World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v6.i12.851

World J Hepatol 2014 December 27; 6(12): 851-859
ISSN 1948-5182 (online)

TOPIC HIGHLIGHT

WJH 6th Anniversary Special Issues (3): Hepatitis B virus

How did hepatitis B virus effect the host genome in the last decade?

Pinar Ozkal-Baydin

Pinar Ozkal-Baydin, Ankara University, Stem Cell Institute, 06520 Ankara, Turkey
Author contributions: Ozkal-Baydin P solely contributed to this paper.
Correspondence to: Pinar Ozkal-Baydin, PhD, Ankara University, Stem Cell Institute, Ceyhun Atuf Kansu Street, Cevizlidere, 06520 Ankara, Turkey. ozkal@medicine.ankara.edu.tr
Telephone: +90-312-5836601
Received: April 25, 2014 Revised: September 23, 2014
Accepted: October 14, 2014
Published online: December 27, 2014

Abstract

The principal reason of chronic liver disease, cirrhosis and hepatocellular carcinoma is chronic viral hepatitis all over the world. Hepatitis B virus (HBV) has some mutagenic effects on the host genome. HBV may be exhibiting these mutagenic effects through integrating into the host genome, through its viral proteins or through some epigenetic mechanisms related with HBV proteins. This review aims to summarize the molecular mechanisms used by HBV for effecting host genome determined in the last decade. The focus will be on the effects of integration, HBV proteins, especially HBV X protein and epigenetic mechanisms on the host genome. These interactions between HBV and the host genome also forms the underlying mechanisms of the evolution of hepatocellular carcinoma.

Key words: Hepatitis B virus; Host genome; Integration; Hepatitis B virus proteins; Epigenetic

Core tip: Hepatitis B virus (HBV) has some mutagenic effects on the host genome. This review aims to summarize the molecular mechanisms used by HBV for effecting host genome determined in the last decade.

Ozkal-Baydin P. How did hepatitis B virus effect the host genome in the last decade? *World J Hepatol* 2014; 6(12): 851-859 Available from: URL: http://www.wjgnet.com/1948-5182/full/v6/i12/851.htm DOI: http://dx.doi.org/10.4254/wjh.v6.i12.851

INTRODUCTION

There are more than 350 million people who are infected by hepatitis B virus (HBV) throughout the world[1]. HBV is the main cause of some liver illnesses, such as chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (HCC)[2]. The World Health Organization classifies HBV in "group 1" as the consequential oncogenic factor after tobacco smoking. It has been estimated that due to late diagnosis and limited treatment options, after lung and stomach cancer, HCC is third prominent cause of cancer related death and nearly 53% of HCC cases has a connection with HBV. The incidence of HCC changes according to geographical conditions. Chronic HBV infection is the most important risk factor for HCC in the world. Other risk factors such as chronic hepatitis C virus (HCV), hepatitis D virus or human immunodeficiency virus infection, aflotoxin B_1 exposure, metabolic factors as obesity and diabetes and alcohol abuse increase the comparative endanger for tumor progression when coexist with HBV infection. Moreover, demographic factors such as Asian or African ancestry, male sex or advanced age are the synergistic effects that have been reported to raise the possibility of HCC in chronic HBV-infected individuals[3-7].

Even though many pathways and factors contributing to HCC development have been identified, many features of hepatocellular carcinogenesis and direct role of viral factors are difficult to define[3]. However, HBV infection is the main risk factor for HCC development. Not only in HCC, but also in chronic HBV patients and chronic HBV carriers some mutagenic effects of HBV on somatic cells

are detected. For example in our study, we proofed the genotoxic effects of HBV on peripheral blood lymphocytes of chronic HBV patients and chronic HBV carriers[8]. Ucur *et al*[9] showed the increased sister chromatid exchange frequency and low mitotoic index; Bolukbas *et al*[10] and Grossi *et al*[11] demonstrated DNA damage using the alkaline comet assay, in peripheral blood lymphocytes. HBV may harm to host DNA in many ways. But simply it can be categorized in at least 3 different mechanisms: First, the viral DNA integration in the host genome can induce chromosome instability, although HBV usually persists as an episome and the integrate genomes are dead and can no longer drive HBV replication. Second, insertional mutations of *HBV* are known to activate many genes and promote genetic alterations in the host genome. The third mechanism is based on viral proteins and gene products of sporadically truncated *HBV* genes from integrated HBV DNA[3,5]. While a variety of manuscripts have been published about these mechanisms separately, here a short review describing effects of HBV on host genome in the last decade is given. The focus of this review will be on integration, proteins and epigenetic mechanisms of HBV.

DIRECT EFFECTS OF INTEGRATION ACTIVATED BY THE HBV DNA ON THE HOST GENOME

Integration is not necessary for the viral replication but it enables viral genomic persistence. Long term chronic inflammation related to continuous cycles of cell death and proliferation increases the amounts of DNA ends in host genomic DNA, thus supporting the viral integration. Cellular topoisomerase I is a crucial factor in the linearization and integration of viral replicative mediators[3,7]. Several kinds of changes in the sequence of HBV genome have been identified, but inverted duplications and the deletions are the most common alterations[12]. Disclosure to oxidative stress or mutagenic agents, loss of DNA repair capacity, high hepatocyte turnover due to inflammation and/or coinfection with other viruses may be the reason of HBV DNA integration[3,7]. In these conditions the genome is more unstable and tend to the devolopment of deletions, single- or double-stranded breaks or rearrangements[12]. HBV integration can induce rearrangements and/or partial deletions at the integration site of the host chromosome[6]. Integration happens by chance in the contex of human genomes and may ocur at the various places of different chromosomes[7]. Translocations, production of fusion transcripts, chromosomal deletions and generalized genomic instability may be caused by these integrations, and alterations probably cause the choosing of hepatocyte clones that have a growth profit[3,12]. Liver carcinogenesis may occur as a result of HBV integration that cause a significant increase in an anti-apoptotic or oncogenic signal[12]. Using a polymerase chain reaction (PCR) based approach (*i.e.*, inverse PCR, Alu PCR, restriction site PCR)[12], it was confirmed that insertion of HBV into cellular genome is an event that happens during HBV infection even after acute self-limiting hepatitis[6]. In 85%-90% of HBV-related HCC, integrated viral DNA has been detected[3,7].

