini S. Hepatitis B treatment: rational combination chemotherapy based on viral kinetic and animal model studies. Antiviral Res 2002;55:381–396.
[26] Pollicino T, Belloni L, Raffa G, Pediconi N, Squadrito G, Raimondo G, et al. Hepatitis B virus replication is regulated by the acetylation status of

hepatitis B virus cccDNA-bound H3 and H4 histones. Gastroenterology 2006;130:823–837.
[27] Chen HS, Kanako S, Girones R, Anderson RW, Hornbuckle WE, Tennant BC, et al. The woodchuck hepatitis virus X gene is important for establishment of virus infection in woodchucks. J Virol 1993;67:1218–1226.
[28] Zoulim F, Saputelli J, Seeger C. Woodchuck hepatitis virus X protein is required for viral infection in vivo. J Virol 1994;68:2026–2030.
[29] Leupin O, Bontron S, Schaeffer C, Strubin M. Hepatitis B virus X protein stimulates viral genome replication via a DDB1-dependent pathway distinct from that leading to cell death. J Virol 2005;79:4238–4245.
[30] Keasler VV, Hodgson AJ, Madden CR, Slagle BL. Enhancement of hepatitis B virus replication by the regulatory X protein in vitro and in vivo. J Virol 2007;81:2656–2662.
[31] Keasler VV, Hodgson AJ, Madden CR, Slagle BL. Hepatitis B virus HBx protein localized to the nucleus restores HBx-deficient virus replication in HepG2 cells and in vivo in hydrodynamically-injected mice. Virology 2009;390:122–129.
[32] Tuttleman JS, Pourcel C, Summers J. Formation of the pool of covalently closed circular viral DNA in hepadnavirus-infected cells. Cell 1986;47:451–460.
[33] Ganem D. Hepadnaviridae: the viruses and their replication. In: Fields B, Knipe D, Howley P, editors. Fields virology. Philadelphia: Lippincott-Raven; 1996. p. 2703–2737.
[34] Wei Y, Tiollais P. Molecular biology of hepatitis B virus. Clin Liver Dis 1999;3:189–219.
[35] Hoofnagle JH, Doo E, Liang TJ, Fleischer R, Lok AS. Management of hepatitis B: summary of a clinical research workshop. Hepatology 2007;45:1056–1075.
[36] Liver EAftSot. EASL clinical practice guidelines: management of chronic hepatitis B. J Hepatol 2009;50:227.
[37] Ikeda H, Sasakia M, Satoa Y, Haradaa K, Zenc Y, Mitsuid T, et al. Large cell change of hepatocytes in chronic viral hepatitis represents a senescent-related lesion. Human Pathol, in press. doi: 10.1016/j.humpath.2009.1006.1009.
[38] Collado M, Blasco MA, Serrano M. Cellular senescence in cancer and aging. Cell 2007;130:223–233.
[39] Realdi G, Alberti A, Rugge M, Bortolotti F, Rigoli AM, Tremolada F, et al. Seroconversion from hepatitis B e antigen to anti-HBe in chronic hepatitis B virus infection. Gastroenterology 1980;79:195–199.
[40] Liaw YF, Chu CM, Su IJ, Huang MJ, Lin DY, Chang-Chien CS. Clinical and histological events preceding hepatitis B e antigen seroconversion in chronic type B hepatitis. Gastroenterology 1983;84:216–219.
[41] Martinot-Peignoux M, Boyer N, Colombat M, Akremi R, Pham BN, Ollivier S, et al. Serum hepatitis B virus DNA levels and liver histology in inactive HBsAg carriers. J Hepatol 2002;36:543–546.
[42] Carman WF, Jacyna MR, Hadziyannis S, Karayiannis P, McGarvey MJ, Markis A, et al. Mutation preventing formation of hepatitis e antigen in patients with chronic hepatitis B infection. Lancet 1989;ii:588–591.
