The International Journal of Biochemistry & Cell Biology 45 (2013) 1987–1996

Contents lists available at SciVerse ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Review

Unraveling the complexity of hepatitis B virus: From molecular understanding to therapeutic strategy in 50 years

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ARTICLE INFO

Article history:
Received 5 May 2013
Received in revised form 18 June 2013
Accepted 21 June 2013
Available online 29 June 2013

Keywords:
Hepatitis B virus
HBx protein
Hepatocellular carcinoma
In vivo model
HBV therapy

ABSTRACT

Hepatitis B virus (HBV) is a well-known hepadnavirus with a double-stranded circular DNA genome. Although HBV was first described approximately 50 years ago, the precise mechanisms of HBV infection and effective therapeutic strategies remain unclear. Here, we focus on summarizing the complicated mechanisms of HBV replication and infection, as well as genomic factors and epigenetic regulation. Additionally, we discuss *in vivo* models of HBV, as well as diagnosis, prevention and therapeutic drugs for HBV. Together, the data in this 50-year review may provide new clues to elucidate molecular mechanisms of HBV pathogenesis and shed new light on the future HBV therapies.

Contents

Abbreviations: AFP, α-fetoprotein; ALDH1, aldehyde dehydrogenase 1; ALL, lymphoblastic leukemia; ALT, alanine aminotransferase; AR, androgen receptor; AST, aspartate aminotransferase; cccDNA, covalently closed circular DNA; CDH1, cadherin-1; C/EBP-β, CCAAT enhancer binding protein β; CHB, chronic hepatitis B; CIITA, class II transactivator; CP, core promoter; CREB, cyclic adenosine monophosphate (cAMP) response element-binding protein; CRHR2, corticotropin-releasing hormone receptor 2; CTLs, CD8T lymphocytes; CXCR1, chemokine (C-X-C motif) receptor 1; DDC, 3,5-diethoxycarbonyl-1,4-dihydrocollidine-treated; DTP, diphtheria–tetanus–pertussis; FAK, focal adhesion kinase; FDA, Food and Drug Administration; FXRA, farnesoid X receptor alpha; HB, hepatitis B; HBeAg, hepatitis B e antigen; HBcAg, hepatitis B core antigen; HBIG, hepatitis B immune globulin; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HBx, hepatitis B X protein; HCC, hepatocellular carcinoma; HDAC1, histone deacetylase 1; hnRNP K, heterogeneous nuclear ribonucleoproteins K; HO-1, Heme oxygenase-1; IFNAR1, type I interferon (IFN-alpha/beta) receptor 1; IL, interleukin; IFN-α, interferon alpha; JAK, Janus kinase; MAPK, mitogen-activated protein kinase; miRNA, microRNA; MMF, macrophagic myofasciitis; MTA1, metastasis-associated protein 1; NHP, non-human primates; OPN, osteopontin; ORFs, open reading frames; PC, precore; PKC, protein kinase C; PEG, polyethylene glycol; PH, portal hypertension; PP1, protein phosphatase 1; PPIs, protein–protein interactions; RNAi, RNA interference; SNPs, single nucleotide polymorphisms; SPP1, secreted phosphoprotein-1; STAT, signal transducer and activator of transcription; TBP, TATA binding protein; TGF, transforming growth factor; TNF, tumor necrosis factor; WHV, woodchuck hepatitis virus; 5-LOX, 5-lipoxygenase.

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http://dx.doi.org/10.1016/j.biocel.2013.06.017

1. Introduction

Hepatitis B virus (HBV) is a member of the hepadnaviridae virus family, whose replication is not directly cytopathic (Liaw and Chu, 2009). However, HBV infection, a global health problem, can lead to a wide spectrum of liver disease ranging from acute to chronic viral hepatitis, which often develops into liver cirrhosis and even hepatocellular carcinoma (HCC) (Urban et al., 2010).

In early 1966, "Australia antigen" (Au) was reported to be linked to liver diseases and subsequently confirmed to be a marker of HBV infection (Ganem and Prince, 2004; Blumberg et al., 1965). In 1979, the HBV genome sequence was revealed and the gene sequence of the hepatitis B virus surface antigen (HBsAg) was identified (Galibert et al., 1979; Valenzuela et al., 1979). In 1981, hepatitis B x protein (HBx), one of the most important HBV proteins, was first identified, while in 1986, Pre-S1 coded sequences in the envelope proteins of HBV were found to be involved in the specific attachment of HBV to liver cells (Neurath et al., 1986; Fattovich et al., 2008). Sodium taurocholate cotransporting polypeptide (NTCP) was demonstrated to be a functional receptor for HBV in 2012 (Yan et al., 2012). Various vaccines and two types of drugs, including interferons and nucleos(t)ide analogs, have been developed to treat HBV infection. A timeline in this paper covers the history of HBV, focusing on a series of key events that may lead to further understanding of HBV (Fig. 1).

2. Hepatitis B virus (HBV) replication and infection

Viral proteins of clinical importance in HBV include the envelope protein, hepatitis B surface antigen (HBsAg), hepatitis B core antigen (HBcAg), and hepatitis B e antigen (HBeAg) (Seeger et al., 1986). The first step in HBV replication involves a non-cell-type-specific primary attachment followed by irreversible binding of the virus to a specific receptor on the plasma membrane of hepatocytes (Schmitt et al., 1999; Bardens et al., 2011). Once in the cytoplasm, the capsid delivers its relaxed circular DNA (rcDNA) previously contained within the virion, into the nucleus by using nuclear pore complexes (NPC) (Kann et al., 2007). Upon arriving in the nucleus, viral rcDNAs, which are incomplete circles, are converted into a covalently closed circular form (cccDNA) (Ng et al., 2005). Unlike the provirus DNA of retroviruses, the cccDNA does not need to be integrated into the host genome, and the cccDNA pool appears to be stable in the absence of cell division (Dandri et al., 2002; Zoulim, 2005; Beck and Nassal, 2007; Lutgehetmann et al., 2010). Subsequently, pgRNA is packaged into newly formed capsids, the crucial step in HBV replication. Assembly and release of the DNA-containing nucleocapsids seem to be required to recruit the nucleocapsid to the site of budding by a balanced co-expression of small and large envelope proteins (Newbold et al., 1995; Liang, 2009). After concomitant degradation of the pgRNA, a single-stranded DNA of minus polarity and a complementary plus-strand DNA are synthesized to form the HBV genome (rcDNA) (Mason et al., 2005). Finally, virions are assembled on and bud from the endoplasmic reticulum (ER) membrane through the engulfing of rcDNA-containing capsids by ER membranes that contain viral envelope proteins (Perlman and Hu, 2003) (Fig. 2).