Most of the integration events reported occur near or within common fragile sites (large genomic regions that are liable to deletions, breaks, chromosomal rearrangements and gene amplifications)[12], in genes managing proliferation control, cellular signal transduction cascades, cell viability[6] or Alu sequences and microsatellites (other repetitive human genomic regions) that are liable to instability in carcinogenesis development and growth. Integrated viral DNA into coding regions or cellular regulatory regions of the genome may alter gene expression (cis-activation) or change the structure and function of the produced cellular proteins which possibly cause malignant transformation[3,7]. Insertion of viral DNA may also cause hepatocellular malign transformation *via* the production of mutated viral proteins such as preS/S proteins or truncated X proteins which may trigger signalling cascades in carcinogenesis (trans-activation)[7]. Moreover, HBV genome has enhancer elements that can activate heterologous promoters in a orientation- and position-independent manner[13]. The study of many groups suggest that the HBV enhancers are able to transactivate cellular genes up to 100 kb distant from the integration site[12].

In the late 1970s and early 1980s, the primer investigations defining cellular genomic regions of HBV integrations were carried out[12]. In humans, the recurrent integration of the viral genome into or in the proximity of a host genome has been suggested for HBV in 1987 and in the development of HCC in 2000s. In fact, woodchuck hepatitis virus causes liver cancer by targeting myc oncogenes (*N-myc*, *c-myc*, and *N-myc2*) and some cases of HBV integration into important cellular genes have been informed in human liver tumors (*e.g.*, retinoic acid receptor beta, cyclin A). However, HBV integration regions targeting host genes have not been defined in the past[13]. But in the last decade, there have been many investigations focusing on the integration sites of HBV and insertional mutagenesis seen in HCC.

Paterlini-Bréchot *et al*[13] have isolated nine DNA integration regions from nine hepatocellular carcinomas, demonstrating that the viral genome make mutations in the important regulatory host genes by using HBV-Alu PCR. These genes have a role in cell proliferation and/or differantiation and/or survival: interleukin (IL)-1R-associated kinase 2 gene, neurotropic tyrosin receptor kinase 2 gene, inositol 1,4,5-triphosphate receptor type 2 (*IP3R2*) gene, p42 mitogen-activated protein kinase 1 (*p42MAPK1*) gene, alpha 2,3 sialytransferase gene, *IP3R1* gene, EMX2-like gene, human telomerase reverse transcriptase (*hTERT*) gene and thyroid hormone uncoupling protein gene (Table 1). Different genes, which were located at the HBV DNA integration region, have a specific key cellular function or share common cell signalling cascades. Also, they found that HBV targets both the telomerase gene

Table 1 Genes targeted by hepatitis B virus DNA integration

Gene	Description	Ref.
NTRK2	Neurotropic tyrosin receptor kinase 2	Paterlini-Bréchot *et al*[13]
IRAK2	Interleukin-1R-associated kinase 2	Paterlini-Bréchot *et al*[13]
MAPK1	Mitogen-activated protein kinase 1	Paterlini-Bréchot *et al*[13]
IP3R2	İnositol 1,4,5-triphosphate receptor type 2	Paterlini-Bréchot *et al*[13]
IP3R1	İnositol 1,4,5-triphosphate receptor type 1	Paterlini-Bréchot *et al*[13]
ST3GAL VI	Alpha 2,3 sialytransferase	Paterlini-Bréchot *et al*[13]
TRUP	Thyroid hormone uncoupling protein	Paterlini-Bréchot *et al*[13]
EMX2-like	Empty spiracles 2-like	Paterlini-Bréchot *et al*[13]
hTERT	Human telomerase reverse transcriptase	Paterlini-Bréchot *et al*[13]
WBSCR1	Williams-Beuren syndrome critical region 1	Kimbi *et al*[14]
AXIN1	Axis inhibitor 1	Minami *et al*[16]
BBX	Bobby sox	Minami *et al*[16]
CTNND2	Catenin delta-2	Minami *et al*[16]
EYA3	Eyes absent 3	Minami *et al*[16]
ODZ2	Odd Oz 2	Minami *et al*[16]
TERT	Telomerase reverse transcriptase	Sung *et al*[18]
MLL4	Mixed-lineage leukemia 4	Sung *et al*[18]
CCNE1	Cyclin E 1	Sung *et al*[18]
FN1	Fibronectin 1	Ding *et al*[21]
SMAD5	SMAD family member 5	Ding *et al*[21]
PHACTR4	Phosphatase and actin regulator 4	Ding *et al*[21]

and *IP3R* gene in two different tumors. This data shows viral integration may target some preferential regions, especially *hTERT* gene.

Kimbi *et al*[14] amplified HBV and chromosomal DNA from the sera of five patients with uncomplicated acute hepatitis B and one with fulminant disease. In one patient with uncomplicated disease, HBV DNA was integrated into host chromosome 7q11.23 in the Williams-Beuren syndrome critical region 1 gene (Table 1). This gene contains a high abundance of Alu repeats, repetitive elements that have shown to be the preferred sites for recombination and for HBV DNA insertion. Moreover, the integrant is within the region commonly deleted in patients with Williams-Beuren syndrome, giving rise to loss of heterozygosity. The investigators also mentioned that clonal expansion of an integrant is required for tumor formation and early integration of HBV DNA might have a role in HBV-induced hepatocarcinogenesis. It is because the integrated viral DNA in early infection is in accordance with the observation, that importation of linear DNA into the nucleus is necessary for insertion of viral DNA into chromosomal DNA, and that such importation is known to occur during the initiation of infection. This data was also examined by Murakami *et al*[15] in the study of detecting the possible persistence of integrated genomes in peripheral blood mononuclear cells (PBMCs) and the exact position of the viral genome, after the clearence of serum HBV surface antigen (HBsAg). Their results showed that HBV genome integrates early during acute viral infections and persists in an integrated form in PBMCs. Another study providing the proof of HBV integration at an early stage of chronic infection in hepatocytes was carried out by Minami *et al*[16]. They examined virus-cellular gene junctions in chronic hepatitis tissues without HCC and by analysing six patients 42 independent viral-host junctions have been obtained and chromosomal locations for 20 of the 42 junctions have been shown. Each integration evidently influenced a single clone in six clones. Among these six genes, axis inhibitor 1 is believed to function as a tumor suppressor; eyes absent 3, homolog of odd Oz 2 and homolog of bobby sox are human homolog of drosophila genes that are critical for organ development, but their roles are still mysterious; catenin delta-2 is an oncogene (Table 1). Moreover, in contrast to the previous claim that HBV integration happens randomly, this study suggests that the integration of HBV into chromosome 3 is preferential.

