iMedPub Journals
http://www.imedpub.com

Annals of Clinical and Laboratory Research
ISSN 2386-5180

2015
Special Issue

Research Advances on Occult Hepatitis B Virus Infection and its Roles in Digestive System Tumor Development: A New Focus in Cancer Prevention

Feifei Shang[4], Weiwei Cao[2], Lei Li[1], Huanling Shi[1], Jing Zhang[1], Bin Tan[1] and Jiyong Liu[3]

1 Gastroenterologist, Department of Gastroenterology, Taian City Central Hospital, China PR
2 Department of Gastroenterology, Texas A&M University Health Science Center, USA
3 Department of Gastroenterology, Shandong Provincial Hospital, China PR
4 School of Basic Medical Sciences, Taishan Medical University, Taian, China PR

Corresponding author:
Lei Li

doctorlilei@yeah.ne

Department of Gastroenterology, Taian City Central Hospital, China

Tel:+8618605380850, +8613521312560

Abstract

Chronic HBV infection is well known as the major risk factor for Hepatocellular Carcinoma (HCC). During the course of chronic HBV infection, some patients may undergo Hepatitis B surface Antigen (HBsAg) sero-clearance and transfer to occult hepatitis B infection (OBI) status. Patients with OBI have negative serum HBsAg, but detectable HBV DNA in liver tissue with or without detectable serum HBV DNA. The underlying molecular mechanisms of OBI remain elusive. Several factors has been suggested, such as host immune factors mediated by epigenetic modification of HBV covalently closed circular DNA (cccDNA) in hepatocytes, and immune escape mutations and other mutations causing inhibited viral replication and surface antigen expression. OBI may maintain the pro-oncogenic properties as the overt infection. Recent studies have demonstrated that OBI patients have increased risk of developing HCC. By comparison between HCC cases arising from OBI patients and chronic HBV patients, HCC patients with OBI often has a milder cirrhotic liver. The mutation profiles were also found different. HBV infection has been linked with an increased risk of other malignancies such as Intra-hepatic cholangio carcinoma (ICC) and pancreatic cancer (PC), yet the implication of OBI in those cancers remains unclear. HBV vaccination has been proven to be the most efficient way to prevent HBV infections including OBI. However escape mutations and primary OBI are often reported among vaccinated populations. In the post vaccination era, the prevalence of OBI and its role in HCC needs to be further studied.

Keywords: Occult hepatitis B infection, HBV, Liver cancer, Pancreatic cancer, HBV vaccine

HBV Disease Spectrum and OBI

More than 2 billion people or one third of the world's population has past or current infection with HBV, among them 350–400 million people are chronic HBV surface antigen (HBsAg) carriers [1]. HBV infection leads to a wide spectrum of infection status includes fulminate liver failure, acute infection, chronic infection, asymptomatic carrier, and occult hepatitis B infection (OBI) [2]. The first four infection status are referred to as the overt HBV infection characterized by presence of HBV surface antigen (HBsAg), in contrast to OBI in which there is undetectable serum HBsAg but detectable HBV DNA in liver tissue.

HBsAg is the major protein on the virion surface playing a key role in virus infection and replication by mediating viral entrance into host cells. In acute infection, HBsAg disappear within 6 months after infection; while in chronic infection, positive HBV HBsAg will present in serum for more than 6 month. A considerable proportion of long-term chronic infections will eventually progresses to liver cirrhosis, HBV related end-stage liver diseases, or hepatocelluar carcinoma. Chronic Hepatitis infection has been well known as a common cause of hepatocellular carcinoma with about 60% of the world's yearly HCC cases is related to chronic hepatitis infection. In all acute HBV infection and some chronic HBV infection, patients undergo sero-clearance of HBsAg, which marks the turning point toward recumbence phase of the illness. However recent recognition of OBI state reveals that the clearance of HBsAg may not be equal to free of HBV and its complications.

OBI draws more and more attention recent years which is defined

as the presence of HBV DNA in liver and/or blood with blood HBsAg tested negative in currently available serologic assays. OBI can only be detected by highly sensitive HBV DNA assays. It is initially described in the mid-1970s when the detection of HBV DNA was first available. However its existence has been debated for many years until 2008 when the definition and diagnosis criteria of OBI were finally determined in the Taormina expert meeting on occult hepatitis B virus[3]. In OBI, the HBV DNA copy number in serum if detectable should be as low as less than 200IU. OBI is asymptomatic clinically but can be re-activated under immune depressed conditions such as oncohematological malignancies under treatment, organ transplantation, hematopoietic stem cell transplantation, rheumatological diseases under treatment, HIV infection, dermatological and inflammatory bowel diseases treated with biologics, solid tumors treated with chemotherapy and so on[4].

The essential point in OBI infection is the suppression of HBV virus to such a low level that the HBsAg cannot be detected. Those HBsAg negative patients who have comparable serum DNA levels usually found in overt infection are defined as "false" OBI. In false OBI, undetectable HBsAg is attributable to mutations on the antibody epitope at the surface protein. Hence, multivalent anti-HBs antibodies are superior to monoclonal antibody in distinguishing "False" OBI from OBI. OBI can be further classified into sero-positive and sero-negative (about 20-25% of OBI cases[5]) according to presence or absence of serum HBV antibodies; or primary and secondary OBI according to whether it is developed after an overt HBV infection. Generally speaking, sero-positive OBI is believed to develop after HBsAg disappearance at the resolution of an overt HBV infection. Sero-negative OBI might develop from either secondary OBI having lost its HBV antibodies or as primary OBI which lacks serum HBV specific antibodies from the beginning of infection [3,6].

The definite diagnosis of OBI relies on detection of HBV DNA in liver tissue which requires invasive procedures and not easily obtainable. Therefore, serum HBV DNA is often used to detect OBI. Thus OBI could be poorly diagnosed for several reasons. First, patients with OBI often show phases of serum HBV DNA negativity alternating with phases of detectible serum HBV DNA[7,8]. As indicated in several studies testing both liver samples and serum samples, the detection rate was much lower in serum samples than liver samples [9-12]. Of note, in a study carried out on OBI HCC patients in Hong Kong, Wong et al compared the HBV DNA detection rate in patients' serum vs liver tissue. HBV DNA was indentified in 24 cryptogenic HCC OBI patients' liver samples, but none can be detected in their cryopreserved serum samples. Serial detections on serum samples might increase OBI detection [3,13]. Secondly, the serum HBV nucleotide test outcomes are affected by assay's sensitivity and specificity. The direct detection assays which do not use PCR typically has a detection limit of 10^3-10^5 templates[14]. Many PCR based commercial HBV DNA kits have a low cut-off of 200-1000 copies/ml (50-200IU/ml), which is still not sensitive enough to detect OBI. In the meanwhile, false-positive diagnosis of OBI can happen due to sample contamination and low assay specificity.

OBI prevalence has been extensively studied in different regions and among cohorts with different characteristics. However, there are discrepant results even in similar population [15-20], which may due to the true difference in OBI prevalence between regions, cohort selection criteria, vaccination status, sensitivity issue and specificity issue in all kinds of nucleotide assays, as well as different types of sample used.

Nevertheless, considering there is no way to clear HBV cccDNA from a infected cell unless it's been killed, the cccDNA or integrated HBV genome probably will persist for a long time in a portion of patients with past HBV infection. Thus the prevalence of OBI should be highly related to the geographic prevalence of HBV infection. High HBV endemic area includes East Asia and sub-Saharan Africa where 41%-90% of the population had prior exposure to HBV with 5–10% of the adult population is chronically infected; Middle East and the Indian subcontinent are intermediate endemic area for HBV. Low endemic area includes Western Europe and North America. Only 5%-20% of the population had previous exposure, less than 1% of the population is chronically infected [21]. Studies on OBI prevalence among blood donors reported 0.1%-1.59% prevalence of OBI in low endemic area, and up to 6% in donors from an endemic area [22,23]. According to studies carried out in high endemic area such as China, Korea, Hong Kong, and Italy, OBI prevalence among liver disease-free individuals had ranged from 10%-16% [5,24-27].

OBI is much more often associated with end stage HCV liver disease and cryptogenic HCC, the prevalence is also higher in cohorts with high HBV exposure factors such as HCV, HIV infection, hemodialysis patients[28], hemophilia patients, and drug abusers [22,23]. In studies carried out in the USA, OBI is present in 50% of HCV end stage liver disease underwent liver transplantation [9], 25% in chronic C patients [10]. In Mediterranean basin and Far East Asia, OBI was present in 30%-50% of patients with chronic HCV infection, and 20%-30% of patients with cryptogenic liver disease [22]. OBI was reported present in 51% of hemophiliac patients in Japan [5], up to 16% in hemodialysis patients in Turkey [29] , 45% of IV drug abusers in Baltimore, and 14% of IV drug abuser in Brazil [30].