The individual course of HBV infection is determined by the interaction between virus replication and the host immune response. It has been widely accepted that HBV infection goes through different, usually successive phases: (1) immune tolerance, (2) HBeAg-positive chronic hepatitis B (CHB) (immune clearance), (3) immune control (low or non-replicative), and (4) HBeAg negative CHB (immune escape) (Chu et al., 2002a; Iloeje et al., 2012). The four phases have been identified on the basis of specific biochemical, serological and virological characteristics, including serum aminotransferase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), HBV DNA and HBsAg protein levels and HBeAg serostatus (Fig. 2).

3. Molecular mechanisms of HBV

3.1. Core regulators in HBV and hepatocellular carcinoma

Core regulators such as hepatitis B X protein (HBx) have been identified and explored in HBV pathogenesis (Fig. 3A). HBx, a small 16.5-kDa polypeptide of 154 amino acids, is well characterized to mediate the pathological effects of HBV (Xie et al., 2012; Cougot et al., 2012). In the cytoplasm, HBx modulates many host processes directly or indirectly, while in the nucleus, this viral protein can stimulate the activity of numerous transcription factors and interact with various members of the general transcription machinery complex (Sung et al., 2009; Zhang et al., 2010; Lucifora et al., 2011). HBx can activate genes targeted by the transcription factor cyclic adenosine monophosphate response element-binding protein (CREB), which is then recruited to HBV DNA in infected cells (Zhang et al., 2013; Murakami, 2001). In addition, HBx can affect several key signaling pathways, such as the Ras-Raf-MAPKs, SAPK/JNK, JAK/STATs and protein kinase C (PKC) pathways (Ma et al., 2011). Activation of the ERK/NF-κB pathway by HBx can lead to the transactivation of interleukin-23 (IL-23), a critical protein in many chronic inflammatory diseases including HB (Xia et al., 2012). Several putative nuclear targets of HBx have also been identified, including CREB transcriptional factors, RNA polymerase subunit RPB5, p53, and TATA binding protein (TBP) (Qadri et al., 2011). Additionally, HBx can induce tumor necrosis factor (TNF)-α-mediated apoptotic destruction of liver cells or directly induce the expression of transforming growth factor (TGF)-β1 (Lee et al., 2001). Recently, new interactions between HBx and HVDAC3 and a novel subcellular distribution of HBx have been identified (Rahmani et al., 2000). Some specific targets, such as aldehyde dehydrogenase 1 (ALDH1) and two calcium ion-binding proteins, S100A6 and S100A4, can be hypermethylated by HBx (Kim et al., 2006). HBx can also repress apoptosis by inhibiting the p53-mediated pathway or caspase-independent pathways involving the repression of mitochondrion-to-nucleus translocation of AIF. HBx may activate the autophagic pathway in HepG-2 cells *via* the

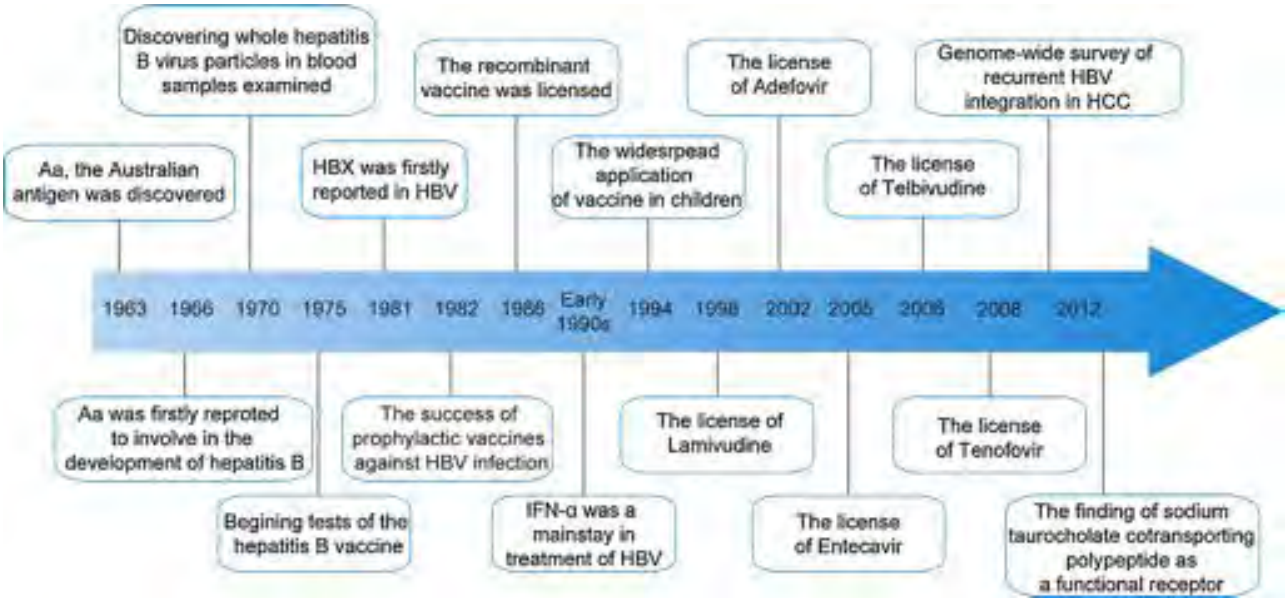

Fig. 1. Timeline: a history of hepatitis B virus.