[43] Paterlini P, Gerken G, Khemeny F, Franco D, D'Errico A, Grigioni W, et al. Primary liver cancer in HBsAg-negative patients: a study of HBV genome using the polymerase chain reaction. In: Hollinger FB, Lemon SM, Margolis H, editors. Viral hepatitis and liver disease. USA: Williams and Wilkins; 1991. p. 605–610.
[44] Rehermann B, Ferrari C, Pasquinelli C, Chisari FV. The hepatitis B virus persists for decades after patients' recovery from acute viral hepatitis despite active maintenance of a cytotoxic T-lymphocyte response. Nat Med 1996;2:1104–1108.
[45] 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.
[46] Honda M, Kaneko S, Kawai H, Shirota Y, Kobayashi K. Differential gene expression between chronic hepatitis B and C hepatic lesion. Gastroenterology 2001;120:955–966.
[47] Honda M, Yamashita T, Ueda T, Takatori H, Nishino R, Kaneko S. Different signaling pathways in the livers of patients with chronic hepatitis B or chronic hepatitis C. Hepatology 2006;44:1122–1138.
[48] Yuen MF, Tanaka Y, Fong DY, Fung J, Wong DK, Yuen JC, et al. Independent risk factors and predictive score for the development of hepatocellular carcinoma in chronic hepatitis B. J Hepatol 2009;50:80–88.
[49] Buendia MA. Genetics of hepatocellular carcinoma. Semin Cancer Biol 2000;10:185–200.
[50] Laurent-Puig P, Zucman-Rossi J. Genetics of hepatocellular tumors. Oncogene 2006;25:3778–3786.
[51] Lee JS, Thorgeirsson SS. Comparative and integrative functional genomics of HCC. Oncogene 2006;25:3801–3809.
[52] de La Coste A, Romagnolo B, Billuart P, Renard CA, Buendia MA, Soubrane O, et al. Somatic mutations of the beta-catenin gene are frequent in mouse and human hepatocellular carcinomas. Proc Natl Acad Sci USA 1998;95:8847–8851.
[53] Hussain SP, Schwank J, Staib F, Wang XW, Harris CC. TP53 mutations and hepatocellular carcinoma: insights into the etiology and pathogenesis of liver cancer. Oncogene 2007;26:2166–2176.
[54] Villanueva A, Chiang DY, Newell P, Peix J, Thung S, Alsinet C, et al. Pivotal role of mTOR signaling in hepatocellular carcinoma. Gastroenterology 2008;135:1972–1983.
[55] Breuhahn K, Longerich T, Schirmacher P. Dysregulation of growth factor signaling in human hepatocellular carcinoma. Oncogene 2006;25:3787–3800.
[56] Legoix P, Bluteau O, Bayer J, Perret C, Balabaud C, Belghiti J, et al. Beta-catenin mutations in hepatocellular carcinoma correlate with a low rate of loss of heterozygosity. Oncogene 1999;18:4044–4046.
[57] Laurent-Puig P, Legoix P, Bluteau O, Belghiti J, Franco D, Binot F, et al. Genetic alterations associated with hepatocellular carcinomas define distinct pathways of hepatocarcinogenesis. Gastroenterology 2001;120:1763–1773.
[58] Cavard C, Colnot S, Audard V, Benhamouche S, Finzi L, Torre C, et al. Wnt/beta-catenin pathway in hepatocellular carcinoma pathogenesis and liver physiology. Future Oncol 2008;4:647–660.
[59] Audard V, Grimber G, Elie C, Radenen B, Audebourg A, Letourneur F, et al. Cholestasis is a marker for hepatocellular carcinomas displaying beta-catenin mutations. J Pathol 2007;212:345–352.
[60] Benhamouche S, Decaens T, Godard C, Chambrey R, Rickman DS, Moinard C, et al. Apc tumor suppressor gene is the "zonation-keeper" of mouse liver. Dev Cell 2006;10:759–770.