The structure of HBV virus

HBV, a prototype member of the hapdnaviridae family, infects human and chimpanzee with liver as its major infection and replication location. The infectious HBV particle in blood is called Dane particle which is 42nm in diameter in contrast to the 22nm diameter empty envelops present in large excess in the sera of patients with overt HBV infection[31]. Dane particle has double shells, an outer shell of lipoprotein envelope comprised of host derived the lipids and three related viral encoded surface proteins. HBsAg is the smallest yet most abundant surface protein on the outer envelope. In OBI, circulating HBV dane particles and empty particles in blood are reduced to such a low level that HBsAg cannot be detected.

Inside the outer envelope of Dane particle is nucleocapsid or core which consist of core protein enclosing a viral polymerase and partially double helix virus DNA genome. HBV genome contains only approximately 3,200 nucleotides in length and four open-

reading-frames (ORF), pre-S/S, pre-C-C, P, and X [10]. The four ORFs are arranged in such a partially overlapping manner so that Pre-S/S is located within the longest ORF P in a frame shift manner, Pre-C-C and X are partially overlapped with P [6].

The four ORF encodes 7 major viral proteins [13]. Three surface proteins (L, M, S protein) including HBsAg (S protein) the smallest surface protein, are encoded by pre-S/S ORF by three alternative start codons. M and S protein are produced in abundance and they are required for packing of virus core into lipoenvelope. L protein is believed to be involved in interaction with host cell surface receptor to mediate the virus entrance into host cell. Mutations in preS-S ORF is involved in the induction of OBI and false OBI. Numerous studies have been carried out in order to find the etiological mutations that contribute to OBI.

Pre-C-C ORF has two differential initiation codons, encoding the core protein (HBcAg), and a secretary protein antigen E (HBeAg) respectively. Core protein or HBcAg is the major structure protein of HBV core within the envelope of dane particle. HBeAg is not required for virus replication [32], but serves as a seromarker for active virus replication. HBcAg is the strongest HBV antigen and it often sustains longest time after resolution of HBV infection. It is often considered as the best biomarker for previous HBV infections, and sometime used as a surrogate marker for OBI when HBV DNA assay is not available.

P ORF encodes a multifunctional viral polymerase. Upon the finish of synthesis, this polymerase binds to pgRNA and initiates the packing of pgRNA into core protein to form virus core. Once within the nucleocapsid, the polymerase reverse transcribes pgRNA into partially double-stranded relaxed DNA genome. At the same time pgRNA is degraded by the same enzyme. HBV reverse transcript polymerase has a high error rate with estimated nucleotide substitution rate at $1.4–3.2 \times 10 - 5$ /site per year [33]. Thus HBV replication is very prone to generate mutations. As a result, HBV exist as quasispecies in its host body.

X ORF encodes the viral regulatory X protein (HBx), which is not packaged into virions during assembly but is expressed after infection in the new host cell [34]. With its multiple regulatory functions, HBx is essential to initiate and maintain virus replication after infection. X ORF and preS-S ORF are the most integrated HBV DNA fragments in host genome. They are also most commonly targeted sequences for detection of HBV DNA assays.

Early classification of HBV was based on the serological characters of surface antigen. HBV was divided into four major subtype adw, ayw, adr, ayr based on two mutually exclusive determinant pairs d/y and w/r, and one common determinant a on the surface antigen[35]. As the whole genome sequencing being available, HBV has been classified to several viral genotypes A, B, C, D, E, F, I, by comparison of whole genome DNA sequences of many isolates of HBV. Each genotype has its own characteristic geographic distribution [33]. Studies on HBV genotype prevalence reveal that infection with certain genotypes may be more prone to manifest as OBI; while some genotypes are more often to be associated with HCC. Genotype C is most popular in Asia and also is more often associated with HCC [36]. Genotype A is an important risk factor for HCC in Africa [37]. Interestingly, a study in Korea demonstrated that despite subtype adr (genotype C) was found in 96% of HBsAg positive patients, while 75% of OBI patients who lost HBsAg spontaneously were infected with the ayw subtype with sequence similar to genotype D (38). Genotype D was also dominant in OBI among Egyptian paediatric hepatitis cancer patients [30].

The life circle of HBV starts with surface protein mediated endocytosis of viral particle into host cell. Once within the host cell, HBV genome DNA was transported into the nucleus. The partially double-stranded circular DNA is converted to covalently closed circular DNA (cccDNA) by the host cell DNA repair system. In host nucleus, cccDNA is stabilized by histone and organizes into mini-chromatin. cccDNA mini-chromosome serves as the template for viral gene transcription, protein expression and DNA replication [39]. Its expression is subjected to epigenetic modifications. cccDNA is transcribed into viral RNAs including a pregenomic RNA (pgRNA) (3.5kb) presenting the full length of DNA genome [40]. pgRNA is packed with viral polymerase into HBV core where it is reverse transcribed into progeny DNA. The HBV core or nucleocapsids has two pathways, one of which enters ER and Golgi complex where they are further packed into lipid envelope and secreted into blood as infectious particles; The alternative one is going back to the nucleus to add on more copies in the cccDNA pool of the host cells [13]. cccDNA probably will remain in the host cell nucleus as stable mini-chromosome or integrated into host genome. In either way, it will stay in the liver host cell nucleus for a long time, and will probably stay there permanently until the host cell had been killed by immune T cells. Compared to chronic HBV infection, OBI has much lower cccDNA copies (0.0052/cell vs <0.0002/cell) in liver cells and lower expression levels measured by pgRNA copies (2.9/cell vs 0.0002/cell) [12].

Despite many studies conducted on factors contributing to OBI, the induction mechanism of OBI status remains unclear. Different mechanisms and factors such as viral mutations, epigenetic modifications, host immune factors are very likely interplay with each other in the development of OBI [41]. It's possible that the development of OBI varies with virus genotypes, geographic population, immune status, treatment status, and co-infection etc.

It is generally believed that the majority of OBI cases represent the state of remaining small amount of HBV virus after the sero-clearance of acute or chronic HBV infection. In a meta-analysis on association between OBI and serological HBV markers, pooled data from 16 epidemiological research studies revealed that OBI is associated with the concomitant presence of anti-HBs and anti-HBc combination which is also an indicator of resolved HBV infection [42]. A study on T-cell response in OBI, indicates that the immune response in OBI is similar to resolved HBV infection, and higher than inactive HBsAg carriers [43]. Under the tight control of host immune system, those remaining HBV in liver cells can only replicate at a very low level [5]. This hypothesis is supported by numerous observations in that OBI cases can be fully reactivated under immune-deficient status. Sequence analysis research on the isolated HBV from OBI and overt infection patients found similar virus clones, lacking significant difference

to explain the development of OBI. What's more, HBV isolates from OBI patients are fully capable in replication in vitro [44,45].

HBV specific T cell response probably plays a key role in keeping tight control of virus infection in OBI. Other immune factors are also implicated as suggested by research studies. In a study comparing cytokines between OBI and overt HBV infections, sFas was found to have significant difference between the two groups [46]. Fas/Fas-ligand (FasL) system is an important apoptosis signaling pathway that is used by cytotoxic T lymphocytes to eradicate HBV from the liver. Anarabidini et al also found significant difference of IL-12 between OBI and overt infection. It was reported that HBV infection is more likely to present as OBI in children than in adults [47]. Vitamin D receptor gene polymorphism was found to be associated with OBI and other HBV infection clinical phenotypes [2].

Inhibition of HBV replication by epigenetic modifications has been well documented [5,48,49,50], including hypoacetylation of cccDNA-bound H3 and H4 histones and methylation of CpG-rich region. Methylation patterns differ between cases of occult and non-occult HBV infection [45]. HBV regulatory protein HBx is essential for histone acytalation and cccDNA-bound histones are rapidly hypoacetylated in cells replicating the HBx mutant [51]. Recently two independent groups using different in vitro culture and in vivo animal systems have demonstrated that α-Interferon (IFN-α) inhibits HBV transcription and replication by reducing acetylation on histone bound cccDNA minichromosomes [52,53]. IFN-α inhibitory pathway involves STAT1 and STAT2 transcription factors and IFN-stimulated response element IRSE on HBV genome [53,53]. Liu et al also found IFN-a can accelerate the decay of cccDNA [52]. Those studies shed some light on the linkage between host immune control and epigenetic modification in the inhibition of virus gene expression and replication from cccDNA.

The HBV genome exhibited higher methylation levels in both premalignant (cirrhosis) and malignant (HCC) tissues in patients with occult chronic hepatitis, suggesting that hypermethylation of the HBV genome is strongly associated with the oncogenesis. This is consistent with the founding that HBV DNA is more frequently present in non-tumor liver tissues of OBI than tumor tissues [12].