Mixed-lineage leukemia (*MLL*) 2 and 4 genes are also suggested as preferential targets for HBV DNA integration[2,17]. In addition to TERT and cyclin E 1 genes, Sung *et al*[18] showed integration at *MLL4* gene as previous studies (Table 1). MLL4 locates on chromosome 19q13.1 where an amplification or a frequent rearrangement has been shown in solid tumors. After integration, site specific expression such as HBV X (HBx)/MLL4 proteins and chimeric HBx/MLL4 transcripts proposing an insertional mutagenesis that could functionally have a connection with liver carcinogenesis[2].

New accesses have been improved to identify unique integration sites in high-throughput manner using the next generation sequencing (NGS) technologies, which prevented biased identification and preferential amplification of unique integration sites. In the researches of Sung *et al*[18], Fujimoto *et al*[19] and Jiang *et al*[20] they used complete genomic sequencing to identify genome wide HBV integration by the advantages in NGS technologies. Considering that, whole genome sequencing is overpriced for sequencing wide amounts of specimen, Ding *et al*[21] have improved an optional method for deep sequencing and amplification that joins ligation mediated PCR to Illumina's paired-end adapters. This effective and cheaper method have been called massive anchored parallel se-

quencing (MAPS) method. By this method, in addition to two familiar recurrent target genes, fibronectin 1 and TERT1, they identified novel target genes for HBV integration such as actin regulator 4, SMAD family member 5 and phosphatase (Table 1). They also found that HBV integration prefered chromosome 17 and mostly integrated into human transcriptional sites.

Occult HBV infections (OHBI) have the presence of HBV DNA but are short of available serum HBsAg. Occult infection mechanism is still mysterious, however, many acceptable pathways, such as maintenance in PBMCs and integration into human genomic regions exist. Bhargava *et al*[22] planned to research the molecular pathways lying beneath the DNA damage response activated as a result of OHBI in host cells in their investigation. They found that OHBI causes DNA damage in peripheral blood lymphocytes. It was also found that there was a strong relationship between OHBI and oxidative stress. On the other hand, Pollicino *et al*[23] reported a case of 43 years old man seronegative for HBV and HCV infections and positive for HFE-haemochromatosis, who developed HCC in the lack of severe liver damage. In this study they tried to evaluate the occult HBV infection. HBV-Alu PCR showed HBV integration. This integrant was placed upstream of the partitioning-defective-6-homolog-gamma gene (*PARD6G*) and this gene had overexpression in tumor tissues when we compare it to non-tumor liver tissues. Being a target of transforming growth factor-beta in the tumor invasion and metastasis, PARD6G is included in the polarized migration of cells, establishment of cell polarization. These two studies show that OHBI lead to deregulation of gene expression and may alter the oncogenic pathways.

HBV integration effectively surveys the human genome, exerting insertional mutation pressure, and thus may expand the oncogenic opportunities for patients infected by HBV. The most dominant HBV integration sites occur *MLL* and *hTERT* genes. Moreover, there are many candidate genes such as 60S ribosomal protein genes, platelet-derived growth factor receptor, calcium signalling related genes[20,24]. Bok *et al*[25] proposed 3 different models for gene activation in HBV DNA integration on chromosome 11q13 in the SNU cell line: (1) viral integration induces genetic changes and activation of gene expression at the integration site without gene amplification; (2) viral DNA induces gene amplification, causing overexpression during integration and rearrangement; and (3) gene activation is related to gene amplification, regardless of viral integration. These models might also be available for all other gene activation mechanisms in HBV DNA integration. The target sites and integration mechanisms will give information for key genes and pathways included in development of not only HBV and but also non-HBV-induced cancers.

EFFECTS OF HBV PROTEINS ON THE HOST GENOME

Several studies have reported about the procarcinogenic effects of HBV proteins or their randomly truncated transcripts after integration. This part will focus on the effects of HBV proteins, especially X protein, on the host genome in the last decade.

HBx protein

HBx, is a X open reading frame encoded small polypeptide of 154 amino acids, usually produced at very limited amounts during chronic and acute HBV infection. HBx can be found in the cytoplasm of infected hepatocytes and at low level in the nucleus. A variety of HBx functions are still enigmatic[3,6].

The clinical importance of HBx starts with the integration of HBV DNA into the chronic HBV carriers' hepotocytes genome. *X* gene is generally preserved in the integrants, and HBx is frequently seen in malignant hepatocytes of chronic HBV carriers[26]. The integrated HBx often have rearranged forms and may show many deletions, truncation with fusion to cellular DNA or point mutations[7]. One significant information derived from the researches of HBV integration was that 3'-end *X* gene was frequently deleted in HCC cells, and this causes the COOH-terminal truncated HBx protein. This protein, rather than the full length HBx, is needed and adequate to cause HCC[27]. So as to understand the relation between HBV integration and HCC development, Wang *et al*[28] isolated and characterized integrated HBV in 14 primer cases of HCC. The findings showed that C-terminal X protein caused by 3'-deleted *X* gene was observed in 10 samples as a result of HBV integration. These deletions lead to the losses of transcription factor Sp1 binding site, p53-dependent transcriptional repression binding site, and growth-suppressive effect domain, causing cell transformation and proliferation. This result proposes that 3'-deleted *X* gene may have a significant role in the HCC development.

HBx has some controversal effects like pro-proliferative effects and induction of cell cycle arrest or prevention and initiation of apoptosis[3,6]. HBx effects the expression of several genes that are included in signal transduction pathways, metastasis, transcriptional regulation, immune response, metabolism, control of the cell cycle, proliferation and the apoptosis[6,26].

HBx changes expression of cellular gene by triggering cytoplasmic signal transduction pathways [*e.g.*, ras, nuclear factor kappa B (NF-κB), src, activator protein-1 (AP-1), Jak/STAT, PI3K/Akt, Wnt] and by binding to nuclear transcription factors [*e.g.*, activating transcription factor 2 (ATF-2), cAMP responsive element-binding protein (CREB), Oct-1, basal transcription factors], and they both help cell growth and survival[1]. HBx localizations (cytoplasm and nucleus) are associated with different functions. HBx, placed in the nucleus, is proposed to interfere directly with transcription factors or to use a function like a transcription factor. A direct relationship between ATF-2 and CREB concluding in their raised DNA binding affinity[6]. HBx placed in the cytoplasm, where it interferes with and stimulates protein kinases, including IKK, protein kinase C, Jak/STAT, PI3K, pro-

tein kinaseB/Akt, and stres activated protein kinase/Jun N-terminal kinase[26].