[61] Hailfinger S, Jaworski M, Braeuning A, Buchmann A, Schwarz M. Zonal gene expression in murine liver: lessons from tumors. Hepatology 2006;43:407–414.
[62] Boyault S, Rickman DS, de Reynies A, Balabaud C, Rebouissou S, Jeannot E, et al. Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. Hepatology 2007;45:42–52.
[63] Matsuzaki K. Modulation of TGF-beta signaling during progression of chronic liver diseases. Front Biosci 2009;14:2923–2934.
[64] Rossmanith W, Schulte-Hermann R. Biology of transforming growth factor beta in hepatocarcinogenesis. Microsc Res Tech 2001;52:430–436.
[65] Fransvea E, Mazzocca A, Antonaci S, Giannelli G. Targeting transforming growth factor (TGF)-betaRI inhibits activation of beta1 integrin and blocks vascular invasion in hepatocellular carcinoma. Hepatology 2009;49:839–850.
[66] Tanaka S, Arii S. Molecularly targeted therapy for hepatocellular carcinoma. Cancer Sci 2009;100:1–8.
[67] Lee JS, Heo J, Libbrecht L, Chu IS, Kaposi-Novak P, Calvisi DF, et al. A novel prognostic subtype of human hepatocellular carcinoma derived from hepatic progenitor cells. Nat Med 2006;12:410–416.
[68] Hsieh YH, Wu TT, Huang CY, Hsieh YS, Hwang JM, Liu JY. p38 mitogen-activated protein kinase pathway is involved in protein kinase Calpha-regulated invasion in human hepatocellular carcinoma cells. Cancer Res 2007;67:4320–4327.
[69] Chen F, Beezhold K, Castranova V. JNK1, a potential therapeutic target for hepatocellular carcinoma. Biochim Biophys Acta 2009;1796:242–251.
[70] Llovet JM, Bruix J. Novel advancements in the management of hepatocellular carcinoma in 2008. J Hepatol 2008;48:S20–S37.
[71] Newell P, Toffanin S, Villanueva A, Chiang DY, Minguez B, Cabellos L, et al. Ras pathway activation in hepatocellular carcinoma and anti-tumoral effect of combined sorafenib and rapamycin in vivo. J Hepatol 2009;51:725–733.
[72] Staib F, Hussain SP, Hofseth LJ, Wang XW, Harris CC. TP53 and liver carcinogenesis. Hum Mutat 2003;21:201–216.
[73] Zucman-Rossi J, Benhamouche S, Godard C, Boyault S, Grimber G, Balabaud C, et al. Differential effects of inactivated Axin1 and activated beta-catenin mutations in human hepatocellular carcinomas. Oncogene 2007;26:774–780.
[74] Calvisi DF, Ladu S, Gorden A, Farina M, Conner EA, Lee JS, et al. Ubiquitous activation of Ras and Jak/Stat pathways in human HCC. Gastroenterology 2006;130:1117–1128.
[75] Pineau P, Marchio A, Nagamori S, Seki S, Tiollais P, Dejean A. Homozygous deletion scanning in hepatobiliary tumor cell lines reveals alternative pathways for liver carcinogenesis. Hepatology 2003;37:852–861.

[76] Marchio A, Pineau P, Meddeb M, Terris B, Tiollais P, Bernheim A, et al. Distinct chromosomal abnormality pattern in primary liver cancer of non-B, non-C patients. Oncogene 2000;19:3733–3738.
[77] Yamashita T, Forgues M, Wang W, Kim JW, Ye Q, Jia H, et al. EpCAM and alpha-fetoprotein expression defines novel prognostic subtypes of hepatocellular carcinoma. Cancer Res 2008;68:1451–1461.
[78] Hoshida Y, Nijman SM, Kobayashi M, Chan JA, Brunet JP, Chiang DY, et al. Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. Cancer Res 2009;69:7385–7392.