Virus Mutation

Sequence analysis have consistently found that mutations are seen in a higher rate in OBI compared to overt infection [38,44,45,55-57], probably due to the chronic course of OBI. Many studies have been focused on the analysis of pre-S-S ORF to identify mutations that contribute to OBI [30,38,56,58-60]. As HBV always present as a mixture of clones in an individual, the change of immune pressure can select specific dominant HBV clones carrying certain mutations. Mutations and deletions that could contribute to OBI have been found in the HBsAg [61-63], pre-S region[45,57,64], RNA splicing [65], core-pre-core, and P region.

In successfully vaccinated individuals, the S escape mutations have been well documented [66-68]. Similar S escape mutations have been reported on the liver transplantation patients receiving HBV IgG prophylaxis[69]. In these cases, the mutations located in *a* determinant of surface protein let virus escape from HBs-Ab recognition, yet viral replication and infection is unaffected. This kind of infection is referred as "false occult HBV infection"[3]. The IgG immune selection pressure plays a key role in development of false OBI. Similar mechanism could happen in OBI cases in spite that the escape infection is harder to be detected due to its occult clinical presentations. It is worth to note that the whole pre-S-S ORF region encoding for surface proteins is completely overlapped by part of the P ORF encoding polymerase. The viral polymerase is essential for virus replication and packing, and prone to harbor lamivudine treatment induced mutations [70]. Mutations on the pre-S-S region selected by IgG pressure are also potential mutations in the P region affecting polymerase function and leading to low replication level in OBI. On the other hand, the treatment associated mutation in the P overlapping segment may also affect pre-S-S ORF and surface antigen expression [71].

Small inoculums cause primary OBI

An unknown small portion of sero-negative OBI might be primary occult infection which is not led by a previous overt infection. Although direct evidence lacks to prove the existence of primary OBI in human, animal experiments on woodchuck model reveals that exposure to small number of WHV virions (less than 10*3) may cause a persistent infection without conferring any protective immunity. This WHV primary infection preferentially engages the immune system and initiates virus-specific T cell response in the absence of antiviral antibody induction in woodchuck model [72]. Anti-HBc-positive patients showed a T-cell response typical of protective memory. In contrast, HBV-specific T cells in anti-HBc-negative patients did not readily expand and produce interferon-gamma *in vitro*, suggesting the possibility of a low-dose infection insufficient to allow maturation of protective memory. If HBV infection is initiated from a low copy number of HBV, the circulating HBV antigens may not reach a high enough level to induce host production of antibodies against HBV. Or host immune response is so strong that the replication of HBV cannot ever reach such a high level to induce antibodies, so the infection remains in body as the sneaking forms of OBI.

Co-infection with other microbes

High OBI prevalence is found in co-infections with other microbes such as HCV, HIV, or Schistosma mansoni [13]. In HCV and HBV coinfection case, the two virus are found to co-localize extensively the in host hepatocytes. HCV core protein and NS2 protein are able to inhibit HBV protein synthesis and virus replication in vitro. Schistosma is a parasite residing in liver, mainly seen in Asia and Africa. Reduction of HBV replication levels by coinfection of Schistosma is mediated by immune response to parasite particularly cytokine IFN-r [13].

OBI and HCC

Chronic HBV infection has been well known as the major risk factor for Hepatocellular Carcinoma (HCC) for a long time. HBV is classified as group 1 human carcinogens by the World Health Organization. More than 80% HCC cases all over the world happen in HBV endemic regions. Approximately 60% of the annual HCC cases are related to chronic HBV infection. This number could be higher in HBV endemic regions. For an example, in a latest large retrospective studies on 610 consecutive patients diagnosed with

HCC in east China from January 2006 to December 2008, 498 (81.6%) were positive for HBsAg, 16 (2.6%) were positive for anti-HCV, and 6 (1%) were positive for HBsAg and anti-HCV, whereas 90 (14.8%) were negative for both HBsAg and anti-HCV. Of the 85 NBNC-HCC patients tested, 59 patients (68.6%) has OBI (HBV DNA detected in liver sample) [73].

OBI has been recognized as a risk factor for HCC by a large body of emerging evidence in epidemiology and molecular biology. A meta-analysis published in 2012 including eight prospective studies demonstrated a 2.44-fold increased risk of HCC in subjects with occult HBV infection, which was consistent with the significantly increased OR of 6.08 from 8 pooled retrospective studies [42]. Prevalence of OBI in HCC and HCV patients has been summarized in the latest review by Huang et al [74]. The prevalence of OBI has ranged from 40.5% to 76.2% in HCV negative HCC patients in Japan, China, Taiwan, Hong Kong, South Africa, Egypt and Italy. In comparison, OBI prevalence in healthy control is 2.4%, 10.6%, 8% in Japan, China, and Taiwan [75]. According to a recent study carried out the US, high OBI prevalence (more than 40%) is identified in the liver samples of HCC patients. OBI appears to have synergetic pro-oncogenic effects with other liver carcinogens such as HCV [76], alcoholics [77], Aflatoxin, and iron overload[37].

The persistence of HBV DNA in hepatocyte nucleus is the fundamental molecular basis of development of OBI, which also plays a critical role in HCC development as well. Evident on human and animals show that after sero-clearance from overt infection, HBV maintains its pro-oncogenic role in the occult status. In OBI, liver necroinflammation will persist for decades or even lifelong after acute episode HBV hepatitis resolution [5]. In a recent cohort study conducted for more than two decades following mothers screened for HBV infection at each birth delivery, HCC development was significantly higher in women who underwent HBsAg sero-clearance (secondary OBI) during follow-up compared to HBV-unexposed women. This finding is in line with experiments on animal models recovered from hepadnaviral hepatitis. Those animals have lifelong persistence of small amounts of replicating virus and mild liver necroinflammation after spontaneous sero-clearance of the viral surface antigen and sero-conversion to the corresponding antibody. Both woodchucks and ground squirrels are still at increased risk of developing HCC after resolution of overt hepatitis [78]. Not only OBI following the resolution of acute hepatitis has increased risk for PCC, sero-negative primary OBI is also found associated with HCC in woodchucks animal models. Primary OBI infection was induced by small number of virions. WHV DNA was integrated in hepatic and lymphatic genomes in 9/10 animals and typical hepatocellular carcinoma (HCC) developed in 2/10 animals [72].

The Mechanism Underling HBV Induced Transformation of Hepatocyte into Cancer Cell can be Summarized in Three Aspects

1. Virus infection caused liver necro inflammation

2. Pro-oncogenic viral protein or transcripts

3. Integration of viral DNA into host cell genome. OBI maintains the all the pro-oncogenic properties typical of the overt infection. The three aspects are summarized as the following.

Virus infection caused liver necro inflammation

In OBI, continuous or recurrent phases of necroinflammation and regeneration of liver cell persists for long time. However whether OBI caused necroinflammation is severe enough to cause liver cirrhosis remains controversial. On one hand there are considerable evidence that OBI is associated with the progression of liver fibrosis and cirrhosis. On the other hand, studies on animal models and human indicating that only small amount of replicating virus and mild-necroinflamation present in OBI. A possible explanation is many OBI cases is converted from a preceding acute or chronic HBV infection when liver damage has already happened [5]. The afterward HBV occult infection will constantly worsen the liver condition. Nevertheless, although cirrhosis is highly associated with HCC, as 80% to 90% of HCCs occur in patients with underlying cirrhosis [37], cirrhosis is not a premalignant condition. Instead cirrhosis and HCC happens in parallel. OBI appears to exert its pro-oncogenic role in the mild or absence of obvious liver cirrhosis such as in cryptogenic HCC [11]. Yet, OBI often serves as a synergetic risk factor for HCC with many other risk factors causing liver cirrhosis such as previous HBV infection, HCV, alcohol, and fatty liver disease etc.

Pro-oncogenic viral protein or transcripts

Viral expression of pro-oncogenic protein such as HBx, truncated S protein, and HBV Spliced Protein [79,80]. **HBx** is the regulatory protein absolutely required for virus active transcription and HBV replication [34]. It does not packed into virion, but expressed in the new host cell to regulate virus and host gene expression. X protein serves as a trans-activator for transcription of genes involved in many signaling pathways which are promoting cellular proliferation and interfering with DNA repair and apoptosis, [81]. X protein has a particularly high detection rate in liver cancer samples collected from HBV positive HCC patients, probably due to colonial selection. It's also more frequently integrated into HCC genome. Some of the pathways X protein has been reported to be involved includes NF-κ B [82], P53, Wnt/-beta Catenin[83,84], Hepatic Transforming Growth Factor (TGF)-beta [85], calcium signaling, telomerase reverse transcription expression, and many others. X gene also involves in aberrant epigenetic modification in hepato-carcinogenesis [86].