HBx is an activator of transcription factor NFκB. HBx stimulated NF-κB promotes liver cells to survive against Fas-mediated apoptosis[26]. However, Zhang *et al*[29] showed another function of NF-κB in their study. Calpain small subunit 1 (Capn4) is included in the HCC metastasis and upregulated in the tissues of HCC. They supposed that HBx might assist migration of hepatoma cell by Capn4. Their results revealed that HBx could upregulate the Capn4 expression at the mRNA and protein levels, and increase Capn4 promoter activity. Interestingly, they found that the inhibition of NF-κB could attenuate the upregulatin of Capn4. Thus, they concluded that HBx upregulate Capn4 through NF-κB/65 to promote migration of hepatoma cells. In another study, Zhou *et al*[30] showed the migration of leukocytes in a NF-κB related pathway. Interferon-γ inducible protein 10 (IP-10) involves in cellular immune damage and inflammatory cell recruitment during virus infection. In their study, Zhou *et al*[30] demonstrated that HBx increases IP-10 expression and the effect of HBx on IP-10 induction is blocked by the addition of the NF-κB inhibitor. Consequently, they reported that HBx affects NF-κB pathway which leads to IP-10 promoter transactivation and then increases leukocyte migration, thus causes immune pathological injury of liver.

HBx interacts with transcription machinery, in addition, there is evidence that HBx involves in the stages of apoptosis. HBx affects the regulation of apoptosis through its role on survivin, caspases, and mitochondria. It has been shown that HBx blocks caspase 3 activity[26]. The elevation of cytosolic calcium signals seems to play a possible role in stimulation of cell proliferation and transcription pathways. Direct interaction of HBx with endoplasmic reticulum (ER) and mitochondria as well as integration events of the X open reading frame were reported to alter intracellular calcium homeostasis[3]. Having a role in caspase-3-dependent pathway, HBx pertubs homeostasis of intracellular Ca^{2+}. This is an important effect in the control of HBx-related apoptosis. HBx possibly have a contact with Bcl-2 during hepatic apoptosis. Proapoptotic activity of HBx bypasses or gets over the Bcl-2 inhibitory effect[26]. Survivin is a apoptosis preventer protein and is overexpressed in a majority of human tumors. HBx can upregulate the expression of survivin in hepatic tumor cells[31]. Moreover, several factors, for example transforming growth factor (TGF)-β, induce PI3K and its downstream target, protein kinase B/Akt, to inhibit apoptosis. HBx downregulates TGF-β-induced apoptosis in hepatocytes by stimulating activity of PI3K[26].

UV-damaged DNA binding protein 1 (DDB1) works as an E3 ubiquitin ligase complex subunit[32] and has been shown to help cell cycle regulation and DNA repair[26,32]. HBx binds to DDB1 and by this way replication of HBV genome is stimulated in the nuclear compartment of cells. HBx needs this nuclear interaction with DDB1 also for interfering with cell viability. It has been demonstrated that HBx triggers lagging chromosomes during mitosis, which then causes arrangement of abnormal mitotic spindles and cells with multinucleus. These formations demand the binding of HBx to DDB1. Thus, this binding may induce genetic instability in regenerating hepatocytes; therefore causes to HCC development[32].

The human *p53* genes' transcriptional repression is caused by HBx and it has capacity to bind to the p53. HBx C-terminal region is needed for sustaining the p53 in the cytoplasm and blocking the p53-mediated apoptosis. However, a tremendous excess of p53 is found when it is compared to HBx in the hepatocytes[6,26]. It seems that the anti and proapoptotic effects of HBx depends on the status of hepatocyte differantiation[3].

Telomerase which adds repetitive DNA sequences to the telomeres, the ends of the chromosomes, is a ribonucleoprotein. By this way it prevents telomere shortening and cell death[33]. Telomerase activation has been implied in immortalization and malignant transformation of cells in vitro and is a vital step in tumor and cellular senescence[3,26]. Although telomerase is a complex comprising a catalytic subunit (hTERT) and an RNA component (hTER), hTERT is the crucial factor of telomerase activity in human cells[34]. High levels of hTERT mRNA in HCC of several grades were found by researchers and they originate in cells which have gone through the molecular changes of the first steps of hepatocarcinogenesis[33]. It has shown that HBx gene can up-regulate the transcriptional expression of hTERT mRNA[35]. In the study of Su *et al*[36], it was found that by transcriptionally repressing its promoter, HBx down-regulated the human telomerase expression. They evaluated human telomerase promoter and identified myc-associated zing finger protein (MAZ) as a transcriptional repressor of the promoter in order to find out the molecular mechanism. It was found that the physical association of HBx with MAZ, supresses human telomerase by enhancing MAZ binding to its consensus sequence in the promoter. In this situation, HBx acts as a transcriptional corepressor.

HBx can also lead to both stabilization of hypoxia-inducible factor-1 and overexpression of vascular endothelial growth factor gene. It seems HBx causes carcinogenesis *via* the alteration of angiogenic pathways[7].

In addition to being involved in angiogenic pathways, HBx can contribute to tumor cell invasion by the way that includes the up-regulation of heat shock protein 90 alpha (HSP90alpha). HSP90alpha isoform is an ATP-dependent molecular chaperone which sustains the effective structure of client oncoproteins in tumor cells. Li *et al*[37] showed that HBx triggers expression of Hsp90alpha at the transcriptional level. HBx is directly included in the HSP90alpha transcriptional activation mediated by c-myc. Moreover, by activation of Ras/Raf/ERK1/2 cascades HBx triggers c-Myc expression, which causes c-Myc-mediated HSP90alpha promoter activation first and then HSP90alpha expression up-regulation. HSP70 and HSP60 have also been shown as a HBx cellular targets[26].

In conclusion, the HBx protein is a multifunctional and very important viral protein in the initiation of he-

patocellular transformation and cell survival during the HBV infection. It interacts with alot of molecules and involves in many cellular pathways. Variations in the role of HBx in hepatocarcinogenesis may be due to hepatocyte differantiation, different functions of truncated X protein and amount of expressed HBx. More information about HBx might highlight many mechanisms involved in HCC and give insights and tools for therapeutic means.

Surface proteins

Large hepatitis B (LHBs) and Middle hepatitis B (MHBs) virus surface proteins are encoded by the preS1/preS2 sequences of HBV. Experimental data revealed that HBV *preS/S* genes truncated at the 3' end and integrated into the cellular genes have a transcriptional activator function and encode proteins that accumulate in the ER. These *preS/S*^{t} genes encoded for C-terminally truncated surface proteins (MHBS^{t}) exhibit regulatory functions, such as the transactivation of host genes involving c-Ha-ras, c-myc, and c-fos oncogenes and the precise activation of the c-Raf-1/MEK/Erk2 signaling essential for AP-1 and NF-κB activation. Described processes result in increased hepatocyte proliferation[3,7].