[79] Woo HG, Park ES, Cheon JH, Kim JH, Lee JS, Park BJ, et al. Gene expression-based recurrence prediction of hepatitis B virus-related human hepatocellular carcinoma. Clin Cancer Res 2008;14:2056–2064.
[80] Liu S, Zhang H, Gu C, Yin J, He Y, Xie J, et al. Associations between hepatitis B virus mutations and the risk of hepatocellular carcinoma: a meta-analysis. J Natl Cancer Inst 2009;101:1066–1082.
[81] Sumi H, Yokosuka O, Seki N, Arai M, Imazeki F, Kurihara T, et al. Influence of hepatitis B virus genotypes on the progression of chronic type B liver disease. Hepatology 2003;37:19–26.
[82] Westland C, Delaney W, Yang H, Chen SS, Marcellin P, Hadziyannis S, et al. Hepatitis B virus genotypes and virologic response in 694 patients in phase III studies of adefovir dipivoxil1. Gastroenterology 2003;125:107–116.
[83] Yotsuyanagi H, Hino K, Tomita E, Toyoda J, Yasuda K, Iino S. Precore and core promoter mutations, hepatitis B virus DNA levels and progressive liver injury in chronic hepatitis B. J Hepatol 2002;37:355–363.
[84] Yang HI, Lu SN, Liaw YF, You SL, Sun CA, Wang LY, et al. Hepatitis B e antigen and the risk of hepatocellular carcinoma. N Engl J Med 2002;347:168–174.
[85] Kao JH, Chen PJ, Lai MY, Chen DS. Basal core promoter mutations of hepatitis B virus increase the risk of hepatocellular carcinoma in hepatitis B carriers. Gastroenterology 2003;124:327–334.
[86] Tanaka Y, Hasegawa I, Kato T, Orito E, Hirashima N, Acharya SK, et al. A case-control study for differences among hepatitis B virus infections of genotypes A (subtypes Aa and Ae) and D. Hepatology 2004;40:747–755.
[87] Orito E, Mizokami M. Hepatitis B virus genotypes and hepatocellular carcinoma in Japan. Intervirology 2003;46:408–412.
[88] Ni YH, Chang MH, Wang KJ, Hsu HY, Chen HL, Kao JH, et al. Clinical relevance of hepatitis B virus genotype in children with chronic infection and hepatocellular carcinoma. Gastroenterology 2004;127:1733–1738.
[89] Chan HL, Wong ML, Hui AY, Hung LC, Chan FK, Sung JJ. Hepatitis B virus genotype C takes a more aggressive disease course than hepatitis B virus genotype B in hepatitis B e antigen-positive patients. J Clin Microbiol 2003;41:1277–1279.
[90] Chan HL, Hui AY, Wong ML, Tse AM, Hung LC, Wong VW, et al. Genotype C hepatitis B virus infection is associated with an increased risk of hepatocellular carcinoma. Gut 2004;53:1494–1498.
[91] Kuang SY, Jackson PE, Wang JB, Lu PX, Munoz A, Qian GS, et al. Specific mutations of hepatitis B virus in plasma predict liver cancer development. Proc Natl Acad Sci USA 2004;101:3575–3580.
[92] Lugassy C, Bernuau J, Thiers V, Krosgaard K, Degott C, Wantzin P, et al. Sequences of hepatitis B virus DNA in the serum and liver of patients with acute benign and fulminant hepatitis. J Infect Dis 1987;155:64–71.
[93] Yaginuma K, Kobayashi H, Kobayashi M, Morishima T, Matsuyama K, Koike K. Multiple integration site of hepatitis B virus DNA in hepatocellular carcinoma and chronic active hepatitis tissues from children. J Virol 1987;61:1808–1813.
[94] Boender PJ, Schalm SW, Heijtink RA. Detection of integration during active replication of hepatitis B virus in the liver. J Med Virol 1985;16:47–54.
[95] Tanaka Y, Esumi M, Shikata T. Frequent integration of hepatitis B virus DNA in noncancerous liver tissue from hepatocellular carcinoma patients. J Med Virol 1988;26:7–14.