PI3K-Akt-mTOR pathway or up-regulation of Beclin-1 expression (Tang et al., 2009; Liu et al., 2012b; Wang et al., 2013). Heme Oxygenase-1 (HO-1) can elicit its anti-viral effect directly in hepatocytes and inhibit HBV infection efficiently at a posttranscriptional step by reducing the stability of HBV core protein (Protzer et al., 2007). Activation of signal transducer and activator of transcription (STAT) family members is critical for regulation of some key cellular processes in HBV infection (Gao et al., 2012). Moreover, cytotoxic CD8T lymphocytes (CTLs) can play a pivotal role in the control of HBV infection (Hofmeyer et al., 2011). Specifically, accumulation of the large envelope protein in the ER of hepatocytes is associated with predisposition to transformation in transgenic animal studies

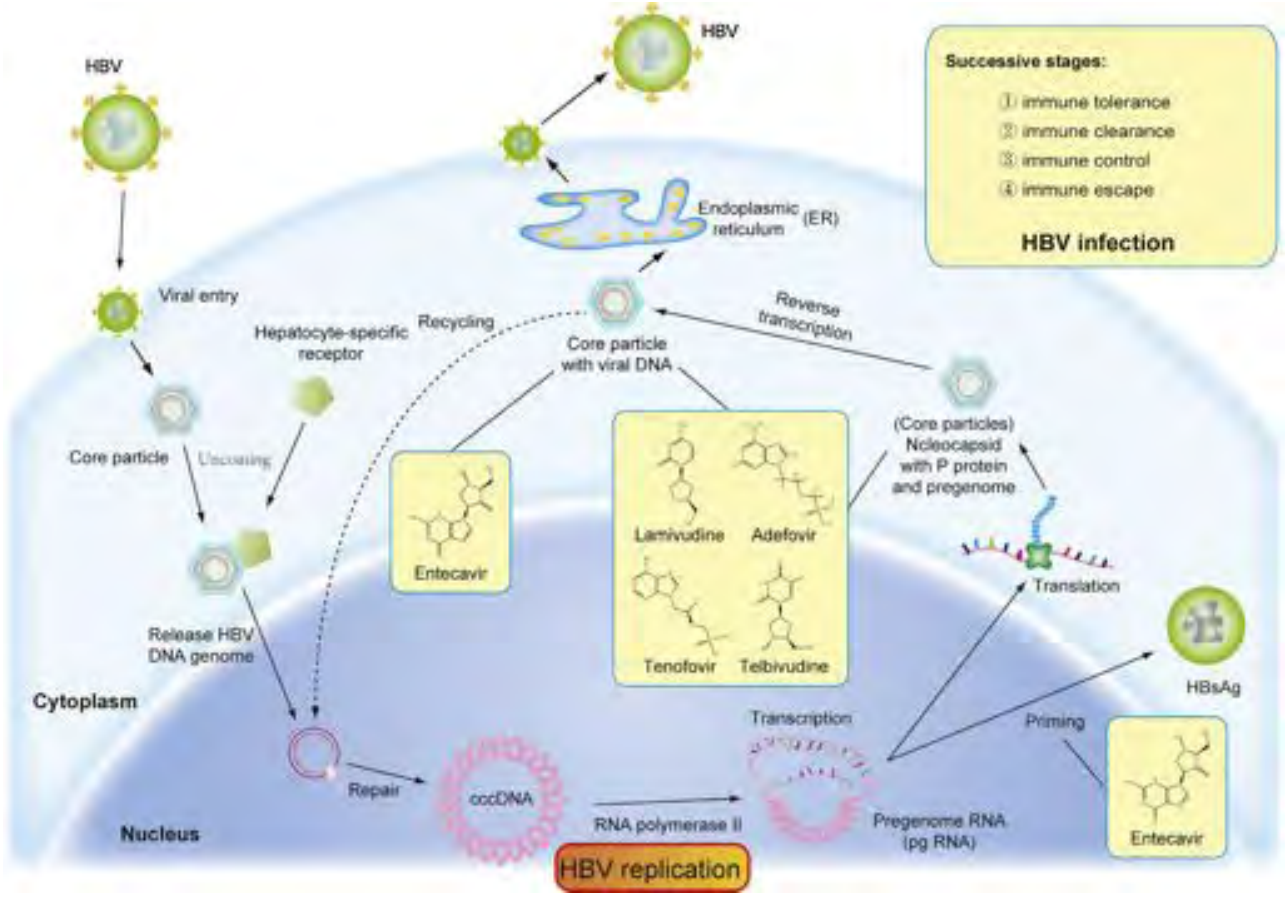

Fig. 2. Different stages of HBV replication and infection.

