Hepatoma Research

Review

Impact of occult hepatitis B virus infection and high-fat diet on hepatocellular carcinoma development

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Abstract

In the areas where viral infections are more common, the incidence of hepatocellular carcinoma (HCC) has declined. This is largely attributed to the availability of vaccines against hepatitis B virus (HBV), antiviral treatments, and a change in diet with healthier foods. However, in the Western world, the HCC incidence rate has risen steadily, probably due to an increased prevalence of metabolic dysfunction-associated fatty liver disease (MAFLD), a condition that has increasingly been considered an important risk factor for HCC. Despite this, in 2019, 41% of HCC cases were diagnosed globally and a majority of diagnosed cases from mainland China were related to HBV. Some HCC cases tested negative for hepatitis B surface antigen (HBsAg) but tested positive for HBV-DNA in blood. This is considered an occult HBV infection (OBI). OBI is related to various mutations in the viral genome, which modifies host immunity, subsequently leading to the long-term persistence of cccDNA in the nucleus of infected hepatocytes. Many OBIs are associated with HBV variants carrying mutations in preS/S genomic regions, which occur either spontaneously or as a result of antiviral treatments. OBIs are also frequently reported in HBV-vaccinated individuals. HBV variants post-HBV vaccination carry mutations in the preS area. This review discusses the relationship between preS variant-related OBIs and the development of HCC in the context of a high-fat diet, one of the preventable behavioral risk factors for MAFLD.

Keywords: Occult HBV infection, preS mutations, hepatocellular carcinoma, high-fat diet, hepatitis B vaccination, metabolic dysfunction associated fatty liver disease

INTRODUCTION

The age-standardized incidence rate of hepatocellular carcinoma (HCC) has decreased consistently in Oceania, sub-Saharan Africa, and Asia, such as in China[1]. This trend was greatly attributed to the strategies for reducing established HCC risk factors. It includes immunization against hepatitis B virus (HBV) using HepB vaccine[2-4], decreased exposure to carcinogen aflatoxins[5], treatment of patients with HBV using nucleos(t)ide analogs[6], and treatment of patients with hepatitis C virus (HCV) using direct-acting antiviral agents[7]. The vegetable-based dietary pattern was observed to reduce the risk of liver cancer, particularly in individuals with a history of chronic liver disease[8,9], and this pattern is considered increasingly more acceptable in China[10].

The HCC incidence rates have increased steadily in the Western world. It is likely associated with the increased prevalence of metabolic dysfunction-associated with fatty liver disease (MAFLD), formerly known as non-alcoholic fatty liver disease (NAFLD). MAFLD is an overarching term describing heterogenous liver diseases associated with known metabolic dysfunctions. It is associated with various factors such as age, gender, genetic predisposition, microbiota, hormonal status, and behavioral factors, including dietary patterns[11]. Epidemiologically, a major HCC etiology is a shift from viral to metabolic liver diseases[12-14]. The MAFLD prevalence has consistently increased in Asia, such as China, over the last two decades[15].

A study indicated that 41.0% of HCC cases diagnosed globally in 2019 were related to HBV[6]. In Mailand of China, 84.4% of diagnosed HCC cases during 2003-2020 were related to HBV. Some of these cases were serologically negative for HBV surface antigen (HBsAg) but positive for HBV-DNA[16]. A global systematic review and meta-analysis of 22 studies that included over 56,000 HCC individuals indicated that 49% of all HCC cases and 40% of HBV-related HCC cases have MAFLD, but only 12% of all HCC cases were associated with solitary MAFLD[14]. An 11-year follow-up study on the Chinese population with 13,032 company employees reported that the prevalence of NAFLD was 17.2% in 2006, which increased to 32.4% in 2016. However, the main clinical outcomes of NAFLD in this cohort population were diabetes, hypertension, and hyperuricemia. No patients with NAFLD progressed into HCC, except for one male patient who developed cirrhosis over the 11 years[17]. HepB vaccine is prophylactic and has no effect on established HBV infection. Antiviral therapies with nucleot(s)ide analogs inhibit viral DNA synthesis and delay the occurrence of HBV-related diseases including HCC but cannot cure HBV infections[18]. Global HBV prevalence in 2022 was estimated to be at 3.2%, corresponding to 257.5 million (216.6-316.4) HBsAg-positive individuals[19]. Primary prevention based on etiology offers the best cost-effective method for decreasing HCC incidence. Thus, it is important to understand the pathophysiology of HCC development in individuals with pre-existing HBV infection in the context of preventable behavioral risk factors that can lead to MAFLD, such as dietary patterns.