[96] Bréchot C, Pourcel C, Louise A, Rain B, Tiollais P. Presence of integrated hepatitis B virus DNA sequences in cellular DNA of human hepatocellular carcinoma. Nature 1980;286:533–535.
[97] Brechot C, Gozuacik D, Murakami Y, Paterlini-Brechot P. Molecular bases for the development of hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC). Semin Cancer Biol 2000;10:211–231.
[98] Tokino T, Matsubara K. Chromosomal sites for hepatitis B virus integration in human hepatocellular carcinoma. J Virol 1991;65:6761–6764.
[99] Dejean A, Bougueleret L, Grzeschik KH, Tiollais P. Hepatitis B virus DNA integration in a sequence homologous to v-erbA and steroid receptor genes in a hepatocellular carcinoma. Nature 1986;322:70–72.
[100] De Thé H, Marchio A, Tiollais P, Dejean A. A novel steroid/thyroid hormone receptor-related gene inappropriately expressed in human hepatocellular carcinoma. Nature 1987;330:667–670.
[101] Wang J, Chenivesse X, Henglein B, Bréchot C. Hepatitis B virus integration in a cyclin A gene in a human hepatocellular carcinoma. Nature 1990;343:555–557.
[102] Wang J, Zindy F, Chenivesse X, Lamas E, Henglein B, Brechot C. Modification of cyclin A expression by hepatitis B virus DNA integration in a hepatocellular carcinoma. Oncogene 1992;7:1653–1656.
[103] Garcia M, de The H, Tiollais P, Samarut J, Dejean A. A hepatitis B virus pre-S-retinoic acid receptor beta chimera transforms erythrocytic progenitor cells in vitro. Proc Natl Acad Sci USA 1993;90:89–93.
[104] Berasain C, Patil D, Perara E, Huang SM, Mouly H, Brechot C. Oncogenic activation of a human cyclin A2 targeted to the endoplasmic reticulum upon hepatitis B virus genome insertion. Oncogene 1998;16:1277–1288.
[105] Paterlini-Brechot P, Saigo K, Murakami Y, Chami M, Gozuacik D, Mugnier C, 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.
[106] Murakami Y, Saigo K, Takashima H, Minami M, Okanoue T, Brechot C, et al. Large scaled analysis of hepatitis B virus (HBV) DNA integration in HBV related hepatocellular carcinomas. Gut 2005;54:1162–1168.
[107] Mikkers H, Allen J, Knipscheer P, Romeijn L, Hart A, Vink E, et al. High-throughput retroviral tagging to identify components of specific signaling pathways in cancer. Nat Genet 2002;32:153–159.
[108] Wu X, Li Y, Crise B, Burgess SM. Transcription start regions in the human genome are favored targets for MLV integration. Science 2003;300:1749–1751.
[109] Horikawa I, Barrett JC. cis-Activation of the human telomerase gene (hTERT) by the hepatitis B virus genome. J Natl Cancer Inst 2001;93:1171–1173.
[110] Ferber MJ, Montoya DP, Yu C, Aderca I, McGee A, Thorland EC, et al. Integrations of the hepatitis B virus (HBV) and human papillomavirus (HPV) into the human telomerase reverse transcriptase (hTERT) gene in liver and cervical cancers. Oncogene 2003;22:3813–3820.
[111] Tamori A, Yamanishi Y, Kawashima S, Kanehisa M, Enomoto M, Tanaka H, et al. Alteration of gene expression in human hepatocellular carcinoma with integrated hepatitis B virus DNA. Clin Cancer Res 2005;11:5821–5826.
[112] Yaginuma K, Kobayashi M, Yoshida E, Koike K. Hepatitis B virus integration in hepatocellular carcinoma DNA: duplication of cellular flanking sequences at the integration site. Proc Natl Acad Sci USA 1985;82:4458–4462.
