Chapter

# Hepatitis B Virus (HBV) - Induced Hepatocarcinogenesis, a Founding Framework of Cancer Evolution and Development (*Cancer Evo-Dev*)

*Wenbin Liu and Guangwen Cao*

## Abstract

In this chapter, we present the founding framework of a novel theory termed as Cancer Evolution-Development (*Cancer Evo-Dev*), based on the current understanding of hepatitis B virus (HBV) induced hepatocarcinogenesis. The interactions of genetic predispositions and HBV infection is responsible for the maintenance of chronic non-resolving inflammation. Under the inflammatory microenvironment, pro-inflammatory factors trans-activate the expression of cytidine deaminases and suppress the expression of uracil DNA glycosylase. The imbalance between the mutagenic forces and mutation-correcting forces facilitates the generations of somatic mutations, viral mutations, and viral integrations into the host genomes. The majority of cells with genomic mutations and mutated viruses are eliminated in survival competition. Only a small percentage of the mutated cells adapted to the hostile environment can survive, retro-differentiate, and function as cancer-initiating cells, representing a process of "mutation-selection-adaptation". *Cancer Evo-Dev* lays the theoretical foundation for understanding the mechanisms by which chronic infection of HBV promotes hepatocarcinogenesis. This theory also plays an important role in specific prophylaxis, prediction, early diagnosis, and targeted treatment of cancers.

**Keywords:** Hepatocarcinogenesis, hepatitis B virus, inflammation, mutation, evolution

## 1. Introduction

Chronic infection of hepatitis B virus (HBV) is a major cause of hepatocellular carcinoma (HCC). Although the strong etiologic relationship between HBV infection and HCC has been supported by substantial evidence, the underlying mechanism is still elusive. There are more than nine thousand studies investigating HBV-induced hepatocarcinogenesis, which yield 143 genes function in 137 pathways [1]. Most of the studies are one-sided investigations, only a few trying to provide a theoretical hypothesis and to promote the system-level understanding of HBV-induced HCC (HBV-HCC). In past decades, continuous attempts have been made to investigate carcinogenesis from an evolutionary point of view. In 1976, Dr. Nowell first proposed that most neoplasms originate from a single cell. Malignant cells are more genetically unstable than normal cells [2]. In 2006, it was pointed out

that cancer clone genetic diversification and sub-clonal selection occurs within the microenvironment, which is similar to the process of Darwinian natural selection [3]. This viewpoint was put forward mainly based on morphological evidence and only a limited number of gene mutations and related signaling pathways were discussed. The widespread application of new generation sequencing promotes the investigation of genetic diversification and clonal selection within tissue ecosystems. It was found that the number of mutations in cancer range from 10 to hundreds of thousands. The majority of mutations are "passengers" and a small part are "drivers" [4]. Cancer cells acquire a variety of critical phenotypes via driver mutations, which compound to enhance the capabilities of self-renewal, migration, and invasion. The mutational spectra in cancers can reflect the characteristics of the mutational process, including the error-prone repair and genotoxic exposure [5]. Interestingly, the cytidine deaminase induced mutation is dominant in most cancers [6]. Cytidine deaminase is upregulated during inflammation and defense against many viruses, including HBV. Epidemiological and experimental evidence identified the co-evolution of HBV and cancer cells during chronic inflammation. In turn, the mutant cells and viruses also affect the inflammatory microenvironment [7]. Thus, there is a similarity between the process of carcinogenesis and Darwinian evolution. Furthermore, the investigation of cancer evolution can draw upon the understanding of developmental processes. Development is referred to the process that a fertilized egg develops into an individual. In humans, the fertilized diploid cell differentiates into various functional and/or structural cells to form different organs and tissues within 40 weeks. This process resembles the process of long-term organic evolution morphologically, from single cell creatures to multicellular creatures, and from aquatic creatures to terrestrial mammals. Some evolutionarily conserved molecules, like Hedgehog, HOX, and Myc are essential for the developmental process, suggesting evolution and development have similar inherent mechanisms [8–11]. The integration of evolution and developmental biology was termed *Evo-Devo* [12, 13]. In this chapter, we present a scientific theory of Cancer Evolution-Development (*Cancer Evo-Dev*) based on the current understanding of HBV-HCC [14]. This theoretical hypothesis can provide an evolutionary insight of profiling HCC risk and developing more reasonable predictive and prognostic strategies.

## 2. Framework of *Cancer Evo-Dev*

The synergetic effects of genetic predisposition and environmental factors contribute to the imbalance of the immune system, resulting in the activation and maintenance of non-resolving inflammation, that functions as the microenvironment for the *Cancer Evo-Dev*. Activated inflammatory signaling pathways can trans-activate the expression of nucleic acid editing enzymes, such as the human apolipoprotein B mRNA-editing enzyme catalytic polypeptides (APOBECs) family, thus promoting viral and somatic mutations. Viral mutants facilitate the malignant transformation of normal cells. Most mutant cells are eliminated under the selective pressure of the inflammatory microenvironment, while a small proportion of mutated cells survive. These survived mutant clones evolve to tumor-initiating cells by altering the original cell signal patterns, promoting epithelial-mesenchymal transition (EMT), or reprogramming the metabolic patterns, etc. Some established cancer markers, such as α-fetoprotein (AFP) and carcinoembryonic antigen (CEA), are usually expressed at the embryonic stage, silenced after birth, and re-expressed in cancer patients. These pieces of evidence imply that the process of *Cancer Evo-Dev* can be characterized as "backward evolution" and "retro-differentiation".

## 3. Chronic inflammation is indispensable for HBV-HCC evolution

As a defense mechanism responding to exogenous infection and injury, acute inflammation is beneficial to humans. However, chronic inflammation, also termed non-resolving inflammation is essential for carcinogenesis. The weak immunity, HBV mutation, and HBV genotype contribute to the chronicity of inflammation. During the development of HBV-induced HCC evolution, non-resolving inflammation is evident. By relieving hepatic inflammation, antiviral therapy can significantly lower the risks of HCC occurrence and postoperative recurrence [15, 16]. Interestingly, the risk of HCC is still significantly higher in the complete responder group of oral-administered antiviral therapy, compared with the subjects with inactive chronic hepatitis B (CHB) [17]. The active inflammation on chronic infection background also indicated postoperative recurrence [18, 19]. The close association between chronic inflammation and the risk of HCC can be explained from the perspective of *Cancer Evo-Dev*. Cancer evolution is based on two conditions: the continuous acquisition of somatic mutations and natural selection acting on the resultant phenotypic diversity [20]. These two conditions were fulfilled by HBV infection-induced chronic inflammation, that induces mutagenic factors such as APOBECs and provides selection pressure.

### 3.1 Chronicity of HBV infection and hepatic inflammation

The oncogenic capability of HBV is closely related to its capacity to induce and maintain chronic inflammation. The chronicity of HBV infection is dependent on 3 aspects: infection occasion, HBV genotypes, and genetic predisposition of the key immune molecules. HBV infection in early childhood is generally believed to be one of the major causes of chronic HBV infection in adulthood. The perinatal infection occurred in 8.7% and 84.2% of infants born to hepatitis B e-antigen (HBeAg)-positive mothers who did and did not receive immunoprophylaxis, respectively. The infection rates were 0.4% and 6.7% for infants born to HBeAg-negative mothers and HBeAg-positive mothers, respectively. Furthermore, the chronicity of HBV infection acquired perinatally was 28.2% and 64.5% for infants born to HBeAg-negative mothers and HBeAg-positive mothers, respectively [21]. This vulnerability of infants may due to the immaturity of the immune system. Although perinatal HBV infection is an important cause of chronic HBV infection, the chronic transformation of acute hepatitis B is the predominant cause of chronic HBV infection in adults. In China, 8.5% of patients with acute hepatitis B develop into chronic HBV infection 6 months after acute infection [22]. The HBV genotype and genetic predisposition of immune molecules contribute to this transformation.

According to sequence divergence of 8% in the whole viral genome, HBV can be classified into eight genotypes (A to H) [23]. Variant genotypes are distributed unevenly around the world, and the predominant one in mainland China is genotype C (68.3%), followed by genotype B (25.5%) [24]. Under selection pressure from the inflammatory microenvironment, the fates of different HBV genotypes are distinct. Genotype B HBV is prone to causing acute infection, whereas genotype C HBV is associated with chronic infection and contributes independently to the development of HCC [22, 25, 26].