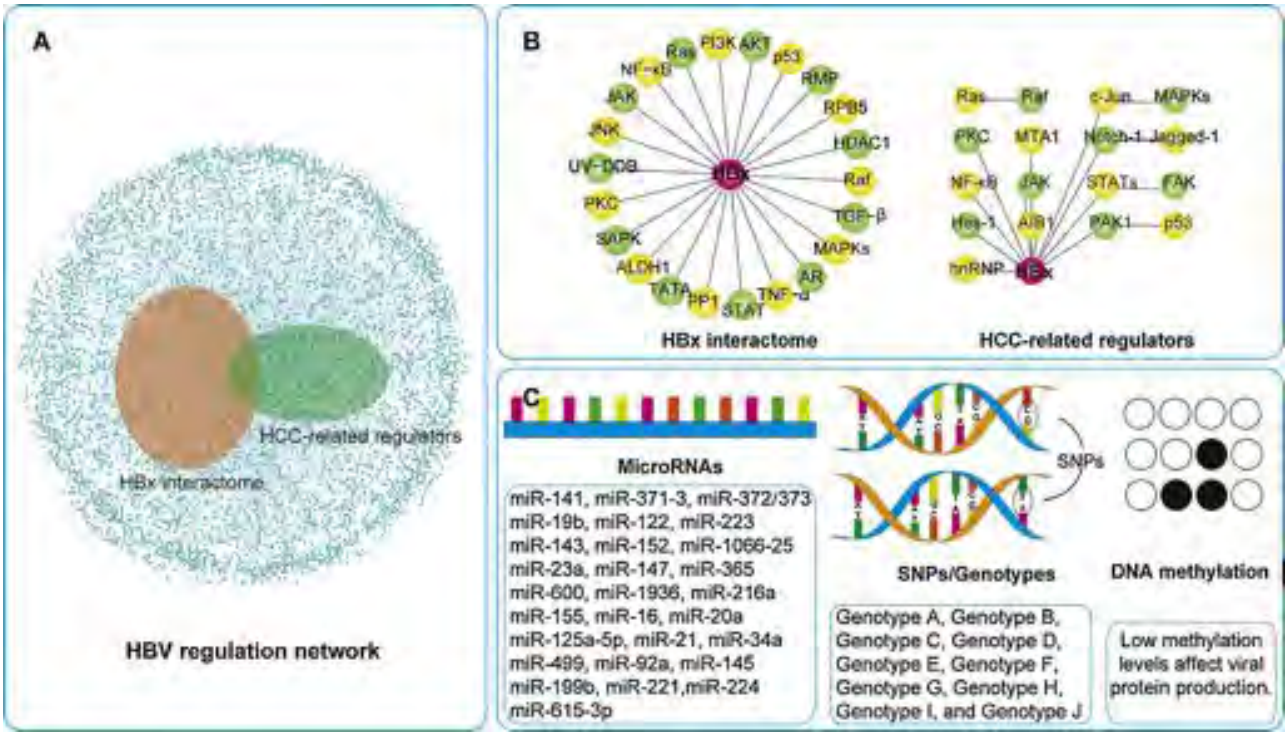

Fig. 3. Regulatory mechanisms of HBV infection. (A) HBV regulation network; (B) HBx interactome and HCC-related modulators; (C) MicroRNAs, genomic factors and epigenetic regulation.

and with severe fulminant hepatitis in human (Chisari et al., 1989). Additionally, integrated HBV DNA can encode a carboxyl terminally truncated protein (Fig. 3B).

HBV core promoter (CP) mutations are known to be associated with an increased risk of HCC due to ubiquitin-mediated proteasomal degradation (Huang et al., 2011). HBx does not bind directly to DNA but causes transcriptional activation through interaction with nuclear transcription factors and modulation of cytoplasmic signaling pathways, including Ras, Raf, c-JUN, Mitogen-activated protein kinases (MAPKs), NF-κB, Janus kinase (JAK)-STAT, focal adhesion kinase (FAK), PKC, and metastasis-associated protein 1 (MTA1) (Feitelson and Lee, 2007). Additionally, HBx can up-regulate the expression of Notch-1, Jagged-1 and Hes-1 at the transcriptional level, suggesting that HBx may promote the progression to HCC by activating the Notch pathway (Wang et al., 2012b). HBx can stabilize AIB1 protein, and in conjunction, these proteins promote cell invasiveness in HCC (Liu et al., 2012a). HBx can activate PAK1, which may promote HCC progression in chronic HBV infection (Xu et al., 2012). The complex cell context-dependent interactions between p53 family members and HBx in the regulation of apoptosis may be essential in HBV-induced HCC therapy (Knoll et al., 2011) (Fig. 3B).

3.2. HBV genotype

Of note, HBV can be classified into 10 genotypes, named A–J, with distinct prevalence of certain genotypes in different geographical regions (Chu and Lok, 2002b). There is increasing evidence that HBV genotypes may correlate with both clinical outcomes of chronic HBV infection and response to treatment. Genotype A is most common in the United States and Northern Europe, B and C in Asia, E in Africa, F/H in Central and South America and D/G are scattered worldwide (Orito et al., 2001; Sumi et al., 2003). Most studies on HBV genotypes have been carried out in Asia, where studies have been restricted to comparisons of patients infected with genotypes B and C (Wang et al., 2010a). It is widely accepted that different HBV genotypes may be associated with different rates of progression from acute to chronic HBV infection (Chen et al., 2007) (Fig. 3C).

3.3. MicroRNAs in HBV and hepatocellular carcinoma (HCC)

MicroRNAs (miRNAs) are small and non-coding RNAs ~22 nucleotides (nt) in length and may regulate approximately 30% of human gene expression (Fu et al., 2012). Recently, numerous studies have highlighted miRNAs as new regulators in HBV. MiR-155 can activate the HBV enhancer II as well as bind to the core and S promoters (Wang et al., 2009). Inhibition of miR-372 results in induction of phosphorylation of CREB and dissociation of CREB from the promoter (Wang et al., 2010b). In addition, miR-1 is able to enhance HBV core promoter transcription activity. miR-19b, miR-122 and miR-223 are associated with HBsAg positive immunoprecipitates, while miR-16 and miR-20a can be detected in control immunoprecipitates (Pedersen et al., 2007). Moreover, hsa-miR-125a-5p, an miRNA expressed in human liver, can down-regulate expression of the HBV S gene (Lakner et al., 2011).