HBV Spliced Protein

Wild-type HBV genome encodes 7 proteins which are translated from 4 unspliced RNA directly transcripted from the virus genome. Some HBV RNA transcripts are subjected to host cell splicing system and generating spliced mRNA. Spliced RNA is not essential for HBV replication, but it is related to chronic course of HBV infection. About one-third of chronic carriers of HBV have HBSP antibodies indicating is commonly associated with HBV chronic infection. HBV spliced protein might be associated with persistence, the severity of chronic hepatitis and HCC [87]. A variety of spliced RNA transcripts have been identified in liver samples infected with HBV and some spliced RNA is specific to

different genotypes [88,89]. Some HBV spliced proteins are reported to promotes cancer cell migration and invasion by Interaction with Cathepsin B [90], and have trans-activation activity [91].

Deficient HBV genome generated by reverse-transcription from spliced RNA can be packed with core protein and secreted as deficient particle with the help of a wild-type virus [92]. There is no research study available on whether HBV spliced HBV transcripts, the proteins generated from the spliced transcripts, and the corresponding defective particles related to OBI. But in vitro studies show spliced transcripts cause decrease in core particle formation, HBs secretion, and HBV DNA synthesis [93].

Truncated preS2/S. Truncated preS2/S is created by a series of truncated mutations at the 3' terminal of preS2/S ORF generated at the integration site. In contrast to the full-length pre-S2/S gene products which did not exhibit trans-activator activity, the truncated protein with deletion mutation within a certain region at 3' terminal exhibits trans-activator activity, which are implicated in the activation of a number of pro-oncogenic genes such as c-myc [94,95], c-Raf-1/Erk2, AP-1, NF-kappaB, and those involved in inactivation of P53 [96]. The deletion were found in one-third of the integrates investigated [96]. A number of epidemiological studies have indicated that Hepatitis B virus pre-S deletion mutations are a risk factor for hepatocellular carcinoma [97-99].

Study on HBV DNA sequence comparisons between HBsAg-positive HCC controls and occult HBV-infected HCC patients, had found different mutation profiles between the two groups in X, pre-S-S gene. OBI has higher mutation frequencies in pre-S-S gene and X gene, yet lower rates of pre-S deletions and core promoter/precore gene mutations, indicating virological factors of HBV related to HCC were different between occult HBV-infected and HBsAg-positive patients [75].

Integration

Integration of virus DNA is well known for its oncogenetic effects and is present in nearly all the HBV related HCC liver samples investigated. Integration probably plays a more important role in OBI related HCC. Mechanism of HBV causing HCC [34,81,100] can be summarized in four aspects:

1. Alteration of expression profiles by insertion of virus transcriptional regulators and promoters [101,102]. HBV has very strong promoter and enhancer element, which can be inserted near a pro-oncogenic gene to up-regulate the expression of those oncogenes. On the other side, integration can also interrupt a tumor suppressor gene and interferes its function.

2. Instability of genome structure mediated by multiples of insertion locations and times [103,104].

3. Integration and expression of virus pro-oncogenetic genes in hepatic cellular genome. The most frequently integrated fragment of virus DNA is X gene and preS/S gene [34,81,100], both of which have been well documented for its trans-activation and pro-oncogencity.

4. Integration mediated generation of deletion mutation or fusion transcripts. Those mutant protein or transcripts have gained pro-oncogenic function or disruption of tumor suppressor such as truncated BHx protein and truncated medium S protein[105]. Interestingly, a latest study found HBV DNA integration generates a chimeric RNA transcript of viral HBx and host LINE1 fusion that have oncogenic function in its RNA form without dependence on the protein. The HBx LINE1 RNA transcript activates Wnt/β-catenin signaling pathway. The importance in cancer development of this chimeric fusion is indicated by its high prevalence in HCC samples tested (in 21 of 90 HCC samples while the next most frequent fusion is 3 out 90) [104,106].

Recent years with the advancing in genome DNA sequencing and data analyzing, several studies of large scale analysis on the sequence of integrated fragments and franking genome regions gives a much deeper view of integration characters and its role in HCC development [103,106-112]. X gene is consistently found as the most frequently inserted sequence especially at the 3'-end with truncation or virus-host chimeric fusion formation. The insertion sites are widespread all over the genome with both gene rich regions and non-gene regions being involved [107,111]. Promoter regions are preferred insertion sites. Significant difference of insertion sites between tumor tissues and non-tumor tissues was reported [107,110]. A functional annotation analysis obtained from 1115 HBV integration sites (1004 unique sites) of 155 HCC patient pooled from 4 studies, revealed that, "compared to those in adjacent non-tumor tissues, ITGs in HCC tumor tissues were significantly enriched in regions related to negative regulation of cell death, transcription regulation, development and differentiation, and cancer related pathways" [107]. Some of the referred pathways or genes involved in HBV DNA integration includes thmixed lineage leukemia2 (MLL2) gene [102,110], human telomerase reverse transcriptase gene (TERT) gene[110, 111], c-MYC, PVT1 and miR-1204 [109], calcium signalling related genes, 60s ribosomal protein encoding genes, and platelet derived growth factor, tumour suppressor genes and genes involved in apoptosis [110].

A study including 49 OBI HCC cases and 20 controls on HBV DNA integration was published in 2015. HBV DNA was integrated in a similar high frequency in OBI HCC cases and HBsAg-positive controls (75.5% vs 80%), in a similar pattern with mainly X and preS/S viral genomic regions involved, and frequent in locations of regulatory and functional genes [108]. Studies on animal models revealed that integration also happens in primary OBI induced in woodchuck models. Comparing OBI with overt chronic HBV, OBI has milder cirrhosis but comparable integration rate, indicating integration might play a more important role in OBI induced HCC [109]. Indeed, HBV DNA integration is an event occurring early in the course of the infection preceding the development of HCC, favoring cell growth and colonial expansion.

In a recent study, the HBV DNA integration between early-onset hepatocellular carcinoma (HCC onset before age of 30) and late-onset HCC (onset after age of 70) was compared. Early-onset HCC accounting for 15 to 20% of total HCC cases in Asia, features reduced cirrhosis development, rapid progress and poor prognosis. A higher prevalence of integration incidence in 8q24 between c-Myc and PVT1 was found in early-onset

HCC compared to late-onset (12.4% vs 1.4%). Over-expression of c-MYC, PVT1 and miR-1204 induced by integration was demonstrated, indicating integration could play a key role in at least a portion of HBV related HCC, especially in those cases with mild cirrhosis [109]. They also reported that HBV genotype B2 is more prevalent in early-onset HCC compared to late-onset HCC. In a whole-genome sequencing study of 88 matched HCC tumor/non-tumor pairs screening for a number of most frequently mutated oncogenes, Wnt/beta-catenin and JAK/STAT pathways are identified likely to be major oncogenic drivers in HCC [113]. Those mutations are generated by HBV integration, or associated with other risk factor such as aflatoxin caused TP53 mutation. Accumulation of mutations maybe the fundamental basis of HBV synergetic carcinogenesis with other risk factors.

OBI&ICC

Intra-hepatic cholangiocarcinoma (ICC) is the second most common primary liver cancer following HCC. It has a much lower incidence than HCC, accounting for 10-15% primary liver cancers [114]. The carcinogenic mechanism and etiology of ICC is still unclear. Potential risk factors include chronic biliary tract diseases, parasitic infection, alcohol use, obesity, fatty liver disease. Recently several studies in HBV endemic regions suggested that chronic HBV infection with detectable circulating hepatitis B surface antigens (HBsAg) may play a role in ICC development [115,116]. The presence of HBV DNA and proteins has been documented in ICC cancer tissues. Meta-analysis study evaluating the potential role of OBI as a risk factor for ICC development found " OBI may represent an important risk factor for ICC" [117].

HBV and Pancreatic Cancer

Extra-hepatic infection

HBV infection has been found in several extra hepatic sites [118-121], such as pancreas [122], kidney, gastric mucosal cells, and mononuclear cells. Extra hepatic HBV infection related diseases including pancreatic cancer [122], necrotizing pancreatitis [123], and insulin secretion disorder diabetes mellitus [124] In the woodchucks convalesce OBI models, the lymphatic system is the site with lifelong virus replication [78]. Woodchuck Hepatitis Virus has been found to infect lymphatic tissue as a primary occult infection site with liver spared when the infectious inoculum is very low [125]. Recently, clinical study suggested that OBI especially primary OBI with sero-negativity might be a potential risk factor for chronic lymphocytic leukaemia [126].