HBV LIFE CYCLE

HBV life cycle is described briefly here as it has been reviewed in detail elsewhere[20,21]. Infectious HBV viral particles that contain the genome of a 3.2 kb relaxed circular (rc) partially double-stranded DNA (rcDNA) attach first to heparan sulfate proteoglycans. Then, they enter hepatocytes via bile acid transporter sodium taurocholate co-transporting polypeptide (NTCP) through an interaction with the preS1 domain at the large envelop protein (L-HBsAg)[22]. Upon entry into the hepatocytes, the HBV viral particle is uncoated and nucleocapsids move to the hepatocyte nuclear pore complex, where rcDNA is released into the nucleus. By using host factors, rcDNA is converted into covalently closed circular DNA (cccDNA)[23,24], which serves as the template for transcription of viral pregenomic RNA (pgRNA) and viral mRNAs, including 3.5, 2.4, 2.1, and 0.7 kb HBV RNA. The 0.7 kb HBV RNA is transcribed from the *X* gene promoter and translated into a multifunctional HBx protein, which is known to activate viral and host gene transcriptions relating to

hepatocarcinogenesis[20]. From 1,783 nt of the HBV genome, a 3.5 kb RNA is transcribed and translated to precore polypeptide which is trafficked into hepatocyte endoplasmic reticulum (ER) via a signal peptide in the N-terminal region. Within ER, the signal peptide and 34 amino acids (AA) of the C-terminal domain are removed, and hepatitis B e antigen (HBeAg) is matured to release from hepatocytes. From 1,818 nt of the HBV genome, a 3.5 kb pgRNA is transcribed and translated to HBV core protein (HBcAg) and viral polymerase (P), and encapsidated with P protein by the core protein dimers to form nucleocapsids particles. Reverse transcription is initiated within the particles and conducted by the P protein to generate rcDNA, or double-strand linear DNA (dslDNA) in some situations. The dslDNA is preferably integrated into the host genome. Some nucleocapsids are shuttled back to the nucleus to maintain a relatively stable pool of cccDNA and some are enveloped by L-HBsAg, which is translated from 2.4 kb HBV RNA. Meanwhile, middle (M-HBsAg) and small (S-HBsAg) envelop proteins are translated from 2.1 kb RNA. Synthesized L-, M-, and S-HBsAg are sorted into the ER for processing and then transferred to the Golgi apparatus. Through two cytoplasmic matrix domains, one located at the preS1-preS2 boundary and another at the C-terminal of S-HBsAg, the L-HBsAg contacts with nucleocapsids to pack tightly and drive the inward budding. The inward-budded nucleocapsids are processed by endosomal sorting complexes required for transport to catalyze the membrane fission and subsequently release outside the cells as virions[25,26]. The domains of L-HBsAg at preS1 and S are required for the generation of infectious viral particles (42 nm in diameter) and spherical particles (20 nm in diameter). The spherical particles contain no HBV genome, which mainly comprises S-HBsAg[20,26].

HCC RISK IN PATIENTS WITH CHRONIC HBV INFECTION COMBINED WITH METABOLIC DYSFUNCTION

MAFLD is a phenotype with complex and diverse causes[11]. Currently, HBV infection in combination with NAFLD leading to the progression of HCC remains unconfirmed. A previous review article summarized the interaction between HBV and NAFLD and the progression of the disease[27]. A study based on a healthy Korean adult cohort reported that HBsAg seropositivity was associated with a lower risk of developing NAFLD[28]. Most studies in the Chinese population also suggest that HBV infection reduces the NAFLD incidence[27]. In an HBV-immunocompetent mouse model with HBV genotype B infection, HBV replication was even attenuated in the context of high-fat diet-induced hepatic steatosis[29]. Many individuals with hepatic steatosis exhibit no liver inflammatory injury, and an individual with NAFLD can oscillate between steatosis and steatohepatitis even over a short timeframe[30].