The genetic predisposition of immune molecules is the third major cause of chronic HBV infection. The single nucleotide polymorphisms (SNPs) in the loci encoding human leukocyte antigen class II (HLA-II) are significantly associated with vaccine response as well as the risk of CHB, HBV-induced liver cirrhosis, and HBV-HCC [27–32]. Interestingly, the allele frequencies of SNPs affecting the expression of HLA-DP and HLA-DQ are variant in different human races. The polymorphic

genotypes that are more frequent in the Han Chinese than in European populations are significantly associated with the increased risk of chronicity of HBV infection as well as the immune selection of HBV mutations related to end-stage liver diseases [21]. These data suggest that the Han Chinese are inherently more apt to progress into chronic infection once exposed to HBV infection than Europeans. This might be partly responsible for the fact that chronic HBV infection, HBV-induced liver cirrhosis, and HBV-HCC are more frequent in Chinese than in European populations. The genetic polymorphisms of HLA-II may facilitate the progression of CHB into HCC through predisposing immune imbalance and maintain HBV infection. Due to the chronicity of inflammation, the mutagenic force that serves as antiviral immunity is prone to injury the human genome, thus induce *Cancer Evo-Dev.*

### 3.2 HBV promote the generation of inflammatory mutations

The APOBECs are powerful endogenous mutagenic factors that can catalyze irreversible cytidine and deoxycytidine deamination to convert bases from cytosine to uracil, creating a cytosine-to-uracil mismatch in minus-strand and reverse-transcript G-to-A (guanosine-to-adenosine) transitions in plus-stranded DNA. APOBEC3s play important roles in the innate immune system [7]. Mutagenesis mediated by APOBEC3s can increase the viral mutation load to a level that exceeds the threshold for viral viability. Accordingly, APOBEC3s can similarly increase the number of somatic mutations to a threshold that exceeds the host's repair ability and starts the *Cancer Evo-Dev*. Three mechanisms prevent the induction of somatic mutations by the APOBEC3s family. First, APOBEC3s rarely express in normal tissues, and short-term activation of APOBEC3s is beneficial for eliminating pathogens. Second, the cytidine deaminase activity of APOBEC3s is applied almost exclusively to single-stranded nucleotides, in which mutagenesis is 200–300 times more efficient than it is in double-stranded DNA. Third, the uracil-induced mutagenesis of APOBEC3s is counteracted by uracil–DNA glycosylase (UNG), that plays an important role in the base-excision repair mechanism [7, 33]. However, genetic susceptibility, viral mutations, and an unbalanced immune system interact with each other to prevent the absolute elimination of HBV, resulting in chronic inflammation accompanied with APOBEC3s expression. During the HBV-induced malignant transformation, inflammatory signaling pathways including interleukin 6 (IL-6)/signal transducer and activator of transcription 3 (STAT3) and tumor necrosis factor α (TNF-α)/ nuclear factor kappa B (NF-κB) are activated, which up-regulate the expression of APOBEC3s [7]. Among the members of APOBEC3s, APOBEC3B was identified as the major subtype responsible for the APOBEC-signature somatic mutations in multiple cancers [34]. The mutagenic effect of inflammatory factors on the HBV genome depends on the degree of the damage to the APOBEC3B–UNG balance. IL-6 can increase the expression of APOBEC3B and decrease the expression of UNG. The functional polymorphisms located in the *APOBEC3B* promoter (rs2267401-G) and *UNG* enhancer (rs3890995-C) predispose the IL-6 induced APOBEC3B-UNG imbalance and increase the risk of HCC [35].

### 3.3 HBV affects the selection pressure of the inflammatory microenvironment

In an inflammatory microenvironment, continuous necrosis and proliferation can help to accumulate somatic mutations, and tumor-initiating cell clones with strong viability are selected. HBV replication directly reflects the selective stress and influences the evolution of HCC. It has been revealed by various studies that HBV DNA load increases the risk of HCC in CHB patients [25, 36]. A high level of HBV DNA load either in serum or liver tissue predicts poor postoperative prognosis

in HCC [37]. Meanwhile, HBV in turn affecting the selective pressure of the inflammatory microenvironment. The innate immune and adaptive immune against HBV are both participate in the selection of malignant cells. During the chronic infection of HBV, APOBEC3B is stimulated and reduces the occupancy of H3K27me3 on the promoter of CC-chemokine ligand 2 (CCL2). By this mechanism, APOBEC3B upregulates the CCL2 to enhance the recruitment of tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs). TAMs and MDSCs suppress the function of $CD8^+$T cells and are associated with a poor prognosis of HCC [38]. HBV can transmit into natural killer (NK) cells through exosomes, thereby inducing the disfunction of NK cells, which promotes the HCC evolution [39, 40]. HBV also induced the exhaustion of HBV-specific $CD8^+$ T cells through impairing the mitochondrial functions, including electron transport, membrane transport, and the transcription of mitochondrial DNA [41]. The glucose metabolism of T cells is reprogramed by HBV, which leads to increased lactate production and decreased migration of T cells [42]. During chronic infection, HBV promotes the recruitment of regulatory T cells (Tregs) through activating the growth factor-beta (TGF-β)/ miR-34a/ CC-motif chemokine ligand 22 (CCL22) axis [43]. The increased Tregs suppress HCC antigen-specific immune responses and HBV antigen-specific immune response at the same time [44]. Thus, the HBV that survives the survival competition can in turn affect the inflammatory microenvironment.

## 4. Roles of HBV mutation during the process of HCC evolution

During HBV-induced hepatocarcinogenesis, viruses also experience the process of evolution. Viral evolution serves as a valuable clue to investigate the mechanism underlying the HBV-HCC [45]. The generation and accumulation of HBV mutations abide by the Darwinian model: mutation-selection–adaptation. In the inflammatory microenvironment, most HBV mutants are eliminated by the antiviral immune response. Only a tiny fraction of mutant viruses that facilitate the regeneration of hepatocytes can survive and gradually develop into the HCC-promoting clones.

### 4.1 The generation of HBV mutation

Two major mechanisms are responsible for the generation of HBV mutation. The first pattern is the replicative errors. During viral replication, the partially double-stranded HBV DNA is generated from an intermediate RNA through the reverse transcription activity of the viral polymerase. Due to lack of proofreading capacity, the HBV genome has a higher mutation rate than other DNA viruses, which is in the range of $1.5 \times 10^{-5}$ to $5 \times 10^{-5}$ nucleotide substitutions per site per year, which can increase after HBeAg seroconversion [46]. The second viral mutation pattern is induced by host cytidine deaminases [7]. The APOBEC family has a dual effect on HBV: reduction of HBV and induction of HBV mutations [47]. The expression levels of APOBEC3s are positively correlated with the quasispecies complexity of HBV [48]. The genetic polymorphisms predisposing the IL-6 induced APOBEC3B-UNG imbalance significantly promote the generation of HCC related HBV mutations [35]. Although many HBV genome fragments, including the Enhancer II (EnhII) / basal core promoter (BCP)/ precore region and the S region, are generally sensitive to editing by members of APOBEC3 [49–53], the sequence encoding HBV X protein (HBx) is more vulnerable. APOBEC3 prefers the HBx region as its editing target and generates carboxylic acid–terminal truncated HBx (Ct-HBx). Although most HBV mutations are random, the directional evolution of HBV occurs under the selective pressures of chronic inflammation. In the immune tolerance phase of

chronic infection, the immune pressure is weak, and most of the individual viruses are wild-type. Immune pressure increases with the progression of chronic inflammation, which facilitates the gradual occurrence of viral mutations, especially in HBeAg-negative individuals [54, 55]. HCC-related HBV mutations are selected by the immune microenvironment before the occurrence of HCC and can be used as predictive markers. Single-nucleotide polymorphisms of the inflammatory signaling pathway genes, including STAT3, NF-κB, HLA-DP, and HLA-DQ have been demonstrated to maintain the chronic infection and to facilitate the selection of HCC-related HBV mutations that contribute to the risk of liver cancer [32, 56, 57]. However, those viral mutants that affect the pre-cancer hepatocytes are less infectious to normal liver cells, which leads to a process of "dead-end" evolution.