HBV surface proteins accumulate in ER. Accumulation of proteins in ER is known to trigger apoptosis in the presence of prolonged and severe stress due to an induction of an oxidative stres[3]. PreS-mutant LHBs might also accumulate in ER and with induction of genomic instability and oxidative DNA damage, they become the reason of stress-signalling pathways. This also causes defective DNA damage response and repair in liver cells expressing HBV surface antigen[38]. Moreover, centrosome multiplication and the overexpression of both cyclin A and cyclooxygenase 2 might be caused by pre-S2 mutant proteins, therefore inducing cell cycle progression, chromosome instability and proliferation of hepatocytes in HBV related HCC[3,7]. Churin *et al*[39] also demonstrated that the expression of HBV surface proteins in the liver of transgenic mice induces phosphorylation of eukaryotic initiation factor 2α (eIF2α) and protein kinase like ER kinase activation. eIF2α phosphorylation resulted in activation of ER stress markers and a proapoptotic protein. Morover, by searching on two groups of mice with different genetic backround, they showed that hepatic HBV surface protein expression induced tumor development and fibrosis depends on host genetic backround.

HBV surface proteins may also alter the expression of some host genes with known functions as proved in the research of Rao *et al*[40]. It has shown that HBV-encoded small surface protein (SHBs) has an influence on hepatic cell expression of host genes related to fatty acid synthesis and decomposition.

Core protein

The HBV core protein (HBc) is a 21-22 kDa protein that affects the human immune response. HBV genome encode for the core protein to form the viral capsid[3]. HBc may interact with the human genome in HBV infected liver cells. HBc was demonstrated to suppress the the human carcinogenesis related genes expression, p53 and interferon beta; inhibits tumor necrosis factor-related apoptosis-inducing ligand induced apoptosis in hepatocytes *via* blocking gene expression of the pathway-associated death receptor[41].

However, it is not known how HBc interacts with human genome. Guo *et al*[41] examined the distribution of HBc binding to promoters in the human genome and assessed its effects on the associated genes' expression. It was shown that HBc antibody immunoprecipitated nearly 3100 human gene promoters. The high CpG density promoters were found most commonly among this set of gene promoters. HBc is able to bind to 41 gene promoters of the WNT/β-catenin signalling pathways and 64 gene promoters of the MAPK pathways. These are two most important pathways involved in HBV-related HCC. Moreover, HBc is able to increase host NF-κB DNA-binding ability, thus in order to enhance or inhibit other host nuclear proteins' transcriptional activator functions, HBc may bind to and interact with them. As a result, HBc can bind to a great amount of human gene promoters throughout the whole human genome. This key finding may represent one of the pathogenic mechanism of HBV infection.

EPIGENETIC EFFECTS OF HBV ON THE HOST DNA

According to the recent researches virus and host interactions also happen epigenetically. Interactions between epigenetic machinery and the viral proteins may cause changes in the epigenetic landscape of the cell thus causing cancer. Histone modifications and DNA methylation are epigenetic mechanisms which have an important role during HBV replication[42].

Bisulfite sequencing is the gold standart technique to analyze the methylation state of individual cytosines. In order to acquire complete DNA methylomes of double-stranded DNA viruses like HBV, Fernandez *et al*[43] joined the use of model organisms and bisulfite genomic sequencing of multiple clones. Most importantly, they found that DNA methylated *HBVgp2* and *HBVgp4* genes, which respectively code for the S and C viral proteins, have lost their expression. It was also shown in their sequencing data that the vast majority of the HBV genomes kept the *HBVgp3* gene coding for the X protein in an unmethylated condition. Interestingly, HBX protein might regulate DNA methyltransferase (DNMT) activity.

The expression of DNMTs, which catalyze the addition of a methyl group to the cytosine ring of the 5'-CpG dinucleotide, is often raised in livers infected with HBV and also in HCC[7,44]. The roles of the major methyltransferases have the following roles: DNMT1 adds methyl groups to the hemimethylated CpG dinucleotides and has an important function in the supply of methylation during cell division; DNMT2 lacks DNA methyltransferase capabilities and seems to take part in adding methyl

groups to the structural RNA; DNMT3a and DNMT3b is able to methylate not only unmethylated, but also hemi-methylated CpG dinucleotides[45]. Several studies have reported that both HCC and HBV-infected cells exhibit increased levels of DNMT1, DNMT3a and DNMT3b and aberrant DNA methylation[44].

It was reported that the overexpression of HBx protein induces transcription of *DNMT1*, *DNMT3a* and *DNMT3b* genes and directly interacts and activates the *de novo* methyltransferase DNMT3a. It suggests that this viral protein may be responsible for methylator phenotype in HBV related HCC[7,44].

In their study, Vivekanandan *et al*[45] have revealed that HBV infection up-regulates DNMTs, which then causes the methylation of HBV DNA by DNMT3a. This methylation causes the production of pregenomic RNA, viral mRNA, and a decreased production in viral protein. However, in the same cells, the up-regulation of DNMTs also causes methylation of host CpG islands overlapping gene promoters related to carcinoma[45]. It was demonstrated that specific gene promoters are methylated in HBV infected cells such as metallothionein-1F, IGFBP3, SUFU, and TIRAP[44,45]. HBx may also inhibits transcription of E-cadherin[46], $p16^{INK4A}$ and glutathione S-transferase P1 *via* CpG methylation of the regulatory elements[7]. Some immunoregulatory genes active against HBV can also be methylated. For instance, HBV replication is able to cause *de novo* methylation and decrease the IL-4 expression, that favors the virus because HBV replication is repressed by the IL-4 expression[45].

In addition to methylation processes, it has also been shown that HBx associates with components of histone modification machinery, such as HDAC and CBP/p300 HAT. So it has an effect on gene expression[42].

In summary, HBV infection up-regulates DNMTs in hepatocytes. This up-regulation causes methylation of viral DNA, however specific and critic genes in the host genome can also be methylated. It is certain that, these modifications are significant factors in the development of HCC.