Most HCCs develop with a background of chronic liver injury, hepatic inflammation, and liver fibrosis. Clinical evidence and a few animal models suggested that hepatic steatosis in patients with chronic hepatitis B (CHB) might reduce HCC risks. Virus *per se* is the most important factor in the development of HBV-related HCC. Among the HBsAg-positive population, the HBeAg positivity significantly increases HCC risk[31]. However, among CHB patients with steatosis, the portions of serum HBeAg positivity and HBV viremia reduced, and the positive staining of intrahepatic HBsAg and hepatitis B core antigen (HBcAg) decreased in liver tissue[32]. A meta-analysis in CHB patients found that hepatic steatosis was not significantly associated with liver fibrosis[33]. CHB patients with MAFLD increased the overall risk of liver fibrosis, although atherosclerosis was protected[34]. A prospective study conducted on CHB patients from Hong Kong reported that reduced hepatic steatosis significantly increased the HCC risk[35]. Analyzing the database of the Korean National Health Insurance Service, which included 1,504,880 adults with HBV infection, it was reported that metabolic syndromes increased the risk for most malignancies but were negatively associated with HCC[36]. However, the 5-year follow-up results from 10,546 treatment-naive CHB patients reported that hepatic steatosis and metabolic dysfunction displayed distinct effects on HCC development[37]. A study based on 5,373 male Taiwanese civil servants indicated that the cumulative

incidence of HCC and liver-related deaths are significantly different in HBV carriers with different metabolic risk factors. Metabolic risk factors and insulin resistance presented the largest effect on HCC risk in CHB patients. More importantly, the association between HCC and lower levels of serum HBV-DNA (less than 10,000 copies/mL) was stronger compared to that in patients with higher levels of HBV-DNA[38]. HBV retention within hepatocytes might play an important role in promoting HCC development [Figure 1]. Indeed, reduced HBV secretion from hepatocytes promotes HCC development, such as the infection of preS/S variants that lead to an imbalance in HBV replication and secretion from hepatocytes[39-41].

OCCULT HBV INFECTION POST HepB VACCINATION AT INFANCY

The definition of occult HBV infection (OBI) was updated in October 2018. It is now defined as the presence of replication-competent HBV (the episomal cccDNA) in liver and/or HBV-DNA in blood of individuals who are seronegative for HBsAg with currently available assays. The pathogenesis of OBI is related to the long-term persistence of cccDNA in the nucleus of infected hepatocytes[42]. The infection with HBV S variants carrying mutations in the *S* gene generates modified HBsAg, which cannot be recognized by some of the commercially available HBsAg assays. The infection with HBV variants carrying mutations in the promoter area of *S*-gene, including preS1 and/or preS2 area, and the HBV splice variants can reduce HBsAg production/secretion that falls below the detection threshold of currently available assays[42-46]. In addition to viral factors, host immunity also plays an important role in controlling virus replication in the "occult" status[42,47]. The gold standard OBI diagnostic method is the detection of HBV-DNA in liver. However, commonly available methods use blood to detect HBV-DNA. Detection of anti-HBc in blood is often used as a surrogate technique[42]. Integrated HBV in the genome of hepatocytes, which is not replication-competent, might be detected in the blood when cell death occurs after spontaneous or treatment-induced HBsAg clearance. The true OBI should contain the replication-competent HBV-DNA[42]. An assay of over-gap PCR amplification can identify the presence of replication-competent HBV-DNA in blood (rcDNA) and liver tissues (cccDNA), distinguishing the replication-competent HBV-DNA from integrated HBV-DNA[48].

HepB vaccine contains only the small S-HBsAg and does not include the preS1 domain that presents in the infectious virion[49]. Since the implementation of HepB vaccination to Chinese infants in 1992, the HBsAg-positive rate has dropped from 10% to 0.32%, 0.94%, and 4.38% for age groups of 1-4 years, 5-14 years, and 15-29 years, respectively[50]. After 10-15 years of vaccination, the vaccination conferred anti-HBs levels waned or reached undetectable levels in a vast majority of individuals[4,51,52]. A study involving 215,627 Chinese children who received HepB vaccination reported that the HBsAg-positive rate was 0.55% (ranging from 0.30%-0.64%) among those aged 1-9 years, and it increased to 1.40% (ranging from 1.00%-2.04%) in age groups older than 9 years. The positive rate of anti-HBc stayed relatively stable (5.69%) between 1-10 years but increased to 7.80% for age groups of 11-16 years[53]. Among individuals immunized with the HepB vaccine, HBV-DNA was frequently detected in blood samples with serological markers indicating HBsAg-negative but anti-HBc-positive status, suggesting the presence of OBI[43,54-57]. HBV rcDNA in blood is in the form of replication-component HBV[20]. To confirm the presence of true OBI, the method of over-gap PCR amplification was used[48]. It reiterated the presence of HBV rcDNA in the blood of few HepB-vaccinated young adults who tested negative for HBsAg but positive for anti-HBc[48,57]. The serum level of rcDNA among the HepB-vaccinated young adults with OBI was between 100 to 1,000 IU/mL[43,57].