### 4.2 The "dead-end" evolution of HBV

Hepatitis B virus belongs to the Hepadnaviridae family and is evolutionarily conservative in the long-term evolution of species [58]. However, the evolution of the HBV genome is evident in infected individuals during chronic infection. Previous research by our group established the wild-type HBV sequences of HBV subgenotypes B2 and C2, based on the whole HBV genome sequenced using 1000 asymptomatic carriers of the HBV surface antigen from community-based epidemiologic surveys. Based on the wild-type HBV sequences, HCC-related mutations and their development patterns were subsequently identified. We also observed that HBV mutations posing a significant HCC risk are located mainly within the BCP and preS regions [59–61]. During the HBV-induced carcinogenic "trilogy" (chronic hepatitis, liver cirrhosis, HCC), the species and frequencies of those mutations often accumulate consecutively and can be used to predict the occurrence and development of liver cirrhosis and HCC [15, 32, 56, 62]. Retrospective and prospective cohort studies have both identified a combination of HBV mutations (C1653T, A1762T/G1764A, and T1753V) that have significant predictive value [32, 63, 64]. Among them, the A1762T/G1764A mutation usually appears in the early stage; other mutations, including T1753V, C1653T, preS deletion, are evident only in a late stage of the evolution [65]. Reaction to chronic HBV infection (characterized by the immune response–induced hepatocyte injury and release of transaminase) is usually accompanied by HBeAg seroconversion and an increase in HBV mutations, indicating the selective effect of immune cells on viral mutants. The deficiency of $CD8^+$ T cell epitopes is one of the main features of HBV mutations. The mutant virus with a low density of $CD8^+$ T cells epitopes can evade immune eradication [66, 67]. The proportion of mutant preS/S region is higher in patients with occult HBV infection than in CHB patients [67, 68]. Therefore, $CD^+8$ T cell is essential for the immune selection of HCC-related HBV mutants.

Hepatitis B virus acquired during infancy or early childhood, or at the early infection stage in adults, is usually the wild type [15, 32, 56]. During the chronic inflammation process, especially after an HBeAg shift from HBeAg-positive to HBeAg-negative, mutant HBV subgroups gradually increase. Although the HCC-related HBV mutants are present in fetal cord blood, neonatal infection is usually caused by wild-type HBV rather than by mutant subgroups. At 1–15 years in HBV-infected children, the frequencies of HCC-related mutations increase with increasing age. However, compared with their mothers, who have been exposed to chronic infection for at least about 25 years, the children have fewer HCC-related HBV mutations [65]. The foregoing results are based on analyses of serum HBV. In individuals with chronic HBV infection, most all HBV is synthesized in hepatocytes and released into the circulation at a pace of up to $10^{11}$ viral particles daily [69]. The immune microenvironment of circulation, tumor tissue, and tumor-adjacent

liver tissue are all necessary for the HBV evolution [48]. Interestingly, HBV evolves more advanced in the sera than in the tumors of HCC patients. The evolutionary similarity between the sera-derived HBV strains and adjacent tissue-derived ones is significantly stronger than that between sera-derived HBV strains and tumor-derived ones [48]. Although tumor-adjacent tissues are pathologically categorized as "normal," they are typical precancerous lesions and have already entered the middle stage of the cancer evolutionary process. The HCCs that relapse more than 2 years after resection are considered to be recurrent HCC and not a result of the initial HCC cell diffusion into remnant liver tissue [18]. The species and frequencies of certain HBV mutations in adjacent tissues are distinct in the different populations. Together with immune markers and expression levels of inflammatory genes, they can therefore be used to predict prognosis in HCC patients receiving curative surgery. For example, HBV mutations in the EnhII/BCP/PreC region, such as A1762T/G1764A, can serve as predictive markers for survival and recurrence [18], indicating that HBV evolution in adjacent tissues continues until the patient dies. Antiviral therapy can block HBV evolution in adjacent tissues by easing inflammation and notably prolongs survival in HCC patients [15].

Taken together, the Hepadnaviridae family members are highly conservative across species [65]. Wild-type HBV has the advantage of infecting hepatocytes, facilitating viral spread from one individual to another, and contributing to the maintenance of its viral species. The HCC-related mutants can cause malignant transformation but have lost the advantage of person-to-person infection. Those mutants are therefore usually eliminated at the death of the carriers, which is termed "dead-end" evolution.

### 4.3 High-risk HBV mutations promote the *Cancer Evo-Dev*

During hepatocarcinogenesis, high-risk HBV mutations are selected by the immune microenvironment. Because of overlapping open reading frames, HBV mutations altering the genes necessary for viral replication are unlikely transferred into their progeny viruses. Natural selection ensures only the fittest survive to pass their genes on to the next generation. Thus, the random natural mutations are therefore constrained to special regions of the HBV genome, especially in the fragment of HBx gene and large envelope protein gene fragment (preS1/preS2/S). These HBV mutations that survive the selective pressure can promote the evolution of HCC, which is supported by many pieces of evidence from epidemiology studies and mechanism studies.

Previous longitudinal studies, especially cohort studies, support that combo HBV mutations including A1762T/G1764A, C1653T, and T1753V in HBx gene in sera can predict the occurrence of HCC [64, 70]. The mutations in the HBV preS fragment, including the preS deletion, accumulate during the process of inflammation-HCC transformation, which is significantly associated with increased risk of HCC [62, 71, 72]. Epidemiological evidence identified the interaction effect between HBV mutations and genetic polymorphisms of immune molecules. For the population with the infection of genotype B HBV, the SNPs of HLA-DP, including rs3077 (T allele), rs2281388 (T allele), rs3135021 (G allele), and rs9277535 (G allele) can promote the HBV persistence and are associated with a higher prevalence of HBV mutation increasing HCC risk. Moreover, the effects of HBV mutations on HCC risk are selectively significant in subjects with these HLA-DP SNPs that promote HBV persistence [32]. For the population with the infection of genotype C HBV, the HLA-DQ SNP, rs9275319 (GG genotype), is significantly associated with an increased prevalence of preS1 start codon mutation, an HCC-risk mutation [63]. The SNPs of STAT3 SNPs appear to promote HCC evolution

in the host with HBV mutations [56]. The interaction effect of STAT3 rs1053004 with T1674C/G and the interaction effect of STAT3 rs4796793 with preS2 start codon mutation are both significantly associated with an increased HCC risk. The T allele of rs223406 impairs the promoter activity of NFKBIA, a key molecule of the NF-κB signaling pathway. The interaction of rs223406 T allele with A1762T/G1764A is significantly associated with an increased risk of HCC [57]. The genetic polymorphisms predisposing the imbalance of APOBEC3B and UNG increase the risk of HCC through through facilitate the generation of APOBEC3B-signature HBV mutations. Furthermore, the positive rate of APOBEC-signature HBV mutations consecutively increased from asymptomatic HBsAg carrier (ASC) to HCC in HBV-infected subjects [35]. This line of evidence highlights the important role of HBV mutation in the process of HCC evolution.

Experimental evidence also confirms that HBV mutation can endow the hepatocytes with a survival advantage. The HBx with A1762T/G1764A–based combo mutations can upregulate the expression of S-phase kinase-associated protein 2 (SKP2) by activating E2F1, a transcription factor, downregulate cell cycle inhibitors, and facilitate the ubiquitin-mediated proteasomal degradation of p21, thereby enhancing the proliferation of HCC cells [73, 74]. Moreover, HBx with A1762T/G1764A–based combo mutations also enhance the cell migration through activating the Wnt/β-catenin signaling pathway [75]. Ct-HBx mutation can promote cell metastasis and invasiveness by activating the C-Jun/matrix metalloproteinase protein 10 signaling pathway [15, 76]. The HBx gene with K130M/V131I mutations enhances HCC evolution by activating the arachidonic acid metabolism and the hypoxia-inducible factor-1a [77, 78]. Besides the mutated HBx gene, the mutated preS1, preS2, and S regions also notably facilitate carcinogenesis [18, 61]. The preS2 region with F141L can significantly downregulate the expression of the p53 pathway and upregulate the expression of cyclin-dependent kinase 4 and cyclin A, thereby promoting proliferation and colony-forming rates [79]. The accumulation of mutant envelop protein in the endoplasmic reticulum (ER) leads to the activation of ER stress signaling pathway [80]. ER stress promotes HCC evolution through generating reactive oxygen species (ROS), inducing oxidative DNA damage, and ultimately increasing genomic instability [81, 82]. Although HBV mutation plays important role in hepatocarcinogenesis, somatic mutation of the human genome is the direct cause of cell evolution.