In the recent ten years, the association of hepatitis B virus and pancreatic cancer (PC) has become a new focus in the field of PC etiology. Just like patients with HCC, cirrhosis of chronic hepatitis B, HBV antigens and hepadnaviral DNA can be found in pancreatic tissue also. In some cancers (cervical cancer, gastric cancer) associated with infectious organisms, direct infection plays an important role in the course of malignant transformation. The host immune reaction to virus may lead to a series of result such as inflammation and even cancer. With HBV DNA integrating into the cellular genome, the function of anti-oncogenes could be disrupted and oncogenes be stimulated. These are the possible mechanisms that hepatitis B virus could cause pancreatic cancer. Many studies including cohort studies and case-control studies have been conducted to evaluate the role of HBV in PC, but the results were controversial. The current meta-analysis showed that hepatitis B virus infection as well as the OBI may increase the risk of pancreatic cancer [127]. Additional studies on larger population size are warranted to elucidate the role of OBI and PC risk.

Inspiration in Prevention

According to World Cancer Report 2014, the most important etiological factor for hepatocarcinogenesis remains to be viral infections, usually involving HBV or HCV, followed by intake of aflatoxin and consumption of alcohol [128]. More than half of new liver cancer cases and death occur in China. In China, nearly ninety percents of liver cancer are closely related to hepatitis B infection. HCC incidence rate is on the rise in most highly developed countries such as the US and Europe in the last three decades, probably due to increased HBV infection. Recently HBV has been found to be a potential risk factor for PC and ICC. All the HBV related cancers are highly malignant, difficult to treat with poor prognosis. Early control of HBV infection and eliminating other synergetic risk factors such as intake of aflatoxin, alcohol, smoking, drinking contaminated water, consumption of moldy peanuts and corn, and contaminated oil, are the best way to control HCC. Control of HCV infection and elimination of aflatoxin intake has lead to a drop in HCC incidence for the last decade in both Japan and China. Identification and treatment of HBV infection may also reduce the incidence of PC, ICC and lymphoma.

HBV vaccination is the most effective way to control HBV infection including OBI and will contribute to reduce HCC incidence in endemic area. Universal HBV vaccination has been applied since 1984 in Taiwan, and 1992 in China mainland. A 20-year follow-up cohort studies on HBV vaccination and HCC development indicated that HBV vaccination can dramatically reduce HCC incident by 70% in pediatric and young adult population who received HBV vaccine after the launch of the vaccination program in Taiwan [129]. In Mainland China, mandatory infant vaccination was implemented in 1992 which reduced carrier rate in children from 10% to less than 1% within two decades. Because the development of HCC usually happens after age of 40 to 50 years old following decades of HBV infection, the dramatic HCC incidence drop due to HBV vaccine probably won't be appreciated until the first vaccinated population reach their middle age.

Although HBV vaccine efficiently reduces the HBs-Ag prevalence in young population, attention needs to be paid for surveillance of vaccine escape mutations and OBI infection. According to a recent study on 2028 vaccinated blood donors, confirmed anti-HBc was present in 21.4% of vaccinated donors, indicating that the vaccinated blood donors are frequently infected with OBI which featured by asymptomatic sero-conversion to anti-HBc, and a boost of anti-HBs serum level, without elevation of ALT level [130]. The infection rate increased with time after the vaccination. The same study found that thirty percent of vaccinated people did not carry detectable anti-HBs 18 to 25 years after vaccination. Another possible reason is that vaccine made of Genotype A2 recombinant protein might not be fully efficient for genotypes B

and C which are prevalent in China [130]. In another study carried out in Taiwan, the prevalence of occult HBV infection has been reported as high as 10.9% in HBV vaccinated children [68]. OBI was reported in 19.4% (6/31) of vaccinated children in Gambia, and in 28% (21/75) of immunized HBsAg-negative Iranian children born to carrier mothers. In a large population based study on OBI prevalence in Taiwan, the estimated OBI frequency per 10^4 HBsAg-negative subjects declined from 160.7 in unvaccinated cohorts to 11.5 in vaccinated cohorts [59].

It's worth to note that in immunized population, the profile of sero-positive HBV antibodies with negative HBV antigens is mostly due to breakthrough primary OBI infections. OBI in immunized children is probably a result from very small quantities of HBV retain in the liver following perinatal exposure to high viral load of carrier mothers, or following frequent exposure [59].

In post-vaccination era, anti-HBc seropositivity is a useful marker for OBI screening in HBsAg-negative subjects [59].

Due to the fact that OBI often has serum DNA negative period, it lacks a reliable serum marker for OBI infection. Especially in anti-HBs positive individuals such as those received vaccination or have previous infection. It was reported that IgG against HBsAg block HBV secretion rather than increase clearance [131,132]. IgG may limit the presence of HBV DNA in serum of OBI. Hence, many researchers have recommended anti-HBc as surrogate for OBI. For the sakes of cancer prevention, besides current chronic HBV infection, previous HBV infection and potential current OBI suggested by HBV DNA or anti-HBc should also be on the watch. In the post vaccination era, special attention should be paid to monitor the development of primary OBI. Positive anti-HBc or increased anti-HBs maybe indicate presence of OBI in vaccinated individuals. However it's possible that some primary OBI lacks the serological biomarkers in serum, making it even harder to be detected. Animal experiments indicate such primary OBI exist with its pro-oncogenic potency and transmission capability, and virus DNA integration into the host's hepatic and immune system genomes are found in majority of primary OBI animals [72]. Well-designed long term clinical studies are warranted to monitor the trend of HBV infection evolution in vaccinated population and the role of OBI on the development of digestive system tumor.

References

1 European Association For The Study Of The Liver (2012) EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. J Hepatol 57: 167-185.

2 Arababadi MK, Nasiri Ahmadabadi B, Kennedy D (2012) Current information on the immunologic status of occult hepatitis B infection. Transfusion 52: 1819-1826.

3 Raimondo G, Allain JP, Brunetto MR, Buendia MA, Chen DS, et al. (2008) Statements from the Taormina expert meeting on occult hepatitis B virus infection. J Hepatol 49: 652-657.

4 Raimondo G, Filomia R, Maimone S (2014) Therapy of occult hepatitis B virus infection and prevention of reactivation. Intervirology 57: 189-195.

5 Raimondo G, Caccamo G, Filomia R, Pollicino T (2013) Occult HBV infection. Semin Immunopathol 35: 39-52.

6 Locarnini S, Zoulim F (2010) Molecular genetics of HBV infection. Antivir Ther 15 Suppl 3: 3-14.

7 Kannangai R, Vivekanandan P, Netski D, Mehta S, Kirk GD, et al. (2007) Liver enzyme flares and occult hepatitis B in persons with chronic hepatitis C infection. Journal of clinical virology: the official publication of the Pan American Society for Clinical Virology 39: 101-105.

8 Chemin I, Guillaud O, Queyron PC, Trépo C (2009) Close monitoring of serum HBV DNA levels and liver enzymes levels is most useful in the management of patients with occult HBV infection. J Hepatol 51: 824-825.

9 Shetty K, Hussain M, Nei L, Reddy KR, Lok AS (2008) Prevalence and significance of occult hepatitis B in a liver transplant population with chronic hepatitis C. Liver transplantation : official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society 14: 534-540.

10 Lok AS, Everhart JE, Di Bisceglie AM, Kim HY, Hussain M, et al. (2011) Occult and previous hepatitis B virus infection are not associated with hepatocellular carcinoma in United States patients with chronic hepatitis C. Hepatology 54: 434-442.

11 Kondo R, Nakashima O, Sata M, Imazeki F, Yokosuka O, et al. (2014) Pathological characteristics of patients who develop hepatocellular carcinoma with negative results of both serous hepatitis B surface antigen and hepatitis C virus antibody. Hepatology research: the official journal of the Japan Society of Hepatology 44:1039-1046.

12 Wong DK, Huang FY, Lai CL, Poon RT, Seto WK, et al. (2011) Occult hepatitis B infection and HBV replicative activity in patients with cryptogenic cause of hepatocellular carcinoma. Hepatology 54: 829-836.

13 Samal J, Kandpal M, Vivekanandan P (2012) Molecular mechanisms underlying occult hepatitis B virus infection. Clin Microbiol Rev 25: 142-163.

14 Datta S, Chatterjee S, Veer V1 (2014) Recent advances in molecular diagnostics of hepatitis B virus. World J Gastroenterol 20: 14615-14625.

15 Kang SY, Kim MH, Lee WI (2010) The prevalence of "anti-HBc alone" and HBV DNA detection among anti-HBc alone in Korea. J Med Virol 82: 1508-1514.

16 Levast M, Larrat S, Thelu MA, Nicod S, Plages A, et al. (2010) Prevalence and impact of occult hepatitis B infection in chronic hepatitis C patients treated with pegylated interferon and ribavirin. Journal of medical virology 82: 747-754.

17 Bae E, Park CH, Ki CS, Kim SJ, Huh W, et al. (2012) Prevalence and clinical significance of occult hepatitis B virus infection among renal transplant recipients in Korea. Scandinavian journal of infectious diseases 44: 788-792.

18 Franz C, Perez Rde M, Zalis MG, Zalona AC, Rocha PT, et al. (2013) Prevalence of occult hepatitis B virus infection in kidney transplant recipients. Mem Inst Oswaldo Cruz 108: 657-660.