Several reports have examined miRNA expression profiles in human HCC tissues by miRNA-based microarray or sequencing and analyzed the miRNA alteration link to HBV infection (Ladeiro et al., 2008). Recent studies have also reported that human serum/plasma contains a number of stable miRNAs that could potentially serve as a novel noninvasive biomarker for disease diagnosis (Chen et al., 2008). HB can also progress into liver cirrhosis that causes predisposition to HCC, and a number of miRNAs have been found during HBV-related cirrhotic stages (Braconi and Patel, 2008) (Fig. 3C).

3.4. Single nucleotide polymorphisms (SNPs) in HBV

The most common type of sequence variations in the human genome is the single nucleotide polymorphisms (SNPs), the

stable substitution of a single base (Carlson et al., 2003). Accumulated evidence in molecular genetics indicates that SNPs in tumorigenesis-related genes are associated with susceptibility to HBV and HCC, especially in Asian countries. The SNPs in chemokine (C-X-C motif) receptor 1 (CXCR1) rs2234671 may associate with chronic HBV infection (Almajhdi et al., 2013). Moreover, the SNPs in rs9277535 non-GG genotype are associated with a high likelihood of spontaneous HBsAg seroclearance (Xie et al., 2013). A single-nucleotide polymorphism (rs2910164) within the miR-146a gene is associated with the risk of acquiring acute-on-chronic hepatitis B liver failure (Cheng et al., 2013). IL-28B rs12979860C/T polymorphism may also confer symptomatic specificity in the progress and extent of hepatitis B infection (Chen et al., 2012). Moreover, gene variants of IL10 and IL20 polymorphisms can influence HBV infection outcome (Truelove et al., 2008). The polymorphisms in cytokine and toll-like receptors (TLR)-2/3 genes have also been found to be related to hepatitis B infection (Wang et al., 2012e). The SNPs in the regulated and normal T cell expressed and secreted (RANTES) gene are associated with HBV, and the SNPs (C-1350T and G-944C) in class II transactivator (CIITA) promoter IV, which plays a pivotal role in immune response, are associated with persistent HBV infection (Zhang et al., 2007; Al-Qahtani et al., 2012). The type I interferon (IFN-alpha/beta) receptor 1 (IFNAR1) 19158C/G polymorphism has been found to be primarily associated with chronic HBV infection (Zhou et al., 2009) (Fig. 3C).

3.5. Epigenetic factor in HBV: DNA methylation

Epigenetics refers to all heritable changes in gene expression and chromatin organization that are independent of the actual DNA sequence, including DNA methylation, RNAs, and histone (chromatin) modifications. DNA methylation is the predominant mechanism employed to inactivate relevant genes in HCC. Thus, a role for epigenetic changes in the regulation of viral protein production likely reflects viral adaptation to host cells (Tong et al., 2009). When HBV infects liver tissue, methylation density varies considerably. In many cases, methylation levels are low and these low levels can affect viral protein production. In addition, methylation of cccDNA affects protein production, and methylated cccDNA can be found in human tissues (Vivekanandan et al., 2008) (Fig. 3C).

4. *In vivo* models in HBV

4.1. Mouse models

The transgenic mouse model is established by introduction of HBV DNA into the mouse germ line, in particular the HBx gene, and has been studied in HCC with a transgenic CD1 mouse model (Heindryckx et al., 2009; Kim et al., 2009). HBx can promote the growth of Huh7 xenograft tumors in mice. Multifocal areas of altered hepatocytes with high levels of HBx protein are found in transgenic mice 4 months after birth, and by months 8–10, the altered hepatocytes have developed into adenomas that expressed high levels of HBx protein (Wang et al., 2012c). HBx can induce intrinsic cellular transformation, as well as promoting expansion and tumorigenicity in 3,5-diethoxycarbonyl-1,4-dihydrocollidine-treated (DDC) HBx transgenic mice (Lu et al., 2012). Some antioxidant enzymes, such as glutathione peroxidase and superoxide dismutase 2, are down-regulated in HBV transgenic mice and correlate with JNK activation. HBV can enhance the Fas-mediated activation of caspase-6, caspase-8 and JNK without enhancing activation of caspase-3 and apoptosis (Wang et al., 2012a). Dysregulation of apoptosis facilitates the escape of abnormal cells from death, suggesting a mechanism by which HBV promotes HCC (Barone et al., 2006). A humanized mouse model made by reconstitution of human primary hepatocytes in the liver of an immunodeficient mouse provides novel experimental opportunities because this model mimics the *in vivo* growth of human hepatocytes and thus facilitating the direct investigation of HBV and HCC (Zhou et al., 2012) (Fig. 4).

4.2. Chimpanzee models

Chimpanzees have served as a model for studying HBV infection for 20 years. Between 3 and 6% of wild-caught chimpanzees are positive for HBsAg, a marker of chronic HBV infection, and as many as 50% are positive for antibodies against HBsAg (anti-HBs), a marker of resolved infection (Vartanian et al., 2002). The HBVs isolated from 11 of 13 chimpanzees with chronic infections appear to be genetically distinct from known human HBV genotypes and appear to represent viruses that produce an infection indigenous to chimpanzees (Hu et al., 2000). Compared with HBV isolated from other primates, the chimpanzee HBV has unique nucleotide and amino acid changes throughout the entire genome; the S gene may allow rapid and precise identification of this strain (MacDonald et al., 2000). This occurrence demonstrates that despite their genetic divergence, human and non-human associated variants of HBV may share hosts in nature. A recent study characterizing HBV variants infecting chimpanzee populations has demonstrated the existence of a novel HBV strain and evidence of recombination between HBV strains circulating in chimpanzees (Sa-Nguanmoo et al., 2009). The S-gene mutant readily reverted back to the wild-type sequence but the Pol-gene mutant was stable during the course of infection (Magiorkinis et al., 2005). Vaccination of naive chimpanzees with a commercial hepatitis B vaccine resulted in the induction of cellular immune responses but did not appear to confer sterilizing immunity against challenge with the Pol-gene mutant and subsequent challenge with a serum-derived wt-HBV (Lyons et al., 2012) (Fig. 4).