## 5. Roles of somatic mutation during the process of HCC evolution

The spontaneous rate of somatic mutations is not high enough to trigger the evolution process. HBV participates in the alteration of the host genome, both directly and indirectly. First, HBV can cause somatic mutations by directly integrating into the human genome. Second, mutant HBV contributes to the maintenance of non-resolving inflammation, that induces long-term up-regulation of APOBECs [7]. Somatic mutations can be classified according to their effects on *Cancer Evo-Dev*. A small proportion of the mutations can lead to advantageous phenotypes that are positively selected during the evolution process and thus are called “driver” mutations. The remaining mutations are “passengers” that contribute very little to carcinogenesis [4]. Due to survival competition and the positive selection of the inflammatory microenvironment, driver mutations accumulate sufficiently to promote malignant transformation. The distribution, combination, and dynamic patterns of driver mutations reflex the pressure of microenvironmental selection and growth advantage of cell subsets. As HCC has many etiological causes and experiences a long evolutionary process, the somatic mutation spectrum is most heterogeneous [6, 83]. The driver somatic mutations affect multiple functions, like signaling pathways, EMT, and energy metabolism.

### 5.1 Somatic mutations alter "stem-ness" signaling pathways

Based on the investigations of whole-exome sequencing, it is found that the somatic mutation in HCC evolution mainly altering six cancer related pathways: signaling pathway related with telomere maintenance, Wnt/b-catenin pathway, P53 and cell cycle pathway, oxidative stress pathway, epigenome modifiers, RAS/RAF/mitogen-activated protein kinase pathway, and PI3K/AKT/mTOR pathways [84]. Among them, the somatic mutation related to telomeres pathway is most frequent. Telomerase is activated in more than 90% HCC patients. Somatic mutation within the promoter of telomerase reverse transcriptase (*TERT*) is the major cause with the prevalence ranging from 54–60%. The second cause is the HBV integration in the *TERT* promoter, which is observed in 10–15% of HCC patients. Interestingly, the mutation of catenin beta 1 (*CTNNB1*) is more frequent in hepatitis C virus induced HCC, indicated a different way of *Cancer Evo-Dev* [85, 86]. The frequencies of mutation in other hot genes range from 5–20%. Although the spectrums and frequencies of altered genes vary greatly among individuals, they are usually clustered to pathways or functional groups that are closely related to stem-ness and embryonic characteristics. In this regard, global mutation rates of functionally related genes are added together to define the mutation rate of a given signaling pathway. Mutation rates of Wnt/β-catenin, p53/cell cycle control, JAK/STAT, and PI3k/mTOR pathways range from 12–72%. Similar outstanding outcomes are also observed in functional gene groups of chromatin remodeling and telomere maintenance. Therefore, it is promising to use combo somatic mutations as predictive and prognostic biomarkers just like gene signatures [19].

### 5.2 Somatic mutations affect HCC evolution through regulating EMT

APOBECs can promote gene demethylation and remove epigenetic memory to stabilize the pluripotent state in embryonic stem cells through deaminating 5-methylcytosine (5mC) or 5-hydroxymethylcytosine (5hmC) [87, 88]. EMT is a landmark event of *Cancer Evo-Dev*, which is driven by transcription factors, like ZEB1, ZEB2, SNAI1, and SNAI2. AID, a member of the APOBECs family, is upregulated by inflammatory signals and induces demethylation of the promoters of ZEB1, ZEB2, SNAI1, and SNAI2. Silencing AID leads to increased methylation of CpG island proximal to the promoters of these EMT regulators, thus inhibits EMT and invasion of cells [89]. AID-induced, CpG methylation-dependent mutagenesis is proven to be a common feature of cancer evolution [90]. Therefore, it is reasonable to postulate that re-expression of embryonic factors in cancers might result from epigenetic reprogramming caused by APOBECs family, that is upregulated by proinflammatory factors.

### 5.3 Somatic mutations reprogram energy metabolism

To support the rapid growth of malignant cells, tumor tissues prefer to use glycolysis for energy production, even in the presence of oxygen. Glucose is more easily to be metabolized to lactate in tumor tissues than in normal tissues. This pattern of energy metabolism was identified in 1920 and was termed as Warburg effect [91]. Warburg effect in TAMs promotes vascular network formation, augments extravasation of tumor cells out of blood vessels, and induces higher levels of EMT at inflammatory foci within the tumor [92]. In the microenvironment with both hypoxia and hypoglycemia, stem cell-, angiogenic-, and EMT-biomarkers, as well as glycoprotein-P content and invasiveness of cancer cells are enhanced [93]. Thus, we believe that the Warburg effect promotes the evolutionary process of cancer under both hypoxia and hypoglycemia conditions. The Warburg effect can provide

essential energy for cell survival in a hostile microenvironment, furthermore, glycolysis generates the raw material for DNA synthesis of progeny cells. HBV infection and somatic mutation are both the possible origin of Warburg phenotype. In HBV-HCC, the major pattern of single nucleotide variants in mitochondrial DNA (mtDNA) is C > T, that is the character of APOBEC induced mutation. This kind of mutation mainly occurs in the D-loop region of mtDNA and promotes the proliferation, invasion, and metastasis of HCC cells [94]. Pyruvate kinase M2 (PKM2), an alternatively spliced variant of the pyruvate kinase gene that is preferentially expressed during embryonic development and in cancer cells, alters the final rate-limiting step of glycolysis, resulting in the cancer-specific Warburg effect [95]. Besides the Warburg effect, HCC cells also enhance other patterns of energy metabolism during evolution. For example, the inactivating mutation of ribosomal S6 kinase 2 (RSK2) can support cholesterol metabolism in HCC [96].

### 5.4 HBV integration

HBV integration is a kind of somatic mutation that is specific to the HBV-induced *Cancer Evo-Dev.* Although the HCC in an individual can be monoclonal, HBV integration is common in most clones, indicating it is the early driver event for HCC evolution [83]. The HBV integration can be detected in 85–90% of HBV-HCC patients [97]. Moreover, the prevalence of HBV integration is 60–75% in HCCs from patients with occult HBV infection, indicating the HBV integration contributes to the occult HBV infection induced HCC [98, 99]. Approximately five thousand HBV integration events have been reported and more than half of them locate in the intergenic regions. Only the HBV integration events within thirteen genes are repeated in diverse studies [1]. *TERT*, mixed-lineage leukemia 4 (*MLL4*), fibronectin 1 (*FN1*), cyclin E1 (*CCNE1*), and cyclin A2(*CCNA2*) are the top five most frequently integrated genes [85, 100–105]. The X and core genes of HBV are the regions that most frequently insert into the human genome [103, 105]. Cis-activation of host genes is an important mechanism by which HBV integration promotes HCC evolution. The highest frequency of HBV integration is observed in the promoter region of *TERT* [85, 100–105]. The HBV integration within the *TERT* promoter leads to an increased mRNA level of *TERT*, that is significantly associated with a poor prognosis of HCC [103, 105]. MLL4 is the second most frequently integrated gene and the HBV integration mainly locate in the introns and exons [85, 105]. Since *MLL* gene family has methyltransferase activity, the HBV integration within *MLL4* may promote HCC evolution in an epigenetic way. As the third most frequently integrated gene, *FN1* is reported to create a microenvironment promoting metastasis of lung cancer [106]. Most HBV integration events within *FN1* are detected in the adjacent tissues of HCC, indicating these mutations may contribute to the microenvironment of the early stage of HCC evolution [85, 101]. HBV integration is associated with an increased expression of *CCNE1*, that is reported to promote hepatic inflammation and hepatocarcinogenesis [107]. The HBV-*CCNA2* chimeric transcript encodes a chimeric protein promoting cell cycle progression [108]. Besides affecting the expression or function of coding genes, HBV integration within the region of long interspersed nuclear elements (LINEs) can generate HBx-LINE1 chimeric transcript acting as long non-coding RNA (lncRNA). This lncRNA increases the activity of the Wnt pathway through decrease the level of miR-122 [104]. The DNA fragment with HBV integration can be used as a circulating biomarker of HCC recurrence. The HBV-host chimera DNA can be detected in more than 90% of HCC patients before surgery. After the surgery, HBV-host chimera DNA can still be detected in 20% of HCC patients, which may come from the mutant hepatocytes at the early stage of evolution and are significantly associated with HCC recurrence [109]. Thus,

most HBV integration occurs randomly. The integration mutations that endow the hepatocytes with survival advantage will have the opportunity of accumulation.

As mentioned above, hepatocarcinogenesis involves the co-evolution of HBV and transformed cells. The interaction between somatic mutation and HBV mutation occurs during this process. The deletion, duplication, and translocation are observed near the insertion site of integrated HBV fragments [84]. The frequency of HBV mutation is positive associated with the level of HBV integration. The prevalence of HBx mutation is significantly higher in patients with HBV integration in *TERT* promoter (35%) than in patients without these integration events (19.8%) [83]. There are studies reporting the selective expression of mutant HBx and preS2 genes in the tumor tissues from patients with occult HBV infection [110]. These pieces of evidence support that the integration and selection of mutant HBV fragments play important roles in the HCC evolution.