19 Ferrari CA, Xavier AP, Vidigal VT, Amaral NS, Diniz PA, et al. (2014) Occult hepatitis B virus infection in liver transplant patients in a Brazilian referral center. Brazilian Journal of Medical and Biological Research 47: 990-994.

20 Kang SY, Kim MH, Lee WI (2014) Occult hepatitis B virus infection in Korean patients with isolated anti-HBc. Arch Virol 159: 227-233.

21 Organization WH (2014) Hepatitis B Fact sheet N°204 Updated July 2014. Available online http://wwwwhoint/mediacentre/factsheets/fs204/en/.

22 Kwak MS, Kim YJ1 (2014) Occult hepatitis B virus infection. World J Hepatol 6: 860-869.

23 Gutiérrez-García ML, Fernandez-Rodriguez CM, Lledo-Navarro JL, Buhigas-Garcia I (2011) Prevalence of occult hepatitis B virus infection. World J Gastroenterol 17: 1538-1542.

24 Fang Y, Shang QL, Liu JY, Li D, Xu WZ, et al. (2009) Prevalence of occult hepatitis B virus infection among hepatopathy patients and healthy people in China. J Infect 58: 383-388.

25 Hui CK, Sun J, Au WY, Lie AK, Yueng YH, et al. (2005) Occult hepatitis B virus infection in hematopoietic stem cell donors in a hepatitis B virus endemic area. J Hepatol 42: 813-819.

26 Kim SM, Lee KS, Park CJ, Lee JY, Kim KH, et al. (2007) Prevalence of occult HBV infection among subjects with normal serum ALT levels in Korea. J Infect 54: 185-191.

27 Raimondo G, Navarra G, Mondello S, Costantino L, Colloredo G, et al. (2008) Occult hepatitis B virus in liver tissue of individuals without hepatic disease. J Hepatol 48: 743-746.

28 Dumaidi K, Al-Jawabreh A (2014) Prevalence of occult HBV among hemodialysis patients in two districts in the northern part of the West Bank, Palestine. J Med Virol 86: 1694-1699.

29 Sav T, Gursoy S, Torun E, Sav NM, Unal A, et al. (2010) Occult HBV infection in continuous ambulatory peritoneal dialysis and hemodialysis patients. Ren Fail 32: 74-77.

30 Raouf HE, Yassin AS, Megahed SA, Ashour MS, Mansour TM (2015) Seroprevalence of occult hepatitis B among Egyptian paediatric hepatitis C cancer patients. J Viral Hepat 22: 103-111.

31 The hepatitis B virus [PMIDQ2995835].pdf

32 Ganem D, Prince AM (2004) Hepatitis B virus infection--natural history and clinical consequences. N Engl J Med 350: 1118-1129.

33 Kao JH1 (2002) Hepatitis B viral genotypes: clinical relevance and molecular characteristics. J Gastroenterol Hepatol 17: 643-650.

34 Lucifora J, Arzberger S, Durantel D, Belloni L, Strubin M, et al. (2011) Hepatitis B virus X protein is essential to initiate and maintain virus replication after infection. J Hepatol 55: 996-1003.

35 Magnius LO, Norder H (1995) Subtypes, genotypes and molecular epidemiology of the hepatitis B virus as reflected by sequence variability of the S-gene. Intervirology 38: 24-34.

36 (2003) Geographic Characterization of Hepatitis Virus Infections, Genotyping of Hepatitis B Virus, and p53 Mutation in Hepatocellular Carcinoma Analyzed by In Situ Detection of Viral Genomes from Carcinoma Tissues: Comparison among Six Different Countries.

37 Kew MC1 (2010) Hepatocellular carcinoma in African Blacks: Recent progress in etiology and pathogenesis. World J Hepatol 2: 65-73.

38 Kim KH, Chang HY, Park JY, Park ES, Park YK, et al. (2014) Spontaneous HBsAg loss in Korean patients: relevance of viral genotypes, S gene mutations, and covalently closed circular DNA copy numbers. Clinical and molecular hepatology 20: 251-260.

39 Bock CT, Schwinn S, Locarnini S, Fyfe J, Manns MP, et al. (2001) Structural organization of the hepatitis B virus minichromosome. J Mol Biol 307: 183-196.

40 Su H, Yee JK (1992) Regulation of hepatitis B virus gene expression by its two enhancers. Proc Natl Acad Sci U S A 89: 2708-2712.

41 Quarleri J1 (2014) Core promoter: a critical region where the hepatitis B virus makes decisions. World J Gastroenterol 20: 425-435.

42 Shi Y, Wu YH, Wu W, Zhang WJ, Yang J, et al. (2012) Association between occult hepatitis B infection and the risk of hepatocellular carcinoma: a meta-analysis. Liver international: official journal of the International Association for the Study of the Liver 32: 231-240.

43 Bes M, Vargas V, Piron M, Casamitjana N, Esteban JI, et al. (2012) T cell responses and viral variability in blood donation candidates with occult hepatitis B infection. J Hepatol 56: 765-774.

44 Pollicino T, Raffa G, Costantino L, Lisa A, Campello C, et al. (2007) Molecular and functional analysis of occult hepatitis B virus isolates from patients with hepatocellular carcinoma. Hepatology 45: 277-285.

45 Vivekanandan P, Kannangai R, Ray SC, Thomas DL, Torbenson M (2008) Comprehensive genetic and epigenetic analysis of occult hepatitis B from liver tissue samples. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 46:1227-1236.

46 Martin CM, Welge JA, Shire NJ, Shata MT, Sherman KE, et al. (2009) Cytokine expression during chronic versus occult hepatitis B virus infection in HIV co-infected individuals. Cytokine 47: 194-198.

47 Escobedo-Melendez G, Panduro A, Fierro NA, Roman S (2014) High prevalence of occult hepatitis B virus genotype H infection among children with clinical hepatitis in west Mexico. Memórias do Instituto Oswaldo Cruz 109:728-737.

48 Pollicino T, Belloni L, Raffa G, Pediconi N, Squadrito G, et al. (2006) Hepatitis B virus replication is regulated by the acetylation status of hepatitis B virus cccDNA-bound H3 and H4 histones. Gastroenterology 130: 823-837.

49 Kim JW, Lee SH, Park YS, Hwang JH, Jeong SH, et al. (2011) Replicative activity of hepatitis B virus is negatively associated with methylation of covalently closed circular DNA in advanced hepatitis B virus infection. Intervirology 54: 316-325.

50 Vivekanandan P, Thomas D, Torbenson M (2009) Methylation regulates hepatitis B viral protein expression. J Infect Dis 199: 1286-1291.

51 Belloni L, Pollicino T, De Nicola F, Guerrieri F, Raffa G, et al. (2009) Nuclear HBx binds the HBV minichromosome and modifies the epigenetic regulation of cccDNA function. Proc Natl Acad Sci U S A 106: 19975-19979.

52 Belloni L, Allweiss L, Guerrieri F, Pediconi N, Volz T, et al. (2012) IFN-Î± inhibits HBV transcription and replication in cell culture and in humanized mice by targeting the epigenetic regulation of the nuclear cccDNA minichromosome. J Clin Invest 122: 529-537.

53 Liu F, Campagna M, Qi Y, Zhao X, Guo F, et al. (2013) Alpha-interferon suppresses hepadnavirus transcription by altering epigenetic modification of cccDNA minichromosomes. PLoS pathogens 9: e1003613.

54 Kaur P, Paliwal A, Durantel D, Hainaut P, Scoazec JY, et al. (2010) DNA methylation of hepatitis B virus (HBV) genome associated with the development of hepatocellular carcinoma and occult HBV infection. J Infect Dis 202: 700-704.

55 Rios-Ocampo WA, Cortes-Mancera F, Olarte JC, Soto A, Navas MC1 (2014) Occult hepatitis B virus infection among blood donors in Colombia. Virol J 11: 206.

56 Kim H, Lee SA, Kim DW, Lee SH, Kim BJ (2013) Naturally occurring mutations in large surface genes related to occult infection of hepatitis B virus genotype C. PLoS One 8: e54486.

57 Chaudhuri V, Tayal R, Nayak B, Acharya SK, Panda SK (2004) Occult hepatitis B virus infection in chronic liver disease: full-length genome and analysis of mutant surface promoter. Gastroenterology 127: 1356-1371.

58 El Chaar M, Candotti D, Crowther RA, Allain JP (2010) Impact of hepatitis B virus surface protein mutations on the diagnosis of occult hepatitis B virus infection. Hepatology 52: 1600-1610.

59 Hsu HY, Chang MH, Ni YH, Chiang CL, Wu JF, et al. (2015) Universal infant immunization and occult hepatitis B virus infection in children and adolescents: a population-based study. Hepatology 61: 1183-1191.