4.3. Woodchuck models

Woodchuck hepatitis virus (WHV) has been used extensively in the modeling of HBV infection and antiviral therapy (Roggendorf et al., 2010). Since 1988, the neonatal chronic WHV infection model has been used primarily to test antiviral nucleoside analogs in chronic HBV infection. The focus of investigations using the woodchuck model has ranged widely, with flexible emphasis on both model development and application in many areas of HBV research, including viral and disease pathogenesis, prevention and treatment of HBV infection, disease sequelae (including HCC) using vaccines, antiviral drugs, and immunomodulators alone and in combination (Korba et al., 2000) (Fig. 4).

4.4. Tree shrews models

Tree shrews, the lowest order of non-human primates, can be used as a reliable and useful animal model for research on HBV infection as well as its relation to HCC (Yang et al., 2005). Tree shrews can be experimentally infected with HBV by inoculation with HBV-positive human serum. The transmission of HBV into tree shrews by inoculation with HBV-positive tree shrew serum is more effective than primary inoculation with HBV-positive human serum. Additionally, successive infections have been passed down through five generations of tree shrews inoculated with HBV-positive sera from an infected animal (Ren and Nassal, 2001). The experimental infection of tree shrews with HBV may be prevented by immunization with a hepatitis B vaccine, which is similar to the results in human studies (Wang et al., 2012d) (Fig. 4).

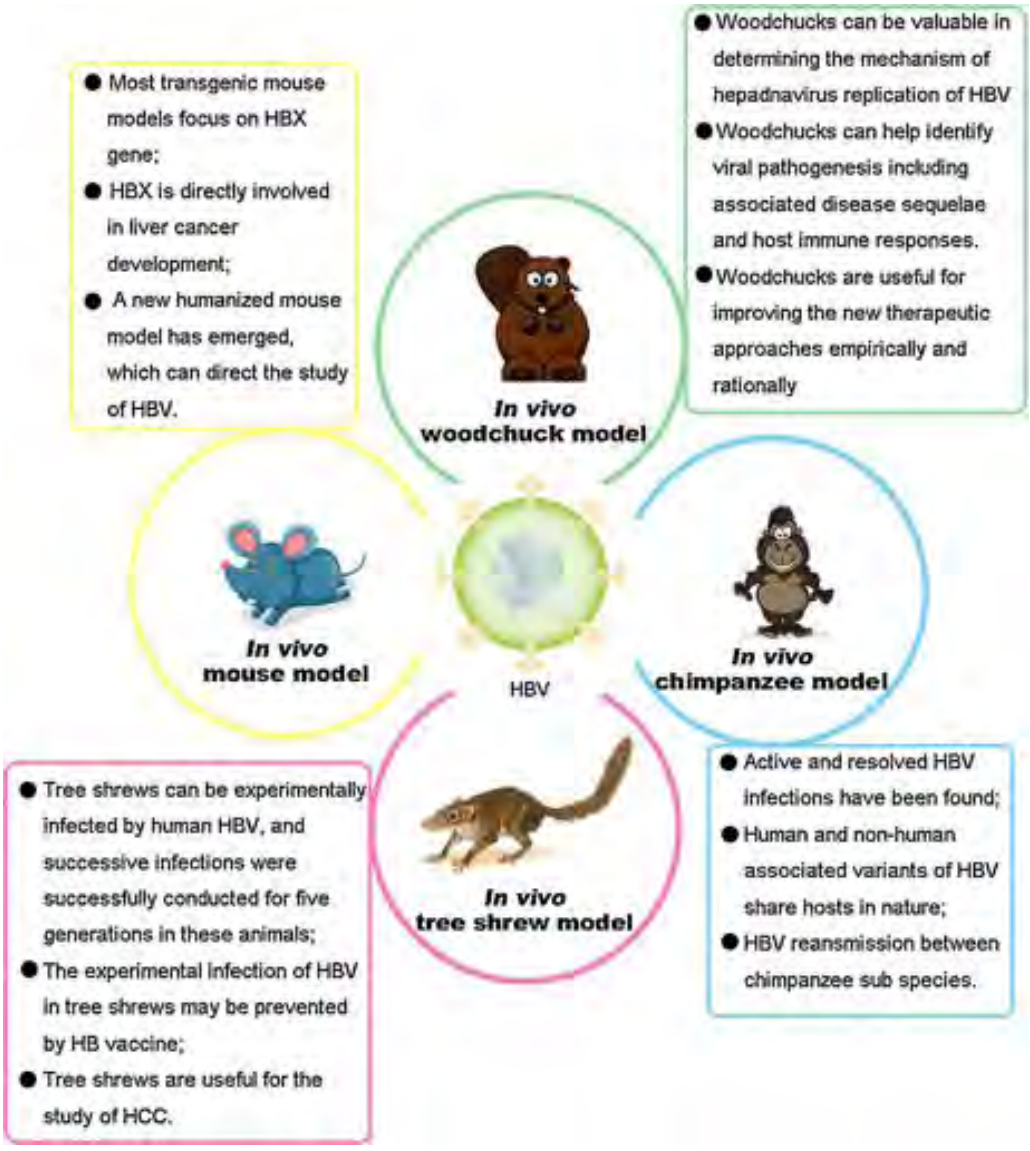

Fig. 4. *In vivo* animal models of HBV.