## 6. Conclusion

Based on studies of HBV-induced hepatocarcinogenesis (a typical evolutionary process), we put forward the theory of *Cancer Evo-Dev*. Under conditions of genetic predisposition, exogenous factors such as viral infection can induce chronic inflammation. The elimination of chronic infection can relieve inflammation, reducing the incidence of cancer and subsequently extending effective survival. As the theory describes, tumor-initiating cells obtain survival advantage during the evolutionary process of mutation-selection–adaptation by activating a "stem-ness" pathway and simultaneously causing evolutionary heterogeneity. Critical molecules in a functional subnetwork that maintains and promotes the *Cancer Evo-Dev* process can be demonstrated using systems biology approaches. The development of high-efficiency inhibitors that will target these critical molecules and block corresponding signal pathways could be a powerful treatment strategy in advanced cancers. The theory of *Cancer Evo-Dev* will serve three purposes: first, the early prevention that reduces the cancer incidence and delays its onset; second, targeted therapy that reduces morbidity and mortality rates. Therefore, this theory can contribute to the realization of "P4 pattern" medicine (predictive, preventive, personalized, and participatory).

## Acknowledgements

This work was supported by grant 2015CB554006 from the National Key Basic Research Program of China, grants 91529305, 81520108021, and 81673250, and 81521091 from the National Natural Science Foundation of China.

## Conflict of interest

The authors declare no conflict of interest.

IntechOpen

## Author details

Wenbin Liu and Guangwen Cao*
Department of Epidemiology, Second Military Medical University, Shanghai, China

*Address all correspondence to: gcao@smmu.edu.cn

IntechOpen

## References

[1] Lee WY, Bachtiar M, Choo CCS, Lee CG. Comprehensive review of Hepatitis B Virus-associated hepatocellular carcinoma research through text mining and big data analytics. Biol Rev Camb Philos Soc. 2019;94(2):353-367. DOI: 10.1111/brv.12457

[2] Nowell PC. The clonal evolution of tumor cell populations. Science. 1976;194(4260):23-28. DOI: 10.1126/science.959840

[3] Merlo LM, Pepper JW, Reid BJ, Maley CC. Cancer as an evolutionary and ecological process. Nat Rev Cancer. 2006;6(12):924-935. DOI: 10.1038/nrc2013

[4] Greaves M, Maley CC. Clonal evolution in cancer. Nature. 2012;481(7381):306-313. DOI: 10.1038/nature10762

[5] Stratton MR. Exploring the genomes of cancer cells: progress and promise. Science. 2011;331(6024):1553-1558. DOI: 10.1126/science.1204040

[6] Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. Nature. 2013;500(7463):415-421. DOI: 10.1038/nature12477

[7] Deng Y, Du Y, Zhang Q, Han X, Cao G. Human cytidine deaminases facilitate hepatitis B virus evolution and link inflammation and hepatocellular carcinoma. Cancer letters. 2014;343(2):161-171. DOI: 10.1016/j.canlet.2013.09.041

[8] Wang S, Zhang J, Jiao W, Li J, Xun X, Sun Y, et al. Scallop genome provides insights into evolution of bilaterian karyotype and development. Nat Ecol Evol. 2017;1(5):120. DOI: 10.1038/s41559-017-0120

[9] Deschamps J, Duboule D. Embryonic timing, axial stem cells, chromatin dynamics, and the Hox clock. Genes Dev. 2017;31(14):1406-1416. DOI: 10.1101/gad.303123.117

[10] Lettice LA, Devenney P, De Angelis C, Hill RE. The Conserved Sonic Hedgehog Limb Enhancer Consists of Discrete Functional Elements that Regulate Precise Spatial Expression. Cell Rep. 2017;20(6):1396-1408. DOI: 10.1016/j.celrep.2017.07.037

[11] Zhong C, Zhou YK, Yang SS, Zhao JF, Zhu XL, Chen HH, et al. Developmental expression of the N-myc downstream regulated gene (Ndrg) family during Xenopus tropicalis embryogenesis. Int J Dev Biol. 2015;59(10-12):511-517. DOI: 10.1387/ijdb.150178xh

[12] Raff RA. Evo-devo: the evolution of a new discipline. Nat Rev Genet. 2000;1(1):74-79. DOI: 10.1038/35049594

[13] Carroll SB. Evo-devo and an expanding evolutionary synthesis: a genetic theory of morphological evolution. Cell. 2008;134(1):25-36. DOI: 10.1016/j.cell.2008.06.030

[14] Cao GW. Cancer Evo-Dev, a novel hypothesis derived from studies on hepatitis B virus-induced carcinogenesis. Hepatoma Res. 2017;3:241-259. DOI: 10.20517/2394-5079.2017.45

[15] Yin J, Li N, Han Y, Xue J, Deng Y, Shi J, et al. Effect of antiviral treatment with nucleotide/nucleoside analogs on postoperative prognosis of hepatitis B virus-related hepatocellular carcinoma: a two-stage longitudinal clinical study. J Clin Oncol. 2013;31(29):3647-3655. DOI: 10.1200/JCO.2012.48.5896

[16] Chen LP, Zhao J, Du Y, Han YF, Su T, Zhang HW, et al. Antiviral treatment to prevent chronic hepatitis B or C-related hepatocellular carcinoma. World J Virol.

2012;1(6):174-183. DOI: 10.5501/wjv.v1.i6.174

[17] Cho JY, Paik YH, Sohn W, Cho HC, Gwak GY, Choi MS, et al. Patients with chronic hepatitis B treated with oral antiviral therapy retain a higher risk for HCC compared with patients with inactive stage disease. Gut. 2014; 63(12):1943-1950. DOI: 10.1136/gutjnl-2013-306409

[18] Chen L, Zhang Q, Chang W, Du Y, Zhang H, Cao G. Viral and host inflammation-related factors that can predict the prognosis of hepatocellular carcinoma. European journal of cancer. 2012;48(13):1977-1987. DOI: 10.1016/j.ejca.2012.01.015

[19] Liu WB, Yang F, Shao DY, Cao GW. Novel predictive and prognostic strategies of hepatitis B virus related hepatocellular carcinoma. Hepatoma Res. 2016;2:331-340. DOI: 10.20517/2394-5079.2016.38

[20] Gatenby RA, Gillies RJ, Brown JS. Of cancer and cave fish. Nat Rev Cancer. 2011;11(4):237-238. DOI: 10.1038/nrc3036

[21] Li Z, Hou X, Cao G. Is mother-to-infant transmission the most important factor for persistent HBV infection? Emerg Microbes Infect. 2015;4(5):e30. DOI: 10.1038/emi.2015.30

[22] Zhang HW, Yin JH, Li YT, Li CZ, Ren H, Gu CY, et al. Risk factors for acute hepatitis B and its progression to chronic hepatitis in Shanghai, China. Gut. 2008;57(12):1713-1720. DOI: 10.1136/gut.2008.157149

[23] Norder H, Courouce AM, Coursaget P, Echevarria JM, Lee SD, Mushahwar IK, et al. Genetic diversity of hepatitis B virus strains derived worldwide: genotypes, subgenotypes, and HBsAg subtypes. Intervirology. 2004;47(6):289-309. DOI: 10.1159/000080872

[24] Yin J, Zhang H, He Y, Xie J, Liu S, Chang W, et al. Distribution and hepatocellular carcinoma-related viral properties of hepatitis B virus genotypes in Mainland China: a community-based study. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2010; 19(3):777-786. DOI: 10.1158/1055-9965.EPI-09-1001

[25] Chan HL, Tse CH, Mo F, Koh J, Wong VW, Wong GL, et al. High viral load and hepatitis B virus subgenotype ce are associated with increased risk of hepatocellular carcinoma. J Clin Oncol. 2008;26(2):177-182. DOI: 10.1200/JCO.2007.13.2043

[26] Chan HL, Hui AY, Wong ML, Tse AM, Hung LC, Wong VW, et al. Genotype C hepatitis B virus infection is associated with an increased risk of hepatocellular carcinoma. Gut. 2004; 53(10):1494-1498. DOI: 10.1136/gut.2003.033324

[27] Kamatani Y, Wattanapokayakit S, Ochi H, Kawaguchi T, Takahashi A, Hosono N, et al. A genome-wide association study identifies variants in the HLA-DP locus associated with chronic hepatitis B in Asians. Nat Genet. 2009;41(5):591-595. DOI: 10.1038/ng.348