60 Powell EA, Gededzha MP, Rentz M, Rakgole NJ, Selabe SG, et al. (2015) Mutations associated with occult hepatitis B in HIV-positive South Africans. J Med Virol 87: 388-400.

61 Biswas S, Candotti D, Allain JP (2013) Specific amino acid substitutions in the S protein prevent its excretion in vitro and may contribute to occult hepatitis B virus infection. J Virol 87: 7882-7892.

62 Torresi J, Earnest-Silveira L, Deliyannis G, Edgtton K, Zhuang H, et al. (2002) Reduced antigenicity of the hepatitis B virus HBsAg protein arising as a consequence of sequence changes in the overlapping polymerase gene that are selected by lamivudine therapy. Virology 293: 305-313.

63 Mingshun Z, Guohong G, Yonglin Y, Xubing C, Qiang F, et al. (2013) Decreased antigenicity profiles of immune-escaped and drug-resistant hepatitis B surface antigen (HBsAg) double mutants 10: 292.

64 Fan YF, Lu CC, Chen WC, Yao WJ, Wang HC, et al. (2001) Prevalence and significance of hepatitis B virus (HBV) pre-S mutants in serum and liver at different replicative stages of chronic HBV infection. Hepatology 33: 277-286.

65 Hass M, Hannoun C, Kalinina T, Sommer G, Manegold C, et al. (2005) Functional analysis of hepatitis B virus reactivating in hepatitis B surface antigen-negative individuals. Hepatology 42: 93-103.

66 Oon CJ, Lim GK, Ye Z, Goh KT, Tan KL, et al. (1995) Molecular epidemiology of hepatitis B virus vaccine variants in Singapore. Vaccine 13: 699-702.

67 Carman WF, Zanetti AR, Karayiannis P, Waters J, Manzillo G, et al. (1990) Vaccine-induced escape mutant of hepatitis B virus. Lancet 336: 325-329.

68 Mu SC, Lin YM, Jow GM, Chen BF (2009) Occult hepatitis B virus infection in hepatitis B vaccinated children in Taiwan. J Hepatol 50: 264-272.

69 Ghany MG, Ayola B, Villamil FG, Gish RG, Rojter S, et al. (1998) Hepatitis B virus S mutants in liver transplant recipients who were reinfected despite hepatitis B immune globulin prophylaxis. Hepatology 27: 213-222.

70 Wakil SM, Kazim SN, Khan LA, Raisuddin S, Parvez MK, et al. (2002) Prevalence and profile of mutations associated with lamivudine therapy in Indian patients with chronic hepatitis B in the surface and polymerase genes of hepatitis B virus. Journal of medical virology. 68: 311-318.

71 Torresi J1 (2002) The virological and clinical significance of mutations in the overlapping envelope and polymerase genes of hepatitis B virus. J Clin Virol 25: 97-106.

72 Mulrooney-Cousins PM, Chauhan R, Churchill ND, Michalak TI (2014) Primary seronegative but molecularly evident hepadnaviral infection engages liver and induces hepatocarcinoma in the woodchuck model of hepatitis B. PLoS pathogens. 10: e1004332.

73 Wu ZF, Xu Z, Li WS, Zhang HB, Yang N, et al. (2015) Impact of occult hepatitis B virus infection on outcome after resection for non-B non-C hepatocellular carcinoma. J Surg Res 193: 153-160.

74 Huang X, Hollinger FB (2014) Occult hepatitis B virus infection and hepatocellular carcinoma: a systematic review. J Viral Hepat 21: 153-162.

75 Chen CH, Changchien CS, Lee CM, Tung WC, Hung CH, et al. (2009) A study on sequence variations in pre-S/surface, X and enhancer II/core promoter/precore regions of occult hepatitis B virus in non-B, non-C hepatocellular carcinoma patients in Taiwan. International journal of cancer Journal international du cancer. 125: 621-629.

76 Kazuki I, Yukio O, Takefumi N, Naoto K, Yukitaka Y, et al. (2007) Antibody to Hepatitis B Core Antigen and Risk for Hepatitis C–Related Hepatocellular Carcinoma: A Prospective Study.

77 Kwon OS, Jung YK, Bae KS, Kim JH, Kim SG, et al. (2012) Anti-hepatitis B core positivity as a risk factor for hepatocellular carcinoma in alcoholic cirrhosis: a case-control study. Alcohol 46: 537-541.

78 Tomasz IM, Ingrid UP, Carla SC, Norma DC, Dion SF, et al. (1999) Occult Lifelong Persistence of Infectious Hepadnavirus and Residual Liver Inflammation in Woodchucks Convalescent From Acute Viral Hepatitis. Hepatology 29: 928-938.

79 Bréchot C (2004) Pathogenesis of hepatitis B virus-related hepatocellular carcinoma: old and new paradigms. Gastroenterology 127: S56-61.

80 Ringelhan M, Heikenwalder M, Protzer U (2013) Direct effects of hepatitis B virus-encoded proteins and chronic infection in liver cancer development. Dig Dis 31: 138-151.

81 Madden CR, Slagle BL (2001) Stimulation of cellular proliferation by hepatitis B virus X protein. Dis Markers 17: 153-157.

82 Lu B, Guo H, Zhao J, Wang C, Wu G, et al. (2010) Increased expression of iASPP, regulated by hepatitis B virus X protein-mediated NF-ÎºB activation, in hepatocellular carcinoma. Gastroenterology 139: 2183-2194.

83 Cha MY, Kim CM, Park YM, Ryu WS (2004) Hepatitis B virus X protein is essential for the activation of Wnt/beta-catenin signaling in hepatoma cells. Hepatology 39: 1683-1693.

84 Lian Z, Liu J, Li L, Li X, Clayton M, et al. (2006) Enhanced cell survival of Hep3B cells by the hepatitis B x antigen effector, URG1, is associated with upregulation of beta-catenin. Hepatology 43: 415-424.

85 Murata M, Matsuzaki K, Yoshida K, Sekimoto G, Tahashi Y, et al. (2009) Hepatitis B virus X protein shifts human hepatic transforming growth factor (TGF)-beta signaling from tumor suppression to oncogenesis in early chronic hepatitis B. Hepatology 49: 1203-1217.

86 Park IY, Sohn BH, Yu E, Suh DJ, Chung YH, et al. (2007) Aberrant epigenetic modifications in hepatocarcinogenesis induced by hepatitis B virus X protein. Gastroenterology 132: 1476-1494.

87 Soussan P, Tuveri R, Nalpas B, Garreau F, Zavala F, et al. (2003) The expression of hepatitis B spliced protein (HBSP) encoded by a spliced hepatitis B virus RNA is associated with viral replication and liver fibrosis. J Hepatol 38: 343-348.

88 Günther S, Sommer G, Iwanska A, Will H (1997) Heterogeneity and common features of defective hepatitis B virus genomes derived from spliced pregenomic RNA. Virology 238: 363-371.

89 Wu HL, Chen PJ, Tu SJ, Lin MH, Lai MY, et al. (1991) Characterization and genetic analysis of alternatively spliced transcripts of hepatitis B virus in infected human liver tissues and transfected HepG2 cells. J Virol 65: 1680-1686.

90 Chen WN, Chen JY, Jiao BY, Lin WS, Wu YL, et al. (2012) Interaction of the hepatitis B spliced protein with cathepsin B promotes hepatoma cell migration and invasion. J Virol 86: 13533-13541.

91 Chen WN, Chen JY, Lin WS, Lin JY, Lin X (2010) Hepatitis B doubly spliced protein, generated by a 2.2 kb doubly spliced hepatitis B virus RNA, is a pleiotropic activator protein mediating its effects via activator protein-1- and CCAAT/enhancer-binding protein-binding sites. The Journal of general virology. 91: 2592-2600.

92 Soussan P, Garreau F, Zylberberg H, Ferray C, Brechot C, et al. (2000) In vivo expression of a new hepatitis B virus protein encoded by a spliced RNA. J Clin Invest 105: 55-60.

93 Park GS, Kim HY, Shin HS, Park S, Shin HJ, et al. (2008) Modulation of hepatitis B virus replication by expression of polymerase-surface fusion protein through splicing: implications for viral persistence. Virus Res 136: 166-174.

94 Lauer U, Weiss L, Hofschneider PH, Kekulé AS (1992) The hepatitis B virus pre-S/S(t) transactivator is generated by 3' truncations within a defined region of the S gene. J Virol 66: 5284-5289.

95 Kekul A, As LU, Meyer M, Caselmann WH, Hofschneider PH, et al. (1990) The preS2 S region of integrated hepatitis B virus DNA encodes a transcriptional transactivator. Nature 343: 457-461.