5. Therapeutic applications in HBV

5.1. HBV diagnosis and prevention

The diagnosis of HBV infection and its associated diseases is based upon a constellation of clinical, biochemical, histological, and serologic findings (Dufour et al., 2000). Many viral antigens and their respective antibodies can be detected in serum after HBV infection, and proper interpretation of the results is essential for the correct diagnosis of the various clinical forms of HBV infection (Kao, 2008). Increased HBV viral level is a risk predictor for the development of cirrhosis and HCC (Liang and Ghany, 2002). Levels of hepatic cccDNA may provide a greater predictive value of response to clinical therapies than alternative measures. Sensitive assays are available that can detect resistant viral variants during therapy before an increase in HBV-DNA level. The method for HBV genotype determination is sequencing followed by phylogenetic analysis of the generated sequences with reference sequences (Ganova-Raeva et al., 2012). Furthermore, detection of GzmH levels may be a potential parameter for the diagnosis of HBV and HCC because low GzmH expression in cytotoxic lymphocytes has been observed in individuals susceptible to HBV infection and HCC (Bosch et al., 2004).

HBV vaccines are HBsAg-based and their protective efficacy is mediated by neutralizing antibodies, which block HBV from entering hepatocytes (Francois et al., 2005). Active prophylaxis against HBV infection is available in the form of a recombinant HBV vaccine. The commercial products available are Recombivax HB and Engerix-B, both of which are highly effective in producing antibodies (Fitzsimons et al., 2005; Rapicetta et al., 2009). In addition, passive immunization is available by pooling serum from patients who have recovered spontaneously from acute HBV and have significant anti-HBs concentration (Chunsuttiwat et al., 2002).

5.2. Current HBV therapeutic drugs

The HBV polymerase has been the main target of anti-HBV drug development. The FDA has licensed two types of drugs to treat HBV infection: two formulations of interferons and five nucleos(t)ide analogs (lamivudine, adefovir, entecavir, telbivudine, and tenofovir) (see in Table 1).

Table 1
Approved and ongoing anti-HBV therapeutic agents.

Mechanism	Aim at HBV		Aim at host
	Inhibition of the peptides processing way	Inhibition of HBV replication	Immune regulation targets
Target	HBx, HBeAg	Inhibits viral DNA polymerase	HBV Core Antigen
Approved drugs	NOV-205 (Approved in Russia)	Lamivudine, Adefovir Dipivoxil, Entecavir, Telbivudine, Tenofovir,	Interferon-α Peginterferon-α 2a
Phase III		Clevudine Emtricitabine	
Phase II	LB80380 (ANA380) Valtorcitabine (Phase I/II) (Sweden)	MIV-210 Elvucitabine (Phase I/II)	IFN-λ (Phase I/II) REP 9AC (Phase I/II)
Phase I	Valtorcitabine (Phase I/II) (Sweden)	Elvucitabine (Phase I/II) Myrcludex-B Pradefovir	IFN-λ (Phase I/II) DV-601 REP 9AC (Phase I/II)

5.2.1. Interferons

IFNs, binding to receptors on cell membranes, are naturally occurring cytokines that act as immunomodulatory, anti-proliferative and antiviral reagents. IFN-α has been approved for HBV treatment, but this interferon requires three injections every week and, unfortunately, has limited treatment success (Janssen et al., 2005). The addition of a polyethylene glycol (PEG) molecule to the IFN extends the half-life of drug and prolongs IFN activity, resulting in an increase in the incidence of HBeAg and HBsAg seroconversion (van Zonneveld et al., 2004). Over the last few years, clinical research has focused on the use of peginterferon (PEG-IFN) administered by injection on a weekly basis and peginterferon-α-2a has been licensed for the treatment of chronic HBV infection (Hayashi et al., 2007).

5.2.2. Nucleos(t)ide analogs

Lamivudine, the first nucleoside analog licensed in 1998, is given in a dosage of 100 mg daily and generally results in normal ALT levels and undetectable HBV DNA in HBeAg-positive patients (50–80%) and HBeAg-negative patients (20–25%) (Liaw et al., 2004). Besides, lamivudine leads to resistance at a rate of approximately 20% of patients per year and then reaches 65–70% after 4–5 years of therapy. Adefovir can incorporate diphosphates into the replicating viral DNA, causes premature DNA chain termination, associated with a 12% rate of HBeAg seroconversion, 21% rate of undetectable serum HBV DNA and 53% rate of improvement in HBeAg-positive patients after 1 year of therapy (Fung et al., 2006). However, resistance becomes a limiting factor with prolonged use and has been demonstrated at 1, 2, 4 and 5 years at a rate of 0%, 3%, 18% and 29%, respectively (Yuen and Lai, 2004). Entecavir is a guanosine nucleoside analog that has selective activity against HBV by competing with the natural substrate deoxyguanosine triphosphate and inhibiting the activities of HBV polymerase (Colonno et al., 2006). Early studies suggested that entecavir-treated patients had a more sustained response to the drug, even after treatment ended, and even in patients with HBeAg-negative HB (Sherman et al., 2008). The cumulative probability of entecavir resistance at years 1 through 5 is 6%, 15%, 36%, 46% and 51%, respectively. Telbivudine is a nucleoside analog that suppresses viral load and decreases liver inflammation; the analog is easily phosphorylated in the body to the active triphosphate form (Lai et al., 2007). The overall rate of telbivudine resistance is 22% in patients with HBeAg-positive CHB and 9% in those with HBeAg-negative CHB. Tenofovir is a methyl derivative of adefovir and exhibits anti-viral activity against lamivudine-resistant HBV, similar to adefovir (van Bömmel et al., 2006). Tenofovir has been shown to be more potent than adefovir in achieving viral suppression defined as histological improvement (67%) and higher rates of HBsAg loss (3.2%) at 48 weeks in patients with HBeAg-positive CHB.