[28] Guo X, Zhang Y, Li J, Ma J, Wei Z, Tan W, et al. Strong influence of human leukocyte antigen (HLA)-DP gene variants on development of persistent chronic hepatitis B virus carriers in the Han Chinese population. Hepatology. 2011;53(2):422-428. DOI: 10.1002/hep.24048

[29] Hu Z, Liu Y, Zhai X, Dai J, Jin G, Wang L, et al. New loci associated with chronic hepatitis B virus infection in Han Chinese. Nat Genet. 2013;45(12):1499-1503. DOI: 10.1038/ng.2809

[30] Png E, Thalamuthu A, Ong RT, Snippe H, Boland GJ, Seielstad M. A genome-wide association study of

hepatitis B vaccine response in an Indonesian population reveals multiple independent risk variants in the HLA region. Hum Mol Genet. 2011;20(19): 3893-3898. DOI: 10.1093/hmg/ddr302

[31] Jiang DK, Sun J, Cao G, Liu Y, Lin D, Gao YZ, et al. Genetic variants in STAT4 and HLA-DQ genes confer risk of hepatitis B virus-related hepatocellular carcinoma. Nat Genet. 2013;45(1):72-75. DOI: 10.1038/ng.2483

[32] Zhang Q, Yin J, Zhang Y, Deng Y, Ji X, Du Y, et al. HLA-DP polymorphisms affect the outcomes of chronic hepatitis B virus infections, possibly through interacting with viral mutations. J Virol. 2013;87(22):12176-12186. DOI: 10.1128/JVI.02073-13

[33] Siriwardena SU, Chen K, Bhagwat AS. Functions and Malfunctions of Mammalian DNA-Cytosine Deaminases. Chem Rev. 2016;116(20):12688-12710. DOI: 10.1021/acs.chemrev.6b00296

[34] Kuong KJ, Loeb LA. APOBEC3B mutagenesis in cancer. Nat Genet. 2013;45(9):964-965. DOI: 10.1038/ng.2736

[35] Liu W, Wu J, Yang F, Ma L, Ni C, Hou X, et al. Genetic Polymorphisms Predisposing the Interleukin 6-Induced APOBEC3B-UNG Imbalance Increase HCC Risk via Promoting the Generation of APOBEC-Signature HBV Mutations. Clin Cancer Res. 2019;25(18):5525-5536. DOI: 10.1158/1078-0432.CCR-18-3083

[36] Yang HI, Yuen MF, Chan HL, Han KH, Chen PJ, Kim DY, et al. Risk estimation for hepatocellular carcinoma in chronic hepatitis B (REACH-B): development and validation of a predictive score. Lancet Oncol. 2011;12(6):568-574. DOI: 10.1016/S1470-2045(11)70077-8

[37] Yeh CT, So M, Ng J, Yang HW, Chang ML, Lai MW, et al. Hepatitis B virus-DNA level and basal core promoter A1762T/G1764A mutation in liver tissue independently predict postoperative survival in hepatocellular carcinoma. Hepatology. 2010;52(6):1922-1933. DOI: 10.1002/hep.23898

[38] Wang D, Li X, Li J, Lu Y, Zhao S, Tang X, et al. APOBEC3B interaction with PRC2 modulates microenvironment to promote HCC progression. Gut. 2019;68(10):1846-1857. DOI: 10.1136/gutjnl-2018-317601

[39] Yang Y, Han Q, Hou Z, Zhang C, Tian Z, Zhang J. Exosomes mediate hepatitis B virus (HBV) transmission and NK-cell dysfunction. Cell Mol Immunol. 2017;14(5):465-475. DOI: 10.1038/cmi.2016.24

[40] Zhang QF, Yin WW, Xia Y, Yi YY, He QF, Wang X, et al. Liver-infiltrating CD11b(−)CD27(−) NK subsets account for NK-cell dysfunction in patients with hepatocellular carcinoma and are associated with tumor progression. Cell Mol Immunol. 2017;14(10):819-829. DOI: 10.1038/cmi.2016.28

[41] Fisicaro P, Barili V, Montanini B, Acerbi G, Ferracin M, Guerrieri F, et al. Targeting mitochondrial dysfunction can restore antiviral activity of exhausted HBV-specific CD8 T cells in chronic hepatitis B. Nat Med. 2017;23(3):327-336. DOI: 10.1038/nm.4275

[42] Masson JJ, Billings HW, Palmer CS. Metabolic reprogramming during hepatitis B disease progression offers novel diagnostic and therapeutic opportunities. Antivir Chem Chemother. 2017;25(2):53-57. DOI: 10.1177/2040206617701372

[43] Yang P, Li QJ, Feng Y, Zhang Y, Markowitz GJ, Ning S, et al. TGF-beta-miR-34a-CCL22 signaling-induced Treg cell recruitment promotes venous metastases of HBV-positive hepatocellular carcinoma. Cancer Cell. 2012;22(3):291-303. DOI: 10.1016/j.ccr.2012.07.023

[44] Zhang HH, Mei MH, Fei R, Liu F, Wang JH, Liao WJ, et al. Regulatory T cells in chronic hepatitis B patients affect the immunopathogenesis of hepatocellular carcinoma by suppressing the anti-tumour immune responses. J Viral Hepat. 2010;17 Suppl 1:34-43. DOI: 10.1111/j.1365-2893.2010.01269.x

[45] Yang F, Ma LT, Cao GW. Hepatocellular carcinoma: co - evolution of hepatocytes and hepatitis B virus. Zhonghua Gan Zang Bing Za Zhi. 2017;25(5):321-324. DOI: 10.3760/cma.j.issn.1007-3418.2017.05.001

[46] Orito E, Mizokami M, Ina Y, Moriyama EN, Kameshima N, Yamamoto M, et al. Host-independent evolution and a genetic classification of the hepadnavirus family based on nucleotide sequences. Proc Natl Acad Sci U S A. 1989;86(18):7059-7062. DOI: 10.1073/pnas.86.18.7059

[47] Noguchi C, Imamura M, Tsuge M, Hiraga N, Mori N, Miki D, et al. G-to-A hypermutation in hepatitis B virus (HBV) and clinical course of patients with chronic HBV infection. J Infect Dis. 2009;199(11):1599-1607. DOI: 10.1086/598951

[48] Yin J, Chen X, Li N, Han X, Liu W, Pu R, et al. Compartmentalized evolution of hepatitis B virus contributes differently to the prognosis of hepatocellular carcinoma. Carcinogenesis. 2021;42(3):461-470. DOI: 10.1093/carcin/bgaa127

[49] Vartanian JP, Henry M, Marchio A, Suspene R, Aynaud MM, Guetard D, et al. Massive APOBEC3 editing of hepatitis B viral DNA in cirrhosis. PLoS Pathog. 2010;6(5):e1000928. DOI: 10.1371/journal.ppat.1000928

[50] Suspene R, Guetard D, Henry M, Sommer P, Wain-Hobson S, Vartanian JP. Extensive editing of both hepatitis B virus DNA strands by APOBEC3 cytidine deaminases in vitro and in vivo. Proc Natl Acad Sci U S A. 2005;102(23):8321-8326. DOI: 10.1073/pnas.0408223102

[51] Beggel B, Munk C, Daumer M, Hauck K, Haussinger D, Lengauer T, et al. Full genome ultra-deep pyrosequencing associates G-to-A hypermutation of the hepatitis B virus genome with the natural progression of hepatitis B. J Viral Hepat. 2013;20(12):882-889. DOI: 10.1111/jvh.12110

[52] Kock J, Blum HE. Hypermutation of hepatitis B virus genomes by APOBEC3G, APOBEC3C and APOBEC3H. J Gen Virol. 2008;89(Pt 5):1184-1191. DOI: 10.1099/vir.0.83507-0

[53] Reuman EC, Margeridon-Thermet S, Caudill HB, Liu T, Borroto-Esoda K, Svarovskaia ES, et al. A classification model for G-to-A hypermutation in hepatitis B virus ultra-deep pyrosequencing reads. Bioinformatics. 2010;26(23):2929-2932. DOI: 10.1093/bioinformatics/btq570

[54] Han YF, Zhao J, Ma LY, Yin JH, Chang WJ, Zhang HW, et al. Factors predicting occurrence and prognosis of hepatitis-B-virus-related hepatocellular carcinoma. World J Gastroenterol. 2011;17(38):4258-4270. DOI: 10.3748/wjg.v17.i38.4258

[55] Hannoun C, Horal P, Lindh M. Long-term mutation rates in the hepatitis B virus genome. J Gen Virol. 2000;81(Pt 1):75-83. DOI: 10.1099/0022-1317-81-1-75