HepB vaccination significantly reduced the risk of HCC by lowering the seroprevalence of HBsAg[3,4,58]. However, the increasing amount of evidence reveals that the individuals who completed the HepB vaccination series during infancy have an increased risk of HBV infection as they age and the infection can
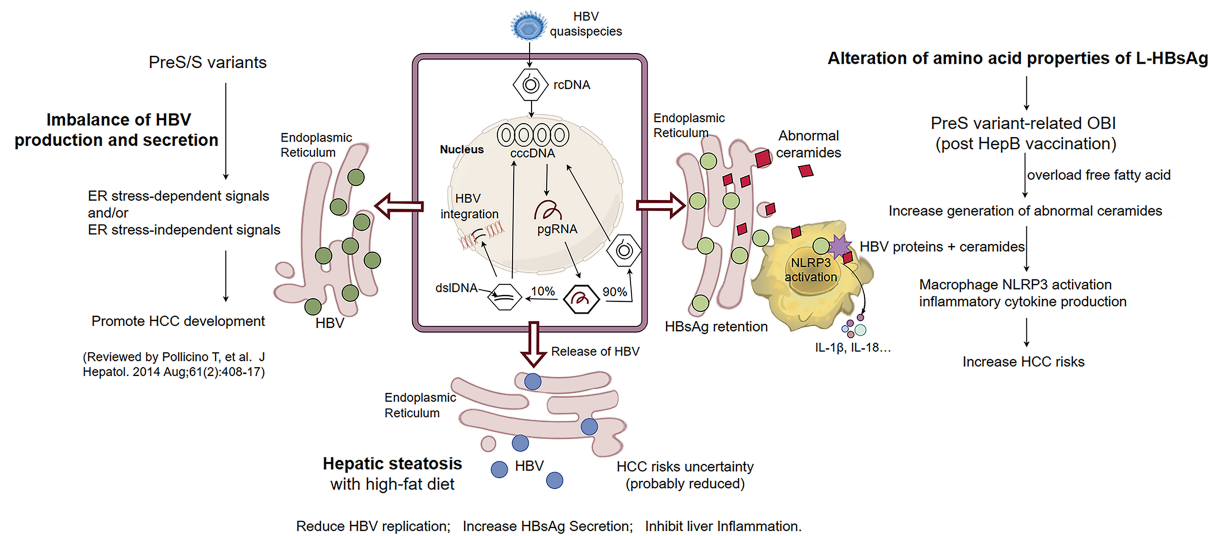
Figure 1. Schematic representation of the impact of HBV infection on HCC development. (1) Hepatic steatosis with high-fat diet can reduce HBV replication, increase HBsAg secretion, and inhibit liver inflammation, and probably reduce the risk of HCC; (2) Some types of HBV preS/S variants can induce an imbalanced HBV production and secretion, leading to ER stress-dependent and/or independent signals to promote HCC development (Reviewed by Pollicino *et al.*[40]); (3) PreS mutations related to OBI post HepB vaccination occur. The OBI-related variants display the alteration of amino acid properties in large-S protein and result in HBsAg retention in hepatocyte ER. PreS variant-infected hepatocytes elevate the generation of abnormal ceramides after overload of free fatty acids. The abnormal ceramides, together with HBV proteins, co-activate NLRP3 inflammasome to increase the production of inflammatory cytokines from liver macrophages, increasing the risk of HCC. HBV: Hepatitis B virus; HCC: hepatocellular carcinoma; HBsAg: hepatitis B surface antigen; ER: endoplasmic reticulum; OBI: occult hepatitis B infection; NLRP3: NOD-like receptor protein 3.

be chronic[43,52,53,59]. A meta-analysis has reported that OBI is significantly associated with an increased risk of HCC[42,60]. Wong reported that 24 of 33 cryptogenic HCC patients (73%) were related to OBI. Among them, HBV was detected more in the non-tumorous than the tumorous tissues. When compared with the HCC patients with overt chronic hepatitis B, intrahepatic pgRNA levels were detected at lower levels. The investigators hypothesized that HBV integration was the likely cause of HCC in OBI patients[61]. In cases of OBI, HBV maintains its pro-oncogenic properties[39]. However, the hepatocarcinogenesis remains to be elucidated.