CONCLUSION

At present, in terms of the experimental approaches used, such as PCRs, gene clonings, microarrays, immunohistochemical methods, it is possible to explore the effects of HBV on the host genome. In the last decade, the investigations about the pathogenic effects of HBV on the host genome have mostly focused on HCC development. During this development period, a dominant oncogene isn't encoded by HBV genome, however it uses multifactorial pathogenic mechanisms. In addition to direct mechanisms, which are mainly represented by integration of HBV DNA into the cellular genes and by the production of proteins with transforming capacities, indirect mechanisms involving HBV proteins disrupting vital molecular pathways may be seen in HCC development. Although it is thought that HBV DNA integrations are random and there is no specific region of integration, it is not fully incidental but instead seems to be partly optional. Special integration sites change the expression of various components of the cell cycle, signalling, transcription and apoptotic pathways. It is also shown that the formation of truncated HBV proteins, the activation of host genes by integrated HBV enhancer sequences and modifications in the epigenetic machinery of the host cell are important carcinogenic mechanisms in HCCs.

Although the main risk factors for HBV-induced hepatocarcinogenesis are well known, we still lack a deeper understanding of molecular pathways disrupted by HBV and the relationship between key molecules in these pathways. Therefore, molecular understanding of the mechanisms in HBV-related HCC is necessary for defining risks and identifying novel therapeutic approaches.

Cloning and fully characterizing HBV integrations, phage library construction and sequencing are still the gold standards. Recently, several new methods have been developed using NGS technologies avoiding optional amplification and biased identification of unique integration sites. For example, complete genomic sequencing and an efficient, cost effective method, MAPS, have been developed in high-throughput manner. In the future, novel easy and not time consuming experimental approaches and new HCC animal models might be developed to invent promising therapeutic approaches. The specific antiviral new drugs reducing the chance of integration, decreasing or preventing the harmful epigenetic effects and blocking HBV proteins related with HCC development might provide basis for future and might give hope to the patients.

REFERENCES

1 **Feitelson MA**, Lee J. Hepatitis B virus integration, fragile sites, and hepatocarcinogenesis. *Cancer Lett* 2007; **252**: 157-170 [PMID: 17188425 DOI: 10.1016/j.canlet.2006.11.010]

2 **Saigo K**, Yoshida K, Ikeda R, Sakamoto Y, Murakami Y, Urashima T, Asano T, Kenmochi T, Inoue I. Integration of hepatitis B virus DNA into the myeloid/lymphoid or mixed-lineage leukemia (MLL4) gene and rearrangements of MLL4 in human hepatocellular carcinoma. *Hum Mutat* 2008; **29**: 703-708 [PMID: 18320596 DOI: 10.1002/humu20701]

3 **Ringelhan M**, Heikenwalder M, Protzer U. Direct effects of hepatitis B virus-encoded proteins and chronic infection in liver cancer development. *Dig Dis* 2013; **31**: 138-151 [PMID: 23797136 DOI: 10.1159/000347209]

4 **Wong N**, Lai P, Pang E, Fung LF, Sheng Z, Wong V, Wang W, Hayashi Y, Perlman E, Yuna S, Lau JW, Johnson PJ. Genomic aberrations in human hepatocellular carcinomas of differing etiologies. *Clin Cancer Res* 2000; **6**: 4000-4009 [PMID: 11051249]

5 **Oyagbemi AA**, Azeez OI, Saba AB. Hepatocellular carcinoma and the underlying mechanisms. *Afr Health Sci* 2010; **10**: 93-98 [PMID: 20811532]

6 **Lupberger J**, Hildt E. Hepatitis B virus-induced oncogenesis. *World J Gastroenterol* 2007; **13**: 74-81 [PMID: 17206756]

7 **Pollicino T**, Saitta C, Raimondo G. Hepatocellular carcinoma: the point of view of the hepatitis B virus. *Carcinogenesis* 2011; **32**: 1122-1132 [PMID: 21665892 DOI: 10.1093/carcin/bgr108]

8 **Ozkal P**, Ilgin-Ruhi H, Akdogan M, Elhan AH, Kaçar S,

Sasmaz N. The genotoxic effects of hepatitis B virus to host DNA. *Mutagenesis* 2005; **20**: 147-150 [PMID: 15843391 DOI: 10.1093/mutage/gei021]

9 **Ucur A**, Palanduz S, Cefle K, Ozturk S, Tutkan G, Vatansever S, Erden S, Karan MA, Erten N, Guler K, Tascioglu C. Sister chromatid exchange and mitotic index in patients with cirrhosis related to hepatitis B and C viruses and in chronic carriers. *Hepatogastroenterology* 2003; **50**: 2137-2140 [PMID: 14696481]

10 **Bolukbas C**, Bolukbas FF, Kocyigit A, Aslan M, Selek S, Bitiren M, Ulukanligil M. Relationship between levels of DNA damage in lymphocytes and histopathological severity of chronic hepatitis C and various clinical forms of hepatitis B. *J Gastroenterol Hepatol* 2006; **21**: 610-616 [PMID: 16638108 DOI: 10.1111/j.1440-1746.2005.04069.x]

11 **Grossi S**, Sumberaz A, Gosmar M, Mattioli F, Testino G, Martelli A. DNA damage in peripheral blood lymphocytes of patients with cirrhosis related to alcohol abuse or to hepatitis B and C viruses. *Eur J Gastroenterol Hepatol* 2008; **20**: 22-25 [PMID: 18090985 DOI: 10.1097/MEG.0b013e3282f163fe]

12 **Bonilla Guerrero R**, Roberts LR. The role of hepatitis B virus integrations in the pathogenesis of human hepatocellular carcinoma. *J Hepatol* 2005; **42**: 760-777 [PMID: 15826727 DOI: 10.1016/j.jhep.2005.02.005]

13 **Paterlini-Bréchot P**, Saigo K, Murakami Y, Chami M, Gozuacik D, Mugnier C, Lagorce D, Bréchot C. Hepatitis B virus-related insertional mutagenesis occurs frequently in human liver cancers and recurrently targets human telomerase gene. *Oncogene* 2003; **22**: 3911-3916 [PMID: 12813464 DOI: 10.1038/sj.onc.1206492]

14 **Kimbi GC**, Kramvis A, Kew MC. Integration of hepatitis B virus DNA into chromosomal DNA during acute hepatitis B. *World J Gastroenterol* 2005; **11**: 6416-6421 [PMID: 16425409]

15 **Murakami Y**, Minami M, Daimon Y, Okanoue T. Hepatitis B virus DNA in liver, serum, and peripheral blood mononuclear cells after the clearance of serum hepatitis B virus surface antigen. *J Med Virol* 2004; **72**: 203-214 [PMID: 14695661 DOI: 10.1002/jmv.10547]