[56] Xie J, Zhang Y, Zhang Q, Han Y, Yin J, Pu R, et al. Interaction of signal transducer and activator of transcription 3 polymorphisms with hepatitis B virus mutations in hepatocellular carcinoma. Hepatology. 2013;57(6):2369-2377. DOI: 10.1002/hep.26303

[57] Zhang Q, Ji XW, Hou XM, Lu FM, Du Y, Yin JH, et al. Effect of functional nuclear factor-kappaB genetic polymorphisms on hepatitis B virus

persistence and their interactions with viral mutations on the risk of hepatocellular carcinoma. Ann Oncol. 2014;25(12):2413-2419. DOI: 10.1093/annonc/mdu451

[58] Schaefer S. Hepatitis B virus taxonomy and hepatitis B virus genotypes. World J Gastroenterol. 2007; 13(1):14-21. DOI: 10.3748/wjg.v13.i1.14

[59] Yin J, Xie J, Liu S, Zhang H, Han L, Lu W, et al. Association between the various mutations in viral core promoter region to different stages of hepatitis B, ranging of asymptomatic carrier state to hepatocellular carcinoma. Am J Gastroenterol. 2011;106(1):81-92. DOI: 10.1038/ajg.2010.399

[60] Yin J, Xie J, Zhang H, Shen Q, Han L, Lu W, et al. Significant association of different preS mutations with hepatitis B-related cirrhosis or hepatocellular carcinoma. J gastroenterol. 2010;45(10): 1063-1071. DOI: 10.1007/s00535-010-0253-1

[61] Liu S, Xie J, Yin J, Zhang H, Zhang Q, Pu R, et al. A matched case-control study of hepatitis B virus mutations in the preS and core promoter regions associated independently with hepatocellular carcinoma. J Med Virol. 2011;83(1):45-53. DOI: 10.1002/jmv.21829

[62] Liu S, Zhang H, Gu C, Yin J, He Y, Xie J, et al. Associations between hepatitis B virus mutations and the risk of hepatocellular carcinoma: a meta-analysis. J Natl Cancer Inst. 2009; 101(15):1066-1082. DOI: 10.1093/jnci/djp180

[63] Ji X, Zhang Q, Li B, Du Y, Yin J, Liu W, et al. Impacts of human leukocyte antigen DQ genetic polymorphisms and their interactions with hepatitis B virus mutations on the risks of viral persistence, liver cirrhosis, and hepatocellular carcinoma. Infect Genet Evol. 2014;28:201-209. DOI: 10.1016/j.meegid.2014.09.032

[64] Yin J, Wang J, Pu R, Xin H, Li Z, Han X, et al. Hepatitis B Virus Combo Mutations Improve the Prediction and Active Prophylaxis of Hepatocellular Carcinoma: A Clinic-Based Cohort Study. Cancer Prev Res. 2015;8(10):978-988. DOI: 10.1158/1940-6207.CAPR-15-0160

[65] Li Z, Xie Z, Ni H, Zhang Q, Lu W, Yin J, et al. Mother-to-child transmission of hepatitis B virus: evolution of hepatocellular carcinoma-related viral mutations in the post-immunization era. J Clin Virol. 2014;61(1):47-54. DOI: 10.1016/j.jcv.2014.06.010

[66] Maman Y, Blancher A, Benichou J, Yablonka A, Efroni S, Louzoun Y. Immune-induced evolutionary selection focused on a single reading frame in overlapping hepatitis B virus proteins. J Virol. 2011;85(9):4558-4566. DOI: 10.1128/JVI.02142-10

[67] Chen BF. Hepatitis B virus pre-S/S variants in liver diseases. World J Gastroenterol. 2018;24(14):1507-1520. DOI: 10.3748/wjg.v24.i14.1507

[68] Huang X, Ma C, Zhang Q, Shi Q, Huang T, Liu C, et al. Impact of "a" determinant mutations on detection of hepatitis B surface antigen (HBsAg) in HBV strains from Chinese patients with occult hepatitis B. Journal of medical virology. 2017;89(10):1796-1803. DOI: 10.1002/jmv.24859

[69] Nowak MA, Bonhoeffer S, Hill AM, Boehme R, Thomas HC, McDade H. Viral dynamics in hepatitis B virus infection. Proc Natl Acad Sci U S A. 1996;93(9): 4398-4402. DOI: 10.1073/pnas.93.9.4398

[70] Sung FY, Lan CY, Huang CJ, Lin CL, Liu CJ, Chen PJ, et al. Progressive accumulation of mutations in the hepatitis B virus genome and its impact on time to diagnosis of hepatocellular carcinoma. Hepatology. 2016;64(3):720-731. DOI: 10.1002/hep.28654

[71] Chen CH, Hung CH, Lee CM, Hu TH, Wang JH, Wang JC, et al. Pre-S

deletion and complex mutations of hepatitis B virus related to advanced liver disease in HBeAg-negative patients. Gastroenterology. 2007;133(5):1466-1474. DOI: 10.1053/j.gastro.2007.09.002

[72] Liu WC, Wu IC, Lee YC, Lin CP, Cheng JH, Lin YJ, et al. Hepatocellular carcinoma-associated single-nucleotide variants and deletions identified by the use of genome-wide high-throughput analysis of hepatitis B virus. J Pathol. 2017;243(2):176-192. DOI: 10.1002/path.4938

[73] Huang Y, Tong S, Tai AW, Hussain M, Lok AS. Hepatitis B virus core promoter mutations contribute to hepatocarcinogenesis by deregulating SKP2 and its target, p21. Gastroenterology. 2011;141(4):1412-1421, 1421 e1411-1415. DOI: 10.1053/j.gastro.2011.06.048

[74] Huang Y, Tai AW, Tong S, Lok AS. HBV core promoter mutations promote cellular proliferation through E2F1-mediated upregulation of S-phase kinase-associated protein 2 transcription. J Hepatol. 2013;58(6):1068-1073. DOI: 10.1016/j.jhep.2013.01.014

[75] Chen Z, Tang J, Cai X, Huang Y, Gao Q, Liang L, et al. HBx mutations promote hepatoma cell migration through the Wnt/beta-catenin signaling pathway. Cancer Sci. 2016;107(10):1380-1389. DOI: 10.1111/cas.13014

[76] Sze KM, Chu GK, Lee JM, Ng IO. C-terminal truncated hepatitis B virus x protein is associated with metastasis and enhances invasiveness by C-Jun/matrix metalloproteinase protein 10 activation in hepatocellular carcinoma. Hepatology. 2013;57(1):131-139. DOI: 10.1002/hep.25979

[77] Chiu AP, Tschida BR, Sham TT, Lo LH, Moriarity BS, Li XX, et al. HBx-K130M/V131I Promotes Liver Cancer in Transgenic Mice via AKT/FOXO1 Signaling Pathway and Arachidonic Acid Metabolism. Mol Cancer Res. 2019;17(7):1582-1593. DOI: 10.1158/1541-7786.MCR-18-1127

[78] Liu LP, Hu BG, Ye C, Ho RL, Chen GG, Lai PB. HBx mutants differentially affect the activation of hypoxia-inducible factor-1alpha in hepatocellular carcinoma. Br J Cancer. 2014;110(4):1066-1073. DOI: 10.1038/bjc.2013.787

[79] Huang J, Deng Q, Wang Q, Li KY, Dai JH, Li N, et al. Exome sequencing of hepatitis B virus-associated hepatocellular carcinoma. Nat Genet. 2012;44(10):1117-1121. DOI: 10.1038/ng.2391

[80] Pollicino T, Cacciola I, Saffioti F, Raimondo G. Hepatitis B virus PreS/S gene variants: pathobiology and clinical implications. J Hepatol. 2014;61(2):408-417. DOI: 10.1016/j.jhep.2014.04.041

[81] Hsieh YH, Su IJ, Wang HC, Chang WW, Lei HY, Lai MD, et al. Pre-S mutant surface antigens in chronic hepatitis B virus infection induce oxidative stress and DNA damage. Carcinogenesis. 2004;25(10):2023-2032. DOI: 10.1093/carcin/bgh207

[82] Wang LH, Huang W, Lai MD, Su IJ. Aberrant cyclin A expression and centrosome overduplication induced by hepatitis B virus pre-S2 mutants and its implication in hepatocarcinogenesis. Carcinogenesis. 2012;33(2):466-472. DOI: 10.1093/carcin/bgr296