HepB-VACCINATED INDIVIDUALS WITH OBI WERE FREQUENTLY ASSOCIATED WITH VARIANTS CARRYING MUTATIONS IN THE HBV preS REGION

Some "vaccine-selected escape mutants", in which one amino acid is replaced by another within the "a" determinant, including G145R, have been identified in children receiving the HepB vaccine and they generated anti-HBs[62,63]. In 2009, Mu reported that 5/46 (10.9%) of children were serologically HBsAg-negative but HBV-DNA positive as tested using nested PCR in at least two regions. A low titer of HBV-DNA was detected in five children. Analysis of the *S*-gene sequencing found no G145R, but C139S vaccine escape mutant was detected. Variations and deletions were more common in the preS region of these OBI isolates[55]. In 2010, Xu reported that 124 out of 2,919 (4.2%) young adults receiving the HepB vaccine at infancy were HBsAg-negative but anti-HBs and anti-HBc-positive. Among these 124 individuals, 37 (29.8%) had HBV-DNA at the levels of 500-10,000 copies/mL and 73 (58.9%) had it below 500 copies/mL. After analysis of 41 HBV isolates, the mutations of "a" epitope in S-HBsAg were found to be few but more frequent in the preS region. All the sequences were recorded in GenBank (from HM191578 to HM191618). Alignment analysis showed that most isolates carried mutations that altered the properties of encoded AA[43,52,57,64]. Most of those were seropositive for anti-HBs and carried the mutations at the preS region, particularly in the preS1 area[57].

HEPATOCYTE INFECTION OF preS VARIANTS CAUSES HBsAg RETENTION WITHIN ER, GENERATING ABNORMAL CERAMIDES

In L-HBsAg, the domains located at the preS1 and S region are required for the generation of infectious viral particles and for HBsAg secretion[26]. Pollicino reported that the ratio of HBsAg to HBV-DNA concentrations was significantly lower in patients with *preS/S* gene variants, which included a 183-nucleotide deletion within the preS1 region, the deletion of preS2 start codon, and an anormal stop signal within the *S* gene[41]. Infection of HepG2 cells with these three specified types of specified *preS-S* gene variants resulted in the reduction of HBsAg secretion, retention of envelope proteins in the ER, and decreased efficiency in virion secretion, alongside the significantly increased accumulation of cccDNA in the nucleus. Both experimental data and human studies show that these specified mutations in the *preS/S* gene cause HBsAg retention within the hepatocyte ER, resulting in ER stress[41]. Infection of preS/S variants has been reported to be associated with fulminant hepatitis, fibrosing cholestatic hepatitis, cirrhosis, and HCC development via various mechanisms related to ER stress[40].

PreS-S of HBV from 35 HCC patients related to OBI were sequenced and recognized to carry extensive mutations in the large S area. Most variants carried mutations with altered AA properties of L-HBsAg, leading to the conversion of hydrophobic properties to hydrophilic ones, and vice versa, along with charge modifications[65]. The mutations of HBV isolated from OBI-related HCC were similar to those detected in people who received HepB vaccination during infancy[43,57]. The preS1 domain of L-HBsAg can be either projected toward cytoplasm or oriented toward ER lumen[20,26]. To address the effects of preS mutations, which changed AA properties of L-HBsAg on infected hepatocytes, the mtPreS1 or mtPreS2 plasmids were generated, which contained 1.3 × HBV genome. As a control, the plasmid of Ref-HBV was generated based on an HBV that was isolated from an HCC patient with overt HBsAg-positive HBV infection. When the hepatocytes were infected with the two types of preS variants, HBsAg secretion from hepatocytes reduced compared to the cells infected with Ref-HBV. HBsAg retention was mainly detected in ER compartment.