16 **Minami M**, Daimon Y, Mori K, Takashima H, Nakajima T, Itoh Y, Okanoue T. Hepatitis B virus-related insertional mutagenesis in chronic hepatitis B patients as an early drastic genetic change leading to hepatocarcinogenesis. *Oncogene* 2005; **24**: 4340-4348 [PMID: 15806150 DOI: 10.1038/sj.onc.1208628]

17 **Tamori A**, Yamanishi Y, Kawashima S, Kanehisa M, Enomoto M, Tanaka H, Kubo S, Shiomi S, Nishiguchi S. Alteration of gene expression in human hepatocellular carcinoma with integrated hepatitis B virus DNA. *Clin Cancer Res* 2005; **11**: 5821-5826 [PMID: 16115921 DOI: 10.1158/1078-0432.CCR-04-2055]

18 **Sung WK**, Zheng H, Li S, Chen R, Liu X, Li Y, Lee NP, Lee WH, Ariyaratne PN, Tennakoon C, Mulawadi FH, Wong KF, Liu AM, Poon RT, Fan ST, Chan KL, Gong Z, Hu Y, Lin Z, Wang G, Zhang Q, Barber TD, Chou WC, Aggarwal A, Hao K, Zhou W, Zhang C, Hardwick J, Buser C, Xu J, Kan Z, Dai H, Mao M, Reinhard C, Wang J, Luk JM. Genome-wide survey of recurrent HBV integration in hepatocellular carcinoma. *Nat Genet* 2012; **44**: 765-769 [PMID: 22634754 DOI: 10.1038/ng.2295]

19 **Fujimoto A**, Totoki Y, Abe T, Boroevich KA, Hosoda F, Nguyen HH, Aoki M, Hosono N, Kubo M, Miya F, Arai Y, Takahashi H, Shirakihara T, Nagasaki M, Shibuya T, Nakano K, Watanabe-Makino K, Tanaka H, Nakamura H, Kusuda J, Ojima H, Shimada K, Okusaka T, Ueno M, Shigekawa Y, Kawakami Y, Arihiro K, Ohdan H, Gotoh K, Ishikawa O, Ariizumi S, Yamamoto M, Yamada T, Chayama K, Kosuge T, Yamaue H, Kamatani N, Miyano S, Nakagama H, Nakamura Y, Tsunoda T, Shibata T, Nakagawa H. Whole-genome sequencing of liver cancers identifies etiological influences on mutation patterns and recurrent mutations in chromatin regulators. *Nat Genet* 2012; **44**: 760-764 [PMID: 22634756 DOI: 10.1038/ng.2291]

20 **Jiang Z**, Jhunjhunwala S, Liu J, Haverty PM, Kennemer MI, Guan Y, Lee W, Carnevali P, Stinson J, Johnson S, Diao J, Yeung S, Jubb A, Ye W, Wu TD, Kapadia SB, de Sauvage FJ, Gentleman RC, Stern HM, Seshagiri S, Pant KP, Modrusan Z, Ballinger DG, Zhang Z. The effects of hepatitis B virus integration into the genomes of hepatocellular carcinoma patients. *Genome Res* 2012; **22**: 593-601 [PMID: 22267523 DOI: 10.1101/gr.133926.111]

21 **Ding D**, Lou X, Hua D, Yu W, Li L, Wang J, Gao F, Zhao N, Ren G, Li L, Lin B. Recurrent targeted genes of hepatitis B virus in the liver cancer genomes identified by a next-generation sequencing-based approach. *PLoS Genet* 2012; **8**: e1003065 [PMID: 23236287 DOI: 10.1371/journal.pgen.1003065]

22 **Bhargava A**, Khan S, Panwar H, Pathak N, Punde RP, Varshney S, Mishra PK. Occult hepatitis B virus infection with low viremia induces DNA damage, apoptosis and oxidative stress in peripheral blood lymphocytes. *Virus Res* 2010; **153**: 143-150 [PMID: 20667493 DOI: 10.1016/j.virusres.2010.07.023]

23 **Pollicino T**, Vegetti A, Saitta C, Ferrara F, Corradini E, Raffa G, Pietrangelo A, Raimondo G. Hepatitis B virus DNA integration in tumour tissue of a non-cirrhotic HFE-haemochromatosis patient with hepatocellular carcinoma. *J Hepatol* 2013; **58**: 190-193 [PMID: 22989571 DOI: 10.1016/j.jhep.2012.09.005]

24 **Murakami Y**, Saigo K, Takashima H, Minami M, Okanoue T, Bréchot C, Paterlini-Bréchot P. Large scaled analysis of hepatitis B virus (HBV) DNA integration in HBV related hepatocellular carcinomas. *Gut* 2005; **54**: 1162-1168 [PMID: 16009689 DOI: 10.1136/gut.2004.054452]

25 **Bok J**, Kim KJ, Park MH, Cho SH, Lee HJ, Lee EJ, Park C, Lee JY. Identification and extensive analysis of inverted-duplicated HBV integration in a human hepatocellular carcinoma cell line. *BMB Rep* 2012; **45**: 365-370 [PMID: 22732223 DOI: 10.5483/BMBRep.2012.45.6.279]

26 **Zhang X**, Zhang H, Ye L. Effects of hepatitis B virus X protein on the development of liver cancer. *J Lab Clin Med* 2006; **147**: 58-66 [PMID: 16459163 DOI: 10.1016/j.lab.2005.10.003]

27 **Ma NF**, Lau SH, Hu L, Xie D, Wu J, Yang J, Wang Y, Wu MC, Fung J, Bai X, Tzang CH, Fu L, Yang M, Su YA, Guan XY. COOH-terminal truncated HBV X protein plays key role in hepatocarcinogenesis. *Clin Cancer Res* 2008; **14**: 5061-5068 [PMID: 18698024 DOI: 10.1158/1078-0432.CCR-07-5082]

28 **Wang Y**, Lau SH, Sham JS, Wu MC, Wang T, Guan XY. Characterization of HBV integrants in 14 hepatocellular carcinomas: association of truncated X gene and hepatocellular carcinogenesis. *Oncogene* 2004; **23**: 142-148 [PMID: 14712219 DOI: 10.1038/sj.onc.1206889]

29 **Zhang F**, Wang Q, Ye L, Feng Y, Zhang X. Hepatitis B virus X protein upregulates expression of calpain small subunit 1 via nuclear factor-kappaB/p65 in hepatoma cells. *J Med Virol* 2010; **82**: 920-928 [PMID: 20419804 DOI: 10.1002/jmv.21753]