[83] Cui X, Wei W, Wang C, Qi Y, Qin X, Huang L, et al. Studies on the correlation between mutation and integration of HBV in hepatocellular carcinoma. Biosci Rep. 2020;40(8). DOI: 10.1042/BSR20201988

[84] Levrero M, Zucman-Rossi J. Mechanisms of HBV-induced hepatocellular carcinoma. J Hepatol. 2016;64(1):S84-S101. DOI: 10.1016/j.jhep.2016.02.021

[85] Sung WK, Zheng H, Li S, Chen R, Liu X, Li Y, et al. Genome-wide survey of recurrent HBV integration in hepatocellular carcinoma. Nat Genet. 2012; 44(7):765-769. DOI: 10.1038/ng.2295

[86] Totoki Y, Tatsuno K, Covington KR, Ueda H, Creighton CJ, Kato M, et al. Trans-ancestry mutational landscape of hepatocellular carcinoma genomes. Nat Genet. 2014;46(12):1267-1273. DOI: 10.1038/ng.3126

[87] Nabel CS, Jia H, Ye Y, Shen L, Goldschmidt HL, Stivers JT, et al. AID/APOBEC deaminases disfavor modified cytosines implicated in DNA demethylation. Nat Chem Biol. 2012;8(9):751-758. DOI: 10.1038/nchembio.1042

[88] Kumar R, DiMenna L, Schrode N, Liu TC, Franck P, Munoz-Descalzo S, et al. AID stabilizes stem-cell phenotype by removing epigenetic memory of pluripotency genes. Nature. 2013; 500(7460):89-92. DOI: 10.1038/nature12299

[89] Munoz DP, Lee EL, Takayama S, Coppe JP, Heo SJ, Boffelli D, et al. Activation-induced cytidine deaminase (AID) is necessary for the epithelial-mesenchymal transition in mammary epithelial cells. Proc Natl Acad Sci U S A. 2013;110(32):E2977-E2986. DOI: 10.1073/pnas.1301021110

[90] Rogozin IB, Lada AG, Goncearenco A, Green MR, De S, Nudelman G, et al. Activation induced deaminase mutational signature overlaps with CpG methylation sites in follicular lymphoma and other cancers. Sci Rep. 2016;6:38133. DOI: 10.1038/srep38133

[91] Koppenol WH, Bounds PL, Dang CV. Otto Warburg's contributions to current concepts of cancer metabolism. Nat Rev Cancer. 2011;11(5):325-337. DOI: 10.1038/nrc3038

[92] Penny HL, Sieow JL, Adriani G, Yeap WH, See Chi Ee P, San Luis B, et al. Warburg metabolism in tumor-conditioned macrophages promotes metastasis in human pancreatic ductal adenocarcinoma. Oncoimmunology. 2016;5(8):e1191731. DOI: 10.1080/2162402X.2016.1191731

[93] Marin-Hernandez A, Gallardo-Perez JC, Hernandez-Resendiz I, Del Mazo-Monsalvo I, Robledo-Cadena DX, Moreno-Sanchez R, et al. Hypoglycemia Enhances Epithelial-Mesenchymal Transition and Invasiveness, and Restrains the Warburg Phenotype, in Hypoxic HeLa Cell Cultures and Microspheroids. J Cell Physiol. 2017;232(6):1346-1359. DOI: 10.1002/jcp.25617

[94] Yin C, Li DY, Guo X, Cao HY, Chen YB, Zhou F, et al. NGS-based profiling reveals a critical contributing role of somatic D-loop mtDNA mutations in HBV-related hepatocarcinogenesis. Ann Oncol. 2019;30(6):953-962. DOI: 10.1093/annonc/mdz105

[95] Garcia-Heredia JM, Carnero A. Decoding Warburg's hypothesis: tumor-related mutations in the mitochondrial respiratory chain. Oncotarget. 2015;6(39):41582-41599. DOI: 10.18632/oncotarget.6057

[96] Chan LK, Ho DW, Kam CS, Chiu EY, Lo IL, Yau DT, et al. RSK2-inactivating mutations potentiate MAPK signaling and support cholesterol metabolism in hepatocellular carcinoma. J Hepatol. 2021;74(2):360-371. DOI: 10.1016/j.jhep.2020.08.036

[97] Pollicino T, Saitta C, Raimondo G. Hepatocellular carcinoma: the point of view of the hepatitis B virus. Carcinogenesis. 2011;32(8):1122-1132. DOI: 10.1093/carcin/bgr108

[98] Saitta C, Tripodi G, Barbera A, Bertuccio A, Smedile A, Ciancio A, et al. Hepatitis B virus (HBV) DNA integration in patients with occult HBV infection and hepatocellular carcinoma.

Liver Int. 2015;35(10):2311-2317. DOI: 10.1111/liv.12807

[99] Wong DK, Cheng SCY, Mak LL, To EW, Lo RC, Cheung TT, et al. Among Patients with Undetectable Hepatitis B Surface Antigen and Hepatocellular Carcinoma, a High Proportion Has Integration of HBV DNA into Hepatocyte DNA and No Cirrhosis. Clin Gastroenterol Hepatol. 2020;18(2):449-456. DOI: 10.1016/j.cgh.2019.06.029

[100] Bonilla Guerrero R, Roberts LR. The role of hepatitis B virus integrations in the pathogenesis of human hepatocellular carcinoma. J Hepatol. 2005;42(5):760-777. DOI: 10.1016/j.jhep.2005.02.005

[101] Ding D, Lou X, Hua D, Yu W, Li L, Wang J, et al. Recurrent targeted genes of hepatitis B virus in the liver cancer genomes identified by a next-generation sequencing-based approach. PLoS Genet. 2012;8(12):e1003065. DOI: 10.1371/journal.pgen.1003065

[102] Jiang Z, Jhunjhunwala S, Liu J, Haverty PM, Kennemer MI, Guan Y, et al. The effects of hepatitis B virus integration into the genomes of hepatocellular carcinoma patients. Genome Res. 2012;22(4):593-601. DOI: 10.1101/gr.133926.111

[103] Toh ST, Jin Y, Liu L, Wang J, Babrzadeh F, Gharizadeh B, et al. Deep sequencing of the hepatitis B virus in hepatocellular carcinoma patients reveals enriched integration events, structural alterations and sequence variations. Carcinogenesis. 2013;34(4):787-798. DOI: 10.1093/carcin/bgs406

[104] Lau CC, Sun T, Ching AK, He M, Li JW, Wong AM, et al. Viral-human chimeric transcript predisposes risk to liver cancer development and progression. Cancer Cell. 2014;25(3):335-349. DOI: 10.1016/j.ccr.2014.01.030

[105] Zhao LH, Liu X, Yan HX, Li WY, Zeng X, Yang Y, et al. Genomic and oncogenic preference of HBV integration in hepatocellular carcinoma. Nat Commun. 2016;7:12992. DOI: 10.1038/ncomms12992

[106] Liu J, Cao L, Meng J, Li Y, Deng P, Pan P, et al. The fibrotic microenvironment promotes the metastatic seeding of tumor cells into the lungs via mediating the ZEB1-AS1/miR-200b-3p/ZEB1 signaling. Cell Cycle. 2020;19(20):2701-2719. DOI: 10.1080/15384101.2020.1826236

[107] Ehedego H, Mohs A, Jansen B, Hiththetiya K, Sicinski P, Liedtke C, et al. Loss of Cyclin E1 attenuates hepatitis and hepatocarcinogenesis in a mouse model of chronic liver injury. Oncogene. 2018;37(25):3329-3339. DOI: 10.1038/s41388-018-0181-8

[108] Chiu YT, Wong JK, Choi SW, Sze KM, Ho DW, Chan LK, et al. Novel pre-mRNA splicing of intronically integrated HBV generates oncogenic chimera in hepatocellular carcinoma. J Hepatol. 2016;64(6):1256-1264. DOI: 10.1016/j.jhep.2016.02.005

[109] Li CL, Ho MC, Lin YY, Tzeng ST, Chen YJ, Pai HY, et al. Cell-Free Virus-Host Chimera DNA From Hepatitis B Virus Integration Sites as a Circulating Biomarker of Hepatocellular Cancer. Hepatology. 2020;72(6):2063-2076. DOI: 10.1002/hep.31230

[110] Hatazawa Y, Yano Y, Okada R, Tanahashi T, Hayashi H, Hirano H, et al. Quasispecies variant of pre-S/S gene in HBV-related hepatocellular carcinoma with HBs antigen positive and occult infection. Infect Agent Cancer. 2018;13:7. DOI: 10.1186/s13027-018-0179-4