Hepatocyte ER is intricately linked to a wide spectrum of cellular functions. It is a critical component in maintaining and restoring metabolic health. Therefore, unsolved ER stress can lead to aberrant metabolism, organelle dysfunction, insulin resistance, and inflammation[66]. Three ER transmembrane proteins have been described as ER stress sensors: (1) activating transcription factor 6 (ATF6), (2) inositol-requiring enzyme 1α (IRE1α), and (3) PRKR-like ER kinase (PERK). Upon activation, multiple mechanisms are involved for reinstating the ER homeostasis, such as unfolded protein response (UPR), ER-associated protein degradation (ERAD), or autophagy[66]. In responding to HBsAg retention, the IRE1α-XBP1 pathway, which is key to lipid metabolic homeostasis[66], was found to be significantly activated in hepatocytes[65]. Abnormal accumulation of ceramides is a hallmark of metabolism-related disorders, and the ceramides exert markedly different effects depending on amide-linked fatty acid chain lengths[67]. Using HPLC-MS/MS, we determined the amounts and species of ceramides associated with various lengths of fatty acyl chains. HBsAg retention within ER, caused by preS variations due to AA property alterations in HBV envelop proteins, disturbed hepatocyte lipid metabolism homeostasis. This led to the generation of abnormal amounts and species of ceramides, mainly the C16:0 ceramide[65]. Modern diets are rich in unhealthy fat and ingestion of excessive fat is one of the major risk factors for MAFLD development. In a cell culture system, we observed that loading free fatty acids into hepatocytes infected with hepatitis B virus (HBV), particularly those with preS variants, significantly increased the production of abnormal ceramides[65].

INFECTION OF HBV preS VARIANTS WITH HIGH-FAT DIET SYNERGISTICALLY PROMOTED AUTOCHTHONOUS HCC RELATING TO LIVER INFLAMMATION MEDIATED BY CERAMIDE AND HBV ANTIGENS IN MURINE MODEL

Individuals infected with HBV variants mutated in the preS-S genomic region are frequent. These variants

might result from Hep vaccination or antiviral treatments, or also may occur spontaneously. As discussed above, some HBV variants carry mutations that modify AA properties of L-HBsAg, leading to HBsAg retention in hepatic ER. The impacts of these types of variant infections on HCC development and their underlying mechanisms were examined in male C57BL/6J mice. The mice that were infected with the same amount of replicating plasmids consisting of 1.3 × HBV genome of mtPreS1, or mtPreS2, or Ref-HBV were followed up by giving normal chow (NC) or high-fat (HF) diet for five weeks. A higher ratio of liver weight to body weight was observed in HBV-infected mice, particularly among those infected with the variants at PreS1 or PreS2 regions. Notably, an elevated ratio of liver weight to body weight was also detected in NC-diet mice after infection of preS variants. Intrinsic genetic lesions are crucial for tumor initiation. Mice were then administered carcinogen diethylnitrosamine (DEN) to induce liver cancer[68]. By 14 weeks post DEN injection, liver cancer did not develop in mice that received empty-vector even if they were fed HF diet. After HBV-plasmid injection, liver cancer developed by 14 weeks in all mice that were given the HF diet but not the NC diet. Compared to mice infected with Ref-HBV isolated from an HCC patient with overt HBV infection, those infected with preS variants derived from OBI patients exhibited a higher liver tumor burden. In a subset of HF-diet mice that received DEN and mtPreS1 variants, no liver cancer was detected following myriocin administration for inhibition of ceramide *de novo* synthesis. Notably, mice that did not receive DEN injections did not develop liver cancer by 14 weeks, even when fed the HF diet.

In cancer development, chronic inflammation has been suggested as a factor in different settings[69-71]. As an endogenous "danger signal" to trigger inflammation, ceramide can trigger activation of NOD-like receptor protein 3 (NLRP3), promoting the development of metabolism-related disorders and liver disease progression[67,72]. Owing to their location at the interface in the tissue, liver macrophages constantly receive signals from hepatocytes[73]. The IL-1β and IL-18 production mediated by NLRP3 activation was enhanced in macrophages after stimulation with HBV proteins in the presence of ceramides. HBV proteins and ceramides displayed synergistic effects on the activation of macrophage NLRP3 and inflammatory cytokine production depending on ceramide concentration. In the livers of HF-diet mice, inflammatory macrophages increased threefold compared with liver cells in NC diet-fed mice. Remarkably, NLRP3 activation after HF diet was significantly activated in liver macrophages of HBV-infected mice, especially those with preS variant infection, generating larger amounts of IL-1β and IL-18[65]. These results from murine model suggest that hepatocyte infection with preS variants altered amino acid properties of L-HBsAg and generated abnormal ceramides. In the presence of HBV proteins, ceramides co-activated NLRP3 inflammasome in liver macrophages to promote autochthonous HCC development [Figure 1]. HBV infection, particularly with variants leading to HBV retention, might synergistically promote HCC development in the context of HF diet.