Some adverse effects do exist for current HBV drugs. The side effects of pegylated interferon include flu-like symptoms, marrow suppression, depression and anxiety, as well as autoimmune disorders (Lee et al., 2012). The disadvantages of lamivudine include the high incidence of antiviral resistance and high risk of relapse after discontinuation of therapy (Dienstag et al., 2003). The major adverse effects of long-term administration of nucleotide or nucleoside RT inhibitors are nephrotoxicity and myopathy (Fleischer and Lok, 2009). Nephrotoxicity is characterized by a gradual increase in serum creatinine and a decrease in serum phosphorus due to the inhibition (or toxicity) of kidney function. At high doses, adefovir and tenofovir have been reported to be associated with nephrotoxicity in chronic hepatitis B patients (Zoulim and Locarnini, 2009). In addition, myopathy characterized by muscle pain, weakness or tenderness can be observed in patients who have received long-term treatment with telbivudine (Delaney et al., 2006).

5.3. Current HBV drugs

Several novel drugs can inhibit the reverse transcription involved in HBV DNA replication for both wild-type and drug-resistant HBV. Lagociclovir valactate (MIV-210) is a prodrug with high oral bioavailability in humans and potent activity against hepatitis B virus (Michalak et al., 2009). Elvucitabine is a nucleoside analog RT inhibitor that showed strong anti-HBV activity in an *in vitro* trial for treatment of chronic HBV (Zhu et al., 1998). Additionally, Valtorcitabine, a well-absorbed prodrug of L-deoxycytidine, has been shown to suppress serum HBV DNA in HBeAg-positive patients (Iino et al., 2005). Emtricitabine, which is similar in structure, efficacy and resistance profile to lamivudine, appears to confer no advantage over lamivudine, while clevudine is distinguished from other oral agents by its sustained suppression of HBV DNA (Lim et al., 2006; Saniova et al., 2006). The entry inhibitor Myrcludex-B can prevent the spread of HBV from infected human hepatocytes *in vivo* and hinder amplification of the cccDNA pool in initially infected hepatocytes (Volz et al., 2012). A cyclic 1-aryl-1, 3-propanyl prodrug of adefovir dipivoxil, Pradefovir, has been developed to avoid much of the renal toxicity of adefovir dipivoxil (Li et al., 2008). Besifovir (LB80380) is a potent oral nucleotide prodrug with a chemical structure similar to that of adefovir and tenofovir, and an *in vitro* study showed that besifovir was effective against HBV strains resistant to lamivudine, adefovir dipivoxil, entecavir, and telbivudine (Yuen et al., 2009).

Some non-nucleoside anti-HBV agents are being developed, and a number of these inhibitors targeting viral antigens or replication have been tested in hepatitis-related cells. REP 9AC is a nucleic acid-based amphipathic polymer that belongs to a new class of anti-viral compounds based on the sequence-independent activity of phosphorothioated oligonucleotides (Wu et al., 2009). REP

9AC facilitates innate immunity against HBV by inhibiting release of subviral particles, including HBsAg, from infected hepatocytes. IFN-λ, with similar biological characteristics to IFN-α and IFN-β, can inhibit HBV replication by preventing the assembly of viral RNA-containing capsids in the cytoplasm (Robek et al., 2005).

5.4. Combination therapeutic strategies

Combination therapies of adefovir and entecavir demonstrated faster and greater suppression of HBV DNA compared to adefovir add-on lamivudine combination therapies for patients with lamivudine-resistance mutations. The former combination therapy was superior to the latter for both initial virological response and long-term suppression activity against HBV. Adefovir and entecavir combination therapies are most effective at discouraging selection of HBV strains that are cross-resistant in LAM-resistance patients (Momin and Richardson, 2012). Extended combination therapy with lamivudine and adefovir is associated with a high rate of long-term virological and biochemical responses (Lai et al., 2005). Similarly, telbivudine and lamivudine in combination cannot achieve additional anti-viral activity over that of telbivudine alone (Akyildiz et al., 2007). Combination therapeutics with agents of differing resistance profiles should limit the emergence of resistance. Resistance is negligible during the early years of treatment with entecavir or tenofovir, demonstrating the superiority of preemptive combination therapies over initial mono-therapy. A complementary drug added after the emergence of viral resistance has been another therapeutic strategy.

6. Conclusions and future perspectives

HBV infection is one of the most important causes of human liver disease, and substantial data have explored the complicated mechanisms of HBV replication and infection and several key modulators in HBV and HCC, as well as genomic factors (*e.g.*, microRNAs, genotypes and SNPs) and epigenetic regulation, all of which may be integrated to form systematically the complicated HBV regulation network. Moreover, *in vivo* models of HBV have been developed for further elucidation of the infection mechanisms of HBV, thus providing new clues for exploiting potential novel diagnostics and preventive or therapeutic drugs against HBV. Currently, there is increasing evidence of the importance of profound, durable therapeutic HBV DNA suppression to slow and reverse HBV infection with the aid of FDA-approved drugs such as interferon alpha, pegylated interferon alpha-2a, lamivudine, adefovir, entecavir, telbivudine, and tenofovir.

The best hope for HBV therapy may lie in discovering novel candidate drugs aimed at the "Achilles heel" of HBV, namely, core regulatory pathways or even the entire network, rather than individual gene or protein components (single target). The next generation of HBV drug discovery should target the HBV network (multi-targets). In summary, a Herculean effort will have to be mounted to explore the intricate mechanisms and novel therapeutic strategies for HBV. Thus, we expect anti-HBV drug regimens to improve in efficacy without engendering resistance and hope that combination therapeutics and even multi-target approaches will make great contributions and address future problems.

Acknowledgements

This work was supported by grants from the National 973 Basic Research Program of China (Nos. 2013CB911300, 2010CB529900 and 2012CB518900), National Natural Science Funds for Distinguished Young Scholar (No. 81225015), Key Projects of the National Science and Technology Pillar Program (No. 2011BAZ02590), the National Science and Technology Major Project (No. 2012ZX09501001-003), and National Natural Science Foundation of China (Nos. 81260628 and 81202403).

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