[113] Tokino T, Fukushige S, Nakamura T, Nagaya T, Murotsu T, Shiga K, et al. Chromosomal translocation and inverted duplication associated with integrated hepatitis B virus in hepatocellular carcinomas. J Virol 1987;61:3848–3854.
[114] Hino O, Shows TB, Rogler CE. Hepatitis B virus integration site in hepatocellular carcinoma at chromosome 17;18 translocation. Proc Natl Acad Sci USA 1986;83:8338–8342.
[115] Hino O, Tabata S, Hotta Y. Evidence for increased in vitro recombination with insertion of human hepatitis B virus DNA. Proc Natl Acad Sci USA 1991;88:9248–9252.
[116] Tsuei DJ, Chang MH, Chen PJ, Hsu TY, Ni YH. Characterization of integration patterns and flanking cellular sequences of hepatitis B virus in childhood hepatocellular carcinomas. J Med Virol 2002;68:513–521.
[117] Andrisani OM, Barnabas S. The transcriptional function of the hepatitis B virus X protein and its role in hepatocarcinogenesis (Review). Int J Oncol 1999;15:373–379.
[118] Bouchard MJ, Schneider RJ. The enigmatic X gene of hepatitis B virus. J Virol 2004;78:12725–12734.
[119] Tang H, Oishi N, Kaneko S, Murakami S. Molecular functions and biological roles of hepatitis B virus x protein. Cancer Sci 2006;97:977–983.
[120] Seifer M, Hohne M, Schaefer S, Gerlich WH. In vitro tumorigenicity of hepatitis B virus DNA and HBx protein. J Hepatol 1991;13:S61–S65.
[121] Gottlob K, Pagano S, Levrero M, Graessmann A. Hepatitis B virus X protein transcription activation domains are neither required nor sufficient for cell transformation. Cancer Res 1998;58:3566–3570.
[122] Kim YC, Song KS, Yoon G, Nam MJ, Ryu WS. Activated ras oncogene collaborates with HBx gene of hepatitis B virus to transform cells by suppressing HBx-mediated apoptosis. Oncogene 2001;20:16–23.
[123] Oishi N, Shilagardi K, Nakamoto Y, Honda M, Kaneko S, Murakami S. Hepatitis B virus X protein overcomes oncogenic RAS-induced senescence in human immortalized cells. Cancer Sci 2007;98:1540–1548.
[124] Oguey D, Dumenco LL, Pierce RH, Fausto N. Analysis of the tumorigenicity of the X gene of hepatitis B virus in a nontransformed hepatocyte cell line and the effects of cotransfection with a murine p53 mutant equivalent to human codon 249. Hepatology 1996;24:1024–1033.

[125] Schuster R, Gerlich WH, Schaefer S. Induction of apoptosis by the transactivating domains of the hepatitis B virus X gene leads to suppression of oncogenic transformation of primary rat embryo fibroblasts. Oncogene 2000;19:1173–1180.
[126] Su Q, Schrôder CH, Hofman WJ, Otto G, Pichlmayr R, Bannasch P. Expression of hepatitis B virus X protein in HBV-infected human livers and hepatocellular carcinoma. Hepatology 1998;27:1109–1120.
[127] Kim CM, Koike K, Saito I, Miyamura T, Jay G. HBx gene of hepatitis B virus induces liver cancer in transgenic mice. Nature 1991;351:317–320.
[128] Reifenberg K, Pudollek HP, Schmitteckert E, Spindler G, Köck J, Schlicht HJ. Long-term expression of hepatitis B virus core-e- and X-proteins does not cause pathologic changes in transgenic mice. J Hepatol 1997;26: 119–130.
[129] Slagle BL, Lee TH, Medina D, Finegold MJ, Butel JS. Increased sensitivity to the hepatocarcinogen diethylnitrosamine in transgenic mice carrying the hepatitis B virus x gene. Mol Carcinog 1996;15:261–269.