EFFECTS OF OCCULT HEPATITIS B VIRUS INFECTION AND METABOLIC DISORDERS ON HCC DEVELOPMENT: IS THERE A SYNERGISTIC EFFECT?

Over the past two decades, NAFLD/MAFLD has been extensively described in populations from different areas. Experts in this area have proposed the term MAFLD and described the disease as an overarching one. They appreciated that MAFLD is a phenotype with complex and different causes and its pathogenesis is heterogeneous[11]. Due to the high prevalence of HBV infection, many studies in the Asian population also address disease progression *per se* to liver and other body organs/systems in individuals with chronic HBV infection, even among those considered "healthy". Patients with MAFLD have a higher incidence of HCC than those without the disease, and HCC can develop in cases of metabolic syndromes with no liver cirrhosis[74-78]. MAFLD, therefore, has received significant attention and is considered an important risk

factor for HCC[11]. Concurrent fatty liver is common in HBV-infected patients and it is an independent risk factor potentiating HBV-associated HCC development[79]. Obesity, one of the components of MAFLD, was reported to be much stronger in individuals with concomitant HCV infection than in individuals with HBV infection[80]. Since few cases were also positive for HCV[60], a few reviewers questioned the OBI as an independent risk factor for HCC but considered the OBI as a cofactor with HCV infection that promotes HCC development[81]. Nevertheless, clinical and laboratory experimental studies with solitary HBV infection confirm the importance of OBI in HCC development[39,42,61,65].

Due to the crucial role of HBx in HCC development, some laboratory experimental studies and animal models suggested that chronic HBV infection can promote hepatic steatosis, which is mainly dependent on HBx-induced expression of liver fatty acid binding protein[82]. However, clinical data showed that hepatic steatosis and metabolic dysfunction have distinct effects on HCC risk[32,37]. Hepatic steatosis was not associated with liver fibrosis[33], a proven HCC risk factor[1]. In cases of hepatic steatosis induced by high-fat diet, HBV replication was even attenuated, accompanied by reduced serum HBeAg positivity, HBV viremia, and positive staining of intrahepatic HBsAg and HBcAg[29,32,37]. Reduced hepatic steatosis even significantly increased the HCC risk[35]. Although HBV infection in combination with NAFLD for the progression of HCC remains debatable, increasing clinical evidence suggests that HBV retention within hepatocytes might play an important role in promoting HCC development [Figure 1]. Our experimental data indicated that preS variant-related OBI increases the risk of HCC in the context of a high-fat diet and these two factors act synergistically[65]. Molecular mechanisms relating to HBV retention within hepatocytes remain to be clarified. Population-based longitudinal studies are required to clarify the effects of OBI in the context of a high-fat diet, one of the preventable behavioral risk factors leading to MAFLD, such as in OBI individuals after HepB vaccination.

SUMMARY

Antiviral therapies with nucleot(s)ide analogs delay the occurrence of HBV-related diseases including HCC, but cannot cure HBV infections due to the persistence of cccDNA in hepatocytes[18]. As an emerging HCC risk factor, MAFLD is influenced by heterogeneous and multiple factors, including dietary patterns[11,83]. Hepatic ER action is significantly challenged by excess lipid exposure derived from diet[66]. Although the evidence of OBI as an independent risk factor for HCC has been debated[81], experimental data strongly suggest that preS variant-related OBI synergistically promotes HCC development in the context of a high-fat diet[65]. Modern diets are rich in unhealthy fat. To reduce HCC risk, individuals infected with PreS variant-related OBI should pay more attention to preventing metabolic disorders by, at least, improving their diet pattern and receiving proper intervention when metabolic disorders appear. People who tested positive for HBsAg have typically received more focused attention for HCC monitoring to help reduce HCC mortality[84,85]. Going forward, individuals with MAFLD who also test positive for anti-HBc should also be closely monitored for HCC screening.

DECLARATIONS

Authors' contributions
Conceptualized the article, conducted the literature review, and prepared the final draft of the manuscript: Qu C
Performed the literature review and contributed to the writing and editing of the manuscript: Qu C, Chen K

Availability of data and materials
Not applicable.

Conflicts of interest

Qu C is an Editorial Board member of *Hepatoma Research*. The other author declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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