30 **Zhou Y**, Wang S, Ma JW, Lei Z, Zhu HF, Lei P, Yang ZS, Zhang B, Yao XX, Shi C, Sun LF, Wu XW, Ning Q, Shen GX, Huang B. Hepatitis B virus protein X-induced expression of the CXC chemokine IP-10 is mediated through activation of NF-kappaB and increases migration of leukocytes. *J Biol Chem* 2010; **285**: 12159-12168 [PMID: 20164184 DOI: 10.1074/jbc.M109.067629]

31 **Zhang X**, Dong N, Yin L, Cai N, Ma H, You J, Zhang H, Wang H, He R, Ye L. Hepatitis B virus X protein upregulates survivin expression in hepatoma tissues. *J Med Virol* 2005; **77**: 374-381 [PMID: 16173017 DOI: 10.1002/jmv.20466]

32 **Martin-Lluesma S**, Schaeffer C, Robert EI, van Breugel PC, Leupin O, Hantz O, Strubin M. Hepatitis B virus X protein affects S phase progression leading to chromosome segregation defects by binding to damaged DNA binding protein 1. *Hepatology* 2008; **48**: 1467-1476 [PMID: 18781669 DOI: 10.1002/hep.22542]

33 **Hytiroglou P**, Theise ND. Telomerase activation in human hepatocarcinogenesis. *Am J Gastroenterol* 2006; **101**: 839-841 [PMID: 16635228 DOI: 10.1111/j.1572-0241.2006.00521.x]

34 **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 [PMID: 11481390 DOI: 10.1093/jnci/93.15.1171]

35 **Qu ZL**, Zou SQ, Cui NQ, Wu XZ, Qin MF, Kong D, Zhou ZL. Upregulation of human telomerase reverse transcriptase mRNA expression by in vitro transfection of hepatitis B virus X gene into human hepatocarcinoma and cholangiocarcinoma cells. *World J Gastroenterol* 2005; **11**: 5627-5632 [PMID: 16237755]

36 **Su JM**, Lai XM, Lan KH, Li CP, Chao Y, Yen SH, Chang FY, Lee SD, Lee WP. X protein of hepatitis B virus functions as a transcriptional corepressor on the human telomerase promoter. *Hepatology* 2007; **46**: 402-413 [PMID: 17559154 DOI: 10.1002/hep.21675]

37 **Li W**, Miao X, Qi Z, Zeng W, Liang J, Liang Z. Hepatitis B virus X protein upregulates HSP90alpha expression via activation of c-Myc in human hepatocarcinoma cell line, HepG2. *Virol J* 2010; **7**: 45 [PMID: 20170530 DOI: 10.1186/1743-422X-7-45]

38 **Chung YL**. Defective DNA damage response and repair in liver cells expressing hepatitis B virus surface antigen. *FASEB J* 2013; **27**: 2316-2327 [PMID: 23444429 DOI: 10.1096/fj.12-226639]

39 **Churin Y**, Roderfeld M, Stiefel J, Würger T, Schröder D, Matono T, Mollenkopf HJ, Montalbano R, Pompaiah M, Reifenberg K, Zahner D, Ocker M, Gerlich W, Glebe D, Roeb E. Pathological impact of hepatitis B virus surface proteins on the liver is associated with the host genetic background. *PLoS One* 2014; **9**: e90608 [PMID: 24594856 DOI: 10.1371/journal.pone.0090608]

40 **Rao ZL**, Dong J, Zhu YY, Chen J, You J, Zheng Q, Jiang JJ. [Hepatitis B surface antigen affects the expression of lipid metabolism-related genes in HepG2 cells]. *Zhonghua Gan Zang Bing Za Zhi* 2013; **21**: 624-630 [PMID: 24119745 DOI: 10.3760/cma.j.issn.1007-3418.2013.08.014]

41 **Guo Y**, Kang W, Lei X, Li Y, Xiang A, Liu Y, Zhao J, Zhang J, Yan Z. Hepatitis B viral core protein disrupts human host gene expression by binding to promoter regions. *BMC Genomics* 2012; **13**: 563 [PMID: 23088787 DOI: 10.1186/1471-2164-13-563]

42 **Poreba E**, Broniarczyk JK, Gozdzicka-Jozefiak A. Epigenetic mechanisms in virus-induced tumorigenesis. *Clin Epigenetics* 2011; **2**: 233-247 [PMID: 22704339 DOI: 10.1007/s13148-011-0026-6]

43 **Fernandez AF**, Rosales C, Lopez-Nieva P, Graña O, Ballestar E, Ropero S, Espada J, Melo SA, Lujambio A, Fraga MF, Pino I, Javierre B, Carmona FJ, Acquadro F, Steenbergen RD, Snijders PJ, Meijer CJ, Pineau P, Dejean A, Lloveras B, Capella G, Quer J, Buti M, Esteban JI, Allende H, Rodriguez-Frias F, Castellsague X, Minarovits J, Ponce J, Capello D, Gaidano G, Cigudosa JC, Gomez-Lopez G, Pisano DG, Valencia A, Piris MA, Bosch FX, Cahir-McFarland E, Kieff E, Esteller M. The dynamic DNA methylomes of double-stranded DNA viruses associated with human cancer. *Genome Res* 2009; **19**: 438-451 [PMID: 19208682 DOI: 10.1101/gr.083550.108]

44 **Andrisani OM**. Deregulation of epigenetic mechanisms by the hepatitis B virus X protein in hepatocarcinogenesis. *Viruses* 2013; **5**: 858-872 [PMID: 23507839 DOI: 10.3390/v5030858]

45 **Vivekanandan P**, Daniel HD, Kannangai R, Martinez-Murillo F, Torbenson M. Hepatitis B virus replication induces methylation of both host and viral DNA. *J Virol* 2010; **84**: 4321-4329 [PMID: 20147412 DOI: 10.1128/JVI.02280-09]

46 **Liu J**, Lian Z, Han S, Waye MM, Wang H, Wu MC, Wu K, Ding J, Arbuthnot P, Kew M, Fan D, Feitelson MA. Down-regulation of E-cadherin by hepatitis B virus X antigen in hepatocellular carcinoma. *Oncogene* 2006; **25**: 1008-1017 [PMID: 16247464 DOI: 10.1038/sj.onc1209138]

P- Reviewer: Arai M, Chung YH, Rodriguez-Frias F, Tetsuya T
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Liu SQ

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com