[130] Terradillos O, Billet O, Renard CA, Lévy R, Molina T, Briand P, et al. The hepatitis B virus X gene potentiates c-myc-induced liver oncogenesis in transgenic mice. Oncogene 1997;14:395–404.
[131] Terradillos O, Pollicino T, Lecoeur H, Tripodi M, Gougeon ML, Tiollais P, et al. p53-independent apoptotic effects of the hepatitis B virus HBx protein in vivo and in vitro. Oncogene 1998;17:2115–2123.
[132] Madden CR, Finegold MJ, Slagle BL. Hepatitis B virus X protein acts as a tumor promoter in development of diethylnitrosamine-induced preneoplastic lesions. J Virol 2001;75:3851–3858.
[133] Madden CR, Finegold MJ, Slagle BL. Altered DNA mutation spectrum in aflatoxin b1-treated transgenic mice that express the hepatitis B virus x protein. J Virol 2002;76:11770–11774.
[134] Wu BK, Li CC, Chen HJ, Chang JL, Jeng KS, Chou CK, et al. Blocking of G1/S transition and cell death in the regenerating liver of Hepatitis B virus X protein transgenic mice. Biochem Biophys Res Commun 2006;340: 916–928.
[135] Tralhao JG, Roudier J, Morosan S, Giannini C, Tu H, Goulenok C, et al. Paracrine in vivo inhibitory effects of hepatitis B virus X protein (HBx) on liver cell proliferation: an alternative mechanism of HBx-related pathogenesis. Proc Natl Acad Sci USA 2002;99:6991–6996.
[136] Hodgson AJ, Keasler VV, Slagle BL. Premature cell cycle entry induced by hepatitis B virus regulatory HBx protein during compensatory liver regeneration. Cancer Res 2008;68:10341–10348.
[137] Rossner MT. Hepatitis B virus X-gene product: a promiscuous transcriptional activator. J Med Virol 1992;36:101–117.
[138] Yen BTS. Hepadnaviral X protein: review of recent progress. J Biomed Sci 1996;3:20–30.
[139] Cougot D, Buendia MA, Neuveut C. Carcinogenesis induced by hepatitis B virus. In: Chan SHH, editor. Translational research in biomedecine. Basel: Karger; 2008. p. 108–136.
[140] Cougot D, Wu Y, Cairo S, Caramel J, Renard CA, Levy L, et al. The hepatitis B virus X protein functionally interacts with CREB-binding protein/p300 in the regulation of CREB-mediated transcription. J Biol Chem 2007;282:4277–4287.
[141] Barnabas S, Hai T, Andrisani OM. The hepatitis B virus X protein enhances the DNA binding potential and transcription efficacy of bZip transcription factors. J Biol Chem 1997;272:20684–20690.
[142] Abramovitch R, Tavor E, Jacob-Hirsch J, Zeira E, Amariglio N, Pappo O, et al. A pivotal role of cyclic AMP-responsive element binding protein in tumor progression. Cancer Res 2004;64:1338–1346.
[143] Lehrmann H, Pritchard LL, Harel-Bellan A. Histone acetyltransferases and deacetylases in the control of cell proliferation and differentiation. Adv Cancer Res 2002;86:41–65.
[144] Kim S, Park SY, Yong H, Famulski JK, Chae S, Lee JH, et al. HBV X protein targets hBubR1, which induces dysregulation of the mitotic checkpoint. Oncogene 2008;27:3457–3464.
[145] Wen Y, Golubkov VS, Strongin AY, Jiang W, Reed JC. Interaction of hepatitis B viral oncoprotein with cellular target HBXIP dysregulates centrosome dynamics and mitotic spindle formation. J Biol Chem 2008;283: 2793–2803.
[146] Rakotomalala L, Studach L, Wang WH, Gregori G, Hullinger RL, Andrisani O. Hepatitis B virus X protein increases the Cdt1-to-geminin ratio inducing DNA re-replication and polyploidy. J Biol Chem 2008;283:28729–28740.