96 Hildt E, Munz B, Saher G, Reifenberg K, Hofschneider PH (2002) The PreS2 activator MHBs(t) of hepatitis B virus activates c-raf-1/Erk2 signaling in transgenic mice. EMBO J 21: 525-535.

97 Fang ZL, Sabin CA, Dong BQ, Wei SC, Chen QY, et al. (2008) Hepatitis B virus pre-S deletion mutations are a risk factor for hepatocellular carcinoma: a matched nested case-control study. J Gen Virol 89: 2882-2890.

98 Yeung P, Wong DK, Lai CL, Fung J, Seto WK, et al. (2011) Association of hepatitis B virus pre-S deletions with the development of hepatocellular carcinoma in chronic hepatitis B. J Infect Dis 203: 646-654.

99 Qu LS, Liu JX, Liu TT, Shen XZ, Chen TY, et al. (2014) Association of hepatitis B virus pre-S deletions with the development of hepatocellular carcinoma in Qidong, China. PLoS One 9: e98257.

100 Guerrieri F, Belloni L, Pediconi N, Levrero M (2013) Molecular mechanisms of HBV-associated hepatocarcinogenesis. Semin Liver Dis 33: 147-156.

101 Shamay M, Agami R, Shaul Y (2001) HBV integrants of hepatocellular

carcinoma cell lines contain an active enhancer. Oncogene 20: 6811-6819.

102 Tamori A, Yamanishi Y, Kawashima S, Kanehisa M, Enomoto M, et al. (2005) Alteration of gene expression in human hepatocellular carcinoma with integrated hepatitis B virus DNA. Clinical cancer research: an official journal of the American Association for Cancer Research 11: 5821-5826.

103 Jiang Z, Jhunjhunwala S, Liu J, Haverty PM, Kennemer MI, et al. (2012) The effects of hepatitis B virus integration into the genomes of hepatocellular carcinoma patients. Genome Res 22: 593-601.

104 Niknafs YS, Chinnaiyan AM2 (2014) RNA identity crisis: hepatitis B walks the LINE. Cancer Cell 25: 259-260.

105 Wang Y, Lau SH, Sham JS, Wu MC, Wang T, et al. (2004) Characterization of HBV integrants in 14 hepatocellular carcinomas: association of truncated X gene and hepatocellular carcinogenesis. Oncogene 23: 142-148.

106 Lau CC, Sun T, Ching AK, He M, Li JW, et al. (2014) Viral-human chimeric transcript predisposes risk to liver cancer development and progression. Cancer Cell 25: 335-349.

107 Li X, Zhang J, Yang Z, Kang J, Jiang S, et al. (2014) The function of targeted host genes determines the oncogenicity of HBV integration in hepatocellular carcinoma. J Hepatol 60: 975-984.

108 Saitta C, Tripodi G, Barbera A, Bertuccio A, Smedile A, et al. (2015) Hepatitis B virus (HBV) DNA integration in patients with occult HBV infection and hepatocellular carcinoma. Liver Int 35: 2311-2317.

109 Yan H,, Yang Y, Zhang L, Tang G, et al. (2015) Characterization of the genotype and integration patterns of hepatitis B virus in early- and late-onset hepatocellular carcinoma. Hepatology 61: 1821-1831.

110 Murakami Y, Saigo K, Takashima H, Minami M, Okanoue T, et al. (2005) Large scaled analysis of hepatitis B virus (HBV) DNA integration in HBV related hepatocellular carcinomas. Gut 54: 1162-1168.

111 Toh ST, Jin Y, Liu L, Wang J, Babrzadeh F, et al. (2013) Deep sequencing of the hepatitis B virus in hepatocellular carcinoma patients reveals enriched integration events, structural alterations and sequence variations. Carcinogenesis 34: 787-798.

112 Sung WK, Zheng H, Li S, Chen R, Liu X, et al. (2012) Genome-wide survey of recurrent HBV integration in hepatocellular carcinoma. Nat Genet 44: 765-769.

113 Kan Z, Zheng H, Liu X, Li S, Barber TD, et al. (2013) Whole-genome sequencing identifies recurrent mutations in hepatocellular carcinoma. Genome Res 23: 1422-1433.

114 Zhou HB, Hu JY, Hu HP1 (2014) Hepatitis B virus infection and intrahepatic cholangiocarcinoma. World J Gastroenterol 20: 5721-5729.

115 Lee TY, Lee SS, Jung SW, Jeon SH, Yun SC, et al. (2008) Hepatitis B virus infection and intrahepatic cholangiocarcinoma in Korea: a case-control study. Am J Gastroenterol 103: 1716-1720.

116 Matsumoto K, Onoyama T, Kawata S, Takeda Y, Harada K, et al. (2014) Hepatitis B and C virus infection is a risk factor for the development of cholangiocarcinoma. Intern Med 53: 651-654.

117 YuQiong LM, Hui W, DaPeng MM, JingYi HM, Hao W, et al. (2013) Occult Hepatitis B Virus Infection in Chinese Cryptogenic Intrahepatic Cholangiocarcinoma Patient Population. J Clin Gastroenterol *48:* 878-882.

118 Dejean A, Lugassy C, Zafrani S, Tiollais P, Brechot C (1984) Detection of hepatitis B virus DNA in pancreas, kidney and skin of two human carriers of the virus. J Gen Virol 65 : 651-655.

119 Barker LF, Maynard JE, Purcell RH, Hoofnagle JH, Berquist KR, et al. (1975) Hepatitis B virus infection in chimpanzees: titration of subtypes. J Infect Dis 132: 451-458.

120 Halpern MS, England JM, Deery DT, Petcu DJ, Mason WS, et al. (1983) Viral nucleic acid synthesis and antigen accumulation in pancreas and kidney of Pekin ducks infected with duck hepatitis B virus. Proc Natl Acad Sci U S A 80: 4865-4869.

121 Korba BE, Gowans EJ, Wells FV, Tennant BC, Clarke R, et al. (1988) Systemic distribution of woodchuck hepatitis virus in the tissues of experimentally infected woodchucks. Virology 165: 172-181.

122 Jin Y, Gao H, Chen H, Wang J, Chen M, et al. (2013) Identification and impact of hepatitis B virus DNA and antigens in pancreatic cancer tissues and adjacent non-cancerous tissues. Cancer Lett 335: 447-454.

123 Cavallari A, Vivarelli M, D'Errico A, Bellusci R, Scarani P, et al. (1995) Fatal necrotizing pancreatitis caused by hepatitis B virus infection in a liver transplant recipient. J Hepatol 22: 685-690.

124 Shi DR, Dong CL, Lu L, Cong WT, Zhou Y (2003) [Relationship between glucose metabolic disorders and expression of insulin receptor in posthepatitic cirrhosis hepatocyte and HBV DNA in pancreatic cells]. Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi 17: 372-374.

125 Michalak TI, Mulrooney PM, Coffin CS (2004) Low doses of hepadnavirus induce infection of the lymphatic system that does not engage the liver. J Virol 78: 1730-1738.

126 Pinato D, Rossi D, Minh M, Toniutto P, Boccato E, et al. (2012) Hepatitis B virus and lymphomagenesis: novel insights into an occult relationship. Digestive and liver disease: official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver 44: 235-238.

127 Li L, Wu B, Yang LB, Yin GC, Liu JY (2013) Chronic hepatitis B virus infection and risk of pancreatic cancer: a meta-analysis. Asian Pac J Cancer Prev 14: 275-279.

128 Candotti D, Lin CK, Belkhiri D, Sakuldamrongpanich T, Biswas S, et al. (2012) Occult hepatitis B infection in blood donors from South East Asia: molecular characterisation and potential mechanisms of occurrence. Gut 61: 1744-1753.

129 Chang MH, You SL, Chen CJ, Liu CJ, Lee CM, et al. (2009) Decreased incidence of hepatocellular carcinoma in hepatitis B vaccinees: a 20-year follow-up study. J Natl Cancer Inst 101: 1348-1355.

130 Zheng X, Ye X, Du P, Zeng J, Zhu W, et al. (2014) High prevalence of anti-hepatitis B core antigen in hepatitis B virus-vaccinated Chinese blood donors suggests insufficient protection but little threat to the blood supply. Transfusion *55:* 890-897.

131 Schilling R, Ijaz S, Davidoff M, Lee JY, Locarnini S, et al. (2003) Endocytosis of hepatitis B immune globulin into hepatocytes inhibits the secretion of hepatitis B virus surface antigen and virions. J Virol 77: 8882-8892.

132 Neumann AU, Phillips S, Levine I, Ijaz S, Dahari H, et al. (2010) Novel mechanism of antibodies to hepatitis B virus in blocking viral particle release from cells. Hepatology 52: 875-885.

This article is part of the Special Issue entitled - Clinical and Health Care, edited by Dr. Nguyen Van Bang, (Hanoi Medical University, Vietnam) and belongs to Volume S1 of Annals of Clinical and Laboratory Research